Poster Session II
Tuesday, December 6, 2011 5:30 PM – 7:30 PM

1. A Zebrafish Model for the Functional Analysis of Genes in Autism

Ellen J. Hoffman*, Antonio Giraldez, Matthew State

Yale University, New Haven, USA

Background: A critical challenge in the genetics of neuropsychiatric disorders is distinguishing deleterious rare mutations from neutral variants, as rare sequence and structural variants have been identified throughout the genomes of both affected and unaffected individuals, including at candidate gene loci. The ability to distinguish rare functional from rare neutral variation is critical for confirming the association of risk genes carrying rare alleles. For this reason, we propose to develop an *in vivo* model that will allow us rapidly to differentiate mutations that alter the function of susceptibility genes from neutral rare variants. This novel approach capitalizes on critical advantages of zebrafish, including visualization of the developing nervous system in transparent embryos, ease of genetic manipulation, and large progenies that facilitate the conduct of large-scale pharmacologic screens. Therefore, we generated zebrafish knockouts of the ASD risk gene, *CNTNAP2*, using the emerging technology of zinc finger nucleases (ZFN). We anticipate that *CNTNAP2* will be particularly informative in this regard, as homozygous disruption of *CNTNAP2* by a single base pair deletion in the Old Order Amish population causes a monogenic syndrome that is highly associated with ASD. In addition, the State laboratory identified a *de novo* chromosome 7q inversion disrupting *CNTNAP2* in a child with cognitive and social delay. Our goals in developing this model are: 1) to leverage any distinctive reproducible and quantifiable phenotype for forward genetic studies that will help to elaborate conserved molecular mechanisms and pathways involving these susceptibility genes; and 2) to test the relative ability of the wild type human gene compared to constructs containing rare mutations identified in affected and unaffected individuals to rescue the identified phenotype.
Methods: We identified the zebrafish orthologs of the human *CNTNAP2* gene by conducting a search of the zebrafish genome (Zv7) in the National Center for Biotechnology Information (NCBI) database. We analyzed the expression patterns of these paralogs in zebrafish embryos at 30 and 48 hours post fertilization by *in situ* hybridization. We utilized ZFN directed against exons 2 and 3 of *CNTNAP2a* and *CNTNAP2b*, respectively, to generate targeted germline deletions in each gene. Founders were generated by injecting mRNA encoding ZFN (Sigma-Aldrich) targeting either *CNTNAP2a* or *2b* into embryos at the one-cell stage. Founders were identified by screening the progeny of incrosses of ZFN-injected adult fish by PCR followed by high-resolution fragment analysis. Founders were outcrossed to wild-type fish, and the heterozygous *CNTNAP2a* and *2b* knockouts were incrossed to generate viable homozygous knockouts.
Results: We identified two zebrafish orthologs of *CNTNAP2*, *CNTNAP2a* and *2b*. *In situ* analysis of these transcripts between 30 and 48 hours after fertilization revealed expression of both paralogs in the CNS, predominantly in the midbrain and hindbrain. These paralogs demonstrate distinct yet partially overlapping expression patterns. Utilizing ZFN targeting each gene, we generated multiple zebrafish founders with deleterious germline mutations in both *CNTNAP2a* and *2b* and outcrossed these founders to wild-type fish, producing viable heterozygous

CNTNAP2a and *2b* knockouts. These mutations are predicted to be damaging as they occur early in the coding regions of each gene and produce a frameshift, resulting in a premature stop codon and truncation of the protein in or immediately after the N-terminal discoidin domain. In total, we have successfully generated zebrafish founders with deleterious germline mutations in both *CNTNAP2* paralogs. We are currently conducting a battery of morphological and behavioral assays to identify quantifiable phenotypes in CNS structure and larval neural circuits in mutant fish.
Discussion: Our experiments lay the foundation for the use of zebrafish as a model system for elucidating the function of susceptibility genes in ASD. We have identified the zebrafish orthologs of the ASD susceptibility gene, *CNTNAP2*, demonstrated its expression in the zebrafish CNS, and successfully generated the first ZFN-induced deletions in each *CNTNAP2* paralog. This model has tremendous promise for illuminating common pathways involving ASD susceptibility genes and rapidly assessing the functional consequences of rare sequence variation in a risk gene. Future applications of this research include large-scale pharmacological screens to identify novel therapeutic targets aimed at the mechanisms underlying the core deficits of ASD.
Disclosure: E. Hoffman: none. **A. Giraldez:** none. **M. State:** none.

2. Histidine Decarboxylase Deficiency produces Tourette Syndrome Phenomenology and Dopamine Dysregulation in Humans and Mice

Lissandra C. Baldan Ramsey, Kyle Williams, Jean-Dominique Gallezot, Michael Crowley, George Anderson, Bennett L. Leventhal, Hiroshi Ohtsu, John H. Krystal, Linda Mayes, Ivan de Araujo, Yu-Shin Ding, Matthew W. State, Christopher Pittenger*

Yale University, New Haven, USA

Background: Tourette syndrome (TS) is a neurodevelopmental disorder characterized by tics and sensory gating abnormalities. Available treatments are limited, and its neurobiological underpinnings are not well understood. Pathophysiological analysis of such conditions in animal models can provide key insight; however, modeling complex neuropsychiatric conditions presents daunting challenges. TS has a heritability of 30-60%, but causative mutations and risk alleles have proven elusive, attesting to a complex, heterogeneous genetic architecture. In this setting, the investigation of rare, highly penetrant mutations is of particular value. A recent study of a dense 2-generation TS pedigree identified a rare segregating nonsense mutation, *Hdc* W317X, in the *l-histidine decarboxylase (Hdc)* gene (Ercan –Sencicek *et al.*, *NEJM* 2010). The causal connection between a reduction in HDC activity and the symptoms of TS remains unclear. Here we analyze mice lacking one or both copies of the *Hdc* gene and TS patients carrying the *Hdc* W317X mutation to establish that reduction in histamine (HA) biosynthesis produces key phenomenological and neurochemical features of TS.
Methods: 9 TS patients carrying the *Hdc* W317X mutation were characterized clinically and in prepulse inhibition (PPI). Adult male HDC knockout mice, backcrossed multiple generations onto the C57Bl/6 genetic background, were tested in parallel behavioral assays (stereotypy; PPI). Striatal dopamine in knockout mice was assessed by *in vivo* microdialysis. Dopamine D2/D3 receptors were quantified *in vivo* in patients carrying the *Hdc* W317X mutation by positron emission tomography (PET) with the D3-preferring ligand PHNO, and *in vitro* in mice using the D2/D3 ligand raclopride.

Results: All patients carrying the *Hdc* W317X mutation had current and/or past tics; patients also had a deficit in auditory PPI, as has been shown in TS more generally. Heterozygous and homozygous knockout mice had reduced brain histamine and exhibited parallel behavioral symptomatology, with enhanced stereotypy after amphetamine administration and a deficit in PPI. Mice showed increased dopamine in the dorsal striatum during the active (dark) phase of the circadian cycle, when histamine was high in wild-type controls. Both patients and mice showed an elevation in D2/D3 receptor binding in the substantia nigra; this is consistent with the upregulation of D3 receptors in this structure seen in other contexts after chronic dopamine elevation.

Discussion: Parallel behavioral abnormalities in patients and mice establish a casual link between disruption of the *Hdc* gene and TS-like behavioral abnormalities, validating the unexpected connection first revealed by genetic linkage in a single family. Dysregulation of striatal dopamine represents a plausible pathophysiological link between histamine deficiency and functional abnormalities in the basal ganglia circuitry previously implicated in TS. Alterations in D2/D3 receptors, present both in patients carrying the *Hdc* W317X mutation and in haploinsufficient and knockout mice, further support the generality and relevance of this abnormality. These data establish new mouse model of TS, which has clear face and construct validity. While *Hdc* mutations appear to be a very rare cause of TS, we anticipate that further mechanistic investigations in this model may shed light on the pathophysiology of the disorder more generally.

3. Poor and Unstable Sustained Attentional Performance in Sign-Trackers: An Animal Model of Poor Top-Down Cognitive Control of Attention

Giovanna Paolone, Christopher C. Angelakos, Paul J. Meyer, Terry E. Robinson, Martin Sarter*

University of Michigan, Department of Psychology, Ann Arbor, USA

Background: Sustained attentional performance requires the detection of target cues, the filtering of non-target stimuli, continued orientation toward the source of target cues, and the suppression of competing cognitive activities and of alternative and competitive behaviors. A weakening of such "top-down", "executive" or "cognitive control" mechanisms results in distractibility and attentional lapses, impulsivity and, generally, attentional fatigue. Such impairments in attention are the cardinal cognitive symptoms of ADHD, schizophrenia and other disorders. Modeling these symptoms in animals has remained rare, yet would be of significance for neuropsychopharmacological research. "Sign-tracking" rats (STs) are screened from outbred populations based on high propensity to attribute incentive salience to conditional stimuli, relative to"goal-trackers" (GTs). STs more readily develop addiction-like behavior and are prone to impulsive action, and therefore,

we hypothesized that they may also have poor attentional control. Indeed, impulsivity may be conceptualized as a consequence of such poor top-down control of attention. We therefore characterized the attentional capacities of STs and GTs using our translational Sustained Attention Task (SAT; e.g., Demeter *et al.*, 2008, 2011).

Methods: Sprague-Dawley rats were screened using a Pavlovian Conditioned Approach (PCA) procedure and designated STs or GTs, as described previously (e.g., Lovic *et al.*, 2011). Animals were then trained on the SAT (see Demeter *et al.*, 2008). This task consists of random sequences of unpredictably occurring visual signals of variable duration as well as trials during which no signal is presented (blanks). Following a signal or non-signal event two levers are extended into the chambers for 4 sec, and subjects report the presence or absence of a signal by pressing the appropriate lever. Hits and correct rejections are rewarded while misses and false alarms trigger a variable intertrial interval. Daily sessions lasted 64 min and consisted of approximately 318 trials total. Animals were extensively trained until their performance stabilized and then over-trained for several additional weeks. Measures of performance included the percent hits/misses, correct rejections/false alarms, and errors of omission. In addition, the SAT combines the individual measures into a single score that varies from 1.0 (perfect performance) to zero (random response selection).

Results: Both STs and GTs learned and performed the task. However, relative to GTs, STs had marked difficulty sustaining attentional performance. In terms of overall SAT scores, as well as the relative number of hits, STs performance was significantly lower than GTs. On this task all rats show periods of very high and very poor levels of performance, defined by SAT scores >0.4 and <0.2, respectively. These periods typically last about 3 min. The frequency of periods of particularly high levels of performance did not differ between the two groups. However, the number of poor performance periods, during which performance dropped to levels characterized by almost random response selection, were significantly more frequent in STs. Importantly, poor performance in STs was not associated with increased omissions, suggesting that these periods of attentional fatigue were not confounded by loss of motivation to perform. Finally, the analysis of key performance measures over three sessions separated by 7 days indicated the reliability of the group differences.

Discussion: Compared to GTs, STs have a lower capacity for sustained attentional performance. Specifically, the performance of STs is characterized by an increased frequency and longer duration of periods of extreme attentional fatigue. Importantly, STs do not simply disengage from the task, as indicated by low and stable errors of omission. Compared to GTs, STs' performance shifts more frequently between high and extremely poor levels of performance. In STs these shifts manifested early into the session. These findings are consistent with the hypothesis that STs have weaker top-down control of attention. Preliminary data indicating lower performance-associated levels of cortical cholinergic neuro-transmission is consistent with this hypothesis. Thus, STs may serve as valuable model to study the neuronal mechanism of poor attentional control and for drug discovery efforts. Effects of psychostimulants and nicotinic compounds are being investigated.

4. Further Support for a Brain-Selective Activity of 17β-Estradiol Prodrug (DHED) in the Mouse

Michal Arad*, Laszlo Prokai, Katalin Tatrai-Prokai, Istvan Merchenthaler, Todd D. Gould

Department of Psychiatry, University of Maryland School of Medicine, Baltimore, USA

Background: Hormonal replacement therapy with estro-gens has been suggested as a treatment for hormone-related

neuropsychiatric diseases. Although antidepressant, anxiolytic and antipsychotic capacities of various estrogens have been repeatedly shown in animals and humans, many women cannot benefit from chronic estrogen treatments due to potentially detrimental peripheral side effects. Previously we have shown that a novel bioprecursor/prodrug of 17β-estradiol (called DHED by its chemical acronym) is selectively activated into 17β-estradiol only in the mammalian brain but not in the blood and peripheral tissues. Therefore, 17β-estradiol central action after systemic DHED treatment is restricted only to the brain without exposing the periphery to the hormone. The current study was designed to test the utility of our brain-selective prodrug approach to modify depression- and anxiety-like behaviors as well as gene expression changes in the mouse.

Methods: Female C57BL/6J mice were ovariectomized at seven weeks of age and were then allowed to recover for seven days. Hormonal treatment (s.c.) of vehicle, DHED (3 μg/kg/5 ml) or 17β-estradiol (25 μg/kg/5 ml) was then initiated and lasted throughout the experiment. Mice were tested in several procedures measuring depressive- and anxiety-like behaviors (first test was conducted following the 10th injection): Tail Suspension Test (TST), Learned Helplessness (LH), Open Field (OF), and Elevated Plus Maze (EPM). Animals were administered individually 2 hours prior to each procedure. Two hours following the 18th injection brains, pituitaries and uteri were collected and kept under $-80°C$ until assessments. Using real-time PCR, hippocampal mRNA levels of BDNF as well as pituitary mRNA levels of galanin were assessed.

Results: Chronic administration of DHED increased the time mice spent in the center of the OF and in the open arms of the EPM, without affecting locomotor activity in both procedures. DHED treatment resulted also in reduced immobility time in the TST and reduced despair-like behavior in the LH procedure. As expected, in all procedures the effects of DHED treatment closely resembled the effects of the parent 17β-estradiol. Moreover, as predicted by the CNS-specific prodrug activation to the parent 17β-estradiol, chronic administration of DHED had no effect on uterus weight, or pituitary mRNA levels of galanin, unlike the robust effect seen on these targets following treatment with 17β-estradiol. Hippocampal mRNA levels of BDNF did not differ between DHED, 17β-estradiol, and control groups. Data of mRNA levels of BDNF in the prefrontal cortex, progesterone receptor in the preoptic area, as well as complement 3 and progesterone receptor in the uterus, will also be presented.

Discussion: These results provide a further support for a brain-selective action of DHED, since it had antidepressant- and anxiolytic-like action on behavior without having any estrogenic effects on the uterus or pituitary. Given that estrogen treatment was associated with increased risk for breast and uterine cancer in some women, the use of the unique CNS-specific DHED prodrug that is devoid of 17β-estradiol-related side effects in the periphery, while keeping the behavioral estrogenic-like capacity may provide a novel and promising treatment for women who suffer from psychiatric disorders.

Disclosure: M. Arad: None. **L. Prokai:** None. **K. Tatrai-Prokai:** None. **I. Merchenthaler:** None. **T. Gould:** None.

5. Depressive-Like Effects of Blocking Astrocytic Glutamate Uptake in the Prefrontal Cortex

Catherine John, Karen Smith, Ashlee Van't Veer, Bruce Cohen, Dost Öngür, Anita Bechtholt-Gompf*

Harvard Medical School - McLean Hospital, Belmont, USA

Background: Studies of Major Depression in humans have observed both dysregulation of glutamatergic neurotransmission and fewer glial cells in brain - deficits that may be functionally related. Astrocytes regulate glutamate levels by removing glutamate from the synaptic cleft through the GLT-1 transporter. We previously demonstrated that decreased uptake of glutamate by astrocytes through central blockade of GLT-1 induces anhedonia, cognitive impairments and c-Fos expression in brain areas that regulate motivation and affective states, including the prefrontal cortex (PFC) of rats. Given the role of the PFC in regulating mood and executive function and a deficit of glia in the PFC of depressed patients, we hypothesized that blockade of glutamate uptake by astrocytes in the PFC alone would produce depressive-like symptoms in rats.

Methods: We microinjected the astrocytic glutamate transporter (GLT-1) inhibitor, dihydrokainic acid (DHK), into the PFC and examined effects on mood using intracranial self-stimulation (ICSS) and sucrose intake, indices of anhedonia and dysphoria in rodents. Data were analyzed using repeated measures ANOVAs.

Results: At lower doses, intra-PFC DHK produced modest increases in the minimum frequency that would maintain ICSS (T0), reflecting a depressive-like effect and higher doses resulted in complete cessation of responding for 15-30 min. To further interpret these findings we conducted three more tests to clarify whether this total cessation of responding was related to an anhedonic state (tested by sucrose preference), a nonspecific result of motor impairment (measured by the tape test) or seizure activity (measured with EEG). In the sucrose preference test, the highest dose of DHK increased latency to begin drinking without altering total sucrose intake ($p < .05$). The tape test, which provides an index of fine motor control, showed no difference between rats treated with vehicle or the highest dose of DHK. Similarly, no evidence of seizure activity was observed after administration of the high dose of DHK.

Discussion: The modest decrease in reward followed by complete cessation of ICSS responding suggests a dose-dependent anhedonic-like effect of intra-PFC DHK, as substantiated by increased latency to drink in the sucrose test. It is unlikely that intra-PFC DHK impairs performance or induces seizures, since DHK treatment did not significantly alter fine motor coordination or induce seizure-like activity. Overall, these studies suggest that blockade of astrocytic glutamate uptake in the PFC is sufficient to produce anhedonia, a core symptom of depression. These findings are consistent with the results from post mortem human studies suggesting that reduction glia cell density and number in the PFC, as observed in depressed patients, contributes to the symptoms of psychiatric illness.

Disclosure: C. John: None. **K. Smith:** None. **A. Van't Veer:** None. **B. Cohen:** None. **D. Öngür:** Part 4: Received drug for a study from Sanofi-Aventis. **A. Bechtholt-Gompf:** None.

6. Estrogen increases Stress Resilience and Hippocampal Synaptic Physiology in the Learned Helplessness Rodent Model of Major Depression

Teruko M. Bredemann*, Lori L. McMahon

UAB Psychology, Birmingham, USA

Background: Psychiatric research on estrogen related mood disorders shows that some women are more likely to develop Major Depression when ovarian hormones are low or fluctuating, such as during puberty, postpartum, or menopause. In addition to mood symptoms, depressive episodes also include learning and memory impairments that have been linked to hippocampus. Stress decreases the ability of excitatory synapses in hippocampus to express long-term potentiation (LTP), a cellular correlate of learning and memory. However, high levels of estrogen, such as those during proestrus, enhance LTP and improve memory. Because the beneficial effects of estrogen are in opposition to the harmful consequences of stress, we hypothesize that hormonal fluctuations in women could leave hippocampal circuits vulnerable

to the detrimental effects of stress and increase susceptibility to major depression. In this study, we used the Learned Helplessness (LH) preclinical model of depressionto determine whether the absence of 17 β estradiol (E2) is causal to the development of depression like behavior in ovariectomized (OVX) female rats. We hypothesized that the absence of E2 in OVX rats would increase acquisition of LH and exacerbate the damaging effects of inescapable shock (IES) on hippocampal LTP. Furthermore, because E2 has protective effects in hippocampus, we hypothesized that treatment with proestrus levels of E2 would prevent the acquisition of LH and minimize the negative effects of IES on hippocampal LTP. Finally, to determine the clinical relevance of E2 treatment for depression like behavior, LH females were treated with E2 and retested for LH behavior to determine whether E2 facilitates learning during escape testing.

Methods: To induce Learned Helplessness (LH), OVX Sprague Dawley rats were subjected to IES should define above on 2 consecutive days prior to shuttle box escape testing on day 3. During escape testing, animals exposed to 30 escapable foot shocks (0.65 mA), 20 s apart. Motion detection was used to record the time latency for each trial during which a shuttle response was made. Animals that fail $\geqslant 5$ of the last 10 trials met criteria for LH, while those that learn to escape shock are considered resilient (RES). OVX rats were treated with either E2 or vehicle (VH; cottonseed oil) to determine whether E2 treatment prevents the acquisition of LH. To achieve proestrus levels of E2, OVX rats received two subcutaneous injections (24 hrs apart) of E2 at 10 μg per 250 g of weight. Cottonseed oil (10 μg/250 g) was used for vehicle controls. Following behavioral assessment, we examined the effects of IES on LTP in both LH and RES animals to determine whether there are deficits in LTP in LH and RE animals.

Results: Proestrus levels of E2 significantly increased resilience to IES in OVX rats. Only 22% of OVX rats treated with E2 (n = 70) reached criteria for LH compared to 43% in the VH-treated group (n = 85, p < 0.05). Additionally, E2 treatment reversed previously established LH in 10 of 16 OVX rats treated before retesting for LH behavior, while all (n = 17) VH treated LH rats failed all trails during retesting. To determine whether deficits in hippocampal synaptic function are associated with expression of LH, we measured LTP at CA3-CA1 synapses in slices from VH or E2-treated LH and RES rats. Excitingly, the magnitude of LTP is slices from both E2 (127 ± 2%, n = 10) and VH (120 ± 1%, n = 9) treated RES animals was significantly(p < 0.05) increased compared to E2 (114 ± 2%, n = 4) and VH (107 ± 1%, n = 5) treated LH animals. These data suggest that resilience to IES is associated with increased hippocampal LTP.

Discussion: Collectively, these results indicate that proestrous levels of E2 increase resilience to stress in OVX rats subjected to IES in the LH model of depression and that the effects of E2 on hippocampal synaptic physiology can protect against hippocampal related learning deficits associated with depression-like behavior. Therefore, estrogen therapy in women may protect against the cognitive deficits associated with depression and serve to reverse depressive behaviors associated with alterations in ovarian hormone levels.

Disclosure: T. Bredemann: None. L. McMahon: None.

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7. Susceptibility and Resilience to Chronic Social Defeat-Induced Anhedonia in Rats: Effects of Chronic Fluoxetine Treatment

Andre Der-Avakian*, Athina Markou

University of California, San Diego, La Jolla, USA

Background: Exposure to chronic or traumatic stress can precipitate the development of mood disorders, such as major depression, that are characterized by anhedonia, defined as loss of interest in pleasurable or rewarding events. However, individual responses to stress vary, with some individuals being susceptible to the detrimental effects of stressor exposure (e.g., resulting in psychopathology), and others being resilient in the face of adversity presumably by engaging more adaptive coping strategies than susceptible individuals. Current monoamine-based antidepressant medications are only partially effective in alleviating symptoms such as anhedonia, highlighting the need for more effective treatments. The goal of the present study was to determine the beneficial effects of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), in a novel stress-induced anhedonia procedure in rats used to model certain key etiological and pathophysiological aspects of major depression.

Methods: Male Wistar rats were first trained on the intracranial self-stimulation (ICSS) procedure that is used to assess changes in brain reward function. Elevations in the primary measure derived from this procedure, ICSS reward thresholds, reflect an anhedonic response. Then, rats were exposed to three weeks of daily social defeat and subordination or no stress. ICSS reward thresholds were assessed daily immediately after social defeat sessions and for one week after termination of the stressor. During the final week of stress exposure and for one week after termination of the stressor (14 days total), rats were administered 5 mg/kg (i.p.) fluoxetine or vehicle daily after each ICSS session.

Results: Repeated social defeat elevated ICSS reward thresholds, reflecting anhedonia during stressor exposure, in approximately 50% of all rats exposed to chronic social defeat (i.e., susceptible rats). The remaining subjects responded similarly to non-stressed controls (i.e., resilient rats) and showed no stress-induced changes in ICSS thresholds. Chronic fluoxetine treatment lowered stress-induced ICSS threshold elevations in approximately 50% of susceptible rats (i.e., responders), but did not lower thresholds in the other subset of susceptible rats (i.e., non-responders). Interestingly, there was a negative correlation between severity of stress-induced anhedonia and anti-anhedonic response to fluoxetine. Fluoxetine had no effect in resilient or control rats' reward thresholds.

Discussion: These results indicate that chronic exposure to social defeat induces an immediate and enduring anhedonic phenotype in only a subset of susceptible rats, whereas the remaining rats display resilience to the anhedonia-inducing effects of chronic exposure to this stressor. In addition, fluoxetine treatment attenuated the anhedonic response to stress in only a subset of susceptible rats, and responsivity to fluoxetine was determined by the severity of the anhedonic response. These patterns of results parallel the human condition whereby reactivity to stress varies across the population and may contribute to the development of stress-induced psychopathology in a subset of vulnerable individuals. Furthermore, traditional antidepressant medications do not lead to remission in all individuals, and even in individuals who achieve remission, anhedonia often persists. These data suggest that anhedonia severity may be an important variable in predicting the effectiveness of SSRI antidepressant treatments.

Disclosure: A. Der-Avakian: None. A. Markou: Part 1: Intracellular Therapeutics, Inc. Lundbeck Research USA, Inc. Bristol-Myers Squibb Co. F. Hoffman-La Roche Inc. Abbott GmbH and Co. Pfizer Astra-Zeneca, Part 4: Intracellular Therapeutics, Inc. Lundbeck Research USA, Inc. Bristol-Myers Squibb Co. F. Hoffman-La Roche Inc. Abbott GmbH and Co. Pfizer Astra-Zeneca.

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8. Paternal Transmission of Stressed-Induced Pathologies

David M. Dietz*, Quincey LaPlant , Vincent Vialou, Emily L. Watts, Georgia E. Hodes, Scott J. Russo, Jian Feng, Ronald S. Oosting, Eric J. Nestler

Department of Pharmacology and Toxicology University of Buffalo, Buffalo, USA

Background: Major depressive disorder (MDD) is a common and disabling disorder with an overall lifetime risk estimated to be

~15% in the general U.S. population and has been show to be highly heritable, with roughly 40% of the risk being genetic. There has been recent interest in the possibility that epigenetic mechanisms might contribute to the trans-generational transmission of MDD. In these studies we focused on possible paternal transmission of depressive- and anxiety-like behaviors using the social defeat stress paradigm.

Methods: Male mice exposed to chronic social defeat stress, or control non-defeated mice, were bred with normal female mice and their offspring were assessed behaviorally in a battery of measures. Plasma levels of corticosterone and vascular endothelial growth factor (VEGF) were also assayed. To directly assess the role of epigenetic mechanisms, we used *in vitro* fertilization (IVF); behavioral assessments were conducted on offspring of mice from IVF-control and IVF-defeated fathers.

Results: We show that both male and female offspring from defeated fathers exhibit increased measures of several depression- and anxiety-like behaviors. The male offspring of defeated fathers also display increased baseline plasma levels of corticosterone and decreased levels of VEGF, both implicated in depression models. However, most of these behavioral changes were not observed when offspring were generated through IVF.

Discussion: These results suggest that, while behavioral adaptations that occur after chronic social defeat stress can be transmitted robustly from the father to his male and female F1 progeny, only subtle changes might be transmitted epigenetically based on our IVF findings.

Disclosure: D. Dietz: None. **Q. LaPlant :** None. **V. Vialou:** None. **E. Watts:** None. **G. Hodes:** None. **S. Russo:** None. **J. Feng:** None. **R. Oosting:** None. **E. Nestler:** None.

9. A Functional Role for Interleukin 6 in Susceptibility to Depression

Georgia E. Hodes*, Viktoria Steizhammer, Sam A. Golden, Daniel J. Christoffel, Jane Magida, Wolfgang Kluge, Carol A. Tamminga, Subroto Ghose, Erik H.F. Wong, Chi-Ming Lee, Sabine Bahn, Scott J. Russo

Mt. Sinai School Medicine, New York, USA

Background: The pro-inflammatory cytokine Interleukin-6 (IL-6) is increased in patients with major depressive disorder (Dowlati et al., 2009). It is currently unknown whether IL-6 levels are altered as a result of a depressive episode or whether IL-6 is functionally involved in the etiology of depression.

Methods: Using multiplex enzyme-linked immunosorbent assays (ELISA) we examined circulating levels of cytokines in humans experiencing their first depressive episode or those with chronic major depressive disorder (CMDD). We then conducted further validation of blood and brain levels of IL-6 after repeated social defeat stress, a mouse model of depression. We also tested whether alterations of IL-6 levels in the Nucleus Accumbens (NAc) were sufficient to induce susceptibility to stress. IL-6 was infused into the NAc and depression-like behavior was examined following a one-day micro-defeat, a stress paradigm that does not induce depression-like behavior in control animals. Additionally, to further understand the mechanism of IL-6 induction in NAc, we examined gene expression profiles of inflammatory signaling pathways in mice exposed to social defeat and in post-mortem tissue from subjects with major depression.

Results: We found a similar increase in circulating levels of IL-6 in both susceptible mice exposed to repeated social defeat stress and in CMDD patients ($p < 0.05$), but not in resilient mice or in patients experiencing a single depressive episode ($p > 0.05$). Standard antidepressant treatment did not alter IL-6 levels in either susceptible mice or CMDD patients ($p > 0.05$). A more detailed analysis of the time course of IL-6 induction in susceptible mice showed a 150 fold increase in blood levels of IL-6 30 min after their first social defeat compared to animals that displayed resiliency to social stress ($p < 0.05$). We also found that IL-6 levels were elevated in the blood ($p < 0.05$) and NAc ($p < 0.05$) of susceptible mice 48 hours after the last social defeat. Providing a functional role for this induction, we found that infusion of IL-6 directly into the NAc increased susceptibility to a micro-defeat ($p < 0.05$). Surprisingly, transcription of IL-6 and its receptors was decreased in the NAc of both susceptible and resilient mice, and in postmortem NAc from humans with depression ($p < 0.05$). These data indicate that the source of elevated NAc IL-6 protein is not from local production and we are currently examining what the source may be.

Discussion: Based on these results, we feel that repeated social defeat stress has strong validity as a model for CMDD, especially when examining the role of cytokines in depression. Our mouse model indicates that elevations in IL-6 blood levels immediately after a single stressful experience may be a good biomarker for susceptibility to stress. A single IL-6 infusion in the NAc prior to a sub-threshold micro-defeat induced depression associated behavior indicating a functional role for IL-6 in susceptibility to stress. While IL-6 protein levels in the NAc were increased in animals susceptible to the effects of social defeat stress, gene expression data indicated that local transcription of IL-6 was decreased, which suggests that the source is likely from the periphery or other brain structures. Overall, our data suggests that individual differences in the IL-6 response to a stressful experience may mediate the development of depression. Given the higher levels of IL-6 in the blood of patients with CMDD and the lack of regulation by traditional antidepressants, IL-6 may be a novel target for drug development with great potential for use in subsets of patient with dysregulated inflammatory pathways.

Disclosure: G. Hodes: None. **V. Steizhammer:** None. **S. Golden:** None. **D. Christoffel:** None. **J. Magida:** None. **W. Kluge:** None. **C. Tamminga:** Part 1: Pure tech ventures- *ad hoc* consultant Eli Lilly- *ad hoc* consultant Sunovion- *ad hoc* consultant Astellas- *ad hoc* consultant Merck- *ad hoc* consultant Intracellular technologies- advisory board American psychiatric association- deputy editor, Part 2: Pure tech ventures Eli Lilly Sunosion Asrellas Merck, Part 3: None, Part 4: None. **S. Ghose:** None. **E. Wong:** Part 1: Full time employee of AstraZeneca, Part 5: Primary employer is AstraZeneca pharmaceuticals. **C. Lee:** Part 1: Full time employee of AstraZeneca pharmaceuticals, Part 5: Primary employer is AstraZeneca pharmaceuticals. **S. Bahn:** Part 1: Consultant for Myriad RBM Inc, Part 2: Consultant for Myriad RBM Inc. **S. Russo:** None.

10. Behavioral Stress-Induced Activation of FoxO3a in the Cerebral Cortex of Mice and the Underlying Signaling Mechanisms

Wenjun Zhou, Ligong Chen, Xiaohua Li*

University of Alabama at Birmingham, Birmingham, USA

Background: FoxO3a is a transcription factor that responds to apoptotic and stress signals. It is highly expressed in brain, regulated by neurotrophins, serotonin, and psychotropics, and FoxO3a deficiency is associated with an antidepressant phenotype in animals. However, little is known about the response of FoxO3a to behavioral stress and its impact in the associated behavioral changes.

Methods: In this study, the model of inescapable foot shocks (IES) was used to induce behavioral stress in mice, followed by testing learned helplessness (LH) and social interaction. The activity of FoxO3a in the cerebral cortex after IES was measured by its phosphorylation and nuclear/cytosolic distribution. The activity of Akt and glycogen synthase kinase-3 (GSK3) in response to IES was

measured by their phosphorylation state, and interaction between GSK3beta and FoxO3a was measured in the cerebral cortex and *in vitro* by immunoprecipitation (IP) and bioluminescent renascence energy transfer (BRET). Additionally, the effect of GSK3 inhibitors on FoxO3a activity was tested *in vitro* and *in vivo*.

Results: A single session of IES in mice reduced FoxO3a phosphorylation at the Akt-regulating serine/threonine residues and induced prolonged nuclear accumulation of FoxO3a in the cerebral cortex, both indicate activation of FoxO3a in brain. The response of FoxO3a is accompanied by a transient inactivation of Akt and a prolonged activation of GSK3beta. Noticeably, FoxO3a formed a protein complex with GSK3beta in the cerebral cortex, and the interaction between the two proteins was stronger in IES-treated mice. Inhibition of GSK3 was able to abolish IES-induced LH behavior, disrupt IES-induced GSK3beta-FoxO3a interaction, and reduce nuclear FoxO3a accumulation. *In vitro* approaches further revealed that the interaction between GSK3beta and FoxO3a was strongest when both were active, FoxO3a was phosphorylated by recombinant GSK3beta, and GSK3 inhibitors effectively reduced FoxO3a transcriptional activity. Importantly, IES-induced LH behavior was markedly diminished in FoxO3a-deficient mice that have minimal FoxO3a expression and reduced levels of FoxO3a-inducible genes.

Discussion: Behavioral stress caused a prolonged activation state of FoxO3a in the cerebral cortex, and this activation is facilitated by its interaction with GSK3beta. Inhibition of GSK3 or reducing FoxO3a expression promotes resilience of mice to stress-induced behavioral disturbance by disrupting the GSK3beta-FoxO3a signaling mechanism. Future studies shall investigate the significance of this signaling mechanism in developing drugs to treat behavioral stress-induced disorders, such as depression and PTSD.

Disclosure: W. Zhou: None. **L. Chen:** None. **X. Li:** Part 4: Otsuka, Novartis, Ortho-McNeil Janssen.

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11. Neurocircutiry in the Learned Helplessness Model of Depression Revealed by Whole Brain c-fos Expression
Martine M. Mirrione*, Nora Ruth, Bo Li, Fritz A. Henn

Cold Spring Harbor Laboratory, Cold Spring Harbor, USA

Background: Understanding dysfunctional neurocircuitry underlying major depression in humans is essential for identifying new targetable pathways for treatment. We employ the learned helplessness (LH) model to examine brain regions correlated to behavioral changes expressed in animals which may be akin to depression in humans. In our previous studies using metabolic neuroimaging, we have shown the habenula, substantia nigra, ventral tegmental area (VTA), and dorsal raphe are associated with the helplessness phenotype, and that habenula activity correlates with degree of helplessness. However, the low resolution of this method precludes a detailed analysis of these small regions. In this study, we employ cellular level microscopy and c-fos expression to evaluate LH circuitry. Immediate early genes such as c-fos are upregulated following periods of prolonged neuronal activity and therefore can be an alternate marker for mapping neuronal activity.

Methods: Twenty wild type male Sprague Dawley rats underwent training (120 inescapable, unpredictable foot-shocks) and testing (15 escapable, predictable foot-shocks) for the learned helplessness paradigm. This yielded 10 learned helpless (LH-"depressed"), 6 non-learned helpless (NLH), and 4 intermediate phenotypes. Exactly 90 minutes following the test, rats were transcardially perfused with 4% paraformaldehyde (including naïve controls). Brains were excised, post fixed in 30% sucrose, and sectioned coronally at 40 μm on a microtome. Free-floating sections (every 120 or 240 μm) were taken for c-fos immunofluorescence. Images of the whole brain (up to 112 slices) were taken at 10x using tile

software on a Zeiss microscope and are co-registered to an atlas traced from Paxinos and Watson, 2007 using Atlas Tracer and Atlas Fitter Software developed by C.D. Kopec 2010. C-fos expression can be quantified using the accompanying Cell Counter program run in Matlab. Adjacent sections were evaluated for GAD67, VGlut1, 2, or 3, tyrosine hydroxylase, anti-dopamine, and/or anti-serotonin to determine the cellular identity of the c-fos expressing cells in regions of interest.

Results: Preliminarily analysis of LH (n = 2) and NLH (n = 2) whole brain c-fos expression indicates a network of brain regions underlying the learned helplessness behavior, which includes the medial prefrontal cortex, nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), habenula, hypothalamus, paraventricular thalamus, dorsal raphe, periaqueductal gray, and locus coruleus. The extent of activity in each brain region of either LH or NLH animals will be quantified. Interestingly, the c-fos expression in the habenula of LH animals is localized to the medial portion of the lateral habenula. In the dorsal raphe, the dorsal, ventral, and lateral portions show c-fos expression, in addition to the caudal raphe, however there is little expression in the median raphe. Preliminary observation of cell identity in the raphe suggests that c-fos positive cells are serotonergic, and some co-express VGlut3. There is also c-fos expression in the core of the NAc, anterior BNST, and septum. However, there does not appear to be any c-fos expression in the ventral tegmental area, or substantia nigra of LH animals.

Discussion: The habenula-dorsal raphe-medial prefrontal cortex interconnecting pathways have previously been implicated in the LH phenotype and major depression by several studies. Here we extend those findings by examining neuronal activity in the entire brain of both LH and NLH animals. This will allow us to identify new regions that may have been previously overlooked, differentiate regions that are related to either LH or NLH behavior, and begin to identify the cell types involved in the behavior. Our preliminary data suggests complex, regional, c-fos expression associated with the LH phenotype.

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Disclosure: M. Mirrione: None. **N. Ruth:** None. **B. Li:** None. **F. Henn:** Part 1: Astra Zeneca.

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12. Stress Exposure produces a Switch from Appetitive to Aversive Signaling by Corticotropin-Releasing Factor in the Nucleus Accumbens
Julia Lemos, Matthew Wanat, Jeffrey Smith, Beverly Reyes, Elisabeth Van Bockstaele, Charles Chavkin, Paul Phillips*

University of Washington, Seattle, USA

Background: Stressors motivate an array of adaptive responses ranging from "fight or flight" to an internal urgency signal facilitating long-term goals. However, traumatic or chronic uncontrollable stress promotes the onset of Major Depressive Disorder where acute stressors lose their motivational properties. An emerging neurobiological substrate of clinical depression is the nucleus accumbens which has the capacity to mediate a diverse range of stressor responses by interfacing limbic, cognitive and motor circuitry. Here, we studied the actions of the stress-related neuropeptide, corticotropin releasing factor (CRF) in the nucleus accumbens on dopamine transmission and behavior.

Methods: Inmmunohistochemistry and transmission electron microscopy were used to study anatomical interactions between CRF and dopamine terminals, and fast-scan cyclic voltammetry was used to assess the effect of CRF on dopamine transmission in the nucleus accumbens of mice. Subjective effects of intrathecal administration of CRF into the nucleus accumbens was tested using a place-conditioning paradigm. These experiments were

carried out in stress-naïve mice, or those exposed to a forced-swim-stress paradigm to elicit a depressive phenotype.

Results: Within the nucleus accumbens, CRF-containing fibers were interdigitated with dopamine-containing fibers. Both CRF R1 and R2 receptors were co-localized with tyrosine hydroxylase expression, indicating that they are expressed on dopamine-containing terminals. In the nucleus accumbens of stress-naïve mice, CRF increased dopamine release through co-activation of CRF R1 and R2 receptors. However, severe-stress exposure completely abolished this effect without recovery for at least 90 days. This loss of CRF's capacity to regulate dopamine release in the nucleus accumbens was accompanied by a switch in the perception of CRF from an appetitive to an aversive stimulus, indicating a diametric change in the emotional response to acute stressors.

Discussion: Here we demonstrate a specific defect in the regulation of dopamine transmission in the nucleus accumbens in the genesis of depression. We show that severe stress disables the capacity of CRF to positively regulate dopamine, removing CRF's appetitive qualities, leaving a negative perceptual bias. Indeed, depressive disorders produce a profound change in the perception and behavioral response to acute stressors and other arousing environmental stimuli that elicit CRF signaling. Taken together, our findings provide a neurobiological mechanism for the affective shift from engagement of the environment to withdrawal following severe stress, central to the manifestation of Major Depressive Disorder.

Disclosure: J. Lemos: None. M. Wanat: None. J. Smith: None. B. Reyes: None. E. Van Bockstaele: None. C. Chavkin: None. P. Phillips: Part 1: My spouse is an employee of Amgen, Inc., Part 2: My spouse is an employee of Amgen, Inc., Part 3: My spouse is an employee of Amgen, Inc., Part 5: My spouse is an employee of Amgen, Inc.

13. Neuropeptide Y System Gene Expression in the Non-human Primate Amygdala is Associated with Anxious Temperament.

Patrick H. Roseboom*, Steven A. Nanda, Andrew S. Fox, Jonathan A. Oler, Steve E. Shelton, Ned H. Kalin

University of Wisconsin School of Medicine and Public Health, Madison, USA

Background: Neuropeptide Y (NPY) is a putative endogenous anxiolytic neurotransmitter that may play an important role in adaptive and maladaptive responses to stress, as well as in mediating psychopathology. Using a well-established, non-human primate model of anxious temperament (AT), we examined whether alterations in the monkey amygdala NPY system are associated with AT. AT is of interest because it is a phenotype that is identifiable early in life that is characterized by excessive shyness, worrying, and avoidant behavior and increases the risk of developing anxiety and depression. We focused on the central nucleus region of the amygdala since our previous work established that metabolic activity in this region is predictive of AT.

Methods: Using Affymetrix GeneChip® rhesus macaque genome arrays, we assessed gene expression from the dorsal amygdala region in 24 young male rhesus monkeys phenotyped for AT. Robust regression analysis was performed with correction for multiple comparisons across all annotated transcripts that are part of the neuroactive ligand pathway in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Results: As hypothesized, individual differences in gene expression were negatively correlated with individual differences in AT. Specifically, the expression of NPY receptor 1 (NPY1R; $t = -4.34$, $p = 0.016$) and NPY receptor 5 (NPY5R; $t = -3.49$, $p = 0.035$) identified in the microarray analysis were significantly negatively

correlated with AT. To validate these findings and fully investigate all members of the NPY system expressed in this region, we performed quantitative real time polymerase chain reactions (qRT-PCR) in the same RNA samples used for microarray analysis. The associations between AT and expression levels of NPY1R ($r = -0.71$, $p < 0.0001$) and NPY5R ($r = -0.55$, $p < 0.01$) were confirmed. In contrast, two other members of the NPY system detected in the dorsal amygdala were not significantly correlated with AT [NPY ($r = -0.02$, $p = 0.93$) and NPY2R ($r = -0.32$, $p = 0.15$).

Discussion: These findings suggest that two of the NPY receptors in the central nucleus region of the amygdala are selectively associated with expression of AT, such that higher levels of NPY1R and NPY5R gene expression are associated with less AT. Increased expression of the genes encoding for these receptors may facilitate NPY signaling in the amygdala promoting adaptive responses and resilience in response to potentially anxiety provoking situations. This work was supported by NIH grants MH084051, MH091550 and MH046729, Meriter Hospital and the University of Wisconsin HealthEmotions Research Institute.

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14. Effect of Chronic Unpredictable Stress on Cortical GABAergic Neurons

Ashley Lepack, Mounira Banasr, Ronald Duman, Gerard Sanacora*

Yale University, New Haven, USA

Background: The past decade has highlighted the involvement of the amino acid neurotransmitter systems in the neurobiology of mood disorders. Although much of the recent attention has focused on glutamatergic mechanisms, GABAergic involvement in the pathophysiology and treatment of mood disorders is supported by several lines of evidence including; 1) animal studies showing stress-related changes in GABAergic function, 2) the ability of GABA agonists and antagonists to modulate behavioral models of depression in rodents, 3) GABAergic effects of existing antidepressant medications, 4) preliminary evidence of clinical antidepressant efficacy associated with GABAergic drugs, and 5) demonstration of GABAergic abnormalities and genetic associations in patients with mood disorders. In addition to early studies reporting reduced levels of GABA in the plasma and serum of depressed patients, several spectroscopic imaging studies have demonstrated reduced GABA content in cortical regions of individuals with major depressive disorders (MDD). A series of more recent postmortem studies have provided evidence to suggest MDD is associated with specific pathological processes involving GABAergic neurons in cortical brain regions. As stress is generally accepted to be a major contributor to the pathogenesis of mood disorders, the aim of this study is to characterize the effects of chronic unpredictable stress (CUS), a well documented animal model of depression, on the function of GABAergic neuronal subpopulations in the rat prefrontal cortex.

Methods: Male Sprague Dawly Rats were subjected to a five week period of CUS or normal handeling according to previously published methods and protocols approved by the Yale IACUC. Following this 5-week treatment period, animals were sacrificed and the tissue was isolated for analyses. Protein levels of GAD65 & GAD67, calbindin-D28k, parvalbumin and calretinin were determined using western blot analysis in both the prefrontal cortex and hippocampus.

Results: Animals subjected to CUS showed significantly reduced expression of critical proteins involved in the function of

GABAergic neurons such as calbindin-D28k and GAD67 protein levels (approximately 20% decrease for both) in the frontal cortex. CUS also induced a non-significant trend toward decreased parvalbumin protein levels. No change in cortical GAD65 or calretinin expression was observed in animals subjected to CUS. Preliminary data suggest differential changes in the hippocampus, with no changes in GAD65 or GAD67 being evident.

Discussion: The findings suggest that stress may have deleterious effects on a subset of GABAergic neurons. Interestingly, these changes are very similar to those observed in recent postmortem studies of brains from patients with MDD where reduced cortical GAD67 expression and calbindin- D28k-positive cells were reported. The findings are also consistent with other work form our laboratory, demonstrating significantly reduced levels of frontal cortex GABA synthesis in rats following CUS, and preliminary data suggesting reductions in GABA synthesis in depressed patients. In an attempt to better identify the subset of GABA neurons affected by the CUS, we are currently examining the effects of CUS on other markers of GABAergic neurons including somatostatin. In sum, our results demonstrate that stress can induce alterations in GABAergic neurons and provide support to the hypothesis that GABAergic anomalies contribute to the pathogenesis and pathophysiology of depression.

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15. A Role for Dopamine Transmission in the Acute Rescue of a Depression Phenotype induced by Chronic Mild Stress

Kay Tye*, Julie Mirzabekov, Sung-yon Kim, Melissa Warden, Ilana Witten, Karl Deisseroth

Stanford University, Stanford, USA

Background: Although 13% of Americans are diagnosed with Major Depression at some time during their lives, the pathology of depression and other mood disorders is poorly understood, and available anti-depressant medications have only moderate efficacy, with a 54% response rate, and must be taken for 4-6 weeks to show improvements in human patients. Studies involving animal models of depression are paramount to increasing our understanding of the neural perturbations underlying depression and are critical to the development of improved therapeutic interventions with increased efficacy on a shorter time-scale and fewer side-effects.

Methods: Here, we used a mouse model of inducing depression using a chronic mild stress (CMS) paradigm in which mice were exposed to unpredictable stressors twice daily for 8-12 weeks, and reliably produces anhedonia as well as reduced sexual, aggressive and locomotor behavior. The CMS paradigm has been demonstrated to have good predictive validity, in that anti-depressants successfully reverse the behavioral changes induced by the CMS treatment. To identify the critical circuit elements that contribute to the behavioral symptoms of depression, we used *in vivo* optogenetic techniques in combination with a well-validated depression assay, the tail suspension test (TST). In the TST, mice are exposed to an inescapable stressor (hanging from their tail),

and the proportion of time spent performing escape behavior relative to the time spent immobile reflects the depression-related phenotype of that animal. Porsolt (1978) has compared the immobility in depression assays to "behavioral despair" and anti-depressants have been shown to decrease the proportion of time spent immobile relative to the time spent struggling. Since two main symptoms of depression include a reduction in the ability to experience hedonic pleasure and a decrease in motivation to participate in activities, we hypothesized that dopamine transmission which has been linked to motivation and reward processing may be critically involved in a depression-related phenotype.

Results: Here, we selectively transduced dopamine neurons in the ventral tegmental area with channelrhodopsin2 (ChR2) or a control virus that only carried a fluorophore (eYFP) in mice treated with CMS and controls. When we compared the performance of these four groups of mice (ChR2 CMS, $n = 13$; eYFP CMS, $n = 10$; ChR2 Non-CMS, $n = 9$; eYFP Non-CMS, $n = 8$), we found that at baseline, the groups that underwent CMS spent significantly less ($\sim 50\%$) time engaging in escape behavior relative to controls ($p < 0.01$). However, upon sparse bursts of illumination, the depression-like phenotype was rescued in the ChR2 CMS mice, but not the eYFP CMS mice.

Discussion: We therefore identify a role for dopamine transmission in acutely, on the order of seconds, rescuing a stress-induced depression phenotype .

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16. Can a Model with Predictive Validity for Clinical Efficacy in Treatment-Resistant Depression be Derived from the Differential Behavioral Effects of Drugs Across Mouse Strains?

Jeffrey M. Witkin*, Kurt Rasmussen, David Bleakman, Karen Baker, Nicholas J. Brandon, Zoe A. Hughs, Michelle P. Kelly

Lilly Research Labs, Indianapolis, USA

Background: A significant percentage of patients suffering from major depressive disorder (MDD) are not fully served by existing antidepressants such as selective serotonin, or serotonin norepinephrine reuptake inhibitors (SSRIs or SNRIs, respectively) [1, 2]. Only about one third of patients respond to current antidepressants and less than one third display symptom remission. Another third of MDD patients are classified as suffering from treatment resistant depression (TRD) as they fail to respond to multiple therapeutic interventions. The TRD population represents a significant unmet medical need in a disease area that affects millions of people each year [2]. There are a handful of mechanisms with clinically proven efficacy in TRD that include the NMDA receptor antagonist, ketamine [3,4], the selective NMDA NR2B antagonist, CP-101606 [5], and the combination of fluoxetine and olanzapine [6]. To address this unmet need, Pfizer and Eli Lilly are collaborating on the development of animal models to predict efficacy in TRD. The initial focus of the collaboration is to determine whether there are inbred mouse strains that are naturally non-responsive to standard antidepressants and then whether such a strain could have utility in identifying potential therapies for TRD.

Methods: All animal studies were performed in accordance to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Pub. 85-23, revised 1996) and under the approval of either the Pfizer Groton or Lilly Research Labs Institutional Animal Care and Use Committees (IACUC). Five mouse strains were evaluated in the tail suspension test. Studies were performed in parallel in two separate laboratories by scientists at Pfizer and Eli Lilly using different

automated tail-suspension apparatus. Immobility time of male SWR/J-0689, C3H/HeJ-0659, C57BL/6J-0664, A/J-0646 (Jackson Labs), and CD-1 mice (Charles River) was measured after administration of vehicle, imipramine (3.2-32 mg/kg, sc), fluoxetine (10-56 mg/kg, sc), or bupropion (3.2-32 mg/kg, sc) to determine sensitivity to standard antidepressants. To investigate responsiveness to agents efficacious in TRD, effects of ketamine (3.2-56 mg/kg, ip) and CP-101606 (3.2-32 mg/kg, sc) were studied. In order to rule out a difference in pharmacokinetics across strains, plasma and brain exposures were measured. Furthermore, *ex vivo* target occupancy methods were used to assess occupancy of the serotonin and dopamine transporters (SERT and DAT) in brain after treatment with standard antidepressants.

Results: The concordance of findings across labs was generally good. Both labs identified responsive (e.g. C57BL/6J) and non-responsive (e.g. CD-1) strains of mice. In contrast to the inactivity or marked reduced potency of imipramine, bupropion, and fluoxetine in CD-1 mice, ketamine and CP-101606 had prominent antidepressant-like effects in CD-1 mice. Levels of drug exposure in plasma and brain were generally dose dependent and did not explain differences in efficacy of drugs across strains. Target occupancy at DAT and SERT after standard antidepressants was also generally comparable across strains.

Discussion: These preliminary data demonstrate the value of collaborative studies to test the robustness and reliability of findings across labs. The data indicate that the pharmacokinetic profiles of the standard antidepressants are generally comparable across strains and do not explain the degree of responsiveness. Further work is required to identify possible physiological mechanisms underlying the differential sensitivity of mouse strains to conventional antidepressants. A host of assumptions and issues surrounding the interpretation and utility of such findings for the development of a murine model for predicting therapeutic agents for TRD will be discussed.

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17. The Serotonin Syndrome in Monoamine Oxidase (MAO) AB Knockout Mice: Enhanced Behavioral Responses induced by 5-hydroxy-L-tryptophan (5-HTP) and Tramadol

Meredith A. Fox*, Micaella G. Panessiti, Teresa J. Tolliver, Kevin Chen, Jean C. Shih, Dennis L. Murphy

National Institute of Mental Health, NIH, Bethesda, USA

Background: The serotonin syndrome typically occurs following administration of combinations of serotonin-enhancing drugs, including antidepressant medications such as serotonin reuptake inhibitors (SRIs) and monoamine oxidase (MAO) inhibitors. We have previously shown that serotonin transporter (SERT)-deficient mice, which have depleted tissue levels but enhanced extracellular levels of serotonin, display enhanced baseline serotonin syndrome behaviors (Fox *et al.*, 2007; 2008). In addition, we have shown that SERT-deficient mice have exaggerated serotonin syndrome behavioral responses following administration of single serotonin-enhancing drugs, including the serotonin precursor 5-hydroxy-L-tryptophan (5-HTP), the MAO AB inhibitor tranylcypromine, and the atypical opioids tramadol and meperidine (Fox *et al.*, 2007; 2008; 2009). These behavioral effects have been shown to be due to enhanced increases in serotonin levels in these mice following 5-HTP (Fox *et al.*, 2008). In the current studies, we assessed serotonin syndrome behaviors and temperature responses in MAO AB wildtype (WT) and knockout (KO) mice, which have also been shown to have altered serotonergic systems (Chen *et al.*, 2004).

Methods: We assessed serotonin syndrome behavioral and temperature responses to 5-HTP (80 mg/kg) and tramadol (60 mg/kg) in MAO AB WT and KO mice. In addition, monoamines and their metabolites were assessed in five brain regions from MAO AB WT and KO mice at baseline and following 5-HTP administration.

Results: At baseline, overall serotonin syndrome behaviors were enhanced in MAO AB KO mice compared to their WT counterparts. 5-HTP significantly increased serotonin syndrome behaviors in MAO AB KO compared to vehicle, whereas 5-HTP was without effect in MAO AB WT mice. 5-HTP decreased temperature to a similar degree in both MAO AB WT and KO mice. Tramadol induced serotonin syndrome behaviors in both MAO AB WT and KO mice compared to vehicle, and this response was exaggerated in MAO AB KO mice compared to MAO AB WT mice. Tramadol decreased temperature in both MAO AB WT and KO mice, although this effect was decreased in MAO AB KO mice. Based on initial HPLC findings in striatum, baseline serotonin levels were increased in MAO AB KO mice compared to MAO AB WT mice, and 5-HIAA levels and serotonin turnover rates (5-HIAA/serotonin) were decreased in MAO AB KO mice. Following 5-HTP, serotonin levels were increased in both MAO AB WT and KO mice compared to vehicle, and this increase was markedly exaggerated in MAO AB KO mice compared to their WT counterparts. Whereas 5-HTP increased both 5-HIAA and serotonin turnover rates in MAO AB WT mice, 5-HTP had no effect on these measures in MAO AB KO mice.

Discussion: Overall, these findings of enhanced baseline and serotonin syndrome behavioral responses to 5-HTP and tramadol in MAO AB KO mice are similar to findings previously reported in SERT-deficient mice. Regarding the temperature change induced by these drugs, whereas SERT knockout (-/-) mice have exaggerated hypothermic responses to 5-HTP compared to SERT wildtype (+/+) mice, the hypothermic response in MAO AB WT and KO mice was similar in degree. However, similar to SERT-deficient mice, MAO AB KO mice also had a reduced hypothermic response to tramadol (Fox *et al.*, *unpublished data*). The current findings extend our model of possible genetic vulnerability to the development of the serotonin syndrome from functional variants in SERT to variants in MAO. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH).

18. Behavior-Based Neuroactive Drug Discovery in the Zebrafish

David Kokel*, Randall Peterson

Harvard Medical School, MGH, Boston, USA

Background: Because psychotropic drugs cause changes in behavior, we can use changes in behavior to discover psychotropic drugs. The original prototypes of most psychotropic drugs were discovered in humans, rodents and other model organisms. Most of these discoveries were made by chance, but the process of behavior based drug discovery can be made more systematic and efficient.

Methods: To enable a systems-level approach to neuropharmacology, we developed a fully automated platform for analyzing the behavior of embryonic zebrafish. This platform is based on a robotic microscope that captures digital video recordings of animals in each individual well of a 96-well plate before, during, and after a series of stimuli. Overall, we analyzed a total of $\sim 20,000$ uncharacterized compounds, including thousands of control wells.

Results: We find that systems-level analysis of behavioral phenotypes genterate testable hypotheses about the molecular mechanisms of poorly understood drugs and behaviors. To analyze systematically the thousands of beahvioral recordings obtained from a large-scale chemical screen, we transform these behavioral recordings into barcodes, providing a concise and interpretable summary of the observed phenotypes in each well. After small molecule-induced behavioral effects have been converted to a barcode, computational tools can be used to identify and organize candidate hits. Hierarchical clustering of these behavioral barcodes based on their phenotypic similarities revealed that candidate hits fall into numerous clusters. We find that clusters of barcodes with similar phenotypes are often enriched with functionally related compounds. We hypothesized that behavioral barcodes could be used to predict mechanisms of action for novel neuroactive compounds. To test this possibility, we looked for clusters containing both well-characterized and completely uncharacterized molecules. For example, we identified fifteen molecules that all caused a distinctive slow-to-relax (STR) phenotype. Several of these compounds, are known to inhibit acetylcholinesterase (AChE), whereas two other compounds, STR-1 and STR-2, are structurally unrelated molecules with no known activity in animals. We find that both compounds inhibit AChE, and that STR-2 specifically inhibits AChE *in vivo*, but not *in vitro*. These observations demonstrate that *in vivo* behavioral phenotyping in zebrafish can identify novel neuroactive compounds and their mechanisms of action, including molecules that require bioactivation and would likely have been missed using *in vitro* screening assays.

Discussion: By combining the *in vivo* relevance of behavior-based phenotyping with the scale and automation of modern drug screening technolgies, systematic behavioral barcoding represents not only a means of discovering psychotropic drugs but also provides a powerful, systematic approach for unraveling the complexities of vertebrate behavior.

Disclosure: D. Kokel: None. R. Peterson: None.

19. Roles of GSK-3 and HDAC Inhibition in Beneficial Effects of Combined Lithium and Valproate Treatment in Transgenic Mouse Models of Huntington's Disease

Chi-Tso Chiu*, De-Maw Chuang

National Institute of Mental Health, National Institutes of Health, Bethesda, USA

Background: Huntington's disease (HD), an inherited neurodegenerative disorder, is caused by mutation on a gene that encodes a polyQ stretch in the disease-causing protein called huntingtin. This mutation results in a gradual death of neurons particularly in the striatum and cortex. Transcriptional dysregulation also plays a central role in the pathology of this disease. HD patients suffer from various symptoms, such as memory loss and emotional deterioration, in addition to jerky and uncontrollable movements. HD is lethal and currently there is no treatment that can halt the disease progression. Lithium has been the first-line drug used for the treatment of bipolar disorder for more than half a century. Valproate (VPA), an anticonvulsant drug, is also effective in treating this disorder. Significant attention has recently focused on their neuroprotective and neurotrophic abilities. Emerging evidence suggests that these effects of lithium and VPA are mediated through inhibition of brain glycogen synthase kinase 3 (GSK-3) and histone deacetylases (HDACs), respectively.

Methods: The present study investigated the putative benefits and potential underlying mechanisms of co-treatment with lithium and VPA in both N171-82Q and YAC128 mouse models of HD. These two mouse strains exhibit neurological and behavioral abnormalities similar to those found in human HD patients, but have different genetic backgrounds and pathological progressions. For drug treatment, mice received either control chow, or an equivalent diet containing lithium carbonate (3 g/kg), VPA (25 g/kg), or both. These doses were chosen based on a pilot dose-response study indicating that blood concentrations of both drugs were within the therapeutic levels for patients with bipolar disorder. The general motor activity, motor coordination, anxiety-like and depressive-like behaviors of animals were measured by a battery of well-validated behavioral tests: the open-field test, the accelerating rotarod test, the elevated zero-maze test, the forced swim test, and the tail suspension test. Biochemical alterations in the brain associated with drug treatment were measured by Western blot analysis and real-time PCR.

Results: N171-82Q mice exhibited poor motor coordination and die prematurely, whereas YAC128 mice showed a slower progression in motor impairments and have a normal lifespan. We found that co-treatment with lithium and VPA produced greater benefits in both HD models. First, co-treatment with lithium and VPA more effectively alleviated impaired locomotion and depressive-like behaviors in both models of HD mice. In addition, this co-treatment also more successfully improved motor skill learning and coordination in N171-82Q mice, and suppressed anxiety-like behaviors in YAC128 mice. Importantly, co-treatment markedly prolonged median survival of N171-82Q mice from 31.6 to 41.6 weeks. Associated with the onset of behavioral symptoms, we observed a progressive decrease in levels of GSK-3β Ser9 phosphorylation and histone H3 acetylation in the striatum and cerebral cortex of untreated transgenic HD mice, indicating a hyperactivity of GSK-3β and HDACs. Combined treatment with lithium and VPA, however, consistently decreased the activities of these enzymes, and caused a sustained elevation in striatal as well as cortical BDNF and HSP70, two proteins that are deficient in the brains of HD subjects but important for neuronal growth and protection.

Discussion: Dysfunction of GSK-3 is implicated in many neuropsychiatric disorders, and activation of this kinase is linked to apoptotic cell death induced by multiple insults. HDACs, on the other hand, play a key role in the homeostasis of histone acetylation on chromatin and regulating transcription. Imbalances in protein acetylation and transcription are associated with a wide variety of brain disorders. Our findings implicate the hyperactivity of GSK-3 and HDACs in the pathogenesis of HD, and support the hypothesis that the behavioral benefits of this combined treatment in the mouse models of HD may be mediated via potentiated inhibition of these enzymes. Moreover, enhanced BDNF expression is known to protect neurons from neurochemical insults associated with HD, while HSP overexpression not only reduces the formation of huntingtin aggregates but also suppresses neurodegeneration and toxicity associated with this disease. These

results suggest that BDNF and HSP70 are likely downstream mediators involved in lithium and VPA co-treatment. In conclusion, our data provide a clinical relevance for the combined treatment of HD with these two drugs, which are already FDA-approved medications with a long history of safe use in humans.
Disclosure: C. Chiu: None. **D. Chuang:** None.

20. Hippocampal Neuroplasticity is Altered in an Animal Model of NMDA Receptor Hypofunction, the Serine Racemase Knockout Mouse

Darrick T. Balu*, Amy Truong, John P. Corradi, Angela M. Cacace, Joseph T. Coyle

McLean Hospital/Harvard University, Belmont, USA

Background: There is substantial evidence, both pharmacologic and genetic, that hypofunction of the *N*-methyl-D-aspartate receptor (NMDAR) is a core pathophysiological mechanism underlying schizophrenia. In addition, there are abnormalities in hippocampal structure and function in schizophrenia, including reductions in the expression of brain derived neurotrophic factor (BDNF). Previous studies from our laboratory have demonstrated that the serine racemase knockout (SR-/-) mouse, a genetic model of NMDAR hypofunction, exhibits deficiencies in hippocampal long-term potentiation (LTP) and subtle impairments in behaviors dependent on this brain region. Our current experiments aimed to identify potential mechanisms underlying these hippocampal abnormalities.
Methods: Golgi impregnation was used to analyze dendritic spine density of granule cells in the dentate gyrus (DG) of the hippocampus. We used microarray analysis (Affymetrix mouse Genome HT_MG-430A array that contains $>22,600$ probe sets to analyze the expression of $>14,000$ mouse genes) to probe global gene expression changes and Ingenuity Pathway Analysis (IPA) software to gain insight into network and cellular functions that were preferentially affected in SR-/- mice. Quantitative PCR (qPCR) was used to measure mRNA and microRNA (miR) levels, while Western blot and enzyme-linked immunosorbent assay (ELISA) were used to measure protein changes.
Results: Dendritic spine density on DG granule cells from SR-/- mice was significantly reduced as compared to neurons from wild-type (WT) mice (20%, $p < 0.05$). Using a gene expression microarray, we found a total of 502 differentially expressed genes in the hippocampus of SR-/- mice. The top diseases and disorders associated with the observed gene changes included immunological disease and genetic disorders. The most affected molecular and cellular functions included cellular assembly and organization, cellular growth and proliferation, as well as cell migration. SR-/- mice displayed gene expression changes consistent with altered nervous system development and function. One notable change identified in the microarray was reduced expression of BDNF in SR-/- mice. We validated this reduction in hippocampal BDNF mRNA using qPCR (25%, $p < 0.05$), which was then further confirmed at the protein level (20%, $p = 0.01$). Although the total protein amount of tropomyosin receptor kinase B (TrkB), the high affinity receptor for BDNF, was unchanged in the hippocampus of SR-/- mice, the amount of phosphorylated TrkB was reduced (35%; $p < 0.05$). Finally, the expression of the primary (15%; $p < 0.05$), precursor (40%; $p < 0.05$), and mature (40%; $p < 0.05$) transcripts of miR-132 were significantly reduced in SR-/- mice, which is consistent with our findings of reduced spine density and NMDAR hypofunction.
Discussion: These data suggest that numerous aspects of hippocampal neuroplasticity, including cell migration and proliferation, as well as processes related to neurite outgrowth

and dendritic morphology are perturbed in SR-/- mice. Moreover, the dendritic and electrophysiological changes in SR-/- mice could be due to impaired BDNF signaling and reduced levels of miR-132. These findings highlight the glycine modulatory site (GMS) and other downstream signaling pathways as potential therapeutic targets for mitigating the neuroplastic and dendritic perturbations associated with brain disorders, such as schizophrenia.
Disclosure: D. Balu: None. **A. Truong:** Part 5: Bristol-Myers Squibb. **J. Corradi:** Part 5: Bristol-Myers Squibb. **A. Cacace:** Part 5: Bristol-Myers Squibb. **J. Coyle:** Part 1: Dr. Coyle is a consultant for Biovail, Abbott, Puretech, and Bristol-Myers Squibb, and own stock in Abbott. A patent owned by Massachusetts General Hospital for the use of D-serine as a treatment for serious mental illness could yield royalties for Dr. Coyle., Part 4: Recipient of an unrestricted grant from Bristol-Myers Squibb.

21. Evidence that Mutation in Neuregulin 1, a Schizophrenia Susceptibility Gene, alters Glucose Tolerance in Animals

Nancy M. Bivens*, Jay A. Gingrich

Columbia University/NYSPI, New York, USA

Background: Neuregulin 1 (Nrg1) alleles have been associated with schizophrenia and Nrg1 mutant animals show psychosis related phenotypes. Nrg1 is an EGF-like growth factor implicated in muscle glucose metabolism as well as in brain development and synaptic function. Nrg1 and its receptor ErbB4 have recently been described as showing signals of positive selection in human populations, although it is unclear whether this would relate to energy balance, neuronal functions, or other growth factor related functions of Nrg1. We hypothesized that Nrg1 mutant animals would show impaired glucose tolerance. This would have relevance for patients with schizophrenia, as diabetes is very common, likely due to effects of antipsychotic medications.
Methods: Male animals heterozygous for Nrg1 Immunoglobulin domain isoform null mutation (Nrg1IGhet) were fed a normal diet and tested as adults. The mutant animals were on a c57Bl/6 background, and were tested against wild-type (WT) littermate controls. Each group consisted of eight to ten animals. Blood glucose was tested with a clinical glucometer immediately prior to and 30, 60, and 120 minutes after i.p. administration of a 1.5 g/kg glucose load. Fasting glucose was also recorded for animals treated chronically (two months) with clozapine at 10 mg/kg in drinking water (a blood level of 59 ng/ml Norclozapine).
Results: Contrary to the hypothesized result, Nrg1IGhets showed improved glucose tolerance with an average maximum blood glucose of 165 mg/dl at 30 minutes after glucose load, while wild type littermates had a max of 220 mg/dl at 30 minutes after glucose load ($p < .05$). There were no significant differences between WT and heterozygous animals for baseline fasting glucose. There were also no significant differences in baseline fasting glucose between animals treated chronically with clozapine and those treated with vehicle, regardless of genotype.
Discussion: This result suggests that Nrg1 genotypes may impact risk for diabetes, for instance, reducing risk in low expression situations. It also provides alternate frameworks for interpreting the psychosis and seizure related phenotypes found both in Nrg1 hypomutant animals and in Nrg1 overexpression mutants. It is of particular relevance to understanding neurological phenotypes produced by acute peripheral administration of recombinant Nrg1, as a strong immediate effect on blood glucose levels may mediate many downstream effects. Nrg1 effects on blood glucose metabolism may also be of interest in determining populations at higher risk for side effects of antipsychotic medications.
Disclosure: N. Bivens: None. **J. Gingrich:** None.

22. Early Developmental Elevations of Kynurenic Acid, an Endogenous Negative Modulator of Alpha7 Nicotinic Receptors: A Novel Animal Model of the Cognitive deficits in Schizophrenia

Ana Pocivavsek, Kathleen S. Alexander, David Bortz, Hui-Qiu Wu, Robert Schwarcz, John P. Bruno*

Depts. of Psychology & Neuroscience, The Ohio State University, Columbus, USA

Background: Cognitive deficits (i.e. attention, cognitive flexibility, working memory) are a core feature of schizophrenia (SZ). While the pathophysiology of the disease is complex and heterogeneous, three features appear to have gained considerable support. First, anatomical and neurochemical impairments across a broad, distributed neural system involving the prefrontal cortex (PFC), hippocampus (HIPPO), and nucleus accumbens (NAC) contribute to these cognitive deficits. Second, these chemoanatomical dysfunctions emerge during early development as a complex interaction between genetic vulnerabilities and environmental events. Finally, dysregulation of the tryptophan metabolite kynurenine results in elevated levels of kynurenic acid (KYNA), a negative modulator of alpha7 nicotinic receptors, in the brains of SZ patients. In recognition of these features, we have developed a novel animal model in which KYNA levels are elevated in brain during a critical stage of pre- and postnatal neurodevelopment. Neurochemical measures were assessed in PFC and HIPPO at various stages of development, and the integrity of prefrontally-mediated cognitive flexibility and hippocampally-mediated spatial working memory was tested in adult offspring.

Methods: KYNA levels were elevated chronically from gestational day 15 to weaning on postnatal day (PD) 21 by adding the KYNA precursor kynurenine (100 mg/day) daily to a chow wet mash fed to pregnant and nursing Wistar rat mothers. At the time of weaning, rats were fed normal rat chow until tested for neurochemical or behavioral outcomes. Control litters received unadulterated wet mash. Serum kynurenine levels were determined in the mothers and male offspring at the time of weaning and at the same time point tissue homogenate KYNA levels were determined in forebrain homogenates of the offspring. Extracellular basal levels of KYNA and glutamate were measured in PFC and HIPPO, using microdialysis, in male offspring after they reached adulthood ($>$ PD 56). The mesolimbic stimulation of prefrontal glutamate, thought to be critical for the top-down control of attentional processing, was quantified using an amperometric microelectrode array following intra-NAC infusions of NMDA (0.05-$0.30\,\mu g/0.4\,\mu L$). Finally, the integrity of cognitive flexibility using an attentional set-shifting task and spatial working memory using the Morris water maze were assessed in separate groups of offspring that had reached adulthood.

Results: The kynurenine supplementation regimen markedly elevated kynurenine levels in the serum of mothers (24-fold increase) and offspring (8-fold increase) at the time of weaning. Furthermore, KYNA levels in forebrain homogenates were elevated at PD21 (110% increase). These effects persisted in adults as extracellular levels of KYNA in HIPPO and PFC were significantly increased by 21% and 11%, respectively. Basal levels of glutamate were decreased by 17% in HIPPO but unchanged in PFC. Interestingly, the mesolimbic modulation of stimulated prefrontal glutamate release in adults was significantly reduced following the early exposure paradigm. In response to an NAC infusion of $0.15\,\mu g$ of NMDA, controls displayed a $2.27\,\mu M$ increase in PFC glutamate levels in contrast to a $0.56\,\mu M$ increase in rats exposed to kynurenine. Behaviorally, adult rats treated with kynurenine during early development exhibited a delayed acquisition of spatial memory in the Morris water maze as well as marked impairments in the reversal and extra-dimensional shift stages of the attentional set-shifting task. Deficits in attention, cognitive flexibility, and working memory are characteristic impairments seen in SZ.

Discussion: Collectively, these data strongly support the protocol of early developmental exposure to kynurenine, and subsequent increases in brain KYNA, as a novel and valid animal model to study pathology and cognitive deficits in SZ. Moreover, the results provide additional support for the KYNA hypothesis of SZ and indicate that drugs designed to counteract the effects of high levels of KYNA may prove useful as cognition-enhancing therapies in SZ.

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23. Inhibition of COMT Reverses the Novel Object Recognition Deficit in COMT-Val Transgenic Mice

Gregory V. Carr*, Francesco Papaleo, Daniel R. Weinberger

National Institute of Mental Health, Bethesda, USA

Background: Catechol-O-methyltransferase (COMT) is critically involved in the catabolism of dopamine (DA) in the prefrontal cortex and prefrontal DA is an important modulator of cognition. A large body of evidence shows that alterations in COMT function affect performance in many different cognitive tasks. A functional COMT polymorphism (Val158Met) has been a popular subject of investigation due to its effect on COMT activity and cognitive performance. COMT-Val, which has greater enzyme activity, is associated with decreased cognitive performance in humans and overexpression of COMT-Val in mice produces deficits in attentional set-shifting, novel object recognition, and working memory in a T-maze task. In contrast, the centrally-active COMT inhibitor tolcapone improves verbal episodic memory in humans with the val/val genotype. These data combined with the known role of prefrontal DA in normal cognitive function suggest that the inhibition of COMT is a potential mechanism by which schizophrenia-related cognitive deficits could be ameliorated. Unfortunately, long-term tolcapone use in the clinic is complicated by the risk of drug-induced liver toxicity, therefore, the development of less toxic COMT inhibitors would serve to improve treatment options. Here, we describe an animal model utilizing COMT-Val transgenic mice and their endogenous deficit in novel object recognition as a potential screen for novel COMT inhibitors.

Methods: We tested COMT-Val transgenic mice and their wild-type littermates in a novel object recognition task previously validated in our laboratory. On Day 1 of testing, mice were given one hour to explore the open field testing arena (no objects) and their locomotor activity was recorded. On Day 2, mice were placed back in the open field for ten minutes and allowed to freely explore two identical copies of the sample object. Immediately following this sample phase, mice were removed from the open field and given an injection of either vehicle (20% (2-hydroxypropyl)-beta-cyclodextrin in saline) or tolcapone (50 mg/kg). One hour later, mice were returned to the open field and were allowed to freely explore a copy of the sample object and a novel object for five minutes. The preference index for the novel object (novel object exploration time / total object exploration time * 100) was subsequently calculated.

Results: In agreement with our previously published work (Papaleo *et al* J. Neurosci 2008), wild-type mice exhibited a significant preference for the novel object while COMT-Val mice showed no preference for the novel object. The difference in novel object preference was not due to alterations in basic exploratory behavior because there were no differences in total exploration time. Only the distribution of exploration time between the novel and familiar object was affected by genotype. Tolcapone treatment had no effect on novel object recognition in wild-type mice. On the other hand, tolcapone reversed the novel object recognition deficit present in the COMT-Val mice. The tolcapone-treated COMT-Val mice exhibited novel object recognition similar to that seen in wild-type mice.

Discussion: This study identifies an animal model of cognitive function that may serve as an ideal screen for the development of novel COMT inhibitors. The novel object recognition task is a rapid (two days) behavioral task that is well suited for drug discovery programs. No training is required for mice to reach optimal performance levels. Additionally, potentially stressful food and water restrictions are not required because the task utilizes the natural preference of rodents to explore novel objects. One potential drawback of the novel object recognition task is that wild-type mice perform at near optimal levels at baseline, therefore, the identification of gain of function therapies would be difficult. Deficits can be induced through the use of additional drug treatment, but this can confound the effects of the target compound. Here, we use the endogenous novel object deficits found in COMT-Val mice as the baseline for testing the effects of COMT inhibition. Additionally, COMT-Val mice, which display increased COMT activity, have a neurobiological deficit that is 1) directly addressed by COMT inhibition and 2) implicated in the development of cognitive dysfunction and schizophrenia. Future studies will investigate the level of COMT activity required for optimal performance in the novel object recognition test.

Disclosure: G. Carr: None. **F. Papaleo:** None. **D. Weinberger:** None.

24. Decreased Synaptogenesis, increased Excitability of Cortical Neurons and Impaired Working Memory in Transgenic Mice expressing the Schizophrenia Associated KCNH2 3.1 Isoform

Jingshan Chen*, Peixiong Yuan, Qingjun Tian, Feng Yang, Grace Zhang, Jiemin Jia, Yun Wang, Jing Du, Paul Glineburg, Gregory Carr, Francesco Papaleo, James Pickel, Zheng Li, Daniel Weinberger

Clinical Brain Disorders Branch, Genes, Cognition and Psychosis Program, NIMH, NIH, Bethesda, USA

Background: Schizophrenia is one of the most challenging mental disorders with little understanding of pathophysiology and few effective treatments for core symptoms. Recent discovery of a novel schizophrenia susceptibility gene, KCNH2, provides some clues for understanding pathophysiology and offers a potential target for development of a new class of antipsychotic drugs. KCNH2 is a voltage-gated potassium channel critical for repolarization of neurons and cardiac myocytes. A novel primate-specific and brain-selective isoform of KCNH2, KCNH2 3.1, has been identified. It is expressed at higher levels in the postmortem brain of schizophrenic patients than those of normal controls and is linked to risk associated genotypes. The most remarkable feature of KCNH2 3.1 is that it lacks the first two exons encoding the PAS domain of the full length KCNH2, an important domain for slow deactivation during repolarization. Without the PAS domain, KCNH2 3.1 closes the channel too quickly during the deactivation phase, and increases the excitability and firing frequency of neurons, which could be a pathophysiological change in schizo-phrenic patients.

Methods: To test the roles of the KCNH2 3.1 in electrophysiology and synaptogenesis of cortical neurons and cognitive functions, we developed inducible transgenic mice carrying the human KCNH2 3.1 transgene. The transgenic mice were analyzed by quantitative RT-PCR, electrophysiological whole-cell recording, confocal microscopy of synaptogenesis, and T-maze working memory test.

Results: Quantitative RT-PCR analyses of gene expression showed high levels of KCNH2 3.1 mRNA in frontal cortex of the transgenic mice. Electrophysiological analyses of cortical neurons in brain tissue sections revealed shortened tail currents and increased excitability of layer V neurons in frontal cortex. Confocal microscopic analyses of synaptogenesis in cortical neurons demonstrated that the number of mature mushroom-shaped synapses was significantly decreased in the transgenic mice, which was reversed by *in vivo* treatment with clozapine, an effective antipsychotic drug and also a potent KCNH2 antagonist.

Discussion: Our results suggest that increases of neuronal excitability and decreases of mature synapses by KCNH2 3.1 might be the pathophysiological changes underlying the mechanism of cognitive dysfunction in schizophrenia and specific modulators of KCNH2 3,1 could improve cognitive function by reversing the deficits caused by KCNH2 3.1 over-expression as a new class of antipsychotic drugs.

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25. Development of a Discrete Trials Task to Assess Serotonergic Modulation of Interval Timing in Mice

Adam L. Halberstadt*, Jared W. Young, Mark A. Geyer

University of California San Diego, La Jolla, USA

Background: The perception of time is essential for survival and is required for the precise organization of sequences of activity as well as the anticipation of behavioral outcomes and future events. One form of temporal perception is interval timing, which refers to the discrimination of durations, typically in the seconds to minutes range. A variety of reports indicate that schizophrenia is associated with timing deficits, and it has been proposed that impaired temporal processing is a core deficit of schizophrenia that contributes to cognitive dysfunction, hallucinations, and inap-propriate behavior. There is also evidence that the serotonergic system, which is believed to play a role in the neuropathology of schizophrenia, regulates temporal perception and timing behavior. For example, serotonergic hallucinogens such as lysergic acid diethylamide (LSD) and psilocybin markedly alter the subjective experience of time and disrupt interval timing. Unfortunately, very little is known about the neural substrate(s) that are involved in serotonergic modulation of timing. Characterizing the mechanism through which the serotonergic system regulates timing will increase our understanding of the linkage between serotonin (5-HT) and schizophrenia, and will provide insight into the mechanism of action of hallucinogenic drugs.

Methods: We have developed a discrete trials interval timing task in mice that can be used to elucidate the neural and receptor mechanisms underlying the modulation of interval timing by both endogenous 5-HT and hallucinogenic drugs. This is a translational paradigm that is very similar to tasks used to assess timing in rats and humans. Development of the task in mice allows examination of the genetic contributions to performance, and facilitates the use of optogenetic challenges. In the discrete trials task, a lamp is illuminated for a variable duration, and then two levers are presented. Responding on lever A is reinforced if the stimulus duration is shorter than 6.5 s, and responding on lever B is reinforced if the stimulus duration is longer than 6.5 s. C57BL/6J

mice were trained to discriminate between short (2.5 and 5.0 s) and long (7.5 and 10 s) stimulus durations, and then challenged with a wider range of test stimuli (1.25-11.75 s). To determine whether the performance of the task is sensitive to the effects of serotonergic ligands, we also examined whether the hallucinogen and 5-HT2A/2C receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) alters performance of the task.

Results: We found that mice can learn to reliably discriminate between the short and long duration training stimuli, responding on the correct lever $>80\%$ of the time for the two extreme stimulus durations (2.5 and 10 s). Challenge studies demonstrated that the proportion of lever B responding increased with the stimulus duration. Administration of DOI (0.5-1.0 mg/kg, IP) markedly altered %B lever responding ($F(14,28) = 5.90$, $p < 0.0001$). The effect of DOI was not associated with a significant change in the response rate, suggesting that the effect reflects a disruption of interval timing, as opposed to nonspecific effects on operant responding. DOI also increased the Weber fraction, a measure of timing precision.

Discussion: These experiments demonstrate that mice can be trained to perform an interval timing task, and indicate that timing in mice is altered by serotonergic hallucinogens. Development of timing tasks that are sensitive to hallucinogen effects is highly desirable because timing behavior is translatable, whereas many behavioral paradigms currently used to assess the effects of hallucinogens in rodents have no human counterpart, and thus it is not clear how the behaviors relate to the subjective effects of hallucinogens. Our goal is to use this behavioral paradigm to investigate the regulation of interval timing by the serotonergic system, and to determine the neural site(s) involved in this effect. It is possible that the disruption of temporal perception induced by hallucinogens could be developed as an animal model relevant to schizophrenia, potentially facilitating the development of novel agents with antipsychotic activity.

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26. Enhanced Cue-Induced Relapse to Cocaine Seeking in the Neonatal Ventral Hippocampal Lesion (NVHL) Rat Model of Schizophrenia

Rose-Marie Karlsson*, Daniel Kircher, Yavin Shaham, Patricio O'Donnell

University of Maryland, School of Medicine, Baltimore, USA

Background: Close to half of the patients with schizophrenia have a lifetime diagnosis of substance abuse. These psychiatric comorbidities are commonly believed to be due to self-medication; however, recent studies suggest that this vulnerability may instead be due to a common pathophysiology. In humans and experimental animals, relapse to drug seeking can be triggered by environmental cues, a process that is dependent on an intact medial prefrontal cortex (mPFC), which has also been proposed to be affected in patients with schizophrenia. One of the most comprehensively studied animal models of schizophrenia is the neonatal ventral hippocampal lesion (NVHL) model; this early neuroanatomical manipulation causes a dopamine-mediated disinhibition of PFC output. Based on this, we hypothesized that NVHL rats would show enhanced cue-induced reinstatement of cocaine seeking.

Methods: At post-natal (PD) day 7-9 male pups (15-20 g) received either bilateral excitotoxic lesion to the ventral hippocampus via injection of ibotenic acid or sham surgery. Upon reaching adulthood (PD >56), NVHL and SHAM rats were implanted with a chronic indwelling jugular catheter and were trained for 10 days to self-administer cocaine (3-h/day, Fixed-Ratio-1 20-sec timeout reinforcement schedule). During training, active lever-presses resulted in a cocaine infusion (0.75 mg/kg/injection) and the presentation of a light-tone compound cue for 5 sec. Response to inactive lever was recorded but had no programmed consequences. Next, the rats underwent extinction training for a minimum of 10 sessions (3 h/day) or until they had less than 15 active lever presses for 2 consecutive days. During this time, neither active nor inactive lever press produced any programmed consequences (i.e. no infusion, light or tone cues). During the subsequent reinstatement test, active lever-presses resulted in the presentation of the tone-light compound cue, but not cocaine.

Results: For intake of cocaine during the self-administration there was significant effect of session ($F_{9,198} = 15.2$, $P < 0.001$) due to increases in cocaine intake over days, but no effect of lesion status or session x lesion status (P values > 0.05). For the extinction training, there were significant effects of session ($F_{9,198} = 26.0$, $P < 0.001$) and session x lesion status interaction ($F_{9,198} = 2.3$, $P < 0.05$). *Post-hoc* analysis revealed that NVHL had significantly more active lever presses during the first day of extinction ($P < 0.01$). Additional analysis revealed that NVHL rats took significantly longer to reach the extinction criterion set *a priori* ($\chi^2 = 4.7$, df $= 1$, $P < 0.05$). Finally, when the cue-induced reinstatement session was compared to the last day of extinction training, there was a significant effect of lesion status ($F_{1,22} = 5.8$, $P < 0.05$), session ($F_{1,22} = 80.7$, $P < 0.001$), lever ($F_{1,22} = 76.1$, $P < 0.001$), and lesion status x session x lever interaction ($F_{1,22} = 8.0$, $P < 0.01$). *Post-hoc* analysis revealed that NVHL rats had a significantly increased active lever responding to the cues ($P < 0.001$).

Discussion: While we did not detect any difference in cocaine intake during training between the two groups, NVHL rats showed both a heightened extinction and cue-induced reinstatement responding compared to SHAM controls. These data suggest that NVHL rats are more vulnerable to cue-induced relapse to cocaine seeking. Additional studies using this animal model could shed light on the mechanisms involved in the prevalence of schizophrenia and drug addiction.

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27. Vesicular Monoamine Transporter 1 (VMAT1) Null-Mutant Mice show Neurodegenerative Changes in Hippocampus - Implications for Schizophrenia and Bipolar Disorder beyond the Monoamine Hypothesis

Pushpinder K. Multani, Marcel Estévez, Rachel Hodge, Glenn A. Doyle, Falk W. Lohoff*

University of Pennsylvania, Philadelphia, USA

Background: Genetic variants in the human vesicular monoamine transporter 1 (VMAT1) gene on chromosome 8p have been associated with increased risk for bipolar disorder and schizophrenia. Given recent evidence of VMAT1 brain expression and its biological role in presynaptic monoaminergic neurotransmission, we used a knock-out (KO) mouse model to investigate biochemical and behavioral consequences of VMAT1 deficiency. Because of high VMAT1 expression in particular in the dentate gyrus region and robust evidence of neurocognitive deficits in patients with bipolar disorder/schizophrenia likely due to hippocampal abnormalities, we have investigated neurodegenerative changes in hippocampus of VMAT1 KO mice and assessed behavioral phenotypes related to memory and cognition.

Methods: VMAT1 null-mutant mice were generated using gene targeting. Formalin-fixed Coronal (40 μm thick) sections from

adult male VMAT1 KO and wild type (WT) mice (8-12 weeks old, n = 5/genotype) were used for immunohistochemistry with an active caspase-3 specific antibody (Cell signaling #9662). Neurons that stained positive for caspase-3 in the dentate gyrus were counted on images captured and quantified using Image Pro Plus software. Groups were compared statistically using the Student's t-test. Hippocampal tissue lysates were further investigated to determine the protein profile of active caspase-3 by Western immunoblot. Western analysis for caspase-3 in the hippocampus from WT and KO mice (8-12 weeks old, n = 5/genotype) was done using standard protocols. Immunoblots were quantified using Image J software. Mice were behaviorally assessed for baseline contextual fear conditioning and spatial object recognition.

Results: VMAT1 KO mice displayed a significant (p < 0.001) increase of active caspase-3 labeling in neurons of the dentate gyrus as compared to WT controls. Western analysis confirmed our IHC findings of caspase-3 activation, as we observed increased cleaved 17kda product in the hippocampal lysate of VMAT1-/- mice compared to the WT controls. VMAT1 KO mice showed significant deficits in spatial object recognition but not in the contextual fear conditioning test.

Discussion: Our findings indicate that deletion of VMAT1 leads to apoptotic cell death in hippocampus with subsequent neuro-degenerative changes and cognitive deficits in mice. Given the important role of the hippocampus in a variety of psychiatric disorders, VMAT1 represents a novel target for investigation in particular in disorders with neurocognitive symptom domains. Future studies are necessary to elucidate the exact mechanism for increased cell death in VMAT1 null-mutant mice and its behavioral consequences.

Disclosure: P. Multani: None. **M. Estévez:** None. **R. Hodge:** None. **G. Doyle:** None. **F. Lohoff:** None.

28. Effects of Genetic Reduction of Activity-Dependent BDNF on Cortical Slow-Wave Activity

Keri Martinowich*, Robert Schloesser, Nicholas Hardy, Dennisse Jimenez, Bai Lu, Daniel Weinberger

National Institute of Mental Health, Bethesda, USA

Background: Abnormal sleep is a prominent feature of neuropsychiatric disorders, but the relationship to disease pathophysiology is unclear. Normal brain function and generation of sleep are linked by common neurotransmitter systems and shared circuitry. Thus, behavioral manifestations of neuropsychiatric disorders and disordered sleep may share similar mechanisms of origin. The most well-established physiological marker of sleep intensity is slow wave activity (SWA), the electroencephalogram power measured between 0.5-4.0 Hz. SWA is regulated homeostatically; power in the delta frequency increases as a function of preceding waking. SWA reflects the extent of synaptic strength and efficacy, which promote neuronal synchronization. Prolonged waking increases markers of synaptic plasticity, including brain-derived neurotrophic factor (BDNF), and subsequently, SWA. The most consistent electrophysiological sleep abnormality reported in schizophrenia is decreased SWA. A role for BDNF, via its effects on GABAergic inhibitory interneuron circuitry, has been implicated in schizophrenia. Neuronal excitability and synaptic efficacy are regulated by GABAergic interneurons and developmental disruption of inhibitory circuitry is linked to schizophrenia. We previously reported that a genetic mouse model of reduced activity-dependent BDNF signaling (BDNFK-IV) shows impairments in inhibitory interneuron function and sleep behavior. Follow up studies were designed to assess SWA in BDNFK-IV animals at baseline and in response to homeostatic challenge.

Methods: We utilized EEG recordings to monitor SWA. Surgeries were performed to chronically implant electrodes and animals were allowed at least two weeks for recovery before recordings. Animals were acclimated to attachment of a flexible tether for 24 hours before conducting a 24 h baseline recording. In a subsequent experiment, animals were subjected to total sleep deprivation for 8 hours, a homeostatic challenge known to increase SWA during rebound sleep, before initiation of recordings. Experimental local field-potential (LFP) waveforms were manually scored as wake, slow-wave sleep, or repetitive eye movement (REM) sleep based on the EEG and electromyogram waveforms. LFPs were further processed to analyze power spectra and sleep latencies using tools that were developed with Python and PyLab add-on in conjunction with commercially available software.

Results: Analysis of our recordings showed that compared to wild-type animals, BDNFK-IV animals exhibit a significant decrease in SWA during non-REM sleep. In addition, the magnitude of induction in delta power as well as the latency to sleep is impaired following total sleep deprivation compared to wild-type counterparts.

Discussion: Our previous results showed deficits in inhibitory interneuron signaling as reflected by deficits in frontocortical inhibitory post-synaptic potentials and immunodetection of the calcium-binding protein, parvalbumin in BDNFK-IV animals. In addition, BDNFK-IV animals show substantial deficits in fronto-cortical expression of several neuropeptides, including cortistatin, neuropeptide Y, somatostatin, corticotropin binding hormone releasing peptide and tachykinin-1, which, within the cortex are exclusively localized to inhibitory interneurons. Interestingly, expression of several of these peptides is also regulated in response to prolonged waking, suggesting a role in sleep homeostasis. Our studies demonstrate a role for neuronal activity-dependent BDNF in regulation of sleep behavior as well as SWA. Future studies will aim to examine causal relationships between effects of activity-dependent BDNF signaling on development of inhibitory circuits and the ability of synaptic plasticity to promote neuronal synchronization.

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29. Further Validation of a New Animal Model of Schizophrenia: Whole Transcriptome Sequencing by RNA-Seq reveals Expression Changes of Genes in mPFC Relevant to Schizophrenia

Charles D. Nichols*, David A. Martin, Danuta Marona-Lewica, David E. Nichols

LSU Health Sciences Center, New Orleans, USA

Background: Schizophrenia is a highly debilitating psychiatric disorder that remains poorly understood despite decades of research. The lack of understanding of the complex etiology of schizophrenia has been a major factor in the failure of modern pharmacotherapy to treat many aspects of the illness effectively. There is need for an integrative animal model that encompasses the positive, negative, and cognitive symptoms of schizophrenia. Current animal models to study this disorder are problematic because they employ blockade of the behavioral effects of dopaminergic agonists such as apomorphine or amphetamine, or they utilize acute administration of drugs whose effects are thought to resemble psychosis, such as phencyclidine, events that do not occur with the *onset* of psychosis. Therefore, an animal model that more closely mimics the behavioral, neurochemical, and genetic aspects of schizophrenia than current models is highly desirable. As a new rodent model of schizophrenia we recently proposed rats treated for three months with low doses of the powerful hallucinogenic drug, lysergic acid diethylamide (LSD). This treatment gradually induces aberrant behaviors beginning at about six weeks that are stabilized by three months, which persist at full strength for at least many months *in the absence of drug.*

Significantly, the aberrant behaviors are relevant to both the positive and negative symptoms of schizophrenia (hyper-reactivity, social withdrawal, anhedonia, etc.), and can be temporarily reversed by treatment with antipsychotics. Here, using next generation sequencing, we present data on the molecular genetic effects in the mPFC of rats chronically treated for three months with LSD, followed by a four week wash-out. To our knowledge, this study is the first to use Next Generation Sequencing technologies to examine the effects of a drug within the brain.

Methods: Male Sprague-Dawley rats were treated with 0.16 mg/kg LSD ip every other day for 90 days. Behavioral analysis was performed 30 days after the last LSD treatment to verify persistent behavioral changes in the LSD-treated group compared to the saline-injected control group. Brains were collected and total RNA isolated from the medial prefrontal cortex of each rat and subjected to RNA-Seq analysis on an Illumina genome analyzer. Bioinformatics were performed utilizing Galaxy as a front-end for Bowtie and Tophat, followed by statistical comparison between animals and treatment groups using DESeq. Functional cluster analysis utilized NIH's Database for Annotation, Visualization, andIntegrated Discovery. Validation and expression refinement were performed by QPCR.

Results: Analysis of RNA-Seq data indicates that over 600 genes are significantly altered in expression by greater than 20%. Unlike microarray data, which are only semi-quantitative at best, Next Generation Sequencing is believed to be as quantitative as QPCR. Functional clustering indicates that genes implicated in synaptic plasticity are highly represented among our candidate pool. Importantly, there is substantial overlap between our results and previous studies examining post-mortem schizophrenic brain for certain key genes and pathways including dopaminergic, glutamatergic, serotonergic, and GABAergic systems *that have all been strongly implicated in the processes underlying schizophrenia.* Additional interesting and relevant gene expression changes include those for dysbindin, GAD2, discs-large, BDNF, NPY, SOD1, GST, THIO2, and ErbB4, which are potentially linked to the pathogenesis of schizophrenia.

Discussion: Administration of low doses of LSD for three months produced significant changes in gene expression in the mPFC. Importantly, many of these changes are conserved with those observed in schizophrenia, as well as other animal models of the disease. We hypothesize that changes in the brain neurochemistry of LSD-treated animals reflect permanent shifts in homeostasis that manifest as long-lasting, perhaps permanent alteration in behaviors. A potential key feature and advantage of our model over other existing models is that, once the relatively short LSD treatment regimen is completed, one has a viable discovery platform, *in the absence of drug,* for up to 18 additional months. Our results demonstrating aberrant behaviors conserved with schizophrenia, conserved molecular genetic changes in the brain underlying these behaviors, and the ability of antipsychotics to reverse these behaviors provides face, construct, and predictive validity for our model in the study of schizophrenia. Future studies will utilize this model as a discovery platform for novel therapeutic targets and strategies.

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30. Validation of the Cesarean Section Birth Model of Schizophrenia in Rat at the Molecular Level

Gabriela Novak*, Theresa Fan, Susan George

University of Toronto, Toronto, Canada

Background: Rats born by cesarean section have long been used as an animal model of schizophrenia. Rodents born by this method show a number of key schizophrenia-like phenotypes, including altered prepulse inhibition (PPI), amphetamine sensitivity and increased proportion of dopamine 2 receptors (D2Rs) in their high affinity state (D2High). Recently, studies using transgenic mice with inducible D2R gene expression selective to the striatum, have shown that a temporary upregulation of D2Rs in the striatum during development leads to permanent changes in dopaminergic signaling of the prefrontal cortex, characteristic of schizophrenia. The first two postnatal weeks of life in rat is a known period of susceptibility, when stress and other insults lead to alterations in neurodevelopment relevant to neuropsychiatric disease. This parallels human striatal development during second trimester, a period during which stress or infection in the mother is known to increase predisposition to schizophrenia in the offspring. Hence, we wanted to investigate the early postnatal expression of DRs in the c-section model of schizophrenia. In the present study we examined the developmental changes in expression of dopamine receptors (D1Rs and D2Rs) and of related pathways during the first three weeks of life in rats born by cesarean section.

Methods: We used quantitative real-time PCR to measure the expression levels of D1Rs and D2Rs in the striatum of animals born by cesarean section. Striatal tissues were collected from postnatal day 2 through postnatal day 22, every 2-3 days. Each time point is represented by three independent striatal samples from the c-section group and three from a control group born vaginally. Both groups were from timed pregnancies, of the same gestation age. To avoid prematurity, c-section was performed only after two dams from the group delivered vaginally. All pups were fostered. The data were standardized to total RNA, as well as to b-glucuronidase, a housekeeping gene. In order to investigate other pathways of the D2R signalling network, the expression of CaMKIIa, CaMKIIb and DARPP-32 genes was investigated. Gene expression of lineage-specific markers and developmental markers was investigated for indication of lineage-specific effects or maturation delays.

Results: We found a temporary upregulation of D2Rs in the striatum of these animals, lasting throughout the second week of life. There was no change in D1R expression or in the expression of related signaling molecules, such as CaMKII or DARPP-32, indicating that the upregulation of D2Rs may be one of the earliest alterations present and, possibly, the primary change leading to the schizophrenia phenotype observed in these animals.

Discussion: Observations in transgenic mice show that such temporary upregulation of D2R expression, specific to the striatum, is sufficient to produce schizophrenia-like dysregulation of the prefrontal cortex. Specifically, it was shown to decrease inhibitory neurotransmission in the PFC and reduce sensitivity of dopaminergic modulation of inhibitory postsynaptic currents in this region. This effect was D2R specific and lead to a functional deficit in the GABAergic system. A deficiency in the GABAergic system is thought to be secondary to alterations in the dopaminergic system in schizophrenia. Hence, the observation that the D2Rs are upregulated in the striatum of c-section born rats and that this occurs during a key neurodevelopmental period linked to schizophrenia susceptibility further validates this rat model of schizophrenia at the molecular level, providing us with a valuable and minimally invasive rat model of the disease. This offers numerous advantages and expands our repertoire beyond the use of mice models.

Disclosure: G. Novak: None. **T. Fan:** None. **S. George:** None.

31. Rat Strain Sensitivity to Startle-EnhancingEeffects of D1 Stimulation vs. Gating-Disruptive Effects of D2 Stimulation

Neal R. Swerdlow*, Steve T.C. Pham, Daniel Keolasy, Samantha R. Hines

University of California San Diego, La Jolla, USA

Background: Outbred rat strains differ in the behavioral sensitivity to dopamine (DA) agonists. For example, we reported significantly

greater sensitivity to the prepulse inhibition (PPI)-disruptive effects of D2-family agonists in Sprague-Dawley (SD) vs. Long Evans (LE) rats; this sensitivity is heritable, and appears to reflect differential activation of DA signaling in the nucleus accumbens (NAC). Our studies utilizing a mixed D2/D1 agonist, apomorphine (APO), have often detected startle-enhancing effects of APO in LE rats vs. no effects, or even startle-suppressing effects, in SD rats. Here, we tested whether these strains differ in sensitivity to the startle-enhancing effects of D1 stimulation, and whether this phenotype is separable from changes in PPI.

Methods: Startle magnitude and PPI were assessed in male SD and LE rats after treatment with the D1-preferential agonist, SKF 81297 (0.3, 1.0, 3.0 mg/kg sc), using 85 dB(A) prepulses and 95-120 db(A) startle pulses over a 70 dB(A) background. Startle-enhancing effects of SKF 81297 were also tested in LE rats after pretreatment with the D1-preferential antagonist, SCH23390 (0.05 mg/kg sc).

Results: SKF 81297 significantly potentiated startle in LE but not SD rats, and had no significant effect on PPI in either strain. ANOVA of startle magnitude revealed significant interactions of dose x strain and dose x strain x pulse intensity (p's < 0.0001); post-hoc comparisons detected startle-potentiating effects of SKF 81297 only in LE rats, at 1.0 and 3.0 mg/kg doses. These startle-potentiating effects of SKF 81297 (1.0 mg/kg) were prevented by pretreatment with SCH23390.

Discussion: Strain differences in the DAergic regulation of startle phenotypes exhibit a complementary pattern, with SD > LE sensitivity to the PPI-disruptive effects of D2 stimulation, and LE > SD sensitivity to the startle-enhancing effects of D1 stimulation. In the case of PPI, this differential D2 sensitivity appears to reflect differences in NAC gene expression and D2-signaling, but the neural basis for differential D1 startle sensitivity is not known. We reported LE > SD sensitivity to the startle-enhancing effects of mild restraint stress; based on this and convergent pharmacological data, we are now examining the role of the amygdala in the differential expression of D1-potentiated startle in SD vs. LE rats. Supported by NIH grants: MH068366, MH059803 and MH042228

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32. DISC1 Partners with Serine Racemase to Modulate D-serine Production by Astrocytes

Mikhail Pletnikov*, Ting Martin Ma, Jun Nomura, Bagrat Abazyan, Sofya Abazyan, Akira Sawa, Solomon H. Snyder

Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, USA

Background: Disrupted-In-Schizophrenia-1 (DISC1) is a strong candidate gene for psychiatric disorders. The majority of studies have focused on neuronal functions of DISC1. A recent report has identified expression of DISC1 in glial cells, including astrocytes (Saurav, 2010). Our study sought to elucidate roles for DISC1 in astrocytes, as abnormal astrocytic functions may contribute to psychiatric disease.

Methods: To study DISC1 functions in astrocytes, we used a mouse model of GFAP promoter-driven selective and inducible expression of mutant DISC1 in astrocytes to impact endogenous DISC1 in a dominant-negative manner. We evaluated the effects of mutant DISC1 on glutamate uptake, D-serine production, and expression of the major astrocytic markers in primary astrocytes and the brain of transgenic mice at different time points during postnatal development. In addition, GFAP-DISC1 transgenic mice were assessed in a series of behavioral tests relevant to aspects of schizophrenia.

Results: Astrocytic expression of mutant DISC1 did not produce gross developmental abnormalities in mice but was associated with elevated anxiety, mild cognitive deficits and exacerbated responses to a NMDA antagonist, MK-801, in adult DISC1 transgenic mice. Mutant DISC1 had no significant effect on glutamate uptake, levels of GFAP, GLT-1 or connexins in primary astrocytes or brain tissue. In contrast, we found significantly decreased expression of endogenous mouse DISC1 and serine racemase (SR), leading to diminished levels of D-serine in primary astrocytes derived from mutant DISC1 newborn mice. Our biochemical experiments suggest that DISC1 may partner with SR to modulate production of D-serine.

Discussion: Our model system may facilitate studies of DISC1 function in astrocytes to address roles for glial cells in the pathophysiology of schizophrenia.

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33. Initial Behavioral and Neurochemical Characterization of Perinatal Ketamine Administration in Mice

Susan B. Powell*, Luis F. Garcia, Jared W. Young, M. Margarita Behrens

University of California San Diego, La Jolla, USA

Background: Decreased number of GABAergic neurons is a consistent finding in schizophrenia postmortem studies, where the subpopulation of parvalbumin-expressing (PV) fast-spiking inhibitory neurons is specifically affected. Deficits in cortical fast-spiking inhibitory systems may underlie the psychotic features and cognitive deficits associated with schizophrenia-related disorders. Our initial studies indicated that NMDA-R antagonists produce oxidative stress in brain through activation of the IL-6/Nox2 pathway in adulthood. We have recently confirmed that this pathway is also responsible for the effects of perinatal administration of NMDA-R antagonists on PV-expressing GABAergic interneurons. In the current studies we (1) assessed the effects of perinatal ketamine on PV-interneurons during adolescence, (2) examined whether the loss of PV-interneurons following perinatal ketamine administration was due to death of the cells, and (3) began an initial characterization of the effects of perinatal ketamine on behaviors relevant to schizophrenia (e.g. locomotor activity, startle reactivity/plasticity, and cognitive flexibility).

Methods: C57BL/6J and G42 mice were bred in our facility. Ketamine (30 mg/kg, SC) was administered on postnatal days 7, 9, and 11 (perinatal ketamine). PV-expressing neurons were labeled and counted as described in (Dugan et al., 2009; PLoS One 4(5):e5518)and expressed as percent of WT saline conditions. Activity was measured in six 30.5 x 61 cm Plexiglas chambers with floor and wall holes by use of photobeams to register holepokes, horizontal activity, and rearing (Halberstadt et al., 2009; Neuropsychopharmacology. 34(8):1958-67). Startle and PPI testing were performed in SR-AB startle chambers, using an experimental session that was specifiedpreviously (Asp et al., 2010; Int J Neuropsychopharmacol. 13(4):475-85). Male mice were tested in the attentional set shifting task (ASST) as described in (Young et al., 2010; Cogn Affect Behav Neurosci. 10(2):243-51) in which odor and platform texture were used as the relevant dimensions. Total trials to criterion and mean correct latency over 7 stages of discriminations (e.g. reversals, intra- & extra-dimensional shifts), were used as the dependent measures.

Results: To test whether the reduced number of PV-interneurons was due to their death, we used a mouse line expressing green fluorescent protein exclusively in PV + interneurons (G42 line x C57BL/6J) and exposed them to perinatal ketamine. Analysis of PV

and GFP co-expression at 5-6 weeks old showed a similar decrease in PV expression as we had observed in WT adult animals; however, there was no decrease in GFP-expressing neurons, suggesting that the cells were still alive, but their developmental maturation was affected (overall ANOVA; $F(3,12) = 12.278$, $p < 0.001$; saline vs. ketamine in WT mice, $p < 0.05$). The initial behavioral characterization revealed subtle differences in behavior resulting from perinatal ketamine administration. Ketamine-treated mice, particularly females, spent less time in the center of an open field during the second half of the session, (time x drug; $F(1,95) = 4.10$, $p < 0.05$), suggesting that ketamine-treated mice show less habituation to the open field. To examine the sensitivity of the mice to an NMDA antagonist in adulthood, we challenged the mice with vehicle or one of two doses of MK801 (0.1 & 0.3 mg/kg). Ketamine-treated female mice were more sensitive to the startle increasing effects of MK801 (sex x ketamine x MK801; $[F(2,138) = 3.58$, $p < 0.05]$) but not differentially sensitive to the PPI-disruptive effects of MK801. In order to examine the effects of perinatal ketamine exposure on cognitive function, we first tested the mice in the ASST. Preliminary data indicate that ketamine-treated mice had increased trials to criterion compared to saline-treated mice during the intradimensional shift ($F(1,28) = 5.61$, $p < 0.05$), suggesting a failure to form an attentional set.

Discussion: Taken together these data suggest that the effects of ketamine administered during the second postnatal week on PV interneurons are evident during adolescence and are not due to cell death. This reduction in PV in the prefrontal cortex is accompanied by behavioral differences in startle response, open field activity, and set shifting performance. Hence, the GABAergic inhibitory system, specifically the PV + interneurons, may be uniquely sensitive to various environmental perturbations during early development.

Disclosure: S. Powell: Part 1: Service contract with Ferring Pharmaceuticals, Part 4: Service contract with Ferring Pharmaceuticals. L. Garcia: None. J. Young: Part 1: Service agreement with Cerca Insights, Part 4: Service agreement with Cerca Insights. M. Behrens: None.

34. Behavioural Evaluation of Positive AMPA Receptor modulators CX1739 and CX1837 in Rodent Models of Sustained Attention and Non-Spatial Working Memory

Mohammed Shoaib*, Mark Varney

Institute of Neuroscience, Newcastle University, Newcastle, United Kingdom

Background: Cognitive impairments in neuropsychiatric patients are thought to be due to glutamate dysfunction in the CNS. Specifically, the cognitive deficits associated with schizophrenia (CDS) have been highlighted as potential targets for intervention using glutamatergic compounds. The present study reports on the effect of a series of positive AMPA receptor modulators (or "AMPAKINES") on two cognitive domains; sustained attention and working memory.

Methods: In a 5-choice serial reaction time task (5CSRTT), male hooded Lister rats ($n = 14$) were trained to a performance criterion before acute treatment with AMPAKINES (CX1739 & CX1837) given 20 min prior to tests. The odour span task (OST) was used to assess effects on non-spatial working memory. Once rats ($n = 8$) met performance criterion on the OST, sub-chronic exposure to ketamine (10 mg/kg daily for 5 days) impaired their performance to detect a novel odour from an increasing series of odours that were placed randomly on the platform to obtain food reward.

Results: Significant improvements on accuracy and reductions on anticipatory responses in the 5CSRTT were obtained with CX1739 (3 & 10 mg/kg) and CX1837 (0.3-1.0 mg/kg) similar to effects observed with the norepinephrine-reuptake inhibitor, atomoxetine

(1.0-10 mg/kg) under the same conditions. On OST performance, acute treatment with CX1739 (0.3-10.0 mg/kg IP) or CX1837 (0.1-1.0 mg/kg) dose-dependently restored the ketamine-induced impairments. The same dose range for both AMPAKINES had no effect in the vehicle-treated groups. Furthermore, these improvements on working memory in ketamine-treated subjects were still apparent following repeated tests with CX1739 (3 mg/kg IP) and also in subjects that had a history of chronic exposure to clozapine (3.0 mg/kg daily for 21 days).

Discussion: The results with these AMPAKINES highlights potential therapeutic roles for their use to improve cognition states associated with impulsivity as measured in the 5CSSRT and also the deficits in working memory following sub chronic ketamine-exposure, improvements that do not show tolerance with repeated use and are also apparent when used as adjuncts with neuroleptics for treating CDS. (research supported by Cortex Pharmaceuticals, Irvine, US)

Disclosure: M. Shoaib: Part 1: Janssen Research & Development, Part 2: None, Part 3: None, Part 4: Janssen Research & Development, Part 5: N/A. M. Varney: None.

35. Dopamine Transporter Knockdown Mice exhibit Poorer Within-Session Risk Learning in a Mouse Iowa Gambling Task consistent with Bipolar Mania Patients

Jared W. Young*, Jordy van Enkhuizen, Mark A. Geyer

University of California, San Diego, La Jolla, USA

Background: Patients with Bipolar Disorder (BD) mania exhibit a range of symptoms that contribute to many of the difficulties they face throughout their lives. During manic episodes, patients exhibit risk-taking behaviors that are detrimental to their well-being, including high-risk gambling. This behavior can be quantified using the Iowa Gambling Task (IGT) and is not currently treated adequately, as evidenced by the fact that poor IGT performance is also observed during periods of euthymia. The IGT requires subjects to learn within a session to select options that provide lower reinforcement because there is less associated risk and more gained overall. BD patients do not learn this rule to the same level as healthy comparison subjects. Animal models of this impaired learning are required in order to test for novel treatments for this symptom. We have described dopamine transporter (DAT) knockdown (KD) mice that exhibit an aberrant profile of exploratory behavior that is consistent with BD mania patients when assessed in the mouse and human Behavioral Pattern Monitors (BPM) respectively. Moreover, the reduced DAT levels in unmedicated subjects with BD support the etiological validity of this model. To further examine the similarity of this model to mania, we 1) replicated our findings of abnormal exploration in the BPM using mice backcrossed onto the C57BL/6 strain, and 2) tested the risk learning behavior of these mice in a mouse version of the within-session learning IGT.

Methods: After testing in the BPM, male DAT wildtype (WT) and KD ($n = 28$ and 31 respectively) littermates were trained to holepoke for a single food reward in any 1 of 4 locations. The reward contingencies were altered for a single 60 min IGT session whereby 2 locations provided 2 rewards but also long punishment durations (flashing light), while the other 2 locations continued to provide only 1 reward but with short punishment durations. Holepokes into each location were rewarded and punished randomly on similar schedules. Good, intermediate, and poor learners were quantified by taking % good choices (% holepokes into the low reward holes from total holepokes) of the third trial period from the % good choices from the first trial period and stratifying them as 1) > 0.5, 2) between 0.5 and -0.5, and 3) < 0.5 standard deviations from the mean respectively. IGT performance was analyzed using a repeated measures ANOVA with gene and

group as between-subjects factors and trial period as the within subject factor. BPM performance was analyzed using a one-way ANOVA with gene as the between-subjects factor. Significant main effects and interactions were subjected to Tukey *post hoc* analyses. **Results:** DAT KD mice exhibited increased activity ($F_{(1,52)} = 46.3$, $p < 0.0001$) and exploration ($F_{(1,52)} = 4.9$, $p < 0.05$), as well as more straight-line patterns of movement ($F_{(1,52)} = 6.0$, $p < 0.05$) in the BPM. When tested in the IGT, there were more WT (39%) than KD (29%) mice in the good learners group, despite % good choices not differing between the 3 groups during trial period 1 ($F_{(2,53)} = 1.5$, ns). Although both WT ($F_{(2,20)} = 13.3$, $p < 0.0001$)and KD ($F_{(2,16)} = 11.0$, $p < 0.005$)mice exhibited risk-related learning in the IGT, WT mice exhibited a trend for more % good choices when compared with KD mice ($F_{(1,18)} = 3.9$, $p = 0.064$). The % good choices of WT and KD mice in the intermediate ($F_{(1,21)} = 2.8$, $p = 0.11$)and poor ($F < 1$, ns) groups did not differ significantly. **Discussion:** Mice with reduced expression of the DAT exhibit poor risk-related learning in a mouse IGT consistent with subjects with BD in the human version of the IGT. The increased risk preference of these mice in a within-session risk-learning paradigm supports their use as a model of BD. Despite changing the background strain of these mice (C57BL/6 compared with 129/S previously), the DAT KD mice continued to exhibit an abnormal pattern of exploration in the mouse BPM consistent with that of patients with BD mania in the human BPM. These data provide further support for the use of these mice in testing novel compounds to treat BD mania.

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36. Progression of Drug Cue-Induced Phasic Dopamine Release from Limbic to Sensorimotor Striatum mediates Action Selection of Drug-Taking Behavior in a Rodent Model of Drug Addiction

Ingo Willuhn*, Barry J. Everitt, Paul E.M. Phillips

Departments of Psychiatry & Behavioral Sciences and Pharmacology, University of Washington, Seattle, USA

Background: Dopamine neurotransmission in the ventral striatum is strongly implicated in the acute reinforcing effects of drugs of abuse. After repeated drug intake, the dorsolateral striatum is thought to become increasingly involved in the control of drug taking and the automation of this behavior. Dopamine neurotransmission in the dorsolateral striatum is potentially regulated by ventral striatal circuitry via serial striatonigrostriatal connection. Therefore, we simultaneously characterized phasic dopamine signaling in these two brain regions using an animal model of drug addiction. Changes in dopamine release were measured over the course of weeks in rats self-administering cocaine. Furthermore, we tested whether dopamine release in the dorsolateral striatum is dependent upon ventral striatal circuitry. Finally, we blocked dopamine signaling in the dorsolateral striatum to further investigate its role in the control of drug taking. **Methods:** Multiple carbon-fiber microelectrodes for fast-scan cyclic voltammetry or guide cannulas for microinfusion of the dopamine receptor antagonist alpha-flupenthixol were chronically implanted in the striatum of rats bearing indwelling intravenous catheters for drug self-administration. Animals had access to cocaine for one hour per day for 20 days. During a self-administration session, a nose poke into the active hole elicited a cocaine infusion (FR1, 0.5 mg/kg/infusion) that was accompanied by a 20-second presentation of an audiovisual stimulus (drug cue). Additional responses during this time-out period

or nose pokes into the inactive hole (control) were without consequences. **Results:** Throughout self-administration training, we observed phasic dopamine release in the ventral striatum associated with contingent and non-contingent presentation of the drug cue. In contrast, cue-related dopamine signals in the dorsolateral striatum developed only during later stages of training. Unilateral lesion of the ventral striatum with quinolinic acid inhibited the development of such dorsolateral dopamine signals in the ipsilateral, but not contralateral, hemisphere without affecting drug intake. Bilateral blockade of dopamine receptors in the dorsolateral striatum with alpha-flupenthixol increased drug intake both early in training, before dorsolateral dopamine signaling were detected, as well as later in training, when dopamine signals were present. However, the efficiency of drug intake (successful / total nose pokes) was affected at the late time point only. **Discussion:** Our results demonstrate that phasic dopamine signaling in the striatum in response to the presentation of drug cues is dynamic and region specific, developing in the ventral then dorsolateral striatum sequentially. This progression of dopamine signaling from limbic to sensorimotor regions of the striatum requires intact ventral striatal circuitry and plays a role in the action selection of drug-taking behavior. Overall, these data implicate recruitment of sensorimotor striatal circuitry for dopamine-mediated encoding of drug cues in the development of long-term efficient performance of the drug-taking response and, thus, such recruitment may contribute to automated, habitual drug intake observed in addicts.

Disclosure: I. Willuhn: None. **B. Everitt:** None. **P. Phillips:** None.

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37. Dreaded Decision-Making: Revealing a Role for the 'Direct' Pathway in Reward Preference

Susan Ferguson*, Paul Phillips, Bryan Roth, John Neumaier

Seattle Children's Research Institute, Seattle, USA

Background: A central feature of many neuropsychiatric disorders, such as drug addiction and obsessive-compulsive disorder, is the development of aberrant reinforcement learning and decision-making processes. Dysregulation of the striatum is thought to contribute to these disorders; however, the striatum is a heterogeneous structure containing distinct populations of GABAergic medium spiny projection neurons (MSNs) that differ in their neuropeptide composition and form two major efferent pathways. MSNs that contain the neuropeptides dynorphin and substance P are part of the striatonigral, or 'direct', pathway whereas MSNs that contain the neuropeptide enkephalin are part of the striatopallidal, or 'indirect', pathway. The roles of these specific striatal sub-types in reinforcement learning and decision-making are not yet known. As a first step toward addressing this question, we used a novel chemical-genetic approach to determine how modulating activity of the striatonigral pathway would change preferences in a high versus low reward decision-making task. **Methods:** Briefly, we developed viral vectors that use the preprodynorphin promoter to target expression of hemagluttin-tagged $G_{i/o}$-coupled DREADD (<u>D</u>esigner <u>R</u>eceptor <u>E</u>xclusively <u>A</u>ctivated by a <u>D</u>esigner <u>D</u>rug) receptors or hemagluttin-tagged G_s-coupled DREADD receptors to striatonigral neurons. Activation of $G_{i/o}$-DREADD receptors allows for transient reduction of neuronal excitability whereas activation of G_s-DREADD receptors allows for transient increases in neuronal excitability using the pharmacologically inert synthetic ligand clozapine-*N*-oxide (CNO). **Results:** After viral infusion and DREADD receptor expression into the dorsomedial striatum of Long Evans rats, we found that decreasing activity of striatonigral neurons impaired the acquisition of a high-reward preference in a decision-making task for small versus large magnitude natural rewards (i.e., 1 or 4 food

pellets) whereas transiently increasing activity of striatonigral neurons produced the opposite effect. However, altering activity of striatonigral neurons had no effect on performance in this decision-making task once the high-reward preference was established.

Discussion: These findings demonstrate that the 'direct' pathway is an important modulator of decision-making processes related to reward choice. They also support the idea that striatal dysregulation contributes to the development of aberrant reinforcement learning and decision-making that is common among neuro-psychiatric disorders.

Disclosure: S. Ferguson: None. **P. Phillips:** None. **B. Roth:** None. **J. Neumaier:** None.

38. Melatonin Receptor Deletion abrogates Methamphetamine-Induced Locomotor Sensitization and Reward in C3H/HeN Mice

Margarita L. Dubocovich, Shannon J. Clough, Anthony J. Hutchinson, Iwona Stepien, Randall L. Hudson*

Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, USA

Background: Psychostimulant-induced sensitization and reward have profound implications for abuse potential in humans (Phillips *et al.*, *Neuromethods.* 50:267,2011). Behaviors induced by drugs of abuse are time-of-day dependent, suggesting the involvement of circadian mechanisms (Abarca *et al.*, *PNAS* 99:9026, 2002). Melatonin, a hormone synthesized in the pineal gland following a circadian rhythm with high levels at night (Reiter R., *Mol Cel Endocrinol* 79:C153,1991) modulates cocaine-induced sensitization and reward in a time-dependent manner (Uz *et al.* *Neuropsychopharmacol.* 28:2117,2003; Kurtuncu *et al.* *Eur J Pharmacol* 489:203,2004). This study reports the involvement of the MT_1 and MT_2 melatonin receptors in methamphetamine (METH)-induced sensitization and reward in wild-type (WT) C3H/HeN mice and mice with genetic deletion of the MT_1 and/or MT_2 receptors (MT_1KO, MT_2KO, MT_1/MT_2KO).

Methods: *Locomotor sensitization* was induced in WT, MT_1KO, MT_2KO and MT_1/MT_2KO C3H/HeN mice by 6 daily doses of METH (1.2 mg/kg, ip) or vehicle (VEH) in a novel environment. The expression of sensitization was assessed following 4 days of withdrawal by challenge with METH (1.2 mg/kg, ip). Sensitization was assessed by measuring locomotor activity as distance traveled using the LocoScan System (Clever Inc, Reston, VA). *Conditioned Place Preference* (CPP) measured as distance travel within the chambers was induced by 6 days of alternating treatments of VEH and METH (1.2 mg/kg, ip), following drug-free habituation and pre-test sessions in WT, MT_1KO, MT_2KO C3H/HeN mice. Mice were tested for place preference 1 day after the last conditioning session. All data was analyzed using Student's *t*-test or Two-Way Analysis of Variance (ANOVA) with Bonferroni correction for paired comparisons.

Results: METH treatment during the day (ZT 5-7; ZT 0 = lights on) induced gradual locomotor sensitization from day 1 to 6 when compared with VEH treatment. A robust and statistically significant difference between VEH- and METH-pretreated WT, MT_1KO and MT_2KO mice was observed on day 6, however no sensitization was observed in MT_1/MT_2KO mice. Following 4 days of withdrawal, expression of sensitization by a challenge dose of METH (1.2 mg/kg, i.p.) during the first hour post treatment was significantly higher in WT (71.4 ± 15.1 m, n = 8), MT_1KO (61.9 ± 12.3 m, n = 16), and MT_2KO (73.26 ± 12.1 m, n = 9) mice pretreated with METH than in those pretreated with VEH, but sensitization was completely blocked in MT_1/MT_2KO mice (15.2 ± 11.9 m, n = 12, $p < 0.05$).

Next, we assessed the rewarding properties of METH using mice in a CPP paradigm. Mice were tested for place preference one day after the last conditioning session, in which mice received alternating daily treatments of METH or VEH in chambers with distinct walls and floor texture. A preference score was derived by subtracting the duration a mouse spent in the VEH-paired chamber from the duration spent in the METH-paired chamber during the post-test. WT mice exhibited a significantly higher preference score when receiving METH (226.0 ± 35.5 s, n = 11, $p < 0.001$) compared to VEH controls (-36.3 ± 28.1 s, n = 12). In contrast when MT_1KO or MT_2KO mice were subjected to the same paradigm the METH group and VEH group showed no significant differences in preference score.

Discussion: METH induced sensitization in the C3H/HeN mice was abrogated by deletion of both MT_1 and MT_2 melatonin receptors. These data suggest that melatonin receptor activation may be required for the expression of METH-mediated sensitization following withdrawal in mice during the day. The rewarding properties of METH, as measured by the conditioned place preference paradigm, are dependent on either the MT_1 or MT_2 melatonin receptors. These results, along with reported data (Wang *et al.*, *Eur J Neuroscience* 22:2231,2005) suggest that melatonin receptor activation may facilitate METH effects in the CPP paradigm through the potential involvement of two alternate signaling pathways, one tied to reward (MT_1) and the other to learning and memory (MT_2).

Disclosure: M. Dubocovich: Part 1: Consultant 2009 - Adolor 2010-2011 - Takeda Pharmaceuticals North America. **S. Clough:** None. **A. Hutchinson:** None. **I. Stepien:** None. **R. Hudson:** None.

39. Circuit-Specific Spine Modifications with Fear Memory

Jeff D. Sanders*, Dilip Jeste, Mark Mayford

UCSD, San Diego, USA

Background: Dendritic spines are the primary sites for excitatory synaptic contact on pyramidal neurons in the brain. The structure and density of dendritic spines is altered in many disorders across the lifespan that affect cognitive function. These include developmental disorders such as autism and fragile-X syndrome as well as age-related conditions of cognitive impairment. The size and density of spines have been found to change in a number of synaptic and behavioral plasticity paradigms leading to the suggestion that they may form a structural substrate for long-term memory. If spine changes underlie long-term information encoding we would expect that the effects would be long-lasting, circuit-specific, and sensitive to the contingencies in a behavioral learning paradigm.

Methods: In order to test this idea we used a transgenic mouse that expressed a dendritic marker (GFP-GluR1) under the control of the c-fos promoter that is responsive to neural activity. This allowed us to specifically analyze the morphology of the neural circuits that were activated during learning relative to inactive circuits within the same brain area. We examined structural changes in the hippocampus at twenty-four hours following contextual fear conditioning, unpaired shock training, or contextual exposure alone.

Results: Twenty- four hours after contextual fear conditioning or unpaired shock training there is a decrease in total spine density ($F(2,374) = 7.7333$, $p = 0.00051$). Examination of the total population of active versus inactive cells, across experimental groups, reveals a lower spine density on active cells ($F(1,374) = 15.679$, $p = 0.00009$). Spines on active cells of fear-conditioned animals, but not animals exposed to unpaired shock or context alone, are significantly fewer than spines on inactive cells ($p < 0.005$). We further analyzed mushroom, branched, thin and stubby spine subtypes. Mushroom spine density is decreased in animals exposed to fear conditioning or unpaired shock training

$(F_{(2,374)} = 14.844, p = 0.00000)$, and mushroom and branched spine density is decreased on active cells, $(F_{(1,374)} = 4.7025, p = 0.03075)$ and $(F_{(1,374)} = 7.628, p = 0.00736)$, respectively. Furthermore, branched spines display a decrease that is specific to activated cells of fear-conditioned mice $(p < 0.005)$.

Discussion: Prior data from our laboratory have shown that AMPA receptors are selectively enriched in mushroom spines twenty-four hours after fear conditioning. Considered in the context of our current findings, these data suggest that circuit-specific decreases in mushroom spines may be a homeostatic adaptation to increased AMPA receptor insertion during learning. These data further point to an important role for homeostatic decreases in branched spines to neural activity, and reveal that branched spines are pruned with learning in a circuit-specific manner. Collectively, these findings imply that spine density perturbations in psychiatric disorders may reflect an altered system of activity-related synaptic refinement characteristic of new learning.

Disclosure: J. Sanders: None. D. Jeste: None. M. Mayford: None.

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40. Human Hair Follicle Derived Induced Pluripotent Stem Cells (iPSC) and Their Differentiation into Dopaminergic Neurons as a Model to Study Neurdevelopmental Abnormalities in Schizophrenia

Dorit Ben-Shachar*, Odil Robicsek, Isabelle Petit, Nava Salman, Rachel Karry, Daniel Aberdam

Technion IIT&Rambam Medical Center, Haifa, Israel

Background: Reprogramming of somatic cells into induced pluripotent stem cells (iPSC) and differentiating them into different neuronal lineages is a new powerful technology that could offer an attractive tool to model neurodevelopmental disorders. Neuropsychiatric disorders, as many other complex disorders, necessitate cellular models with relevance to their underlying biological mechanisms and pharmacotherapy. The biological basis of psychiatric disorders is still enigmatic, despite tremendous advances in science and medicine. Several lines of evidence support a neurodevelopmental, multifactorial etiology in psychiatric diseases, specifically in schizophrenia, suggesting embryonic insults that impact brain developmental events. We hypothesized that keratinocytes isolated from hair follicles of schizophrenia patients could be an ideal source of somatic cells for reprogramming, due to their accessibility as well as common neuroectodermal origin with neurons.

Methods: iPSC lines from schizophrenic patients (DSM-IV criteria; $n = 4$) and from healthy subjects ($n = 2$) were produced from hair follicle (HF) keratinocytesby infection with lentiviral particles expressing the polycistronic plasmid STEMCCA (Oct4, Sox2, Klf4 and c-myc).iPSC pluripotency was characterized by the expression of pluripotency markers using immunofluorescence and q-RT-PCR and by PluriTest assay, a novel bioinformatic assay for pluripotency that eliminates the need of animal use for teratoma assay. Characterized pluripotent HF-iPSC were differentiated into $Pax6^+/nestin^+$ neuronal precursors and then further differentiated into beta3-tubulin$^+$/TH$^+$ dopaminergic cells and forebrain beta3-tubulin$^+$/TBR1$^+$ glutamatergic cells using SMAD and TGFb-inhibition protocol or prolonged spontaneous EB differentiation, respectively. Schizophrenia-related abnormalities in dopamine turnover rate and mitochondrial function were assessed at different developmental stages.

Results: We have established culture conditions to isolate and expand keratinocytes from as few as 5 HF at anagen phase, from both healthy controls and schizophrenia patients.HF could be stored for at least 48 h in DMEM medium without a decrease in cell recoveryKeratinocytes infected with lentiviral construct carrying STEMCA produced several colonies of reprogrammed cells. Pluripotency of iPSC was confirmed by immunofluorescence

staining of TRA-1-81, TRA-1 60 and SSEA-4 and mRNA expression of Oct3/4, SOX2, NANOG and DNMT3B, as well as by in-vitro differentiation into the 3 embryonic germ layers. PluriTest assay showed high pluripotency score, similarly to hES and fibroblast-derived iPSC, of our iPSC lines. iPSC from both schizophrenic patients (Sch-iPSC) and from healthy subjects (CTL-iPSC) were differentiated into neuronal cells. While CTL-iPSC displayed a neuronal morphology and formed network-like structures, Sch-iPSC cells were much bigger, did not present morphological features of neurons and seemed less developed. Immunofluorescence staining revealed that a large proportion of differentiated CTL-iPSC were beta3-tubulin$^+$/TH$^+$. Flow cytometry analysis confirmed that 70% of differentiated CTL-iPSC, yet only 3.6% of Sch-iPSC were beta3-tubulin-positive. Analysis of extracellular monoamines by HPLC revealed that differentiated CTL-iPSC released dopamine and its metabolite HVA significantly more than Sch-iPSC. Levels of serotonin and its metabolite (5HIAA) were similar in both groups. Both CTL-iPSC and Sch-iPSC similarly differentiate into forebrain beta3-tubulin$^+$/TBR1$^+$ neuron-like cells. However, Sch-iPSC-derived neurons did not expressed TBR1, a marker of final stage of differentiation of forebrain neurons. Mitochondrial function including respiration, membrane potential ($\Delta\psi_m$), and mitochondrial network dynamics were abnormal at various developmental stages in schizophrenia derived cells including keratinocytes, iPSC, neuronal precursors and differentiated neurons.

Discussion: The results of this study show neurodevelopmental impairments in schizophrenia in a novel in-vitro cellular model. These cells show morphological and functional abnormalities, which have been implicated in schizophrenia. Thus, the non-invasive painless means of HF keratynocytes-derived iPSC may provide new insights into neuron development in health and disease and may serve as a tool for the design of alternative therapeutics and for personalized pharmaco-genetic studies to optimize drug therapy for the benefit of the patients.

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41. GABA$_A$ and GABA$_B$ Receptor Subunits display Altered Expression in Cerebella of Subjects with Schizophrenia, Bipolar Disorder, and Major Depression

S. Hossein Fatemi*, Timothy D. Folsom

Department of Psychiatry, University of Minnesota, Minneapolis, USA

Background: Gamma-aminobutyric acid (GABA) acts as the main inhibitory neurotransmitter in the central nervous system. GABA signaling is important for multiple processes including learning and memory and GABAergic dysfunction has been implicated in multiple psychiatric disorders. In the current study we have examined expression of GABA$_A$ and GABA$_B$ receptor subunits in the cerebellum, a brain area that has been implicated in the pathology of schizophrenia, bipolar disorder, and major depression.

Methods: Postmortem, lateral cerebellar samples were derived from subjects with schizophrenia ($N = 15$), bipolar disorder ($N = 15$), major depression ($N = 15$) and matched controls ($N = 15$). SDS-PAGE and western blotting were used to measure protein levels. We measured protein levels of GABA$_A$ receptor alpha 1 (GABRα1), GABA$_A$ receptor alpha 2 (GABRα2), GABA$_A$ receptor alpha 3 (GABRα3), GABA$_A$ receptor beta 1 (GABRβ1), GABA$_A$ receptor beta 2 (GABRβ2), GABA$_B$ receptor 1 (GABBR1), and GABA$_B$ receptor 2 (GABBR2). All protein measurements for subjects with schizophrenia, bipolar disorder, major depression, and control subjects were normalized against b-actin. Group

differences were analyzed statistically using student's t-test. Significant differences were defined as those with a p value < 0.05. Confound effects (i.e., age and PMI) were examined using Pearson's correlation test.

Results: We observed significant increases in protein for GABRα1 in cerebella of subjects with schizophrenia ($p<0.049$), bipolar disorder ($p<0.046$) and major depression ($p<0.022$). Similarly GABRα2 was also increased in brains of subjects with schizophrenia ($p<0.0084$), bipolar disorder ($p<0.026$), and major depression ($p<0.0054$). In contrast, GABRα3 was not significantly altered in the three diagnostic groups. GABRβ1 protein was reduced in cerebella of subjects with schizophrenia ($p<0.0006$) and bipolar disorder ($p<0.021$) while GABRβ2 was significantly reduced in cerebella of subjects with bipolar disorder ($p<0.014$). GABBR1 showed reduced expression in cerebella of subjects with schizophrenia ($p<0.0007$), bipolar disorder ($p<0.012$), and major depression ($p<0.023$). Similarly GABBR2 also showed reduced expression in cerebella of subjects with schizophrenia ($p<0.0001$), bipolar disorder ($p<0.0063$), and major depression ($p<0.0020$).

Discussion: Our results are the first to demonstrate widespread altered expression of GABA$_A$ and GABA$_B$ subunits in the cerebella of subjects with schizophrenia and mood disorders. It is likely that altered GABA receptor expression contributes to the cognitive and behavioral deficits associated with these disorders.

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42. Chandelier Cell Inputs to Pyramidal Neurons in Schizophrenia and Development

Kenneth N. Fish*, Gil D. Hoftman, Robert A. Sweet, David A. Lewis

University of Pittsburgh, Pittsburgh, USA

Background: Parvalbumin (PV)-containing chandelier GABA neurons (Ch cells) provide synapses exclusively onto the axon initial segment (AIS) of pyramidal neurons (PYR) in distinct vertical arrays termed cartridges. By targeting the site of action potential generation, Ch cells are ideally positioned to be master regulators of PYR output. In primate prefrontal cortex (PFC) development, the density of cartridges detected by GABA membrane transporter-1 (GAT1) immunoreactivity peaks prior to the onset of puberty, and then declines markedly to adult levels. In PFC of subjects with schizophrenia, the relative density of axon cartridges that can be detected by immunoreactivity for GAT1 is also reduced. Lower GAT1-immunoreactive cartridge density in disease and development is interpreted as resulting from reduced protein expression in morphologically intact Ch cartridges, though this has never been evaluated directly.

Methods: Multi-label fluorescence confocal microscopy is used to assess innervation of AIS in monkey PFC at developmental time points spanning ages 3-160 months in order to define the postnatal developmental trajectories of pre- (PV) and postsynaptic (GABA$_A$ receptor γ2 subunit) proteins in Ch to PYR connections and the terminal density at the AIS.

Results: Preliminary studies indicate that the number of Ch terminals/AIS decreases drastically between 3 months and adulthood in monkey PFC. In addition, qualitative assessment suggests that the level of PV expression in Ch terminals first increases and then decreases over development. A more complete assessment of the above findings will be presented.

Discussion: Our findings provide evidence of morphological developmental changes in Ch to PYR innervation. We have previously observed that Ch terminals in PFC are unique in that they express high levels of GAD67, but not GAD65 (Fish *et al.*, 2011). These findings have led us to the hypothesis that Ch terminals are particularly vulnerable to reductions in GAD67 expression (as observed in schizophrenia), with the result being an increase in the number of Ch terminals lost in development. Preliminary analysis of 2 matched subject pairs (schizophrenia and control) is consistent with this hypothesis, as Ch terminal/AIS density was significantly decreased in schizophrenia. Future studies further addressing this hypothesis are needed, as supportive findings would reinforce the need for the development of therapeutics aimed at stopping excessive Ch terminal loss, rather than therapeutics solely focused on pharmacologically manipulating the residual synapses.

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43. The Role for NDEL1 in nNOS Signaling for Schizophrenia: Implications for Cortical Development and Prefrontal Cortex-Mediated Cognitive Behaviors

Atsushi Saito, Yu Taniguchi, Sandra P. Zoubovsky, Sun-Hong Kim, Balakrishnan Selvakuma, Vladimir M. Pogorelov, Peter Yoon, Solomon H. Snyder, Akira Sawa, Mikhail V. Pletnikov, Atsushi Kamiya*

Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, USA

Background: Higher brain function and behavior are influenced by neuronal circuit formation during brain development. Many genetic risk factors for schizophrenia, such as Disrupted-in-Schizophrenia-1 (DISC1) and neuronal nitric oxide synthase (nNOS), have key roles in neurodevelopment. Consequently, disturbances in brain development are suggested to underlie the pathology of such devastating conditions. Although roles for these factors have been reported at the molecular level, there are limited studies on whether they act in common molecular pathways that contribute to disease pathology. In this study, we explored the role of NudE-like 1 (NDEL1), a schizophrenia-associated protein interactor of DISC1, in nNOS signaling for the development of the prefrontal cortex and resultant behaviors. Given that nNOS and NDEL1 are highly expressed in the cortical plate of developing cerebral cortex, NDEL1 may function as a downstream effecter of nNOS signaling for cortical development, which may also contribute to the NO-mediated establishment of neuronal circuits responsible for long-lasting behaviors.

Methods: We examine the role of S-nitrosylation of NDEL1 via nNOS signaling for cortical development and their underlying molecular mechanisms by using cortical neuron cultures with RNAi approaches and brains from nNOS KO mice. To manipulate NDEL1 function in the developing cerebral cortex, we use a Cre/loxP-mediated inducible expression system with *in utero* electroporation.

Results: We found that NDEL1 is *S*-nitrosylated in nNOS signaling. S-nitrosylation of NDEL1 is required for dendritic development. We also found the interaction of NDEL1 with nNOS, which is mediated by DISC1. Furthermore, our data from the behavioral characterization of nNOS KO mice suggest that prefrontal cortex-mediated cognitive functions are impaired in nNOS KO mice.

Discussion: We are currently exploring the impact of nNOS/NDEL1 signaling *in vivo* by *in utero* electroporation with an inducible gene expression system. With this technique, we can dissect the temporal requirement for the studies of NDEL1 in nNOS signaling in cortical development as well as explore the molecular basis of disease animal models for further testing of resultant behaviors. Our results will provide us with important clues for the possible involvement of nNOS/NDEL1 signaling in the etiology of schizophrenia. Further investigation of the molecular interaction between nNOS, DISC1, and NDEL1 is expected to address how

genetic disturbances in nNOS-mediated signaling contribute to the underlying mechanisms of impaired cognition in schizophrenia.

44. Identification of Cellular Signatures for Schizophrenia and Bipolar Disorder in iPSC-Derived Neuronal Cells

Rakesh Karmacharya*, Steven D Sheridan, Kraig Theriault, Sabine Bavamian, Jennifer Wang, Elizabeth G.J. O′Brien, Sigrun Gustafsdottir, Katherine L Madden, Donna McPhie, Roy H. Perlis, Dost Ongur, Alykhan Shamji, Anne E. Carpenter, Bruce M. Cohen, Stuart L. Schreiber, Stephen J. Haggarty

Harvard University, Cambridge, USA

Background: The study of major neuropsychiatric illnesses such as schizophrenia and bipolar disorder has been hindered by the lack of access to live neuronal tissue from patients. Recent advances in the generation of induced pluripotent stem cells (iPSCs) and neural progenitor cells (NPCs) from human fibroblasts present an exciting opportunity to generate neuronal cultures with the patients' genetic makeup.

Methods: We are using defined genetic factors to reprogram fibroblasts to generate iPSCs from patients with schizophrenia and bipolar disorder as well as from matched controls. These iPSCs are validated using step-wise appearance of stem-cell specific markers. We differentiate these iPSCs into NPCs which are further differentiated along the neuronal lineage. We will acquire high-content images of NPC-derived neuronal cells and label them with neuronal subtype-specific markers as well as a range of cellular stains. We will analyze the high-content images using new algorithms that allow us to quantify a range of cellular and sub-cellular features (e.g. numbers, lengths, branching patterns of dendrites, quantity/localization of mitochondria etc.). In addition to acquiring high-content images of neuronal cultures under normal conditions, we intend to collect images of neuronal cultures treated with a set of small molecule perturbagens. We will use an annotated set of 300 small molecules comprised of known inhibitors/activators of various signaling pathways as well as small molecules/drugs that are known to modulate neuronal and glial biology. While disease-related phenotypic features may not be readily visible at baseline, perturbation of specific signaling pathways may expose cellular deficits inherent to the biology of the disease neurons. We will use machine-learning algorithms to identify features that distinguish neurons derived from schizophrenia and bipolar disorder iPSCs from neurons derived from healthy controls. If a specific small molecule elicits different phenotypic responses in disease neuronal cells *vis-à-vis* control cells, we will carry out RNAi knockdown of the target to recapitulate the different effects observed with small molecules. The identification of differential responses to small molecule/RNAi will also give important clues on the underlying pathways that may be aberrant in the diseased neuronal cells. Using this methodology, we will carry out unbiased phenotypic profiling of the neuronal cells and extract "disease signatures" that can be used for high-throughput screens.

Results: We have created iPSCs from patient fibroblasts by retroviral transduction of four genes (OCT4, SOX2, KLF4 and C-MYC). We validated the creation of iPSCs using step-wise appearance of stem-cell specific markers. We have differentiated the iPSCs to establish self-renewing NPCs that grow as adherent monolayers. We have further differentiated NPCs along neuronal and glial lineages to develop a reliable and reproducible protocol to generate neuronal cultures from NPCs in multi-well format that can be used to carry out high-throughput characterization and small-molecule screening.

Discussion: Understanding the cellular pathophysiology underlying schizophrenia and bipolar disorder can lead to the identification of new therapeutic targets and the development of improved treatment for schizophrenia and bipolar disorder. Recent advances in stem cell research and chemical biology provide new avenues to investigate disease biology and develop new therapeutic leads for schizophrenia and bipolar disorder. We present an approach to generate and characterize patient iPSC-derived neuronal cells in the presence of a range of small molecule perturbations in order to identify disease-specific cellular signatures. We discuss our approach to use these "disease signatures" to screen for small molecules that modulate or normalize the disease-related cellular signatures in order to develop new leads for therapeutic development.

45. DARPP-32 Transcripts are Upregulated in the Prefrontal Cortex of Major Psychiatric Disorders and Associated with Genetic Variants

Yasuto Kunii*, Thomas M. Hyde, Tianzhang Ye, Chao Li, Daniel R. Weinberger, Joel E. Kleinman, Barbara K. Lipska

National Institute of Mental Health, Bethesda, USA

Background: Dopamine- and cAMP-regulated phosphoprotein of molecular weight 32 kDa (DARPP-32 or *PPP1R1B*) has been implicated in the pathogenesis of schizophrenia and bipolar disorderbecause of itsrole as a molecular integrator ofdopaminergic and glutaminergic signaling.Previously reported resquencing of DARPP-32 identified a frequent haplotype associated with the expression of DARPP-32 in the dorsolateral prefrontal cortex, cognition and frontostriatal function using imaging in normal controls (Meyer-Lindenberg *et al.*, 2007). In this study, we investigated the expression of two major DARPP-32 transcripts, full-length and truncated, in the dorsolateral prefrontal cortex (DLPFC) in a large cohort of patients with schizophrenia, bipolar disorder and major depression as well as controls across the lifespan. We also analyzed associations of expression of these transcripts with genetic variants of DARPP-32.

Methods: We examined the expression of DARPP-32 transcripts in the postmortem DLPFC of 324 normal controls, including 42 fetal samples, 176patients with schizophrenia, 61patients with bipolar disorder and 138 subjects with major depression disorder by quantitative real-time polymerase chainreactionmethodsusing Taqman ABI assays. We examinedassociations withgenetic variation in DARPP-32 for 58 SNPs genotyped using 1 M Illumina BeadArrays. We used ANCOVAs with post-hoc Bonferroni corrections to analyze the data.

Results: Developmental expression patterns of full length DARPP-32 (FL-DARPP-32) and the splice variantencoding truncated-DARPP-32protein (t-DARPP-32)differed markedly. Expression of FL-DARPP-32 was high during the prenatal period, dropped at birth, and then increased gradually throughout the postnatal life until old age. In contrast, t-DARPP-32 was expressed at very low levels prenatally, increased sharply from birth to pubescent ages and remained relatively stable throughout the rest of life. There were dramatic differences in the expression of both transcripts between the diagnostic groups. FL-DARPP-32 was significantly increased in major depression as compared to all other groups ($p = 7.3 \times 10^{-4}$). The expression of t-DARPP-32 as well as the ratio of t-DARPP-32 to FL_DARPP32 were increased in schizophrenia ($p = 3.4 \times 10^{-7}$) and bipolar disorder ($p < 1.0 \times 10^{-17}$) as compared with normal controls. Expression of t-DARPP-32 in bipolar

disorder patients was also significantly higher than in patients with schizophrenia ($p = 3.3 \times 10^{-12}$) and patients with major depression ($p < 1.0 \times 10^{-17}$). We did not detect the effects of nicotine, antipsychotic medication, antidepressants or lithium on the expression levels of the two transcripts. We analysed 58 SNPs in the DARPP-32 regionand found that 4 SNPs predicted expression of t-DARPP-32, including2 SNPs(rs90974 and rs3764352)that were previously associated with schizophrenia, cognitive and imaging phenotypes ($p < 0.001$).

Discussion: Our data show that the expression levels of FL-DARPP-32 and t-DARPP-32are altered in patients with schizophrenia and affective disorders.Although preliminary analysis did not find the effects of medication on the expression of DARPP-32 splice variants, it cannot be precluded that the differences between diagnostic groups are due to psychotropic drugs. The increased ratio of truncated/full length DARPP-32 may lead to an attenuation of dopamine signaling in the DLPFC because t-DARPP-32 lacks the Threonine 34 phosphorylation site and proteinphosphatase inhibitory domain, which are critical for dopamine signaling in thebrain.In addition, t-DARPP-32may interfere with PKA inhibitionby FL-DARPP-32via adominant negative mechanismand then activate phosphoinositide 3-Kinase/Akt pathway signaling (Guet al. 2009).Therefore, the data suggest that this pathway may contribute to the pathophysiology of schizophrenia and affective disorders. Moreover, we foundthat2 SNPs, rs907094 and rs3764352, which were associated with schizophrenia,performance on several cognitive tests and frontostriatal functions, predicted expression of t-DARPP-32. Our results suggest that variation in *PPP1R1B* affects splicing of DARPP-32 and identify potential molecular mechanisms of the pathogenesis of mental disorders.

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46. Ultrastructural Features of the Anterior Cingulate Cortex in Postmortem Brain from Subjects with Schizophrenia and Controls

Rosalinda Roberts, Joy Roche, Adrienne Lahti*

University of Alabama, Birmingham, USA

Background: Schizophrenia (SZ) is a serious mental illness that manifests itself with psychotic symptoms such as delusions, hallucinations, disorganized thought and behavior as well as negative symptoms and cognitive deficits. The anterior cingulate cortex (ACC) is one or several brain regions that are abnormal in SZ. Imagingand electrophysiologicstudies show impairments in ACC function during error or conflict monitoring in SZ. Our positron emission tomography (PET) and regional cerebral blood flow (rCBF) imaging studies have revealed that limbic brain networks, including the ACC, are related to psychosis and treatment response to antipsychotic drugs (APD). Postmortem studies indicate several abnormalities in the ACC, including increases in glutamatergic axons and decreases in pyramidal cell density. The purpose of the present study was to compare the synaptic organization in the anterior cingulate cortex in SZs and controls.

Methods: Postmortem human brain tissue was obtained from the Maryland and Alabama Brain Collections. The tissue was collected with family permission within 8 hours of death from adult SZ subjects and controls (NCs) (n = 4 per group). The demographics of the NCs and SZs were, respectively: 1) age, 51.0 ± 24.6y and 54.5 ± 8.5y; 2) race, 2C&2AA and 1C&3AA; 3) sex, 2F&2 M and 1F&3 M, and 4) PMI, 7.4 ± 0.48 h and 5.6 ± 1.49 h. None of these parameters were significantly different between groups. Coronal blocks from the ACC were immersed in a cold solution of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (PB), pH = 7.4 and kept at $4°C$ until vibratomed at 40 µm. One series from each case was prepared for electron microscopic analysis. Total synaptic density, various synaptic subtypes (axospinous, axodendritic, asymmetric and symmetric and combinations thereof) and mitochondria in axon terminals were counted using stereology techniques from layers III and V-VI. The average volume counted from each layer in each case was 887 µm³ and 880 µm³ from NCs and SZs, respectively.

Results: In the ACC, the proportions of asymmetric (glutamatergic) to symmetric (inhibitory) synapses were similar in NCs and SZs: asymmetric, 90% in NCs and 88% in SZs; symmetric synapses, 10% in NCs and 12% in SZs. Another way to look at synapses is by whether they form with spines or dendrites. In NCs, 86% were axospinous and 14% were axodendritic; these proportions were similar between layers III and V-VI. In SZs, there was a shift in proportion of synapses formed with spines vs. dendrites compared to NCs: 77% were axospinous ($p < 0.037$), and 22% were axodendritic ($p < 0.039$); these proportions were similar between layers. In both layer III and layers V-VI, there was an equivalent decrease in total synaptic density of about 80% in the SZ vs. the NCs. Axospinous synapses (both asymmetric and symmetric) were equivalently affected by about 30%, while axodendritic synapses were equal to or increased in proportion (up to 138%) in the SZ vs. the NCs. Both layers III and layers V-VI showed similar changes between SZ and NCs. These results were not significant, probably due to small sample size and a lot of individual variability, particularly in the SZ group. The proportion of axon terminals containing at least one mitochondrion was 32% in NCs and 28% in SZs, for all layers combined. The percentage of asymmetric axospinous terminals in layer III in SZ that contained mitochondria was reduced to 76% of that of NCs ($p < 0.05$). The percentage of symmetric axospinous synapses in all layers combined that contained at least one mitochondrion was 70% of that of NCs ($p < 0.03$). This change was confined to layers V-VI where only 45% of this remaining type of synapse contained at least one mitochondrion ($p < 0.014$).

Discussion: Overall decrease in synaptic density in the SZs is consistent with the reduced neuropil hypothesis.Fewer axospinous synapses in SZ is probably a reflection of spine loss that has been reported in other studies. The decrease in density of axospinous synapses together with a lower proportion of remaining terminals that contain mitochondria suggests a decrease in cortical synaptic efficiency. The increase in proportion of axodendritic synapses suggests that when spines are lost, at least some terminals may end up forming a synapse on the dendritic shaft. By examining the morphological characteristics of synapses such as symmetry and postsynaptic targets, it is possible to gain insight as to the origin of the neurons forming the synapses. These changes suggest alterations in multiple cortical connections.

Disclosure: R. Roberts: None. **J. Roche:** None. **A. Lahti:** Part 1: Pfizer, Part 4: Pfizer.

47. Mitochondrial Variants and Subjects with Psychatric Disorders

Marquis P. Vawter*, Brandi Rollins, Firoza Mamdani, Fabio Macciardi, William E. Bunney, Pedro A. Sequeira

Functional Genomics Laboratory, Department of Psychiatry & Human Behavior, University of California, Irvine, USA

Background: Several lines of evidence support a role of mitochondrial dysfunction in mood disorders and schizophrenia,

including postmortem brain, imaging and, genetic studies. Previously we have investigated mitochondrial DNA (mtDNA) variants in brain and blood using high-density resequencing arrays. In this study, we have now investigated mtDNA variants using next generation sequencing of 12 - 48 samples in parallel. Further, we have performed association analyses of 6,040 controls, schizophrenia, and bipolar subjects from the WTCCC2 and GAIN cohorts using selected mtSNPs.

Methods: Mitochondrial DNA (mtDNA) mutations were investigated using a combination of Illumina GAII and Hi-Seq instruments, with 12 and 48 bar coded samples in a lane, respectively. The dorsolateral prefrontal cortex (DLPFC) from 69 subjects were sequenced for the entire mtDNA genome, in five of these subjects, eleven brain regions and peripheral blood were also compared. The levels of heteroplasmic and homoplasmic mutations in the DLPFC were measured in a sample comprising 14 bipolar disorder (BD), 14 schizophrenia (SZ), 15 major depressive disorder (MDD), 6 methamphetamine users (METH), and 20 control (CO) subjects. For the association study, the cel files from Affymetrix 6.0 SNP chips were analysed in one batch for mtDNA alleles using multidimensional scaling (MDS) to correct for original batch and hybridization intensity and compared by logistic regression in a total sample of 6,040.

Results: Full mtDNA genome resequencing was completed with over 1000x and 5000x coverage on the Illumina GAII (12 samples/lane) and Hi-Seq (48 samples/lane) runs. Preliminary analysis shows twelve novel or rare homoplasmic mutations previously not identified in the mtDNA haplogroups. We found the presence of heteroplasmic mutations such as deletions and insertions, which are currently being validated, across multiple brain regions. The transition and transversion rates in the five groups are being studied and will be presented, although we have found consistent anomalies in both Illumina platforms for base calls at certain positions which will be presented. For association, there were no genome wide mtDNA SNPs that achieved significance. There were, however, five mtDNA SNPs predominantly in the hypervariable control region that showed nominal association to SZ or BD (p < 0.05)after correction by MDS, and also remained nominally associated after stratification by major ethnic haplogroup.

Discussion: Current experiments reinforce the findings that accumulation of heteroplasmic events in post-mitotic tissue such as brain is rarely observed at the gross tissue homogenate level. To further address possible heteroplasmic mutations will require robust and precise cellular level analysis. A very preliminary finding of associated SNPs in mtDNA to SZ or BD that are located in a hypervariable region will require further investigation in other SZ and BD cohorts, as well as some functional validation of these SNPs. This study reinforces that brain shows heteroplasmic accumulation of mitochondrial variants and that some of those heteroplasmic variants show association with psychiatric populations compared to controls.

48. Prenatal Stress decreases Expression of Transcription Factors in GABAergic Neuron Progenitors and GABAergic Progenitor Migration

Hanna E. Stevens*, Flora M. Vaccarino

Yale Child Study Center, New Haven, USA

Background: Prenatal stress has been linked to behavioral and emotional problems in childhood as well as affective and psychotic disorders in adulthood. Multiple lines of evidence implicate GABAergic neuronal populations in these disorders. The study of prenatal stress in model animal systems has demonstrated functional changes that implicate an impact of prenatal stress on GABAergic systems. GABAergic neurons may be affected during prenatal stress through effects upon the proliferation, differentiation, or migration of their precursors. Here we combine a well-validated animal model of prenatal stress with techniques for examining GABAergic progenitors. This study addresses important gaps in the knowledge base regarding developmental contributions to neuropsychiatry.

Methods: Wild type female CD-1 mice were mated to CD-1 males heterozygous for a green fluorescent protein (GFP) knocked-in at the GAD67 locus. Half of pregnant mice underwent 3 daily episodes of acute restraint stress under bright lights from embryonic day 12 (E12). All females were injected with BrdU on E12, E13, E14 or E15 in order to birthdate GABAergic progenitors and analyze their development. Brain tissue of offspring was collected on E13, E14, E15, and postnatal day (PND) 0. Some tissue was homogenized to permit isolation of RNA and conducting quantitative PCR for *dlx1, dlx2, nkx2.1, fgf2, bdnf,* and *erbb4*. Some tissue was fixed and sectioned and immunohistochemistry and *in situ* hybridization were used to localize GABAergic progenitors, BrdU labeled cells, proliferative cells, apoptotic cells and transcription factors, *dlx1, dlx2, nkx2.1, ngn2,* and *mash1*. With immunostained tissue, cell numbers and brain region volumes were assessed using unbiased stereological techniques and student t-tests for statistical significance.

Results: After one day of stress, the stereological count of GAD67GFP + cells in E13 dorsal telencephalon were significantly lower in prenatally-stressed offspring. In addition, at E14, the leading edge of GAD67GFP + cells in the tangential migratory pathway of prenatally-stressed animals was consistently less advanced—83.4% of the total circumference in E14 telencephalon of control embryos and 69.1% in prenatally-stressed embryos. Differences in the circumferential distribution were greater in posterior than in anterior regions. GABAergic progenitors also follow a short path of radial migration after they reach the cortical plate and become incorporated into different layers. The distribution of GAD67GFP + cells across the developing cortical plate at E15 was significantly changed by prenatal stress. Lastly, we observed an altered distribution of GAD67GFP + cells in PND0 gray and white matter of the dorsal telencephalon which is consistent with a disruption of the migration of these cells in their typical pathway. Measures of E12 BrdU labeled GAD67GFP + cells and apoptotic cells at E13 show that survival of these early born cells is not deficient in prenatally stressed embryos either in ventral or dorsal telencephalon. Proliferative cells at E14 were not decreased in the ventral telencephalon of prenatally stressed offspring. However, at E15, the density of proliferative cells in the ganglionic eminence was reduced. *In situ* hybridization demonstrated that the expression of transcription factors implicated in GABAergic progenitor migration was reduced in the telencephalon of prenatally-stressed offspring. Quantitative PCR confirmed that *nkx2.1*, a critical transcription factor for cortical GABAergic neuron migrationand its downstream target, *erbb4*, showed a trend of reduced expression in the brains of prenatally-stressed mice.

Discussion: These results demonstrate that prenatal stress has an impact on early events in the development of GABAergic cell populations. Multiple lines of evidence implicate a delay in GABAergic progenitor migration as a mechanism for abnormal GABAergic systems. The mechanism of this effect of prenatal stress on migration is at least in part through the alteration of transcription factors and their downstream targets. These findings elucidate how the environmental risk factor of prenatal stress may converge with known genetic risk factors in the development of disorders such as schizophrenia.

49. The Phosphodiesterase Isoform 4a5 (PDE4a5) is the Critical Mediator of Hippocampus-Dependent Cognitive Impairments induced by Sleep Loss

Robbert Havekes*, Jennifer H.K. Choi, Vibeke M. Bruinenberg, George S. Baillie, Alan J. Park, Jonathan P. Day, Leonardo A. Guercio, Edward Linton, Hannah Schoch, Sara J. Aton, Peter Meerlo, Miles D. Housley, Ted Abel

Department of Biology, University of Pennsylvania, Philadelphia, USA

Background: In a previous study by our laboratory, we showed that 5 hours of sleep deprivation led to elevated protein levels of PDE4a5 in the hippocampus and an increase in PDE4 activity leading to an attenuation of the cAMP pathway. However, it has not been determined whether the elevated hippocampal PDE4a5 levels and activity are *the* key mediator of the memory deficits observed after brief sleep deprivation.
Methods: We used Adeno Associated Virusses (AAV) in combination with a camkII promoter fragment to express either of the following transgenes in excitatory neurons of the hippocampus: 1) a catalytically inactive dominant negative form of PDE4a5 (PDE4a5DN), 2) wild-type PDE4a5 (PDE4a5WT), or 3) enhanced green fluorescent protein (eGFP). Mice bilaterally injected with eGFP virus into the hippocampus served as controls. Four weeks after the bilateral injection of AAV, mice were trained in hippocampus-dependent or hippocampus-independent learning paradigms. From a different cohort of mice, tissue from the hippocampus and other brain regions was collected for biochemical and electrophysiological studies.
Results: We found that overexpression of the PDE4a5DN selectively in the hippocampus reversed the sleep loss-induced memory deficits in the object-location memory task. Overexpression of the PDE4a5DN did not affect memory formation under non-sleep deprivation conditions.
Overexpression of PDE4a5WT increased hippocampal PDE4 activity and reduced cAMP levels in the hippocampus, but not in the cerebellum and prefrontal cortex. Furthermore, overexpression of PDE4a5WT impaired forskolin-mediated potentiation in CA1 Schaffer collaterals and reduced AMPA receptor phosphorylation at the GluR1 Serine 845 site, two phenomena also observed after sleep deprivation. Behaviorally, we found that overexpression of hippocampal PDE4A5 protein levels impaired the formation of long-term memories in the contextual fear conditioning task and object-location memory task. In contrast, long-term memory formation in hippocampus-independent tasks was not affected.
Discussion: Together, these findings suggest that PDE4a5 in the hippocampus is *the* critical mediator of the memory and plasticity deficits observed after 5 hours of sleep deprivation. Studies are currently underway to determine which downstream targets of the cAMP pathway are affected by the sleep deprivation-induced increase in PDE4a5 activity, but not by sleep deprivation under conditions of PDE4a5DN expression. Elucidation of the downstream targets of PDE4a5 may provide a new therapeutic approach to counteract the cognitive effects of sleep deprivation.

50. Managing Functional and Cognitive Decline in Patients with Mild-to-Moderate Alzheimer's Disease: A 48-week, Randomized, Double-blind Evaluation of 13.3 mg/24 h (15 cm²) *versus* 9.5 mg/24 h (10 cm²) Rivastigmine Patch

Jeffrey Cummings*, Lutz Frölich, Sandra E. Black, Serge Bakchine, Giuseppe Bellelli, José L. Molinuevo, Reto W. Kressig, Pamela Downs, Angelika Caputo, Sibel Tekin, Christine Strohmaier

Cleveland Clinic Lou Ruvo Center for Brain Health, Las Vegas, USA

Background: Progressive symptomatic decline in Alzheimer's disease (AD), even with current therapies, is inevitable. The acetyl- and butyrylcholinesterase inhibitor rivastigmine is widely approved for the symptomatic treatment of mild-to-moderate AD. To test the hypothesis that patients who demonstrate functional and cognitive decline on the 9.5 mg/24 h (10 cm²) rivastigmine patch can benefit from an increased dose, a study was designed to evaluate the efficacy and safety of the higher dose rivastigmine patch (13.3 mg/24 h delivery rate; 15 cm²).
Methods: This was a 48-week, multicenter, double-blind study in patients with mild-to-moderate AD (Mini-Mental State Examination [MMSE] score of 10–24). Patients who demonstrated functional and cognitive decline after 24 to 48 weeks of treatment with 9.5 mg/24 h rivastigmine patch (initial open-label phase), were randomized to receive the 9.5 mg/24 h or 13.3 mg/24 h patch in a 48-week, double-blind treatment phase. Functional decline was assessed according to investigator judgement; cognitive decline was defined as a decrease in MMSE > 2 points from previous visit or 3 points from baseline. Co-primary outcomes were change from randomized baseline at Week 48 on the Alzheimer's Disease Cooperative Study–Instrumental Activities of Daily Living (ADCS-IADL) scale and the Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-cog). Secondary outcomes included the incidence of adverse events (AEs), serious AEs (SAEs), and the discontinuation rate due to AEs. Hypotheses on the co-primary outcomes (ADCS-IADL and ADAS-cog) were investigated by means of an analysis of covariance model adjusted for country and the last available corresponding score prior to randomization.
Results: In total, 567 of 1584 enrolled patients met the decline criteria and were randomized into the 48-week, double-blind phase. Baseline demographics and characteristics were similar between treatment groups. Functional decline (change from baseline on the ADCS-IADL) was significantly less in the 13.3 mg/24 h rivastigmine patch group than the 9.5 mg/24 h group from Week 16 to 48 ($p < 0.05$). Cognitive decline (change from baseline on the ADAS-cog) was significantly less in the 13.3 mg/24 h rivastigmine patch group than the 9.5 mg/24 h group at Week 24 ($p = 0.027$), but not at Week 48 ($p = 0.227$). Incidences of deaths and SAEs were similar between groups. Overall, AEs were reported in 75.0% of patients who received the 13.3 mg/24 h rivastigmine patch and in 68.2% who received the 9.5 mg/24 h patch. In both groups, AEs occurred more frequently in the first 24 weeks (Weeks 0–24: 13.3 mg/24 h, 64.6%; 9.5 mg/24 h, 54.8%), than the second 24 weeks (Weeks 25–48), where the total number of AEs were comparable. The most frequent AEs during the 48-week treatment phase in the 13.3 mg/24 h patch group were nausea (Weeks 0–48: 12.1% vs 4.9% with the 9.5 mg/24 h patch; Weeks 0–24: 9.6% vs 3.5%; Weeks 25–48: 4.1 % vs 1.6 %), vomiting (Weeks 0–48: 10.4 % vs 4.6 %; Weeks 0–24: 8.9% vs 2.8%; Weeks 25–48: 2.5% vs 2.4%), and falls (Weeks 0–48: 7.5% vs 6.0%; Weeks 0–24: 4.3% vs 3.5%; Weeks 25–48: 3.7% vs 2.8%). Overall, discontinuations due to AEs were lower in the 13.3 mg/24 h group than 9.5 mg/24 h group (9.6% and 12.7%, respectively). The only AEs leading to discontinuation that occurred in 1% of patients in either the 13.3 mg/24 h patch group or the 9.5 mg/24 h group were vomiting (1.4% and 0.4%, respectively), application site pruritus (1.1% and 1.1%), and aggression (0.4% and 1.1%).

Discussion: In the current study, the efficacy and safety of 13.3 mg/24 h *versus* 9.5 mg/24 h rivastigmine patch was evaluated in patients experiencing functional and cognitive decline after pre-treatment with the lower dose. Patients randomized to the 13.3 mg/24 h patch showed significantly less decline in instrumental activities of daily living (Weeks 16–48) and cognition (Week 24) than those maintained on the 9.5 mg/24 h patch. Treatment with rivastigmine 13.3 mg/24 h patch was generally well tolerated, with no new safety findings compared to studies involving the lower dose patch.

Disclosure: J. Cummings: Part 1: Dr Cummings has provided consultation to Abbott, Acadia, Acerra, ADAMAS, Anavex, Astellas, Avanir, Baxter, Bristol-Myers Squibb, Eisai, Elan, EnVivo, Forest, Genentech, GlaxoSmithKline, Janssen, Lilly, Lundbeck, Medivation, Medtronics, Merck, Merz, Neurokos, Novartis, Pfizer, Prana, QR, Sonexa, Takeda, and Toyama pharmaceutical companies. Dr. Cummings had provided consultation to Bayer, Avid, GE, MedAvante, Neurotrax, and UBC. Dr. Cummings owns stock in ADAMAS, Prana, Sonexa, MedAvante, Neurotrax, Neurokos, and QR pharma. Dr Cummings has acted as a speaker/lecturer for Eisai, Forest, Janssen, Novartis, Pfizer, and Lundbeck., Part 2: n/a, Part 3: n/a, Part 4: n/a, Part 5: n/a. **L. Frölich:** Part 1: Dr Frölich is a member of the board for Eisai, Elan/Weyth, Eli Lilly, GE Health Care, Janssen-Cilag, Lundbeck, Merz Pharma, Novartis, Pfizer, and Schering-Plough. Dr Frölich has acted as a consultant to AstraZeneca, Merz Pharma, Myriad, Neurochem, Novartis, Pfizer, and Schering-Plough. He has provided expert testimony for Apotex Inc. Dr Frölich has received honoraria from Allergan, Eisai, Elan/Weyth, Janssen-Cilag, Lundbeck, Merz Pharma, Neurochem Inc., Novartis, and Pfizer. Dr Frölich has received patment for educational presentations/services on speakers′ bureaus from Novartis. He has had travel/accommodation expenses covered or reimbursed by Merz Pharma., Part 2: n/a, Part 3: n/a, Part 4: Novartis and Pfizer have provided grants/have pending grants., Part 5: n/a. **S. Black:** Part 1: Dr Black has a financial relationship in the form of contract research funds to the Cognitive Neurology and Stroke Research Units from Pfizer (Wyeth Research), Pfizer Canada Inc., GlaxoSmithKline, Lundbeck, Roche Pharmaceuticals and Novartis Pharmaceuticals. Dr Black has received Speaker′s honoraria for CME from Pfizer and Eisai and had received honoraria for *ad hoc* consulting from Pfizer, Elan and Wyeth Pharmaceuticals, Bristol-Myers Squibb and Roche Pharmaceuticals.. **S. Bakchine:** Part 1: Dr Bakchine has acted as Study Coordinator and received honoraria from Novartis. **G. Bellelli:** None. **J. Molinuevo:** None. **R. Kressig:** None. **P. Downs:** Part 5: Novartis Pharmaceuticals Corporation. **A. Caputo:** Part 5: Novartis Pharma AG. **S. Tekin:** Part 2: Stock options of Novartis Pharmaceuticals received annually during employment since 2009., Part 5: Novartis Pharmaceuticals Corporation. **C. Strohmaier:** Part 5: Novartis Pharma AG.

51. Efficacy and Tolerability of 5 mg Lu AA21004 in an 8-Week European Trial of Adults with Generalized Anxiety Disorder

Leszek Bidzan, Atul R. Mahableshwarkar* Paula Jacobsen, Mingjin Yan, David V. Sheehan

Takeda Global Research and Development Center, Deerfield, USA

Background: Lu AA21004 is a multimodal antidepressant that functions *in vitro* as a $5\text{-}HT_3$ and $5\text{-}HT_7$ receptor antagonist, $5\text{-}HT_{1B}$ receptor partial agonist, $5\text{-}HT_{1A}$ receptor agonist and inhibitor of the 5-HT transporter. *In vivo* nonclinical studies have demonstrated that Lu AA21004 enhances levels of the neurotransmitters serotonin, noradrenaline, dopamine, acetylcholine and histamine in specific areas of the brain. Lu AA21004 has demonstrated anxiolytic-like activity in animal models suggesting it may be an effective treatment for anxiety disorders. The goal of this study was to evaluate the efficacy and tolerability of 5 mg Lu AA21004 after 8 weeks of treatment in subjects with generalized anxiety disorder (GAD).

Methods: Adults aged $\geqslant 18$ years, diagnosed with GAD (DSM-IV TR) with a HAM-A total score $\geqslant 20$, a HAM-A score $\geqslant 2$ on both Item 1 (anxious mood) and Item 2 (tension), and a MADRS total score $\leqslant 16$ were eligible for this multicenter, randomized, double-blind, placebo-controlled, parallel-group study. After screening, subjects were randomized to receive either Lu AA21004 5 mg QD or placebo QD for 8 weeks. Efficacy assessments were made at Weeks 1, 2, 4, 6, and 8. The primary efficacy endpoint was change from Baseline in HAM-A total score after 8 weeks of treatment compared with placebo. A sequential testing procedure beginning with the primary endpoint followed by key secondary endpoints was used to control Type I error at 0.05. The sequence for testing was as follows: change from Baseline in HAM-A total score at Week 8 (mixed model for repeated measurements, MMRM), change from Baseline in HAD anxiety subscore at Week 8 (MMRM), CGI-I at Week 8 (MMRM), change from Baseline in SDS total score at Week 8 (MMRM), HAM-A response rate at Week 8 (last observation carried forward, LOCF), change from Baseline in HAM-A total score at Week 8 in subjects with HAM-A total score $\geqslant 25$ (MMRM), and change from Baseline in SF-36 social functioning subscore at Week 8 (MMRM). As soon as the test regarding an endpoint was not significant at the level of 0.05, the testing procedure was stopped for all subsequent endpoints. The statistical plan called for comparisons between Lu AA21004 5 mg and placebo on the full analysis set (all randomized subjects who received at least 1 dose of study drug and had at least 1 valid post baseline assessment of primary efficacy) using MMRM. HAM-A response rates were analyzed by logistic regression, adjusting for baseline HAM-A score and treatment using LOCF data. Confirmatory analyses of the primary and key secondary endpoints were performed using an analysis of covariance (ANCOVA) based on LOCF data. Adverse events (AEs) were assessed throughout the 8-week double-blind treatment period and at a follow-up call 4 weeks after completion of the treatment period.

Results: A total of 301 subjects (mean age 45.1 years) were randomized at 47 sites in Europe (Estonia, Germany, Latvia, Lithuania, Poland, Romania, Russia, and Ukraine) and Russia and 254 completed the 8-week treatment period (128/150 Lu AA21004 5 mg and 126/151 placebo). There was a significant difference in change from Baseline in HAM-A total score between the Lu AA21004 5 mg (-14.30) and placebo (-10.49) groups at Week 8 ($p < 0.001$ MMRM and ANCOVA) with separation from placebo at Week 2 ($p < 0.05$ MMRM and ANCOVA). There were significant differences between Lu AA21004 5 mg and placebo in all of the key secondary endpoints tested in the sequential testing procedure ($p < 0.05$ for all using MMRM). Analyses with LOCF data confirmed significant differences from placebo in all key secondary endpoints except the SDS total score at Week 8 ($p = 0.102$). The majority of AEs were considered by the investigators to be mild to moderate in intensity. The most common AEs were nausea, headache, and dizziness. Clinical chemistry, hematology, and urinalysis results were generally within normal ranges and no clinically meaningful differences between the 2 treatment groups were observed.

Discussion: In this study of adults with GAD from Europe and Russia, treatment with Lu AA21004 5 mg significantly improved the mean change from Baseline in HAM-A total score at Week 8 compared with placebo. All of the pre-defined secondary endpoints were significantly improved with 8 weeks of Lu AA21004 treatment, suggesting Lu AA21004 may be an effective treatment for GAD in this population.

Disclosure: L. Bidzan: Part 1: Eli Lilly, Janssen Cilag, Lundbeck, Novartis, Pfizer, KRKA, and Sanofi Aventis, Part 4: Eli Lilly. **A. Mahableshwarkar:** Part 5: Takeda Global Research and Development Center. **P. Jacobsen:** Part 5: Takeda Global Research and

Development Center. **M. Yan:** Part 5: Takeda Global Research and Development Center. **D. Sheehan:** Part 1: Jazz Pharmaceuticals AstraZeneca Pfizer, Inc. National Anxiety Foundation Lilly Research Laboratories Applied Health Outcomes/ xCENDA Targacept Cypress Bioscience United BioSource (UBC) Takeda Pharmaceuticals International Society for CNS Drug Development (ISCDD) INC Research Labopharm-Angellini Sanofi-Aventis Neuronetics NovaDel Sagene PharmaNeuroBoost, Part 2: Medical Outcome Systems, Part 4: Abbott Laboratories Merck Sharp & Dohme Ltd American Medical Association National Institute of Drug Abuse Anclote Foundation National Institute of Health (NIH) Astra Zeneca Novartis Pharmaceuticals Corp. Bristol-Myers Squibb Pharmaceuticals Company Parke-Davis Burroughs-Wellcome Pharmaceutical Company Pfizer Cephalon Quintiles Eisai America, Inc. Sandoz Pharmaceuticals Corporation Eli Lilly & Company Sanofi-Aventis Forest Laboratories Sanofi-Synthelabo Recherche (L.E.R.S.) Glaxo-Wellcome SmithKlineBeecham Pharmaceuticals GlaxoSmithKline Tampa Gen.Hosp.-University Psychiatry Ctn. International Clinical Research (ICR) TAP Pharmaceuticals Janssen Pharmaceutica Products, L.P. The Upjohn Company Jazz Pharmaceuticals Warner Chilcott Pharmaceutical Company Kali Duphar Laboratories, Inc. Worldwide Clinical Trials Labopharm Wyeth-Ayerst Pharmaceutical Company Mead Johnson Zeneca Pharmaceuticals Medicinova.

52. Cognitive Behavioral Therapy augments the Efficacy of Paroxetine in Partial Responders with Social Anxiety Disorder

Carlos Blanco*, Richard Heimberg, Thomas Rodebaugh, Franklin Schneier, Debra Ledley, Keng-Han Lin, Brigette Erwin, Michael Liebowitz

Columbia University, New York, USA

Background: Social anxiety disorder is a prevalent and debilitating disorder, and both cognitive-behavioral therapists and pharmacotherapists have developed efficacious treatments. However, it is not clear that simultaneously applied combination treatments provide an increment in treatment success over either type of treatment alone, and the literature is quite mixed on this issue. Because medications for social anxiety disorder (e.g., selective serotonin reuptake inhibitors, SSRIs) are more widely available than cognitive behavioral therapy (CBT), we sought to investigate whether patients with social anxiety disorder who demonstrate only a partial response to an initial trial of an SSRI would benefit from an additional trial of CBT in the context of continuing medication.
Methods: One hundred fifty patients with social anxiety disorder presenting for treatment at either the Adult Anxiety Clinic of Temple University or the Anxiety Disorders Clinic of the New York State Psychiatric Institute were enrolled in Phase I of the study, in which they received 12 weeks of open-label treatment with the SSRI paroxetine. Patients who showed at least minimal response were advanced to Phase II, in which they were randomized to receive only medication continuation or medication continuation plus CBT for an additional 16 weeks. Here we focus on those 61 patients who were advanced to Phase II, having achieved at least minimal response, but who failed to achieve criteria for remission of their disorder, and report Phase II results.
Results: In Phase II, continuation of paroxetine alone resulted in maintenance of gains, both on categorical measures of response and continuous clinician-administered and self-report measures, but there was little evidence for further change over the course of Phase II for these patients. However, augmentation of paroxetine with CBT was associated with significant further improvement. Patients receiving CBT in Phase II improved significantly on 5 of 7 measures during Phase II. Compared to patients receiving paroxetine alone, they were also significantly more likely to be classified as treatment responders (Clinical Global Impression Improvement Scale, CGI-I, rating of 1 or 2), 87.5% vs. 58.6%, Fisher's Exact Test, $p = .02$, and to meet criteria for remission (CGI-I = 1), 34.4% vs. 10.3%, Fisher's Exact Test, $p = .03$. Improvement was significantly greater for patients receiving CBT than for those receiving paroxetine alone for two measures (Social Phobia Scale, Brief Fear of Negative Evaluation Scale, both $ps < .05$) and approached significance for a third (Social Interaction Anxiety Scale, $p = .07$).
Discussion: These results suggest a role for the combination of paroxetine and CBT for the treatment of partial responders to an initial open trial of paroxetine alone.
Disclosure: C. Blanco: None. **R. Heimberg:** Part 3: Royalties from books published by Oxford University Press. **T. Rodebaugh:** None. **F. Schneier:** Part 1: GlaxoSmithKline. **D. Ledley:** None. **K. Lin:** None. **B. Erwin:** None. **M. Liebowitz:** Part 1: Astra Zeneca, Wyeth, Pfizer, Takeda, Pherin, Lilly, Otsuka, Eisai, GSK, Avera, Tikvah, Endo, Indevus, Servier, Part 2: ChiMatrix LLC Liebowitz Social Anxiety Scale Pherin Pharmaceuticals, Part 4: Allergan, Pfizer, GSK, Astra Zeneca, Forest, Tikvah, Avera, Eli Lilly, Novartis, Sepracor, Horizon, Johnson and Johnson. Pherin, PGX Health, Abbott, Jazz, MAP, Takeda, Wyeth, Cephalon, Indevus, Endo, Ortho-McNeil, Gruenthal, Otsuka, Gruenthal, Purdue Fredrick.

53. Efficacy and Tolerability of 5 mg Lu AA21004 in an 8-Week US Trial of Adults with Generalized Anxiety Disorder

Anthony J. Rothschild*, Atul R. Mahableshwarkar, Paula Jacobsen, Mingjin Yan, David V. Sheehan

University of Massachusetts Medical School and UMassMemorial HealthCare, Worcester, USA

Background: Lu AA21004 is a multimodal antidepressant that functions *in vitro* as a 5-HT$_3$ and 5-HT$_7$ receptor antagonist, 5-HT$_{1B}$ receptor partial agonist, 5-HT$_{1A}$ receptor agonist and inhibitor of the 5-HT transporter. *In vivo* nonclinical studies have demonstrated that Lu AA21004 enhances levels of the neurotransmitters serotonin, noradrenaline, dopamine, acetylcholine and histamine in specific areas of the brain. Lu AA21004 has demonstrated anxiolytic-like activity in animal models suggesting it may be an effective treatment for anxiety disorders. The goal of this study was to evaluate the efficacy and tolerability of 5 mg Lu AA21004 after 8 weeks of treatment in subjects with generalized anxiety disorder (GAD).
Methods: Adults aged ≥ 18 years, diagnosed with GAD (DSM-IV TR) with a HAM-A total score ≥ 20, a HAM-A score ≥ 2 on both Item 1 (anxious mood) and Item 2 (tension), and a MADRS total score ≤ 16 were eligible for this multicenter, randomized, double-blind, placebo-controlled, parallel-group study in the US. After screening, subjects were randomized to receive either Lu AA21004 5 mg QD or placebo QD for 8 weeks. Efficacy assessments were made at Weeks 1, 2, 4, 6, and 8. The primary efficacy endpoint was change from Baseline in HAM-A total score after 8 weeks of treatment compared with placebo. A sequential testing procedure beginning with the primary endpoint followed by key secondary endpoints was used to control Type I error at 0.05. The sequence for testing was as follows: change from Baseline in HAM-A total score at Week 8 (mixed model for repeated measures, MMRM), change from Baseline in HAD anxiety subscore at Week 8 (MMRM), CGI-I at Week 8 (MMRM), change from Baseline in SDS total score at Week 8 (MMRM), HAM-A response rate at Week 8 (last observation carried forward, LOCF), change from Baseline in HAM-A total score at Week 8 in subjects with HAM-A total score 25 (MMRM), and change from Baseline in SF-36 social functioning subscore at Week 8 (MMRM). As soon as the test regarding an endpoint was not significant at the level of 0.05, formal statistical testing was stopped for all subsequent endpoints.

The statistical plan called for comparisons between Lu AA21004 5 mg and placebo on the full analysis set (all randomized subjects who received at least 1 dose of study drug and had at least 1 valid post baseline assessment of primary efficacy) using MMRM. HAM-A response rates were analyzed by logistic regression, adjusting for baseline HAM-A score and treatment using LOCF. Adverse events (AEs) were assessed throughout the 8-week double-blind treatment period and at a follow-up call 4 weeks after completion of the treatment period.

Results: A total of 304 subjects (mean age 41.2 years) were randomized at 33 sites in the US and 239 completed the 8-week treatment period (125/152 Lu AA21004 5 mg and 114/152 placebo). There was no statistically significant difference in change from Baseline in mean HAM-A total score between Lu AA21004 5 mg (-12.57) and placebo (-13.16) at Week 8. There were no differences in any of the secondary endpoints between Lu AA21004 5 mg and placebo. The majority of AEs were considered mild to moderate in intensity. Overall, the most common AEs were nausea, headache, dry mouth, dizziness, somnolence, diarrhea, and nasopharyngitis. Clinical chemistry, hematology, and urinalysis results were generally within normal ranges and no clinically meaningful differences between the 2 treatment groups were observed.

Discussion: In this study of adults with GAD in the US, Lu AA21004 5 mg was not significantly different from placebo in reducing HAM-A total scores from Baseline at Week 8 and did not demonstrate efficacy on any secondary endpoints. Additional studies will be needed to determine the efficacy of Lu AA21004 5 mg for GAD in this population.

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54. Venlafaxine Treatment of Compulsive Hoarding

Sanjaya Saxena*, Jennifer Sumner, Brandon Barrios

UCSD Department of Psychiatry, La Jolla, USA

Background: Compulsive hoarding is a common, chronic, and often disabling disorder that can be difficult to treat. Compulsive hoarding appears to a distinct clinical syndrome, separate from obsessive-compulsive disorder (OCD), with different neurobiology, etiology, and epidemiology. "Hoarding Disorder" is now being considered for inclusion as a new disorder in the DSM-5. Only one study to date prospectively measured response to standardized pharmacotherapy in patients with compulsive hoarding, finding that hoarders responded as well to paroxetine as did patients with non-hoarding OCD. However, paroxetine was not tolerated well by older hoarding patients in that study, and overall response was moderate. Therefore, we conducted an open-label trial of venlafaxine, a serotonin/norepinephrine reuptake inhibitor (SNRI) medication that is better tolerated in older populations, for compulsive hoarding.

Methods: 15 patients (13 female, 2 male; mean age: 52.3 +/- 7.8 yrs.) meeting the proposed DSM-5 diagnostic criteria for Hoarding Disorder were enrolled. All comorbid Axis I disorders except Dysthymia, Generalized Anxiety Disorder, and Major Depressive Disorder were excluded. All subjects were free of psychotropic medication for at least 12 weeks prior to the study. All subjects were treated with extended-release venlafaxine (Effexor XR) for 12 weeks, according to a standardized protocol. No other psychotropic medications, cognitive-behavioral therapy, professional organizers, or cleaning crews were permitted during the study. To measure the severity of compulsive hoarding, the UCLA Hoarding Severity Scale (UHSS) and Saving Inventory-Revised (SI-R) were administered before and after treatment.

Results: 14 of the 15 patients enrolled (93%) completed venlafaxine treatment (mean final dose: 175 +/- 74 mg/day) for 12 weeks. Subjects showed significant improvements in compulsive hoarding symptoms, with a mean 37% decrease in UHSS scores and a mean 32% decrease in SI-R scores (both p < .001). Ten of the 14 completers (71%) were classified as responders to venlafaxine, based on 30% reduction in hoarding symptom severity, as well as a rating of at least "much improved" on the CGI-I. Comorbid depressive, anxiety, and OCD symptoms also improved markedly.

Discussion: The results of this open trial suggest that extended-release venlafaxine may be quite effective for treatment of compulsive hoarding and is well tolerated in this population. Taken together, the results of the paroxetine and venlafaxine studies indicate that the "conventional wisdom" that compulsive hoarding does not respond well to pharmacotherapy is wrong. Placebo-controlled trials of venlafaxine for compulsive hoarding are now warranted.

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55. Alzheimer Disease Trials Simulations to Test New Research Criteria, Biomarkers, and Other Proposed Methodological Improvements

Lon S. Schneider*, Richard E. Kennedy, Gary R. Cutter

USC Keck School of Medicine, Los Angeles, USA

Background: The vast majority of Alzheimer disease clinical trials have not shown efficacy. The most probable reason for failed trials is that the tested drug is ineffective. Nevertheless, phase 2 and 3 trials sponsored by pharmaceutical companies and NIH are carefully crafted for particular diagnoses, methods, and outcomes, with the intention to detect particularly small effects after 18 months to 2 years of treatment. Reactions to the null results of these many trials have been to consider the methods flawed by design and to then make subtle changes to the methods, for example, by enrolling patients presumed to be earlier in their illness course as diagnosed with new, research criteria such as 'MCI due to AD' or 'prodromal AD,' requiring positivity on certain biomarkers, using different outcomes, or longer treatment durations. Although these and other methods haven't been

validated for this purpose, it is, nevertheless, assumed that such changes would make the trials in question more efficient. As the proposed, new designs have not been adequately tested, the changes represent in many ways leaps of faith based on assumptions and sparse data. Appropriate modeling and testing with simulations, however, can be used to *empirically* investigate methodological assumptions and test the likelihood that a particular trial design would be successful in its intent and that a drug effect would be detected if indeed there is one to detect. As part of the aims of an NIH grant, we present examples of trials simulations using 'MCI due to AD' inclusion criteria with and without biomarkers to illustrate how trials methods can be assessed empirically and prospectively.

Methods: We have been testing various, recommended, advances in AD clinical trials designs using a meta-database combining ADNI and ADCS observational data and clinical trials and then simulating proposed trial designs. We randomly resample the database obtaining samples for 1000 trials for each trial scenario, testing sets of inclusion criteria, with and without biomarkers, changing trials lengths, sample sizes, and dropouts, and effect sizes, and calculated statistical power for the scenarios.

Results: In one set of simulations the addition of a low $A\beta42$ CSF biomarker requirement resulted in minimal or no increase in the power of the trials compared to enrolling MCI due to AD or prodromal AD without requiring biomarker criteria. Larger mean differences between placebo and treatment were offset by increased outcome variability in the biomarker-defined groups. In another model comparing MCI due to AD with early AD, therewas substantial overlap in clinical characteristics and many neuropsychological tests. About half the MCI group was indistinguishable clinically from the mild AD group showing substantial, nearly 100%, overlapping frequency of positive biomarkers and similar rates of decline.

Discussion: There was no particular evidence that including "MCI due to AD" or "prodromal AD" patients or those who had positive CSF biomarkers in clinical trials and treating them for 18 to 24 months would result in more efficient clinical trials or would achieve the desired results of treating earlier patients or more clinically homogeneous patients. These results illustrate how simulations using a large trials meta-database can test trials designs empirically and prospectively and lead to better trials.

56. Evaluation of Mibampator for Agitation and Aggression in Alzheimer's Disease

Paula Trzepacz*, Thomas Konechnik, Tammy Forrester, Brian Willis, Catherine Shuler, Linda Tabas, Jeffrey Cummings, Constantine Lyketsos

Eli Lilly and Company, Indianapolis, USA

Background: Neuropsychiatric symptoms (NPS) are universal in Alzheimer's disease (AD) yet there are no approved drugs targeted to this indication. Agitation and aggression (A/A) are common and associated with increased caregiver burden and patient institutionalization. Abnormalities of glutamatergic homeostasis have been put forth as important in Alzheimer's disease (AD) pathology and AMPA receptors are reduced in AD, and this reduction might underlie the development of A/A. AMPA potentiator agents augment glutamatergic synaptic responses that control fast synaptic transmission by binding to allosteric sites on neurons and slowing the desensitization process, thereby enhancing AMPA receptor signaling. Mibampator (N-[(2R)-2-[4′-[2-[(methylsulfonyl)amino]ethyl][1, 1′-biphenyl]-4-yl]propyl]-2-propanesulfonamide) is a positive allosteric modulator of the AMPA receptor. The objective of this Phase 2 study was to investigate the efficacy and safety of mibampator in community-dwelling Alzheimer's disease patients with A/A symptoms.

Methods: 132 patients were randomized 1:1 to receive twice-daily doses of 3 mg mibampator (n = 63) or placebo (n = 69) administered orally during a 12-week double-blind placebo-controlled trial. A one-time dose reduction to 1 mg twice-daily was permitted for intolerability. Patients were $\geqslant 60$ years with probable AD, non-delirious, and had clinically significant persistent A/A symptoms disruptive to daily living or that put them or others in harm's way. The Neuropsychiatric Inventory (NPI) was administered to measure behavioral symptoms, and item scores from four domains of the NPI (agitation /aggression, disinhibition, irritability/emotional lability, aberrant motor behavior) were summed as a measure of A/A (NPI-4-A/A subscale). Patients were required to have an NPI 10-item total score (NPI-10) $\geqslant 10$ at study entry and an item score 4 on at least one of the four domains of the NPI-4-A/A at both baseline visits. Randomization was stratified by site and neuropsychiatric symptom (NPS) severity (NPI-10 < 30 vs NPI-10 $\geqslant 30$). The primary outcome measure was the NPI-4-A/A. Secondary efficacy measures included the Cohen-Mansfield Agitation Inventory (CMAI), CGI-S-A/A for overall agitation and aggression, NPI-D-4-A/A for caregiver distress, Cornell Scale for Depression in Dementia, Frontal Systems Behavior (FrSBe) inventory, ADCS-ADL, ADAS-Cog, and CGI-S-GF for global functioning. A likelihood-based, mixed-effects model repeated measures analysis (MMRM) was performed for primary and secondary measures, with fixed categorical effects for treatment, investigator, NPS severity, visit and treatment-by-visit as well as continuous fixed covariates of baseline score and baseline score-by-visit interaction. Safety was assessed by monitoring treatment emergent adverse events, changes in laboratory tests, vital signs and ECGs.

Results: Baseline values were similar between mibampator and placebo groups with means (SD) for age 77.2(8.2) vs 77.7(7.6), MMSE 16.0(6.1) vs 18(5.3), NPI-10 31.9(16.7) vs 29.7(13.2), and NPI-4-A/A 18.8(8.7) vs 18.1(8.2). Both groups improved on NPI-4-A/A by approximately 5 points at the first post-randomization visit (week 3) and sustained that improvement. The mean change from baseline to endpoint on the primary outcome was not significantly different between groups after 12 weeks of treatment (p = 0.685). The FrSBe was the only secondary efficacy outcome for which the mibampator group had a significantly greater overall improvement than the placebo group (p = 0.007). Treatment-emergent adverse events (TEAEs) were similar between groups (p = 0.862) with 57.14% in mibampator and 55.07% in placebo experiencing at least one adverse event. The majority of TEAEs were rated mild in severity in both groups. There was 1 death in the placebo group due to intracranial hemorrhage. SAEs were: pancreatic pseudocyst, non-cardiac chest pain, pneumonia, diverticulitis, spinal column stenosis, presyncope, transient ischaemic attack, and psychotic disorder. No SAEs were attributed to drug.

Discussion: Mibampator was not better than placebo on the NPI-4-A/A subscale, the primary outcome measure. At the dose used, mibampator may have not achieved glutamatergic potentiation.

Or, if achieved, such potentiation did not affect most behavioral symptoms in AD.

57. Medication and Parent Training in Children with Pervasive Developmental Disorders and Serious Behavior Problems: Effectiveness and Tolerability of Aripiprazole in Risperidone Nonresponders

Kimberly A. Stigler*, Christopher J. McDougle, Laurence Scahill, Michael G. Aman, L. Eugene Arnold, Cynthia Johnson, Ben Handen, Ben Vitiello

Indiana University School of Medicine, Indianapolis, USA

Background: Pervasive developmental disorders (PDDs) are neuropsychiatric disorders characterized by social and communication impairments, as well as repetitive interests and activities. In addition, interfering symptoms of irritability (tantrums, aggression, self-injury) are commonly observed in children and adolescents with PDDs. The effective treatment of severe irritability in this diagnostic group often involves the use of an atypical antipsychotic in combination with behavioral therapy. A recent multisite study found that combined treatment (COMB) with risperidone and parent training in behavior management was superior to medication alone (MED) in reducing serious behaviors such as irritability, noncompliance, and hyperactivity in children with PDDs. Given that risperidone is not uniformly effective, the investigators employed a unique design that permitted risperidone nonresponders to switch to aripiprazole and continue study participation. Here, we extend the initial findings of the multisite study by investigating the effectiveness and tolerability of aripiprazole in the subset of participants who were risperidone nonresponders.

Methods: This is a 24-week multisite, randomized, controlled study of COMB versus MED in children and adolescents ages 4 to 13 years with PDDs and associated severe irritability. Eligibility was determined by a Clinical Global Impressions-Severity (CGI-S) score of $\geqslant 4$ (moderately ill) and an Aberrant Behavior Checklist-Irritability (ABC-I) subscale score of $\geqslant 18$. All subjects initially were randomized 3:2 to the COMB or MED group and received 8 weeks of risperidone treatment. Drug response was determined by a CGI-Improvement (I) scale score of 1 or 2 (very much or much improved) and $\geqslant 25\%$ improvement on the ABC-I subscale. At week 8, subjects who were nonresponders to risperidone were cross-tapered onto aripiprazole (starting dose $= 2.5$ mg/day) and continued in the multisite study. Participants received 10 to 20 mg/day of aripiprazole. Primary outcome measures included the CGI-I scale and ABC-I subscale.

Results: Twelve children, ages 4-11 years (mean age, 6.5 $\pm$ 2 years), were deemed risperidone nonresponders and entered the aripiprazole trial (COMB, n $=6$; MED, n $=6$). Mean intelligence quotient scores ranged from 40 to 119 (mean, 73). The final mean dosage of aripiprazole was 15 mg $\pm$ 3.4 mg/day. At study endpoint, 7 (71%) of 12 subjects were considered responders to aripiprazole. Five (71%) of the responders were in the COMB group. The mean endpoint CGI-I score was 2 $\pm$ 0.6 in the COMB group (p $\leqslant 0.001$) versus 3 $\pm$ 1.3 in the MED group (n.s.). Mean ABC-I subscale scores improved from 26.6 $\pm$ 3.8 at baseline to 13.4 $\pm$ 6.3 at endpoint in the COMB group (p $\leqslant 0.006$) versus 31.2 $\pm$ 5.2 at baseline to 23 $\pm$ 9.8 at endpoint in the MED group (n.s.). Analyses of the other ABC subscales revealed significantly greater improvement in the mean ABC-Hyperactivity subscale score from baseline to endpoint in the COMB group (p $\leqslant 0.02$). Although not significant, there was a trend toward greater improvement in the ABC-Social Withdrawal subscale score in the COMB group (p $= 0.08$). Aripiprazole was generally well tolerated. The most common adverse effects reported were mild and included increased weight, fatigue, cough, headache, vomiting, and increased appetite. Extrapyramidal symptoms included mild sialorrhea (n $=10$), mild muscle twitching (n $=1$), and mild tremor (n $=1$). No clinically significant changes in heart rate or blood pressure were recorded. Subjects gained weight after switching to aripiprazole, with mean age- and sex-adjusted BMIs increasing from 17 $\pm$ 2.1 at baseline to 20.3 $\pm$ 2.6 at endpoint (p $\leqslant 0.0001$).

Discussion: Preliminary data suggest that aripiprazole may be well tolerated and effective for irritability in risperidone nonresponders, particularly when combined with parent training in behavior management. Aripiprazole's mechanism of action as a partial DA D_2 and 5-HT$_{1A}$ agonist, and 5-HT$_{2A}$ antagonist may have contributed to its effectiveness in this group. Larger-scale research studies are needed to better understand these findings.

58. A Placebo-Controlled Trial of Riluzole for Treatment of Childhood-Onset Obsessive Compulsive Disorder

Lisa Joseph, Paul Grant, Susan Swedo*

PDN Branch, NIMH, Bethesda, USA

Background: Obsessive-Compulsive Disorder (OCD) affects 1 - 2% of children and adolescents, causing significant distress and

impairments of functioning. Anti-obsessional medications (typically SSRIs) and cognitive-behavioral therapy are beneficial to many, but not all children. Preliminary data from studies of adults suggest that riluzole, a drug that affects glutamate within the CNS, may be effective for patients who have failed to respond to other medications. The purpose of this study was to conduct a randomized, placebo-controlled trial of riluzole in children and adolescents with OCD to determine if riluzole would reduce symptoms to a greater extent than placebo.

Methods: Participants were 60 children and adolescents (44 males, 16 females; mean age 14.4 ± 2.3 yrs) with moderate to severe OCD (CYBOCS mean total score = 28.17 ± 3.7). All had failed to respond adequately to standard therapies (on average, children were taking 2 psychotropic medications at baseline). 17 of the 60 subjects had an autism spectrum disorder in addition to OCD. Subjects were randomly assigned to receive capsules containing placebo (PLA) or riluzole (RIL; 50 mg q 12 hrs) for 12 weeks. Symptom ratings were completed at biweekly intervals; the final blinded ratings (at 12 weeks) of obsessive-compulsive symptoms, anxiety and depression were used for analyses. Those completing the 12 weeks double-blind trial were offered open-label RIL and all elected to take RIL for the next nine months (total study duration = 1 year).

Results: 51 children completed the double-blind phase of the study. The drug was generally well-tolerated, but there was one serious adverse event (a child taking multiple drugs developed pancreatitis; he recovered fully after RIL and other drugs were D/C'ed) 5 children had modest transaminase elevations during RIL administration. An intent-to-treat analysis (using last data point recorded) found no significant differences in ratings of OCD, depression or anxiety between the 30 children randomized to PLA and the 30 in the RIL group. Analysis of data from those completing 12 weeks of study drug (RIL n = 22; PLA n = 29) revealed significantly greater improvements in OCD symptoms during RIL administration (CY-BOCS total score from baseline to 12 weeks: RIL 27.3 ± 3.7 to 20.6 ± 6.4; PLA 29.0 ± 3.5 to 23.3 ± 4.7). RIL was also superior to PLA on ratings of compulsions (CY-BOCS Compulsions: RIL 13.8 ± 1.9 to 10.4 ± 3.7; PLA 14.6 ± 1.9 to 11.9 ± 2.5). Patients continued to improve throughout the 9 months of open-label RIL administration, with all subjects reporting less OCD, anxiety and depression at 1 yr than at baseline.

Discussion: This small study of the efficacy and safety of riluzole for children and adolescents with OCD demonstrated some benefits, particularly following open-label administration for 9 months. However, the differences between riluzole and placebo were statistically significant only when examined for the cohort of children who completed the 12 weeks double-blind trial. The lack of significant differences on the intent-to-treat analysis appears to be due, at least in part, to the larger number of children withdrawn from the active study group due to adverse effects, including one episode of pancreatitis. Despite these limitations, the results of this trial are encouraging and warrant replication and extension to a cohort of children without a history of multiple treatment failures. It would also be interesting to test the efficacy of RIL as a solitary treatment, rather than as an "augmenting agent" added to a current, non-effective treatment regimen (as was done in this study).

Disclosure: L. Joseph: None. P. Grant: None. S. Swedo: None.

59. Selegiline Transdermal System (STS) in Patients with Recurrent Unipolar Major Depression: A Post-Hoc Analysis of 2 Randomized, Double Blind Studies

Donald Robinson*, Kimberly B. Portland

Worldwide Drug Development, Shelburne, USA

Background: Major depression is a chronic and recurring disorder. Depression is recurrent in 75% to 80% of depressed patients, and becomes chronic (i.e., lasting 2 years or longer) in 15% to 20% of depressed patients.[1,2] Selegiline transdermal system (STS) has been shown to be an effective and well-tolerated acute and maintenance treatment for MDD.[3-6] STS delivers sustained blood levels of monoamine oxidase inhibitor (MAOI) directly into systemic circulation, avoiding the need for a tyramine-restricted diet at the 6 mg/day dose. The objective of this post-hoc analysis was to examine efficacy of STS versus placebo in patients with recurrent unipolar major depression.

Methods: Data from two pivotal, short-term (one 6 week and one 8 week), randomized, double-blind, placebo-controlled clinical trials of STS[3,4] were pooled for this analysis (N=433). Single versus recurrent depressive episodes was coded by the investigator at the time of baseline assessment. An analysis of covariance (ANCOVA) model, controlling for baseline severity and study, was used to evaluate the effect of treatment as measured by the 28-item Hamilton Rating Scales for Depression (HAM-D) and the Montgomery-Åsberg Depression Rating Scale (MADRS) at study endpoint. χ^2 analysis was used to assess differences in endpoint remission rates between STS and placebo.

Results: Almost three quarters of the patients met criteria for 'recurrent depression' (70.4%; n = 305). Patients with recurrent depression had a mean baseline HAM-D17 score of 23.1 (± 2.4). In patients with recurrent depressive episodes, patients receiving STS showed significantly greater improvement at endpoint on MADRS and HAM-D28 total scores versus patients receiving placebo ($p < 0.001$). Additionally, remission rates as defined by endpoint HAM-D28 and MADRS were significantly greater in patients with recurrent depression treated with STS than placebo (MADRS remission rates: STS = 27.9%, placebo = 13.3%, $p < 0.01$; HAM-D28 remission rates: STS = 21.8%, placebo = 10.1%, $p < 0.01$). Remission rates were higher for patients who had experienced recurrent depressive episodes than for single-episode patients.

Discussion: STS is an effective treatment for patients with recurrent episodes of unipolar major depression. Interestingly, treatment effects were not as strong in patients with single episode depression.

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Disclosure: D. Robinson: Part 1: Dey Pharma, LP, Forest Laboratories. **K. Portland:** Part 1: Dey Pharma, LP, Part 2: Dey Pharma, LP, Part 3: Dey Pharma, LP, Part 5: Dey Pharma, LP.

60. A Translational Approach to Evaluate the Efficacy and Safety of the Novel AMPA Receptor Positive Allosteric Modulator Org 26576 in Adult Attention-Deficit/Hyperactivity Disorder
Pilar Cazorla*, Rene Kroon, Mohammed Shahid, Frank Tarazi, Jacques Schipper, Mark Stein, Armin Szegedi, Lenard Adler

Merck, Rahway, USA

Background: Glutamate dysregulation has been postulated to play a role in the neurobiology of attention-deficit/hyperactivity disorder (ADHD). Modulation of this neurotransmitter pathway may provide improved therapeutic options when compared to psychostimulants which have significant limitations such as abuse liability. Most significantly, clinical efficacy with the AMPA receptor positive allosteric modulator (AMPA-PAM) Cx717 has previously been shown in adults with ADHD. This hypothesis was further investigated following a translational approach that included both preclinical and clinical testing with Org 26576, a novel AMPA PAM.

Methods: Neonatal rat 6-hydroxydopamine (6OHDA) lesion-induced hyperactivity was used as preclinical model to evaluate Org 26576 activity at several doses (1, 3, 10 mg/kg i.p.). Seventy-eight ADHD adults entered a multi-center, double-blind, placebo-controlled, 2-period crossover clinical trial. All subjects received placebo for two weeks in between periods. After one-week placebo lead-in, sixty-seven subjects were randomized into one of four treatment sequences: Sequence A (n = 15) Org 26576 (100 mg bid) for 3 weeks, followed by 5-weeks placebo; Sequence B (n = 16) 5 weeks placebo followed by 3 weeks Org 26576 (100 mg bid); Sequence C (n = 18) Org 26576 flexible dose (100-300 mg bid) for 3 weeks, then 5-weeks placebo; Sequence D (n = 18) 5 weeks placebo followed by 3 weeks Org 26576 (100-300 mg bid). The Adult ADHD Investigator Symptoms Rating Scale (AISRS) was used to assess changes in ADHD symptomatology.

Results: Org 26576 produced a dose-dependent inhibition of hyperactive behavior in 6-OHDA-lesioned rats without stimulation of locomotion in sham lesioned rats. In the clinical setting, Org 26576 (100 mg bid) was superior to placebo in improving symptoms of ADHD in adults as measured by for the primary endpoint, the change from baseline (CFB) in AISRS total score. The estimated difference (Org 26576 100 mg bid minus placebo) was -5.70 (97.5% CI: (-10.911, −0.4881); p = 0.0147). These results were supported by a number of secondary analyses. Org 26576 flexible dose (100-300 mg bid) did not show superiority versus placebo in the primary endpoint (CFB + 0.99, p = 0.59) or any secondary endpoints. Org 26576 was generally well tolerated in all treatment sequences. The most frequently reported AEs were nausea, dizziness and headache. The frequency and severity of AEs as well as the percentage of discontinuations due to drug-related AEs were higher in the Org 26576 (100-300 mg bid) group than in the Org 26576 (100 mg bid) group. Tolerability was comparable between the Org 26576 (100 mg bid) and placebo groups.

Discussion: In summary, the described preclinical findings were also mirrored in the clinical setting. The neonatal 6-hydroxydopamine dopaminergic lesion induced hyperactivity rat model is a widely employed animal model for screening agents useful in the treatment of ADHD and is viewed to carry considerable predictive validity. Administration of stimulants such as methylphenidate inhibits rat hyperactive behavior in this model. In the current study Org 26576 showed a similar profile to stimulants suggesting the potential for efficacy in ADHD. The improvement in ADHD symptomatology observed with Org 26576 (100 mg bid) in ADHD adults is in line with the preclinical results and results previously reported for the ampakine Cx717 in a clinical trial of comparable design. An efficacy signal was identified in the treatment of adult ADHD with the novel AMPA PAM Org 26576 at a fixed dose of (100 mg bid). However, these results should be interpreted in view of the fact that higher flexible doses did not confirm the findings. It is however unclear whether the observed data in the Org 26576 (100-300 mg bid) represent an artifact or are the result of an inverse U-shaped dose response. These results might serve as the basis of future clinical research with Org 26576 and related AMPA PAM compounds, which may represent a potential alternative and improved treatment to ADHD.

61. Magnetic Seizure Therapy (MST): Introduction to a Promising Treatment for Geriatric Depression in Development
Stefan B. Rowny*

Columbia U/NYSPI, New York, USA

Background: ECT is widely considered to be the most efficacious treatment and is often the treatment of choice in the severely depressed geriatric individuals. There is compelling evidence for the efficacy of ECT in depressed elders. However, it is frequently under-utilized because of historical stigma and the increased risk of medical and adverse cognitive side effects, especially in the case of co-existing dementia and other conditions that may increase its risks. Thus, the search for novel safe and effective ways of treating severe depression, particularly after medications fail, continues.

Methods: Magnetic Seizure Therapy (MST) is a promising new approach to reducing the cognitive side effects of ECT through enhanced control over the site of seizure induction. With ECT, the application of electricity through the scalp and skull presents inherent limitations in the ability to target the stimulus precisely and leads to significant inter-individual variation in delivered dose. MST overcomes this limitation because with electromagnetic induction, the induced electric field is limited to superficial layers of the cortex, thereby sparing deeper brain structures from exposure to the electric field and enabling the induction of seizures that have a superficial focus and secondary generalization. Studies report that MST induces more focal seizures, with fewer side effects than ECT. There is also initial evidence for antidepressant effects of MST. However, the feasibility and safety of MST in the elderly has not been specifically studied, and this would be important since they represent the population most likely to benefit from this novel intervention.

Results: Contrasting the neurobiological effects of MST and ECT, two interventions that both induce therapeutic seizures but differ

so markedly in cognitive side effects, presents an unparalleled opportunity to define the brain mechanisms responsible for seizure-induced amnesia, and would facilitate the development of a targeted seizure therapy to minimize these adverse effects. There have been less than a handful of studies on the differential effects of MST and ECT on neurophysiology, and no functional imaging studies on MST outside of a single case report with [(99 m)Tc]-HMPAO SPECT. This study will provide the first data examining the linkages between the effects of MST versus ECT on functional brain activation and clinical outcomes. Results will shed light on the mechanism of action of convulsive therapy, providing the first test of whether regional differences in the neural response to seizures result in differential clinical effects on depression and cognitive function.

Discussion: MST was developed at Columbia by Srah Lisanby, MD, through projects supported by NIH and foundations. Our modeling data show that MST results in much more superficial fields than all forms of ECT. MST was also less affected by variation in scalp and skull thickness and brain atrophy than ECT (up to 29% variance for MST compared with up to 82% variance for ECT), a result important for the geriatric population. These modeling results match our empirical intracerebral recordings in rhesus monkey that demonstrate MST-induced fields spare the temporal lobe, and the resultant seizures are more focal with less deep generalization than seen with ECT. This enhanced control represents a means to focus the treatment in targeted cortical structures thought to mediate antidepressantresponse and to reduce spread to medial temporal areas implicated in the amnestic effects of ECT. We have performed a series of parallel studies in nonhuman primates and in humans on the physiological, cognitive, neuroanatomical, medical, and antidepressant effects of MST. Work to date demonstrates that MST has fewer cognitive side effects, but still shows antidepressant benefits. However, the feasibility and tolerability of MST in a geriatric sample has never been studied. Contrasting the neurobiological effects of ECT with MST provides a unique strategy to differentiate the neural underpinnings of efficacy (which both ECT and MST should show) from those related to amnesia (which should be greater in ECT than MST). Results will inform the development of more targeted treatments, guided by knowledge of mechanisms.

Disclosure: S. Rowny: None.

62. Some Urban Legends Of CNS Clinical Trial Methodology: Unsuccessful Solutions to the Problem of Failed Trials
Janet B.W. Williams*, Danielle Popp, Scott Reines, Michael J. Detke

MedAvante, Hamilton, USA

Background: The problem of failed trials in CNS is well recognized (Khin, 2011). As the number of failed trials has grown, drug developers have attempted several strategies to improve signal detection and reduce the failure rate. We present five common strategies and evaluate the evidence for their effectiveness.

Methods: 1. Increase sample size to increase statistical power. If one assumes statistical power increases with sample size and effect size is fixed, it appears reasonable to assume that increasing sample size will increase effect size in a given study. Liu et al., (2008) examined four phase III depression trials to evaluate this assumption. 2. Choose the "right" sites. There is a belief that selecting sites with proven track records across several studies will continue to yield positive results. Gelwicks et al., (2002) analyzed data from 21 clinical trial sites that participated in at least two different trials and randomized at least 30 subjects across trials. 3. Use the most experienced raters. It seems logical that employing more experienced raters will minimize variability and improve

signal detection. Kobak et al., (2009) examined the relative impact of experience and calibration by calculating interrater agreement across three groups of raters: an experienced and calibrated cohort, an experienced but non-calibrated cohort, and an inexperienced cohort. Thirty subjects with MDD were assessed in independent interviews by two different raters on the same day using the SIGH-D. 4. Increase rater training. A frequently cited cause of trial failure is inadequate rater training. There is the potential for huge variability across raters in a single trial, which negatively affects study power and signal detection. Some believe increasing the intensity of rater training will reduce variability. Demitrack et al., (1998) trained 85 raters on the HAM-D in an intensive, six hour iterative training session with four video-tapes and discussion between each tape. 5. Certain regions of the world have better signal detection. Many researchers believe that greater signal detection can be obtained outside the US. Khin et al., (2011) conducted a meta-analysis of 81 randomized double-blind clinical trials of antidepressants that were submitted to the FDA between 1983 and 2008, including both US and ex-US studies.

Results: 1. Increase sample size to increase statistical power. In three positive studies of antidepressants, treatment effect was observed before the first 100 patients per treatment arm were enrolled. Continuing to enroll patients did not maintain the significance of the effect in most cases, and in one study it actually appeared to turn a positive result into a negative one. Also, treatment effect size in both US and non-US depression trials has been decreasing over time despite, or even perhaps because of, a steady increase in sample size per treatment arm. 2. Choose the "right" sites. Site performance across consecutive studies was inconsistent, with no strong correlation within sites across studies on randomization rates, protocol completion percentage, percentage of placebo responders and drug-placebo difference. 3. Use the most experienced raters. The highest interrater agreement was achieved by experienced and calibrated raters (r = 0.93), followed by inexperienced raters (r = 0.77). Experienced but non-calibrated raters achieved the lowest interrater agreement (r = 0.55). 4. Increase rater training. ICCs across the four training tapes ranged from 0.65-0.79 and did not improve across the six hours of training. 5. Certain regions of the world have better signal detection. A meta-analysis of 81 randomized double-blind clinical trials of antidepressants documented increasing placebo response across both US and ex-US regions, and a similar decrease in effect size for both US and ex-US studies.

Discussion: Strategies for improving signal detection in CNS clinical trials are often used without clear evidence of their efficacy. Increasing sample sizes, targeted site selection, using experienced but non-calibrated raters, increasing rater training, and conducting trials ex-US have all proven largely unsuccessful in increasing the success rate. These "urban legends" are widely touted, but evidence to support them is mixed at best. This review highlights the importance of examining the effectiveness of methodological solutions for increasing signal detection to improve clinical trials as well as to inform future drug development decisions.

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Part 3: MedAvante, Inc.; Eli Lilly, Inc., Part 4: None., Part 5: MedAvante, Inc.

63. Randomized Sham Controlled Double-blind Trial of Repetitive Transcranial Magnetic Stimulation (rTMS) for Adults with Severe Tourette Syndrome

Angeli Landeros-Weisenberge, Maria Motlagh, Antonio Mantovani, James F. Leckman, Sarah H. Lisanby*

Duke University, Durham, USA

Background: Tourette syndrome (TS) is a childhood onset neuropsychiatric disorder characterized by motor and vocal tics that are often preceded by premonitory urges. Recent studies have suggested that the cortico-striato-thalamocortical sensorimotor circuit may be altered in TS resulting in an inability to gate sensory information effectively given that tics are often executed to alleviate premonitory sensory urges. Because of the superficial localization of the Supplementary Motor area (SMA) it would be an obvious site to stimulate with rTMS as a means to access the sensorimotor CSTC.This study was a pilot randomized sham controlled, double-blind feasibility study to assess the therapeutic benefits and safety of rTMS delivered to the SMA on tic symptoms, to follow up our previously reported open-label positive results with rTMS in TS.

Methods: Twenty subjects from two sites (Yale Child Study Center and Columbia University) were randomized to receive either active or sham rTMS using a parallel-group trial design using 1 Hz trains on the SMA lasting 30 minutes (1,800 pulses per day) at 110% of the motor threshold, once a day. The subjects included had a primary diagnosis of TS, a Yale Global Tic Severity Scale (YGTSS) score $\geq$ 20, and on stable psychiatric medication for at least four weeks prior to the start of rTMS treatments. SMA targeting was done using the International 10-20 coordinate system.We delivered 15 sessions of active or sham rTMS targeting the bilateral SMA to blinded subjects over a three week period (Phase 1). We then delivered 15 sessions of active rTMS to the same target location to all twenty subjects (Phase 2). Blinded clinical assessments of tic severity and other comorbid symptoms were done by independent evaluators at the end of each five day blocks of rTMS stimulation. Our primary outcome was change in YGTSS tic severity during the first three weeks of treatment.

Results: We treated a total of 20 subjects (16 male, 4 female) with a mean age of 33.7 years (SD 12.2) and a mean YGTSS score of 36 ± 9 despite taking medications to treat their tics. Nine patients received active rTMS and eleven received sham stimulation during the first three weeks of treatment. There was no difference between active and sham-stimulation on YGTSS total tic score (t = −0.34, df = 18, p = 0.7) or any secondary measures. The active rTMS group improved by an average of 6.22 (SD = 8.02) compared to 5.18 (SD = 5.51) in the sham stimulation group. The active treatment group experienced improvement on some secondary measures (YGTSS impairment score) when treatment was continued for an additional three weeks in the unblinded phase of the trial. Seventeen of the twenty subjects completed the six week trial, no significant adverse events were reported.

Discussion: This sham controlled trial did not replicate the previous benefits reported in earlier studies using low frequency rTMS targeting the SMA using the International 10-20 coordinate system in the treatment of severe TS. Future directions include the use of improved target selection (e.g. via MRI-guided TMS), altered dosing paradigm (e.g. longer course of treatment given the suggestion of improvement after 6 weeks), self-reports and videotaping as complementary assessments.

Disclosure: A. Landeros-Weisenberge: None. **M. Motlagh:** None. **A. Mantovani:** None. **J. Leckman:** None. **S. Lisanby:** None.

64. A Multi-Center Investigation of Folate plus B12 Supplementation in schizophrenia

Joshua L Roffman*, Steve Lamberti, Eric Achtyes, Eric A. Macklin, Gail Galendez, Lisa Raeke, Noah J. Silverstein, Dan Tuinstra, Michele Hill, Donald C. Goff

Massachusetts General Hospital / Harvard Medical School, Boston, USA

Background: Negative symptoms, which include apathy, impoverished speech, flattened affect, and social withdrawal, cause substantial morbidity and functional impairment among patients with schizophrenia. These problems are compounded by the lack of effective treatments for negative symptoms. Previous work from our group and others suggests a link between negative symptom severity and altered folate metabolism in schizophrenia. Moreover, a common genetic variant in MTHFR, which plays a key role in folate metabolism, may contribute to this pattern: the hypofunctional 677T allele of MTHFR, each copy of which reduces MTHFR activity by 35%, has been associated with increased schizophrenia risk and specifically with increased severity of negative symptoms and related neurocognitive dysfunction. A previous pilot investigation by our group suggested that folate supplementation may improve negative symptoms in schizophrenia, but only among patients who carried the 677T allele. Here, we conducted a large multi-site investigation to determine whether folate plus B12 supplementation, by itself and in concert with MTHFR 677C > T genotype, influenced symptom severity among medicated schizophrenia patients.

Methods: We enrolled outpatients with schizophrenia at three sites (Massachusetts General Hospital, University of Rochester, and Michigan State University). Patients were taking stable regimens of antipsychotic medication upon enrollment and were continued on their medications throughout the study. A total of 140 patients were randomized, double-blind, in a 2:1 ratio to receive 16 weeks of daily treatment with either 2 mg folic acid plus 400 mcg B12 or placebo. Fasting serum folate values and clinical measures including the Scale for the Assessment of Negative Symptoms (SANS) and Positive and Negative Syndrome Scale (PANSS) were obtained at screening and at 2, 4, 8, 12, and 16 week follow-up visits. For data analysis, linear mixed models that included treatment status (active versus placebo), MTHFR genotype (C/C versus T allele carrier), and baseline folate levels were used to examine changes in SANS total score, PANSS total score, and PANSS positive symptoms over time. Alpha (2-tailed) was set at 0.05.

Results: Preliminary analyses are as follows, with additional analyses to be presented at the meeting. The two treatment groups did not differ on any demographic or clinical variable at baseline. Sixteen week retention was 78%. Serum folate levels rose significantly in the active treatment group. SANS total scores improved significantly in the folate group and significantly more compared to the placebo group, which did not show a change in negative symptoms. PANSS total scores improved significantly in the folate group, but not significantly more compared to placebo. No effects of treatment were seen for PANSS positive symptoms. MTHFR genotype influenced each main outcome variable. For SANS, genotype effects differed by treatment group, as only T allele carriers showed significant improvement compared to placebo. For PANSS total and PANSS positive symptoms, C/C patients improved significantly across both treatment groups on average and significantly more than T carriers, who did not improve, regardless of treatment.

Discussion: Folate plus B12 supplementation confers a specific benefit for negative symptoms of schizophrenia, for which no other treatment is available. Confirming our hypothesis and replicating previous results from a smaller sample, these effects were especially pronounced in patients who carried the low-

functioning 677T variant of MTHFR, which has previously been associated with increased negative symptom severity and related cognitive impairment. Studies of wider-scale implementation of folate plus B12 supplementation in schizophrenia are warranted, as are prospective investigations of whether folate and B12 status influence schizophrenia risk, especially among individuals who are genetically predisposed to altered folate metabolism.

65. MK-6096, a Dual Orexin Receptor Antagonist, improves Subjective Measures of Sleep and Functioning in Adults with Primary Insomnia

Kathryn M. Connor*, Xin Zhao, Erin Mahoney, Saheeda Jackson, Jill Hutzelmann, Ellen Snyder, Duane Snavely, Jay Pearson, David Michelson, Darryle Schoepp, Thomas Roth, W. Joesph Herring

Merck Research Laboratories, North Wales, USA

Background: Orexinergic activity originating in the lateral hypothalamus plays a critical role in sleep/wake regulation. Drugs that influence orexinergic tone may be useful in the treatment of sleep disorders. We evaluated MK-6096, a potent and selective dual orexin receptor antagonist (DORA), for the treatment of primary insomnia using both objective (polysomnographic (PSG)) and subjective (electronic diary (eDiary)) assessments. Primary results from this study have been previously reported and demonstrated significant dose-related effects of MK-6096 on PSG measures of sleep induction and maintenance. In this report, we describe results from subjective assessments of sleep parameters, next day effects, and overall function.

Methods: A randomized, double-blind, placebo-controlled, adaptive, 2-period (4-weeks per treatment period, separated by a 2-week washout) cross-over PSG study was performed to assess the efficacy and tolerability of MK-6096 (2.5, 5, 10, and 20 mg) administered nightly for the treatment of primary insomnia. Patients completed daily assessments, via an eDiary, to evaluate the impact of treatment on sleep onset and maintenance, as well as on parameters related to next day effects. In addition, change in overall functioning over 4 weeks of treatment was assessed using the Sheehan Disability Scale (SDS).

Results: 324 patients administered at least one dose of study medication: 318 received MK-6096 (either 2.5 mg [N = 79], 5 mg [N = 78], 10 mg [N = 80], or 20 mg [N = 81]) and 315 received placebo. All doses of MK-6096 were superior to placebo based on changes from baseline in subjective time to sleep onset (sTSO; minutes) at week 1 (LS mean [95% CI]: 2.5 mg = −7.8 [-14.1, −1.4], 5 mg = −8.4 [-14.7, −2.0], 10 mg = −16.0 [-22.3, −9.7], 20 mg = −14.6 [-20.7, −8.5]) and these differences were sustained through week 4 (2.5 mg = −7.9 [-13.6, −2.1], 5 mg = −9.4 [-15.2, −3.6], 10 mg = −16.8 [-22.5, −11.1], 20 mg = −18.1 [-23.6, −12.5]). All doses of MK-6096 except 2.5 mg were superior to placebo at weeks 1 and 4 based on changes from baseline in subjective

total sleep time (sTST; minutes) (week 1: 2.5 mg = 5.0 [-6.2,16.2], 5 mg = 16.2 [5.0,27.5], 10 mg = 19.1 [8.0,30.3], 20 mg = 29.4 [18.6,40.3]; week 4: (2.5 mg = 12.5 [2.1,22.9], 5 mg = 22.6 [12.1,33.1], 10 mg = 30.8 [20.4,41.3], 20 mg = 37.0 [26.9,47.2]). For other patient-reported outcomes, significant dose-related effects were consistently observed at all time points for higher doses of MK-6096 (10 and 20 mg) for improved sleep quality, and feeling refreshed and rested the following morning. Dose-related improvement in overall daytime functioning was also observed based on changes in the SDS total score, with significant improvement at higher doses of MK-6096 compared to placebo. MK-6096 was generally safe and well-tolerated.

Discussion: MK-6096 is efficacious for the treatment of primary insomnia, based on improvement in subjective sleep measures. These effects are observed as early as week 1 and are sustained through 4 weeks of treatment. MK-6096 is also associated with improvement in overall functioning. MK-6096 is generally well-tolerated in adults with primary insomnia.

66. Power Spectral Analysis of the Dual Orexin Receptor Antagonist Suvorexant (MK-4305) in Patients with Primary Insomnia and in Healthy Subjects

W. Joseph Herring*, Junshiu Ma, Ellen Snyder, Vladimir Svetnik, Jill Hutzelmann, Kenneth Liu, Christopher Lines, Thomas Roth, Darryle Schoepp, David Michelson

Merck Sharp & Dohme Corp, Whitehouse Station, USA

Background: Orexinergic activity originating in the lateral hypothalamus plays a critical role in sleep/wake regulation. Drugs that influence orexinergic tone may be useful in the treatment of sleep disorders. Suvorexant (MK-4305) is a novel potent and selective dual orexin receptor antagonist (DORA) being studied for the treatment of insomnia. We previously reported results from a Phase 2B study which demonstrated significant dose-related effects of suvorexant on sleep induction and maintenance parameters in primary insomnia patients. The DORA mechanism is distinct from other sedative-hypnotic agents currently used in the treatment of insomnia. Most current treatments induce changes in spectral power of EEG during sleep that are specific to each different mechanism. Here we report on the power spectral profile of suvorexant in the Phase 2B study, and compare its profile to that of other hypnotic agents in healthy volunteer studies.

Methods: 1524 polysomnographies (PSGs) from 254 primary insomnia patients were collected in a randomized, double-blind, placebo-controlled, 2-period cross-over multi-center Phase 2B study of suvorexant, which was orally-administered 30 minutes before bed time. The patients were randomized into four subgroups, with each receiving one of the four suvorexant doses (i.e. 10, 20, 40, and 80 mg) in one period and placebo in the other. After removing signal artifacts, the power spectra of the EEG signal in the C3-A2 channel of each PSG recording during non-REM (NREM) and REM sleep were calculated separately using Welch's method. The power spectra is a function at the frequency domain (frequencies ranging from 1 Hz to 32 Hz), and can be represented as the linear combinations of a set of B-spline functions. Functional Linear Mixed Effect (LME) statistical models were fit with power spectra data from the four subgroups and two sleep stages (i.e. NREM and REM sleep) separately. In each LME model, the fixed effects included (1) frequency, (2) treatment, (3) treatment and frequency interaction, (4) patient gender, (5) gender and frequency interaction, (6) period, and (7) site country, while the random effects had a two-level grouping structure comprising of study site and patients recruited by the site. The resultant drug effect measurements are spectral profiles, which were estimated from the fitted LME models. Each profile is the ratio of the drug's effect on EEG spectra relative to placebo at each frequency. The EEG spectral profiles of trazodone 150 mg, zolpidem 10 mg, gaboxadol 15 mg, and suvorexant 50 mg in healthy subjects were also calculated in a similar way using data from three other separate studies.

Results: The day 1 spectral profiles of suvorexant at four doses during both NREM and REM sleep stages were flat and close to 1.0 at all frequencies. Except for the highly variable low-frequency range (i.e. 1 Hz – 2 Hz), the estimated EEG effects of suvorexant at all four doses during NREM sleep were no larger or smaller than 7% of those of the placebo, and during REM sleep no larger or smaller than 10% of those of the placebo. Thus, suvorexant had no effect on sleep EEG spectra relative to placebo. The spectral profile of suvorexant in healthy subjects was similar to that observed in primary insomnia patients. In contrast, the other three drugs did change EEG spectral profiles during sleep in healthy subjects. For NREM sleep, compared with the placebo, trazodone 150 mg significantly increased ($>20\%$) the EEG spectra at the gamma frequency band (24-32 Hz) and decreased ($>10\%$) the lower sigma band (12-15 Hz), zolpidem 10 mg decreased ($>20\%$) the spectra around the theta band (5-9 Hz) and increased ($>10\%$) the sigma band (12-15 Hz), while the investigational hypnotic gaboxadol 15 mg increased ($>20\%$) the delta and theta frequency bands (1 Hz-8 Hz).

Discussion: Suvorexant did not effect the EEG power spectral profile, in contrast to the three comparator hypnotics. These findings suggest the possibility that focused pharmacologic antagonism of the orexin pathway might lead to improvements in sleep induction and maintenance without inducing significant perturbations in power spectral frequencies normally observed during sleep.Given that these data indicate the DORA mechanism can mediate improvements in sleep without spectral changes, the question arises as to what the functional significance is of the various EEG changes seen with current hypnotics.

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67. Lowered CSF levels of Nociceptin in Females with Fibromyalgia Syndrome

Lars Tanum*, Morten Vinje, Gunnar Ordeberg, Fred Nyberg

Akershus University Hospital & University of Oslo, Oslo, Norway

Background: Nociceptin (orphanin) exerts a potent anti-nociceptive action through binding to an opiode-like receptor complex. Nociceptin is widely expressed in the nervous system and has been postulated to play a possible role in development or maintenance of chronic pain conditions. In this study we investigated if the level of nociceptin was altered in a population of females suffering from fibromyalgia syndrome.

Methods: Thirty-six females with a diagnosis of fibromyalgia syndrome according to the ACR 1990 criteria (American College of Rheuma-tology), but with no other axis-1 disorder according to DSM-IV, were included in the study and compared with 13 healthy female volunteers. CSF from both groups was collected in the morning and immediately frozen. All analyses were performed at the Department of Pharmaceutical Biosciences, University of Uppsala, Sweden.

Results: Significant lower CSF-levels of nociceptin were found in female patients with fibromyalgia compared to healthy female controls. Controls had a mean value of 98 femtomol/ml (SD 15.7) while fibromyalgia patients had a mean value of 78.9 femtomol/ml (SD 16.8) ($p < 0.05$).

Discussion: Lowered level of nociceptin in female fibromyalgia patients may contribute to increased pain involving development of hyperalgesia and allodynia generally observed in such patients. To our knowledge, nociceptin levels in cerebrospinal fluid has not previously been compared with healthy controls. The role of nociceptin in fibromyalgia and other widespread chronic pain conditions should be further explored.

Disclosure: L. Tanum: Part 1: Lecturer for Astra Zeneca, Bristol Myers Squibb, H.Lundbeck, Glaxo Smith Kline, Sanofi Aventis, Eli Lilly, Part 2: Total honorarium for all lectures equals approximately 50.000 $ per year, Part 3: No financial involvement, Part 4: One grant in 2010 from the National Research Council in Norway (a governmental institution)., Part 5: Not employed by any commercial company. **M. Vinje:** Part 1: Lecturer for MundiPharma, Pfizer and Eli Lilly, Part 2: Total honorarium is below the equivalent of 10.000$ per year, Part 3: No financial involvement, Part 4: No grants, Part 5: Nor employed by any commercial company. **G. Ordeberg:** None. **F. Nyberg:** None.

68. Naltrexone plus Aripiprazole compared to Naltrexone Alone and Placebo in the Treatment of Alcohol Dependence - A Double Blind Pilot Study

Raymond Anton*, Alicia Baros, Patricia Latham, Pat Randall, Scott Stewart, Derick Vergne, Konstantin Voronin

Medical University of South Carolina, Charleston, USA

Background: Naltrexone, a specific opioid antagonist, is effective for the treatment of alcohol dependence but it is not universally

effective. Since both the brain opioid and dopamine systems may play both independent and complementary roles in the maintenance of addiction, medications that work on both systems, when given together, might show greater efficacy than either one alone. Previous work has shown that the dopamine stabilizer aripiprazole might have efficacy in reducing drinking and craving. This double blind random assignment clinical trial evaluated the efficacy of adding aripiprazole to naltrexone, versus naltrexone alone and matching placebos for the treatment of moderately severe alcohol dependence.

Methods: After signing IRB approved informed consent, 65 medically-stable individuals who met DSM-IV criteria for alcohol dependence and who drank at least 10 drinks/day (men) or 8 drinks/day (women) and who did not meet criteria for other drug dependence (except nicotine), after 4 days of abstinence, were randomized to naltrexone (50 mg/day) alone (n = 14 men, 7 women), naltrexone plus aripiprazole (up to 15 mg/day) (n = 12 men, 9 women) or matching double placebo (n = 16 men, 7 women) for 16 weeks. All received medical management and up to 9 visits (similar to the COMBINE Study procedures) on which they were assessed for daily drinking (TLFB), craving (OCDS), and had %CDT and GGT collected (5 times).

Results: Intent to treat analysis using a mixed linear model found an overall significant effect of medication group by time (study week) for percent days abstinent (p = 0.016) and percent heavy drinking days (p = 0.001) as well as a trend for drinks per drinking day (p = 0.10). There were no significant differences on any of these drinking outcome variables between the placebo and naltrexone-alone group, but there was a significant difference between the combined naltrexone-aripiprazole group and the placebo/naltrexone-alone groups indicating greater percent days abstinent (p = 0.005) and less heavy drinking days (p = 0.009) in subjects treated with the combination of both medications. However, although not significant, there were less 16-week study completers in the naltrexone-aripiprazole group (33%) compared to the naltrexone alone (57%) and placebo group (57%). Medications appeared to be well-tolerated with the number subjects reporting any adverse event being similar in across groups.

Discussion: This pilot exploratory study indicated that adding aripiprazole to naltrexone improved overall drinking outcomes, but at a cost of increased study dropout. Further sensitivity analysis and exploration is needed to evaluate the nature, and relationship, of adverse effects to study dropout and drinking. Subgroups of individuals who might have responded to aripiprazole (such as those with more impulsivity or less side effects) need further exploration. The reason(s) for the lack of difference in the naltrexone-alone and placebo groups is not clear. Genetic analysis of the *OPRM1* gene will be undertaken to evaluate whether this contributed to lack of response. Most likely, the low number of subjects in this pilot study could have led to a type II error. Nevertheless, this preliminary data continues to suggest a role for aripiprazole in the treatment of alcohol dependence, while issues of dosing and/or subgroup response need further clarification.

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69. Associations Between Aspects of Verbal Memory and Brain Structure in Late Life Depression

Melissa Lamar*, Rebecca Charlton, Aifeng Zhang, Anand Kumar

University of Illinois at Chicago, Chicago, USA

Background: Verbal memory deficits attributed to late life depression (LLD) may result from executive dysfunction that is more detrimental to list-learning than story recall. Despite this behavioral dissociation, little work has been done investigating related neuroanatomical dissociations in verbal memory performance.

Methods: We compared list-learning to story-based memory performance in 25 non-demented individuals with LLD (age ~ 66.3 ± 7.8) and 41 non-demented/non-depressed healthy controls (HC; age ~ 67.6 ± 5.3). We correlated results from these analyses with volumes of frontal, temporal and parietal regions associated with verbal memory.

Results: The LLD group showed significantly lower verbal memory performance but only during long-delay free recall on the list-learning task when compared to the HC group [$F(1,63) = 5.9$, $p = .01$], ; story-based memory performance did not differ from HC. Despite equivalent brain volumes across regions, only the LLD group showed a network of associations to verbal memory performance; associations restricted to the list-learning task. Significant positive correlations between long-delay free recall performance and volumetric measures of prefrontal ($r = .46$, $p = .02$), temporal ($r = .42$, $p = .04$) and parietal ($r = .55$, $p < .01$) regions implicated a network of associations involving brain structures important for working memory, subjective organization and mental manipulation as well as encoding and consolidation of to-be-remembered information.

Discussion: This study is the first to demonstrate neuroanatomical dissociations in verbal memory performance in individuals with LLD and provides structural evidence for the executive dysfunction known to be detrimental to list-learning performance in this group. While brain atrophy – particularly hippocampal and temporal lobe degeneration – may not contribute to memory performance in normal aging, it appears to negatively impact long-term memory in the presence of the specific disease-related neuropathology of LLD.

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70. Clinical Assessment Methodology for Alzheimer's Disease Prevention Trials: A Global and Multi-Axial 2 Year Study of Pre-Mild Cognitive Impairment (Pre-MCI) Subjective Cognitive Impairment (SCI)

Barry Reisberg*, Ricardo Osorio, Asif Khan, Kamalika Roy, Carol Torossian, Isabel Monteiro, Salman Anwar, Melanie B. Shulman, Iryna Lobach

Aging and Dementia Clinical Research Center and Alzheimer's Disease Center, New York University Langone Medical Center, New York, USA

Background: There is increasing interest in the development of prevention interventions for Alzheimer's disease(AD). For such studies, there is a need for assessment methodologies for subject characterization and outcome. The Global Deterioration Scale (GDS, Reisberg *et al. Am. J Psychiatry*, 1982) describes three GDS stages prior to dementia in AD. The terminology "mild cognitive impairment" (MCI) was originally coined for GDS stage 3 (Reisberg *et al.*, *Drug Dev. Res.*, 1988). Our 1986 estimate of a 7 year mean duration of the MCI stage in AD (Reisberg, *Geriatrics*, 1986) is consonant with the observed MCI duration reported in

subsequent worldwide studies from clinic populations. The GDS also identifies a pre-MCI stage of subjective cognitive impairment (SCI, GDS stage 2), in which subjective symptoms occur in the absence of objective clinically manifest overt or subtle cognitive deficits. In 1986, we estimated that the SCI stage of eventual AD lasts a mean of 15 years prior to MCI. This temporal estimate, was supported by a subsequent 9-year longitudinal study (Prichep, et al., *Neurobiol Aging*, 2006). Since SCI appears to occur in ~ 25 to 55% of persons aged 65 years and is: (a) a source of concern for these persons, and (b) a precursor of AD dementia, there is a need for adequate assessment of SCI for prevention trials. Herein we report on a 2 year, prospective study of global and multi-axial assessment of SCI symptomatology.

Methods: Subjects from a large, previously reported, 7-year outcome study (Reisberg, et al. *Alzheimer's & Dementia*, 2010), were selected for the present investigation. Selection criteria for the present study included: (1) presence of SCI (GDS stage 2) at baseline; (2) otherwise robust health; and (3) follow-up 1.5 to 3.0 years after baseline. Assessments were performed using the GDS and the Brief Cognitive Rating Scale (BCRS). BCRS axes 1 to 5 (Reisberg & Ferris, *Psychopharmacol Bull*, 1988) assess: (1) concentration and calculation; (2) recent memory; (3) remote memory; (4) orientation; and (5) daily functioning. The BCRS axis scores are enumerated to be optimally concordant with the corresponding GDS stages. Objectives were to examine outcome and stability of SCI after a 2 year interval.

Results: From the prior 7 year outcome study, of 166 SCI subjects, 102 had follow-ups within the selection time-window. Of these, 3 subjects developed possible CNS confounding conditions and were excluded. The 99 studied subjects had a mean age of 68.05 ± 8.9 years, an education level of 15.56 ± 2.5 years, and 62% were women. At baseline, the mean score on the 5 BCRS axes was 1.79 ± 0.33. Hence, as per design, the BCRS axis scores were closely concordant with the GDS stage at baseline. Subjects were followed over a mean of 2.12 ± 0.3 years. The mean GDS score at follow-up, 2.14, was greater than at baseline (p < 0.01) and the change rate of the GDS stage per annum was 6.68%, consistent with a stage lasting 15 years. At follow-up, 8 of 99 subjects (8.08%) reverted to an unimpaired stage score of GDS =1, whereas, > 90% of subjects continued to exhibit subjective and /or overt deficits. Subjects who converted to an unimpaired status at follow-up were younger (p = 0.0001), than those who continued to manifest subjective and/ or overt deficits, however, there were no baseline differences in education or gender. The mean BCRS score at baseline for those subjects who reverted to no cognitive impairment at follow-up was lower, i.e., 1.51 ± 0.25, than the mean baseline scores of participants who manifested subjective and/or objective deficits at follow-up, i.e., 1.81 ± 0.32 (p = 0.001). Similarly, the mean BCRS scores at the 2 year follow-up for subjects free of deficits (i.e., GDS = 1), was 1.11 ± 0.20. This was lower than that of subjects manifesting subjective and/or overt deficits at follow-up, i.e., 1.95 ± 0.40 (p < 0.001).

Discussion: Prior studies had indicated that older persons with subjective cognitive deficits are more likely than those without these deficits to progress in subsequent years to MCI or dementia. The present study indicates that clinical measures previously applied for pivotal worldwide studies of dementia medications (specifically, the GDS and, for certain analyses, the BCRS, used in pivotal trials for rivastigmine and memantine), can demonstrate statistically significant differences in severity after a 2 year period in an SCI cohort. Therefore, prevention trials over a 2 year period, in pre-MCI, SCI subjects, with conventional clinical assessments appear to be feasible at the current time utilizing promising pharmacological interventions.

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71. The Potent M₁ Receptor Allosteric Agonist GSK1034702 improves Episodic Memory in the Nicotine Abstinence Model of Cognitive Dysfunction in Humans

Pradeep J. Nathan*, Jeannette Watson, Jesper Lund, Gary L. Peters, Chris M. Dodds, Philip Lawrence, Graham D. Bentley, Barry V. O'Neill, Jonathan Robertson, Paul Maruff, Marc Laruelle, Edward T. Bullmore

Medicines Discovery and Development, GlaxoSmithKline Clinical Unit, Cambridge, United Kingdom

Background: Hippocampal dependent episodic memory deficits are a core feature of neurodegenerative disorders. Muscarinic M₁ receptors play a critical role in modulating learning and memory and are highly expressed in the hippocampus. GSK1034702 is a potent allosteric agonist at M₁ receptors. We examined for the first time, the effect of GSK1034702 on cognitive function, and in particular episodic memory, in healthy smokers using the nicotine abstinence model of cognitive dysfunction.

Methods: The study utilized a double blind, randomised, placebo controlled, cross-over design in which 20 otherwise healthy male nicotine abstinent smokers were tested following 3 acute treatment conditions(placebo, 4 and 8 mg of GSK1034702). Treatment conditions were separated by a minimum 1 week wash-out period. Cognitive function was assessed using the Cogstate neuropsychological tests of episodic memory, attention, working memory, and executive function.

Results: Compared to the baseline (nicotine on-state), 12 hours of nicotine abstinence significantly reduced immediate (95% CI, -1.13, -.0.11; p = 0.019) and delayed recall (95% CI, -1.14, -.0.11; p = 0.02). None of the other cognitive tasks were modulated by nicotine abstinence. GSK1034702 (8 mg) significantly attenuated (i.e. improved) the nicotinine abstinence induced impairments in immediate recall but not delayed recall (95% CI, 0.16, 1.38; p = 0.014). GSK1034702 had no effects on baseline cognition function.

Discussion: These findings suggest that the M₁ receptor allosteric agonistGSK1034702 improves episodic memory, in particular, the acquisition of new information (i.e. encoding). The findings are consistent with the role of the cholinergic muscarinic receptor system in memory encoding/acquisition and provide evidence that M₁ receptor agonists may have therapeutic benefits for disorders associated with impaired learning.

Disclosure: P. Nathan : Part 1: I am a employee of GlaxoSmithKline Pharmaceuticals, Part 2: I am a employee of GlaxoSmithKline Pharmaceuticals and hold shares in the company., Part 3: I am a employee of GlaxoSmithKline Pharmaceuticals, Part 4: I am a

employee of GlaxoSmithKline Pharmaceuticals and my research is funded by GlaxoSmithKline., Part 5: I am a employee of GlaxoSmithKline Pharmaceuticals. I also work for the University of Cambridge. **J. Watson:** Part 5: At the time of this work I was employed by GSK Pharmaceuticals. **J. Lund:** Part 1: I have been a full time employee of the following biotek and pharmaceutical companies during that period GlaxoSmithKline 2009-2010 Neurosearch A/S 2010-2011 LEO Pharma 2011-present, Part 2: None except my salary from the above positions, Part 3: None None except my salary from the above positions, Part 4: none, Part 5: I have been a full time employee of the following biotek pharmaceutical companies during that period GlaxoSmithKline 2009-2010 Neurosearch A/S 2010-2011 LEO Pharma 2011-present. **G. Peters:** Parts 1-5: I work for GlaxoSmithKline Pharmaceuticals and hold shares in the company. **C. Dodds:** Parts 1-3, 5: I work for Glaxosmithkline. **P. Lawrence:** Parts 1-3, 5: I work for GlaxoSmith-Kline Pharmaceuticals and hold shares in the company, Part 4: I work for GlaxoSmithKline Pharmaceuticals and have obtained grants from GlaxoSmithKline for my reserach. **G. Bentley:** Part 5: GlaxoSmithKline. **B. O′Neill:** Parts 1-3, 5: I work for GlaxoSmith-Kline Pharmaceuticals and hold shares in the company., Part 4: I work for GlaxoSmithKline Pharmaceuticals and hold shares in the company. I have received funding from GlaxoSmithKline for my research. **J. Robertson:** Part 5: GlaxoSmithKline. **P. Maruff:** Part 1: I am a full time employee of CogState Ltd, Part 2: I have shares in CogState ltd, Part 5: CogState Ltd. **M. Laruelle:** Part 1: None, Part 2: None, Part 3: None, Part 4: Marc Laruelle has recieved grants and currently have grants from pharmacetuical companies, the University and granting bodies., Part 5: No. **E. Bullmore:** Part 1: GlaxoSmithKline - half-time employee and share holder Brain Resource Company - share holder, Part 2: GlaxoSmithKline - half-time employee and share holder University of Cambridge - half-time employee, Part 3: GlaxoSmithKline - half-time employee and share holder, Part 4: Not applicable, Part 5: I wourk half-time for GlaxoSmithKline and half-time for the University of Cambridge.

72. Maternal Pre-Pregnancy Body Mass Index is Associated with ADHD Symptoms and Impaired Inhibitory Control in 6-9 Year Old Children

Sonja Entringer*, Claudia Buss, Elysia P. Davis, Pathik D. Wadhwa, Curt A. Sandman

University of California Irvine, Orange, USA

Background: Attention deficit hyperactivity disorder (ADHD), the most commonly-diagnosed neurobehavioral disorder of childhood, is a complex condition that results from the interplay of genetic and environmental risk factors. A growing body of evidence suggests that exposure to adverse intrauterine conditions may increase the risk to develop ADHD later in life. In several independent pregnancy cohorts, maternal pre-pregnancy obesity – a condition that is associated with an increased inflammatory milieu during pregnancy and that may therefore cause fetal neurodevelopmental disruption - was associated with ADHD symptoms. So far, no study has characterized alterations in neurocognitive function that may underlie ADHD symptoms in the offspring in association with maternal pre-pregnancy body mass index (BMI). Thus, the aim of the present study was to test the hypothesis that the association between pre-pregnancy BMI and ADHD symptoms is mediated through alterations in child inhibitory control, one of the key neuropsychological deficits manifested in ADHD.
Methods: A prospective longitudinal pregnancy-offspring cohort (N = 174 mother - child pairs) was followed-up when the children were 6-9 years (mean ± SD: 7.3 ± 0.9) of age. Pre-pregnancy BMI was abstracted from the medical chart. ADHD symptoms

were assessed by maternal report of behavioral and emotional problems with the Child Behavior Checklist (CBCL). A continuous performance task (Go/ No-go Task) was administered to assess child inhibitory control. All analyses were adjusted for the following potentially confounding variables: pregnancy character-istics and birth outcomes (obstetric risk, length of gestation and birth weight percentiles), child characteristics (child BMI percen-tile, sex and age at assessment) and maternal characteristics (maternal race/ethnicity, maternal intelligence scores, years of school completed, maternal depression at child follow-up).
Results: A higher pre-pregnancy BMI was significantly associated with a higher number of ADHD symptoms ($F(2, 158) = 3.99$, $p = 0.02$) and with impaired performance on the Go/ No-go Task ($F(2, 157) = 3.67$, $p = 0.02$) of the children after controlling for potential confounding variables. Furthermore, a mediation model revealed that the association between higher pre-pregnancy BMI and higher number of ADHD symptoms was mediated by impaired inhibitory control (inefficient/ less attentive processing, Sobel Test: $t = 2.03$, $p < 0.05$).
Discussion: To the best of our knowledge this is the first study that showed alterations in neurocognitive function that may underlie ADHD symptoms in the offspring of mothers who were obese prior to pregnancy. The results of this study add further evidence to the growing awareness that pre-disease pathways for neurodevelop-mental disorders may have their foundations very early in life.
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73. Influence of COMT *val158met* on Resting State Functional Connectivity over Adolescence

Aarthi Padmanabhan*, Kai Hwang , David Montez, Beatriz Luna

Laboratory of Neurocognitive Development, University of Pittsburgh Medical Center, Pittsburgh, USA

Background: Accumulating evidence has lead to the predominant view that the majority of neuropsychiatric disorders are develop-mental in nature. This understanding has shifted the focus of neuroscience research to the neurodevelopmental basis of psychiatric disorders in order to develop early intervention plans and treatments. An emphasis has been given to studying adolescence, a period when risk-taking and vulnerability for major psychopathology peaks. Human and animal studies suggest that brain systems such as the striatum and prefrontal cortex (PFC) continue to develop over adolescence. Specifically, there are behaviorally relevant changes in functional and structural connectivity between the prefrontal cortex and striatum from childhood to adulthood. Frontostriatal connectivity is believed to support cognition and motivation through inhibitory control of striatum by PFC neurons and reciprocal striatal efferents to PFC. Immaturities in connectivity patterns, may contribute to suscept-ibility for the emergence of psychopathology. Frontostriatal systems are strongly modulated by dopamine (DA) neurotrans-mission. DA function is involved in the pathogenesis of neuropsychiatric disorders with a developmental basis such as schizophrenia, mood disorders and drug abuse disorders . Animal studies indicate that DA availability peaks during the adolescent period. Catechol-O-methyltransferase (COMT), an enzyme influ-encing DA turnover in the PFC, modulates frontostriatal circuit function through a single nucleotide polymorphism (SNP) resulting in a methionine (*met*) to valine (*val*) substitution in its coding gene. The *val* allele results in low synaptic dopamine levels, and is associated with poorer executive function. The *met* allele, which results in high synaptic dopamine, is associated with relatively better cognitive processing. Due to overall increases in DA transmission in adolescence, there may be a distinct influence of COMT in adolescence on DA related functioning compared

to adulthood. This study investigates the modulation of resting state functional connectivity by the COMT val158met SNP by age interactions in DA related circuitry. We are interested in resting state functional connectivity because variability in DA processing may influence intrinsic connectivity between frontal and striatal systems, influencing behavior and vulnerability for psychopathology.

Methods: Data were acquired at the University of Pittsburgh Medical Center with a Siemens 3-T Tim Trio (Erlangen, Germany). Functional images were acquired using an echo-planar sequence sensitive to blood oxygen level dependent (BOLD) contrast [T2*]. 98 participants were recruited (aged 10-19, 22 met/met, 43 val/met, 30 val/val). Participants were asked to close their eyes, but not fall asleep. Physiological parameters were monitored and recorded throughout the scanning session. Whole brain time-series correlation analyses were run with seed regions in the left and right ventral striatum (nucleus accumbens) that were anatomically defined. Individual participant correlation maps were entered into a random effects model coding genotype as a function of number of *val* alleles (0, 1, 2) and age and the inverse of age (age^{-1}) as continuous variables.

Results: An age^{-1} effect was found in medial and inferior PFC, ACC, and thalamus demonstrating an initial steep increase in connectivity strength from childhood to mid-adolescence and a subsequent flattening in late adolescence. An age^{-1} by COMT genotype interaction was noted in several regions including midbrain, thalamus, medial and lateral PFC, hippocampus and parahippocampal gyrus and anterior insula. These results indicated that individuals homozygous for the met allele demonstrate a steep increase in connectivity strength, flattening by mid adolescence. Conversely, val/met heterozygotes and val/val homozygotes demonstrate the opposite, overall showing a decline in connectivity, with the sharpest decrease between childhood and mid-adolescence.

Discussion: Overall, genotype by age interactions suggest that the developmental trajectory of DA related function is non-linear, and that a genetically-mediated brain phenotype characterized in adolescence may differ significantly from that in adulthood and childhood. Understanding developmental changes in the effects of DA genetic variability can inform basic processes underlying vulnerabilities to psychopathology. This has strong implications regarding the variability observed in vulnerability to psychopathology and in adolescent risk taking behavior.

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74. Impulsivity in Bipolar Disorder: Relationships with Neurocognitive Dysfunction and Substance Use History

Katherine Burdick*, Robyn Powers, Serge Sevy, Anil Malhotra

Mount Sinai School of Medicine, New York, USA

Background: Impulsivity, defined as a predisposition toward rapid, unplanned actions without regard to negative consequences, is also a stable personality trait. Several psychiatric disorders are characterized by impulsiveness, including ADHD, substance use disorders, and bipolar disorder (BD). During acute mania, BD patients demonstrate an increase in impulsive behaviors such as uncontrolled spending, promiscuity, and suicide attempts. Although mood symptoms exacerbate impulsivity in BD, data suggest that self-reports of impulsivity are elevated even during euthymia, which implies a trait-like characteristic. Further, neurocognitive processes linked to impulsivity (e.g. attention, inhibition) are also impaired in BD patients across all phases of the illness, including affective remission.The high frequency of comorbidities associated with impulsivity, including impulse control disorders and substance abuse/dependence in BD further highlight the clinical relevance of this dimension of the illness; however, few studies have focused on the potential association between trait impulsivity and neurocognitive performance in patients with BD.

Methods: We evaluated the nature of impulsivity in ninety-eight BD patients and its relationship with symptoms, cognitive dysfunction, and history of substance use disorders. All subjects were diagnosed with the SCID; mood ratings were conducted at the time of testing. We assessed self reports of trait-impulsivity (Barrett Impulsiveness Scale: BIS) and impulsive behaviors on the Iowa Gambling Task (IGT). A comprehensive neurocognitive battery was also completed. Ninety-five demographically-matched healthy controls (HC) were included. BD patients were compared with HC participants to evaluate differences in impulsivity and neurocognition using ANOVA. We tested for correlations among BIS scores, IGT performance, and neurocognition in BD. Because of data indicating a significant influence of substance abuse history on both impulsivity and neurocognition, alongside the very high rates of substance use disorders in BD, we carried out duplicate analyses dividing the bipolar sample based on prior history of substance abuse/dependence to evaluate its effect on these relationships.

Results: The BD patients were 40.4 +/-12.1 years old; 53% were female; and they had an estimated premorbid IQ of 98.9 +/-10.6. The HCs were well-matched demographically. Results from the ANCOVA revealed highly significant group differences on trait-impulsivity. BD patients had higher BIS Total scores (Mean = 67.2 +/- 1.3) vs. HCs [Mean = 54.0 +/- 1.0; (F = 69.1; p = 1.7 x 10^{-17})] and on all 3 subscales: Attention (F = 61.9; p = 2.6 x 10^{-13}; Motor (F = 39.7; p = 2.0 x 10^{-9}; Non-planning (F = 40.5; p = 1.4 x 10^{-9}). In addition, IGT performance was significantly impaired in the BD patients, with a main effect of Group (F = 4.6; p = 0.036); Block (F = 6.1; p < 0.001); and a Group x Block interaction effect (F = 4.0; p = 0.004). Specifically, when assessing card-by-card strategy [(expectancy-valence (EV) model)], BD patients showed a tendency toward less consistent, more erratic choices (Reliability index; F = 8.7; p = 0.004) and the symptomatic BD patients allocated significantly more attention to losses vs. gains on the IGT than did the euthymic BD patients (F = 7.4; p = 0.009). Correlations revealed that depression was positively correlated with trait-impulsivity (BIS Total: r = 0.29, p = 0.004; Attention: r = 0.35, p = 0.001; and Motor: r = 0.21; p = 0.04) and with an increased tendency to attend more readily to losses vs. gains on the IGT (r = 0.28, p = 0.04). There was no significant relationship between mania ratings (CARS-M) and trait-impulsivity or any index on the IGT. Although we found no significant correlations between BIS scores and neurocognitive performance in the full BD sample, when sub-grouped based on substance abuse histories, significant relationships among higher BIS scores and impaired performance on several cognitive tests were revealed in BD subjects *without* substance abuse histories but not in BD subjects *with* substance abuse histories.

Discussion: Our data support prior findings of increased trait-impulsivity and impairment on behavioral tasks of impulsiveness in BD, even during euthymia. These results furthersuggest that depressive symptoms but not manic symptoms appear to influence trait-impulsivity and behavioral strategies employed during an emotional decision-making task in BD. Finally, we note a differential relationship between impulsiveness and neurocognitive functioning in BD that is dependent upon history of substance abuse.

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75. Motivational Saliency Signal in Ventral Striatum is Modulated by Genetic Variation in the ARC Gene Region

Caroline F. Zink*, Sam A. Colalillo, David N. Blitzer, M. Ryan Haynes, Kuan H. Wang, Daniel R. Weinberger

National Institute of Mental Health, NIH, Bethesda, USA

Background: In humans, reward anticipation has been shown to reliably and robustly evoke activation in the ventral striatum (vSTR). Using fMRI, we have previously demonstrated that activation in the vSTR during reward anticipation corresponds to motivational saliency, as cues associated with greater motivational value, rather than reward value *per se*, evoke greater vSTR signal than cues associated with less motivational value. Based on animal research, such a signal in the vSTR is likely generated by dopamine neurotransmission and/or dopamine modulation of glutamatergic neurotransmission; however, this information cannot be ascertained using traditional fMRI techniques in humans. In the current study, we used "imaging genetics" to investigate the influence of dopaminergic and glutamatergic related genetic variations on the motivational saliency signal in the vSTR.

Methods: During fMRI (3T scanner), 80 healthy participants performed an adapted Monetary Incentive Delay task, in which reward cues informed subjects of upcoming target difficulty in order to manipulate motivation. On each trial, subjects responded, via single button press, to a visually presented target. On reward trials, each successful response resulted in the receipt of $2 (represented visually). Just prior to target appearance, participants were shown one of two cues predicting potential reward: one associated with relatively higher motivational value (a shorter response window) and one associated with relatively lower motivational value (a longer response window). A control cue was not predictive of a reward or difficulty. The imaging data were subjected to an event-related, random-effects analysis, with a particular interest focused on neural activity associated cue presentation. Individual first level contrasts were created for the main effect of motivational value (high motivational value cue > low motivational value cue) and were then entered into a second-level regression analysis to assess the influence of selected genetic variations in dopaminergic and glutamatergic system genes on related neural activity. Gender, age, and IQ were entered into the model as regressors of no-interest to remove these potential confounds. Statistical maps were thresholded at $p < 0.05$, corrected for multiple comparisons across voxels.

Results: Participants responded significantly faster to the cues associated with higher motivational value compared to lower motivational value and no motivational value (non-reward predicting control cues). In concordance with previous investigations, high motivational value cues elicited significantly greater activation in the vSTR compared to cues associated with low motivational value. Using a regression analysis, we found that a single nucleotide polymorphism (SNP) downstream, yet in close proximity, to the ARC gene (rs9324593), predicted the vSTR response to motivational salience, with the G allele being associated with significantly greater vSTR BOLD signal.

Discussion: ARC protein expression is activity dependent and is involved in the endocytosis of AMPA receptors. While the effect of the rs9324593 SNP on ARC protein is still under investigation, these results highlight a genetic variation with potential functionality in the glutamatergic system that may account for individual differences in the neural coding of motivational value. It is striking that of the genetic variations studied here, a genetic variation related to the glutamatergic system, rather than the highly hypothesized dopaminergic system, accounted for the most variability in vSTR reactivity to motivational value. These findings shed light on the genetic influence on neural activity underlying the evaluation of motivationally valuable stimuli, which is highly relevant to the elucidation of the neurobiology of mental illnesses that involve deficits in the proper evaluation of motivationally important stimuli, including schizophrenia and drug addiction.

76. Sensory and Motor Contributions to Visuomotor Impairments in Individuals with Autism

Matthew Mosconi*, Suman Mohanty, Lauren Schmitt, Edwin Cook, David Vaillancourt, John Sweeney

University of Texas Southwestern Medical Center, Departments of Psychiatry and Pediatrics, Dallas, USA

Background: Sensorimotor disturbances are present in the majority of individuals with autism. It remains unclear whether these deficits reflect impaired processing of sensory feedback for action planning, or fundamental deficits in motor control.

Methods: Twenty-six individuals with autism and 26 age- and IQ-matched healthy controls performedsustained precision grip force tasks in which the amplitude of the required target force (motor manipulation) and the precision of visual feedback (sensory manipulation) each were varied. They viewed a white bar that moved upwards with increased grip force toward a fixed green target bar. Subjects were instructed to sustain a constant force in order to stabilize the white bar at the level of the green bar. During the motor manipulation, the green target bar was set to 5, 25, 45, 65 or 85% of individual subjects' maximum force contraction. During the sensory manipulation, the vertical distance the white bar moved per Newton of force applied was set to visual angles of .02, .06, .19, .62, 2.02, 6.66 and 21.13 deg. When the visual angle was small, the white bar moved a smaller distance for every Newton of force applied, increasing the information fidelity of sensory feedback. All trials were 15 sec in duration and were followed by 15 sec of rest. Subjects completed dominant and non-dominant hand testing separately.

Results: The mean force did not differ between subjects with autism compared to controls. However, subjects with autism showed reduced control of their motor output as demonstrated by greater force variability during the trial. This impairment was more robust at greater force amplitudes, especially for the non-dominant hand. Changes in the precision of sensory feedback did not affect force control impairments in individuals with autism; force variability was increased to similar degrees across visual angles in individuals with autism compared to controls. Increased variability of sustained force was associated with clinical ratings of communication impairment and motor stereotypies in individuals with autism.

Discussion: We examined whether deficits in visuomotor control in autism result from disruptions to sensory feedback processing and/or feedforward motor systems. Our results provide clear evidence that visuomotor impairments are due to deficits in producing motor output, and this deficit appears to be independent of the quality of sensory feedback. Further, motor impairments appear to be related to altered development of language skills and stereotypies, indicating a relationship with core clinical features of the disorder. This pattern of motor deficit implicates dysfunction of lobules V-VI and Crus I/II of the cerebellum which generate efferent motor commands to multiple cortical regions, including motor, premotor, and parietal cortices. Our finding that changes in the quality of visual feedback do not affect the degree of motor control impairment in autism suggests that motor cortices which scale their activity according to the precision of visual feedback may be relatively spared. Combined with findings from multiple postmortem studies of autism documenting reduced Purkinje cell size and density, these results suggest that cerebellar abnormalities may underlie the dyspraxia and poor fine motor

control that are present in the majority of individuals with this disorder.

77. Evidence of Neurodevelopment Differences in Prodromal and First Episode Psychosis Subjects in the Prepulse Inhibition Paradigm

Kristin Cadenhead*, Jean Addington, Tyrone Cannon, Barbara Cornblatt, Thomas McGlashan, Diana Perkins, Larry Seidman, Ming Tsuang, Elaine Walker, Scott Woods, Robert Heinssen

UCSD, La Jolla, USA

Background: Schizophrenia and other psychotic disorders are neurodevelopmental disorders that fully emerge in late adolescence or early adulthood. A greater understanding of neurodevelopmental abnormalities can lead to early intervention and prevention of the functional disabilities that are a core feature of the psychotic disorders. Prepulse inhibition (PPI) of the startle response is a biobehavioral marker used in the study of brain disorders such as schizophrenia characterized by abnormalities of sensory gating. Typically PPI is fully mature by early adolescence and can also be studied in developmental animal models. Studies of schizophrenia patients demonstrate deficits in PPI when compared to healthy subjects.

Methods: Age effects on PPI were assessed in two cohorts of early psychosis subjects from the Cognitive Assessment and Risk Evaluation (CARE) Program at UCSD (85 Normal Comparison [NC], 85 At Risk [AR] and 75 First Episode [FE] subjects) and the North American Prodromal Longitudinal Studies (NAPLS) Consortium (108 NC, 160 AR) using ANOVA, correlational and age regression analyses. The paradigm included 115 dB acoustic startle stimuli and 86 dB prepulse stimuli against a 70 dB background with 3 prepulse interstimulus intervals (ISIs - 30, 60 and 120 ms).

Results: Significant age effects on PPI were observed in both samples (CARE $F_{[1,233]} = 11.06$, $p < 0.001$, NAPLS $F_{[1,265]} = 3.8$, $p < 0.05$). Significant Age X PPI correlations were observed in the 30 and 60 ms ISI conditions in both samples across all subjects (CARE $r = 0.24$-0.24, $p < 0.05$; NAPLS 0.13-0.17, $p < 0.05$) but these correlations were driven by the AR (CARE $r = 0.28$-0.39, $p < 0.05$; NAPLS $r = 0.25$-0.27, $p < 0.05$) and FE (CARE $r = 0.22$-0.28, $p < 0.05$) samples and not the NC's (CARE $r = 0.13$-0.15, ns; NAPLS -0.01-0.04, ns). In addition, there were significant group differences in the age regression analysis within the NAPLS sample ($t = 2.0$, $p < 0.05$, $\beta = 0.51$).

Discussion: In cross-sectional analyses of two large cohorts, there are significant age effects on PPI that are present within the early psychosis subjects but not healthy subjects. Further longitudinal study of the development of PPI in psychotic disorders and related animal models may lead to important insights into neuropathological changes and the potential for pre-emptive intervention early in the course of illness.

78. Superior Temporal Gyrus and Frontal 50 and 100 ms Auditory Abnormalities in Schizophrenia

Yu-Han Chen*, J. Christopher Edgar, Mingxiong Huang, Michael A. Hunter, Amanda Olson, Emerson M. Epstein , Jose M. Cañive

University of New Mexico, Albuquerque, USA

Background: Electroencephalography (EEG) and magnetoencephalography (MEG) studies show smaller 100 ms auditory amplitudes in individuals with schizophrenia than controls (Boutros *et al.*, 2004; Clementz *et al.*, 2003; Turetsky *et al.*, 2009). Although MEG source localization studies show superior temporal gyrus (STG) auditory processing abnormalities in schizophrenia at 100 ms (Edgar *et al.*, 2008; Smith *et al.*, 2010), EEG, corticography, and fMRI studies also suggest involvement of other areas during the early ∼50 and ∼100 ms interval (e.g., Korzyukov *et al.*, 2007; Weiland *et al.*, 2008). To investigate cortical brain area involvement in early auditory encoding processes, distributed source localization examined first-click (S1) activity in the paired-clicks paradigm. Study goals were (1) to examine activation in STG and non-STG brain regions from 50 to 100 ms, and (2) to determine schizophrenia (SZ) and healthy control (HC) group differences in 50 and 100 ms activity throughout the cortex.

Methods: The standard paired-click task was administered to 19 SZ and 21 HC subjects. MEG data was obtained using a 306-channel Vector-View system. T1-weighted structural MRI for each subject was obtained for magnetic source analysis. Vector-based Spatial-temporal Analysis using L1-minimum-norm (VESTAL; Huang *et al.*, 2006) provided 3D maps of S1 activity for 50 and 100 ms. Group statistics on the VESTAL 3D maps were performed using a cluster-based statistic test implemented in FSL (Threshold-Free Cluster Enhancement; Smith and Nichols, 2009). Within-group one-sample t-tests compared post-stimulus 50 ms (M50) and 100 ms (M100) activity to baseline. Between-group t-tests examined M50 and M100 post-stimulus group differences.

Results: As expected, bilateral M50 and M100 STG activity was observed in both groups. In addition, inferior frontal gyrus (IFG)/ frontal pole (FP) activity was also observed in both groups during the 50 ms interval. During the 100 ms interval, IFG/FP activity was observed in HC but not SZ. Activation was not observed in other cortical brain areas during the 50 to 100 ms analysis period. With regard to group differences, HC had stronger left STG M50 and M100 activity than SZ. In addition, whereas HC had stronger right IFG/FP activity than SZ, individuals with SZ had stronger right superior frontal gyrus (SFG) activity than HC.

Discussion: Replicating our prior STG findings (Smith *et al.*, 2010), less 100 ms S1 STG activity was observed in SZ than HC, again indicating problems encoding auditory stimuli in SZ. Present findings indicated that early auditory encoding abnormalities are not specific to STG primary/secondary auditory cortex, as group differences in frontal areas were also observed. Interestingly, as in Weiland *et al.*, (2008), frontal activity in many subjects was concurrent with, or even preceded, STG M50 and M100 activation. Thus, present findings suggest that STG auditory abnormalities are not the first node in a cortical auditory processing network, but instead indicate that there are abnormalities in multiple nodes of a

concurrently activated auditory network. Studies examining functional connectivity between STG and frontal areas are of interest.

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79. Contributions of Social Anhedonia and Social Anxiety to Impaired Social Adjustment in the Putative Schizophrenia Prodrome

Victoria Cressman, Scott A. Schobel, Sara Steinfeld, Judy L. Thompson, Shelly Ben-David, Alex Crumbley, Scott A. Small, Holly Moore, Cheryl M. Corcoran*

Dept. of Psychiatry, Columbia University College of Physicians and Surgeons, New York, USA

Background: Impairment in social function is characteristic of schizophrenia, accounting for much of its morbidity, yet largely refractory to drug treatment. Social impairment is also prevalent in subjects at clinical high risk (CHR) for psychosis, prior to an index episode of psychosis. However, the core component processes underlying poor social adjustment in schizophrenia and its risk states remain unknown. In this study, we hypothesized that social anxiety and social anhedonia may contribute to poor social adjustment, and we explored the neural correlates of these processes in a CHR cohort.

Methods: Subjects were part of a longitudinal study of the schizophrenia prodrome. CHR cases (N = 37) were ascertained using the Structured Interview for Prodromal Syndromes. Age- and sex- similar healthy controls (CON; N = 22) were recruited from the same source community. Social anhedonia, social anxiety, and social adjustment were measured, respectively, with the Chapman Revised Physical and Social Anhedonia Scales (c.f. Chapman et al 1994), the Social Anxiety Scale for Adolescents (SAS-A) (La Greca and Lopez 1998), and the Social Adjustment Scale Self-Report Scale (SAS-SR) (Weissman and Bothwell 1976). ANOVAs controlling for age and sex were used to compare CHR and control subjects on outcomes of these scales. Partial correlations and linear regression models were used to assess social anhedonia and social anxiety as predictors of social adjustment. In addition, for a subgroup of patients who had undergone high spatial-resolution functional magnetic resonance imaging (Schobel et al 2009) within 1 year of psychological testing, we assessed correlations between the psychological measures and basal metabolism, as indicated by average cerebral blood volume (CBV), in forebrain regions known to mediate aspects of motivation or anxiety. Regions included striatal subregions, the amygdala and related basal forebrain, and the orbitofrontal and subcallosal cingulate cortices.

Results: CHR patients showed significantly worse social adjustment than controls (SAS-SR Total Scores [mean ± S.E.M.]: 2.4 ± 0.11 vs. 1.4 ± 0.11), as well as significantly higher levels of social anhedonia (CHR: 16.6 ± 1.5, CON: 8.0 ± 1.6) and social anxiety (CHR: 52.6 ± 2.5, CON 39.0 ± 2.8), exhibiting levels comparable to those reported in schizophrenia patients. Social impairment was significantly associated with both social anhedonia and social anxiety (Partial correlation r's > 0.4, p < 0.05), with social anhedonia being the more robust predictor across different regression models controlling for age, gender and physical anhedonia. In the subset of patients for whom basal brain metabolic activity (CBV) data were available, social anhedonia and social anxiety appeared to have dissociable correlations with basal metabolism in selected striatal and amygdala-related basal forebrain regions (as shown by uncontrolled Pearson's correlations). Regression models including the extended amygdala, alone or combined with nucleus accumbens or dorsal amygdala, significantly predicted social anhedonia. Social impairment did not correlate with basal metabolism in any of the brain regions examined.

Discussion: Social impairment is characteristic of schizophrenia and its risk states. Here we provide evidence that social anhedonia and social anxiety, as measured with psychological scales, are elevated to clinically significant levels in CHR subjects and may independently contribute to social impairment in these patients. Moreover, these psychological characteristics were linked to metabolic activity within the striatal complex and extended amygdala. While further work is needed to characterize the neurocognitive processes mediating social anhedonia and social anxiety, the current study provides a rationale for targeting these processes in intervention for social impairment in the schizophrenia prodrome.

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*Holly Moore and Cheryl Corcoran contributed equally as principal investigators

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80. Functional Activation during Probabilistic Reinforcement Learning in Schizophrenia: Relationship to Anhedonia/Avolition

Erin C. Dowd*, Deanna M. Barch

Washington University, St. Louis, USA

Background: Motivational impairments are critical features of schizophrenia that significantly impact functional capacity and are resistant to treatment. Here, we use a reinforcement learning task to examine whether impairments in using rewarding outcomes to guide future choices may contribute to andhedonia/avolition in schizophrenia.

Methods: 20 stable, medicated outpatients with schizophrenia and 29 healthy controls underwent functional magnetic resonance imaging (fMRI) during a probabilistic stimulus selection reinforcement learning task. In this task, three stimulus pairs with different probabilistic reinforcement ratios were presented, and participants learned by trial-and-error to choose the more frequently reinforced member of each pair. Correct responses earned monetary rewards. Patients, but not controls and siblings, completed a full-length practice during a prior session, and all subjects completed a practice block before entering the scanner. During the scan session, participants completed 6 blocks of 60 learning trials, followed by a test phase outside the scanner in which the pairs were recombined and no feedback was given.

Results: Behaviorally, accuracy was examined with reinforcement ratio X block X group ANOVAs, which revealed significant main effects of ratio (p < .001) and block (p < .001), reflecting better performance for higher reinforcement ratios and an improvement in performance over time. There were no main effects or interactions with group. On the test phase, patients performed more poorly than controls the AB pair (p < .05), and on transfer measures of negative RL (frequency of avoiding the least reinforced stimulus; p < .03). fMRI data was examined separately at the time of choice and at the time of feedback. Effects of learning phase were examined using an accuracy regressor, coding the mean accuracy for each stimulus pair

during each 12-trial block, to determine the effect of accuracy on activation. At the time of choice, whole-brain analyses revealed an effect of learning phase in bilateral dorsolateral prefrontal cortex, anterior insula, and posterior parietal cortex, wherein these regions activated more strongly early than late in learning in both groups. At the time of feedback, striatal region of interest analyses revealed a choice X group interaction in the accuracy effect in bilateral caudate. In both groups, responses to feedback in these regions were driven by feedback type early in learning, with greater responses to positive than negative feedback regardless of choice type. Later in learning, controls showed responses that were modulated by choice as well as by outcome. Patients failed to show this modulation, and continued responding primarily to feedback type even during the later stages of learning. Voxelwise correlations with Chapman Physical Anhedonia scores revealed a negative relationship with activity at the time of correct vs. incorrect choices in left caudate. The same region also showed a negative relationship between physical anhedonia and activity in response to unexpected positive feedback.

Discussion: These resulst suggest that controls show a development of expectancy in striatal activation over the course of learning that is impaired in patients. Further, in patients, higher anhedonia is associated with reduced striatal reward anticipation, as well as with reduced striatal responses to unexpected reward.

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81. *Toxoplasma gondii* Exposure affects Neural Processing Speed as Measured by Acoustic Startle Latency in Schizophrenia and Controls

Brad Pearce, Sydney Hubbard, Hilda N. Rivera, Patricia P. Wilkins, Marylynn C. Fisch, Wendy Hasenkamp, Robin Gross, Nancy Bliwise, Jeffrey L. Jones, Erica Duncan*

Emory University, Atlanta, USA

Background: In the United States, approximately 15% of the population has serological evidence of infection with the intracellular parasite, *Toxoplasma gondii* (TOXO). The parasite is neuroinvasive, and the majority of people infected are expected to harbor TOXO cysts in their brains. However, this persistent infection does not typically cause overt neurological symptoms in persons with normal immune systems. The evidence for an association between TOXO seroprevalence and schizophrenia (SCZ) has accumulated over several decades. A recent meta-analysis that included 23 studies demonstrated a highly significant (p value < 0.000001) elevation in the rate of TOXO seropositivity in SCZ patients compared to controls (Torrey *et al.*, 2007). The effect size (odds ratio, 2.73) is greater than that for most gene effects in SCZ, including recent meta-analyses and genome-wide association studies. In rodents TOXO infection results in robust neurochemical and behavioral changes, and the TOXO cysts have a particular affinity for brain areas implicated in SCZ: nucleus accumbens and amygdala. Animal models have also demonstrated that TOXO infection alters levels of dopamine and modulates glutamatergic neurotransmission; both of these neurotransmitter systems are strongly implicated in the pathophysiology of SCZ. In humans TOXO infection has been suggested to be linked to slowed reaction times and an increased rate of motor vehicle accidents. Furthermore, slowed processing speed has been consistently demonstrated in SCZ. The acoustic startle reflex and its modulations have been extensively studied in SCZ and in animal models of psychiatric disease. Startle paradigms allow for the measurement of startle latency, the time required for the startle reflex to occur after the presentation of the startling stimulus. Startle is mediated by a well-characterized three-synapse subcortical neural circuit: as such it provides a measure of neural processing speed. Longer latency of the startle reflex has been found in SCZ. In light of the

above findings, we hypothesized that startle latency would be prolonged in subjects with TOXO exposure.

Methods: We assayed concentrations of IgG antibodies to TOXO to determine seropositivity and serointensity in 167 SCZ subjects and 114 healthy controls (CONT) in order to identify the TOXO status of our subjects. Of this larger set, 81 SCZ subjects and 54 CONT were tested with an acoustic startle session designed to evaluate baseline startle magnitude, prepulse inhibition of startle, and latency of startle. The paradigm, of standard design, consisted of pulse alone trials to evaluate baseline startle, and prepulse + pulse trials with interstimulus intervals of 30, 60 or 120 milliseconds (msec) between the prepulse and pulse stimuli.

Results: A Poisson regression on TOXO serointensity indicated that SCZ subjects had higher TOXO IgG titres than CONT subjects (Wald Chi-Square on diagnosis while controlling for age $= 285.68$, $p < 0.001$). In a mixed model ANOVA with trial type as a within subjects factor, TOXO status and diagnosis as between subjects factors, and age as a covariate, peak latency was significantly longer in SCZ than CONT subjects ($F(1,131) = 12.82$, $p < 0.001$). In pairwise comparisons, peak latency was longer in the 30 msec trials in SCZ subjects who were TOXO positive than TOXO negative ($p = 0.05$). Similarly, peak latency was longer in the 30 msec trials in CONT subjects who were TOXO positive than TOXO negative ($p = 0.05$). This pattern of longer (i.e. slower) latency in SCZ than CONT subjects and in TOXO positive than TOXO negative subjects was seen in onset latency also, although results only reached trend level significance.

Discussion: This is the first report of slowing in acoustic startle latency associated with TOXO seropositivity in SCZ and CONT subjects. This finding in startle latency, a measure of neural processing speed, is in accord with findings of psychomotor slowing in rodents and in nonpsychiatric humans with TOXO seropositivity. While these studies are correlative, they support a potential role of TOXO in some behavioral symptoms of SCZ, and could lead to the development of novel treatment targets for the subset of SCZ patients who are TOXO seropositive. Supported by: VA Merit Review Grant (E Duncan), NIMH (1R21MH083138-01A1, B Pearce), NIDA (1R01DA018294-01A2, E Duncan).

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82. The Effects of Cannabis Dependence on Cognitive Function in Males with Schizophrenia

Rachel A. Rabin, Konstantine K. Zakzanis, Zafiris J. Daskalakis, Tony P. George*

Departments of Psychiatry and Institute of Medical Sciences, University of Toronto, Toronto, Canada

Background: Cannabis is the most commonly used illicit drug among patients with schizophrenia, with nearly 25% of those suffering from schizophrenia diagnosed with a co-morbid cannabis use disorder. While cognitive impairment is common in this disorder, wherein approximately 80% of patients present with deficits across cognitive domains, the moderating role of cannabis on cognition remains unclear. This gap in the literature is important to address given that greater cognitive deficits are associated with poorer prognosis and functional outcome. The primary aim of this study was to examine cognitive performance and symptomatology as a function of cannabis use patterns in schizophrenia. A secondary aim was to determine the effects of cumulative cannabis exposure on cognitive function in schizophrenia.

Methods: Using a cross-sectional design, we examined cannabis use and cognitive performance among male patients with current

cannabis dependence (n = 18) and those with no current cannabis use disorders (n = 29). Subsequently, we divided non-current users into patients with historical cannabis dependence but abstinent for at least six months (n = 21), and those with no lifetime cannabis use (n = 8) to explore whether cognitive alterations associated with cannabis use are best characterized as state or trait phenomena. In addition, we examined the relationship between cumulative cannabis exposure and cognitive function in current and former dependent patients. All participants were nicotine dependent cigarette smokers, who were given smoking breaks to minimize nicotine withdrawal during the 3 h testing procedure.

Results: There were no differences in cognitive performance between patients with schizophrenia regardless of current cannabis-using status. When parsed according to historical cannabis use (current, former or never-user), lifetime users demonstrated better performance in tests assessing speed of processing. Notably, in patients with current dependence, robust relationships emerged between cumulative years of cannabis exposure and cognitive performance particularily on frontal executive and verbal learning tasks; these associations were absent in former cannabis users.

Discussion: Based on these preliminary cross-sectional data, cannabis use status has minimal effects on neurocognitive function in schizophrenia. However, cumulative cannabis exposure significantly impairs cognitive performance on tests mediated by the prefrontal cortex in current, but not former users. Accordingly, our results suggest that the state dependent negative effects of cannabis use may be reversed with sustained abstinence, which has significant implications for treatment of cannabis dependence in persons with schizophrenia.

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83. White Matter Disruption in Adolescents with Childhood Maltreatment History

Uma Rao*, Hao Huang, Tejasvi Gundapuneedi

Meharry Medical College, Nashville, USA

Background: Childhood maltreatment, including emotional abuse, physical abuse, sexual abuse and neglect, is widespread in the United States. Childhood maltreatment has been known to produce long-lasting impairments in behavioral, cognitive and social functioning, but their underlying mechanisms are not well-understood. The developing brain is highly sensitive to the effects of early-life stress and childhood maltreatment has been associated with alterations in the size or functional activity of a variety of brain regions, possibly resulting in the above-described impairments. Diffusion tensor imaging (DTI), a type of magnetic resonance imaging (MRI), is capable of delineating *in-vivo* microstructural changes of white matter tracts noninvasively by measuring the water diffusion in these tracts. The directional dependence in water diffusion can provide quantitative measures on structural integrity of the white matter. Fractional anisotropy (FA), a DTI-derived metric, reflects aspects of membrane integrity and myelin thickness, and decreased FA is usually associated with disruption of the white matter. The present investigation was undertaken to examine white matter tract integrity in adolescents exposed to childhood maltrreatment.

Methods: In an ongoing longitudinal study, to date, 19 adolescent volunteers (5 males, 14 females) with no personal history of a psychiatric illness, but experienced maltreatment (MALTX) prior to age 10 years, and 13 adolescent volunteers (6 males, 7 females) with no personal or family history of a psychiatric disorder (CONT) were recruited. After completing structured psychiatric diagnostic assessments, the participants underwent DTI studies on a 3T Philips Achieva MRI System. Information on early-life adversity also was gathered from the adolescent and parent. A commonly used analysis method in DTI is voxel-based morphometry (VBM) which reveals the voxel-wise disruption of white matter after registering FA maps of all subjects to a template space. Tract-based spatial statistics method (TBSS, FMRIB Center, Oxford, United Kingdom), a software package specifically designed for the analysis of diffusion-weighted images, was used for the whole brain voxel-wise analysis. The significant clusters with p < 0.0001 (uncorrected) in the skeleton voxels of white matter were identified. In order to avoid false positive results, only clusters with continuous voxels greater than15 and averaged FA values greater than 0.2 were retained with home-made IDL (ITT, Boulder CO) programs. In addition to voxel-wise analysis through TBSS, the integrity of individual white matter tracts were assessed by using all skeleton voxels of a specific tract as region of interest (ROI). This approach tested if the structure of a specific white matter tract was altered entirely. For the tracts which had a general integrity change, the along-the-tract FA values for each group were measured and compared between the two groups to reveal the structural profile of these tracts.

Results: The groups did not differ significantly with respect to age (15.9 ± 2.8 years in MLATX, 16.0 ± 2.8 in CONT), gender, ethnicity/race, pubertal status, socioeconomic status, intelligence quotient (IQ), and psychosocial functioning. The MALTX group scored higher on early-life adversity than CONT (p .0001).

The MALTX group had significantly lower FA values in several white matter tracts including the left and right superior longitudinal fasciculus (SLF-L and SLF-R), right cingulum bundle projecting to the hippocampus (CGH-R), left inferior fronto-occipital fasciculus (IFO-L), and forceps major (F-major). However, only SLF-L (with four separate clusters) showed significant reduction in FA values at the tract level (p .05), suggesting that large portions of the skeleton voxels in this tract had decreased FA values in the MALTX group. None of the socio-demographic characteristics correlated significantly with FA values in any of the white matter tracts. After controlling for the socio-demographic features, early-life adversity scores correlated negatively with FA values from all the above-described tracts (r values ranging from -.33 to -.64; p values ranging from .07 to .0001).

Discussion: These preliminary results indicate that childhood maltreatment is associated with alterations in the integrity of major white matter tracts in the brain. The white matter track disruptions were associated the magnitude of early-life adversity in both maltreated and control groups. Longitudinal assessments will determine whether the observed microstructural white matter changes are associated with increased vulnerability for developing psychopathology in this sample.

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84. Impaired Auditory Object Formation in Schizophrenia as Revealed by Theta-Gamma Oscillatory Entrainment Dynamics

David I. Leitman*, Daniel H. Wolf, Jonathan McDaniel, Yin Li, Julie Rosen, James Loughead, Raquel E. Gur, Ruben C. Gur, Bruce I. Turetsky

University of Pennsylvania, Philadelphia, USA

Background: How we form auditory objects from the auditory scene – for example, identify the sound of a hammer at a construction site or the voice of a colleague at a cocktail party – has been a topic of concerted research for many years. In schizophrenia, basic auditory sensory deficits can contribute significantly to higher order cognitive dysfunction. Better understanding of auditory object processing could provide a useful window into how sensory and cognitive disturbances converge within the illness.

Methods: We studied auditory object formation using a series of alternating tones in an ABA paradigm. Tones A and B were presented at a rate of 5 Hz and 2.5 Hz respectively. In the Streaming condition, these tones were separated in frequency by an octave, yielding the perception of two distinct aural streams. In the Integration condition, tones were separated by a single semitone yielding the percept of one triplet or gallop. The electrophysiological sequelae of passive listening to streaming and integration tone sequences were examined in 35 controls and 30 patients with schizophrenia using a 64-channel scalp array. Complex temporal spectral analysis was conducted offline on a cluster of nine frontocentral electrodes. Prior work has shown that rhythmic auditory stimulation entrains endogenous low frequency brain oscillatory activity (theta 3-8 Hz). Here we hypothesized that whether two streams or an integrated triplet is perceived depends upon the cognitive/attentional modulation of this ongoing oscillatory entrainment. We further hypothesized that two cognitive processes work on differing timescales during the formation of auditory objects: a global process which maintains the stability of the percept of two streams or one integrated stream over multiple tone units (ABA), and a local process occurring on the timescale of each tone, which either binds together individual A or B tones, or separates them in to A and B tone streams. According to this hypothesis, the perception of two separate streams in the Streaming condition and triplets in the Integration condition would be reflected at a global timescale by changes in low theta (2.5 Hz) and mid theta (5 Hz) oscillatory power and coherence. Nested within this timescale, the integration or separation of A and B tones within each sequence would be reflected locally in high frequency gamma oscillatory power changes across ABA tones. In schizophrenia patients, we hypothesized that this oscillatory hierarchy would be disrupted.

Results: Spectral analysis of the electrophysiological signal indicated two peaks in the theta range (2.5 and 5 Hz), roughly mirroring the presentation rate of ABA sequences and A tones alone. In the gamma range (30-80 Hz) we observed a peak at 55.1 Hz. Across conditions, we observed large shifts in the relative power and inter-trial coherence (ITC) of evoked oscillatory activity at 2.5 Hz and 5 Hz. In healthy controls, the Integration condition was associated with a significantly higher 2.5:5 Hz power ratio than the Streaming condition. Higher 2.5 Hz power locked to triplet (ABA) onset suggests that such power is the representation of the formation or binding of the triplet. Examinations of evoked gamma oscillations indicated that Integration is characterized by an initial gamma volley for the initial A tone that is reduced for the next two tones in the triplet. During Streaming however, each tone had equivalent gamma power. In patients, however, these theta and gamma differences between conditions were largely absent and similar to control responses during Streaming.

Discussion: Our results suggest that auditory object formation arises from perceptual/cognitive modulation of low frequency oscillatory entrainment to incoming sensory stimulation on a low-frequency global timescale. Nested within this low frequency global entrainment, modulations in gamma power facilitate the binding of auditory elements into an auditory object. In schizophrenia, this entrainment is less precise and this hierarchical oscillatory structure is poorly formed, suggesting that patients fail to adequate integrate auditory information, over time, into a unified percept. Understanding the neural mechanisms of these oscillatory abnormalities in schizophrenia should provide insight into the perceptual deficits observed in schizophrenia. As perceptual deficits contribute to cognitive dysfunction and disability, observed oscillatory abnormalities may constitute an important endophenotype as well as a target for pharmacological and nonpharmacological interventions.

85. Sensory and Cognitively Mediated Event-Related Potentials Index Symptoms in First Episode Schizophrenia

Elisabetta del Re, Margaret Niznikiewicz, Tracey Petryshen, Robert W. McCarley*

VABHS/Harvard Medical School, Brockton, USA

Background: Schizophrenia (SZ) is characterized by information processing, attention and memory deficiencies. Event-related potential (ERP) studies are effective in real-time parsing such neural processes. The positive going P300b (abbreviated P3b) is an index of cognitively mediated processing, is typically elicited through an oddball paradigm, where infrequent stimuli, so-called targets, are embedded into a series of 'standards', are effortfully recognized, such as by counting. Infrequent, novel distracter sounds when added to the active oddball paradigm produce a novel P3, a sensory measure of attention reorienting. In both paradigms, the standard stimuli produce an additional earlier positive-going sensory ERP, the P200 (abbreviated P2). The P3b component has been repeatedly shown to be reduced in chronic and first episode SZ (FESZ). The novel P3 has been studied much less frequently, being variously reported as reduced and unchanged in SZ. The present study assessed these ERPs in FESZ and age-matched healthy volunteer controls (HC).

Methods: Subjects:16 FESZ and 14 age-matched HC.Correlations of ERP components with the brief scale of psychotic symptoms (BPRS) and with the global assessment of functioning (GAF) were investigated using Spearman's rho because of relatively low Ns. Auditory Oddball: Simple oddball task stimuli were tone-pips (100 ms duration, 75 dB SPL), with 20% (36) infrequent target tones (1.5 kHz) and 80% (144) frequent standard tones (1 kHz). ISI was varied between 800 and 1200 ms, mean of 1 sec. The subjects silently counted the targets. Auditory Novel: The novel oddball task added novel auditory sounds (300 ms duration, 75 dB SPL) such as environmental noises (dog bark, door slamming) to the simple oddball tones just described. Six blocks of six novel sounds were randomly presented six times though the paradigm. Novel auditory oddball sequences consisted of 20% (36) target tones (1.2 kHz), 20% (36) infrequent novel sounds, and 60% (108) standard tones (1 kHz). Variable ISI was 800 to 1200 ms, mean of 1 sec. The subjects silently counted the targets.

Results: Counting accuracy did not differ between HC and FESZ . Both novel P3 and P3b amplitudes were significantly decreased in FESZ. BPRS negative symptoms score was inversely correlated with amplitude of the oddball target P3b at Cz and Pz ($P < 0.05$), indicating that higher ratings of negative symptoms correlated with reduced amplitude of the oddball P3b. There were no BPRS-novel P3 amplitude correlations. Lower GAF scores were associated with lower amplitude of both novel P3 at all midline electrodes and also the P3b target at Pz and Cz ($P < 0.05$). Latency findings. The P2 to standard tones in both paradigms had a shortened latency in FESZ compared to HC. Correlation analysis demonstrated that shorter latency of P2 to novel tones significantly correlated with higher scores of overall BPRS, negative symptoms and anxiety/depression symptoms scores. Lower GAF scores across groups were also positively correlated with shorter latency of the P2 to both novel and oddball tones ($P < 0.01$), indicating that shorter latency of P2 to ERP standard in novelty or oddball paradigms associates with lower functioning scores on the GAF scale. CPZ medication equivalents were not correlated with abnormalities.

Discussion: We conclude deficits of both sensory and more cognitively mediated ERP measures coexist in early stages of the disease, and that these deficiencies associate with distinct symptomatology. Specifically, we found these abnormalities in FESZ compared with HC: 1) Reduced amplitude of novel P3 was present and associated with lower GAF scores. This is, in our view, an important addition to the currently sparse literature. 2) Reduced amplitude of the Oddball P3 was present and associated with more negative symptoms and lower GAF scores, supporting its use in indexing these symptoms. 3) Latency differences were also prominent. Interestingly, P2 latency was reduced in FESZ, and the shorter latency was associated with worse symptoms, (worse BPRS for the P2 to standards and worse GAF scores for the P2 to novels and oddball targets). These may reflect an incomplete analysis of stimulus features, an analysis which the P2 is thought to reflect. Genetic Link. As a part of the Boston CIDAR we are now examining genetic associations. In an initial analysis, being followed up in a larger sample for confirmation, the MTHFR gene showed a nominal association with oddball P3 amplitude at Pz, even when controlling for the 35 independent SNPs evaluated. Since this gene is associated with prefrontal dopamine neurotransmission, the finding appears compatible with a frontally processed task, such as the P3b.

Disclosure: E. del Re: None. **M. Niznikiewicz:** None. **T. Petryshen:** None. **R. McCarley:** Part 1: Consultant to Merck, Janssen, Takeda, Sunovion.

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86. Impaired Context Processing as a Potential Marker of Risk in Clinical-High-Risk Youth

Tara Niendam*, Natalie Hutchison, J. Daniel Ragland, Jong Yoon, Marjorie Solomon, Michael Minzenberg, Cameron Carter

University of California, Davis, Sacramento, USA

Background: Impairment in context processing, or the ability to represent and maintain context in order to guide behavior, has been consistently demonstrated in individuals with schizophrenia at all stages of the illness, and has been associated with decreased activation of prefrontally-mediated brain networks. However, it is unclear whether such deficits are present in individuals at clinical high risk for the illness or predictive of later onset of psychosis. The AX Continuous Performance Test (AXCPT) was used to measure context processing in a longitudinal study of clinical-high-risk for psychosis (CHR), recent onset schizophrenia (RO) and healthy control (HC) participants. We hypothesized that RO and CHR individuals would show impaired context processing, as evidenced by decreased accuracy with increased delay between cue and probe, relative to HCs. Further, we hypothesized the CHR individuals who developed psychosis or continued to experience significant attenuated psychotic symptoms over follow-up would demonstrate impaired context processing at baseline when compared to CHR individuals who later showed remission of their attenuated psychotic symptoms.

Methods: HC (n = 83), RO (n = 80), and CHR (n = 42) participants from the UC Davis Imaging Research Center were identified using the Structured Interview for Prodromal Syndromes (SIPS) and Structured Clinical Interview for DSM-IV (SCID-I/P). Participants performed two versions of the computerized AX-CPT (short delay – 1 second between cue and probe; long delay – 5 seconds between cue and probe) during baseline behavioral testing. Clinical follow up data was obtained on 30 CHR individuals (71% of sample) 22.67 ± 12.24 months after baseline.

Results: After controlling for differences in age, RO and CHR participants demonstrated poorer context processing than HCs on both the short and long versions of the AXCPT. Although all groups showed a decline in context processing for the long compared to the short delay (all p < .01), a significant Group X Delay interaction indicated that the effect of the delay was greater in the RO and CHR

individuals when compared to HCs (both p < .001). Within the CHR sample, 17 individuals (CHR-Poor outcome) continued to show attenuated psychotic symptoms (n = 10) or developed psychosis (n = 7) while 13 individuals (CHR-Improved) demonstrated remission of their attenuated symptoms. When compared to CHR-Improved, showed impaired context processing across versions of the task (p = .001) at baseline. CHR-Poor outcome also demonstrated a significant decline in performance with increasing delay (p = .006) while CHR-Improved did not (p = .28).

Discussion: Results provide evidence that CHR individuals demonstrate impaired context processing at ascertainment when compared to healthy controls in a manner that is similar to individuals with recent onset schizophrenia. Additionally, the subset of CHR individuals who demonstrated poor clinical outcome over follow-up, as evidenced by conversion to psychosis or persistent attenuated psychotic symptoms, showed impaired context processing on the AXCPT at baseline when compared to those whose attenuated symptoms remitted. These findings suggest that impaired context processing may serve as a potential marker of risk for poor clinical outcome in CHR youth.

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87. Metacognitive Assessmrent of Self-Awareness in Schizophrenia

Mujeeb Shad*, Matcheri Keshavan, Perry Mihalakos, Jair Soares, Carol Tamminga

UT Health Science Center Houston, Houston, USA

Background: Impairment in self-awareness (SA) is commonly observed in schizophrenia and is notable for being one of several deficits in executive cognitive function associated with chronic functional and psychosocial impairment. However, despite its clinical significance, only a few studies have examined the neuro-biology SA processing in schizophrenia. The aim of this study was conducted to assess metacognitive function in schizophrenia using a SA fMRI paradigm designed to elicit judgments of self-reference in a simulated social context. We predicted that individuals with schizophrenia would demonstrate an altered functional response to self- and other-referential stimuli as compared to controls.

Methods: Seventeen adult volunteers with DSM IV-based diagnosis of schizophrenia and 15 controls matched for age and premorbid IQ underwent functional neuroimaging on a Philips 3.0 T Achieva system while performing on a SA-task. The SA-task required volunteers to read statements made by others and discern whether each statement having either a neutral trait adjective (e.g., honest) or a negative trait adjective (e.g., suspicious) was about them personally [i.e., judged to be self-referential (SR)] or about someone else [(i.e., judged to be other referential (OR)]. A familiarization interview preceded the SA-task, in which volunteers were first asked to imagine a scenario as read aloud by script: "Imagine that you accidentally overhear a conversation between people who may know you. You do not recognize their voices. You listen for a moment, because you think you may have heard your name used in their conversation. But you are not sure." Then a series of ten equivalence statements were read aloud to the volunteer; some include the volunteer's own first name (self-directed), while others use another person's first name (other-directed). After being read each "overheard" statement, the volunteers were presented the cued epochs, "Do you believe that they are talking about you?" and "Do you believe they are talking about some other person?" Subjects were encouraged to respond quickly with yes/no answers which are recorded. Similar procedure was

repeated later in the scanner. In addition, a pre- and post-scan questionnaire was also administered to clarify whether the volunteers responded to their first names or the cued epoch. The raw fMRI data acquired from each subject were converted to ANALYZE image format. Processing of the fMRI data was conducted using SPM5 software. Preprocessing consisted of realignment, coregistration, and normalization, and smoothing. As the first step, an epoch x valence x group interaction was examined using two-sample *t*-test. Since there was no epoch x valence x group interaction, the negative and neutral statements were collapsed to enhance the power of the study. This was followed by three between-group analyses targeting self- and other-directed stimuli within and across the differently cued epochs; 1) Within-epoch self- vs. other-directed contrast; 2) Between-epoch self-directed contrast; 3) Between-epoch self- vs. other-directed contrast. The premorbid IQ was estimated using the Wechsler Test for Adult Reading and psychopathology was assessed with Positive and Negative Syndrome Scale.

Results: Compared to controls, schizophrenia volunteers revealed a greater activation of posterior cingulate cortex, precuneus and lingual gyri in response to between-epoch self- vs. other-directed contrast and inferior parietal lobule and supramarginal gyrus in response to within-epoch self- vs. other-directed contrast. None of the contrasts produced greater activation in controls than schizophrenia. No effect of education, antipsychotic dose or duration of illness was found on these activations. However, psychopathology (PANSS total scores) was positively correlated with SA task-induced activation in thalamus, parahippocampus gyrus, hippocampus, lingual gyrus and fusiform.

Discussion: The present study supports and advances an emerging body of literature showing altered brain function in schizophrenia subjects in response to self-referential processing. The study provides preliminary support for the hypothetical model of self-awareness deficits in schizophrenia, which will not only enhance our understanding of the neurobiology of self-awareness but also help develop early predictors for treatment response to enhance self-awareness in schizophrenia.

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88. Deficits of Inhibitory Behavioral Control in Schizophrenia and Bipolar Disorder

John Sweeney*, James Reilly, Richard Keefe, Scot Hill, Mark Bushong, Carol Tamminga, Matcheri Keshavan, Godfrey Pearlson, Gunvant Thaker

U T Southwestern, Dallas, USA

Background: Generalized cognitive deficits are established in bipolar patients with psychosis as in schizophrenia. While most cognitive impairments are believed to be greater in schizophrenia, the ability to inhibit behavioral responses may not follow this pattern, as on a clinical basis behavioral impulsivity is a common feature of bipolar disorder.

Methods: A computerized stop signal task was administered to subjects participating in the multisite BSNIP study (Bipolar and Schizophrenia Network for Intermediate Phenotypes). A stop signal task was administered in which a target appeared on the left or right on a computer monitor, and subjects pressed a corresponding left or right button in response. On 40% of trials (stop trials), at a varying interval after the peripheral target appeared, a command appeared in the center of the screen cueing subjects to stop the button press response. On the other 60% of interspersed trials (go trials), subject responded to the peripheral cue with no stop signal presentation. A control task was used to estimate each individual's reaction time (RT) on a block of 100% "go" trials with no stop trials interspersed. The sample included 260 controls, and 4 groups of clinically stable patients: schizophrenia (195), schizoaffective depressed (40), schizoaffective manic (86), and bipolar with psychosis (157). 231 first degree relatives of schizophrenia patients and 205 relatives of bipolar patients also completed testing. Error rate (failure to stop responses on stop trials) was examined, along with the difference in RT from go trials in the control condition from go trials when go and stop trials were interspersed. The BACS test of general neuropsychological ability was also administered.

Results: On the BACS test, generalized cognitive deficits of the schizophrenia group ($z = 1.88$) were twice that of the bipolar group ($z = .83$). Schizoaffective groups were intermediate ($\sim z = 1.40$). On the Stop Signal Task, all patient groups were impaired relative to controls but, the number of errors made did not differ between patient groups. Schizophrenia and schizoaffective groups, but not the bipolar group, all showed a reduced ability to slow reaction times (RTs) on GO trials during the task, relative to their RTs on the baseline control task. In controls and schizophrenia groups, but not the bipolar group, slowing of RT predicted % correct performance. Performance was impaired in schizophrenia but not bipolar relatives. Stop Signal Task performance was minimally related to BACS scores ($r < .20$) in all groups.

Discussion: Unlike many cognitive functions, top down behavioral control of action tendencies is a dimension that is impaired to a similar degree in schizophrenia and bipolar disorder with psychosis. RT and family data suggests that the mechanisms may be different across these disorders, with deficits in behavioral control rather than behavioral preparation being more important in bipolar disorder.

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89. Effects of the Val(158)Met Catechol-O-Methyltransferase Gene Polymorphism on Olfactory Processing in Schizophrenia

Vidyulata Kamath, Paul J. Moberg, Raquel E. Gur, Richard L. Doty, Bruce I. Turetsky*

University of Pennsylvania, Philadelphia, USA

Background: The catechol-O-methyltransferase (*COMT*) val158met polymorphism has received attention in schizophrenia due to its

role in prefrontal dopamine catabolism. Given the rich dopaminergic innervations of the olfactory bulb and the influence of dopamine on the transmission of olfactory signals, we examined the influence of *COMT* genotype status on the olfactory processing impairment observed in schizophrenia.

Methods: Schizophrenia patients ($n = 42$) and demographically comparable healthy adults ($n = 30$) were recruited to the Schizophrenia Research Center at the University of Pennsylvania Medical Center. The University of Pennsylvania Smell Identification Test (UPSIT), a 40-item forced-choice odor identification task, was administered unirhinally (20 items to each nostril). Participants were genotyped for the COMT val158met polymorphism (rs4680) using TaqMan® based assays-on-demand.

Results: The distribution of genotypes was consistent with Hardy-Weinberg equilibrium in bothpatients and controls. There were no significant differences in genotypes between the groups. However, a statistically significant interaction of diagnosis and *COMT* genotype was observed ($F_{2,66} = 5.20$, $p < .01$). Schizophrenia patients who were either Val/Met heterozygotes or Met/Met homozygotes showed impaired odor identification accuracy relative to both Val/Val homozygote patients and control subjects. Patients who were Val/Val homozygotes performed at the same level as control subjects and there was no effect of genotype within the control sample. These findings could not be explained by factors such as antipsychotic medication status, clinical symptomatology, or demographic and illness characteristics.

Discussion: The results of our investigation replicate the well-documented observation of odor identification impairment in schizophrenia, and further suggest that this deficit is influenced by *COMT* genotype status. As the Met allele is associated with decreased dopamine catabolism, and therefore higher dopamine levels, these findings raise the possibility that lower *COMT* activity contributes to increased olfactory impairment in schizophrenia. This relationship is consistent with prior studies in individuals with VCFS and with animal studies demonstrating specific dopaminergic modulation of primary olfactory sensory afferents. However, it is inconsistent with previous studies of the effects of the *COMT* val158met polymorphism on cognitive performance, in which the Val/Val genotype is generally associated with poorer performance. This strongly suggests that the effect of the val158met polymorphism on olfactory performance is a direct reflection of altered dopamine levels within the afferent olfactory neurocircuitry, rather than a nonspecific effect on cognition. It also implies that olfactory impairment in schizophrenia is not a byproduct of antipsychotic medications. Based on these findings, treatment with antipsychotic medications might be expected to actually enhance olfactory performance. Future studies examining the relationship between *COMT* haplotypes and other olfactory measures (e.g., discrimination, threshold) is warranted.

90. Neuroscience-Informed Cognitive Training in Schizophrenia "Normalizes" Brain-Behavior Associations in Auditory and Verbal Working Memory Processes: Evidence from MEG and fMRI

Sophia Vinogradov*, Karuna Subramaniam, Alex Herman, Josh Woolley, Tracy Luks, Melissa Fisher, John Houde, Srikantan Nagarajan

San Francisco Department of Veterans Affairs Medical Center and Department of Psychiatry University of California San Francisco, San Francisco, USA

Background: Schizophrenia patients (SZ) suffer from deficits across a range of auditory and verbal working memory (WM) processes. Neuroscience-informed cognitive training improves performance in some of these processes, and induces changes in brain activation patterns as assessed via MEG and fMRI. In the present study, we examined the association between training-induced behavioral improvements and changes in brain activation in 2 different WM experiments. Our goal was to examine neural responses that are associated with improved WM performance in SZ and to compare them to what is seen in healthy subjects.

Methods: SZ and healthy comparison subjects (HC) performed a syllable reproduction task during MEG recording (auditory WM), and a letter N-back task during fMRI scanning (verbal WM). Patients were then randomly assigned to active computerized cognitive training (SZ-AT), or to a control condition of computer-games (SZ-CG). All subjects underwent a second MEG and fMRI session after the training period.

Results: Experiment 1 (MEG during syllable reproduction—auditory WM): Time-frequency localization analysis revealed that, at baseline, SZ subjects showed lower power in the high gamma band (60-150 Hz) in several brain regions compared to HC during the syllable reproduction task. At baseline, SZ subjects did not show any correlations between gamma-band power and task performance in any brain region, whereas in HC subjects, high-gamma power in posterior superior temporal (pSPT) and ventral premotor (vPM) cortices were correlated with task performance. After cognitive training, high gamma activity in SZ-AT subjects, significantly increased and such increases were not observed in the SZ-CG control subjects. High gamma increase was observed in areas important for audiomotor processing, particularly within pSPT, vPM and areas in inferior frontal gyrus – importantly the resulting brain activation patterns now resembled those seen in HC subjects. High-gamma enhancement in vPM and pSPT was correlated with improvement in task performance and with verbal WM. Experiment 2 (FMRI during letter 2-back, verbal WM): At baseline, during the 2-back WM task, HC subjects showed greatest activation in right dorsolateral prefrontal cortex (DLPFC), which correlated with task performance. SZ patients showed impaired performance, activation in bilateral DLPFC, and no significant associations between brain activation and 2-back performance. After cognitive training, SZ-AT subjects, but not SZ-CG subjects, significantly improved their 2-back WM performance, and showed increased DLPFC activation. Further, after training, right DLPFC signal now demonstrated a significant association with WM performance, similar to what was seen in HC subjects at baseline.

Discussion: The findings from these two experiments indicate that: 1) Intensive computerized training attempts to "normalize" disrupted patterns of neural activity in SZ which are involved in auditory and verbal WM. 2) This pattern of "normalization" is observed both in the time-frequency source localization analysis obtained from MEG, and in the BOLD signal analysis obtained from fMRI; 3) Performance improvements are associated with enhanced neural responses across two different WM tasks. Taken together, these data suggest that intensive computerized training

induces restoration of function in impaired neural systems, rather than relying on the development of compensatory neural mechanisms.

91. Gamma Oscillations to the Gestalt Perception of Coherent and Incoherent Motion in Schizophrenia

Thomas J Whitford*, Shahab Ghorashi, Ryan Mears, Kevin M. Spencer

Veterans Affairs Boston Healthcare System, Boston, USA

Background: The perception of a coherent, unified form from individual visual features (i.e., Gestalt perception) has been related to oscillatory synchronization in the gamma band of the electro-encephalogram (EEG) (30-100 Hz). Schizophrenia patients have been shown to exhibit abnormal patterns of gamma band activity to Gestalt percepts, such as illusory contours and multi-stable figures, but their gamma band response to a more elementary perception – coherent motion – has not been previously investigated.

Methods: Twelve patients with chronic schizophrenia (SZ) and 18 healthy controls (HC) participated in the experiment. The visual stimuli consisted of rectangular black and white gratings (spatial frequency 1.75 Hz), bilaterally presented 6.58 deg. to either side of a central fixation point on the horizontal meridian. The gratings drifted upwards or downwards at a frequency of 1.5 Hz. EEG data were acquired (Biosemi Active 2 system, 71 channels, Independent Components Analysis for artifact correction) and time/frequency analyses performed. The stimulus-locked oscillatory responses to Coherent motion (i.e., when the gratings moved in the same direction) vs. Incoherent motion were compared, both within and between groups.

Results: Both Coherent and Incoherent motion stimuli elicited the early visual-evoked gamma oscillation (70-150 ms post-stimulus from 25 and 50 Hz). The magnitude of this oscillation did not differ between groups. However, while Coherent motion was associated with increased phase locking, relative to Incoherent motion, from 29-40 Hz and 270-330 ms post-stimulus in HC, this later gamma oscillation was reduced in SZ. Within the SZ group, late visual-evoked gamma phase locking was positively correlated with total positive symptom ratings, and disorganization in particular.

Discussion: Schizophrenia is associated with an abnormal gamma oscillatory response to coherent motion. The positive correlation between positive symptoms, including disorganization, and the magnitude of the abnormal oscillation is consistent with previous reports that psychosis is associated with increased gamma activity.

92. Facial Processing in Bipolar Disorder and Schizophrenia: An Event-Related Potential Study

Jonathan K. Wynn*, Kristopher I. Mathis, Carol Jahshan, Lori Altshuler, David Glahn, Michael F. Green

VA Greater Los Angeles Healthcare System, Los Angeles, USA

Background: Schizophrenia patients (SZ) exhibit deficits in several types of social cognitive tasks, with facial affect processing being the most studied. However, much less is known about dysfunction in social cognitive tasks in patients with bipolar disorder (BD), and few studies have made direct comparisons between SZ and BD on facial affect processing tasks. We used event-related potentials (ERPs) to examine separate stages of facial affect processing in BD and SZ patients. Namely, we examined an early stage of facial structure encoding and a later stage of facial affect processing. We also examined in BD patients whether antipsychotic medication or lithium had an effect on the ERP measurements.

Methods: Thirty-one SZ patients, 57 BD patients, and 28 healthy controls (HC) were examined. EEG was recorded while participants performed three separate tasks: gender identification, facial affect identification, and building identification. Standard male and female Ekman faces, portraying one of six different emotions, were used for the first two facial processing tasks. In the first task participants were simply asked to identify the gender and in the second asked to identify the emotion on the face. In the third task they were asked to identify if a building was one- or two-stories tall. In an attempt to examine activity mainly involved with face processing, rather than a mix of basic visual processing and face processing activity, ERPs to the building identification task were subtracted from ERPs to the two face processing tasks. Three ERP waveforms were then examined for both of the resulting waveforms for the face processing tasks: N170 and vertex positive potential (VPP) to examine facial structure encoding, and N250 to examine facial affect processing. We examined ERP amplitude and peak latency. Behavioral performance on the two face processing tasks was also examined. We were able to analyze the effects of antipsychotic medications on the ERP measures by examining 25 BD patients not taking antipsychotic medications compared to 32 BD patients taking antipsychotic medications. Finally, we were able to analyze the effects of lithium on the ERP measures by examining 45 BD patients not taking lithium compared to 12 BD patients taking lithium.

Results: HC and BD performance on the emotion identification task was significantly higher compared to SZ; there were no differences in performance between any of the three groups on the gender identification task. For the N170, SZ exhibited significantly smaller N170 amplitudes during both gender and emotion identification tasks compared to BD and HC; no differences in amplitude between BD and HC were seen. N170 latency was significantly faster in HC compared to both BD and SZ; no difference in N170 latency was seen between the patient groups. For VPP amplitude and latency, the only significant finding was smaller VPP amplitudes in SZ compared to HC and BD. For the N250, SZ exhibited significantly smaller amplitudes during both tasks compared to HC and BD. However, BD had a significantly longer latency compared to HC and SZ. Antipsychotic medication and lithium had no significant effects on ERP amplitudes or latencies for either task.

Discussion: These results show that both BD and SZ exhibit abnormalities in early and later stages of facial and facial affect processing. Neural responses are weaker during structural encoding and affect processing of faces in SZ. However, BD exhibited slower peak responses during both stages of processing compared to HC. Additionally, antipsychotic medications had no significant effects on either ERP amplitude or latency within the BD patients. Similarly, lithium did not have a significant effect on ERP amplitude or latency within the BD patients, though with the small sample size we may have not had the power to detect significant differences. These findings reveal that BD patients exhibit slowing of neural responses similar to SZ patients, but normal strength of activation, during face and facial affect processing. The results also suggest that patient-control differences are not due to antipsychotic or lithium medications.

Abbott Laboratories, Amgen, Cypress, Lundbeck, and Teva Speaker: Otsuka and Sunovion.

93. Trajectory of Comorbidities in Obsessive-Compulsive Disorder

Euripedes C. Miguel*, Maria A. de Mathis, Juliana B. Diniz, Ana G. Hounie, Victor Fossaluza, James Leckman, David Pauls, Roseli G. Shavitt, Maria C. Rosario

Department of Psychiatry, University of São Paulo Medical School, São Paulo, Brazil

Background: Obsessive-compulsive symptoms (OCS) may begin at any point of the life span and at least half of the Obsessive-compulsive disorder (OCD) patients report having initial symptoms before 10 years of age. OCS are not an isolated form of psychopathology with symptoms of other psychiatric disorders being present more frequent than not. Additional psychiatric disorders contribute to the OCD-related burden and are, therefore, an important landmark to understand the impact of OCS on normal development. The main goal of this study is to understand the trajectory of comorbid disorders according to the first manifested psychiatric disorder and their impact in the clinical development of OCD and subsequent psychiatric comorbidities based largely on self-report data obtained at one cross-sectional time point. Understanding OCS within this developmental perspective is essential for guiding interventions in children with early signs of psychopathology.
Methods: Clinical sample of 1001 consecutive OCD patients, aged 9 to 82. Inclusion criterion: main psychiatric diagnosis of OCD according to DSM-IV. Exclusion criterion: comorbid schizophrenia, mental retardation. Clinical assessment: Structured Clinical Interview for Diagnosis of Axis I (SCID-I), and SCID for Impulse-Control disorders, Yale-Brown Obsessive-Compulsive Scale (Y-BOCS), Dimensional Yale-Brown Obsessive-Compulsive Scale (DY-BOCS), Yale Global Tics Severity Scale (YGTSS). Statistical analyses: descriptive analyses: frequencies and percentages for discrete variables and means and standard error for continuous variables. Qui-square test was used for categorical variables, and Kruskal-Wallis and ANOVA tests were used for continuous variables. For all the tests the significant level was considered 5%. To investigate the age at comorbidities onset a Bayesian approach was performed.
Results: Patients that presented separation anxiety disorder (SAD) as the first diagnosis (N = 175, age of onset SAD 5.35; age of onset OCD 12.36) tended to present higher frequency of anxiety disorders (78.3%; p = 0.05), somatoform disorders (13.1%; p = 0.05) and post-traumatic stress disorder (30.3% p = 0.003). OCD patients that presented ADHD as the first diagnosis (N = 50, age of onset ADHD 6.42; age of onset OCD 13.08) had higher frequency of substance abuse (16.0%; p = 0.00001). OCD patients that presented tic disorders (N = 44, age of onset tic disorder 6.86; age of onset OCD 16.18) as first diagnosis had higher frequency of OC spectrum disorders (50.0%; p = 0.03).
Discussion: The first psychopathological manifestations are associated with distinct long-term trajectory of psychiatric disorders in OCD patients. From a developmental perspective OCD does not seem to behave as a unitary disorder but rather as a group of symptoms that interact with other early psychiatric disorders increasing the vulnerability for the development of subsequent specific disorders. Prospective, longitudinal studies beginning in childhood are required to confirm these findings and to evaluate the effect of early interventions in preventing the development of additional psychopathology in OCD children with early onset of psychiatric disorders.

94. The Relationship of Adult Attention Deficit Hyperactivity Disorder to Anxiety Disorders in a Clinical Sample

Michael Van Ameringen*, William Simpson, Beth Patterson

McMaster Univeristy, Hamilton, Canada

Background: Adult Attention Deficit Hyperactivity Disorder (ADHD) is a life-long, chronic disorder which has its onset in childhood and is associated with significant functional impairment. Upwards of 36% - 55% of childhood cases maintain symptoms into adulthood. The rate of lifetime DSM-IV ADHD in the community is 8.1%. ADHD appears to be highly comorbid with other psychiatric disorders. There is a dearth of information about the prevalence of ADHD in anxiety disorder clinical samples.
Methods: Consecutive patients referred to an anxiety disorders clinic in Hamilton Canada, completed the Adult ADHD self-report scale and assessed with a Structured Clinical Interview for DSM-IV, and the ADHD module of the Mini International Neuropsychiatric Interview (MINI).
Results: Of the 264 patients assessed, the rate of lifetime ADHD was 37.5 % with48.5% male;and 51.5 % female, p < .05). ADHD was significantly associated with a primary diagnosis of impulse control disorder (23.2% vs. 13.3%, p < .05) and bipolar disorder (5.1% vs. 0.6%, p < .05), and most commonly associated with social phobia (57.6%, NS) and Major Depressive Disorder (56.6%, NS). Those with ADHD had a significantly higher number of comorbid disorders than those without ADHD (3.8 ± 1.8 vs. 3.1 ± 1.5, p < .001.). Symptom severity measure scores on the Padua Inventory, Yale-Brown Obsessive Compulsive Scale (p < .05), Sheehan Disability Scale (SDS) (p < .05), Anxiety Sensitivity Index (ASI) (p < .05), the QUIDS depression rating scale (p < .001), the Penn State Worry Questionnaire (p < .05) and the Davidson Trauma Scale (p < .001) were significantly higher in the ADHD group. In those with ADHD, who had comorbid generalized anxiety disorder, the Clinical Global Impression- Severity Scores (CGI-S) were higher (p < .05) and those with ADHD and comorbid panic disorder with agoraphobia had higher CGI-S, SDS (p < .05) and Quality of Life and Enjoyment Scale scores (p < .001). Individuals with high ADHD severity (defined by symptom count on the MINI), had a higher number of lifetime comorbid diagnoses (p < .05), and higher scores on the CGI-S, QUIDS and ASI than those with milder ADHD severity. Males were more likely than females to have received ADHD treatment in the past. Seventy-six percent (75/ 99) of those diagnosed with Adult ADHD on the MINI had never received the diagnosis previously, and 17.2% had received ADHD treatment in the past. Of the patients who had received previous ADHD diagnoses, 25% were diagnosed in childhood.
Discussion: The prevalence of lifetime ADHD was higher in our anxiety disorders clinic sample than that found in the general population. Individuals with ADHD had higher rates of impulse control disorder and bipolar disorder, which is consistent with the literature. These individuals also had more severe OCD, depressive, generalized anxiety, and disability symptoms than those without ADHD. Despite meeting DSM-IV criteria for lifetime ADHD, most patients in this sample had never been diagnosed.

The presence of ADHD appears to have a significant impact on the severity and impact of comorbid anxiety disorders.

95. Paternal Age and Risk of Bipolar Disorder in Offspring

Alan S. Brown*, Ian W. McKeague, YuanYuan Bao, Ling Shen, Catherine A. Schaefer

New York State Psychiatric Institute, Columbia University College of Physicians and Surgeons, New York, USA

Background: Advanced paternal age is a well-replicated risk factor for schizophrenia. Bipolar disorder (BD) shares several features with schizophrenia, including susceptibility genes, familial aggregation, presence of psychotic symptoms, and response to antipsychotic medications. To date, however, only two previous studies have specifically investigated the question of an association between paternal age and risk of BD.

Methods: In a follow-up of the Child Health and Development Study (CHDS), a large birth cohort, we investigated the relationship between prospectively documented paternal and maternal age and risk of BD. Cases with BD were identified by registry linkages between CHDS and Kaiser Permanente Medical Plan (KPMCP) and Alameda County Behavioral Health Care Services (ABHCS) on BD diagnoses, and mailed questionnaires to mothers and offspring in the CHDS. Potential cases were interviewed with the SCID for DSM-IV-TR and consensus diagnoses were made by three psychiatrists. Data on paternal age were acquired at birth.

Results: Paternal age modeled as a continuous variable was not associated with risk of BD (OR = 1.00, 95% CI = 0.971, 1.037, p = 0.834). Adjustment for maternal age had little effect on this result (OR = 1.027, 95% CI = 0.977, 1.078, p = 0.295). In the analysis of individual paternal age categories, there was no relationship with BD. Maternal age was also not related to BD (OR = 0.98, 95% CI = 0.946, 1.018, p = 0.31).

Discussion: These findings suggest that advanced paternal age is not a risk factor for BD, or that if such a relationship exists, it is negligible compared to that for schizophrenia. Conceivably, BD is less related to effects of paternal age on *de novo* mutations, epigenetic factors, or other biological or psychosocial anomalies that are related to advanced paternal age. These findings address the Kraepelinian dichotomy between schizophrenia and BD, insofar as they may facilitate efforts to dissect these two disorders from one another at the etiopathogenic level, with potential preventive and treatment implications.

96. Health Behaviors Contribute to Arterial Stiffness Later in the Course of Bipolar Disorder

Simrit Sodhi, Jonathan Linder, William H. Coryell, Del D. Miller, William G. Haynes, Jess G. Fiedorowicz*

University of Iowa, Iowa City, USA

Background: Patients with bipolar disorder face excess mortality from cardiovascular disease, although the biobehavioral mechanisms and time course are unclear. Arterial stiffness is a measure of vascular dysfunction and an important predictor of cardiovascular disease. Arterial stiffness can be measured using pulse wave velocity (PWV) and estimated aortic systolic pressure augmentation index (AI); both are non-invasive and have well validated population norms. We sought to evaluate the impact of health behaviors such as physical inactivity, diet, smoking, obesity and pharmacological treatment on the risk of developing vascular disease in patients with bipolar disorder.

Methods: 62 individuals with bipolar disorder (mean (SD) age 33 (6.7) years (range 20-46); 64% female) consented to non-invasive assessments of arterial stiffness after a two hour fast. We assessed lifetime tobacco exposure (pack*years) by clinical interview, physical activity with the long-version of the International Physical Activity Questionnaire, and diet by the Healthy Eating Index. Medication histories were obtained through systematic review of pharmacy records over the past five years. Expected values for our two primary outcomes, AI and PWV, were calculated from published age-based norms using relevant clinical information (higher values indicate greater arterial stiffness). These expected values were compared to our observed values using paired t-tests. The impact of behavioral risk factors on arterial stiffness was evaluated in multivariate linear regression models.

Results: The AI for participants was greater than expected relative to expected from published population norms, based on age, height, and heart rate (10.5 vs. 7.7, p = 0.04). Pulse wave velocity did not significantly differ between participants and the expected reference values, based on age and blood pressure (6.9 vs. 6.7 m/s, p = 0.14). Participants over the age of 32 (median split) had greater arterial stiffness than expected from age-based population norms for AI (14.2 vs. 8.2, p = 0.0002) and PWV (7.6 vs. 7.0 m/s, p = 0.02). The younger portion of the sample did not differ from expected values on these measures (PWV 6.3 vs. 6.4 m/s, p = 0.94; AI 7.6 vs. 7.4, p = 0.66). Age was highly correlated with retrospectively estimated illness duration (r = 0.72, p = 0.001). Diet was substantially poorer than expected from normative data. In the full sample, multivariate models including age, gender, body mass index and behavioral risk factors (diet, physical activity, tobacco exposure), revealed a significant effect of age and body mass index on arterial stiffness measures. However, behavioral risk factors contributed to arterial stiffness for the older half of the sample. In reduced models to avoid over-fitting in this sub-sample, greater physical activity was significantly associated with a lower augmentation index (partial $R^2 = 0.26$, p = 0.01) and better diet approached significance with slower pulse wave velocity (partial $R^2 = 0.16$, p = 0.055). The effects of physical activity and diet were independent of body mass index, which was also significantly associated with both arterial stiffness measures. The inclusion of exposure to antipsychotics or mood stabilizers to models did not substantially alter these findings.

Discussion: Risk for vascular disease appears to be primarily acquired over the long-term course of affective illness rather than inherent to the mood disorder itself, as evidenced by greater arterial stiffness relative to age-based norms for the older but not younger half of this sample. Maladaptive health behaviors, such as poor diet and physical inactivity, contribute to risk and may be amenable to intervention early in the course of illness, prior to the onset of vasculopathy. Addressing these behavioral risk factors presents a potential opportunity for clinicians working with this at-risk population to impact risk.

97. Maternal Iron Deficiency and Risk of Bipolar and Schizophrenia

Y.-Y. Ho*, Y. Bao, I.W. McKeague, L. Shen, C.A. Schaefer, A.S. Brown

Departments of Psychiatry and Biostatistics, Columbia University College of Physicians and Surgeons, New York, USA

Background: Bipolar disorder (BD) shares several features with schizophrenia (SZ), including susceptibility genes, familial aggregation, presence of psychotic symptoms, and response to antipsychotic medications. We have previously reported an association between maternal iron deficiency and risk of SZ in offspring. In the present study, we investigated the association between maternal hemoglobin level, a robust marker of iron deficiency and risk of BD.

Methods: In a nested case-control study based on a follow-up of the Child Health and Development Study (CHDS), a large birth cohort, we assessed the association between maternal hemoglobin level and risk of BD (72 cases, 572 controls) by logistic regression. Cases with BD were identified by registry linkages between CHDS and Kaiser Permanente Medical Plan (KPMCP) and Alameda County Behavioral Health Care Services (ABHCS), and mailed questionnaires to mothers and offspring in the CHDS. Potential BD cases were interviewed with the SCID for DSM-IV-TR and consensus diagnoses were made by three psychiatrists. Maternal hemoglobin data were extracted from prenatal medical records. The mean maternal hemoglobin concentration during the entire pregnancy was chosen as the primary exposure for association with risk of BD.

Results: Statistical analyses did not detect an association between maternal anemia (defined as hemoglobin 10.0 g/dl) and risk of BD (OR = 1.22, 95% CI = 0.42 - 3.50, p = 0.712).

Discussion: Our findings do not support a relationship between maternal iron deficiency and BD in offspring, in contrast to our previous findings for SZ. These results indicate an etiological distinction between BD and SZ. A comparison of effect sizes of maternal anemia between BD and SZ will also be presented.

Disclosure: Y. Ho: None. **Y. Bao:** None. **I. McKeague:** None. **L. Shen:** None. **C. Schaefer:** None. **A. Brown:** None.

98. Sleep Apnea Risk and Clinical Correlates in Patients with Bipolar Disorder

Isabella Soreca*, Jessica C. Levenson, Meredith J. Lotz, Ellen Frank, David J. Kupfer

University of Pittsburgh, Pittsburgh, USA

Background: In spite of the high prevalence of well documented risk factors for obstructive sleep apnea (OSA), the actual prevalence of sleep-related disordered breathing in bipolar disorder (BD) has not been systematically investigated. The clinical presentation of OSA may overlap substantially with that of major depression including report of poor sleep quality, daytime fatigue, cognitive deficits and somatic symptoms. Since the depressive phase of bipolar disorder is often chronic and difficult to treat and undiagnosed underlying medical conditions may greatly contribute to its unfavorable outcome, we hypothesized that OSA may play a role in this clinical picture. Therefore we sought to determine the risk for OSA in a population of 72 patients with bipolar disorder, type I, in an outpatient maintenance treatment protocol. Participants completed a validated instrument that determines risk for OSA. We then compared the demographic and clinical characteristics of patients at high risk (HR) and low risk (LR) for OSA. We also tested the hypothesis that being at high risk for OSA would be associated with greater severity of the mood disorder, independent of sleep disturbance.

Methods: *Participants:* subjects were enrolled in an outpatient maintenance treatment study. Inclusion criteria of the parent study: being diagnosed with bipolar disorder, type I, free of unstable medical conditions, a BMI 25 and in clinical remission. *Measures:* Mood symptom severity was assessed with the HAM-D and YMRS. Body mass index, waist circumference and blood pressure were recorded at every clinic visit. At baseline participants were also asked to complete the Pittsburgh Sleep Quality Index (PSQI). OSA risk was determined with the Berlin Questionnaire. *Statistics:* Group differences were assessed using T-Test for continuous variables to determine main effects of Berlin risk group category. The relationships between Berlin risk group category and categorical variables were assessed using Chi-square tests. A two-step hierarchical regression analyses was performed to assess the association between HAM-D and YMRS scores and OSA risk, adjusting for relevant covariates.

Results: Of the 72 patients evaluated, 39 (54.1%) fell in the HR category for OSA. The HR group did not significantly differ from the LR in terms of age, but had a higher mean BMI [p = .014]and waist circumference [p = .027]. Participants in the HR group were more likely to be on disability (35.8% versus 12.1%).Participants in the HR group had significantly higher scores on the HAM-D [p = .000] and YMRS [p = .022]. This difference remained significant when the items that assess sleep disturbance were removed from the total score. Despite the fact that all the participants were required to be in remission at entry into the parent study, the HR group had also higher baseline depressive symptoms [p = .037]. The first regression model showed that the sleep apnea risk category explained nearly 15% of the HAM-D variance [Step 1 R^2 = .202; step 2 ΔR^2 = .151, adjusted ΔR^2 = .142, F Change = 10.878, p = .002]. The association between sleep apnea risk category and YMRS was not significant.

Discussion: We found that over half of overweight or obese individuals with bipolar I disorder can be considered at high risk of OSA. This prevalence exceeds what reported in the general population and in overweight or obese individuals. Although all study participants were required to be in remission at study entry, those in the HR group had greater severity of mood symptoms both a study entry and at the point at which the risk for OSA was assessed. This suggests that untreated sleep disturbance and hypoxia resulting from apneic/hypopneic events may contribute to an unfavorable course of the psychiatric illness. Self-reported sleep quality as measured by the PSQI was not significantly different between the two risk groups, suggesting that the most frequently used clinical measures of sleep disturbances may not capture sleep apnea risk. Moreover, in the vast majority of cases, sleep apnea is asymptomatic, therefore suggesting that a screening based solely on subjective sleep quality may not be sensitive to apnea risk. More specifically, these results indicate that a sleep apnea evaluation should be considered for those patients with bipolar disorder who are overweight or obese and have chronic mood symptoms.

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99. Examining the Validity of Cyclothymic Disorder in a Youth Sample: Results from Two Studies

Anna Van Meter, Eric Youngstrom*, Jennifer Youngstrom, Norah Feeny, Christine Demeter, Robert L. Findling

University of North Carolina at Chapel Hill, Chapel Hill, USA

Background: Four subtypes of bipolar disorder (BP) – bipolar I, bipolar II, cyclothymia and bipolar not otherwise specified (NOS)

– are defined in DSM-IV-TR and proposed for retention in DSM-5. However, subthreshold bipolar is under-represented in research, and often described in idiosyncratic ways (Youngstrom, 2009). Though the diagnostic criteria for each subtype are consistent for both adults and children, research investigators and clinicians often stray from the DSM when diagnosing pediatric bipolar disorder (PBD) (Leibenluft et al., 2003). Cyclothymic disorder is rarely described in research or subsumed in a generic "NOS" category, and little is known about its presentation in children. However, epidemiological studies indicate that cyclothymia is not uncommon and causes marked impairment among young people (Lewinsohn, Klein, & Seeley, 1995, 2000). Many youth with cyclothymia are not receiving appropriate treatment and may be misdiagnosed with other conditions.

Two separate studies investigated the clinical correlates of cyclothymic disorder and compared it to other bipolar and nonbipolar diagnoses using a variation of the validation method proposed by Robins and Guze (1970).

Methods: For both studies, eligible youths were 5 to 18 years. Diagnoses were made according to strict DSM-IV criteria, based on a KSADS interview with the caregiver and youth. Study 1 ($N = 827$) recruited from an urban community mental health center and an academic research clinic, and included $n = 52$ with cyclothymic disorder. Study 2 ($N = 894$) recruited from a psychiatric research center with a focus on bipolar disorders, $n = 53$ with cyclothymic disorder. Both studies compared cyclothymic disorder to other BP spectrum disorders and to all non-bipolar disorders. Additionally, Study 2 investigated whether having a parent with bipolar disorder changed correlates. Key features investigated in these studies include irritability, comorbid diagnoses, age of onset, sleep disturbance, and family history of mental illness.

Results: Study 1: Cyclothymia could be differentiated from the rest of the sample on all key features, exhibiting high levels of irritability associated with both depressive and elevated mood ($p < .001$), high rates of comorbid ADHD (83%) and anxiety (37%), onset before 10 years old ($p < .0001$), sleep disturbance ($p < .01$), and high rates of familial psychiatric disorder (100%). Cyclothymic disorder also shares many characteristics with other bipolar subtypes, situating it firmly on the bipolar spectrum. However, cyclothymia could be reliably differentiated from other BP subtypes by higher irritability (p < .05; BP I and II), ADHD comorbidity (p < .05; BP I, II and NOS), and sleep disturbance (p < .01; BP II). Study 2 provided strong replication. When compared to youth with non-bipolar disorders, cyclothymia had higher levels of irritability ($p < .005$), more comorbidity ($p < .0005$), greater sleep disturbance ($p < .005$), and were more likely to have a family history of bipolar disorder ($p < .0005$). Cyclothymia was associated with a significantly younger age of onset compared to unipolar depression ($p < .0005$) or bipolar II ($p = .05$). There were no statistically-significant findings in the examination of youth with and without parental bipolar disorder, though trends regarding age of onset and irritability were found.

Discussion: Results show that cyclothymic disorder shares many characteristics with other bipolar subtypes and belongs on the bipolar spectrum. Additionally, there is evidence of important differences in the subthreshold subtypes of bipolar disorder; unfortunately, most studies of pediatric bipolar disorder fail to differentiate between cyclothymia and the various definitions of NOS, in spite of the fact that these subtypes may represent the greatest opportunity for preventative intervention, and are likely the most prevalent presentation of bipolarity. Prospective studies, including family history, genetic, and biologic data, are crucial to the goal of better understanding the etiology and course of subthreshold bipolar disorders.

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Findling: Part 1: Dr. Findling receives or has received research support, acted as a consultant and/or served on a speaker's bureau for Abbott, Addrenex, Alexza, AstraZeneca, Biovail, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Johnson & Johnson, KemPharm Lilly, Lundbeck, Merck, Neuropharm, Novartis, Noven, Organon, Otsuka, Pfizer, Rhodes Pharmaceuticals, Sanofi-Aventis, Schering-Plough, Seaside Therapeutics, Sepracore, Shire, Solvay, Sunovion, Supernus Pharmaceuticals, Transcept Pharmaceuticals, Validus, and Wyeth., Part 2: Dr. Findling has received income sources and equity of $10,000 per year from Shire., Part 3: None, Part 4: Abbott, Addrenex, AstraZeneca, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Johnson & Johnson, Lilly, Merck, Neuropharm, Otsuka, Pfizer, Rhodes Pharmaceuticals, Schering-Plough, Shire, Supernus Pharmaceuticals, and Wyeth., Part 5: N/A.

100. Altered Anxiety-Like Behavior in BDNF Val66Met Mice is Rescued with Early-Life Fluoxetine

Iva Dincheva* Jianmin Yang, Tina Marinic, Kevin Bath, Helena Frielingsdorf, Theresa Teslovic, James Kocsis, Barbara Hempstead, Francis Lee

Weill Medical College of Cornell University, New York, USA

Background: The Val66Met single nucleotide polymorphism (SNP) in the human brain-derived neurotrophic factor (BDNF) gene is associated with alterations in brain anatomy and memory in humans, however its role in affective disorders still remains unclear. Previously, we generated a variant BDNF mouse (BDNF$^{Met/Met}$) that reproduces the phenotypic hallmarks of human Met allele carriers such as alterations in hippocampal anatomy and hippocampal dependent memory. Variant BDNF$_{Met}$ is expressed in mouse brains at normal levels, however its secretion from neurons is decreased. In addition, BDNF$^{Met/Met}$ mice displayed an increased anxiety-like phenotype that emerges in adulthood, which was not previously established in human carriers. This behavior is not normalized by the selective serotonin reuptake inhibitor, fluoxetine, administered in adulthood. We hypothesized that this behavioral consequence of the genetic variant BDNF$_{Met}$ observed in the adult central nervous system was due to reduced BDNF availability during postnatal development. We tested whether transiently increasing BDNF levels via fluoxetine administration during an early-life "sensitive period" when BDNF levels are peaking may prevent the emergence of the increased anxiety-like behavior in BDNF$^{Met/Met}$ mice in adulthood.

Methods: BDNF$^{Val/Val}$ and BDNF$^{Met/Met}$ mice received fluoxetine (160 mg/L in drinking water) in three age groups: P21-P42, P40-P61, and P60-P81. Anxiety-like behaviors were tested after a 3-4 week wash-out period. Hippocampal BDNF levels and neuronal morphology were also assessed.

Results: Early-life (P21-P42) fluoxetine treatment significantly reduced anxiety-like behaviors in BDNF$^{Met/Met}$ mice in adulthood. Fluoxetine given at other time periods to either BDNF$^{Val/Val}$ or BDNF$^{Met/Met}$ mice had no significant effects on anxiety-like behaviors. BDNF levels increased at the end of each treatment period, but were normalized with wash out. The highest levels of total BDNF levels were achieved when fluoxetine was administered during the P21-P42 period.

Discussion: Fluoxetine administration during a peri-adolescent period (P21-P42) with subsequent wash–out leads to a rescue of an adult-onset anxiety-like phenotype in BDNF$^{Met/Met}$ mice. This transient drug administration led to peak BDNF levels, which may have increased BDNF availability during this developmental window in BDNF$^{Met/Met}$ mice. The findings suggest that correctly timed interventions which raise BDNF levels during a defined postnatal period can rescue subsequent anxiety-like phenotypes in a mouse model of the BDNF Val66Met SNP.

101. Convergent Functional Genomics of Anxiety Disorders: Translational Identification of Genes, Biomarkers, Pathways and Mechanisms

Helen Le-Niculescu, Yokesh Balaraman, Sagar Patel, Mikias Ayalew, Ronald Kuczenski, Anantha Shekhar, Nicholas Schork, Mark Geyer, Alexander Niculescu*

IU School of Medicine, Indianapolis, USA

Background: Anxiety disorders are prevalent and disabling yet understudied from a genetic standpoint, compared to other major psychiatric disorders such as bipolar disorder and schizophrenia. The fact that they are more common, diverse, and perceived as embedded in normal life may explain this relative oversight. In addition, as for other psychiatric disorders, there are technical challenges related to the identification and validation of candidate genes and peripheral biomarkers. Human studies, particularly genetic ones, are susceptible to the issue of being underpowered, due to genetic heterogeneity, the effect of variable environmental exposure on gene expression, and difficulty of accrual of large well phenotyped samples. Animal model gene expression studies, in a genetically homogeneous and experimentally tractable setting, can avoid artifacts and provide sensitivity of detection. Subsequent translational integration of the animal model datasets with human genetic and gene expression datasets can ensure cross-validatory power and specificity for illness.

Methods: We have used a pharmacogenomic mouse model (involving treatments with an anxiogenicdrug- yohimbine, and an anti-anxiety drug—diazepam) as a discovery engine for identification of anxiety candidate genes as well as potential blood biomarkers. Gene expression changes in key brain regions for anxiety (prefrontal cortex, amygdala, hippocampus) and blood were analyzed using a Convergent Functional Genomics (CFG) approach, which translationallyintegrates our new data with published human and animal model data, as a translational strategy of cross-matching and prioritizing findings.

Results: Our work identifies top candidate genes (such as FOS, GABBR1, NR4A2, DRD1, ADORA2A, QKI, RGS2, PTGDS, HSPA1B, DYNLL2, CCKBRand DBP), brain-blood biomarkers (such as FOS, QKI and HSPA1B), pathways (such as cAMPsignaling) and mechanisms for anxiety disorders -notably signal transduction and reactivity to environment.

Discussion: Overall, this work complements our previous similar work (on bipolar mood disorders and schizophrenia) conducted over the last decade. It concludes our programmatic first pass mapping of the genomic landscape of the triad of major psychiatric disorders using CFG. These studies permitted us to uncover the significant genetic overlap between anxiety and these other major psychiatric disorders, notably the under-appreciated overlap with schizophrenia, which suggests schizo-anxiety as a possible new nosologicalentity.

102. Maternal Behavior and DNA Methylation of Nr3c1 and Egr1 Genes Show Sex Differences and are Altered by Sex Composition of the Litter

Therese A. Kosten*, Wen Huang, David A. Nielsen

Baylor College of Medicine, Houston, USA

Background: Early life events can influence vulnerability to various mental disorders. In rats, early life events have enduring neural, hormonal, and behavioral effects. Recent research shows such events induce changes in gene expression resulting in stable phenotypic alterations through DNA methylation. Specifically, greater frequency of anogenital licking received as a pup relates to lower DNA methylation of Nr3c1 (Gr17), the glucocorticoid receptor gene, and Erg1 (NGF1), that encodes a transcriptional activator of Nr3c1 and increases its transcription, in adult hippocampus (HP), an area that plays an important role in modulating stress responses. We recently showed DNA methylation levels of another gene (Oprm1) showed sex differences and was altered by manipulating the sex composition of the litter. Here, we test for sex differences and if altering sex composition of the litter affects frequency of anogenital licking and DNA methylation levels of Nr3c1 and Erg1 promotor regions in HP and nucleus accumbens (NAc).

Methods: Sprague-Dawley rats were bred and litters culled to 8 pups on post-natal day 1 (PN1). Litters were either same sex (all male or all female; n's = 10-12) or mixed-sex (4 male, 4 female; n = 20). On PN4, 7, and 10, dams were removed from the cage and placed in an observation tank. One pup was placed with the dam 30-min later for a 10-min session that was video-recorded. A rater blind to sex and condition rated the sessions to determine frequencies of anogenital licking and passive nursing and to record times the pup moved towards the dam. Brain tissue samples were taken from HP, NAc, and cerebellum (CB) after sacrifice on PN35. DNA from these tissues was bisulfite-treated and percent cytosine methylation levels of the Nr3c1 and Egr1 gene promoters quantified.

Results: Frequency of anogenital licking was greatest for males from mixed-sex litters (P < 0.05). Male pups also tended to spend more time moving towards their dam than female pups (P < 0.07). There was no effect of sex or litter sex composition on passive nursing (P > 0.10). Sex and litter sex composition interacted to alter DNA methylation levels across CpG sites for both Nr3c1 and Egr1 genes and at specific CpG sites in HP and NAc (P's < 0.05). Overall DNA methylation levels of Egr1 in CB were differentially affected by sex and sex composition of the litter as well (P < 0.05). These factors interacted in HP to alter methylation of a CpG site found within a cyclic-AMP response element (CRE); this site was hypermethylated in males but hypomethylated in females from same-sex litters compared to mixed-sex litters (P < 0.001). Trends for sex and interaction with litter sex composition at this CpG site were seen in NAc (P's < 0.10). But in NAc, trends reflected an opposite effect from HP – hypomethylation in males from same-sex litters and hypermethylation in males from mixed-sex litters.

Discussion: Results confirm the sex-dependent effects of maternal behavior in rats. Male pups are licked more in the anogenital region than female pups but only if males are in mixed-sex litters. Male pups from same-sex litters are licked the least, especially on PN3, even though litter sex composition does not alter the tendency of male pups to spend more time moving towards the dam relative to female pups. Frequency of anogenital licking received is linked to stress responsivity in adult and associated with DNA methylation levels of Nr3c1 and Egr1 genes in HP. By manipulating the sex composition of the litter and performing assessments in rats of both sexes, we tested if greater frequency of anogenital licking associates with lower DNA methylation levels. That is, DNA methylation levels should be lowest for mixed-sex males and highest for same-sex males and perhaps have little effect in females. Indeed, this was the case for Nr3c1 in NAc, but not in HP as expected. Yet, being raised in same-sex litters had opposing effects on DNA methylation levels of

Egr1 in HP for the sexes, increasing it in males and decreasing it in females. Although these results for *Egr1* in HP were anticipated, based on maternal care findings, the group that received the least amount of anogenital licking (same-sex males) showed the least amount of methylation of *Egr1* in NAc. Thus, the relationship between maternal care and DNA methylation of genes involved in stress responsivity is sex-dependent, varies by brain area, and is altered by the early life event of manipulating sex composition of the litter. Support: DA020117.

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103. Effect of APOE Genotype on Brain Functional Connectivity During Episodic Memory Encoding in Healthy Aging
Joseph C. Masdeu*, Qiang Chen, Venkata Mattay, Philip Kohn, John Muse, Bhaskar Kolachana, Lisa Nichols, Daniel R. Weinberger, Karen F. Berman

National Institutes of Health, Bethesda, USA

Background: The apolipoprotein E (APOE) e4 allele confers the highest genetic risk for Alzheimer's disease. Because this disorder begins with neuronal loss in the medial temporal lobe (MTL), critical for episodic memory mechanisms, the effect of APOE e4 on memory-related neural activation has been extensively studied. We recently found that healthy individuals carrying this genotype do not show the same age-related decrease in MTL activation that e2 carriers and e3 homozygotes do (Nichols et al., 2010) raising the speculation that functional connectivity with aging may also differ among APOE genotypes. Age-related shifts in the pattern of activation and in functional connectivity have been described. For example, activation shifts from posterior regions, including MTL, to the frontal lobes (e.g. Davis et al., 2008), and increased connectivity in task-related areas is associated with good performance in aging, suggesting a compensatory role (Grady et al., 2010). If task-related connectivity were also modified by APOE genotype, fronto-MTL connectivity might be expected to increase as a compensatory mechanism in older e4 carriers. Few studies have evaluated how APOE genotypes affect task-related connectivity, particularly in healthy aging. We determined the effect of APOE genotype on task-related connectivity in a large sample of healthy individuals with ages spanning adulthood.
Methods: One-hundred eighty six (188) right-handed healthy Caucasian volunteers aged 18-69 years were genotyped for APOE and underwent 3T BOLD fMRI during visual scene encoding. The fMRI time series were pre-processed using standard procedures in SPM5. The resulting β images were analyzed using a psychophysiological interaction (PPI) analysis implemented in SPM5. PPI with the entire brain was obtained from seed regions in the DLPFC, anterior and posterior hippocampi, and parahippocampal gyri of both hemispheres. The first level maps were then taken to a second-level random-effects analysis to examine differences across genotype groups in functional coupling of the seed regions to other brain areas as a function of the episodic memory task. In addition to whole-brain analyses, we analyzed connectivity of the frontal seeds with a MTL volume of interest (VOI) and of the MTL seeds with a frontal VOI, both VOIs derived from the SPM Pick Atlas tool.
Results: The three APOE genotype groups studied (e2/3, n=27; e3/3, n=117 and e3/4, n=44) did not differ statistically in age (34±13, 31.6±11, and 32.4±12 years) sex (62, 59, and 59 percent women) or performance (83±9, 86±8 and 87±9 percent correct responses). Thus, differences in the fMRI data across genotypes reflected differences in brain information processing rather than demographics or task performance. PPI connectivity of the left DLPFC with the left posterior parahippocampus (MNI x,y,z=24,36,9;

p=0.032, VOI-FWE-corrected) increased with aging in the e4 carriers, but not in the other two genotypes. A similar relationship was found for connectivity of the right anterior hippocampus with the right lateral prefrontal cortex (33,45,3; p=0.032 FWE) and with the right DLPFC (33,9,21; p<0.0005 uncorrected) and anterior portion of the right cingulate gyrus (12,30,21; p<0.0003) as well as in the connectivity of the right posterior parahippocampus with the opercular portion of the right inferior frontal gyrus (39,36,3, p<0.0007). The opposite relationship (fronto-MTL PPI connectivity tending to decrease with aging in the e4 carriers as compared to the other two genotypes) was not found at corrected significance or even at a trend level in fronto-temporal networks.
Discussion: Our findings support the hypothesis that e4 carriers have increased fronto-MTL PPI connectivity with aging and are in agreement with studies showing greater resting MTL connectivity in e4 carriers (Westlye et al., 2011; Filippini et al., 2009). Because faulty neural repair has been extensively documented in e4 carriers and more recently they have been found to have increased amyloid deposition years before the clinical onset of memory impairment (Mahley et al., 2005; Castellano et al., 2011), increased activation observed in the MTL of elderly carriers has been postulated to reflect inefficient local MTL neural mechanisms (Nichols et al., 2010). Similarly to the activation changes observed in e4 carriers with aging, increased fronto-temporal PPI connectivity in the context of adequate task performance may reflect compensation for a less efficient neural substrate in this genotype.
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104. CDH2 Gene Variants in Obsessive Compulsive Disorder and Tourette Syndrome
Pablo R. Moya*, Jens R. Wendland, Kiara C. Timpano, Liza M. Rubenstein, Zaker Rana, Ruby Fried, Marzena Galdzicka, Edward I. Ginns, Nicholas H. Dodman, Dennis L. Murphy

Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, USA

Background: Obsessive-Compulsive Disorder (OCD) is a chronic, severe neuropsychiatric disorder characterized by intrusive ideations, images or urges (obsessions) and rigid, ritualistic behaviors (compulsions) usually associated with anxiety or dread. OCD has a prevalence of 2-3% of the world population. There is significant evidence, from twin and family studies, indicating that OCD has a genetic component. Similarly, Canine Compulsive Disorders comprise time-consuming, repetitive behaviors causing distress and functional impairment, apparently derived from normal behaviors such as grooming, predatory behavior, eating/suckling or locomotion. Thus, these disorders have compelling parallels with human OCD. A recent report of a genome-wide association analysis of compulsive behaviors in Doberman Pincher dogs indicated a highly significant association with the *Cdh2* region on canine chromosome 7. Therefore, we searched for gene variants in the human orthologue *CDH2* that could contribute to OCD. Unusual phenotypical features found associated with some of the *CDH2* variants in OCD patients prompted us to extend our study in Tourette syndrome (TS) probands.
Methods: We initially sequenced the *CDH2* gene in a subsample of 160 OCD probands and 160 healthy controls from our DNA collection. Sequencing methodology, assay design, amplification and Sanger sequencing were carried out by the NIH Intramural Sequencing Center (NISC). Next, we performed direct genotyping using TaqMan SNP Genotyping 5́ nuclease Assays to confirm those sequencing results in the 320 original sample set as well as to extend genotyping in additional OCD probands and controls

(N = 520). We also genotyped *CDH2* variants in a collection of TS probands, their relatives and additional controls (N = 604).

Results: We successfully sequenced 15 *CDH2* exons; exon 1 could not be amplified despite multiple primer re-designs. We detected four non-synonymous SNPs; two of them being novel variants. No variant was nominally significantly different between the two groups. One novel SNP (N706S; c.2117A > G) was found exclusively in OCD/TS patients and not in any of the 620 controls. One non-synonymous variant (845S) was found to be associated with OCD + TS (43%) compared to N845 (11%) in the sequenced OCD sample and likewise in the additional genotyped OCD samples; this missense variant was also overrepresented in TS probands. Combination of all OCD/TS probands with 845S compared to those with N845 yielded a highly significant contrast and odds ratio ($X^2 = 9.36$; p = 0.002; OR = 6.03) indicative of a strong relationship between *CDH2* N845S and TS spectrum disorders.

Discussion: This is the first clinical study of the neuronal cadherin gene, *CDH2*, in human neuropsychiatric disorders. Sequence analysis of *CDH2* verified the existence and confirmed the relatively low heterozygosity of two missense SNPs (A118T and N845S) previously identified. Our study also revealed the existence of two novel additional missense SNPs (N706S and N485S). Other cadherins have been studied in small samples (<100 patients) of bipolar disorder, ADHD, schizophrenia and OCD patients without notable findings. Cadherins constitute a super-family of adhesion molecules that feature an amino-terminal tandem series of extracellular components (ectodomains) followed by a single anchoring transmembrane domain and a carboxy-terminal cytoplasmic region (150 amino acids aprox) that links cadherins to the underlying cytoskeleton via sequential binding of beta-catenin to alpha -catenin and then to actin. The novel *CDH2* N845S variant found in the present study lies in the highly-conserved cytoplasmic domain; damage to the integrity of this cytoplasmic domain leads to loss of adhesive function of the entire cadherin molecules. Further research is warranted to evaluate the impact of this (as well others) single point mutation on the citoplasmic domain functionality. While laboratory-induced variants in murine genes have recently been found to be associated with rare variants of orthologous human genes with OCD in two models, the present findings may be the first example of the value of using spontaneous mutations in canine genome to search for genetics variants in human neuropsychiatric disorders.

105. Variation in the OxytocinReceptor Gene is Associated with Increased Anxiety in Individuals with a History of Exposure to Early Life Stress

Amanda J. Myers*, Leanne Williams, Justine M. Gatt, Erica McAuley-Clark, Carol Dobson-Stone, Peter Schofield, Charles B. Nemeroff

University of Miami, Miller School of Medicine, Department of Psychiatry & Behavioral Sciences, Miami, USA

Background: Oxytocin (OXT) is synthesized in the hypothalamus, transported to the posterior pituitary and released into the bloodstream. OXT has been shown to have effects on memory (de Wield et al., 1993; Lerer et al., 2008), anxiety (Heinrichs et al., 2003) and social interaction (Uvnas-Moberg, 1998; Kosfeld et al., 2005). Previous work had demonstrated associations between the OXT receptor gene (*OXTR*) haplotypes and affectivity (Lucht et al., 2009) or depressive temperament (TEMPS-A scale, Kawamura et al., 2010). In this study, we attempted to replicate these findings, as well as assess whether *OXTR* variants were associated with increased stress and anxiety phenotypes using the Depression Anxiety Stress Scale (DASS).

Methods: Samples were obtained from the BRAINnet Database resource (www.brainnet.net). Individuals were assessed using the DASS, which is based on a dimensional construct of depression and anxiety. Additionally, individuals were assessed for the number of traumatic events they had been exposed to in early life using the Early Life Stress Questionnaire (ELSQ). Samples were genotyped using 10 single nucleotide polymorphisms (SNPs) to capture the haplotypic variance of the *OXTR*. Analysis was performed using SPSS v18 (Predictive Analytics SoftWare) using general linear models and t-tests. Quality metrics for samples included: 1. Removing samples with no phenotype and 2. Removing samples with call rates across all SNPs < 50%. Quality metrics for SNPs included: 1. Excluding SNPs with genotyping rates < 90% across all samples, 2. Excluding SNPs that were out of Hardy-Weinberg equilibrium (HWE) and 3. Excluding SNPs with a minor allele frequency less than 5%.

Results: Six hundred and seventy-one samples were analyzed using 7 SNP variants. The three SNPs that were dropped were eliminated because they were out of HWE. Testing to see whether there was an interaction of early life stress (ELS) events and either the DASS anxiety, DASS stress or DASS depression scores demonstrated a significant difference in scores between individuals that had experienced ELS (> 1 trauma) and those that had not (p-value = 7.604E-09 for stress, 3.040E-06 for anxiety, 2.245E-11 for depression). Thus, DASS scores are significantly elevated for people with more than 1 ELS event. We then tested whether that interaction was modified by gender, age or years of education. None of the effects were significant except for the relationship between the DASS anxiety measure and age or education. For age, increases in age increased DASS anxiety sores (p-value = 0.009, Beta = 0.78) and for education, increases in education lowered DASS anxiety scores (p-value = 0.001, Beta = −0.75). Three *OXTR* SNPs were significant considering a model with the DASS anxiety score as the outcome, the number of ELS events as an interactor and correcting for age and education. None of the *OXTR* SNPs were significant considering models using DASS stress or depression scores as the outcome. We performed a haplotype test using the three SNPs and the same model and this was also significant considering DASS anxiety as the outcome. Finally, we examined variation across the *OXTR* in an addition set of ∼ 400 human brain samples which were neuropathologically and clinically normal to determine whether the variants that we mapped which interact with ELS and result in differences in anxiety cause differences in the expression of *OXTR*. We mapped several variants which cause changes in the *OXTR* transcript expression; however, these variants all mapped to a haplotype block which was independent from the original effect.

Discussion: We have found that variation in *OXTR* in the context of early life trauma can change anxiety levels later in life. We did not replicate some of the original *OXTR* findings showing an association between *OXTR* SNPs and depression measures.

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106. Resting Dorsal Anterior Cingulate Cortex Metabolism Mediates the Effect of a *FKBP5* Haplotype on Posttraumatic Stress Symptoms in Monozygotic Twins Discordant for Combat Exposure

Roger K. Pitman*, Joshua L. Roffman, Lisa M. Shin, Avital M. Fischer, Stephen A. Haddad, Richie Siburian, David G. Brohawn, Rachel Yehuda, Jordan W. Smoller

Massachusetts General Hospital, Boston, USA

Background: An endophenotype is a biological marker that mediates the effect of a gene on a disorder. An endophenotype should be present even if the illness is inactive. An endophenotype in a PTSD twin should also be present in his or her trauma-unexposed co-twin. In a study of identical twin pairs discordant for combat exposure in Vietnam, we found that resting metabolism, as measured by regional cerebral metabolic rate for glucose (rCMRglu), in the dorsal anterior cingulate cortex/midcingulate cortex (dACC/MCC) brain region, was greater in the co-twins of PTSD veterans than in the co-twins of non-PTSD veterans, making it a candidate PTSD endophenotype. The *FKBP5* gene has been found to be associated with PTSD. We tested whether dACC/MCC rCMRglu in co-twins mediates the effect of *FKPB5* on PTSD symptom severity in twins.

Methods: Subjects were 32 identical Caucasian twin pairs discordant for combat exposure in Vietnam. Combat severity was quantified by means of a retrospective self-report scale. Lifetime combat-related PTSD symptoms were quantified by the Clinician-Administered PTSD Scale (CAPS). Childhood traumatization was quantified by the Childhood Trauma Questionnaire. dACC/MCC rCMRglu was quantified by positron emission tomography using 18F-fluorodeoxyglucose. Five single nucleotide polymorphisms (SNPs) in the *FKBP5* gene were examined. Path analysis was performed by multiple regression. Mediation analyses were performed by a computer bootstrap version of the Sobel test.

Results: Because few subjects were homozygous for the minor allele at any of the five SNPs, minor homozygous subjects were combined with heterozygous subjects in a dominant model. Point-biserial correlations revealed that each SNP was significantly associated with both resting dACC/MCCrCMRglu in the combat-unexposed co-twin and lifetime CAPS score in the combat-exposed twin, with one small exception. Each of the 10 subject pairs that was a carrier of a T allele at rs1334894 (designated rs1334894-T) was also a C allele carrier at rs3800373, an A allele carrier at rs9296158; a T allele carrier at rs1360780, and a T allele carrier at rs9470080, all on the same chromosome, thereby forming a haplotype of the 5 SNPs, which were all minor alleles. Hence the results obtained for rs1334894-T equally apply to this haplotype. None of the predictor or outcome measures significantly correlated with Childhood Trauma Questionnaire score. The major significant results were as follows: 1) rs1334894-T negatively predicted PTSD symptoms in combat-exposed twins ($\beta = -0.38$); 2) rs1334894-T negatively predicted dACC/MCCrCMRglu in combat-unexposed co-twins ($\beta = -0.45$); 3) dACC/MCCrCMRglu in combat-unexposed co-twins positively predicted combat severity in combat-exposed

twins ($\beta = 0.43$); 4) Combat severity positively predicted lifetime CAPS score in combat-exposed twins ($\beta = 0.41$); 5) dACC/MCCrCMRglu in combat-unexposed co-twins positively predicted lifetime CAPS score in combat-exposed twins, mostly directly ($\beta = 0.39$) but also indirectly through combat severity ($\beta = 0.18$); 6) dACC/MCCrCMRglu in combat-unexposed co-twins was a significant mediator of the association between rs1334894-T and lifetime CAPS score in combat-exposed twins (effect $= -0.25$, $z = -2.2$, $p = 0.03$); 7) Combat severity was a significant mediator of the relationship between dACC rCMRglu in combat-unexposed co-twins and lifetime CAPS score in combat-exposed twins (effect $= 0.21$, $z = 2.1$, $p = 0.03$); 8) After removing (indirect) mediating effects, the direct effect of rs1334894-T on lifetime CAPS score was no longer significant ($\beta = -0.08$).

Discussion: These results support the conclusion that resting dACC/MCC glucose utilization is a PTSD endophenotype of the 5-SNP *FKBP5* haplotype studied here. dACC/MCC rCMRglu in the combat-unexposed co-twins significantly mediated the effect of the haplotype on PTSD symptom severity in the combat-exposed twins, mainly directly, but also indirectly through combat severity. However, establishing dACC/MCC rCMRglu's role as a true endophenotype would require showing that it plays its mediating role within the same subjects. This hypothesis could be tested within more abundant, trauma-exposed singletons (non-twins). Thus a role for twin studies might be to identify putative endophenotypes for studies in larger singleton samples. The sign of the effect of the *FKBP5* SNPs on PTSD symptoms observed here was opposite to that found in studies in which these SNPs interact with childhood traumatization to influence PTSD. This merits further investigation.

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107. Sex Differences in PTSD: Dissociation of the Roles for *SRD5A2* and *PACAP* Polymorphisms in a Highly Traumatized Civilian Population

Charles F. Gillespie, Lynn M. Almli, Bekh Bradley, Kristina B. Mercer, Elisabeth B. Binder, Karen N. Conneely, Joseph Cubells, Alicia K. Smith, Kerry J. Ressler*

Emory University, Atlanta, USA

Background: Traumatic events that produce extreme fear and horror are common, but not all individuals develop posttraumatic stress disorder (PTSD) as a result of such exposure. What mediates risk and resilience in the development of stress-related psychopathology is a critical question. Biological factors, such as genotype and neurobiology, interact with environmental factors, such as childhood background and trauma load, to affect vulnerability and resilience in the aftermath of trauma exposure. Additionally, burgeoning evidence suggests that differential biological factors may mediate risk in males and females following trauma exposure.

Methods: Study participants (N = 1443) were traumatized African-American patients of low socioeconomic status with high rates of lifetime trauma exposure recruited from the primary care clinics of a large, urban hospital. PTSD symptoms were measured with the post-traumatic stress symptom scale (PSS). A non-synonymous, single nucleotide polymorphism (SNP) in the gene coding for steroid 5-α-reductase type 2 (SRD5A2) is associated with reduced conversion of testosterone to dihydrotestosterone (DHT). Because SRD5A2 participates in the regulation of testosterone and cortisol metabolism, hormones shown to be dysregulated in patients with PTSD, we examined whether the V89L variant (rs523349) influences risk for post-traumatic stress disorder (PTSD). We also examined differential association between estrogen level, PACAP

gene expression, and PAC1 receptor polymorphisms that may differentially associate with PTSD in females but not males.

Results: With the SRD5a2 genotype, we initially found a significant sex-dependent effect of genotype in male but not female subjects on symptoms. Associations with PTSD symptoms were confirmed using a separate internal replication sample with identical methods of data analysis, followed by pooled analysis of the combined samples (N ~ 1500, sex x genotype interaction $p < 0.002$; males: $n = 536$, $p < 0.001$). Additionally, we report an extension of our prior work (*Nature*, 470(7335): 497-7)suggesting that the *PACAP* gene is differentially sensitive to estrogen and stress, and work demonstrating a possible functional polymorphism that lies within an estrogen response element within the PAC1 receptor.

Discussion: These data support the hypothesis that functional variation within *SRD5A2* and *PACAP* influence, in a sex-specific way, the severity of post-traumatic stress symptoms and risk for diagnosis of PTSD. Together this work suggests that understanding differential sex-specific risk mediators will further our understanding and potential therapeutic approaches to fear-related disorders in humans, such as PTSD.

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108. The Impact of APOE2 Genotype on APOE mRNA Expression, APOE Protein Level, and the Transcriptome in Human Post-Mortem Cortical Tissue

Concepcion Goldberg*, Thomas M. Hyde, Shufen Chen, Joel E. Kleinman, Peter Davies, Terry E. Goldberg

The Feinstein Institute for Medical Research, Manhasset, USA

Background: APOE is triallelic at two loci in its exon 4. While the E4 allele is associated with increased risk for Alzheimer's disease (AD), the E2 allele is associated with diminished risk for AD and as such can be considered neuroprotective. The E3 allele is neutral for neurodegeneration. Here we examined the impact of E2 genotype, in contrast with E3 genotype, in gene expression, protein level, and transcriptional profiling studies of human postmortem temporal cortex.

Methods: We studied 4-6 E2/E3 genotype cases and 9-13 E3/E3 genotype cases using tissue from lateral temporal cortex (BA 21) and in Study 1 only, both BA 21 and sensorimotor cortex (BA 1/2/3). Mean age of the two groups was 53 and 42 years. Mean RIN was 7.99 for E3/E3 cases and 7.88 for E2 cases. No case had diagnosable AD neuropathology. More details about the sample can be found in Conejero-Goldberg et al., 2011.

Results: Study 1: Using qPCR we compared APOE expression in the E2 and E3 groups. We did not find significant isoform differences. We did, however, observe regional differences, such that lower levels of APOE transcripts were present in BA 1/2/3 than in BA 21. Study 2: Using a polyclonal antibody for APOE we compared protein levels of E2 and E3 using Western blots. We identified 3 bands: band 1 at 38-kDa, band 2 at 36-kDa and band 3 at 34-kDa. Significant differences were observed using repeated measures ANOVA for bands 2 and 3. The effect size between the E2 and E3 group for band 2 was 2.14 and for band 3 was 1.03 (both

large). Study 3: We compared the downstream transcriptional impact of E2 in comparison to E3 using Illumina microarrays. 24,835 transcripts met quality control standards. We found 58 transcripts that differed between the two genotypically determined groups significantly at $p < .001$. In addition to these individual transcripts, we found differences in several GO processes, including GTPase energetics and catabolism.

Discussion: We found a mismatch between mRNA expression levels and protein levels in APOE2 and E3 contrasts. While mRNA expression levels did not differ, protein levels were higher in E2 cortices, suggestive of posttranslational modifications. These results are consistent with transgenic mouse models in which human APOE was expressed. Speculatively, protein level may be related to E2s neuroprotective effects. Furthermore, APOE2 also had multiple significant downstream transcriptional effects compared to E3 on the transcriptome.

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109. Epigenetic Modulation of Leukocyte Glucocorticoid Receptor in Healthy Adults: Effects of Childhood Parenting Experiences

Audrey R. Tyrka*, Lawrence H. Price, Carmen Marsit, Oakland C. Walters, Linda L. Carpenter

Department of Psychiatry and Human Behavior, Brown Medical School, Providence, USA

Background: A history of early adverse experiences is an important risk factor for adult psychopathology. Changes in stress sensitivity and functioning of the hypothalamic-pituitary-adrenal (HPA) axis may underlie the association between stress and risk for psychiatric disorders. Preclinical work in rodents has linked low levels of maternal care to increased methylation of the promoter region of the glucocorticoid receptor (GR) gene, as well as to exaggerated hormonal and behavioral responses to stress. Recent studies have begun to examine whether early-life stress leads to epigenetic modifications of the GR gene in humans.

Methods: We examined the degree of methylation of a region of the promoter of the human GR gene (NR3C1) in leukocyte DNA from 99 healthy adults. Participants reported on their childhood experiences of parental behavior, parental death or desertion, and childhood maltreatment. On a separate day, participants completed the dexamethasone /corticotropin-releasing hormone (Dex/CRH) test, a standardized neuroendocrine challenge test.

Results: Disruption or lack of adequate nurturing, as measured by parental loss, childhood maltreatment, and parenting relationships, was associated with increased NR3C1 promoter methylation ($p < .05$). In addition, NR3C1 promoter methylation was linked to attenuated cortisol responses to the Dex/CRH test ($p < .05$).

Discussion: These findings suggest that childhood maltreatment or adverse parenting experiences may lead to epigenetic modifications of the human GR gene. Alterations in methylation of this gene could underlie the associations between childhood adversity, alterations in stress reactivity, and risk for psychopathology.

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110. Genetic Variation in the Pituitary Adenylate Cyclase-Activating Polypeptide 1 Receptor, Type I (PAC1) Gene is Associated with Increased Anxiety and Depressive Symptoms in Adolescent Females with High Levels of Childhood Stress
Suman Baddam*, Rene Olvera, Carolina Livi, Douglas Williamson

University of Texas Health Science Center at San Antonio, San Antonio, USA

Background: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a 38 amino acid neuropeptide highly conserved in mammals that stimulates cAMP formation in anterior pituitary cells. PACAP and the specific type 1 receptor (PAC1) mRNA are expressed at highest levels in the hypothalamus and are also highly expressed in extra-hypothalamic regions in cerebral cortex, hippocampus, thalamus, striatum, nucleus accumbens, substantia nigra, locus ceruleus, and pineal gland (Vaudry et al., 2009). PACAP is known to be neuroprotective and neurotropic (Vaudry et al., 2009). It also plays a central role in stress responses including the sympathoadrenomedullary and hypothalamic-pituitary-adrenal systems (Hashimoto et al., 2011). In animal studies, PACAP knockout mice (PACAP KO) showed increased immobility and decreased swim time – a proxy for depression-like behavior. Administration of Risperidone, Ritanserin (a selective 5-HT$_2$ antagonist) and intra-cerebroventricular PACAP 38, ameliorated the depression-like symptoms in the PACAP KO mice (Hashimoto et al., 2009) implicating that this pathway operates through the serotoninergic system. PACAP KO mice showed increased jumping activity, hyperactivity and depression-like behavior, which further worsened after social isolation. Interestingly, enriched environment in the early development period improved the deficit in social interaction and depression-like symptoms (Ishihama et al., 2010). Chronic variable stress leads to increased mRNA expression of the PAC1 receptor as well as increased BDNF and TrkB in the BNST (Hammack et al., 2009). PACAP 38 peptide infused into BNST increases the startle amplitude dose dependently suggesting an increase in anxiogenic behavior that was sustained after 7 days (Hammack et al., 2009). PAC1 knockout mice exhibit elevated locomotor activity and strongly reduced anxiety like behavior (Otto et al., 2001). In a community-based study in Japan, a single nucleotide polymorphism (SNP) in the intronic region of the PAC1 gene (rs1893154) was associated with MDD in adults (Hashimoto et al., 2010). Further, Ressler et al. identified that CC homozygotes of the rs2267735 SNP were associated with increased PTSD symptoms in females (Ressler et al., 2011). Extending from these studies, we hypothesized that childhood trauma and female gender would be associated with increased anxiety and depressive symptoms in adolescents with CC genotype of SNP rs2267735 in the PAC1 gene.
Methods: A total of 332 adolescents (Male: Female – 159:163, White: Hispanic: Others - 186: 99: 47) aged 12-15 years were recruited from the greater San Antonio area and included in a prospective study examining genes, environment, and brain systems contributing to

depression and alcohol use disorders. All subjects completed the Mood and Feelings Questionnaire (MFQ), Childhood Trauma Questionnaire (CTQ) and Self-Report for Childhood Anxiety Related Disorders (SCARED) and provide blood samples for DNA extraction at baseline. We genotyped the rs2267735 SNP located within the intron region NM_001118.3 of ADCYAP1R1 (PAC1 gene) and tested for C and G alleles from DNA isolated from blood using real time PCR. Multivariate analysis of variance with MFQ score and SCARED score as the dependent variable with genotype, gender and childhood trauma as fixed factors and age as a covariate.
Results: Allele frequencies conformed to Hardy Weinberg Equilibrium with the $x^2 = 0.46$, NS with C allele frequency in the sample of 0.51. A three-way interaction was found between genotype, childhood trauma and gender for anxiety ($F_{1, 309} = 4.1$, $p = 0.01$) and depressive symptoms ($F_{1, 309} = 4.47$, $p = 0.01$). On further analysis, the CC homozygotes were observed to be associated with increased anxiety symptoms ($F_{1, 38} = 6.9$, $p = 0.01$) and depressive symptoms ($F_{1, 38} = 11.6$, $p = 0.002$) in females and no association was observed in males.
Discussion: Consistent with the extant literature, the PACAP-PAC1 signaling system appears to be involved in the expression of anxiety and depressive symptoms during adolescence particularly among females with high levels of childhood stress. Our results extend the findings of Ressler et al. to establish a gene-environment interaction for anxiety and depressive symptoms specific for females moderated by the PAC1 gene. Further studies by our group are underway to elucidate the neurobiological pathways underlying the expression of the gene and its role in the future development of anxiety and depressive disorders.
Disclosure: S. Baddam: None. **R. Olvera:** None. **C. Livi:** None. **D. Williamson:** None.

111. Systems Biological Analyses implicate Glutamate Signaling Abnormalities in Autism and in Intellectual Disability: Implications for Psychopharmacology
Joseph D. Buxbaum*, Avi Ma′ayan, Yan Kuo, Ruth Dannenfelser, Catalina Betancur

Mount Sinai School of Medicine, New York, USA

Background: Autism spectrum disorders (ASDs) are characterized by deficits in social communication and by repetitive behaviors and/or restricted interests. Numerous rare genetic variants of major effect have been identified in ASDs. To understand how such etiological heterogeneity translates into common neurobiological pathways, we used gene enrichment and pathway analyses.
Methods: We made use of a manually collated list of genes where mutations have been shown to be associated with high risk for ASD. These genes include those that are well known (e.g., NRXN3, NRXN4, SHANK2, SHANK3, FMR1, UBE3A, MECP2) and many other less common genes. To extend the findings to an additional neurodevelopmental disorder related genetically to ASD, we also curated a list of almost 200 genes where mutations lead to intellectual disability (ID). We then used unbiased enrichment analyses, making use of data from large-scale proteomic studies, to determine whether there was evidence for enrichment of ASD genes in synaptic and subsynaptic compartments. As a further step we took advantage of emerging whole exome sequencing data in ASD to determine whether there was an enrichment of de novo variation in synaptic and subsynaptic compartments.
Results: We observed strong enrichment of ASD genes in the murine or human synaptic proteome ($P < 1.8 \times 10^{-4}$, hypergeometric test). Remarkably, much of this enrichment could be traced to just two subsynaptic proteomes, that of the NMDA-receptor complex (NRC) and that of the AMPA-receptor complex (ARC). In contrast, the metabotropic glutamate receptor pathway, previously

hypothesized to be broadly implicated in ASD based on Fragile X syndrome, did not show such enrichment. In validation experiments we found that these pathways were also enriched for genes mutated in intellectual disability (ID). As we had two independent datasets (one for ASD and one for ID) we were able to assess whether using existing protein-protein interaction (PPI) databases we were able to identify known neurodevelopmental genes. In these analyses, genes that were associated with ASD were significantly enriched for ID genes and vice versa. This provided a strong rationale for using PPI databases to assess novel ASD findings. We therefore made use of emerging data from a large-scale NIMH and NHGRI-funded whole exome sequencing study (MH089025, Mark Daly, communicating PI, Joseph Buxbaum, Bernie Devlin, Richard Gibbs, Gerard Schellenberg, James Sutcliffe, collaborating PI's), examining de novo mutations in ASD trios to determine whether there was an enrichment of genes that were associated with the NRC and ARC genes found in ASD and ID. We developed a distance metric (D_i) that assessed the average distance between a novel gene and the prior list of ASD and ID genes, using a background PPI network, comparing case to control variants. Even with data from only a modest number of trios available to date, we found a significant ($P < 0.02$, t-test) reduction in average distance for case variants, indicating that amongst the de novo variants there is an enrichment of genes that closely associated with prior ASD genes, particularly those in the NRC and ARC complexes.

Discussion: These studies highlight several important points. First, ionotropic glutamate receptor signaling appears to be broadly disrupted in both ASD and ID. Second, in spite of diverse molecular origins in specific cases of ASD and/or ID certain compartments and pathways are recurrently impacted. Identifying pathways impacted by multiple independent mutations indicates that novel therapeutics that target such pathways can have broader benefit. Third, the findings underscore the role of synaptic function in these developmental disorders and provide a strong rationale for using neurobiological approaches in model systems to understand pathophysiology. Finally, these studies indicate that systems biological approaches using unbiased datasets can be useful for identifying additional genes in ASD and ID.

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112. Rare Copy Number Variants in Tourette Syndrome Disrupt Genes in Histaminergic Pathways and Overlap with Autism

Thomas V. Fernandez*, Stephan J. Sanders, A. Gulhan Ercan-Sencicek, Youeun Song, James F. Leckman, Robert A. King, Donald L. Gilbert, Gary A. Heiman, Jay A. Tischfield, Pieter J. Hoekstra, Matthew W. State

Yale Child Study Center, New Haven, USA

Background: Cellular and molecular mechanisms underlying Tourette Syndrome (TS) pathophysiology remain uncertain, although multiple lines of evidence point to involvement of dopaminergic (DA) neurotransmission and abnormal cortico-striatal-thalamic-cortical circuits. More recent post mortem data has highlighted abnormalities in striatal GABAergic interneurons. Three decades of research in TS have led to widespread agreement that genes play a significant role in TS etiology, likely via heterogeneous architecture. While the major emphasis over the last decade has been on the contribution of common genetic variations, several recent findings have highlighted the importance of studying rare, highly penetrant variants (SLITRK1, CNTNAP2, NLGN4). Most recently, we characterized a highly penetrant rare loss-of-function mutation in the gene HDC in a dense TS pedigree, implicating histaminergic (HA) neurotransmission in the genesis or modulation of tics. The emergence of microarray technologies

that can detect sub-microscopic structural variation have revealed extensive copy number variation (CNV) across the human genome and provided new opportunities for genome-wide assessment of rare variation. Studies in schizophrenia (SCZ) and autism spectrum disorders (ASD) have shown an over-representation of rare CNVs, particularly genic de novo variants, and have highlighted molecular mechanisms, via pathway analyses, that likely play a role in these conditions. Moreover, recent replicated findings show that more than one developmental neuro-psychiatric disorder may share the same rare structural variant as a risk factor (e.g. 16p11.2, 22q11.2, 1q21, NRXN1, SHANK3 in both SCZ and ASD), supporting the hypothesis of shared biological pathways in these conditions. A prior genome-wide CNV study in TS identified rare variants at NRXN1 CTNNA3 loci, leading the authors to hypothesize an overlap of risk with both ASD and SCZ.

Methods: We conducted acase-control study of 460 individuals with TS, including 148 parent-child trios, and 1131 controls. CNV analysis was undertaken using 370K to 1 M probe arrays, and genome-wide genotyping data was used to match cases and controls for ancestry. Transmitted and de novo CNVs present in < 1% of the population were evaluated for differences in overall burden and relative frequencies of RefSeq genes mapping within their boundaries in TS versus controls. We also looked for overlap of rare CNVs with those reported in ASD, SCZ, and intellectual disability (ID). Finally, using multiple pathway analysis algorithms, we determined whether RefSeq genes mapping within rare exonic CNV intervals in TS were over-represented in one or more biological processes or pathways compared to all known genes.

Results: While there was no significant increase in the number of de novo or transmitted rare CNVs in cases versus controls, pathway analysis using multiple algorithms showed enrichment of genes within histamine receptor signaling pathways as well as axon guidance, cell adhesion, and synaptic structure and function processes. Genes mapping within rare CNVs in TS showed significant overlap with those previously identified ASD, but not ID or SCZ. Three large, multigenic, likely-pathogenic, de novo events were identified, including one disrupting multiple GABA receptor genes.

Discussion: Our analysis of rare CNVs in TS supports recent findings implicating HA neurotransmission in the etiology or modulation of tics. Presynaptic HA receptors also regulate a variety of other neurotransmitters, including DA. Late-stage clinical development of HA receptor antagonists and inverse agonists raises possibilities for novel treatment approaches. A single large multigenic de novo CNV in TS highlights the potential involvement of GABAergic mechanisms as well. In light of two recent post mortem studies highlighting the GABA system in TS, further study of genes within this interval and attention to the GABA system will be of particular interest in large-scale sequencing and follow-up CNV studies. Our results also reinforce the notion of shared genetic risks among clinically-distinct syndromes, in this case ASD and TS. Consistent with this, several case reports and cohort studies point to an increased rate of comorbidity between ASD and TS or tic disorders. Finally, we identify three novel, large, rare, genic, de novo CNVs that are likely pathogenic in the individuals in which they were identified, based on their de novo status and high gene content relative to controls.Our data clearly demonstrate the value of pursuing rare variant and CNV analyses in TS, and highlight the pressing need for studies of larger cohorts to replicate, clarify, and extend these findings.

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specific rare mutations in the gene CNTNAP2 and autism spectrum disorders.

113. Whole Exome Sequencing of Autism Families Reveals *De Novo* Mutations in Integrin-Related Genes

James S. Sutcliffe*, Ben M. Neale, Aniko Sabo, Emily L. Crawford, Nicholas G. Campbell, Ana Carneiro, Eric Boerwinkle, Joseph D. Buxbaum, Edwin H. Cook, Bernie Devlin, Richard A. Gibbs, Gerard D. Schellenberg, Mark J. Daly

Vanderbilt University, Nashville, USA

Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that exhibits deficiencies in communication, reciprocal social interaction and patterns of rigid-compulsive and stereotyped behaviors. Substantial data points to a predominantly genetic etiology, although common variant association studies have yielded few if any consistent findings, and they suggest that common susceptibility variants will exert only modest main effects on disease risk. In contrast, compelling data have emerged in recent years pointing to rare copy number variants (CNVs) as a major class of autism risk, with *de novo* CNVs occurring in $\sim$6-8% of ASD cases. The role of rare risk variants in ASD has extended to whole exome sequencing projects, such as the NIH ARRA Autism Sequencing Consortium, a multi-center group conducting whole exome and genome sequencing studies to identify ASD risk loci.
Methods: As a complement to large-scale case-control sequencing, we conducted a multi-center whole exome sequencing project on whole blood DNA from approximately 200 parent-child ASD trios. Sequence reads were mapped back to the reference genome, variants called in all family members and filtered to identify high-confidence variants in the probands but absent in either parent. Putative *de novo* variants (DNVs) were validated using Sanger sequencing in the trio and other family members as available. Pathway analyses have been conducted using loci found to harbor *de novo* coding variants to uncover relevant gene networks potentially related to ASD risk.
Results: Slightly less that one validated exonic DNV was identified per trio. Pathway analysis of genes harboring nonsynonymous, nonsense or splice mutations revealed DN mutations in multiple loci directly involved in integrin function as one of the most significant (and obvious) gene networks affected by DNVs. Integrins function as integral membrane, dimeric receptor structures comprised of alpha and beta subunits that bind to extracellular matrix proteins such as fibronectin. Integrins are involved in a range of cellular functions including neuronal migration as but one example. We identified a *de novo* nonsense mutation in the *ITGA5* gene and a predicted damaging DN mutation at a highly conserved fibronectin (FN1) residue. Further, two independent *de novo* nonsense mutations were identified in the gene encoding Reelin (*RELN*), a long-time ASD candidate that binds to alpha5/beta1 integrins. Further, a missense DNV was found in the gene (*NISCH*) encoding nischarin, dysregulation of which alters actin organization at the extracellular matrix and impacts cellular migration. While no DNVs were seen in *ITGB3*, a common coding variant (Leu33Pro) in integrin beta3 results in allele-specific effects on serotonin transporter (SERT) activity and regulation, previously associated with ASD. Ongoing pathway analyses reveal additional relationships between DNV-affected proteins and integrins or integrin-related molecules described above.
Discussion: ASD has a highly heterogeneous genetic etiology, and only a fraction of the genetic underpinnings have been uncovered. Whole exome and whole genome analyses offer the promise to identify individual risk loci and corresponding pathways that are important to our understanding disease pathogenesis. These studies have identified integrin-related genes as one major theme in ongoing exome sequencing efforts. Existing data already point to integrin-related proteins such as *RELN*, and identification of multiple *de novo* mutations in such genes provide compelling support for dysfunction and/or dysregulation of this pathway as one major biological theme in ASD etiology.

114. COMT Val158Met Variant and Functional Haplotypes Associated With Childhood ADHD History in Women With Bulimia Nervosa

Allan S Kaplan*, Zeynep Yilmaz, Clement C. Zai, Robert D. Levitan, James L. Kennedy

Center for Addiction and Mental Health, Toronto, Canada

Background: Up to one third of patients with bulimia nervosa (BN) report a history of ADHD symptoms, and both disorders may also be associated with dopaminergic abnormalities. COMT gene, coding for an enzyme responsible for the degradation of dopamine, may play a part in the etiology of ADHD and BN. This study aimed to (1) examine if certain variants of the COMT genetic markers (rs6269, rs4633, rs4818, rs4680)are more common in BN versus controls; (2) assess transmission of COMT alleles in BN families; and (3) explore the role of COMT genotypes and haplotypes in bulimic women with childhood ADHD history.
Methods: Eligibility criteria for 241 probands were: 1) DSM IV diagnosis of current or lifetime BN, purging subtype 2) age between 18 and 65 years 3) Maximum lifetime BMI $< 35\,\text{kg/m}^2$, and 4) European Caucasian descent. 75 BN probands and their 148 unaffected first degree relatives were genotyped for COMT rs4680 (Val158Met) and three adjacent markers, rs6269, rs4633, rs4818. The remaining 166 probands were paired with ethnicity matched female nonpsychiatric controls. We also investigated if COMT variants and haplotypes were associated with childhood ADHD history in a subgroup of 86 BN probands who completed the Wender Utah Rating Scale (WURS).
Results: Among 241 BN probands, the mean age was 26, and the mean BMI was $22\,\text{kg/m}^2$. Among the 86 BN probands who completed the WURS, 23% scored above the clinical cutoff score of 45 for childhood ADHD, a rate much higher than that found in the general adult population. Our genetic analyses showed that cases and controls did not differ in COMT allele and haplotype frequencies. In contrast, specific alleles of all four COMT markers and the medium-activity haplotype were preferentially transmitted to the offspring with BN. COMT Val158 allele was overrepresented and the medium-activity haplotype was underrepresented in BN with childhood ADHD history ($p = 0.010$).
Discussion: This study is the first to assess transmission pattern of COMT alleles and haplotypes in BN probands and their first degree relatives, as well as to explore the possible role of COMT functional genotypes and haplotypes in BN. The findings of this study suggest a possible role for COMT variants and related haplotypes in BN and its subphenotypes, specifically BN comorbid with ADHD. If replicated, these preliminary findings may have implications for prevention and treatment of BN that emerges in the context of childhood ADHD, including pharmacological treatments with dopamine agonists.

115. The Hypo-Functional 7-Repeat Allele of DRD4 Predicts both Objective and Reported Fat Intake in 4- to 6- Year Old Girls

Robert Levitan, Patrícia Silveira, André Portella, James Kennedy*, Hélène Gaudreau, Caroline Davis, Meir Steiner, Claudio Soares, Stephen Matthews, Laurette Dubé, Michael Meaney

Centre for Addiction and Mental Health, Toronto, Canada

Background: The prevention and treatment of overeating and obesity continues to be a major challenge. While the ease of availability of highly palatable foods is a major factor in this regard, not all individuals overeat or become obese in this environment. The current project examines individual differences in palatable food intake at a critical time in human development. The current analysis studies the relationship between dopamine genes and eating behaviour in children from 4 to 6 years of age. A particular focus is the hypo-functional 7-repeat (7R) allele of the dopamine-4 receptor gene (DRD4), which associates with overeating and obesity in several female overeater populations (e.g. Levitan et al., 2004, 2006, 2010). Recent imaging work suggests that weaker activation of the brain's reward circuitry may play a role in these associations (Stice et al., 2010).

Methods: The current sample consists of children taking part in a longitudinal cohort study (Maternal Adversity, Vulnerability and Neurodevelopment) based in Canada. The mothers of the children, oversampled based on maternal depression and low SES, were recruited at 13-120 weeks of pregnancy, and the children have been followed intensively since birth.

Key outcome measures include:

1. A laboratory-based snack test at 48 months of age which provides an objective measure of food preferences and overall caloric intake.

2. A food frequency questionnaire at 48 and 72 months, based on maternal report, which assesses naturalistic food intake and feeding behavior.

3. The childhood eating behaviour questionnaire (CEBQ; Wardle et al., 2001) which measures eating styles likely to promote over- or under- weight.

Results: To date, 118 children have completed the 48 month snack test in the lab. Of these, 47 (39.8%) carry the 7R allele, while 71 (60.2%) do not. Controlling for total caloric intake, there is a significant gender X genotype interaction in predicting fat intake during the snack test ($F = 7.16$, df = 4, 113, $p = .009$). Girls who carry the 7R variant are consuming 31.2 % more fat during this snack than are non-carriers (mean = 12.2 vs. 9.3 grams respectively) while in boys, 7R carriers are eating 19.2% less fat than are non-carriers (mean = 11.3 vs. 13.5 grams respectively). This suggests that the 7R allele increases objective fat intake in girls but not boys by four years of age. Strikingly, when intake is measured using the 48 and 72 month food diaries, which were completed two years apart, the gender X 7R interaction is even more robust, accounting for 21 % of the variance in fat intake ($F = 11.3$, df = 3, 42; $p = .002$; N = 46). Based on these diaries, girls who carry the 7R allele are consuming 39.8% more fat in their natural environments at 48 months than are non-7R carrier girls (820.5 vs. 587.0 calories/day respectively). Importantly, this difference remains robust at 72 months of age (881.5 calories as fat in 7R carriers vs. 609.2 calories as fat in non-carriers, a 44.7 % difference). In boys, 7R carriers are consuming 40.1 % less calories from fat than are non-carriers at 48 months, and 25.4 less calories from fat at 72 months based on these diaries. The consistency of these data across both laboratory based measures and food diaries, and the consistency of the food diary data over a two year time span, adds greatly to these findings. These findings are also highly consistent with earlier work in female adult overeater populations (see above).

Discussion: These converging results show a highly robust association between the hypo-functional 7R allele of DRD4 and fat intake in girls, but not boys. The consistency of these findings across different outcome measures and across a two year age span adds greatly to their validity and potential impact. Pending replication and detailed anthropomorphic outcomes later in childhood, these results may identify a significant subgroup of young girls at higher risk for childhood obesity and/or eating disorders. If so, this could inform novel prevention strategies implemented as early as birth in this subgroup.

116. Exploring an Association Between Genetic Variation in Lipid Metabolism Genes and Mental Disorders and Suicide Attempts

Enrique Baca-Garcia*, M. Mercedes Perez-Rodriguez, Maria A. Oquendo, Jorge Lopez-Castroman, Concepcion Vaquero-Lorenzo, Pablo Fernandez-Navarro

Department of Psychiatry at Fundacion Jimenez Diaz Hospital and Autonoma University, Madrid, Spain

Background: Alterations in lipid metabolism have been linked with impulsive aggression and suicidal behavior, although several studies have been unable to replicate this finding. The inter-action among apolipoprotein E(Apo-E), leptin and neuropeptide Y plays a key role in lipid metabolism and food intake regulation. We aimed to explore an association between 25 single nucleotide polymorphisms (SNPs) in 5 candidate genes related to lipid metabolism (ApoE, leptin [LEP], leptin receptor [LEPR], leptin receptor overlapping transcript [LEPROT], and neuro-peptide Y [NPY]) and psychiatric disorders and suicide attempts.

Methods: 25 functional SNPs from the ApoE, LEP, LEPR, LEPROT, and NPY genes were selected and genotyped in 1,251 Caucasian subjects. 812 (65%) were psychiatric patients with at least one DSM-IV Axis-I disorder. 463 (37%) had a history of at least one suicide attempt. All analyses were performed with the GWASpi software for genetic association studies. We tested 25 SNPs in 5 candidate genes related to lipid metabolism (ApoE, leptin [LEP], leptin receptor [LEPR], leptin receptor overlapping transcript [LEPROT], and neuropeptide Y [NPY]) for individual associations with psychiatric disorders and suicide attempts. We used Bonferroni correction for multiple comparisons (threshold $p = 0.05/25 = 0.002$).

Results: All the SNPs were in Hardy-weinberg equilibrium. One snp (rs3806318 in the LEPROT gene) was associated with having at least one psychiatric disorder (Chi-square 10.75, uncorrected $p = 0.004$, OR = 2.32) and with a history of suicide attempts (Chi-square 7.67, uncorrected $p = 0.021$, OR = 2.17). However, the associations did not reach significance after correcting for multiple comparisons.

Discussion: We found an association between a SNP in the leptin receptor overlapping transcript gene, a gene implicated in food intake and lipid metabolism, and mental disorders and suicide attempts. However, the association did not survive correction for multiple comparisons. Nonetheless, given the increasing evidence pointing to alterations of lipid metabolism related to impulsive aggression and suicidal behaviors, future studies in larger samples are needed to clarify the role of lipid metabolism in impulsive aggression and suicidal behaviors.

117. Interaction Between *FKBP5* and Childhood Trauma Increases Risk for Aggressive Behavior

Laura Bevilacqua*, Vladimir Carli, Marco Sarchiapone, Danielle K. George, David Goldman, Alec Roy, Mary-Anne Enoch

Laboratory of Neurogenetics, NIAAA, NIH, Rockville, USA

Background: Childhood trauma may predispose to numerous psychopathologies in adulthood, including aggressive and violent criminal behavior. Childhood trauma and aggressive behavior have both been shown to impact stress reactivity in adulthood by altering hypothalamic-pituitary-adrenal (HPA) axis function. Acute stress activates hypothalamic release of corticotropin-releasing hormone (CRH) from the hypothalamus to the anterior pituitary where it stimulates the secretion of the adrenocorticotropic hormone and ultimately cortisol. FKBP5 is a protein that has a role in regulating the negative feedback that blocks release of CRH. This mechanism is necessary for normal function of the HPA axis and prevents prolonged or excessive activation. Previous studies identified variation in the *FKBP5* gene associated with high protein expression and with increased glucocorticoid resistance and thus less dexamethasone suppression, as seen in controls. Since childhood trauma predicts aggressive behavior and since both have been associated with abnormal HPA axis response, we hypothesized that there would be an interaction between genetic variation in *FKBP5* and childhood trauma in predicting aggressive behavior.

Methods: A cross-sectional study of 583 male Italian prisoners (age 40.6 ±11) incarcerated in the penitentiary district of Abruzzo-Molise in central Italy was performed.All prisoners self-identified as Caucasians. Four *FKBP5* single nucleotide polymorphisms (SNPs) used in previous studies (rs3800373, rs9296158, rs1360780, and rs9470080) were genotyped together with 132 ancestry informative markers. Three *FKBP5* diplotypes were derived from two major putatively functional haplotypes regulating protein expression and previously associated with glucocorticoid receptor sensitivity. A comprehensive analysis of aggression and impulsivity was undertaken using the Brown-Goodwin Lifetime History of Aggression (BGHA) interview, the Buss-Durkee Hostility Inventory (BDHI), and the Barratt Impulsiveness Scale (BIS). A history of childhood trauma was investigated with the Childhood Trauma Questionnaire (CTQ). The interaction between the *FKBP5* diplotypes and childhood trauma on measures of aggression was analyzed. The three diplotypes, age and DSM-IV lifetime Axis I diagnosis were included as independent variables together with the diplotype x CTQ interaction term. Analyses were replicated with a second behavioral measure of aggression: violent behavior exhibited during incarceration that lead to disciplinary reports of physical aggression or assault against other inmates or prison officers. Individual SNP analysis was performed.

Results: Childhood trauma had a significant effect on BGHA and BDHI scores, but not on BIS scores. We observed a significant influence of the *FKBP5* high expression diplotype on both a lifetime history of aggressive behavior (BGHA) (p = 0.012), and on violent behavior in jail (p = 0.025), but only in individuals exposed to childhood trauma, in particular to physical abuse. Moreover, there was a crossover effect such that in prisoners not exposed to childhood trauma, carriers of the high expression diplotype were less aggressive. However there was no G x E effect on indirect aggression (general hostility, expression of anger) ascertained from the BDHI, nor on impulsive personality traits, ascertained from the BIS. No main effect of the *FKBP5* diplotypes was observed.

Discussion: In conclusion, this study reports a significant interaction between childhood trauma, particularly physical abuse, and genetic variation in *FKBP5* in predisposing to overt aggressive behavior in a male population, rather than to hostile/impulsive personality traits. This observation may ultimately contribute to the identification of biological markers that could have a role in clinical practice in preventing aggressive behavior in at risk individuals who were exposed to early life trauma.

118. Association of an Intronic Deletion in the Corticotropin-Releasing Hormone Receptor Gene with Depression in African Americans

Charles B. Nemeroff*, Manuel Ramirez-Restrepo, Anzhelika Engel, Kristina Butze Mercer, Kerry Ressler, Amanda J. Myers

University of Miami, Miller School of Medicine, Miami, USA

Background: It is well known that hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA) is a hallmark of major depressive disorder (MDD) and in part due to a hyperactivity of corticotropin-releasing hormone (CRH) circuits(Nemeroff *et al.*, 1984; Reul *et al.*, 2002). Previous work has demonstrated that there is a synergistic effect between early life stress, variants in the corticotropin releasing hormone receptor 1 gene (*CRHR1*) and development of major depressive disorder(Bradley *et al* 2008). Previously, we imputed additional variants in the region using data from both the Human Hapmap Project (www.hapmap.com, ASW data) as well as the 1000genomes project (www.1000genomes.org, YRI data) to map additional polymorphisms that might contribute to the relationship between *CRHR1* genotypes which interact with prior child abuse to increase risk for depression (Myers *et al.*, 2010-PS-1250-ACNP). We have tested these new variants to map whether they are associated with increased risk for depression in the context of childhood maltreatment.

Methods: All samples were of African-America descent (self-report). The majority of participants were of low socio-economic status (~60% with <$1000/month family income) and had high rates of childhood and/or lifetime trauma. Depression was measured using the Beck's Depression Inventory (BDI) and the history of childhood abuse and neglect was measured using the Childhood Trauma Questionnaire (CTQ). All of the procedures in this study were approved by the institutional review boards of Emory University School of Medicine and Grady Memorial Hospital, Atlanta, Georgia. To capture novel variants within the *CRHR1* gene we used both the data from the ASW Hapmap population, which are individuals with African ancestry living in Southwest USA along with the YRI 1000 genomes dataset which includes African subjects of Yoruban descent. From our imputations we mapped 133 new single nucleotide polymorphisms (SNPs) near *CRHR1*. Seventy of the 133 SNPs were 1. within +/- 10000 bp of the gene and 2. in complete LD with the protective haplotype mapped in Bradley *et al*. Additionally, approximately 30% of SNP variants appear to change TFBS consensus sequences. A subset of this set was picked for further genotyping. Additionally, Sanger sequencing was performed to discover additional variants within *CRHR1*.

Results: We mapped a significant effect with rs8072451considering a model withBDI total score as the outcome, and CTQ score as an interaction term. Further sequencing around this variant showed that it was in complete linkage disequilibrium with a 5 basepair deletion mapping 57 bp away from rs8072451 just upstream from exon 3.

Discussion: We have found additional SNPs within *CRHR1* which are associated with depression in the presence of childhood maltreatment in African Americans.

Method and devices for transdermal delivery of lithium (US 6,375,990B1)Method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by *ex vivo* assay (US 7,148,027B2) Scientific Advisory Boards: American Foundation for Suicide Prevention (AFSP), CeNeRx BioPharma, National Alliance for Research on Schizophrenia and Depression (NARSAD), NovaDel Pharma, Inc., PharmaNeuroBoost, Anxiety Disorders Association of America (ADAA), AstraZeneca Pharmaceuticals Board of Directors: AFSP, NovaDel Pharma, Inc., Mt. Cook Pharma, Part 2: American Psychiatric Publishing NovaDel Pharma CeNeRx BioPharma Xhale PharmaNeuroBoost AstraZeneca Revaax, Part 3: None, Part 4: National Institutes of Health (NIH) Agency for Healthcare Research and Quality (AHRQ), Part 5: No. **M. Ramirez-Restrepo:** None. **A. Engel:** None. **K. Mercer:** None. **K. Ressler:** Part 1: Dr. Ressler has received awards and/or funding support within the last two years from Burroughs Wellcome Foundation, NARSAD, NIMH, NIDA. He is also a founding member of Extinction Pharmaceuticals and Therapade Technologies, which have provided no equity or income, but in which he is a stakeholder. **A. Myers:** None.

119. The Brain Epigenome: Mapping Brain Relevant Gene Regulatory Elements Using Next Generation Sequencing
Cathy Barr*

The Toronto Western Research Institute and The Hospital for Sick Children, Toronto, Canada

Background: Current evidence indicates that psychiatric and neurological disorders are likely to result from genetic variation that changes gene expression. However, the role of variation in gene regulation as a contributor to complex traits disorders has been largely ignored because of the difficulty in identifying the location of gene regulatory elements outside of the proximal promoter.
Methods: We used a genome-wide approach to identify brain-relevant gene regulatory elements, in two cell lines, a neuroblastoma and a glioblastoma, and in human brain tissue. We used chromatin immunoprecipitation (ChIP) to modified histones and transcription factors combined with high throughput sequencing (ChIP-sequencing) to identify the position of brain-relevant regulatory elements across the genome.
Results: This approach was very successful, and we now have genomic maps of putative regulatory elements for genes expressed in these cell lines including genes associated with psychiatric disorders (e.g. *DISC1, NTRK3, DTNBP1, SNAP25*). We also mapped putative gene regulatory elements in gene "deserts" in the region of positive markers from GWAS. We are currently screening the putative regulatory regions in associated genes for genetic variation and testing the relationship to the respective psychiatric disorder in DNA from families.
Discussion: The use of ChIP combined with high throughput sequencing is a powerful approach to map gene regulatory elements allowing gene findings for psychiatric disorders to move forward to functional studies. Further, understanding how risk genes are differentially regulated is crucial for future therapeutic interventions and the understanding of genetic variation in response to these interventions.
Disclosure: C. Barr: None.

120. Length of CAG Repeat in Huntingtin Below Disease Threshold Predicts Volume of Specific Brain Regions
Peggy Nopoulos*, Eric A. Epping, Tom Wassink, Bradley Schlaggar, Joel Perlmutter

University of Iowa, Iowa City, USA

Background: Simple sequence repeats (SSRs) in the genome contribute to normal variation in behavioral traits, but when expanded beyond a threshold, cause neurologic disease. Huntington Disease (HD) is caused by an expansion of greater than 39 CAG repeats in the gene Huntingtin (*HTT*). However, the effects of CAG repeats on brain structure below disease threshold have not been studied. The current study estimates the relationship between brain structure and CAG repeat lengths below disease threshold in the Huntingtin gene.
Methods: Two cohorts of children ages 6-18 years of age were recruited for a study on brain structure in children at risk for HD: 1) children with a parent or grandparent with HD and 2) normal healthy children recruited from the community. Blood or saliva was obtained from all participants, for research purposes only, to determine CAG length of the Huntingtin gene. Brain MRI scans were obtained and processed for quantitative volumetrics. The sample consisted of 49 female children with CAG repeats below disease threshold.
Results: The length of CAG repeat within the normal range (16-30) predicted the volume of specific brain regions: the cerebellum, striatum, and frontal/temporal cortex. Specifically, higher CAG repeats were associated with greater volumes of the striatum, frontal, and temporal cortex. At the same time, greater CAG repeats were associated with reduced volumes of the cerebellum.
Discussion: Length of CAG repeats within the range of normal variation and below disease threshold are directly related to the structure of specific brain regions. In the context of disease, it may be that there is a continuous spectrum of effect such that larger CAG repeats (greater than 39 and above) that cause disease may actually first create very large structures which are then unstable, and deteriorate/atrophy over time which would then manifest in symptoms of the disease. Some have suggested that SSRs have provided the variability needed for the changes in brain development in the primate lineage contriubting to human brain evolution. This study supports the notion that this type of genetic variant, and in particular within the genes known to cause degenerative brain disorders, may well have had a role in the evolution of the human brain. These findings may be relevant to the pathoetiology of not only Huntington's disease but to all other polygluatmine diseases and possibly other neurodegenerative diseases.
Disclosure: P. Nopoulos: None. **E. Epping:** None. **T. Wassink:** None. **B. Schlaggar:** None. **J. Perlmutter:** None.

121. Daily Meditation in Distressed Dementia decreases NF-kappa B Signaling and Increases Interferon Response Factor Transcription
Helen Lavretsky*, Steve W. Cole, Jesusa M. G. Arevalo, Michael R. Irwin

UCLA Semel Institute, Los Angeles, USA

Background: Primary caregiving for a family member with serious medical illness is both highly stressful and associated with leukocyte gene transcriptional alterations that may contribute to an increased risk of physical illness in the caregiver. The present study sought to determine whether a daily meditation intervention might reverse the pattern of increased pro-inflammatory cytokine production and reduced innate antiviral response previously observed in people confronting significant life adversity.
Methods: 39 healthy adult primary caregivers of dementia patients were randomized to 8 weeks of either daily Kirtan Kriya meditation or parallel relaxation music control, and genome-wide transcriptional profiling was carried out peripheral blood mononuclear cell samples obtained at baseline and post-intervention.
Results: In analyses of covariance controlling for age, sex, race, years of education, BMI, duration of distress and depression, and history of depression, 19 gene transcripts showed systematic up-

regulation over time (Group x Time interaction 20% difference across groups) and 45 genes showed systematic decreases in expression in the meditation group as compared to control. Up-regulated genes included immunoglobulin-related transcripts (e.g., IGJ, IGLL3) and multiple un-named genes of unknown function (LOC genes), whereas down-regulated transcripts included pro-inflammatory cytokines (IL8) and activation-related immediate-early genes (JUN, FOSB). Transcript origin analyses identified plasmacytoid dendritic cells and B lymphocytes as the primary cellular context of these transcriptional alterations (both p <.001), and promoter-based bioinformatic analysis implicated reduced NF-kappaB signaling and increased activity of Interferon Response Factor 1 in structuring those effects (both p <.05).

Discussion: The present results thus suggest that an 8-week structured meditation intervention may reverse the pattern of increased NF-kappaB-related transcription of pro-inflammatory cytokines and decreased IRF-related transcription of innate antiviral response genes previously observed in healthy individuals confronting caregiving stress and other forms of significant life adversity.

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122. Linkage Analyses of Twelve Endophenotypes for Schizophrenia from the Consortium on the Genetics of Schizophrenia (COGS)

Tiffany A. Greenwood*, Robert Freedman, Michael F. Green, Raquel E. Gur, Keith H. Nuechterlein, Ann Olincy, Allen D. Radant, Nicholas J. Schork, Larry J. Seidman, Larry J. Siever, Neal R. Swerdlow, Debby W. Tsuang, David L. Braff

University of California San Diego, La Jolla, USA

Background: The Consortium on the Genetics of Schizophrenia (COGS) has undertaken a large multi-site study to characterize the genetic architecture of 12 endophenotypic measures for schizophrenia as a powerful strategy aimed at furthering the understanding of the complex genetic basis of schizophrenia. We have now completed the genotyping of 296 families segregating schizophrenia and have evaluated the 12 heritable endophenotypes for linkage. The heritability analyses of these endophenotypes will be presented separately.

Methods: Each of the 296 families consisted of a proband with schizophrenia, both parents, and at least one unaffected sibling to allow for maximum contrast in the analyses of these quantitative endophenotypes. A total of 1,286 subjects were genotyped within these families. Of these, 1,004 subjects were also assessed for the following twelve primary neurophysiological and neurocognitive endophenotypes: Prepulse inhibition (PPI), P50 suppression, the antisaccade task, Degraded-Stimulus Continuous Performance Test (DS-CPT) d', Letter-Number Span (LNS) re-ordered, and the California Verbal Learning Task (CVLT-II) total score, as well as Abstraction and Mental Flexibility (ABF), Face Memory (FMEM), Spatial Memory (SMEM), Spatial Processing (SPA), Sensori-motor Dexterity (S-M), and Emotion Recognition (EMO) from the University of Pennsylvania Computerized Neuropsychological Battery (Penn CNB). Genotyping was performed in two phases using the Illumina Linkage Panel 12 and HumanLinkage-24 SNP arrays. Merlin was used to conduct linkage analyses of the 6,056 SNPs that were successfully assayed between the two arrays, accounting for linkage disequilibrium between markers and accommodating important covariates (e.g., age and sex) for each endophenotype as necessary.

Results: Linkage analyses of all twelve endophenotypes collectively identified many regions of 'suggestive' evidence for linkage (LOD > 2.2), including chromosomes 1p36 (EMO), 2p25 (SPA), 2q24 (S-M), 3p14 (antisaccade), 5p15 (PPI), 8q24 (CVLT), 10q26 (DS-CPT and FMEM), 12p12 (FMEM), 14q23 (LNS), 16q23 (SPA), 19q13 (CVLT), and Xp11 (DS-CPT).

Discussion: Although none of the regions identified in these analyses met the standard criteria for genomewide significance, the observance of many regions meeting genomewide suggestive criteria is encouraging. These results complement the candidate gene association analyses that we have previously conducted in a portion of this sample. Subsets of these families are also being further characterized through more extensive exonic sequencing, CNV, epigenetic, and gene expression studies. We anticipate that these data collectively will further our understanding of the genetic mechanisms underlying these endophenotypes and ultimately aid in the identification of genes contributing to the expression of the endophenotypes, as well as to risk for schizophrenia.

Disclosure: T. Greenwood: Part 1: Dr. Greenwood has received unrelated support for consulting services from INFOTECH Soft. **R. Freedman:** Part 1: Dr. Freedman has a patent through the Department of Veterans Affairs on DNA sequences in CHRNA7. **M. Green:** Part 1: Dr. Green has received unrelated support for consulting services from Abbott Laboratories, Dainippon Sumitomo Pharma, Otsuka, Sanofi-Aventis Pharmaceuticals, Takeda, and Teva, and he has been a speaker for Janssen Cilag and Lundbeck. **R. Gur:** Part 1: Dr. Gur has received unrelated research support from Pfizer and AstraZeneca. **K. Nuechterlein:** Part 1: Dr. Nuechterlein has received unrelated support for consulting services from Wyeth/Pfizer., Part 4: Dr. Nuechterlein has received unrelated research support from Ortho-McNeil Janssen Scientific Affairs. **A. Olincy:** Part 4: Dr. Olincy has received unrelated research support from Lundbeck Pharmaceuticals. **A. Radant:** None. **N. Schork:** None. **L. Seidman:** None. **L. Siever:** None. **N. Swerdlow:** Part 1: Dr. Swerdlow has received unrelated compensation for consulting services funding from Neurocrine. **D. Tsuang:** None. **D. Braff:** None.

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123. Preliminary Evaluation of Resting-State Functional Connectivity in Children with Bipolar Disorder or Attention Deficit Hyperactivity Disorder

Daniel P. Dickstein*, Brooke L. Reidy, Matthew F. Pescosolido, Thania Galvan, Sharad Sikka, Maarten Mennes, Michael P. Milham

Bradley Hospital PediMIND Program, East Providence, USA

Background: When working with children, clinicians and researchers alike struggle to disentangle the phenomenology of bipolar disorder (BD) and attention deficit hyperactivity disorder (ADHD). Many of their Diagnostic and Statistical Manual 4th edition (DSM-IV) diagnostic criteria overlap, and they are frequently comorbid. Therefore, neural markers that might indicate where the disorders differ, and where they overlap, are much needed. Previously, we have shown that pediatric BD involves neural alterations in a dorsolateral prefrontal cortex (DLPFC)-amygdala-striatal (accumbens) circuit, including both structural and task-independent, spontaneous, resting-state functional connectivity (RSFC) changes. Now, we extend that work by evaluating RSFC in children with primary BD vs. primary ADHD, both in comparison to typically-developing controls (TDC).

Methods: Participants ages 7-17 years old were enrolled in an IRB-approved study conducted at Bradley Hospital and Brown University following informed parental consent and participant assent. Psychopathology was evaluated using the Child Schedule for Affective Disorders Present and Lifetime (K-SADS-PL) administered by the same board-certified child psychiatrist (DPD). Our sample included N = 21 BD, N = 20 ADHD, and

N = 24 TDC participants, the latter without psychopathology in themselves or first-degree relatives. All participants completed a task-independent resting state functional magnetic resonance imaging (fMRI) scan while awake looking at the word "relax". For registration purposes we also collected a high-resolution anatomical image. After standard preprocessing we calculated participant-level functional connectivity maps for the same 3 seed regions of interest as used in our prior work (Dickstein DP Biol Psych 2010). The three seeds included left DLPFC, amygdala, and nucleus accumbens. Functional connectivity maps were obtained by whole-brain voxel-wise correlations of each voxel's BOLD time series with the time series of the seed ROI (correlation maps were Fisher-Z transformed to improve normal distribution). After participant-level analyses we conducted group-analyses using a one-way ANOVA model to assess the effect of diagnosis on the RSFC maps. Age and sex were included as covariates. Resulting maps were corrected for multiple comparisons using Gaussian-Random field correction (p < 0.05, z > 2.3).

Results: We observed significant alterations in RSFC between the left DLPFC and the left operculum, with ADHD participants exhibiting decreased connectivity compared to BD and TDC participants. In addition, BD participants exhibited greater connectivity between the left amygdala and the right insula and medial frontal cortex, when compared to the ADHD or TDC participants. Finally, we found significant RSFC alterations between the accumbens and several regions. BD participants exhibited greater connectivity with right medial frontal cortex and left posterior cingulate, while participants with ADHD exhibited greater connectivity in medial frontal cortex and the frontal pole.

Discussion: Although preliminary, our data suggest that pediatric BD and ADHD differ with respect to RSFC. Given its role in attention and executive function, it is interesting to note that results using our DLPFC seed seem to be driven by the ADHD group. Given its role in emotional processing, it is interesting to note that our results using our amygdala seed seem to be drive by the BD group. Given it role in reward and addiction, it is interesting that both BD and ADHD participants have findings linked to the accumbens. Future study is needed to determine the role of development and gender on these findings.

Disclosure: D. Dickstein: None. B. Reidy: None. M. Pescosolido: None. T. Galvan: None. S. Sikka: None. M. Mennes: None. M. Milham: None.

124. Serotonin Transporter Binding and Treatment Response to Fluoxetine in Individuals Recovered from Anorexia Nervosa

Ursula F. Bailer*, W. Gordon Frankle, Rajesh Narendran, Michael L. Himes, Chester A. Mathis, Walter H. Kaye

Medical University of Vienna, Department of Psychiatry and Psychotherapy, Division of Biological Psychiatry, Vienna, Austria

Background: Anorexia nervosa (AN) may become a chronic disorder with a high morbidity and mortality rate for many individuals because there is no proven treatment that effectively reverses negative mood states and core eating disorder symptoms. Some studies have suggested that the selective serotonin reuptake inhibitor (SSRI) fluoxetine, when administered after weight restoration, may reduce relapse and residual symptoms in AN. Positron emission tomography (PET) imaging and genetic studies indicate there is evidence of alterations in serotonin (5-HT) transporter (5-HTT) activity in AN. This study investigated how altered 5-HTT function may be related to SSRI response in individuals recovered (REC) from AN.

Methods: We used PET and [^{11}C]DASB to assess 5-HTT binding potential (BP$_{non-displaceable\ (ND)}$) in striatal regions; subcortical

regions including thalamus, insula, midbrain, medial temporal lobe; and the neocortical regions (i.e., the dorsolateral prefrontal, medial frontal, orbital frontal, temporal, occipital cortices and anterior cingulate cortex). Analysis of the PET data was performed using the simplified reference tissue method (Lammertsma, 1996) with the cerebellum as a reference region for non-displaceable uptake. 14 REC AN (8 restricting type, 6 binge/purge type [age: 31 ± 8; BMI: 21.3 ± 1.3 kg/m^2]) were studied. General, as well as eating disorder-specific psychopathology, was assessed at baseline and after 8 weeks of treatment with fluoxetine (32 ± 14 mg/day). Initial levels of symptoms, as well as the response to the medication at the end of 8 weeks, were correlated with [^{11}C]DASB$_{ND}$ at baseline.

Results: AN with lower premedication (baseline) [^{11}C]DASB BP$_{ND}$ values in the medial frontal ($r = .65$, $p = .02$) and dorsolateral prefrontal cortex ($r = .60$, $p = .03$) had the greatest reduction in Spielberger state anxiety self-ratings after 8 weeks of fluoxetine, whereas those who had an increase in anxiety after fluoxetine had increased [^{11}C]DASB BP$_{ND}$ values at baseline. There was no relationship between Spielberger state anxiety at baseline and [^{11}C]DASB$_{ND}$ in these regions, and no significant change ($p = .87$) between Spielberger state anxiety values at baseline (36.3 ± 9.7) and after treatment (34.3 ± 12.3).

Discussion: [^{11}C]DASB is not displaced from 5-HTT sites by physiologically relevant 5-HT concentrations. Thus [^{11}C]DASB BP$_{ND}$ presumably reflects 5-HTT density and/or affinity. It has been postulated that reduced [^{11}C]DASB BP$_{ND}$ may reflect reduced function of the 5-HTT, and therefore is associated with increased extracellular 5-HT (Meyer, 2007). SSRIs bind to 5-HTT and raise extracellular 5-HT by inhibiting its reuptake. Since those with the most substantial reduction in anxiety after fluoxetine had lower [^{11}C]DASB BP$_{ND}$, it is possible that this subgroup had more elevated extracellular 5-HT. There is evidence that SSRIs cause an increase of 5-HT in the frontal cortex (Blier, 1994) that leads to a cascade of effects, such as down-regulation of 5-HT$_{1A}$ autoreceptor function. This is thought to be necessary for therapeutic response. While speculative, it may be that some individuals with AN are unable to mount a 5-HT response to SSRIs due to altered 5-HTT function. It is important to note that other studies have implicated altered 5-HT frontal lobe function to be associated with anxious symptoms in AN. For example, a positive correlation was found between harm avoidance and 5-HT$_{1A}$ binding in the medial orbital frontal cortex in REC restricting type AN (Bailer, 2005). A better understanding of altered 5-HTT function may be of heuristic value in developing more effective treatments for these difficult and deadly disorders.

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125. Food Motivation Circuitry Dysfunction during Hunger and Satiety: From Active Anorexia Nervosa to Extreme Obesity

Laura Holsen*, Elizabeth Lawson, Cary Savage, Laura Martin, Amanda Bruce, Rebecca Lepping, Eunice Ko, Justine Blum, Nikos Makris, Anne Klibanksi, William Brooks, Merlin Butler, Jennifer Zarcone, Jill Goldstein

Brigham and Women's Hospital, Boston, USA

Background: Disordered eating is a significant public health issue associated with conditions ranging from self-imposed starvation to obesity. Anorexia nervosa (AN) is an eating disorder characterized by severe weight loss, significant endocrine abnormalities, and food reward circuitry brain dysfunction. Conversely, the phenotype of Prader-Willi syndrome (PWS) includes extreme hyperphagia and obesity. Previous studies on eating behavior, body composition, appetite-regulatory peptide levels, and neural substrates of hyperphagia in PWS indicate the potential of this genetic syndrome to serve as an extreme model of obesity. Thus far, functional neuroimaging studies on food reward and motivation have not examined the full spectrum of disordered eating, and research on the pathophysiology of AN has failed to improve prognosis. We describe a series of fMRI studies which investigate brain activity deficits in food reward circuitry regions during hunger and satiation states in women with AN, healthy controls, individuals with simple (non-PWS) obesity (OB), and individuals with PWS.

Methods: Study 1 sample: Female participants [12 women with AN, 11 age-matched healthy-weight control women (HWC)] viewed high-calorie food and non-food (household objects) images while undergoing functional MRI (fMRI) scanning on a 3T Siemens Trio MR scanner before (pre-meal) and after (post-meal) eating a 400 kcal meal. Stimuli and hunger ratings were also acquired. Study 2 sample: Fourteen individuals with PWS, 14 BMI- and age-matched individuals with OB, and 15 age-matched healthy-weight controls (HWC) viewed food and non-food (animals) images while undergoing fMRI on a 3T Siemens Allegra MR scanner before (pre-meal) and after (post-meal) eating a 500 kcal meal. Data analysis: Data from both studies were analyzed using SPM8 to examine specific between-groups contrasts (Study 1: High-calorie food > objects, AN vs. HWC; Study 2: Food > animals, PWS vs. OB vs. HWC) separately in the pre- and post-meal states. Regions of interest (ROIs) included: hypothalamus (HYPO), nucleus accumbens (NAc), amygdala (AMYG), hippocampus (HIPP), insula (aINS), orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC).

Results: Study 1: In response to high-calorie foods *pre-meal*, AN women demonstrated significant hypoactivation in comparison to HWC women in the HYPO [effect size (d) = 0.93], AMYG (d = 0.84), HIPP (d = 1.35), OFC (d = 1.05), and aINS (d = 1.30). Voxel-wise, FWE-corrected results held in the AMYG and aINS even after controlling for % ideal body weight (%IBW). Similar hypoactivation in AN women was also demonstrated post-meal in the AMYG (d = 0.95) and aINS (d = 1.07), even after controlling for %IBW. Study 2: In response to food images, both OB and PWS groups, compared to HWC, showed *pre-meal* hyperactivation in the HYPO and AMYG, and post-meal hyperactivation in the HYPO. In a direct between-group contrast, individuals with PWS exhibited hyperactivation vs. OB post-meal in the AMYG (d = 0.68) and HIPP (d = 0.68), and hypoactivation vs. OB in the DLPFC (d = 0.95) and OFC (d = 0.72).

Discussion: Findings suggest that subcortical and cortical regions associated with hunger, satiation, and food processing demonstrate significant dysfunction in disorders involving abnormal food intake. Specifically, both before and after eating, we found reduced activity in limbic and paralimbic regions in anorexia nervosa, a disorder of self-starvation, while on the other end of the spectrum,

elevated activation of these same regions was seen in Prader-Willi syndrome, a condition associated with hyperphagia. These results provide evidence of a continuum relating food intake behavior (and outcomes) and food reward circuitry activity, and thus suggest novel neurobiological circuits to target for modulation in treatment of anorexia and obesity.

126. Differential Activation of Ventral and Dorsal Striatum by Motivational Value and Motiavtion Salience

Daniel W. Hommer*, Ashley Smith, Jodi Gilman, Reza Momenan

LCTS/NIAAA/NIH, Bethesda, USA

Background: The striatum plays a major role in motivation and learning. What is less clear is whether the striatum functions differently during behavior motivated by reward and behavior motivated by punishment. Bromberg-Martin *et. al.* (2010) recently reviewed the function of dopamine neurons during reward and non-reward and suggested that there are two types of dopamine neurons. One type codes for motivational value. The other type codes for motivational salience. The value coding neurons project to the nucleus accumbens and are excited by reward and inhibited by non-reward (particularly when reward is expected). The salience coding neurons are excited by any motivationally important stimuli (signaling either reward or punishment) and project to more extensive and dorsal regions of the striatum. We used a risky decision-making task to test the hypothesis that blood oxygenation level dependent (BOLD) signal contrasts of motivational value (notification of gain versus loss) would be localized to the nucleus accumbens, while contrasts of motivational salience (notification of results of risky versus non-risky choice) would localize to the more dorsal regions of the striatum.

Methods: We performed fMRI on 32 healthy adult volunteers (27.0 ± 4.1 years old, 15 males) while they played a risk-taking task (modeled after (Matthews, *et. al.* 2004)). Participants were scanned on a 3T General Electric MR scanner with a 16-channel head coil. Images were analyzed using Analysis of Functional Neural Images (AFNI) software. The risk-taking task required participants to choose between a 'safe' box (safe choice) where they were guaranteed to win $0.25, or a 'mystery' box (risky choice) which presented the possibility of winning $1 or $5, but also the risk of losing those amounts. Each trial of the task consisted of four events (2 s each in duration, interval between them jittered 2-6 s): 1) cue presentation, 2) choice 3), a delay, and 4) feedback, notification of the outcome. In the scanner participants played two,12-minute runs of the task. Our analyses in this report focus exclusively on the feedback (notification of outcome). We contrasted the non-salient notification of outcome following safe choice with the more salient notification of outcome following risky choice. We also compared notification of gain or loss following risky choice with notification of the guaranteed safe outcome. Finally to directly examine effect of outcome value we contrasted notification of gain with loss.

Results: All contrasts reported were thresholded for family-wise errors at an alpha of < .01, a volume of > 40 voxels and a t > 5. The contrast between salient and non-salient outcome (notification of results of risky versus non-risky choice) revealed activations in bilateral anterior insular cortex, right pre-genual cingulate and para-cingulate corticies extending into mesial BA8, bilateral caudate and ventral striatum, right medial dorsal thalamus and peri-aqueductal gray. The contrast between notification of loss following risky choice and notification of safe choice

outcome revealed a pattern of activation similar but less extensive than the risky versus safe outcome. Activation during outcome of wins following risky choice showed a similar pattern to the outcome activation patterns reported above; however, the focus of striatal activation appeared to be more ventral. The contrast of notification of wins versus losses following risk choice showed significant activation restricted exclusively to the right nucleus accumbens suggesting that this region is particularly involved in motivational value. This same region of the nucleus accumbens was also sensitive to the value of the monetary outcome. In contrast, a region of the caudate was selectively sensitive to monetary value of losing outcomes.

Discussion: The functional distinction between dopamine neurons that code for general motivational salience and those that code for value (gain or loss) motivation can be detected in the striatal terminal areas of these neurons by measuring BOLD during notification of certain versus uncertain outcomes in healthy humans.

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127. Functional and Structural Neuroimaging of Blast mTBI in Iraq and Afghanistan Veterans

Elaine R. Peskind*, Eric C. Petrie, Donna J. Cross, Kathleen Pagulayan, David Hoff, Kim Hart, Matthew Tarobochia, Murray A. Raskind , Cynthia Mayer, Vasily Yarnykh, Satoshi Minoshima

VA Puget Sound Health Care System, Seattle, USA

Background: Disagreement exists regarding the extent to which persistent post-concussive symptoms (PPCS) reported by Iraq and Afghanistan combat Veterans with repeated episodes of mild traumatic brain injury (mTBI) from explosive blasts represent structural or functional brain damage or are epiphenomena of comorbid depression or posttraumatic stress disorder (PTSD). Brain imaging in this population may clarify this issue.

Methods: Subjects were 32 male Iraq and Afghanistan war (OIF/OEF) Veterans with blast-induced mild traumatic brain injury (mean age 31.5 ± 9.4 years [mean ± SD, range 25-60]) and 16 non blast-exposed OIF Veterans (mean age 33.1 ± 7.2 years [range 22-46]). Neurocognitive assessments on normal controls were within the expected performance for age and education and Mini Mental State Exam (MMSE) scores ranged between 28-30. Although there was individual variability within the mTBI group, there were no group differences on any neurocognitive measure between blast and non-blast exposed OIF/OEF Veterans. However, 9 of 32 mTBI subjects had MMSE scores below 28, as compared to 0 of 13 OIF/OEF controls. 74% of the blast-exposed OIF/OEF Veterans met DSM-IV criteria for posttraumatic stress disorder (PTSD). The functional imaging technique was [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET). The structural imaging technique was Cross-Relaxation Imaging (CRI) that determines macromolecular protein fraction.

Results: Fluorodeoxyglucose positron emission tomography [FDG-PET] revealed a pattern of significant hypometabolism in the posterior cingulate cortex (3.6%, p = 0.005) and bilaterally in the parietal cortex (2.6%, p = 0.01) in mTBI compared to OIF/OEF deployed, non-blast exposed controls. A separate analysis within the blast exposed OIF veterans comparing those with and without PTSD showed no differences in glucose metabolism between OIF/OEF Veterans with blast-exposure who did or did not have PTSD, suggesting that changes in brain metabolism in the blast exposed mTBI Veterans were not the result of PTSD. Cross relaxation imaging [CRI] revealed highly significant reductions (p values all < 0.0001) in the macromolecular bound pool proton fractions in whole brain white matter, gray matter, and mixed white-gray

matter histogram components, also consistent with diffuse axonal injury.

Discussion: Both structural CRI and functional FDG-PET imaging findings suggest a picture of diffuse axonal injury associated with focal cortical hypometabolism in blast mTBI. In particular, the robust CRI findings using a highly simplified analytic method suggest that whole brain histogram parameters of the macromolecular proton fraction have potential as prospective quantitative biomarkers of blast-induced mTBI with clinical diagnostic utility.

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128. Gonadal Steroid Hormones affect Hippocampal Activation during Spatial Navigation in Women

Shau-Ming Wei*, Philip D. Kohn, J. Shane Kippenhan, Erica B. Baller, Gabriela Alarcon, Peter J. Schmidt, Karen F. Berman

National Institutes of Health, Bethesda, USA

Background: Animal studies have defined a critical role for the hippocampus in the planning, encoding, replaying, and consolidation of environmentally relevant spatial information. In addition, there is considerable pre-clinical evidence that the ovarian steroid hormones estrogen and progesterone affect function of the hippocampus and facilitate hippocampal-dependent processes, including spatial navigation. However, documentation of these effects in humans has remained elusive both because function of the hippocampus offers particular challenges to neuroimaging and because well-controlled studies investigating ovarian hormones' effects in humans are scarce. Here, using an incisive hormone manipulation protocol, together with a 3D spatial navigation paradigm designed for neuroimaging such that the "active" and "control" conditions differed only in the rate at which subjects navigated in the experimental environment, we searched for hormone-related changes in neural activation during spatial navigation.

Methods: *Participants and Hormone Manipulation Protocol:* In the course of a six-month pharmacological protocol, seven healthy, regularly menstruating women (mean age = 31.4) with no psychiatric history underwent functional magnetic resonance imaging (fMRI) during each of three hormonal conditions : (1) ovarian suppression (i.e. temporary menopause) induced by the gonadotropin-releasing hormone agonist leuprolide acetate (Lupron), (2) Lupron plus estradiol replacement, and (3) Lupron plus progesterone replacement. *Navigation Task:* Participants moved interactively in a richly decorated, virtual 3-D house using four buttons in order to locate 20 apples. Each button press resulted in a smooth turn or forward or backward movement that lasted 800 ms. Each button press moved or rotated the subject at one of two fixed speeds (fast or slow) which alternated between 30 second blocks, thus systematically manipulating the amount of visuospatial information being processed per unit time. Subjects completed four five-minute runs of the task during each hormonal condition. Three different 3-D houses were created to prevent practice effect from the repeated fMRI scan sessions, and the order of houses was counter-balanced across conditions. Standard SPM5 preprocessing and analyses were employed to compare blocks with fast movement (more spatial information processing) to those with slow movements (less informational input). First level results were submitted to a full factorial analysis at the second level. A whole brain random-effects analysis was performed using subjects as a dependent measure and hormone condition as repeated measures, and results were examined with an exploratory statistical threshold of $p < 0.005$, uncorrected.

Results: There was no significant difference in performance across hormone conditions (mean apples located = 17.7; $F(2)$ = 0.31). Analysis examining the main effect of task (comparing fast to slow movement) revealed significant activation in bilateral hippocampus, as well as in the visual cortex, bilateral superior parietal cortex, basal ganglia, middle and superior temporal gyri, and middle and superior frontal gyri. A main effect of hormone condition was found in anterior hippocampus and amygdala bilaterally, as well as in the left middle and superior temporal gyri and anterior cingulate; post-hoc analyses revealed that, compared to ovarian suppression (Lupron alone), there was significantly more activation during both estrogen and progesterone replacement in all of these regions except anterior cingulate; during estrogen compared to progesterone replacement, there was greater activation in the right hippocampus and amygdala, as well as in the left putamen, anterior and posterior cingulate, and midbrain; the opposite hormonally-related activation pattern (progesterone > estrogen) was observed in the left superior and middle temporal gyri as well as in the left middle frontal gyrus.

Discussion: Our finding of greater hippocampal activity in the presence of estrogen is consistent with substantial pre-clinical evidence that estrogen enhances hippocampal synaptic plasticity. Preclinical effects of progesterone on hippocampal function have been less clear, but our preliminary data indicate that progesterone, like estrogen, affects hippocampal activity during spatial navigation in humans. Further analyses of functional connectivity between hippocampus and other brain regions will advance our understanding of the circuit-level repercussions of these hormonal effects on hippocampal function during spatial navigation.

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129. A Study of D2/D3 Dopamine Receptor Distribution in a Rare Form of Tourette Syndrome using [^{11}C]PHNO and Positron Emission Tomography

Jean-Dominique Gallezot, Wendol Williams, Keunpoong Lim, Minq-Qiang Zheng, Erin Loring, Richard E. Carson, John Krystal, Matthew State, Yu-Shin Ding*

Yale University School of Medicine, New Haven, USA

Background: Use [^{11}C]PHNO-PET to evaluate the distribution of D2/D3 dopamine receptors in members of a family with a Tourette syndrome (TS) who carry a rare mutation in the L-histidine decarboxylase (HDC) gene, and compare them with gender, age and weight matched healthy controls.

Methods: Three members of a family with TS and a mutated HDC gene and 9 healthy controls (HC) were included in the study: 2 males and 1 female (age = 29 ± 15; BMI = 30 ± 5) were part of the TS group; 6 males and 3 females (age = 29 ± 11; BMI = 28 ± 7) were included in the HC group. Each subject underwent one [^{11}C]PHNO scan on a HRRT scanner. Injected activities were 292 ± 129 MBq with a total injected mass of 0.028 ± 0.004 μg/kg (max = 0.032 μg/kg). Specific radioactivity was 58 ± 27 MBq/nmol at the end of synthesis and 32 ± 15 MBq/nmol at injection time. Head motion was tracked during the scan using a Vicra polaris optical tool. Images were reconstructed using the MOLAR algorithm (with 2 iterations and 30 subsets) with all corrections. Regions of interest (ROI) were delineated in the caudate, putamen and pallidum using the AAL template and nonlinear wrapping between the template MRI and each subject MRI. A region corresponding to the substantia nigra was also added to the template. Binding potentials were quantified using cerebellum as the reference region and SRTM for ROI-based kinetic modeling, and SRTM2 for computation of parametric images.

Results: [^{11}C]PHNO binding potentials (BP_{ND}) estimated with SRTM were higher in the caudate (+11%), putamen (+19%) and pallidum (+48%), but not significantly, and more markedly in the substantia nigra (+139%, po.01, Mann-Whitney rank-sum test) for members of the TS family as compared to HCs. Results were similar when SRTM2 was used to compute parametric images: increases were +8%, +17%, +30% and +93% (po.01, Mann-Whitney rank-sum test), respectively.

Discussion: [^{11}C]PHNO is a dopamine receptor radioligand that binds to both D2 and D3 subtypes with a higher affinity for the D3 subtype (approx. 30-50 fold) [1]. Due to the relative distribution of these two subtypes, [^{11}C]PHNO BP_{ND} in caudate and putamen is mostly due to the contribution of D2 subtype, and the BP_{ND} in the pallidum and substantia nigra is mostly due to the contribution of the D3 subtype. The fact that the highest increases of [^{11}C]PHNO BP_{ND} seen in this study were in the pallidum and substantia nigra may indicate that the observed effect is due to changes in D3 receptors. Further studies would be useful to investigate whether the observed effect is linked to the rare mutation, or if it can also be found in patients with idiopathic Tourette syndrome.

References:
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130. Free-Water Atlas Application to Neuro-Inflammation in Mild Traumatic Brain Injury

Martha E. Shenton*, Pasternak Ofer, Bouix Sylvain, Pelavin Paula, Zafonte Ross, Kubicki Marek

Harvard University and VA Boston, Boston, USA

Background: Acute traumatic brain injury (TBI) may lead to neuroinflammatory processes that induce excess osmosis of water into the extra-cellular space. Advances in diffusion imaging allow the estimation of free-water volume from DTI data, which serves as a new contrast mechanism, sensitive to extra-cellular water molecules. A secondary process of chronic inflammation may cause longer term symptoms, as toxins begin to damage myelin and neuronal tissue. Identification and quantification of inflammation is therefore important for diagnosis, treatment, and for understanding etiology, especially in cases of mild TBI, where CT and/or anatomical MRI scans are often negative.

Methods: Here we investigate extra-cellular abnormalities by constructing a free-water atlas based on 43 healthy volunteers and comparing individual TBI patients with this atlas. We tested 9 mild TBI cases (GCS 13-15) that were symptomatic, chronic, and had negative CT/MRI findings. Diffusion data were acquired from a 3T GE scanner and free-water maps were generated. Statistical inference was performed using a z-test, comparing a free-water map of a single subject with the template free-water-map and the voxel-wise standard deviation of all subjects in the atlas space.

Results: The TBI population showed increased free-water in different locations, likely dependent upon the type and orientation of the injury. Moreover, each TBI subject had at least one brain region with higher contrast than the atlas (fig. 1). For controls the contrast was similar to the atlas.

Discussion: These findings reveal free-water increases in TBI subjects, suggesting that the extra-cellular volume may be a marker of abnormal tissue, with neuroinflammation as a probable

mechanism that explains these changes. An immediate application for this method is to identify lesions in a single TBI subject, although the method can be applied to find other types of lesions such as those found in Multiple Sclerosis, Alzheimer's disease and cancer.

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131. Neural Correlates of Reappraisal Training to Reduce Negative Emotional Reactivity in Borderline Personality Disorder

Harold W. Koenigsberg*[1], Bryan Denny, Jin Fan, Xun Liu, Sarah Jo Mayson, Stephanie Guerreri, Kevin N. Ochsner, Antonia S. New , Marianne Goodman, Larry J. Siever

Mount Sinai School of Medicine, New York, USA

Background: Borderline personality disorder (BPD), a disorder characterized by emotional instability, interpersonal volatility, and a suicide rate of 10%, occurring in 2 to 5.9 % of the population, is quite difficult to treat. Recent work has shown that neural processing of emotion in BPD is aberrant. When BPD patients attempt to apply a common and highly adaptive emotion regulation strategy, cognitive reappraisal-by-distancing, they do not engage the anterior cingulate and intraparietal sulci as healthy subjects do and do not downregulate amygdala activity. We examine whether this impairment in emotion regulation can be reversed by focused training.

Methods: 10 BPD subjects were instructed to reduce their reactions to aversive images by using a reappraisal-by-distancing strategy. On each of 5 consecutive training days, they were shown 48 different negative images, each for 10 seconds. Half of the images were preceded by an instruction to simply look at the picture and half to distance. Subjects rated emotional reactions to each image after carrying out the instruction. Event-related fMRI images were obtained on day 1 (pre-training) and day 5 (post-training) as subjects viewed aversive images and applied the reappraisal-by-distancing strategy to half of the images and passively viewed half of the images. BOLD activation differences for distancing vs. passively viewing were compared pre- and post-training.

Results: With training, BPD subjects significantly reduced their subjective negative reactions to aversive pictures in the look condition (day 1 - day 5: $t(9) = 2.21$, $p < .05$). After training compared to pre-training, subjects showed a decrease in dorsomedial prefrontal cortex (DMPFC) BOLD activation and an increase in left caudate BOLD activation during reappraising-by-distancing. In addition, the degree to which subjects improved post-training in their ability to reduce negative responses by distancing correlated with an increase in caudate activity during distancing ($r = -0.585$, $p = 0.075$).

Discussion: BPD subjects who receive intensive training in reappraisal by distancing develop an increased capacity to reduce their negative subjective response to aversive images, even when simply looking at the images, and show an increase in left caudate and a decrease in DMPLC distancing-related BOLD activation after training vs. before training. The degree to which subjects decrease their negative emotional reactions post- vs. pre-training is proportional to the extent to which they show a post-training increase in distancing-related left caudate activation. These observations suggest that BPD patients may be trained to decrease their negative responses to aversive stimuli and that this trained enhancement in downregulating negative responses is associated with changed patterns of neural activation during the distancing operation.

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132. Distinct Patterns of Altered Default Mode Functional Connectivity in Borderline and Schizotypal Personality Disorders

Daniel R. Rosell*, Maarten Mennes, King-Wai Chu, Larry J. Siever, Erin A. Hazlett

Mount Sinai School of Medicine, Dept. of Psychiatry, New York, USA

Background: Borderline (BPD) and Schizotypal (SPD) personality disorders are associated with significant functional impairment and disproportionate utilization of clinical resources. The pathophysiologic bases of BPD and SPD, however, remain poorly understood, greatly limiting rational development of therapeutics and clinical biomarkers. In recent years, resting state fMRI has emerged as a powerful technique for investigating the myriad functional networks in the brain. One of the most commonly investigated functional brain networks is the default mode network (DMN), which is associated with mental processes such as mentalization, prospection, visualization, and episodic memory. These cognitive processes are of particular relevance to BPD and SPD. Moreover, a number of brain regions implicated in BPD and SPD by volumetric and task-based fMRI studies – dorsomedial prefrontal cortex (dmPFC), posterior (PCC) and anterior (ACC) cingulate, and temporal cortex – are among the major nodes of the DMN. Here, we set out to examine the functional integrity of the DMN in BPD and SPD. Concurrently examining these two personality disorders with similar levels of impairment but distinct phenomenological manifestations will enhance the specificity of our neurobiological findings.

Methods: We examined three age- and sex-matched groups of unmedicated participants without current major depression or active substance abuse who completed 3T fMRI scans on a Siemens Allegra scanner at Mt. Sinai: 30 BPD patients, 30 SPD patients and 30 healthy controls (HC; without an Axis I or II diagnosis). All participants passively viewed a series of unpleasant, neutral, and pleasant pictures (Hazlett et al., under review). We removed the task-induced variance from the event-related data, so that the remaining residuals could be considered as "continuous resting-state" data (Fair, 2007; Fox, 2006). We achieved this by modeling the task-related BOLD response to regress-out the associated variance. Subsequently, we carried out seed-based functional connectivity (FC) analyses on this "resting state" signal using 9 DMN nodes (Fair, 2008). For each participant we calculated whole-brain voxel-wise correlations for the time series of each seed, creating subject-level FC maps (Fisher's Z transformed). Group-level analyses were then carried out using a random-effects ordinary least squares model (min Z > 2.3; cluster significance: p < 0.05, corrected).

Results: No regions exhibited less FC in the BPD group compared to the HC group. Compared to the HC group, the BPD group exhibited greater FC between two DMN regions [PCC and left lateral parietal cortex (L lat-PC)] and the dorsal ACC (dACC) and medial superior frontal cortex (msFC). In the SPD group, frontal DMN regions exhibited less FC with the PCC, compared to the control group. Additionally, compared the the HC group, the SPD group also exhibited decreased FC between the R lateral temporal cortex (lat-TC) and the PCC. In the SPD group, the PCC exhibited less FC with the right dorsolateral prefrontal cortex (R DLPFC), and greater FC with the R insula.

Discussion: In the BPD group, the PCC and L lat-PC exhibited greater FC with dACC/msFC compared to the HC group. The dACC/msFC is a core node of the cingulo-opercular cognitive-control network, which is involved in the adjustment and long-term maintenance of salience and strategies related to goal-directed behavior. The decreased coordination between posterior DMN regions and the dACC/msFC in the BPD group is consistent with impairments in effortful regulation of self-experience leading

to the characteristic manifestations of affective lability, impulsivity, and interpersonal instability. In contrast, the decreased connectivity in the SPD group between dorsal frontal regions of the DMN and the PCC may lead to impairments in processing affectively salient information related to self, possibly accounting for the suspiciousness, social anxiety, and referential thinking observed in SPD. The decreased connectivity of the ventromedial PFC (vmPFC) and R lat-TC with the PCC in the SPD group may contribute to alterations in mental imagery and account for the perceptual disturbances characteristic of SPD. The differential connectivity in SPD between the PCC and the DLPFC (which is part of the fronto-parietal attentional network) and the insula (which is part of the cingulo-opercular network) may contribute to impairments in working memory and context processing, characteristic of SPD.

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133. Relationship of Symptom Domains and Diagnostic Severity to PET Scan Imaging in Borderline Personality Disorder

S. Charles Schulz*, Jazmin Camchong, Ann Romine, Michael Kuskowski, Jose V. Pardo, Kathryn Cullen, Kelvin O. Lim

University of Minnesota, Minneapolis, USA

Background: The purpose of this report is to describe the relationship between clinical rating assessments of borderline personality disorder (BPD) and PET-FDG regional parameters.
Methods: Subjects were recruited by newspaper ads, screened and then underwent SCID I and II assessment. After informed consent, they underwent PET-FDG scanning in a medication-free state. The scans were registered, intensity normalized and z transformed. Correlations were performed on a voxel-by-voxel basis and corrected for multiple comparisons with Buss Burke Hostility Index and the Zanarini-BPD rating scale.
Results: Fourteen subjects met BPD criteria and completed scans and rating scale administration. Scans showed a significant negative correlation between glucose metabolism in frontal brain areas and the BDHI (p < 0.05). Correlations of brain metabolic changes and diagnostic behavioral rating scales(ZAN-BPD) were seen mostly in posterior areas.The assessment of the statistical relationship of the BDHI to brain regions was substantially more robust than the correlations of the total ZAN-BPD score correlations.
Discussion: This exploratory study illustrates regional metabolic values that are highly related to hostile behavior and replicate earlier studies. The substantially greater correlations of the BDHI compared to the ZAN-BPD provides information about the brain underpinning of BPD.
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134. Resting State Functional Connectivity in 22Q11.2 Deletion Syndrome, a Recurrent Genetic Mutation Associated with Schizophrenia

Matthew Schreiner, Katherine Karlsgodt, Maria Jalbrzikowski, Jennifer Ho, Carolyn Chow, Carrie Bearden*

University of California, Los Angeles, Los Angeles, USA

Background: 22q11.2 Deletion Syndrome (22qDS), also known as Velocardiofacial Syndrome, is a genetic disorder arising from a ∼3 Megabase (Mb) deletion at the 22q11.2 locus. It is one of the most prevalent recurrent genetic mutations known to man, affecting around 1/4000 children. Notably, this syndrome is associated with a greatly increased risk of developing psychotic illness; as such, 22qDS offers a valuable model for investigating the pathophysiology of schizophrenia in a disorder with known genetic etiology. While structural brain anomalies have previously been reported in this syndrome, to our knowledge no studies have yet examined the intrinsic functional architecture of the brain in 22qDS. Cortical dysconnectivity is thought to be a critical factor in the expression of psychotic symptoms; in particular, prior studies of patients with schizophrenia have detected reduced functional connectivity between core components of the Default Mode Network (DMN), believed to reflect deficits in spontaneous, endogenously generated mentation. Here we used a seed-based approach to examine resting-state functional connectivity (RSFC) within key DMN regions- the posterior cingulate cortex (PCC) and ventromedial prefrontal cortex (vmPFC)- in patients with 22qDS and demographically matched, typically developing controls.
Methods: Study participants included 26 children and young adults: 9 individuals with a molecularly confirmed diagnosis of 22q11.2 deletion (4 female; Mean Age ± SD = 15.9 ± 4.9) and 17 healthy controls (11 Female, Mean Age ± SD = 15.1 ± 5.9). Functional and structural MRI scans were conducted in a 3 Tesla Siemens Trio MRI system. During the course of the 6-minute resting state scan subjects were instructed to close their eyes and relax. Imaging data were analyzed with FMRIB Software Library (FSL) tools. Functional preprocessing steps undertaken to remove potentially spurious sources of activation included skull stripping, spatial smoothing, motion correction, bandpass temporal filtering (0.005 Hz < f < 0.1 Hz) and regression of the global signal and 6-motion parameters, as well as age and sex. Images were registered to Montreal Neurologic Institute (MNI) space and average ROI timeseries were extracted from 6 mm-diameter spherical seeds placed at MNI coordinates for the PCC and vmPFC. These timeseries were correlated with the rest of the brain on a voxel-by-voxel basis at the individual subject level and the group level to generate correlation maps. The resulting statistical maps were overlaid onto the MNI standard brain and cluster-thresholded for significance at p < 0.05.
Results: Consistent with prior studies, typically developing controls showed robust positive correlations between the PCC and other DMN regions (medial frontal cortex, anterior cingulate cortex, and cerebellar regions). Spontaneous low-frequency oscillations in the PCC of healthy controls were negatively correlated with task-positive regions (i.e., dorsal and superior aspects of the frontal and parietal lobes, supplementary motor cortex). Between-group differences indicated an altered pattern of FC with the PCC in 22qDS patients, with controls showing greater connectivity among structurally distant DMN regions. 22qDS patients also showed stronger anti-correlations between the PCC seed, the right middle temporal gyrus, and the superior parietal lobule. Results from the between-group vmPFC seed-based analysis indicated significantly increased local functional connectivity in the frontal cortex in 22qDS patients vs. controls, primarily lateralized to the right hemisphere. In addition, a group-by-age interaction for FC of the PCC suggested an altered developmental trajectory of DMN connectivity in 22qDS youth.
Discussion: Our findings indicate increased local connectivity, with corresponding decreases in long –range connectivity, across DMN regions in 22qDS. These findings are consistent with recent studies showing abnormalities of DMN connectivity in patients with schizophrenia. The results suggest that faulty integration of default-mode regions may be associated with increased vulnerability to psychosis in this population. Further investigation into the degree to which atypical FC in the DMN and other resting state networks is associated with phenotypic variability in 22qDS (i.e., social cognition, psychotic symptoms) is warranted, and studies are currently underway to address these questions. Additionally, longitudinal studies are now in progress, which will allow us to further interrogate intriguing cross-sectional findings suggesting

an abnormal developmental trajectory of resting-state networks in 22qDS.

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135. Preparing for Conflict: Increased Activation of the Dorsal Striatum in Subjects at Ultra-High Risk for Psychosis

Tiziano Colibazzi*, Guillermo Horga, Zhishun Wang, Pengwei Wang, Kristin Klahr, Cheryl Corcoran, Bradley Peterson

Columbia University, New York, USA

Background: Disruptions of frontal, striatal and temporo-limbic circuits are well documented in schizophrenia. fMRI and PET studies have shown abnormalities in frontostriatal circuits in prodromal patients. Using a non-verbal equivalent of the Stroop task that requires subjects to inhibit a prepotent response to attend to a more task-relevant one, we had previously shown that individuals at ultra-high risk (UHR) for psychosis exhibited lower functional activity in the striatum and right dorsolateral prefrontal cortex during conflict trials. We now aimed at understanding the effect of preceding conflict on brain activity during preparation to subsequent trials. More generally, our goal was to understand how the history of preceding conflict is encoded in the brain and possibly allows adaptation to future conflict.

Methods: Our sample consisted of 26 UHR subjects enrolled in the COPE clinic (NYSPI) and 26 age- and gender matched healthy controls with mean ages of 20.9 and 20.3, respectively. Risk for psychosis was assessed through the scale for prodromal symptoms (SOPS) embedded in the structured interview for prodromal symptoms (SIPS). Individuals were deemed at ultra high-risk for psychosis if they belonged to any of the following categories: 1) subjects with attenuated psychotic symptoms; 2) subjects with intermittent psychotic symptoms or 3) subjects with concurrent genetic risk and functional deterioration. Participants were scanned at baseline on a 3T GE scanner using functional magnetic resonance imaging, while performing a non-verbal variant of the Stroop task, the Simon spatial compatibility task. Stimuli were presented using E-Prime software. Right- or left-pointing white arrows were displayed either on the left or right half of a black screen. Stimuli were congruent (C) if the arrow pointed in the same direction as their position on the screen or incongruent (I) if pointing in the opposite direction as their position on the screen. Participants were instructed to press a right button if the arrow pointed right and a left button if the arrow pointed left, regardless of the position of the arrow on the screen. Only correct trials were included in the analyses. There were a total of four conditions: incompatible (I) or compatible (C) trials, preceded either by incompatible (i) or compatible trials (c). After preprocessing, first level analyses were conducted individually using a modified version of the general linear model. Time series were modeled using the canonical hemodynamic response function convolved with each condition of the Simon task. Least square regressions were used to estimate parameters for each condition in every subject and these estimates were summed up across the 3 runs to generate contrasts. To investigate activation during blank trials and its relation to the preceding active (congruent or incongruent) trials, we created a multi-level multiple regression model. We chose a combination of a threshold p value of less than 0.025 and cluster size of 25 that allowed us address type I error by eliminating random activation of contiguous voxels.

Results: Both UHR subjects and healthy controls engaged in the Simon spatial compatibility task. Within each group, differences in reaction times (RTs) among different types of trials were significantly different $(cI > iI > iC > cC)$. Between-group differences in reaction times for the same condition were not significant. During the inter-trial period following an incongruent trial, both

groups activated dorsal striatal networks, although UHR subjects engaged them to larger extent than healthy controls.

Discussion: We have previously demonstrated that while they were responding correctly to conflict trials, UHR subjects failed to activate fronto-striatal circuits to the same degree than controls. We now suggest that during the preparatory phase that follows conflict, UHR subjects need to activate dorsal striatal circuitry more extensively in order to maintain behavioral performance on subsequent trials. We suspect that this striatal over-activation during preparation could reflect an attempt to compensate for lower striatal activity during task performance itself. Furthermore, because the basal ganglia are involved in selecting actions based on updated representations of the context, we speculate that this contextual updating may be deficient in UHR subjects.

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136. Altered Language Network Activity in Young People at Genetic High-Risk for Schizophrenia

Heidi W. Thermenos, Susan W. Gabrieli, Gul A. Jabbar, Richard Juelich, John D.E. Gabrieli, Martha Shenton, Marek Kubicki, Alan Francis, Duwors Robert, Matcheri S. Keshavan, Larry J. Seidman, Lynn E. DeLisi*

Harvard Medical School, Boston, USA

Background: Schizophrenia is highly heritable; and in addition, verbal abnormalities are core features of the illness. There is evidence that language processing is not only deviant in people with chronic schizophrenia, but also in their well siblings at genetic high-risk for illness. Deviations in the structural and functional brain pathways for language may underlie the symptoms of schizophrenia. We thus studied multiple language related modalities in people at genetic high risk for schizophrenia.

Methods: Approximately 80 subjects were ascertained who were between the ages of 18 to 30: 32 controls with no family history of schizophrenia or other psychotic illness(LRC) and 48 individuals from independent families with a first-degree and at least one additional other relative with schizophrenia(GHR). All subjects had extensive structured clinical interviews, a battery of neuropsychological tests examining language functioning, memory and executive functioning, and MRI scans that consisted of structural, diffusion tensor imaging and an fMRI sequence in which subjects viewed directly related, indirectly related, and unrelated prime-target word-pairs as they performed a lexical decision task, in which they decided whether each target was a real word or a non-word. Scans were performed at the Massachusetts Institute of Technology on a Siemens Tim 3.0 Tesla scanner using a 32 channel head coil. DTI and structural scans were examined for volumetric and white matter tract differences between the high-risk group and controls.

Results: Approximately 50% of those with a positive family history exhibited major DSM-IV Axis I psychopathology, mainly recurrent major depression and some Axis II traits such as social anxiety. However, less than 5% would be considered prodromal. In general,cognitive measures did not differ between groups, although oral soliloquys showed an increase in word associations in the GHR group compared with LRCs. In preliminary analyses, no white matter tracts relevant to language functioning were found to distinguish those at genetic high-risk from controls. However, some gray matter volumetric changes were detected, particularly in hippocampus. In the fMRI analyses, we examined semantic priming-related suppression and enhancement of brain activation. All subjects performed the lexical decision task at > 90% accuracy. There was a main effect of priming condition on reaction time (RT), but no effect of group and no group x

condition interaction on RT. LRCs exhibited significantly greater semantic priming-related suppression of activity than GHR subjects in the right inferiorfrontal gyrus and cerebellum (p < 0.005, cluster-level FWE-corrected for multiple comparisons). Mean parameter estimates extracted from the inferior frontal gyrus cluster revealed that controls exhibited a monotonic decrease in activation with increasing level of prime-to-target semantic relatedness that was not seen in GHRs. By contrast, GHRs exhibited significantly greater priming-related *enhancement* of activation than LRCs in the left superior and middle temporal gyrus (p < 0.005, cluster-level FWE-corrected for multiple comparisons). Mean parameter estimates extracted from this cluster revealed exaggerated activity in the superior and middle temporal region in GHR during the Directly-related condition (relative to other conditions) that was not seen in LRC. Analyses of all MRI, neuropsychological testing and MRI data taking into account evidence of psychopathology are ongoing.

Discussion: Our preliminary data thus far suggest that some alterations in language pathways may be present in people at high genetic risk for developing schizophrenia. Whether these alterations indicate who among this group will eventually develop psychosis or have functional deficits in attaining social, educational and occupational goals is unknown, but can be clarified ultimately in longitudinal follow-up studies.

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137. Alterations of Reward Anticipation in Initially Antipsychotic Naïve Schizophrenia Patients Before and After Antipsychotic Monotherapy with Amisulpride

Mette Nielsen, Egill Rostrup, Sanne Wulff, Henrik Lublin, Shitij Kapur, Birte Glenthoj*

Center for Neuropsychiatric Schizophrenia Research and Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research, Copenhagen University Hospital, Psychiatric Center Glostrup, Glostrup, Denmark

Background: Core schizophrenic symptoms, such as anhedoni, apathy and delusional beliefs, are suggested to be caused by a dysfunction of the brain reward system. Several studies have found alterations of the reward processing in schizophrenic patients, but it still remains unclear which of these changes are caused by the disease process and which are caused by the medication. To investigate these issues, we have conducted a study looking at reward disturbances in antipsychotic naïve schizophrenia patients before and after their first antipsychotic treatment.

Methods: 24 antipsychotic naïve schizophrenia patients and 24 age and gender matched healthy controls have been examined with functional Magnetic Resonance Imaging (fMRI) while playing a variant of the Monetary Incentive Delay task. The psychopathology of the patients was rated with PANSS and GAF. All examinations were repeated after six weeks where the patients were treated with the dopamine D2/3 antagonist amisulpride.

Results: *Baseline:* During the reward anticipation phase, a voxel-wise whole brain analyses showedwidespread group differences in response to cues signalling the need to act to secure a reward. Of particular interest these areas included the Ventral Tegmental area (VTA), the bilateral Striatum and the Anterior Cingulate Cortex (ACC). The schizophrenia patients showed an attenuation of the activation in this contrast compared to the healthy controls. *Follow up:* At follow up, patients were medicated with a mean daily dose of 302 mg of amisulpride. The mean total PANSS score were significantly improved from 83 to 68 and the mean GAF improved significantly from 41 to 52. Looking at the fMRI with a voxel-wise

approach, we did not find any significant effect of time, neither any group x time interaction in a repeated measure ANOVA. Looking at regions of particular interests in relation to medication effect, there was a significant group x time interaction in the right ventral striatum (p = 0.03). In this area, the schizophrenic patients had an increase of the signal in the contrast over time, while the healthy controls had a decrease. The fMRI results are still preliminary and represent work in progress.

Discussion: Our baseline results suggest that changes in reward processing in relation to reward anticipation is a primary disturbance in schizophrenia.The attenuation of the signal at baseline in the network of VTA, VS and ACC might be a result of an increased dopamine turnover in the patients, which decreases the signal to noise ratio and thereby drowns out the BOLD signal. At follow up, there was a significant group x time interaction in the right VS. This is of great interest, since amisulpride is thought to have a limbic selectivity and thereby bind most effectively to the D_2receptors in this area. Patients showed an increase over time of signal in the contrast, which might indicate, that the D2 antagonist tend to normalize the function of the brain reward system.

138. Decreased Short-Distance fMRI Connectivity, Functional Pruning and Network Randomization in Childhood-Onset Schizophrenia

Aaron F. Alexander-Bloch, Jay Giedd, Ed Bullmore, Nitin Gogtay*

University of Cambridge, Brain Mapping Unit, Cambridge, United Kingdom

Background: The human brain is a computationally complex network embedded in three-dimensional anatomical space. Many aspects of its organization can be attributed to an economical principle that limits the distance (or wiring cost) of connections. The brain's profile of short- and long-distance connections reflects the diverse properties of the complex network, both segregative and integrative. Schizophrenia has been associated with disturbances in many of these properties, including decreased modularity, a breakdown of the brain's normal functional community structure (Liu *et al.*, 2008; Lynall *et al.*, 2010; Alexander-Bloch *et al.*, 2010). We hypothesized that changes in complex network properties reflect alterations in the relationship between anatomical distance and functional connectivity, with schizophrenia showing a specific reduction in short-distance functional connectivity.

Methods: Participants with childhood-onset schizophrenia (COS; N = 19) and also healthy participants (N = 20) were recruited for the NIH study of COS and normal brain development. The institutional review board of the National Institutes of Health approved the study and written informed consent and assent were

obtained from parents and children respectively. Subjects had 2 sequential 3 minute EPI scans, the mean time series from ∼300 regions were wavelet-filtered to 0.05-0.1 Hz, and sequential scans were concatenated. The absolute wavelet correlation was the measure of functional connectivity, and functional connections were thresholded to generate sparse networks, composed of a small percentage of the strongest functional connections. Anatomical distance was estimated as the Euclidean distance between the centroids of brain regions. We systematically explored relationships between fMRI functional connectivity, complex network topology, and the anatomical distance between regional nodes.

Results: Participants with schizophrenia showed a loss of short-distance functional connectivity compared to healthy participants. Functional connectivity was disproportionately reduced for pairs of regions < 40 mm apart. In thresholded networks where the number of edges was controlled, this resulted in a longer mean connection distance in schizophrenia. As exemplified by the distance between the 10% strongest edges in every subject, this difference was highly significant (healthy participant average connection distance = 54 mm; COS average connection distance = 59 mm; t-statistic = −4.2708; $P < .0002$). Between-group differences in connection distance were localized specifically to multimodal cortical hubs in lateral temporal, parietal, dorsal prefrontal and medial prefrontal/cingulate regions. Complex network properties were highly correlated with average connection distance, and predictably, differed between the clinical groups. Average connection distance had a main effect on modularity ($F = 33.2$; $P < 0.00001$), clustering ($F = 6.6$; $P < .02$), and small-worldness ($F = 8.8$; $P < .006$); schizophrenia had a main effect on modularity ($F = 17.9$; $P < .0002$), clustering ($F = 11.5$; $P < .002$) and small-worldness ($F = 4.5$; $P < .05$); and there were no interaction effects between distance and clinical status. In other words, longer connection distances were generally associated with reduced modularity, clustering and small-worldness, with the corollary that these topological metrics were significantly abnormal in the COS group. Simulation studies confirmed that a change in the distance of network connections, of the type observed in the COS data, could cause the fMRI network phenotypes of decreased modularity, decreased clustering and decreased small-worldness.

Discussion: We present novel evidence for a specific decrease in short-distance functional connectivity in schizophrenia. In addition, we show how this simple alteration has knock-on effects on the topological properties of the brain network, which could contribute to disturbances in information processing in affected individuals. This link between distance and topology is encouraging, because a mechanistic explanation of decreased short-distance connectivity seems more tractable than the basis of purely topological disturbances. For example, it has been observed previously that short-range functional connectivity is "pruned" in favor of long-range connectivity during normal development (Fair, 2009; Supekar, 2009). Our results are consistent with schizophrenia as a disorder of excessive pruning in this sense, and suggest that such pruning could underlie the disruption of complex network properties.

Disclosure: A. Alexander-Bloch: None. **J. Giedd:** None. **E. Bullmore:** Part 5: ETB is employed half-time at GloxoSmithKline and half-time at University of Cambridge. **N. Gogtay:** None.

139. Associations between Cannabis Use and Longitudinal Structural Brain Changes in Biological Relatives of Schizophrenia Patients

Beng-Choon Ho*

University of Iowa, Iowa City, USA

Background: Epidemiologic studies indicate that adolescent cannabis use is associated with a two-fold increased risk for developing schizophrenia. Animal studies have shown adolescence to be a sensitive time period during which the brain may be especially vulnerable to the adverse effects of cannabis. Chronic tetrahydrocannabinol (THC) administration in adolescent rats, but not adult or pre-pubescent THC exposure, leads to enduring cognitive deficits in adulthood, including learning and memory impairment.It is hypothesized that THC-associated cognitive deficits may be related to apoptosis and other neuromodulatory processes following cannabinoid receptor activation. *Despite clear evidence that THC induces neural cell death, most human structural neuroimaging studies have been inconclusive regarding the harmfulness of* cannabis exposure. *Recent studies indicate that* genetic susceptibility for schizophreniaconfers increased vulnerability to cannabis' deleterious effects on brain structure.

Methods: We examined longitudinal changes in MRI brain volumes and DTI measures involving 19 first-degree relatives of schizophreniapatients and 11 healthy controls (HNV) without family history of schizophrenia. These 30 adolescents/young adults each underwent a MRI brain scan at intake and a follow-up scan approximately 2 years later. None of the HNV has cannabis use. Seven relatives of schizophreniapatients have a history of adolescent cannabis use while the remaining 12 relatives of schizophrenia patients have no lifetime cannabis exposure. Group differences in within-subject brain volume changes were analyzed using voxel-based morphometry as implemented in SPM8. Longitudinal changes in fractional anisotropy (FA) used tract-based spatial statistics.

Results: We found statistically significant group-by-time interaction effects on longitudinal volume and FA changes within brain regions rich in cannabinoid receptors and brain areas previously implicated in schizophrenia. These include parahippocampus, cerebellum, superior temporal gyrus, anterior cingulate and prefrontal cortices. Biological relatives with adolescent cannabis use showed the greatest longitudinal brain changes followed by biological relatives without cannabis use.

Discussion: Our findings suggest that adolescent cannabis exposure contributes to abnormalities in brain structure developmental trajectory during late adolescence/early adulthood. Additional longitudinal studies are needed to fully elucidate the impact of adolescent cannabis use on brain structure in humans. Future studies utilizing sophisticated methods in complex brain network analysis will further enhance our understanding regarding neurobiological mechanisms underlying adolescent cannabis as a risk factor for schizophrenia.

Disclosure: B. Ho: Part 4: Janssen Ortho McNeil Scientific Affairs, Part 5: None.

140. A Functional MRI Study of the Nerocognitive Effect of Quetiapine Compared to Haloperidol in Schizophrenia

Hisham M. Ibrahim*, C. Munro Cullum, Robert W. Greene, Carol A. Tamminga

University of Texas Southwestern, Dallas, USA

Background: Conventional treatment paradigms for schizophrenia have typically focused on reducing positive symptomatology; however, it is increasingly apparent that negative and cognitive symptoms are also important treatment targets. While traditional antipsychotics have little or even a detrimental effect on neurocognitive impairment in patients with schizophrenia, some data suggest that cognitive function may be improved during treatment with atypical antipsychotics.This double-blind study compares the cognitive effects of quetiapine, as measured by a battery of neuropsychological tests, and parallel regional cerebral blood flow changes, using functional MRI (fMRI), with the cognitive effects of holoperidol.

Methods: The patients (N = 20) were randomly assigned to one of two study groups: haloperidol 5-10 mg/day (n = 9), and quetiapine

600-1200 mg/day (n = 11). The study had three segments: an initial 3-day inpatient washout period followed by a 2-week inpatient segment during which study medications were escalated to the specified dose followed by a 6-month outpatient follow up. Efficacy measures, neurocognitive assessments and fMRIs using the N-back task specifically targeting the prefrontal cortex were obtained at three times: (1) at the end of the washout period, (2) at the end of the inpatient segment (week 2), and (3) at the end of the study (week 26). The neurocognitive assessment battery was designed to evaluate new learning and memory, working memory, and several aspects of attention/executive function. The Positive and Negative Syndrome Scale (PANSS) was used to rate the symptoms of schizophrenia. Extrapyramidal Symptoms were assessed by using the Simpson Angus Scale (SAS) and the Barnes Akathisia Scale (BAS).

Results: Quetiapine was significantly better than haloperidol in improving speed of processing (WAIS-coding scale) at Week 2 (p = 0.02) but not week 26 (p = 0.14). There were no other significant differences between the two study drugs on any other cognitive measure. Within-group differences analysis revealed significant time effects in verbal fluency (Boston Naming test) at Week 2 (p < 0.01) and week 26 (P < 0.05) for both study drugs. PANSS total scores were significantly lower at Week 2 and week 26 in both treatment groups, but there were no significant differences on PANSS scores between quetiapine and haloperidol groups at any study time point. There were no significant changes over time or between-groups differences on the Barnes akathisia and SAS scales. Additional data (including fMRI data with N-back) will be reported at the time of the presentation.

Discussion: This pilot study suggests that both quetiapine and haloperidol reduce severity of symptoms and may improve verbal fluency in schizophrenia. Quetiapine treatment may be associated with significant improvement in speed of processing compared to haloperidol. Additional fMRI data are needed to compare the cognitive effects of quetiapine and haloperidol.

Disclosure: H. Ibrahim: None. **C. Cullum:** None. **R. Greene:** Part 4: ASTRA ZENECA PHARMACEUTICALS. **C. Tamminga:** Part 1: Acadia Pharmaceuticals, Inc. Intracellular Therapies, Inc. Lundbeck, Inc. Pure Tech Ventures, Part 3: Acadia Pharmaceuticals, Inc. Intracellular Therapies, Inc. Lundbeck, Inc. Pure Tech Ventures. **C. Tamminga:** Part 1: Acadia Pharmaceuticals, Inc. Intracellular Therapies, Inc. Lundbeck, Inc. Pure Tech Ventures, Part 3: Acadia Pharmaceuticals, Inc. Intracellular Therapies, Inc. Lundbeck, Inc. Pure Tech Ventures.

141. Antipsychotic Treatment Enhances the Cortical Substrate for Attention and Behavioral Planning in First Episode Schizophrenia

Sarah K. Keedy*, James L. Reilly, Margret S.H. Harris, Tin Khine, Sunil Shreshtha, Cherise Rosen, Robert Marvin, Peter J. Weiden, John A. Sweeney

University of Illinois, Chicago, USA

Background: Few studies have systematically investigated how antipsychotic medication impacts functional brain systems to enhance cognition and behavior. Studies in first episode schizophrenia patients performing externally-elicited and internally-generated saccades in the laboratory have demonstrated significant changes after antipsychotic treatment. In our prior fMRI studies of externally-elicited saccades in untreated first episode patients, we demonstrated dorsal cortical abnormalities in the visual attention system, with the most consistent finding being reduced activation in frontal cortex. Post-treatment, these dorsal cortical deficits were no longer observed. The present study sought to replicate these findings for externally-elicited responses and extend them by also examining brain systems supporting similar but internally-generated responses. For this, we employed a predictive saccade task, which is behaviorally similar to an externally-elicited saccade task, but more robustly engages prefrontal, anterior cingulate, parietal, and anterior medial temporal cortex due to greater reliance on internal representations to guide responses.

Methods: Unmedicated first episode schizophrenia patients participated in fMRI studies before and after 5 weeks of second generation antipsychotic treatment (n = 12 treated with a mean of 2.5 mg [SD = 1.6] risperidone; n = 2 treated with 15 mg olanzapine). During each scan session they performed blocks of a visually-guided (externally-elicited) saccade task alternating with blocks of a predictive (internally-generated) saccade task, with interspersed fixation/rest periods. For each saccade task, a white dot appeared in a new location every 1sec and participants were instructed to follow it with their eyes. For externally-elicited saccade blocks, the target location was unpredictable. For internally-generated saccade blocks, shifts in target locations were highly predictable, alternating for 20 consecutive trials between two locations, resulting in saccades anticipating target appearance. Demographically matched healthy controls also performed the tasks twice at 5 week intervals.

Results: Task performance was equivalent between groups. As expected, participants had faster responses during the predictive saccade task compared with the externally-elicited saccade task. For the externally-elicited saccade task, brain activation deficits in schizophrenia patients prior to treatment were normalized after treatment, including frontal and parietal attention systems. Similarly, for the internally-generated saccade task, schizophrenia patients displayed abnormalities prior to treatment in frontal and parietal attentional systems as seen in the externally-elicited saccade task, and also had reduced function in prefrontal cortex and additional parietal and anterior temporal regions. After treatment, these deficits were largely normalized. Post-treatment there was some remaining activation deficit in anterior temporal cortex. There also was a reduction in parahippocampus activation not seen pre-treatment, and in dorsolateral prefrontal cortex, where activation was actually increased before treatment. By comparison, healthy control participants displayed no increases in activation over time.

Discussion: Initiation of antipsychotic treatment in first episode schizophrenia patients was associated with normalized function in visual attention systems, and in prefrontal systems that support the ability to plan and quickly enact voluntary behavior. However, there also was evidence for reduced activation in temporal structures supporting spatial learning and memory. This potentially negative observation occurred in the context of clear systems-level enhancement of function in cortical networks supporting attention and behavioral planning after antipsychotic treatment in acutely ill first episode schizophrenia patients.

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142. White Matter Geometry and Gender Effects in Adolescent-Onset Schizophrenia

Marek Kubicki*, Peter Savadjiev, Morgan, E. Hough, Thomas Whitford, Martha, E. Shenton, Tim J. Crow, Anthony C. James

Harvard University, Boston, USA

Background: The normal human brain is characterized by a pattern of gross anatomical asymmetry known as the brain torque, thought to arise from the lateralization of function, especially

the lateralization of language (e.g., Toga and Thompson, 2003). A particular aspect of the torque is that it underlies an important sexual dimorphism: men's brains tend to be more asymmetric than women's (McGlone 1980). This fact, along with the well known gender differences in brain development and onset of psychosis, have led to a theory that schizophrenia is a disorder with origins in brain development, which is reflected in an abnormal pattern of brain torque (Geschwind et al., 1984, Crow et al., 2007). This theory rests on the assumption that abnormal neurodevelopmental processes result in changes in brain torque that may also lead to psychosis (Crow et al., 2007), due to alterations in interhemispheric connections involved in the processing of language. Furthermore, these alterations are expected to be gender-specific, thus possibly providing an explanation for the sex difference in psychosis, i.e., that psychosis onset in males occurs earlier in life, with generally worse outcomes than in females (Crow et al., 2007). Most previous studies of brain torque focus on volume measurements, or on cortical surface complexity measures based on sulco-gyral pattern (e.g., Narr et al., 2001). However, these features are too variable to detect reliable differences on their own. In this work, we investigate the geometry of interhemispheric white matter connections and its relation to Crow's theory, with a particular focus on gender. In our experiments, we use a recently introduced white matter dispersion index (DI; Savadjiev et al., 2010) to describe the geometrical structure of white matter directly from diffusion tensor imaging (DTI) data. We work with adolescents under the age of 18, where we expect structural abnormalities in white matter geometry to be due to abnormal development rather than to progressive degeneration.

Methods: Diffusion tensor images were acquired from 26 healthy male controls, 26 healthy female controls, 40 male schizophrenia patients and 21 female schizophrenia patients. All subjects were between the ages of 13 and 18. The DI was computed as described in Savadjiev et al., (2010). The resulting data was analyzed using Tract-Based Spatial Statistics (TBSS; Smith et al., 2006), in order to investigate voxelwise differences in DI between the SZ and control groups across all major white matter fasciculi. Firstly, all subjects' DI and fractional anisotropy (FA) images were aligned into a common space using the nonlinear registration tool FNIRT (FMRIB Centre, University of Oxford; www.fmrib.ox.ac.uk/analysis/techrep). The aligned FA images were averaged to create a mean FA image, which was then thinned to create a mean FA skeleton. The skeleton represented the centers of all white matter tracts that were common to all subjects. Each subject's aligned DI data was then projected onto the skeleton, and the resulting data was used to perform voxelwise statistics between subjects.

Results: Results show a significant diagnosis by gender interaction in the anterior left corpus callosum. Compared to male controls, both male patients and female controls showed an increased DI in the left anterior corpus callosum. The same increases were observed when male patients and female controls were compared with female patients.

Discussion: The corpus callosum develops in the antero-posterior direction, with development of posterior parts peaking in the third and fourth decades of life. It is therefore interesting that with our cohort of adolescent-onset schizophrenia patients and age-matched controls, we observe differences in white matter geometrical structure mainly in the anterior parts of the corpus callosum. Furthermore, the locations of these differences are consistent with Crow's theory on how the brain torque would change with diagnosis, as well as with gender. We observe that the trajectory of torque changes is reversed for women compared to men, with female patients sharing features with male controls, and male patients with female controls. To the best of our knowledge, this is the first study to focus on white matter geometry differences in the context of brain torque and schizophrenia.

143. 18-F-Fallypride Binding Potential and Age in Patients withSschizophenia

Monte Buchsbaum*, Douglas Lehrer, Brad Christian, Jogeshwar Mukherjee

University of California, San Diego, USA

Background: Abnormalities in the dopaminergic system are implicated in schizophrenia. Schizophrenia is an illness characterized by a triphasic and variable lifetime course, typically beginning in adolescence, with either a fulminant or insidious course, peaking in young adulthood, and waning in later life. While physical strength may wane with age, social skills and some cognitive functions may improve. This sphinx-riddle pattern may be more prominent in older patients with schizophrenia with a diminution of psychotic symptoms, fewer hospitalizations, and improved psychosocial functioning. Response to antipsychotic medications may diminish over the lifetime. Morphometric analysis of brain structure volumes has suggested that young patients may resemble older healthy subjects. Identification of aging trajectories in dopamine binding is an important prerequisite in understanding this course.

Methods: [F-18]Fallypride is a highly selective, high affinity PET ligand well suited for measuring D2/D3 receptor availability in both the extrastriatal and striatal regions of the brain. The generation of parametric images yielding binding potential (BP) permits the use of statistical parametric mapping for analysis. Resting [F-18]fallypride PET studies were acquired on 33 drug naïve schizophrenics (mean age 28.8, range 18-53) and 18 normal controls (mean age 28.9 years, range 19-48) The fallypride BP images of each subject coregistered to their own RI, and cortical Brodmann areas and subcortical regions were assessed using standard techniques.

Results: Normal volunteers showed significant decreases in binding potential with age in most cortical areas. These decreases were typically in the 10% per decade range. A few regions showed increases with age, including Brodmann area 25. In contrast, patients with schizophrenia tended to have lower binding potential in the first decade (ages 18-28) than normal controls and then show little further decrease. This pattern was most marked in the anterior cingulate (Brodmann area 23) where normals decreased from age 18-50 while patients showed amost no change with age. Similar aging patterns were shown in the superior temporal gyrus (Brodmann area 22). The exception in orbitofrontal cortex (Brodmann area 25) was noteable with both normals and patients increasing binding potential with age in this region.

Discussion: These findings suggest that extrastriatal differences in dopamine D2/D3 receptor availability can be detected using a high affinity D2/D3 radioligand, such as [F-18]fallypride. The reductions in fallypride BP with age are consistent with maturing of the dopamine system. Diminished need for antipsychotics in older patients with schizophrenia may be consistent with binding potential approaching normal volunteer levels as patients reach the 5th decade of life. Dopaminergic activity may have different aging trajectories in some cortical areas and the implications for changing psychopharmaceutical strategies may be of interest.

144. Effects of a Novel H3 Antagonist on fMRI Activation during Working Memory in Schizophrenia

Daniel Wolf*, James Loughead, Kosha Ruparel, Janina Seubert, Mark Elliott, Christian Kohler, Bruce Turetsky, Ruben Gur, Raquel Gur

University of Pennsylvania Department of Psychiatry, Philadelphia, USA

Background: Cognitive deficits in schizophrenia are common, early-onset, persistent, and disabling. Working memory deficits, associated with abnormal activation in brain networks mediating executive function, are consistently found in schizophrenia. Current treatments are largely ineffective for cognitive deficits, creating a critical need for novel therapeutic targets and agents. H3 antagonism represents a promising potential therapeutic mechanism for cognitive deficits in schizophrenia and other neuropsychiatric disorders. Histamine H3 receptors in CNS mediate autoreceptor inhibition of histamine release, and also inhibit the release of other neurotransmitters including dopamine, norepinephrine, and acetylcholine. H3 receptor antagonists thus increase release of these neurotransmitters, and have been shown to have pro-cognitive effects in animal models of learning and memory. There is a need to apply novel therapeutics for cognitive enhancement in schizophrenia in integrative paradigms that incorporate clinical, neurocognitive performance and neurophysiological measures in order to evaluate early signals of efficacy. We therefore performed this fMRI study as part of a Phase 1B pilot study (NCT01346163) of PF-03654746, a selective H3 receptor antagonist, evaluated as an add-on treatment for its effect on Cognitive impairment associated with schizophrenia (CIAS). We hypothesized that treatment with an H3 antagonist would lead to improvements in working memory function and to increased activation in prefrontal brain regions involved in executive function.

Methods: Subjects with complete fMRI data were 15 patients (9 female) with DSMIV schizophrenia, stably treated with second-generation antipsychotics. MRI scanning was performed following an acute dose of PF-03654746 (1 mg), and again after 2 weeks of daily administration (0.5-1 mg flexible titration). The study design was double blind, placebo-controlled, counterbalanced within-subject crossover, with a 7-day washout between drug and placebo phases. The study thus included 4 identical MRI sessions. During fMRI scanning, subjects performed a fractal n-back task (0, 1, 2, and 3-back block design), as well as an auditory p300 task and a resting ASL perfusion scan. *A priori* region-of-interest analysis of n-back BOLD data focused on executive regions previously shown to have memory load-dependent activation in this task, including dorsolateral prefrontal cortex (DLPFC) and dorsal anterior cingulate (ACC). Percent signal change data were extracted from these ROIs for each subject and statistically analyzed for effects of memory load (n-back level), drug, and time (acute vs. chronic) using a mixed model in SAS.

Results: As expected, accuracy was lower and reaction time slowed as working memory load increased. These behavioral measures showed no effect of drug, time or their interaction. We also documented the expected BOLD signal increase with working memory load in a network of regions including DLPFC and ACC. There was a significant drug x time interaction in ACC ($p = .001$) and DLPFC ($p < .0001$), driven by an increase in activation for chronic drug relative to chronic placebo without a difference for acute drug vs. acute placebo. There were no significant main effects of drug or time, nor were there interactions of drug or time with memory load. BOLD activation in DLPFC and ACC correlated positively with working memory efficiency under the chronic drug condition but not under chronic placebo.

Discussion: The H3 antagonist PF-03654746 was generally well-tolerated. At the low dose used in this pilot study, the drug did not produce robust effects on clinical measures or behavioral measures of cognition. However, fMRI was sensitive to drug effects after chronic administration, demonstrating increased activation in executive regions during a working memory task, which correlated positively with performance efficiency. These results encourage further investigation of this H3 antagonist at higher doses, and support the use of fMRI for sensitive detection of drug effects during drug development.

145. Brain Circuits that Link Schizophrenia to High Risk of Cigarette Smoking

Lauren V. Moran*, Hemaltha Sampath, Yihong Yang, Thomas J. Ross, Gunvant K. Thaker, Elliot A. Stein, L Elliot Hong

Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, USA

Background: Smoking remains one of the leading causes of morbidity. The highest risk for smoking occurs in individuals with severe mental illness, especially schizophrenia (SZ). Recent data suggest that a dorsal anterior cingulate (dACC) – ventral striatum circuit appears to be a key pathway associated with nicotine addiction (Hong *et al.*, 2009). Since ventral striatal and anterior cingulate abnormalities are commonly described in SZ, using resting state fMRI, we test the hypothesis that dACC – ventral striatum circuits are key paths for SZ/nicotine addiction comorbidity. The test of this hypothesis relies on demonstrating that (1) circuits associated with nicotine addiction are fundamentally similar in normal control (NC) and SZ smokers; (2) impairment in smoking-related circuits occurs in SZ compared to NC, regardless of smoking status, and must therefore also be demonstrated in SZ *non-smokers;* and(3) to confirm that such circuit impairments are related to the genetic liability of SZ and not due to medication effects, we will see abnormalities in first-degree relatives of SZ patients.

Methods: Fifty-four SZ patients (36 smokers) and 65 normal controls (37 smokers), matched on age, gender and smoking status, and 24 first degree relatives without SZ participated in the study. Smokers were current daily smokers for 1 year; non-smokers were current non-smokers who never smoked daily for 1 month. For smokers, nicotine addiction severity was assessed by the Fagerstrom Test for Nicotine Dependence (FTND). Resting-state fMRI was acquired for five minutes with eyes open. Using individualized, anatomically identified dACC as the "seed" region of interest (ROI), we performed whole-brain resting state functional connectivity (rsFC) by calculating the correlation coefficients between the average time course extracted from the dACC ROI and each voxel in the brain. Control for multiple comparisons was performed by family-wise error correction ($p_{uncorrected} < 0.001$ and minimal cluster size of $405\,mm^3$, corresponding to $p_{corrected} < 0.05$). Linear mixed effects models were used to analyze mean rsFC with diagnosis as the GROUP factor, and FTND as a covariate for analyses on smokers.

Results: Eighteen dACC circuits were identified that differentiated NC from subjects with SZ ($p_{corrected} < 0.05$). Using these NC-SZ difference circuits as a mask in a subsequent analysis in all *smokers*, seven circuits were negatively correlated with FTND in both NC smokers and smokers with SZ (FTND effect, all $p < 0.001$),

indicating an overlap in functional circuits involved in SZ and smoking. To determine that SZ pathology is inherently associated with reduced rsFC in these circuits independent of smoking, we repeated the above analyses by first identifying circuits that were significantly different between *nonsmoker* SZ and NC ($p_{corrected} < 0.05$). Using this mask, we found that all of the 7 circuits were significantly associated with FTND in smokers: dACC-right ventral striatum/posterior insula (FTND effect $t = -4.3$, $p < 0.001$), dACC-left fusiform gyrus ($t = -5.0$, $p < 0.001$), dACC-right inferior parietal lobule ($t = -4.5$, $p < 0.001$), dACC-right amygdala ($t = -4.5$, $p < 0.001$), dACC-medial temporal gyrus ($t = -4.6$, $p < 0.001$), dACC-right para-hippocampal gyrus ($t = -4.0$, $p < 0.001$), and dACC-midbrain ($t = -3.6$, $p < 0.001$). Importantly, the largest cluster ($4482 \, mm^3$) included both right ventral striatum and the right posterior insula. Finally, we compared rsFC in these seven circuits in first-degree relatives compared to normal controls and found two circuits with significantly decreased connectivity strength in relatives compared with NC: dACC-right ventral striatum/posterior insula ($p = 0.008$) and dACC-right inferior parietal lobule ($p = 0.005$), suggesting that these two circuits are not related to a potential medication effect but rather reflect the genetic liability to SZ.

Discussion: As predicted, decreased connectivity in circuits between the dACC and ventral striatum/posterior insula indexes overlapping functional circuitry associated with smoking and SZ and may explain the high rates of nicotine addiction in SZ. We found reduced connectivity in this circuit in association with nicotine addiction severity in both NC and SZ smokers, and decreased connectivity in SZ non-smokers and first-degree relatives. Thus, functional connectivity in this dACC-ventral striatum/posterior insula circuit is inherently reduced in SZ, and may contribute to the high rates of SZ/nicotine dependence comorbidity.

References: Hong LE *et al. Arch Gen Psychiatry* 2009;66:431-4.
Disclosure: L. Moran: None. **H. Sampath:** None. **Y. Yang:** None. **T. Ross:** None. **G. Thaker:** None. **E. Stein:** None. **L. Hong:** None.

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146. Water and Metabolite Transverse T2 Relaxation Time Abnormalities in the White Matter in Schizophrenia

Fei Du, Alissa Cooper, Bruce Cohen, Perry Renshaw, Dost Ongur*

McLean Hospital, Belmont, USA

Background: Multiple lines of evidence suggest that microstructural abnormalities in the white matter are important in the pathophysiology of schizophrenia. Diffusion MRI approaches which can provide evidence on tissue structure have been widely used to probe these abnormalities *in vivo*, but transverse relaxation times (T2) may provide additional insights since they are determined by molecule-microenvironment interactions not revealed by diffusion MRI. T2 of water – located both intra and extracellularly – and N-acetylaspartate (NAA – located intracellularly) reflect related but distinct processes due to their differential localization and interactions with other molecules. No study to date has quantified both water and metabolite T2s in the same psychiatric subject cohorts.
Methods: In this study, we collected water and NAA T2 data from 16 healthy subjects (HC), and sex, age, and parental SES matched 16 patients with schizophrenia (SZ) at 4 Tesla in a 9cc voxel in the right prefrontal white matter using a standard PRESS sequence at 4 echo times: 30, 90, 120, and 200 milliseconds. MRS data fitting and T2 calculations involved LC Model and home-grown software, respectively.
Results: The SZ group had longer water but shorter NAA T2 relaxation times when compared with the HC group. This pattern resulted in a statistically significant metabolite x group interaction ($F(18,1):4.980$, $p = 0.039$). There was also a strong and negative

correlation between water T2 and PANSS scores within the SZ group ($R = -0.683$, $p = 0.010$). Notably, there was no correlation between CPZ equivalents and water T2 ($R = -0.379$, $p = 0.201$) or NAA T2 ($R = 0.392$, $p = 0.262$). In addition, there was no correlation between NAA T2 and water T2 ($R = -0.258$, $p = 0.272$).
Discussion: Prolongation of water T2 and shortening of NAA T2 is consistent with an impoverishment of white matter macromolecule structures (including myelin) and abnormal intra-axonal milieu and volume in SZ. This pattern provides additional evidence for white matter abnormalities in schizophrenia. Much of the neuroimaging evidence in this domain comes from DTI studies which highlight abnormal white matter integrity but cannot point to specific biological abnormalities. Our work suggests that additional and complementary information can be obtained using MRS approaches. The water T2 abnormality suggests a specific myelination abnormality, while the NAA T2 abnormality suggests intra-axonal abnormalities.
Disclosure: F. Du: None. **A. Cooper:** None. **B. Cohen:** None. **P. Renshaw:** Part 1: Dr. Renshaw is a consultant to Novartis, GlaxoSmithKline, Kyowa Hakko, and has received research support from Eli Lilly, GlaxoSmithKline, and Roche. **D. Ongur:** Part 1: Dr. Ongur is PI on a research contract with Rules Based Medicine Inc.

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147. Mechanisms underlying Hippocampal Dysfunction in Schizophrenia and Related Psychotic Disorders

Scott A. Schobel*, Nashid Chaudhury, Cheryl M. Corcoran, Martin Styner, Beatriz Paniagua, Jeffrey A. Lieberman, Holly Moore, Scott A. Small

Columbia University Medical Center, New York, USA

Background: Hippocampal hypermetabolism characterizes schizophrenia and related psychotic disorders. Here, we hypothesized that elevations in extracellular glutamate underlie disease-associated increases in hippocampal metabolism in early stages of psychotic disorder and that elevations in extracellular glutamate drive hippocampal tissue loss upon progression to first episode psychosis. To test this hypothesis, we exploited a high-resolution variant of functional magnetic resonance imaging (fMRI) that can map hippocampal subregional metabolism in patients and in animal models. To translate observed functional imaging abnormalities into underlying neurochemical mechanisms, we applied a glutamate biosensor to measure evoked changes in extracellular glutamate in mice exposed to acute ketamine challenge (30 mg/kg), a pharmacological condition that reliably produces positive, negative, and cognitive symptoms of acute psychosis in humans.
Methods: High-resolution functional and structural MRI in a prospective longitudinal cohort of individuals at clinical risk for psychotic disorders imaged at baseline ($n = 25$) and at 2.5 years prospective follow-up for clinical outcomes ($n = 20$) and a combined high-resolution structural and functional MRI/glutamate biosensor approach in C57b6 WT mice exposed to acute, as well as chronic ketamine challenge (30 mg/kg).
Results: Using this cross-species imaging approach, we observed that as compared to individuals at clinical risk for psychosis who did not progress to psychosis over 2.5 years clinical follow-up, those individuals who did progress to psychotic disorders exhibited hypermetabolism in the CA1 subfield ($F_{1,19} = 6.2$, $p = .03$) and subiculum ($F_{1,19} = 5.8$, $p = .03$), replicating an imaging profile we have previously described in schizophrenia. Hippocampal hypermetabolism at baseline in left anterior CA1 is associated with subsequent hippocampal volume reduction in progressors to first episode psychosis ($r = .7$, $p = .003$). Morphometric shape change localizes this volume reduction to the left anterior CA1 and subiculum subregions. In mice, with acute ketamine challenge (30 mg/kg, i.p.), we again observed a selective increases in

metabolism within the CA1 subfield ($F_{4,13} = 4.1$, p = .02) and subiculum ($F_{4,13} = 3.8$, p = .03). Second, using an *in vivo* glutamate biosensor, we found that acute ketamine challenge (30 mg/kg i.p.) selectively produced increases in extracellular glutamate in the CA1 ($t_{11} = 2.4$, p = .03) and subiculum ($t_{10} = 2.5$, p = .03); there were no changes observed in medial entorhinal cortex or dentate gyrus. Pretreating mice over five-days with glutamate-reducing agents LY379268 (10 mg/kg), gabapentin (600 mg/kg), or lamotrigine (10 mg/kg) showed that relative to saline pretreatment, each drug pretreatment significantly decreased the post-ketamine extracellular glutamate response (LY379268: $t_{10} = 2.4$, p = .04, lamotrigine: $t_{10} = 2.9$, p = .02, gabapentin: $t_{10} = 3.1$, p = .01); as well as the evoked CBV response (LY379268: $t_8 = 9.1$, p < .001, gabapentin: $t_8 = 3.9$, p = .004, lamotrigine: $t_7 = 1.9$ p = .09).

Discussion: Our study results confirm that hypermetabolism in the CA1 subfield and the subiculum are characteristic of schizophrenia and related psychotic disorders, and are inducible by acute ketamine challenge in rodents using the same imaging paradigm. Moreover, the pharmacological model shows that this hypermetabolism is mediated by increases in extracellular glutamate, and can be blocked by diverse glutamate-limiting therapeutic strategies. Longitudinal imaging results in patients across the transition to psychosis suggest that hippocampal hypermetabolism drives a pattern of hippocampal volume loss that is maximal in the left anterior body of the structure. These findings have immediate implications for a glutamate-limiting preventive therapeutic strategy in patients at clinical high risk for psychotic disorders who exhibit hippocampal hypermetabolism.

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148. Proton Spectroscopy Studies in Young Relatives at Genetic High Risk for Schizophrenia: Evidence for Glutamatergic Alterations

Neeraj Tandon*, Alan N. Francis, J.N. Stout, Kunal M. Sanghavi, Vaibhav A. Diwadkar, Nicolas R. Bolo, Jeffrey A. Stanley, Matcheri S. Keshavan

Harvard Medical School - Beth Israel Deaconess Medical Ctr, Boston, USA

Background: *In vivo* proton (1H) Magnetic Resonance spectroscopy (1H MRS) has shown abnormalities in metabolite concentrations in young first-episode patients with schizophrenia. Our study investigated whether these abnormalities are due to the vulnerability to the disease. Subjects at risk for schizophrenia were compared with healthy controls using 1H MRS. We hypothesized alterations in N-acetyl aspartate (NAA) and glutamate + glutamine (Glx) in subcortical and gray matter brain regions in our study group.

Methods: We obtained multi-voxel, short-TE 1H MRS measurements at 1.5 Tesla in 30 consenting young first-degree relatives at risk for Schizophrenia (HR, mean age = 13.8) and 36 age matched healthy controls (HC, mean age = 13.1) recruited at Wayne State University. Relative ratios (to Cr + PCr) of metabolites were examined in cortical and subcortical regions (nominal voxel size: 4.5 cm^3) and processed in LCModel.

Results: We observed marked increases in Glx (f = 8.47, p = 0.0060) and decreases in NAA (f = 10.79, p = 0.0022) in the thalamus in HR subjects as compared to controls. In the inferior parietal lobule, Glu + Gln was reduced in HR as compared to

controls (f = 7.05, p = 0.012), with NAA showing nonsignificant decreases (p = 0.104). NAA was also found to be reduced in the caudate (p = 0.050) and anterior cingulate (p = 0.089). Cognitive performance as measured by the Wisconsin Card Sort Test was inversely correlated with Glx (r = − − −.352, p = 0.003) and positively correlated with NAA (r = .484, p = 0.021). Schizotypal symptoms as measured by the Chapman scale were positively correlated with Glx (r = .579, p = 0.001) and negatively correlated with NAA (r = −.499, p-0.025). No significant differences were found in the inferior prefrontal cortex.

Discussion: Our results suggest that localized changes in brain metabolite levels may underlie not only vulnerability to schizophrenia but also associated cognitive impairment. Altered levels of Glx may reflect disparities in glutamatergic neurotransmission and altered NAA may reflect abnormalities in neuronal integrity.

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149. Dissociable Effects of *NRG3* Genotype on Prefrontal Cortex Physiology in Healthy Volunteers, Schizophrenia Patients, and Their Unaffected Siblings

Heike Tost*, Joseph H. Callicott, Radhakrishna Vakkalanka, Katherine Gambale, Roberta Rasetti, Venkatta S. Mattay, Daniel R. Weinberger, Amanda J. Law

Clinical Brain Disorders Branch, National Institute of Mental Health, Bethesda, USA

Background: NRG3 is a member of the neuregulin family of epidermal-growth factor proteins and a specific ligand for the ErbB4 receptor kinase that plays pleiotropic roles in neurodevelopment. Several studies have linked genetic variation in NRG3 to neurodevelopmental disorders including schizophrenia (Chen *et al.*, 2009, Kao *et al.*, 2010; Morar *et al.*, 2010). Fine mapping of the 10q22-23 locus in schizophrenia has identified genome-wide significant association between delusion severity and polymorphisms in intron 1 of *NRG3* (rs10883866, rs10748842 and rs6584400; Chen *et al.*, 2009). Recent evidence confirmed association with schizophrenia *per se* and demonstrated that a molecular mechanism of association involves rs10748842, a polymorphism in *NRG3* which strongly predicts prefrontal cortex expression of a novel splice isoform of NRG3 (Kao *et al.*, 2010). Recent behavioral data (Morar *et al.*, 2010) has extended these findings, reporting differential effects of rs10883866 and rs6584400 (risk variants in allelic identity with rs10748842) on measures of cognition in schizophrenia patients and healthy controls (Morar *et al.*, 2010). The neurobiological basis of this phenomenon is unclear. Here we examined the effects of rs10748842on prefrontal cortex physiology in a large sample of healthy subjects, schizophrenia patients, and their unaffected sibling using functional magnetic resonance imaging (fMRI) examination of working memory performance.

Methods: All participating subjects were recruited as part of the NIMH Clinical Brain Disorders Branch "Sibling Study", an ongoing investigation of neurobiological abnormalities related to genetic risk for schizophrenia (PI: DR. Weinberger). 611 subjects were studied: 410 healthy participants (195 males, mean age = 31.3 ± 9.1 years), 78 individuals with DSM-IV schizophrenia spectrum disorder (64 males, mean age = 32.7 ± 9.7 years), and 123 healthy siblings of schizophrenia patients (53 males, mean age = 36.7 ± 10.2 years). Brain function was studied with fMRI and a well-established *n*-back working memory paradigm that robustly engages the prefrontal cortex. FMRI data were acquired on a GE 3 Tesla Signa scanner using a gradient-echo EPI sequence (TR = 2,000 ms, TE = 30 ms, voxel dimensions: 3.75 × 3.75 ×

6 mm). Data preprocessing was performed in SPM5 (http://www.fil.ion.ucl.ac.uk/spm/) including image realignment, spatial normalization to standard MNI space, and smoothing. *NRG3* rs10748842 genotypes were determined by Taqman assay. Genotype distributions did not deviate from Hardy-Weinberg equilibrium ($P > 0.05$). Effects of *NRG3* genotype were examined using general linear model in SPM5 with random-effects group statistics at the second level. First, we examined the effects of rs10748842 on prefrontal physiology in healthy volunteers using a multiple regression model with genotype as covariate of interest and age, sex, and *n*-back task performance as nuisance covariates. Genotype by group interaction effects were explored using ANCOVA with group (control, sibling, patient) and genotype as covariates of interest and age, sex, and *n*-back task performance as nuisance covariates. Significance was measured at $P < 0.05$ family-wise error corrected for multiple comparisons across the whole brain.
Results: We observed significant genotype-dependent differences in healthy controls manifesting as a relative increase (or a more "inefficient" prefrontal physiology) in signaling in the right dorsolateral prefrontal cortex (DLPFC) during *n*-back performance in rs10748842 minor allele carriers (MNI: x = 39, y = 33, z = −3; $T = 4.8$, $P < 0.008$ whole-brain corrected). Genotype by group interaction analysis revealed significant dissociable patterns of DLPFC signaling in rs10748842 minor allele carriers. These manifested as a relative increase in signaling in healthy volunteers and unaffected siblings, but a relative decrease (or a more "efficient" prefrontal physiology) in schizophrenia patients (MNI: x = 33, y = 39, z = 15; $T = 4.5$, $P < 0.037$ whole-brain corrected).
Discussion: These data provide novel evidence that a schizophrenia-associated polymorphism in NRG3(rs10748842)impacts prefrontal cortical physiology during working memory performance, a well-established intermediate phenotype for schizophrenia. Given the opposite association of genotype and efficiency of prefrontal physiology in patients vs. controls, examination of interactions of NRG3 genotype with other risk genes and with neuroleptic medication is warranted.
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150. Greater Neuronal Response During Automatic Semantic Processing in Schizophrenia

Jason R. Tregellas*, Lisa B. Wilson, Shireen Shatti, Donald C. Rojas

University of Colorado School of Medicine, Aurora, USA

Background: A core feature of schizophrenia is a disturbance of associative processes. These disturbances have been investigated in behavioral semantic priming studies with inconsistent results. Compared to behavioral studies, functional magnetic resonance imaging (fMRI) offers a more direct measure of brain activity and a possible measure of the neural mechanisms underlying the semantic priming differences observed in individuals with schizophrenia. To date, no fMRI studies have investigated semantic priming in schizophrenia under experimental conditions that measure automatic, as opposed to strategic, processing. The present study's focus was to investigate hemodynamic responses during indirect semantic priming at a short stimulus onset asynchrony (i.e., 350 ms), conditions which are considered to be a particularly sensitive measure of automatic spreading activation during semantic processing and of the associative disturbances in schizophrenia.
Methods: Seventeen subjects with DSM-IV schizophrenia and fifteen comparison subjects underwent functional scanning at 3 Tesla while performing a lexical decision task on directly related,

indirectly related, unrelated, and word/non-word pairs. A random-effects region of interest (ROI) analysis within *a priori* temporal and frontal regions was performed using SPM5.
Results: Whereas comparison subjects exhibited hemodynamic suppression in response to priming, individuals with schizophrenia exhibited hemodynamic enhancement. Relative to the comparison group, these enhancements were observed in the left fusiform (p = 0.03) and superior temporal gyri (p = 0.05) for indirectly related word pairs relative to unrelated pairs.
Discussion: Greater priming-related responses within temporal regions may reflect increased and prolonged automatic spreading activation during semantic processing in schizophrenia.
Disclosure: J. Tregellas: None. L. Wilson: None. S. Shatti: None. D. Rojas: None.

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151. Neuroinflammation in Temporal Cortex in Patients with Schizophrenia Measured with (*R*)-[¹¹C]PK11195 and PET

Bart N. van Berckel*, Thalia van der Doef, Maqsood Yaqub, Matthijs M. Bossong, Ronald Boellaard, Neeltje E. van Haren, Bert D. Windhorst, Adriaan A. Lammertsma, Wiepke Cahn, René S. Kahn

Rudolf Magnus Institute for Neurosciences, Department of Psychiatry, Utrecht, Netherlands

Background: The pathophysiology of gray matter loss in schizophrenia has not been clarified. Gray matter loss is thought to be secondary to neuronal damage, which is closely associated with microglial activation. Recently, microglial activation has been reported in schizophrenia using the positron emission tomography (PET) tracer [(*R*)-[¹¹C]PK11195 (van Berckel *et al.*, 2008; Doorduin *et al.*, 2009). This study reports the regional distribution of (*R*)-[¹¹C]PK11195 binding in patients with schizophrenia and controls.
Methods: (*R*)-[¹¹C]PK11195 binding potential (BP_{ND}) was assessed in ten recent onset schizophrenia patients (age: 24 ± 2 y) and ten age and sex matched controls (age: 23 ± 4 y). Psychopathology was measured with the Positive and Negative Syndrome Scale (PANSS). Dynamic (*R*)-[¹¹C]PK11195 PET scans were acquired on an ECAT EXACT HR+ PET scanner. Parametric (*R*)-[¹¹C]PK11195 BP_{ND} maps were generated with receptor parametic mapping and extraction of the reference tissue was performed with supervised cluster analysis. Subsequently, gray matter regions of interest (ROI) were delineated on a T1-weighted structural MRI scan with an automatic procedure resulting in the following regions: frontal, temporal, parietal, and occipital cortex, and cerebellum.
Results: An overall significant difference in (*R*)-[¹¹C]PK11195 bindingpotential was found between the patients with schizophrenia and healthy controls (F(5) = 5.7, p = 0.005).The region-of-interest analysis revealed significantly higher (*R*)-[¹¹C]PK11195 BP_{ND} in the temporal cortex in schizophrenia patients in comparison to healthy controls (F(1) = 5.5, p = 0.03; Figures 1 and 2). There were no significant differences in mean (*R*)-[¹¹C]PK11195 BP_{ND} in the other areas tested (frontal cortex: SCZ = 0.10 ± 0.06, controls = 0.11 ± 0.09, F(1) = 0.14, p = 0.71; occipital cortex: SCZ = 0.33 ± 0.14, controls = 0.33 ± 0.12;F(1) = 0.28, p = 0.87; parietal cortex: SCZ = 0.13 ± 0.06, controls = 0.15 ± 0.11; F(1) = 0.60, p = 0.45; cerebellum:SCZ = 0.16 ± 0.14, controls = 0.16 ± 0.07; F(1) = 0.03, p = 0.87).
Discussion: Our results support the presence of microglial activation in the temporal cortex in schizophrenia. This finding is in agreement with MRI studies indicating profound gray matter loss in the temporal cortex in schizophrenia patients.
Disclosure: B. van Berckel: None. T. van der Doef: None. M. Yaqub: None. M. Bossong: None. R. Boellaard: None. N. van Haren: None. B. Windhorst: None. A. Lammertsma: None. W. Cahn: None. R. Kahn: None.

152. Subtyping Schizophrenia using a Multimodal Neuroimaging Approach

Aristotle Voineskos*, George Foussias, Jason Lerch, Tarek Rajji, Nancy Lobaugh, Gary Remington, James Kennedy, Bruce Pollock, Benoit Mulsant

Centre for Addiction and Mental Health, Toronto, Canada

Background: A major obstacle to the identification of neurobiological correlates of disease in schizophrenia has been the substantial heterogeneity present in this syndrome. Leveraging clinical homogeneity by dividing schizophrenia patients into 'deficit' and 'nondeficit' categories is an approach that aims to facilitate identification of neurobiological disease markers. Successful discovery of novel biomarkers of disease in such schizophrenia subgroupscould reduce heterogeneity, and lead to neurobiologically-based disease subtyping in schizophrenia.

Methods: We completed high-resolution magnetic resonance and diffusion tensor imaging in 77 schizophrenia patients and 79 healthy controls. 18 deficit patients, identified using the proxy scale for the deficit syndrome were individually matched to 18 nondeficit patients and 18 healthy controls. Diffusion-based measures of white matter tracts and cortical thickness were evaluated in each individual.

Results: Deficit syndrome patients, when compared to nondeficit patients and healthy controls, demonstrated disruption of the white matter tracts that are essential for the emotional and social functions that are characteristically impaired in deficit patients. No differences were present between nondeficit patients and healthy controls. Deficit and non-deficit patients exhibited cortical thickness reductions in the same brain regions compared to healthy controls. Results were similar in both the individually matched sample and in the overall sample.

Discussion: The striking alignment of shared cortical thickness deficits among schizophrenia patients, but white matter disruption found only in deficit syndrome patientsprovides compelling *in vivo* evidence for: (i) white matter tract disruption as a core neurobiological feature of the deficit syndrome and (ii) reductions in cortical thickness as a common feature of patients with a diagnosis of schizophrenia.

153. Neural Compensation to Maintain Cognitive Performance in Schizophrenia

Derin Cobia, Mathew Schroeder, Katherine Blizinsky, John Csernansky, Lei Wang*

Northwestern University Feinberg School of Medicine, Chicago, USA

Background: Previously we have found that cortical and subcortical structures within specific neural networks show progressive deformities in schizophrenia patients. However, we have also found that their cognitive performance and psychopathology remained stable despite these neuroanatomical changes. The focus of the present study is to continue to improve our understanding of the characteristics of progressive neuroanatomical changes in schizophrenia, and to seek new information about compensatory processes that may preserve cognitive function and behavior in the face of such changes.

Methods: Structural MRI, functional MRI (resting-state and during the N-back working memory task) and diffusion weighted MRI were collected on a 3-T scanner at baseline and after 2 years from 10 schizophrenia subjects and 10 healthy control subjects. Neurocognitive performance, cortical thickness, functional activation, resting connectivity and fractional anisotropy (FA) were assessed at both time points.

Results: Neurocognitive deficits, including deficits in N-back performance were observed in the schizophrenia subjects, and these deficits remained stable during the interval period.

Significant differences (after multiple comparisons correction) in annual change (slopes) of cortical thickness for each point on the cortical surface were calculated, showing that the schizophrenia subjects experienced greater rates of cortical thinning over time, compared to healthy individuals, in the posterior parietal cortex, lateral and medial orbital gyri, anterior cingulate cortex, and medial aspects of the superior frontal gyrus. BOLD fMRI data collected while the subjects were performing the N-back working memory task (0-, 1-, and 2-back) demonstrated significant group-by-time interactions in two cortical regions – i.e., the left dorsolateral prefrontal cortex and the right temporo-parietal junction. Both of these cortical regions have been implicated in the performance of working memory tasks before. In both cases, these interactions were strongly significant ($p < .005$) (minimum cluster size 160 mm3). And again in both cases, neural activation as assessed with BOLD suggested that the schizophrenia subjects required increased activation from the initial to the follow-up time point. Changes in FA as a measure of white matter structural integrity were focused on the superior longitudinal fasiculus (SLF), because of its role in transmitting information between cortical regions, especially regions of the prefrontal cortex and more posterior structures, such as the temporo-parietal junction, where we had observed increases in cortical activation in the schizophrenia subjects. In this white matter region, we observed a significant effect of group and time but no difference in the rate of change between the schizophrenia and control subjects.

Discussion: These findings support our hypothesis that compensatory changes in the degree of neural activation and the coherence of white matter pathways may be occurring in synchrony with progressive changes in gray matter structures that help to maintain cognitive performance over time periods of ~ 2 years in subjects with schizophrenia. These findings shed new light on our understanding of the pathophysiology of schizophrenia and how the illness may develop after the first psychotic episode. Obviously, these findings need to be confirmed in a larger sample, and over a longer time period. Current efforts to improve cognitive function in patients with schizophrenia would benefit from a more thorough understanding of the compensatory processes available to the brain. Also, some patients with schizophrenia may undergo cognitive and functional deterioration over the same or perhaps longer time intervals. Ultimately, the majority of patients with schizophrenia are still disabled by their disorder, and at present, there is no treatment that can prevent this disability or restore patients to function once they have become disabled.

154. A Novel High Resolution fMRI Method to Measure Substantia Nigra Function Reveals Up-Regulation of Its Activity During Conditions Requiring Greater Cognitive Control

Jong H. Yoon*[1], Anthony Grandelis[1], Christian La[2], Steffan Soosman[1], Cameron S. Carter[1], Michael J. Minzenberg[1]

University of California Davis School of Medicine, Sacramento, USA

Background: The substantia nigra (SN) is a mid-brain nucleus thought to play a critical role in cognition and the pathophysiology of a variety of neuropsychiatric symptoms and conditions, including psychosis and schizophrenia. Therefore, the study of its functional properties could have a major impact on our understanding of the neural basis of cognition, as well as of the pathophysiology of neuropsychiatric diseases and symptoms. The relatively small size of the SN, however, presents significant challenges in measuring its activity using traditional approaches. Here we have applied a novel set of methods to reliably measure SN activity using fMRI and to test the hypothesis that the SN is engaged in the execution of cognitive control.

Methods: 10 healthy subjects performed a cognitive control task while undergoing high-resolution fMRI – single-shot, T2*-weighted echo planar imaging (EPI) sequence (TR 2000 ms, TE 34 ms, flip angle 75 degrees, FOV 224 mm x 244 mm with 25 contiguous slices in the axial oblique plane, with a voxel size of 1.8 mm x 1.8 mm x 1.9 mm). Images underwent slice time correction and spatial realignment in SPM5 to correct for in-scanner movement. The images did not undergo spatial normalization (a procedure that is commonly applied so that images match a template brain.) Instead, all analyses were conducted in the individual's "native space." This was done to preserve as much spatial resolution as possible, so that for each individual, we could manually draw the SN based on its distinct signal qualities apparent in EPI images. This SN mask was used to derive the trial averaged BOLD time series estimates for hypothesis testing. Only correct trials were analyzed.

Results: We were able to identify the SN and reliably draw it in all subjects. The SN displayed event-related BOLD responses for cue and probe events in both the low and high cognitive control conditions. The magnitude of the BOLD signal was greater at all time points during the high cognitive control condition compared to the low cognitive control condition. Paired t-tests revealed significantly greater BOLD signal precisely during scans reflecting maximal BOLD signal responses to cue and probe events, $P < .05$, two-tailed.

Discussion: We have developed a novel set of methods to measure the functional property of the SN using fMRI. Given the theorized importance of the SN in many brain processes and neuropsychiatric conditions, the adoption of these methods has the potential to elucidate the neural mechanisms giving rise to basic brain functions, as well as to neuropsychiatric conditions. The application of these methods in our study revealed greater activity of the SN in the condition requiring greater cognitive control. This result suggests that the SN may be a critical structure supporting the execution of cognitive control. The further study of the functional properties of the SN may yield clues to the neural mechanisms behind impairments in cognitive control found in conditions such as schizophrenia.

155. Brain Network Analysis and Global-Local Information Processing in Body Dysmorphic Disorder

Jamie D. Feusner*, Donatello Arienzo, Jesse Brown, Johnson GadElkarim, Liang Zhan, Alex Leow

UCLA, Los Angeles, USA

Background: Body dysmorphic disorder (BDD) is characterized by preoccupation with perceived defects of appearance, causing significant distress and disability. Previous studies suggest abnormalities in information processing characterized by greater local relative to global processing. No study in BDD has used a brain network analysis to probe abnormalities of regional or whole-brain network organization. The advantage to this approach is that it allows for quantitative analyses of complex brain networks to provide information about organizational systems as a whole. In graph theory a network is a set of nodes and their connections. The clustering coefficient for a node is the ratio of the number of actual connections among the first-degree neighbors to the number of all possible connections. Complex networks may consist of a number of modules, each containing several interconnected nodes. Modularity is a statistic that quantifies the degree to which the network may be subdivided into such clearly delineated modules. Global efficiency is the inverse of the shortest path length, averaged across all pair of nodes. The purpose of this study is to characterize the whole-brain network organization of white matter in BDD relative to that in healthy controls, and to relate this organization to clinical measures and performance on a behavioral test of global and local information processing. We hypothesized that individuals with BDD would have greater modularity and higher mean clustering, reflecting a network that consists of highly localized sub-networks. We also hypothesized that greater modularity and lower global efficiency would correlate with longer response times on a global-local visuospatial task.

Methods: Participants: males and females with 14 DSM-IV BDD (mean age 26.8 ± 4.96, 7M/7F), and 16 healthy controls (mean age 27.4 ± 5.32, 8M/8F). MRI: we obtained diffusion-weighted MR imaging data on a 3-T Allegra (Siemens) scanner using single-shot spin-echo echo-planar imaging (EPI) (field of view = 240 mm; voxel size = 2.5x2.5x3.0 mm, with 0.75 mm gap; TR/TE = 7400/96 ms; flip angle 9°). We collected axial slices along 34 gradient-sensitizing directions with b = 1000s/mm² and one minimally diffusion-weighted scan. Tractography: We computed whole-brain deterministic DTI tractography, and conducted cortical and subcortical parcellation to yield 113 regions of interest (ROIs) based on the Harvard-Oxford probabilistic atlas. For each pair of ROIs, the number of fibers connecting them was determined to derive the brain network connectivity matrix. These matrices were thresholded at a sparsity of 0.15, then binarized, and analyzed using the Brain Connectivity Toolbox to obtain several graph theory metrics of interest: global mean clustering coefficient (*mcc*), modularity (*mod*), and global efficiency. We performed 2-tailed 2-sample t tests to compare *mcc* and *mod* between the healthy control and BDD groups. Within the BDD group, we calculated Pearson's correlation coefficients between the metrics and BDD symptom severity (BDD-YBOCS) and depression severity (HAMD-17). Behavioral task: we correlated *mod* and global efficiency with the response time from the Navon task, a visuospatial processing task consisting of identifying letters that are either small (local) or large (global).

Results: The BDD group showed significantly higher *mcc* (.514 ± .009 for BDD, .504 ± .009 for controls, t = 2.77, df = 28, $P = .0098$, Cohen's d = 1.04). There were no significant differences between groups for *mod* (.583 ± .014 for BDD, .576 ± .014 for controls, t = .84, df = 28, $P = .4$). BDD symptom severity correlated with *mcc*

(r = .6, P = .02) and inversely with global efficiency (r = −.6, P = .015). The BDD group was significantly slower on the Navon task (.949 ± .185 for BDD, .796 ± .0997 for controls, t = 2.60, df = 23, P = .0158 Cohen's d = 1.03). *Mod*, global efficiency, and *mcc* did not correlate significantly with response times on the Navon task in either group.

Discussion: This study represents the first brain network analysis in BDD. Abnormally high clustering coefficient in individuals with BDD, correlating with symptom severity, suggests a disturbance in their topological organization of brain white matter networks. There were denser local connections, which is often associated with greater local processing. However, there were no associations detected between these network metrics and response times on a global-local visuospatial task. These results are similar to other findings in functional networks in attention-deficit/hyperactivity disorder and obsessive-compulsive disorder. A limitation of this study is the small sample size.

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156. The 5-HT2A Receptor and Serotonin Transporter in Ecstasy Users: a PET Study With [11C]MDL 100907 and [11C]DASB
Ragy R. Girgis*, Nina B. Urban, Peter Talbot, Lawrence S. Kegeles, Judy L. Thompson, Xiaoyan Xu, W. Gordon Frankle, Mark Slifstein, Anissa Abi-Dargham, Marc Laruelle

Department of Psychiatry, Columbia University and New York State Psychiatric Institute, New York, USA

Background: 3,4-methylenedioxymethamphetamine (MDMA), the main psychoactive component of the recreational drug ecstasy, is a potent serotonin (5-HT) releaser. In animals, MDMA induces toxicity of 5-HT neurons, leading to 5-HT depletion. The goal of this study was to investigate both presynaptic (5-HT transporter, SERT) and postsynaptic (5-HT2A receptor) markers of 5-HT transmission in current adult chronic MDMA users. The hypothesis of the study was that MDMA use would be associated with lower SERT density and concomitant upregulation of 5-HT2A receptors.

Methods: Positron emission tomography (PET) studies using the SERT ligand [11C]DASB and the 5HT2A receptor ligand [11C]MDL 100907 were evaluated in 13 current MDMA users and 13 matched healthy controls. MDMA users were young adults with a mean duration of ecstasy use of less than 8 years, reporting mostly moderate, but regular, exposure to MDMA, and who abstained from MDMA for at least two weeks before the scans. SERT and 5HT2A receptor availability (binding potential, BPND) were analyzed using a two-tissue compartment model (2TCM) with arterial input function.

Results: Current recreational MDMA use was significantly associated with lower SERT BPND (medial prefrontal, occipital, and temporal cortex < 0.05) and higher 5HT2A receptor BPND (dorsolateral prefrontal and parietal cortex < 0.05) in cortical, but not subcortical regions.

Discussion: In light of the animal literature, the most parsimonious interpretation of these results is that repeated exposure to MDMA in humans, even in moderate amounts, leads to damage in the terminals of the 5-HT neurons innervating the cortex. It is conceivable that alterations in mood, cognition and impulse control associated with these changes might contribute to sustain the MDMA-taking behavior. The reversibility of these changes upon abstinence remains to be firmly established.

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Therapies. A. Abi-Dargham: Part 1: Sunovion, Shire, Otsuka. M. Laruelle: Part 5: Recent employer was Glaxo Smith Kline.

157. Insula Activation During Inhibitory Control Mediates the Effect of *GABRA2* Genotype on Anxiety in a Family Sample Enriched for Alcoholism
Mary M. Heitzeg*, Sandra Villafuerte, Barbara J. Weiland, Preeti G. Samudra, Margit Burmeister, Robert A. Zucker, Jon-Kar Zubieta

The University of Michigan, Ann Arbor, USA

Background: Alcoholism, other substance abuse and related externalizing disorders have been associated with variants in *GABRA2*, a gene encoding the alpha 2 subunit of the γ-Aminobutyric acid (GABA) A receptor. However, the neural mechanism through which *GABRA2* influences risk remains largely unstudied. GABA is an inhibitory neurotransmitter believed to be critical for the maintenance of an excitation-inhibition homeostatic balance. A lack of inhibitory control is a general liability factor for a range of externalizing and substance use problems. Therefore, we hypothesized that *GABRA2* genetic variation may be linked to individual differences in neural function involved in inhibitory control, and these differences in turn would relate to substance use problems and related, risk-conferring traits. Furthermore, it has been suggested that trait anxiety may mediate the linkage between *GABRA2* genetic variation and addiction (Enoch, 2008, *Pharmacol Biochem Behav.* 90:95-104). Therefore, we investigated the relationship of *GABRA2* genotype and brain activation to anxiety as well as substance use and externalizing behavior.

Methods: We studied 18-21 year olds (n = 45) recruited from the Michigan Longitudinal Study of alcoholic (2/3 of sample) and control families using functional MRI during a go-no-go task. Sample size for each genotype (SNP rs279826) was: 11 AA, 26 AG, 8 GG. First-level contrasts were made in SPM2 comparing activation during correct inhibition to infrequent stimuli with activation during frequent "go" trials. A second-level ANOVA was used to investigate genotype effects. ANOVA results were masked with whole-group activation (correct inhibition > go) and deactivation (go > correct inhibition), thresholded at p < .005, uncorrected. Statistical significance was established at voxel-level uncorrected p < 0.005 and voxel-extent > 30. Measures of risk conferring traits (externalizing behavior and anxiety) were collected with the Adult Self Report (ASR; Achenbach & Rescorla, 2003), and substance use and problems via a drinking and drug history. Performance was measured by false alarm rate (i.e., failure to inhibit to an infrequent target) and reaction time to frequent "go" trials.

Results: An ANOVA revealed no main effect of genotype on performance, ASR measures or substance use and problems. However, exploratory t-tests revealed greater anxiety in A homozygotes compared with G homozygotes. No other differences in performance, traits or substance measures between these two groups emerged. Analysis of functional imaging data revealed that homozygotes for the A allele had greater activation in the right insula and bilateral inferior parietal lobe and greater deactivation in the subgenual anterior cingulate, the medial and medial-orbital superior frontal gyrus and the right fusiform gyrus during correct inhibitions versus go trial conditions. Effect sizes in these clusters were extracted for further analyses including correlations with performance, ASR measures and substance measures. Activation in the right insula correlated with anxiety. No other correlations reached significance after correction for multiple comparisons. Mediation analysis revealed a significant indirect effect of *GABRA2* genotype on anxiety via insula activation.

Discussion: This work uncovers a neural pathway through which *GABRA2* genotype may influence risk for alcoholism related to anxiety. The literature has been unclear regarding which of the two major yin yang haplotypes confer risk for alcoholism. The G allele of SNP rs279826 is a tag allele for the haplotype that has been associated with externalizing problems throughout adolescence (Dick *et al.*, 2009, *Arch Gen Psychiatry*, 66:649-57) and with treatment-seeking alcoholics with no comorbid drug dependence (Covault *et al.*, 2004, *Am J Med Genet B Neuropsychiatr Genet*, 129B:104-9) . In contrast, the A allele of SNP rs279826 is a tag allele for the haplotype that has been associated with alcoholism with comorbid drug dependence (Agrawal *et al.*, 2006, *Behav Genet*, 36:640-50) and "anxious-type" alcoholism (Enoch *et al.*, 2006, *Am J Med Genet B Neuropsychiatr Genet*, 141B:599-607). It has been suggested that both of the two abundant yin yang haplotypes are risk factors and that trait anxiety may mediate their linkage (Enoch, 2008). This study supports a differentiated association of the A allele with anxiety, not with externalizing, and suggests that this association is mediated by insula functioning, here observed during the inhibition of prepotent responding. This work was supported by NIH grants K01 DA020088, R01 DA027261, R01 AA12217, and R37 AA07065.

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158. Differential Effects of an NMDA Receptor Antagonist on Response Inhibition-Related fMRI Activity in Individuals With and Without a Family History of Alcoholism

Sharna Jamadar*, Rachel Jiantonio, Shashwath Meda, Michael Stevens, Elise DeVito, Marc Potenza, John Krystal, Godfrey Pearlson

Olin Neuropsychiatry Research Center, Institute of Living, Hartford, USA

Background: Individuals with a family history of alcoholism (family history positive, FHP) show increased rates of alcoholism and are more impulsive than those without a family history of alcoholism (family history negative, FHN). Genetic susceptibility to alcoholism is associated with increased impulsivity and enhanced NMDA receptor function. Consistent with this, FHP individuals manifest impaired fMRI activity on the Go/No-Go task, a response inhibition task that indexes one aspect of impulsivity. Baseline disordered NMDA/DA interactions in subcortical regions may underlie risk for alcoholism. NMDA receptors are among the highest affinity targets for alcohol in the brain. Chronic alcohol intake results in up-regulation of NMDA receptors and reduced responses to NMDA receptor antagonists; this pattern is also seen in FHP individuals. NMDA receptor antagonists reduce alcohol craving in alcohol-addicted populations, possibly by influencing impulse control. Such findings raise questions about how the relationship between NMDA receptor function and response inhibition may vary in FHP and FHN individuals.

Methods: We investigated whether the NMDA receptor antagonist, memantine, differentially affects Go/No-Go brain activity in matched FHP (n = 15) and FHN (n = 15) individuals. On two separate days, participants received 40 mg memantine or identical appearing placebo 4-hrs prior to fMRI testing in a double-blind placebo-controlled within-subjects design. During fMRI participants responded to Go stimuli (85%) and withheld responses to No-Go stimuli (15%). Reaction time (RT) was analyzed for Go and No-Go false alarm trials. Accuracy showed no significant effects of group or drug. fMRI results were examined for No-Go correct rejects > baseline and No-Go false alarms > baseline; our design did not permit effective modeling of the Go haemodynamic response. Whole-brain SPM results were thresholded at $p < .001$ (k = 30 voxels); region-of-interest (ROI) results were thresholded with a small volume correction FWE $p < .05$.

Results: There were no differences between groups on Go-RT or No-Go false alarms on placebo. Go-RT was increased on memantine vs. placebo in FHN but not FHP. The only difference between FH groups during placebo was an increase in fMRI activity for No-Go correct rejects in the right anterior cingulate and left inferior parietal lobule for FHP vs. FHN. Memantine challenge had little effect on No-Go correct reject fMRI activity for FHN; in FHP activity in the left anterior cingulate and left caudate was reduced relative to placebo. For No-Go false alarms, memantine challenge in FHN decreased activity in a distributed motor network encompassing left superior and middle frontal gyri, anterior cingulate, posterior cingulate, inferior temporal, fusiform, and lingual gyri; bilateral supplementary motor area; and right precuneus and middle temporal gyrus relative to placebo. In the FHP group, memantine challenge had an attenuated effect, with decreased activity only in left insula and precuneus; bilateral putamen and caudate; and right posterior cingulate and superior temporal gyrus relative to placebo.

Discussion: The major finding of this study was that memantine had a larger effect on Go/No-Go RT and fMRI activation in FHN than in FHP individuals. During memantine challenge, FHN individuals showed an increase in RT for both Go correct hits and No-Go false alarms and a decrease in fMRI activity in a distributed cortical motor network for No-Go false alarms relative to placebo. Little change was seen in fMRI activity for No-Go correct reject fMRI activity in memantine vs. placebo in FHN. These results suggest that response execution, but not response inhibition, is affected by NMDA receptor antagonism in healthy FHN individuals. Memantine challenge had an attenuated influence on RT and fMRI measures in the FHP group as compared to the FHN group. In contrast to the FHN group, memantine challenge had no effect on RT for Go correct hit or No-Go false alarm trials relative to placebo. Memantine challenge also had an attenuated effect on fMRI activity for No-Go correct rejects: memantine reduced activity in the left anterior cingulate and caudate relative to placebo. For No-Go false alarms, memantine challenge was associated with a reduction in activity largely in striatal and parietal regions, with no significant effect in frontal regions. These results are consistent with hypotheses of enhanced NMDA receptor function in these individuals and suggest that FHP individuals do not detect their growing level of intoxication as compared to FHN, and are thus more likely to overshoot their target levels of consumption when drinking.

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159. Association between CHRNA5 Genetic Variation at RSL6969968 and Brain Reactivity to Smoking Images in Nicotine Dependent Women

Amy C. Janes, Jordan W. Smoller, Sean P. David, Blaise de B. Frederick, Stephen Haddad, Aditi Basu, Maurizio Fava, A. Eden Evins, Marc J. Kaufman*

McLean Hospital, Belmont, USA

Background: Nicotine dependence is highly heritable suggesting that genetics may play a key role (Lessov-Schlaggar et al., Biochem. Pharmacol. 75:178, 2008). The genetic polymorphism most associated with development of smoking dependence is the non-synonymous coding single-nucleotide polymorphism (SNP) of the nAChR subunit alpha-5 (CHRNA5) gene rs16969968 (Bierut, Trends Pharmacol. Sci.. 31:46, 2010). The rs16969968 SNP encodes an Asp398Asn polymorphism resulting in an aspartic acid (G allele) change to asparagine (A allele) at the 398th amino acid, leading to a decrease of receptor function (Bierut et al., Hum. Mol. Genet. 16:24, 2008; Saccone et al., Hum. Mol. Genet. 16:36, 2007). However, not all nicotine-dependent smokers carry the same genetic risks, suggesting that other factors may moderate nicotine dependence. Clinical and preclinical studies indicate that cue reactivity plays a role in smoking behavior and nicotine seeking (Cohen et al., Neuropsychopharm. 30:145, 2005; Ferguson & Shiffman, J. Subst. Abuse Treat. 36:235, 2009; Shiffman et al., J. Consult. Clin. Psychol. 64:366, 1996). Enhanced cue reactivity as

measured by fMRI may confer relapse vulnerability in smokers (Janes et al., Biol. Psychiatry 67:722, 2010) and in other drug-dependent populations (Grüsser et al., Psychopharm.175:296, 2004; Kosten et al., Neuropsychopharm. 31:644, 2006). To determine whether rs16969968 moderates fMRI-measured brain reactivity to smoking cues, we compared 2 groups of women whose nicotine dependence severity was equivalent. One group expressed an A allele and the other expressed 2 G alleles.

Methods: Fourteen nicotine dependent women with the 'risk' (A) allele and 10 without the 'risk' allele (G/G) underwent fMRI scanning while viewing images of smoking-related (people smoking, hands holding cigarettes, or cigarettes alone) or neutral (general content-matched but no smoking cues) images (Due et al., Am. J. Psychiatry 159:954, 2002; Gilbert & Rabinovich, International smoking image series (with neutral counterparts), version 1.2. Carbondale, IL, 1999).To compare brain reactivity to smoking vs. neutral images between A and G/G allele smokers, a whole-brain mixed-effects approach was implemented using BrainVoyager QX 1.10.4 (Brain Innovation, Maastricht, Netherlands). Multiple comparisons were cluster corrected to $p < 0.05$. Nicotine dependence severity, as assessed with the Fagerstrom test for nicotine dependence, smoking pack-years, and expired carbon monoxide levels, were equivalent in the A and G/G groups.

Results: We observed a group difference in fMRI reactivity; women *without* the A allele (G/G smokers) showed greater fMRI reactivity to smoking images in brain areas related to memory and habitual behavior such as the hippocampus and dorsal striatum. Relative to the G/G smokers, smokers with an A allele showed no increased activation to smoking vs. neutral images in any brain area.

Discussion: We found smoking cue reactivity differences in nicotine dependent smokers with different CHRNA5 genetic profiles. In comparison to A allele smokers, G/G smokers had greater fMRI reactivity to smoking-related versus neutral images. At first glance, this result may seem counterintuitive, as one might have expected that "risk" allele (A) smokers would have had greater cue reactivity than G/G smokers. However, prior work suggests only that the A allele may enhance risk for initially developing nicotine dependence, possibly by reducing nicotine's aversive effects (Jackson et al., J. Pharmacol. Exp. Ther. 334:137, 2010; Fowler et al., Nature 471:597, 2011). By contrast, G/G smokers (who may be at lower risk for initially developing nicotine dependence but who were equally nicotine dependent as A allele smokers) had greater fMRI smoking-cue reactivity. Thus, G/G carriers who develop nicotine dependence may continue smoking due to heightened smoking cue-reactivity. Together, the data suggest that among nicotine dependent smokers, it may be possible to dissociate genetic risk for initial development of nicotine dependence from cue reactivity sensitivity, which may help maintain nicotine dependence. The ability to dissociate genetic risk from other risks for developing and/or maintaining nicotine dependence may assist in development of novel personalized treatments.

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M. Kaufman: Part 1: Varian (Agilent), Air Products and Chemicals, Inc., Michael J. Fox Foundation for Parkinson's Research, Torrey Pines Institute for Molecular Studies, NARSAD, GSK, Part 4: Varian (Agilent), Air Products and Chemicals, Inc., Michael J. Fox Foundation for Parkinson's Research, Torrey Pines Institute for Molecular Studies, NARSAD, GSK.

160 Functional Connectivity Changes in Stimulant Abusers During Abstinence

Jazmin Camchong, Sheila Specker, Valerie Slaymaker, Angus MacDonald, Bryon A. Mueller, Chris Bell, Ann L. Person, Kelvin O. Lim*

University of Minnesota, Minneapolis, USA

Background: Resting functional connectivity (FC) abnormalities have been identified in stimulant-dependent individuals (SDIs). There is a need, however, to investigate how resting FC changes in time during stimulant abstinence. This is the first study to our knowledge that examines changes in resting FC after abstinence in the same sample of SDIs. We hypothesized that: (1) there will be resting FC changes due to abstinence in SDI and (2) abnormalities found during abstinence would be related to relapse.

Methods: Resting functional magnetic resonance imaging data was examined in 12 abstinent SDIs (5 females, age: $M = 23.7$, $SD = 2.90$) and 15 healthy controls (5 females, age: $M = 23.60$, $SD = 2.69$) to look for FC differences within groups ($\sim$30 and 90 days of abstinence) and between groups (SDIs vs controls). With seed-based FC measures, we examined FC in SDI and control subjects within a top-down control network previously found to be abnormal in SDIs.

Results: Results showed a significant effect of time only in the SDI group. SDI showed (a) decrease in functional connectivity between anterior cingulate cortex (ACC) and left middle temporal gyrus, posterior cingulate and superior frontal gyrus and (b) increase in functional connectivity between ACC and medial frontal gyrus across time ($p < 0.01$). Exploratory analysis showed that SDI that had relapsed after 6 months of abstinence had significantly greater changes in functional connectivity between ACC and medial frontal cortex than the SDI that did not relapse ($p < .02$).

Discussion: The present study shows that resting functional connectivity abnormalities are still present after long periods of abstinence and these abnormalities may be biological predictors of relapse in SDIs.

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161. Monoamine Oxidase a Binding during Acute Alcohol Withdrawal: A [^{11}C] harmine PET Study

Brittany A. Matthews*, Stephen J. Kish, Pablo M. Rusjan, Alan A. Wilson, Isabelle Boileau, Sylvain Houle, Jeffrey H. Meyer

Centre for Addiction and Mental Health, Toronto, Canada

Background: Alcohol dependence is a highly impactful disease, as the World Health Organization attributes 4% of global death and 5% of the global burden of disease to alcohol use disorders. Dysphoric mood is reported during acute alcohol withdrawal, and is highly predictive of relapse. Greater MAO-A binding and/or protein levels, particularly in prefrontal and anterior cingulate cortex, have been associated with major depressive episodes, high risk states for major depressive episodes and sad mood (Arch Gen Psych 2006, 2009, 2010, 2011). The aim of the current study is to investigate whether MAO-A binding is elevated in the prefrontal and anterior cingulate cortex during the

mood lowered state of withdrawal from alcohol containing beverages.

Methods: MAO-A total distribution volume (V_T), an index of brain MAO-A levels, was measured using [^{11}C] harmine positron emission tomography. Eleven medication free, non-smoking subjects with alcohol dependence and no comorbid psychiatric illness/substance abuse were scanned following 12 hours of withdrawal, in addition to eleven healthy controls.

Results: There was a significant elevation in MAO-A V_T in the prefrontal and anterior cingulate cortex in alcohol dependent patients during withdrawal compared to controls (38% and 42% respectively, independent samples t-test, $p < 0.01$ and $p < 0.005$ respectively). A similar change was detected in other brain regions (dorsal putamen, ventral striatum, thalamus, midbrain, hippocampus; $p = 0.01$ to 0.14).

Discussion: This data argues in support for a role of elevated brain MAO-A levels during withdrawal from alcoholic beverages. MAO-A inhibition, which has not been targeted by existing therapeutics, may be a new strategy to counter this withdrawal effect.

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162. Pharmacological MRI of D-amphetamine: Effects on a Go-No/go Task in Cocaine Dependent Subjects

F. Gerard Moeller*, Joel L. Steinberg, Scott D. Lane, Liangsuo Ma, Thomas R. Kosten, Ponnada A. Narayana

University of Texas Health Science Center at Houston, Houston, USA

Background: Cocaine dependence is associated with higher impulsivity as measured by a number of behavioral paradigms. Several studies have also shown lower dopamine function in chronic cocaine users, and dopamine-enhancing agents have shown some efficacy at reducing cocaine positive urines. However, the effect of dopamine enhancing drugs on brain function in cocaine dependent subjects while performing behavioral laboratory impulsivity measures has not been studied. The purpose of this study was to determine the effects of acute administration of the d-amphetamine on brain function during a Go-No/go task in cocaine dependent subjects.

Methods: After written informed consent, cocaine dependent subjects underwent screening for psychiatric and non-psychiatric medical disorders. Ten non-treatment seeking cocaine dependent subjects met inclusion criteria and underwent two fMRI scans during performance of a novel Go-No/go paradigm with two levels of difficulty. In a double blind fashion subjects were administered 20 mg of d-amphetamine on one of the scans and placebo on the other scan.

Results: Within cocaine dependent subjects, d-amphetamine significantly reduced brain activation in the striatum compared to placebo during a successful no-go trial (FWE corrected $p < 0.01$).

Discussion: These findings are consistent with enhancement of dopamine function in cocaine users leading to modification of brain activation in brain regions (i.e., striatum) known to subserve response inhibition.

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163. Methylphenidate Enhances Executive Function while Optimizing Prefrontal Error-Related Processing in Both Health and Cocaine Addiction

Scott J. Moeller*, Patricia A. Woicik, Dardo Tomasi, Nora D. Volkow, Rita Z. Goldstein

Brookhaven National Laboratory, Upton, USA

Background: Error monitoring is a core executive function that allows for successful identification and correction of discrepancies between an intended and executed response. Previous studies that investigated genetic variation [e.g., polymorphisms of catechol-O-methyl transferase (COMT), dopamine D2 receptor] or relevant disease states (e.g., Parkinson's disease, attention deficit/hyper-activity disorder, drug addiction) have suggested dopamine to be involved in error monitoring/processing, possibly through impact on reinforcement learning. Nevertheless, pharmacological studies that can manipulate dopaminergic functioning are needed to appropriately characterize the neurochemistry underlying error monitoring. The current study tested whether methylphenidate (MPH), an indirect dopamine and norepinephrine agonist (that acts by blocking both respective transporters), modulates brain and behavioral responses to error. To further explore the effects of MPH on error-related processing vis-à-vis dopamine, we also included individuals addicted to cocaine, a population characterized by drug-mediated decreases in dopamine receptor availability and release, and functional impairments in prefrontal cortical areas innervated by dopamine.

Methods: After receiving oral MPH (20 mg) or placebo (counter-balanced across subjects), 17 healthy and 17 cocaine addicted individuals completed a classical executive function task (the color word Stroop) during event-related functional magnetic resonance imaging. All trials during which an error occurred were modeled against an active baseline (all correct congruent trials) using Statistical Parametric Mapping. Behavioral measures of task performance (total errors and post-error slowing, the latter being an adaptive, corrective response following an error that is thought to enable more controlled responding to prevent future errors) were also collected.

Results: MPH improved task-related performance in all subjects [fewer errors: $F_{(1,32)} = 8.4$, $p < 0.01$; and increased post-error slowing: $F_{(1,32)} = 6.0$, $p < 0.05$]. In parallel, during MPH all subjects showed reduced dorsal anterior cingulate cortex (dACC) response to error ($Z = 3.6$, $p < 0.05$ cluster-level corrected, small volume correction), a region classically implicated in performance/error monitoring. In the cocaine subjects, MPH also reduced activity in the dorsolateral prefrontal cortex (DLPFC), a region implicated in the implementation of cognitive control, to a level where it no longer differed from the healthy individuals (medication × group interaction: $Z > 3.1$, $p < 0.05$ cluster-level corrected, small volume correction). Finally, across medication conditions and compared with controls, the cocaine subjects showed more error-related response in the cerebellum (group effect: $Z = 3.7$, $p < 0.01$ cluster-level corrected).

Discussion: An indirect dopamine/norepinephrine agonist (MPH) improved executive function in health and in cocaine addiction, paralleled by both common and unique underlying neural responses to error between the groups. The reduced error-related

dACC activation in all subjects suggests more efficient error-related processing during MPH. This result is consistent with studies examining genetic variation in the COMT genotype, where individuals with the Val/Val genotype (associated with reduced extracellular dopamine in the prefrontal cortex) showed less efficient cognitive functioning (greater fMRI response in the ACC) than those with the Met/Met genotype during a working memory task. In contrast, MPH reduced DLPFC activation uniquely in the addicted subjects, possibly pointing to differential implementation of cognitive control (as there were indeed differences in DLPFC function between the groups during placebo). The cerebellar hyperactivity in the addicted individuals possibly reflects compensatory mechanisms that are needed (even with MPH) to achieve comparable task performance. Taken together, these pharmacological fMRI results point to the brain regions that could become potential therapeutic targets in future longitudinal intervention studies, such that the supervised and controlled administration of oral MPH could be used to improve cognitive function and optimize brain response to error.

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164. Assessment of Brain Nicotine Accumulation during Cigarette Smoking: a PET Study with ^{11}C-Nicotine Cigarettes

Alexey G. Mukhin*, Yantao Zuo, Sudha Garg, Rachid Nazih, Behm M. Frederique, Pradeep K. Garg, Jed E. Rose

Center for Nicotine and Smoking Cessation Research, Duke University Medical Center, Durham, USA

Background: Tobacco smoking constitutes one of the primary preventable causes of death in developed countries. Two of the popular hypotheses to explain the addictive properties of cigarette smoking are 1) the existence of puff-associated spikes in brain nicotine concentration and 2) fast brain nicotine accumulation during cigarette smoking. Previously (Rose *et al.*, PNAS 2010) we investigated the dynamics of nicotine accumulation in the smoker's brain after a single puff of cigarette smoke loaded with ^{11}C-nicotine and based on the results concluded that puff-associated spikes in the brain nicotine concentration do not occur during habitual cigarette smoking. We also predicted that brain nicotine concentration gradually increases over the course of smoking a cigarette. To confirm this prediction, here we assessed the brain nicotine accumulation during smoking of whole cigarette containing ^{11}C-nicotine evenly distributed along the tobacco rod.

Methods: The study was performed in 16 regular smokers (9 males and 7 females), smoking 10 or more cigarettes per day. In two different PET sessions, the head of the subject was scanned over 12 minutes after inhalation of a single puff or 7 puffs (45 sec inter-puff interval) of smoke from a cigarette containing [11]C-(S)-nicotine. The high temporal resolution scanning (245 frames over 720 sec) was performed using a GE Discovery VCT scanner. After each brain PET scan a total inhaled ^{11}C-nicotine dose (ID) was measured via whole body scan.

Results: 1) Puff-associated spikes in the brain nicotine concentration did not occur during cigarette smoking. 2) Brain nicotine concentration increased in a nearly linear fashion during 6 min of cigarette smoking (average $R^2 = 0.992$) and reached the max value of 4.6 ± 0.2 %ID/kg tissue at 6-7 min after the first puff; 3) The predicted brain nicotine concentration curves for smoking a full cigarette, calculated by convolution of single puff curves, were practically superimposable on those assessed directly (respective average slopes were 0.87 ± 0.05 and 0.82 ± 0.05 %ID/kg tissue/min; correlation between slopes r = 0.95).

Discussion: The results confirm that puff-associated spikes in brain nicotine concentration do not exist during usual habitual

cigarette smoking. The brain nicotine concentration gradually increases for the duration of cigarette smoking (5-10 minutes). Therefore brain nicotine kinetics during cigarette smoking could be closely mimicked by IV administration and potentially other routes of nicotine administration. This opens a new perspective to study the role of brain nicotine kinetics in smoking dependency and the development of new smoking cessation strategies.

165. History of Early Childhood Trauma is Positively Associated with Ventral Striatal Dopamine Responses to Amphetamine

Lynn M. Oswald*, Gary S. Wand, Dean F. Wong[2] Hiroto Kuwabara, James Brasic, Wichana Chamroonrat, Maria Guevara, Eram Zaidi

University of Maryland, Baltimore, USA

Background: Early childhood trauma has been linked to a range of untoward physical health outcomes in adulthood, as well as heightened vulnerability for psychopathological conditions, such as substance use, anxiety, and depressive disorders. Individuals who have experienced childhood trauma also exhibit dysregulation of the physiological stress response, have higher levels of neuroticism, and report greater life stress than persons without such history. Evidence of the strong relationship between childhood trauma and drug abuse has come from both general population studies and clinical studies with drug abusers. Taken together, findings indicate that early trauma is associated with earlier initiation of drug use, heavier consumption, greater use of substances to cope, and more negative consequences of alcohol or drugs. Although mechanisms remain unclear, findings from preclinical studies indicate that early life trauma can have profound, long-lasting effects on behavior and neurobiological function. Such effects include alterations in neuroendocrine stress response and monoamine systems, which are known to be important in drug abuse. Interestingly, maternally separated rats have greater nucleus accumbens (NA) dopamine (DA) release in response to psychostimulants and enhanced acquisition of drug self-administration as compared to control rats. Such findings suggest that early trauma may increase vulnerability for drug abuse by modulating DA function, leading to altered sensitivity to the reinforcing effects of psychostimulants. Studies extending this line of research to primate models are sparse; results of one PET study in female rhesus monkeys did not replicate the rodent findings. The purpose of the present study was to determine whether childhood trauma is associated with dopaminergic or subjective effects of amphetamine (AMPH) in humans. On the basis of the rodent studies, as well as prior evidence from our group and others which has shown that greater responsivity to acute stressors is associated with enhanced responses to psychostimulants, we hypothesized that subjects who reported greater exposure to adverse events as a child would show increased sensitivity to AMPH.

Methods: Twenty healthy adults (M = 14), ages 18 – 29 years, completed diagnostic screening and a battery of self report measures of personality, mood, anxiety, and stress. Early trauma was assessed with the 27-item self-report, short form Early Trauma Inventory (ETISR-SF), which evaluates four types of trauma (general, physical, emotional, and sexual). Subjects underwent two consecutive 90-minute PET studies with [11C]raclopride (RAC). The first scan was preceded by intravenous saline; the second by 0.3 mg/kg AMPH. Dopamine (DA) release was defined as percent change in RAC BP between scans in the ventral striatum (VS) and left (LVS) and right (RVS) subdivisions. Measures of subjective drug effects were also obtained.

Results: Findings indicated that history of early trauma was positively associated with DA responses to AMPH. Total ETI scores correlated with DA release in the VS ($r = 0.49$; $p = 0.03$) and RVS ($r = 0.49$; $p = 0.03$); scores on the emotional trauma subscale correlated with DA release in the VS ($r = 0.45$; $p = 0.047$) and LVS ($r = 0.65$; $p = 0.002$). The only associations observed between early trauma and baseline D2 receptor availability were on the physical trauma subscale ($r = .45$; $p = .049$ for both VS and RVS). A trend for positive relationships was observed between ETI scores and pleasant subjective drug effects. Total ETI scores were associated with self-reported 'high' ($r = .40$; $p = .08$); physical trauma scores were associated with 'rush' ($r = .41$; $p = .075$) and drug 'liking' ($r = .43$; $p = .058$). Highly significant positive correlations were found between ETI total scores and measures of state/trait anxiety, perceived stress, and life events stress ($p < .03$ in all cases). Scores on measures of anxiety and stress were also positively associated with VS DA (particularly RVS) and subjective responses to AMPH. A series of regression analyses suggested that the relationship between early trauma and DA release may be partially mediated by perceived stress and anxiety.

Discussion: This report provides the first evidence of relationships between early trauma and stimulant-induced DA release in humans. Although causal relationships cannot be definitively established on the basis of this correlational study, findings support notions that early childhood trauma may increase vulnerability for drug abuse by altering the function of dopaminergic incentive-motivational neurocircuitry in the brain.

166. Regional Brain Structural Dysmorphology in Chronic Alcoholism, HIV Infection, and Their Comorbidity

Adolf Pfefferbaum*, Margaret J. Rosenbloom, Torsten Rohlfing, Edith V. Sullivan

SRI International, Menlo Park, USA

Background: HIV/AIDS infection continues to rage at pandemic levels worldwide. Of even higher prevalence are alcohol use disorders, which themselves present an unfortunate liability for risky behavior associated with activities promoting HIV infection. Indeed, estimates indicate high incidence of alcohol ″on board″ when infected, and an estimated 30-60% of HIV infected individuals report alcohol use disorders. Given that each disease can result in significant brain tissue shrinkage, we used a four-group design to examine the effects of each condition alone and their comorbidity on regional brain volumes in relation to control measures.

Methods: The participants were 344 men and women (age-range matched 25-69 years): 110 alcoholics (ALC), 59 with HIV infection, 65 with HIV infection and alcohol dependence (HIV + ALC), and 110 unaffected controls. The affected groups generally had fewer years of education, lower intelligence scores, lower socioeconomic status, and more depressive symptoms than controls. The two alcohol groups reported similar lifetime alcohol consumption levels, which were about 14 times higher than in the HIV or control groups. CD4 cell count, CD4 nadir, and age at infection were statistically similar in the two HIV groups.

MR data were collected on a GE 1.5T scanner. Brain volumes were derived from T1-weighted Inversion-Recovery Prepared SPoiled Gradient Recalled (SPGR) images (94, 2 mm thick slices; TR = 25 ms, TE = 5 ms, flip angle = 30°)and dual-echo Fast Spin-Echo (FSE) images(47, 4 mm thick slices, TR = 4775 ms, TE = min/full, ETL = 8). Brain data analysis was conducted by registration-based parcellation. After removal of non-brain signal using FSL BET applied to both the FSE and SPGR data, the SRI24 atlas was registered to each subject′s SPGR brain data with nonrigid registration. The parcellated SRI24 atlas was then reformatted into native space for each individual′s SPGR data sets. Gray matter/white matter/CSF were segmented using FSL FAST with tissue priors also based on the SRI24 atlas. Segmented volumes were quantified for the following brain regions: gray matter volumes for 10 cortical and allocortical regions,tissue volumes (gray + white matter) for 4 subcortical structures, 2 white matter regions, and 5 CSF volumes. For analysis, regional volumes were expressed as standardized Z-scores, adjusted for normal variation in intracranial volume and age estimated from control data obtained in 59 men and 64 women, age 20 to 74 years. Initial one-way analysis of variance (ANOVA) tested for group effects for each regional volume (p.02). Bonferroni directional correction for 21 regions applied to followup 2-group comparisons with t-tests required p = .005 with alpha at .05 for statistical significance.

Results: In ALC, significant volume deficits involved all four cortical lobes, insula, anterior cingulate cortex, globus pallidus, hippocampus, thalamus, and corpus callosum. Abnormally large CSF volumes in the ALC were present in the cortical sulci, notably the frontal and Sylvian regions, and third ventricle. In HIV, the only regions showing significant differences from controls were the insula, thalamus, and frontal sulci. By contrast, in HIV + ALC, abnormalities were more widespread and included lateral frontal, temporal, parietal, occipital, insular, anterior cingulate cortical regions, thalamus, and corpus callosum; greater than normal volumes were present in the frontal sulci. Repeated measures ANOVA over the cortical regions revealed a group-by-region interaction solely in the ALC (p = .0213), indicating greater volume shrinkage in the anterior than posterior regions. Modest correlations indicated greater third ventricular volumes in ALC and HIV

+ ALC with shorter periods of sobriety, and smaller thalamic volumes with greater lifetime alcohol consumption in ALC.

Discussion: The most profound and consistent volume deficits were identified in the two groups with alcohol use disorders;regions showing overlapping volume abnormalities were the cortical mantle, insular and anterior cingulate cortex, thalamus,corpus callosum, and frontal sulci. By contrast, the HIV only group showed volume abnormalities limited to the insula, thalamus, and frontal sulci. Thus, alcoholism in HIV-infected individuals greatly expanded the scope of deficit likely to occur in HIV infected individuals. That longer periods of sobriety and lower lifetime alcohol consumption levels were predictive of attenuated volume abnormalities should encourage the inclusion of alcohol recovery efforts in HIV/AIDS therapeutic settings. **Support:** AA017347, AA005965, AA012388, AA010723, AA017168, AG017919, EB008381.

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167. Effects of Smoking Cessation on Reward Processing

Lena Rademacher, Katja N. Spreckelmeyer, Sören Krach, Susanne Prinz, Ingo Vernaleken, Gerhard Gründer*

RWTH Aachen University, Aachen, Germany

Background: Research on nicotine addiction indicates greater ventral striatal activity in smokers compared to non-smokers in response to smoking-associated cues but blunted reactivity to non-drug rewards (David et al., 2005; Martin-Soelch et al., 2003). However, it is still unclear how reward processing changes after smoking cessation. The aim of the present study was to examine neural correlates of reward anticipation and cue reactivity in non-smokers and nicotine-dependent smokers before and three months after smoking cessation.

Methods: Twenty-one smokers and 29 non-smokers performed two paradigms on a 1.5 T scanner: Monetary and social reward anticipation were investigated using the Monetary and Social Incentive Delay task (Knutson B et al., Neuroimage 2000; 12: 20-27; Spreckelmeyer K et al., SCAN 2009; 4: 158-165). Participants were given a cue indicating potential reward. In order to receive reward, a target button had to be pushed within a certain time window (adapted for individual reaction time). The degree of potential reward was varied on three levels. The three levels of monetary reward were Euro 0.20, Euro 1.00 and Euro 3.00. Success was acknowledged by presenting the picture of a wallet which either contained the respective amount of money in coins or -in the case of no outcome- was empty. For the three levels of social reward magnitude three types of happy face expressions with increasing intensity level were used. The second paradigm examined cue reactivity by presenting blocks of smoking-related, neutral or sexually arousing pictures. All smokers took part in a smoking cessation course. Ten smokers who succeeded in staying abstinent for three months underwent a second fMRI scan with the same paradigms.

Results: During both monetary and social reward anticipation smokers showed weaker activity of the NAcc compared to non-smokers. However, in response to smoking-associated pictures stronger neural responses were found in the caudate nucleus. For both paradigms no effect of smoking cessation could be detected.

Discussion: The data implies that striatal activation during anticipation of non-smoking rewards is decreased in smokers while reactivity is increased for smoking-associated pictures. The findings further suggest that neural activation during reward processing is not affected by smoking cessation. The data points towards a general dysfunction of the reward system in nicotine-dependent smokers.

Most subjects in this study underwent [18F]FDOPA PET scans parallel to the fMRI studies. These data is currently being analyzed and will be available at the meeting.

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168. Methamphetamine Induced Signal Change in rats: An fMRI Study

Carmela M. Reichel*, Xun Zhu, Saeid Taheri, Xingju Nie, Jane E. Joseph, Ronald E. See

Medical University of South Carolina, Charleston, USA

Background: Methamphetamine (meth) is one of the most prevalent drugs of abuse worldwide. Meth poses unique challenges for treatment due to the highly addictive nature of the drug and multiple associated symptoms. Neuroimaging studies in meth addicts have reported functional neuronal changes in brain regions involved in relapse. However, studies in humans are limited by the inability to control for the premorbid state, the degree of meth use over time, and the extent of withdrawal. With the advent of new techniques for *in vivo* brain imaging in rats, we have begun to address these issues in rodent models. Here, we report on meth-induced blood oxygen level dependent (BOLD) signal change using functional magnetic resonance imaging (fMRI) in the medial prefrontal cortex (PFC), nucleus accumbens (NAC), and striatum (STR) of rats.

Methods: fMRI acquisition: Male Long Evans rats underwent *in vivo* imaging while bolus i.v. injections of meth ($n = 8$) or saline ($n = 7$) were infused. Anatomical and fMRI were performed on a 7T BioSpec dedicated research MR scanner under isoflurane gas (1.5-2.0%). Functional images were obtained with a multislice spin-echo, EPI sequence using the following optimized parameters: TE/TR $= 16.5/2000$ ms; BW $= 250$ KHz; FOV $= 2.5$ X 2.5 cm2; Matrix size $= 128$ X 128 pixels; number of repetition $= 2000$, slice thickness 1 mm, slice gap 0.1 mm. After collecting baseline data for 10 min, rats were administered meth or saline over the next 5-10 min for a total 50 min scan time. Specifically, rats received a total of six 2-sec infusions ($20 \mu g/50 \mu l$ bolus) of meth or saline at 1 or 2 min intervals for a total meth dose of approximately 0.3 mg/kg. Imaging then commenced for an additional 30 min. Data processing: For each rat, preprocessing included motion correction with FSL software (version 4.1.7) using MCFLIRT, followed by non-brain substance removal using BET. For each rat, functional runs were registered to an average template by using a 12-parameter affine transformation of individual rats' high-resolution T1-weighted anatomical images. Regions of interest (PFC, NAC, and STR) were identified manually based on anatomical scans. The time series of signal intensity values within regions of interest were averaged within one-minute intervals. Statistical analysis: Data were group analyzed with separate mixed analysis of variance for each ROI. The between group variable was drug exposure (meth or saline) and the within group variable was the time course averaged into 10 min time bins.

Results: Meth infusions during the imaging session resulted in a negative BOLD signal change. In the STR and NAC, meth caused a significant negative signal change relative to baseline. Further, meth induced signal change was significantly lower than signal change following saline infusions in these regions. Meth also induced a negative signal change in the PFC, but this was not significantly different from baseline or saline.

Discussion: We found a sustained negative BOLD signal change in regions implicated in persistent drug seeking and relapse. Possible interpretations for negative BOLD signal changes in fMRI studies include decreased neuronal activity, changes in cerebral blood volume or cerebral blood flow, or increased neuronal activity due to hemodynamics and metabolism interplay. The origin of meth-induced negative signal change is unclear, but may result from sustained vasoconstriction following meth or non-neural physiological modulators (e.g., respiration). Further investigations underlying the importance of this negative signal change in relation to meth's physiological and neural effects are being pursued. Such studies will enhance our understanding of the neurobiological consequences of meth on *in vivo* brain function.

Disclosure: C. Reichel: None. **X. Zhu:** None. **S. Taheri:** None. **X. Nie:** None. **J. Joseph:** None. **R. See:** None.

169. Acute Baclofen's Impact on Fronto-Limbic Connectivity: An Aid to Modulate Negative Affect in Smokers?

Jesse J. Suh*, Teresa R. Franklin, Ronald N. Ehrman, Kanchana Jagannathan, Zachary D. Singer, Kathleen Marquez, Anita Hole, Marina Goldman, Shing C. B. Lam, Ze Wang, Yin Li, Charles P. O'Brien, Anna Rose Childress

University of Pennsylvania Perelman School of Medicine, Philadelphia, USA

Background: Baclofen (BAC), a GABA B agonist, is a treatment for post-spinal cord injury spasticity with potential secondary indications as an anti-craving/anti-relapse medication in alcohol, cocaine, opiate and nicotine addictions. We previously demonstrated that BAC reduced the number of cigarettes smoked per day in a clinical trial for smoking reduction. Within a laboratory setting using a 3-week medication versus placebo regimen, we also showed that chronic baclofen dampened baseline activity in the anterior cingulate and bilaterally in the ventral striatum, insula and orbitofrontal cortex in smokers. Poor regulatory capacity is pervasive in nicotine addiction, and is a recognized risk factor for relapse. Given that baclofen has been shown to improve mood, helps smokers to reduce smoking and modulates brain activity in regions associated with emotional regulation, we tested whether acute baclofen could improve functional connectivity involved in top-down brain circuitry in emotional regulation. We hypothesized that baclofen would strengthen the functional connectivity between the amygdala (AMYG), and the prefrontal cortex and the anterior cingulate during affect regulation task.

Methods: In a within-subject design, we administered either one 20 mg dose of BAC (onBAC) or no medication (offBAC) to 12 smokers (8 females; 4 males). We used block-design BOLD (blood-oxygen-level dependent) fMRI to measure the brain response to neutral and aversive cues while smokers attempted emotional regulation ("watch"; "up"; "down"). Subjects were instructed to watch, increase or decrease their emotions while watching aversive cues. All subjects identified and used cognitive reappraisal strategies to regulate negative emotions. Data were preprocessed within SPM5. We used the task-related partial least square (PLS) analysis on the preprocessed data from neutral and emotional regulation conditions. Seed voxel PLS analyses then was used to contrast the functional connectivity across conditions and between onBAC and offBAC conditions This analysis technique allows an examination of whether the brain regions that are correlated with the amygdala activity are different between onBAC and offBAC.

Results: Smokers' mean age was 29 (s.d. $= 8.4$) and they had obtained approximately 15 years of education (s.d. $= 2.7$). There was no difference in their experience of sedation under onBAC and offBAC. The first and only significant latent variable (LV) (LV1,

crossblock variance = 31.18%, p < 0.001) differentiated between onBAC and offBAC conditions, during "watch" and "down" modulations. Under offBAC, there was a greater activity in the left amygdala during both the "watch" and "down" regulatory attempts, compared to onBAC. Under onBAC, there was a greater activity in the dorsolateral prefrontal cortex, compared to offBAC. Based on the role of the amygdala in emotional regulation and in our tasks, we used the left amygdala as a seed region (-20, -8, -16; 4 mm sphere) within the functional connectivity to identify the cognitive-emotive networks that are active under onBAC and offBAC conditions during "watch" and "down" attempts. The dominant and only significant LV (accounting for 46.27% of the crossblock correlation, p < 0.001) revealed stronger inverse functional connectivity between the amygdala, and supergenual and dorsal anterior cingulate during "down" attempts under onBAC, compared to offBAC.

Discussion: Condition-specific brain activity in the amygdala under offBAC and in the dorsolateral prefrontal cortex under onBAC during "watch" and "down" conditions suggested baclofen's dampening activity in the amygdala. Enhanced connectivity between the frontal modulatory regions and the amygdala under onBAC during "down" attempts suggests BAC may aid smokers in regulating negative affect that contribute to relapse by improving the fronto-limbic connectivity, and show promise for improving the brain circuitry in smokers' regulatory functioning.

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170. Elucidating Fnctional Relationships between Ventral Striatal Dopamine Release and Prefrontal Cortical-Striatal Activity in Human Reward cCrcuitry with fMRI and [11C]Raclopride PET: New Data

Michael J. Travis*, Erika Forbes, N. Scott Mason, Jorge R. C. Almeida, Natalie D. Velasquez, Eric E. Rodriguez, Michael L. Himes, W. Gordon Frankle, Rajesh Narendran, Mary L. Phillips

Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, USA

Background: Alcohol and other substance use disorders, unipolar depression, bipolar disorder and attention-deficit/hyperactivity disorder (ADHD) are amongst the most significant causes of morbidity and mortality. Abnormal function in reward systems is associated with these major psychiatric disorders. Dopamine (DA) release in the ventral and dorsal striatal regions, is important in reward processing. Understanding the relationship between functional activation in the dorsomedial component of the prefrontal cortex, (MdPFC) and dopamine release in the striatum will both help develop novel treatments and inform preventative strategies

Methods: Subjects were 16 healthy volunteers,6 male and 10 females, 20 to 29 years old. 13 subjects, (6 male, 7 female, mean age 23.9 ± 2.7), underwent an fMRI scan during a monetary reward task and four bolus plus constant infusion [11C]raclopride PET scans, each separated by a week: one at rest/baseline, one during a motor control task, one during the same monetary reward task as for the fMRI and a final [11C]raclopride PET scan after administration of oral amphetamine (0.5 mg/kg) to displace [11C]raclopride. 3 subjects, (3 females, mean age 23 ± 2 years), underwent the same protocol but with no post-amphetamine scan. The reward task was a block design for the fMRI comprising a card guessing game in which outcome was positive or negative for each trial. Upon receiving positive feedback subjects were required to respond via button press (either index or middle finger) to engage

consummatory processes to elicit activation and DA release. There were also control blocks, during which participants were instructed to simply make alternating button presses during the presentation of an 'x'. This task reliably activates the ventral striatum (VST), head of caudate and the cortical region BA9 to reward. For the PET scans the task was split between two scans into the reward component, (reward and control), and the motor component, (control only). Both PET and fMRI data was analyzed as reward vs motor.

Results: fMRI – there were Significant BOLD signal changes in the VST bilaterally to reward versus the motor control task, (p < 0.05, alphasim corrected). Similar signal changes to reward versus control were also seen bilaterally in BA32 and BA9, (p < 0.05, alphasim corrected), both part of the MdPFC. PET – There was significantly increased [11C]raclopride displacement, (decreased [11C]raclopride binding), to the motor vs baseline in the left ventral striatum, (-5.1 ± 5.5% p < 0.01), but there was no significant displacement of [11C]raclopride in the baseline vs reward condition. For motor vs reward there was a significant increase in [11C]raclopride binding, (4.6 ± 6.9, p = 0.02) in the left anterior putamen and the associative striatum, (4.2 ± 7.1, p = 0.03) and a numerical increase in [11C]raclopride binding in all other regions in this condition which did not reach significance There were no significant differences in right striatal regions. Importantly the degree of variability was high in all regions and conditions. Based on the neural circuitry of the reward system, we examined the relationship between the magnitude of the BOLD response in left BA32 and the motor vs reward [11C]raclopride displacement in the left VST. There was a significant inverted U shaped relationship between BA32 bold response and VST dopamine release, (R = 0.61, p = 0.01), not present in other regions.

Discussion: These data extend on our previous report of this data, now with 16 subjects, and suggest a complex relationship between VST dopamine response and magnitude of BOLD response in the MdPFC during reward. When BA32 shows little response there is little or no DA release in the VST. As BA32 BOLD signal increases there is evidence that DA release in the VST initially increases but then decreases. The complexity of this relationship may underlie the conflicting findings from previous studies and may reflect interindividual differences in reward processing. Further work will include functional connectivity analysis of fMRI data, inclusion of pharmacological challenge data and determining the relationship between multi-modal imaging findings and clinical measures such as impulsivity. It is hypothesised that differential patterns of MdPFC activity related to VST dopamine response may be related to risk for and protection against future psychiatric disorder.

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171. Brain Dopamine Responses to the Expectation of Methylphenidate in Non-Drug Abusing Subjects

Gene-Jack Wang*, Nora Volkow, Tomasi Dardo, Frank Telang, Christopher Wong, Jean Logan, Millard Jayne, Joanna Fowler

Brookhaven National Laboratory, Upton, USA

Background: The response to drugs of abuse is affected by expectation. This is modulated in part by dopamine, which is a neurotransmitter involved with reward and expectation of reward. We assessed the effect of expectation on methylphenidate induced

dopamine increases in striatum and midbrain in non-drug abusing subjects and compared their responses between expected and unexpected conditions.

Methods: Nineteen male subjects (42.1 ± 3 years old) were scanned with 4 PET scans and [C-11]raclopride after methylphenidate (0.5 mg/kg iv) or placebo. Brain dopamine D2/3 receptor (D2R) availability was measured under four randomly ordered conditions: (1) expecting placebo and receiving placebo (baseline); (2) expecting placebo and receiving methylphenidate; (3) expecting methylphenidate and receiving methylphenidate; (4) expecting methylphenidate and receiving placebo. D2R availability (non-displaceable binding potential, BP_{ND}) was analyzed with the region of interest and statistical parameter mapping (SPM) methods. SPM significance was set at $p < 0.001$, 200 pixels. Self-report ratings (1-low to 10-high) of methylphenidate effect were recorded.

Results: Unexpected methylphenidate induced greater self-report of anxiety than expected methylphenidate ($p < 0.03$). Methylphenidate increased brain dopamine release in dorsal striatum and ventral striatum when expected (methylphenidate/methylphenidate, dorsal striatum: $19 \pm 10\%$, $p < 0.0001$, ventral striatum: $13 \pm 20\%$, $p < 0.002$) and when unexpected (placebo/methylphenidate, dorsal striatum: $15 \pm 9\%$, $p < 0.0001$, ventral striatum: $13 \pm 17\%$, $p < 0.001$) but the dopamine changes when expecting methylphenidate and receiving placebo were not significant. SPM analyses showed methylphenidate increased dopamine release in dorsal striatum and ventral striatum in both expected and unexpected conditions. However, methylphenidate induced greater dopamine release in substantia nigra in expectation than in unexpected condition.

Discussion: Expected and unexpected methylphenidate induced dopamine release in dorsal striatum and ventral striatum to the same extent. Methylphenidate when it was expected increased dopamine release in midbrain more than when it was unexpected. These findings provide further evidence of dopamine's involvement in processing expectation for uncertainty about a drug's effects. (Supported by NIDA)

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172. Low Striatal Dopamine Receptor Availability Linked to Weight Gain and Caloric Intake during Abstinence from Chronic Methamphetamine Abuse

Todd Zorick*, Mark A. Mandelkern, Timothy Fong, Dara G. Ghahremani, Chelsea Robertson, Brittany Sumerel, Edythe D. London

WLA VA Medical Center, Los Angeles, USA

Background: Methamphetamine (MA) abuse, a prevalent problem with considerable public health and criminal justice costs,resembles other addictive disorders in its association with deficits in striatal dopamine receptor availability. This commonality is thought to be linked with the propensity to develop addictions via the Reward Deficiency Hypothesis. Despite observations of low striatal dopamine receptor availability both in individuals with MA dependence and those who have obesity, empirical measures of eating dysregulation and propensity to obesity among abstinent, MA-dependent individuals have not been reported. According to the Reward Deficiency hypothesis, addicted individuals would seek out alternative rewards (e.g., food) in the absence of their preferred pathological rewarding outlet (e.g., MA abuse) to compensate for their deficient striatal dopamine signaling. To test the applicability of this hypothesis to MA dependence, we measured striatal dopamine $D_{2/3}$ receptor availability as well as caloric intake and

weight gain weight in MA-dependent research participants during early abstinence from MA.

Methods: Twenty non-treatment-seeking, MA-dependent research participants resided on an inpatient research ward, according to a protocol approved by the UCLA IRB. Aside from MA- and nicotine dependence, the participants were otherwise healthy, and suffered from no other comorbid conditions. They were allowed to eat and smoke *ad libitum* during the study, with abstinence from alcohol and illicit substances confirmed by daily breathalyzer and urine drug screening. Complete daily calorie counts were assessed over days 3-7 and 28-32 of the inpatient stays. Participants were weighed on admission and weekly thereafter. Positron emission tomography (PET) scanning with [^{18}F]fallypride was performed on days 4-8 of the hospitalization. As measures of $D_{2/3}$ receptor availability in different striatal regions (caudate nucleus, putamen, nucleus accumbens) were highly correlated (r's $\geqslant$ 0.7), they were averaged for regional correlations of receptor availability with caloric intake and change in body mass index (BMI). Correlations were conducted using Spearman's rank order correlation.

Results: Calorie counts during the first week of hospitalization were complete for 20 participants: 11 men (58%), 13 of whom were ethnically White (68%), average age of 35.6 ± 8.3 years (mean $\pm$ s.d.), mostly cigarette smokers (n = 18, 90%). They had been abusing MA for 13.2 ± 8.6 years on average, and 18.6 ± 8.1 days of the last 30 before admission. On average, they consumed 4277 ± 1121 Kcal/day on days 3-7 of hospitalization. Calorie counts were complete in both the first and fourth week of the hospitalization for 7 participants; and participants consumed 3763 ± 1073 Kcal/day on days 3-7, and 3588 ± 621 Kcal/day on days 28-32, with no significant difference between caloric intake at the two times ($p = 0.71$). Eight participants had BMI measurements through 3 weeks of hospitalization. The average admission BMI was 26.1 ± 3.0 kg/m^2, with an increase to 28.2 ± 3.3 kg/m^2 ($p < 0.001$ for effect of time) by the end of the third week, an average increase of 8.0%. Sixteen participants had both complete calorie counts for days 3-7 after admission, and [^{18}F]fallypride PET scans, while eight subjects had third-week BMI data and PET scans. To control for age-related loss of striatal $D_{2/3}$ receptors, $D_{2/3}$ receptor availability was normalized to a common age, using a published estimate (4.9% decline/decade for nucleus accumbens and putamen, 5.5%/decade for caudate). Both first-week calorie counts and third-week BMI increase were highly correlated with striatal $D_{2/3}$ receptor availability (r = -0.54, p = 0.016 and r = -0.91, p = 0.001, respectively, 1-tailed p values).

Discussion: The MA-dependent participants have higher daily caloric intake, on average, than hospitalized control subjects in similar settings, and gained a larger proportion (8%) of their BMI over 3 weeks than psychiatric inpatients taking obesogenic antipsychotic medications. Taken together with the correlation between these measures of increased eating behavior and low striatal dopamine $D_{2/3}$ receptor availabilty, these observations provide support for the Reward Deficiency hypothesis in MA dependence. While the findings are compelling, this study is limited by small sample size, lack of a control sample, and lack of information about dietary choices made by participants; therefore, confirmatory studies are warranted.

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173. Objective Measurement of Postural Sway in Blast-Related Traumatic Brain Injury

Brent G. Nelson*, Scott R. Sponheim, Kelvin O. Lim

University of Minnesota, Minneapolis, USA

Background: Postural instability can be an important sign of neurological dysfunction in neuropsychiatric disorders such as traumatic brain injury (TBI), Parkinson's disease, and multiple

sclerosis. Studies have shown that a standard clinical exam may not be sensitive enough to detect subtle abnormalities. These neurological soft signs (NSS), non localizing abnormalities in sensory integration, motor coordination and inhibition, are important in the study and management of mild traumatic brain injury. Assessment of NSS is typically assessed by observational methods of subject performance on specific tasks. Non subjective, quantitative measures have the potential to provide more reproducible and sensitive indices. Balance can be measured quantitatively using a force platform. Prior studies have found postural instability increases in TBI. Commercial balance force platforms are effective but not used widely due to issues of cost and portability. In this study, we use an inexpensive force platform (Wii Balance Board - WBB) to evaluate postural sway in patients with TBI and controls.
Methods: Subjects consisted of 8 probands and 8 controls. Probands were selected based on a history of blast exposure. Balance measurements were performed for 60 sec for each of 3 tasks repeated 3 times per subject for a total of 9 measurements. The tasks performed included eyes closed, inspection, and search. All required the subject to stand motionless on a WBB with feet shoulder-width apart. In inspection the subject fixates on blank sheet of paper, gaze within bounds at all times. In search the subject scans a paragraph of text counting the occurrences of a specified letter. During each task custom software was used to collect center of pressure measurements at 50 Hz. Average sway magnitude and sway velocities were calculated in both the mediolateral (ML) and anteroposterior (AP) directions.
Results: Significant differences were seen when comparing eyes closed and search tasks in the anterior/posterior direction for both groups. In probands, average sway decreased by 0.020 (p = 0.009). In controls average sway decreased 0.024 (p = 0.029). No significant differences were found when tasks were compared between groups. Both average sway magnitude and average sway variability were compared.
Discussion: Balance is a complex activity and requires many physiological processes. If any of these processes are impaired, due to pathology or medication effects, disturbances in balance can be seen. Consistent with other studies, we were able to detect reduced sway when subjects were challenged with a visual task. This supports the ability of the Wii Balance Board to detect changes in postural sway. Contrary to our hypothesis, differences in postural sway were not detected between probands and controls. The small sample size and mild injury may contribute to the lack of a significant difference. The placement of feet shoulder width apart may make the task too easy, making it difficult to elicit differences in postural sway.
Disclosure: B. Nelson: None. **S. Sponheim:** None. **K. Lim:** None.

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174. Copy Number Variation and Induced Pluripotent Stem Cells in Schizophrenia: First Results from the Mount Sinai Conte Center Large-Scale Fibroblast Studies identifies Known Recurrent CNVs

Joseph I. Friedman*, Nagahide Takahashi, Guiqing Cai, Elodie Drapeau, Laura Gouder, Andrea Matho, Jared Cooper, Emily D'Antonio, Puneet Dabas, Marina Goldenberg, Gayle Myerson, Vladan Novakovic, Kenneth L. Davis, Joseph D. Buxbaum

Mount Sinai School of Medicine, New York, USA

Background: The study of neuropsychiatric disorders has been significantly hindered by the great difficulty in accessing brain material from living subjects. The very complexity of psychiatric disorders such as schizophrenia makes them very amenable to studies in cellular systems. The use of Inducible pluripotent stem cells (IPSCs) provides an experimental system to study complex neuropsychiatric disorders such as schizophrenia at the cellular level. For mutations, copy number variations (CNVs), and chromosomal abnormalities, each associated with high odds ratios (ORs), one can use IPSCs to understand the functional effect on the cellular level for an observed variant. IPSCs can be differentiated into neural cells of interest allowing for the testing of explicit hypotheses in cells with a defined variants associated with high ORs, and allows for the development of new hypothesis when samples are pooled and analyzed on the level of the transcriptome, proteome, epigenome, etc. The generation and characterization of IPSCs from patients with schizophrenia at Mount Sinai is part of a more comprehensive effort to study the consequences of well characterized genet<ins cite = "mailto:Joseph%20Buxbaum" datetime = "2011-08-24T09:52" > ic defects in patients with schizophrenia on multiple phenotypic dimensions including behavioral, neuropsychological, functional, imaging and neural alterations at the cellular level utilizing IPSCs. Here we report on preliminary findings associated with well-known, recurrent CNVs identified in patients with schizophrenia from our sample.
Methods: Patients with schizophrenia and healthy controls are recruited by the Clinical Assessment Core of Mount Sinai's Silvio O. Conte Center for Neuroscience of Mental Disorders on an ongoing basis. All subjects receive a comprehensive diagnostic assessment with the Diagnostic Interview for Genetic Studies. Additionally, data is collected on symptom severity, neuropsychological functioning, functional skills competence, health status, and family history. A subset of patients have undergone structural magnetic resonance imaging and diffusion tensor imaging. DNA samples are obtained from all subjects via venous blood sampling and are genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0. A portion of these subjects undergo dermal punch biopsy in order to harvest fibroblasts for the generation of IPSCs. Reprogramming of fibroblasts is being done for a select number of cases and controls, using optimized methods with Sendai virus. In parallel, we are developing methods for high-throughput reprogramming of fibroblasts.
Results: To date, 245 subjects (149 schizophrenic, 96 controls) have been phenotyped and have had DNA samples collected for analysis. Of these, 94 (48 schizophrenic, 46 healthy controls) have undergone dermal punch biopsy for the generation of IPSCs and 94 (44 schizophrenic and 50 healthy controls) have undergone MRI and DTI. A 0.9 Mb deletion within 1q21.1, encompassing 10 genes, was identified in two male siblings with schizophrenia. In addition a 0.6 Mb deletion within 15q11.2, encompassing 9 genes was identified in a male participant with schizophrenia. Finally, a 1.6 Mb deletion within 3q29, encompassing 21 genes, was identified in a female schizophrenic participant. None of these CNVs were identified in our healthy controls and all are known recurrent CNVs loci in schizophrenia. Reprogramming of these fibroblasts, together with cells from ancestry-matched controls is ongoing. We will present optimized methods for reprogramming, based on Sendai virus and surface antigen monitoring. Detailed phenotypic characterization of patients with theseCNVs will also be presented. In parallel, we are developing methods for high-throughput reprogramming so that we can study, on a large-scale, gene expression changes in samples without causal CNV changes.
Discussion: Accrual of DNA and IPSCs will continue. Future research with collected DNA and fibroblasts include:

1. Identification of additional common CNVs and novel rare CNVs.
2. Downstream analysis with called CNVs.
3. Developing discovery methods of regions/genes associated with schizophrenia.
4. Reprogramming fibroblasts into IPSCs and differentiation into neural stem cells, neurons and glia.
5. Characterization of IPSC derived neurons and glia on the basis of: 1) gene expression, 2) cell morphology, 3) synapse formation and electrophysiological changes.

175. *In vivo* MicroRNA Detection and Quantitation in Cerebrospinal Fluid

Juan A. Gallego*, Kierstyn Claycomb, Mahima Bhatt, Todd Lencz, Anil K. Malhotra

The Zucker Hillside Hospital, Glen Oaks, USA

Background: MicroRNAs (miRNAs) are small non-coding RNA fragments that are involved in post-transcriptional regulation of messenger RNAs. Due to their ability to simultaneously regulate the transcription of multiple mRNAs, miRNAs are appealing as potential biomarkers in the study of psychiatric diseases, where multiple mechanisms and pathways are likely implicated. Alterations in miRNA expression have been linked to schizophrenia, however those studies were conducted using post-mortem brain tissue or peripheral blood as the source of transcript. Cerebrospinal fluid might provide an *in vivo* biomarker more directly reflecting functional changes in the brain. The ultimate goal of our study is to examine miRNA levels in CSF and peripheral blood in patients with schizophrenia; however for this preliminary methodological report, we focus on the comparison in miRNA detection between cerebrospinal fluid and peripheral blood.
Methods: Four healthy volunteers and four patients with schizophrenia spectrum diagnoses underwent lumbar puncture and a blood draw. Expression of 377 validated microRNAs was assessed from each biofluid type for each subject using microarray technology. Six miRNAs were chosen for validation with qPCR based on the combination of prior literature reports and microarray results.
Results: A large number of miRNAs were detected in cerebrospinal fluid and whole blood, even when using stringent thresholds for expression. At $Ct < 30$, a mean of 86 miRNAs were detected in whole blood, while 60 were detected on average in cerebrospinal fluid (paired $t = 1.19$, $df = 7$, $p = 0.27$). Considering all samples, a total of 248 distinct miRNAs were detected ($Ct < 40$) in one or more of the 8 cerebrospinal fluid samples and a total of 271 different miRNAs were detected in one or more of the whole blood samples. Based on microarray results, a substantial number ($n = 95$) of miRNAs were detected more robustly in cerebrospinal fluid compared to blood. About 1/3 (34.7%) of these were detected exclusively in cerebrospinal fluid samples and 1/3 (13.7%) were detected in three or more cerebrospinal fluid samples. Consistent with microarray results, qPCR found that mir 219-5p and mir 9 were predominantly detected in cerebrospinal fluid, while mir 328 was predominantly expressed in whole blood. mir 132 was predominantly expressed in cerebrospinal fluid on qPCR, although this pattern was not evident in the microarray results. mir 7b was detected in all 16 samples using microarray, but was detected in only one sample in qPCR. mir 137 was not detected using qPCR, despite the fact that was detected in low levels in 3 cerebrospinal fluid samples on microarray analysis. Spearman correlation coefficients comparing normalized cerebrospinal fluid and whole blood values in 35 miRNAs detected in both cerebrospinal fluid and blood samples in all subjects shows that coefficients clustered around 0, indicating no systematic relationship between levels detected in the two fluids
Discussion: miRNAs can be successfully detected in CSF in healthy controls as well as in patients with schizophrenia

spectrum disorders. Our data suggests that the miRNA detection in CSF provides independent information that is not captured by assays derived from peripheral blood. Specifically, we found that 1) some miRNAs were expressed in CSF but not in whole blood; 2) some miRNAs were predominantly (though not solely) expressed in CSF and; 3) miRNAs levels in CSF and blood samples were poorly correlated. These preliminary data suggest that the detection of miRNAs in CSF may be complementary to existing approaches for biomarker identification in the study of psychiatric disorders.

176. Predictors of Inaccurate Self Assessment of Everyday Functioning in Clinically Stable Outpatients with Schizophrenia

Samir Sabbag, Thomas Patterson, Robert Heaton, Philip Harvey*

University of Miami Miller School of Medicineq, Miami, USA

Background: Despite multiple lines of evidence suggesting that people with schizophrenia have substantial problems in self-reporting everyday functioning and cognitive performance, self-report methods are still widely used to assess functioning. This study attempted to identify predictors of accuracy in self report, both in terms of accurate self-assessment and over-estimation of current functioning.
Methods: As part of the larger VALERO study, 195 patients with schizophrenia were asked to self report their every functioning with the Specific Levels of Functioning (SLOF). They were also assessed with performance-based measures of neurocognition and functional capacity, and were assessed for symptomatology with the PANSS and the Beck Depression Inventory. In addition, a friend, relative, or clinician informant was interviewed with the SLOF as well and the interviewer generated best estimate ratings of functioning based on patient and informant report and their observation of the patient. These ratings were generated in three areas: social functioning, everyday activities, and vocational functioning. Differences between the patient self-report and interviewer judgments were then examined statistically. In order to examine whether the interviewer judgments appeared more valid than self-reports, we correlated the performance-based measures with both interviewer and patients self-reports of functioning.
Results: Patients significantly ($p < .001$) overestimated their vocational and everyday functioning compared to the interviewer judgments; social functioning was also over-estimated non-significantly ($p = .17$). Lower everyday functioning on the part of patients was consistently associated with overestimation of their functioning. Patient self-reports were not correlated with any performance-based measures, while interviewer judgments were significantly correlated with patients' performance on both cognition and functional capacity ($p < .005$). In regression analyses controlling for baseline levels of functioning, several predictors of the discrepancy between self and interviewer judgments emerged. Depression was associated across domains with reduced discrepancies compared to interviewer judgments ($p < .001$). Several symptoms as rated on the PANSS were associated with over-estimation of functioning. Hallucinations, suspiciousness, grandiosity, and poor rapport were all significantly ($p < .001$) associated with over-estimation of functioning compared to interviewer judgments. Poorer cognitive and functional capacity performance were also correlated with over-estimation of everyday functioning,

but these results were not statistically significant in the regression models.

Discussion: Consistent with previous studies in schizophrenia, other neuropsychiatric conditions, and non-clinical populations, mild depression (Mean BDI = 15.8) was associated with increased accuracy in self assessment. Several different symptoms of schizophrenia were associated with over-estimation of performance. Similarly, lower scores on performance-based measures also predicted over-estimation of functioning. Thus, we identified bi-directional predictors of mis-estimation of everyday functioning, even when lower baseline scores were considered. These data suggest that it may be possible to screen patients for their ability to self-report their functioning, but that even relatively mild levels of symptoms were associated with consistent mis-estimation of everyday functioning that would likely bias the results of treatment studies.

Disclosure: S. Sabbag: None. **T. Patterson:** None. **R. Heaton:** None. **P. Harvey:** Part 1: Abbott Labs, Bristol-Myers-Squibb, Cypress Bio Science En Vivo, Genentech, Johnson and Johnson, Merck and Company, Novartis, Shire, Sunovion Pharma., Part 4: Astra Zeneca Pharmaceuticals.

177. A Dimensional Approach to the Continuum between Bipolar Disorder with Psychosis and Schizophrenia: The Schizo-Bipolar Scale

Matcheri Keshavan*, David Morris, John Sweeney, Godfrey Pearlson, Gunvant Thaker, Carol Tamminga, Larry Seidman, Shaun Eack

Harvard Medical School, Boston, USA

Background: There is increasing evidence for phenomenological, biological and genetic overlap between schizophrenia and bipolar disorder, bringing into question the traditional dichotomy between them. Neurobiological organization using dimensional approaches may provide a better foundation to represent phenotypic variation in neuropsychiatric disorders.

Methods: To capture the interaction between psychosis and affective symptoms dimensionally, we devised a brief descriptive scale based on the type and relative proportions of psychotic and affective symptoms over the illness course. The scale was administered to a series of 762 patients with psychotic disorders, including schizophrenia, schizoaffective and psychotic bipolar disorder assessed as part of the Bipolar- Schizophrenia Network for Intermediate Phenotypes (B-SNIP) study.

Results: The resulting Schizo-Bipolar Scale scores across these disorders showed neither a clear dichotomy nor a simple continuous distribution. While the majority of cases had ratings close to prototypic schizophrenia or bipolar disorder, a large group (45% of cases) fell on the continuum between these two prototypes.

Discussion: Our data suggest a hybrid conceptualization model with a representation of cases with prototypic schizophrenia or bipolar disorder at the extremes, but a large group of patients on the continuum between them that traditionally would be considered schizoaffective. A dimensional approach, using the Schizo-Bipolar Scale, characterized patients across a continuum of psychopathology and may provide a valuable means to examine the relationships between schizophrenia and psychotic bipolar disorder.

Disclosure: M. Keshavan: Part 4: GSK, Sunovion. **D. Morris:** None. **J. Sweeney:** None. **G. Pearlson:** None. **G. Thaker:** None. **C. Tamminga:** Part 1: Intracellular Therapies (ITI, Inc.) - Advisory Board, drug development > \$10,000, Part 2: Intracellular Therapies (ITI, Inc.) - Advisory Board, drug development > \$10,000, Part 3: Intracellular Therapies (ITI, Inc.) - Advisory

Board, drug development > \$10,000 **L. Seidman:** None. **S. Eack:** None.

178. Ketamine for Treatment-Refractory Obsessive-Compulsive Disorder

Michael H. Bloch*, James F. Leckman, John H. Krystal, Zubin H. Bhagwagar, Gerard Sanacora, Christopher Pittenger

Yale University, New Haven, USA

Background: Roughly one-third of patients with obsessive-compulsive disorder (OCD) experience little clinical benefit from first-line interventions such as pharmacotherapy with selective serotonin reuptake inhibitors (SSRIs) or cognitive behavioral therapy (CBT). Furthermore, the full treatment benefits of first-line interventions are only realized after two to three months. Limited symptom relief and delay of symptom relief from first-line treatments are sources of substantial morbidity and decreased quality of life in OCD patients. Convergent evidence from neuroimaging, genetic and pharmacological studies suggests that glutamate abnormalities may contribute to the pathogenesis/or pathophysiology of OCD. Ketamine is a potent noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, a major type of glutamate receptor in the brain. Several trials have reported that a single dose of ketamine (0.5 mg/kg, delivered intravenously over 40 minutes) had rapid antidepressant effects in depressed patients.

Methods: We conducted a pilot, open-label trial of ketamine (0.5 mg/kg IV over 40 minutes) in patients with treatment-refractory OCD. Patients had severe OCD symptoms (Y-BOCS > 24) despite at least two SSRI trials of adequate dose and duration. Clinical ratings of OCD (Y-BOCS) and depression (HAM-D) were performed at baseline, 1, 2 and 3 hours and 1, 2, 3, 5 and 7 days after ketamine infusion. Response for OCD was defined as a greater than 35% improvement in symptoms from baseline at any time between 1-7 days following infusion. Response in depressive symptoms was defined as a greater than 50% reduction in HAM-D ratings from baseline anytime between 1-7 days following infusion.

Results: We enrolled 8 patients with treatment-refractory OCD (6 with comorbid depression). None experienced a clinical response in OCD symptoms during the week following ketamine infusion. On average, the reduction of OCD symptoms peaked 1 day following infusion (improving by 10 ± 12%; not significant). By contrast, 3 of 6 patients with OCD and comorbid depression experienced a clinical response in their depressive symptoms. Improvement in depressive symptoms peaked 2 days following ketamine infusion with a 45 ± 40% average reduction in symptoms (p = 0.04).

Discussion: We observed a clear dissociation between OCD and depressive symptoms in response to ketamine infusion. This pilot trial demonstrates no effect of ketamine, an NMDA antagonist, in the treatment of OCD. By contrast, we demonstrate a potent, though short-lived, antidepressant effect of ketamine infusion, consistent with previous studies.

Disclosure: M. Bloch: None. **J. Leckman:** Part 1: I have invested in C8 Science., Part 2: Yale University; NIH K award, Part 3: I have invested in C8 Science., Part 4: Talecris Biotherapeutics., Part 5: Yale University. **J. Krystal:** Part 1: Aisling Capital, LLC AstraZeneca Pharmaceuticals Brintnall & Nicolini, Inc. Easton Associates Forest Laboratories (June 2008) Gilead Sciences, Inc. GlaxoSmithKline Janssen Pharmaceuticals Lundbeck Research USA Medivation, Inc. Merz Pharmaceuticals MK Medical Communications F. Hoffmann-La Roche Ltd SK Holdings Co., Ltd Sunovion Pharmaceuticals, Inc. Takeda Industries Teva Pharmaceutical Industries, Ltd. Scientific Advisory Board Abbott

Laboratories Bristol-Myers Squibb Eisai, Inc. Eli Lilly and Co. Forest Laboratories, Inc. Lohocla Research Corporation Mnemosyne Pharmaceuticals, Inc. Naurex, Inc. Pfizer Pharmaceuticals Shire Pharmaceuticals Exercisable Warrant Options Tetragenex Pharmaceuticals (value less than $150) Board of Directors: Coalition for Translational Research in Alcohol and Substance Use Disorders President Elect : American College of Neuropsychopharmacology, Part 2: Editor - Biological Psychiatry, Part 4: Research Support to Department of Veterans Affairs Janssen Research Foundation (Provided drug and some study support to the Department of Veterans Affairs). **Z. Bhagwagar:** None. **G. Sanacora:** Part 1: Dr. Sanacora has received consulting fees form Abbott, AstraZeneca, Bristol-Myers Squibb, Evotec, Eli Lilly & Co., Hoffman La-Roche and Johnson & Johnson, Novartis, and Novum Pharmaceuticals. He has also received additional grant support from AstraZeneca, Bristol-Myers Squibb, Hoffman La-Roche, Merck & Co., and Sepracor Inc. In addition Dr. Sanacora is a co-inventor on filed patent application by Yale University concerning the use of glutamate modulating drugs in the treatment of psychiatric disorders (PCTWO06108055A1)., Part 4: He has received grant support from AstraZeneca, Bristol-Myers Squibb, Hoffman La-Roche, Merck & Co., and Sepracor Inc. **C. Pittenger:** None.

179. Early Life Stress produces Immediate and Sustained Effects on Corticolimbic Endocannabinoid Signaling

Matthew N. Hill*, Lisa Eiland, Tiffany T.Y. Lee, Cecilia J. Hillard, Bruce S. McEwen

University of Calgary, Calgary, Canada

Background: Endocannabinoid signaling is known to be actively recruited by acute stress and impaired following exposure to chronic stress. However, to date there is little information regarding the effects of stress early in life on endocannabinoid signaling and whether this system responds in a comparable fashion to aversive stimuli. Further, unlike the effects of chronic stress in adulthood, which are largely reversible following a washout period, early life stress is known to sculpt the development of many stress responsive systems into adulthood. The aim of the current study was to thoroughly examine the effects of early life stress on corticolimbic endocannabinoid signaling as well as the long-term effect this exposure has on the development of the endocannabinoid system into adulthood.

Methods: Male rats were exposed to daily maternal separation stress (180 min/day) from postnatal day 2 (P2) through postnatal day 12 (P12). Analysis was performed in normally reared and separated rats immediately following the initial separation stressor (P2), immediately following the final separation stressor (P12) as well as under steady state conditions as juveniles (P14), adolescents (P40) and adults (P70).

Results: Analaysis revealed that in response to the initial separation stress, tissue content of the endocannabinoid anandamide (AEA) was reduced in both the prefrontal cortex (PFC) and amygdala. Following 10 days of repeated separation stress, the final stressor resulted in a dramatic suppression of AEA content, in tandem with an elevation in the tissue content of the other endocannabinoid 2-arachidonoylglycerol (2-AG), within both the amygdala and hippocampus; however there was no effect on either ligand within the PFC following repeated stress. At P14, the maternal separation stress resulted in a residual alteration of endocannabinoid content within the amygdala, which was characterized by reduced levels of AEA and elevated levels of 2-AG; however, at this time point the changes which were seen in the hippocampus had normalized. At P40, rats which had been exposed to maternal separation exhibited reductions in CB1 receptor binding site density within both the amygdala and PFC,

while AEA and 2-AG levels were both reduced within the hippocampus. Finally, at adulthood on P70, rats which had been exposed to early life stress exhibited a constitutive downregulation of CB1 receptor binding within both the amygdala and hippocampus, which was accompanied by a robust reduction in the tissue content of both AEA and 2-AG within the hippocampus.

Discussion: Taken together, these data indicate that the endocannabinoid system in corticolimbic circuits is stress responsive even at very early developmental points. Further, these data demonstrate that early life stress produces sustained effects on the endocannabinoid system, particularly within the hippocampus, which largely mirror the effects of chronic stress in adulthood. The extent to which these changes contributes to shifts in emotional behaviour and stress reactivity following early life stress has yet to be determined.

Disclosure: M. Hill: None. **L. Eiland:** None. **T. Lee:** None. **C. Hillard:** None. **B. McEwen:** None.

180. Double Blind Randomized Brief Treatment with Escitalopram associated with Extinction Learning Facilitation in Healthy Subjects

Eric Bui, Scott Orr, Ryan J. Jacoby, Nicole LeBlanc, Mark Pollack, Aparna Keshaviah, Naomi Simon*

Massachusetts General Hospital, Boston, USA

Background: To date, no randomized controlled data supports the efficacy of any pharmacological agent for primary prevention of Acute Stress Disorder and Posttraumatic Stress Disorder (PTSD). Fear conditioning, measured by the differential response to a conditioned stimulus paired with an aversive (CS +) and neutral (CS-) stimulus, provides a useful model in which to study the effect of pharmacological agents on fear acquisition and extinction relevant to PTSD development. The present study aims to examine the impact of 14 days of escitalopram versus placebo treatment on the acquisition and extinction of fear in a classical fear conditioning paradigm as an experimental test of a potential role for SSRI antidepressants to protect against PTSD development in response to trauma exposure.

Methods: Healthy volunteers (aged 20 – 61) were randomized and completed 14 days of treatment with escitalopram 10 mg/day (n = 18) versus pill placebo (n = 20) prior to undergoing a classical fear conditioning paradigm. Participants completed measures of anxiety (Beck Anxiety Inventory, State-Trait Anxiety Inventory, Anxiety Sensitivity Index), depression (Beck Depression Anxiety) and personality functioning (NEO-five) first at baseline and again just prior to undergoing the experimental paradigm. Physiological assessment was completed in a humidity- and temperature-controlled, sound-attenuated room. The CS + and CS- were represented by a yellow circle and a white square, respectively and were displayed on a monitor in front of the participant. The unconditioned stimulus (UCS) was a 500-ms electric shock determined to be "highly annoying but not painful". We hypothesized that 14 days of pretreatment with escitalopram would be associated with a reduced level of fear acquisition and with enhanced extinction learning as measured with skin conductance response (SCR), compared to placebo. In order to improve normality, SCR values were square-root transformed. A series of analysis of variance for repeated measures (ANOVAR) was then conducted to compare SCR values within-subject across trials, and test the between-subject effects of treatment (escitalopram or placebo) and CS stimulus (positive or negative), independently for each phase (habituation, acquisition and extinction).

Results: At baseline, the two groups did not differ significantly on any of the socio-demographic or clinical variables except age,

which was higher in the escitalopram group (p < 0.05). Although overall baseline symptoms were low, repeated measures analysis of variance by group failed to reveal any significant changes in measures of anxiety, depression, or personality attributable to 14-day administration with escitalopram. In the habituation phase, there were no significant differences in SCR by CS type, but mean SCR significantly decreased by trial (F(4, 69) = 7.49, p < 0.0001). Further, escitalopram was also associated with a lower orienting response (p < 0.05). In the fear acquisition phase, although mean SCR significantly differed by CS type (F(1) = 31.69, p < 0.0001) and across trials (F(4, 69) = 4.59, p < 0.01), there were no significant differences by treatment. Finally, in the extinction phase, escitalopram was associated with a decreased difference in SCR between CS+ and CS- over time across trials compared to placebo, as indicated by a significant Drug´CS´Trial interaction in both the early extinction ((F(3, 62) = 3.09, p < 0.05) and late extinction subphases (F(3, 62) = 3.41, p < 0.05), which persisted even after adjustment for age and orienting response (F(3, 60) = 2.99; p < 0.05 and F(3, 60) = 4.07; p < 0.05for the early and late extinction subphases, respectively).

Discussion: Escitalopram administered in double blind fashion for 14-days prior to a fear conditioning paradigm produced no effect on fear acquisition, but facilitated extinction learning. Impairments in extinction learning have been identified as a key component of PTSD pathophysiology. Although this study did not examine differences in extinction retention, these preliminary findings suggest that additional experimental and clinical studies assessing the potential efficacy of SSRIs for PTSD prevention are warranted.

181. Assessing the Long-Term Safety and Tolerability of Desvenlafaxine in Child and Adolescent Outpatients with Major Depressive Disorder

Robert L. Findling*, James Groark, Deborah Chiles, Sara Gardiner, Lingfeng Yang, Karen A. Tourian

Case Western Reserve University, Cleveland, USA

Background: Desvenlafaxine (administered as desvenlafaxine succinate) is the primary active metabolite of the serotonin-norepinephrine reuptake inhibitor venlafaxine and is approved for the treatment of major depressive disorder (MDD) in adults (Boyer et al, Int Clin Psychopharmacol, 2008; Liebowitz et al, Curr Med Res Opin, 2008). It has been demonstrated that PK and dose-ranging studies assessing safety should be performed prior to initiation of definitive efficacy studies of antidepressants in children and adolescents with MDD in order to avoid study failure due to improper dosing regimens (Findling et al, J Child Adolesc Psychopharmacol, 2006). As a result, an exploratory pediatric program for evaluating desvenlafaxine in the treatment of MDD in children (aged 7-11 years) and adolescents (aged 12-17 years) was undertaken. An inpatient single-ascending dose pharmacokinetic (PK) study, followed by 7.5 weeks of outpatient open-label multiple-ascending dose treatment was conducted. As an extension to that PK study, this study set out to explore the long-term safety and tolerability of desvenlafaxine in these populations. The combined PK and safety data from these studies are serving as a basis to inform the desvenlafaxine dosing regimens in phase 3 studies.

Methods: A 6-month, multicenter, open-label, flexible-dose extension study was initiated at day 56 of a preceding 8-week, open-label, ascending dose study. Patients were assigned to receive a flexible desvenlafaxine dose of 10, 25, 50, or 100 mg (child) and 25, 50, 100, or 200 mg (adolescent) with a taper phase of 0 to 2 weeks. Safety and tolerability measures included monitoring of treatment-emergent adverse events (TEAEs), withdrawals due to adverse events (AEs), physical examination including a Tanner Assessment, Columbia Suicide-Severity Rating Scale (C-SSRS), ECG, laboratory tests, and vital signs. Efficacy assessments were considered exploratory and examined by the Children's Depression Rating Scale (CDRS-R) and other measures.

Results: Forty male and female patients were enrolled (20 children [45% male]; 20 adolescents [50% male]). Of the 20 children who took at least 1 dose of desvenlafaxine, dosing exposures (beginning at the baseline visit, patients were given the same daily dose to which they were assigned in the preceding study, or adjusted as clinically indicated) were 0 to 10 mg (n = 3); > 10 to 25 mg (n = 5); > 25 to 50 mg (n = 9); and > 50 to 100 mg (n = 3). For adolescents who took at least 1 dose of desvenlafaxine, dosing exposures were > 10 to 25 mg (n = 1); > 25 to 50 mg (n = 9); > 50 to 100 mg (n = 6); and > 100 to 200 mg (n = 4). Seven patients withdrew from the study due to AEs, including 4 (20%) children and 3 (15%) adolescents. The most common TEAEs in children were abdominal pain upper (15%), and headache (15%)(children), while in adolescents they were somnolence (30%), nausea (20%), abdominal pain upper (15%), and headache (15%). Taper/post-study TEAEs were reported by 1 child (headache) and 1 adolescent (depressive symptoms). There were no deaths during this study. One child experienced a serious AE (negativism, oppositional behavior) that led to withdrawal. Suicidal ideation occurred in 1 adolescent post-baseline. Although sporadic individual laboratory values and vital sign abnormalities were identified, no clinically significant trends were found. None of the patients showed clinically important changes in ECG results. Exploratory efficacy data generally showed improvement in CDRS-R and other efficacy measures from pre-treatment baseline to the end of this long-term study.

Discussion: Desvenlafaxine may be generally safe and well tolerated in children and adolescents following long-term treatment. Data obtained from this study will inform dosing regimens in phase 3 trials.

182. Effects of Serotonin Augmentation on Test Meal Food Intake in Anorexia Nervosa

Barbara E. Wolfe*, Evgeniy E. Filin, Amy Silver-Ritter, David C. Jimerson

Boston College, Chestnut Hill, USA

Background: Background: Central nervous system (CNS) serotonin (5-HT) pathways play a substantive role in post-ingestive satiety. Several lines of evidence suggest that dysregulation of CNS 5-HT pathways may contribute to the pathophysiology of anorexia nervosa (AN). Yet, in controlled trials, pharmacologic treatments have generally failed to demonstrate significant effects in accelerating weight gain. Preclinical studies have shown that combined treatment with fluoxetine plus the synthesis precursor 5-hydroxytryptophan (5-HTP) augments central 5-HT release. To explore the possible behavioral effects of 5-HT augmentation on eating behavior, this study examined the effects of short-term medication administration on test meal response in women with AN (n = 11), in normal weight women with AN in remission (R-AN, n = 28), and in healthy female controls (n = 33).

Methods: Methods: Preliminary analyses include participants randomized in a double-blind design to short-term (1-week) outpatient pretreatment with a selective serotonin reuptake inhibitor (SSRI) or placebo. Following pretreatment, subjects were admitted overnight to a General Clinical Research Center for paired pharmacologic challenge study days with single-dose 5-HTP or placebo. Food intake was measured during a single-item test meal 3 hours following the challenge agent. Subjects were instructed to eat until they felt full. Data for test meal food intake was analyzed by analysis of variance (ANOVA).

Results: Results: Test meal food intake on the placebo study day was compared for subjects randomized to placebo pretreatment, showing a significant effect for group ($p < .01$). In comparison to controls, food intake was lowest for the AN group ($p < .05$), and intermediate for the R-AN group. In these same subjects, there was little change in mean food intake on the active 5-HTP study day ($p = ns$). A second analysis assessed food intake for subjects randomized to SSRI-pretreatment. In comparison to results for placebo-pretreated subjects, using data from the active 5-HTP study day, ANOVA again showed a significant effect for diagnostic group ($p < .05$). SSRI-pretreatment was associated with significantly reduced food intake ($p < .02$).

Discussion: Discussion: These results for SSRI-pretreatment on food intake extend previous findings showing enhanced neuro-endocrine responsiveness to serotonergic challenge following short-term SSRI administration. Longitudinal studies will be helpful in assessing changes in 5-HT-mediated satiety during recovery from AN.

Disclosure: B. Wolfe: None. **E. Filin:** None. **A. Silver-Ritter:** None. **D. Jimerson:** None.

183. Treatment of Pathological Gambling with Tolcapone, a Catechol-O-methyl-transferase (COMT) Inhibitor

Jon E. Grant*, Brian L. Odlaug, Suck Won Kim

University of Minnesota, Minneapolis, USA

Background: Pathological gambling (PG) is a disabling disorder experienced by approximately 1% of adults and for which there exist few empirically validated treatments. Tolcapone, a catechol-O-methyl-transferase (OMT) inhibitor, is currently approved by the FDA for the treatment of Parkinson's disease. The methylation enzyme OMT regulates dopamine levels in the prefrontal cortex. Reduced OMT activity results in improved frontal cortical cognitive performance. Impairments in prefrontally-mediated cognitive functions appear to underlie behavioral dysregulation, namely decision making and inhibitory control. These impairments may increase the risk for making decisions that are impulsive, focused on short-terms gains and lack inhibitory control and thereby place the individual at high risk for continued gambling. This study's aims were to: 1) test the efficacy of a novel pharmacotherapy for PG; 2) examine specific mechanisms in treatment response (i.e., inhibitory control and cognitive flexibility) that may underlie multiple addictive behaviors and thereby provide cognitive measures for objective neurobiological treatment effects in addictive disorders; and 3) collect genetic data regarding the possible COMT polymorphism associated with tolcapone response.

Methods: 14 subjects with DSM-IV PG were enrolled in an 8-week, open-label trial of tolcapone. Tolcapone dosing started at 100 mg/day and was titrated to 100 mg tid based on clinical symptoms. Subjects were assessed with the Yale Brown Obsessive Compulsive Scale Modified for Pathological Gambling (PG-YBOCS), Clinical Global Impressions (GI) scale, the Gambling Symptom Assessment Scale (G-SAS), and measures of depression, anxiety, and psychosocial functioning. Subjects underwent selective neurocognitive testing (Stop-signal task [SST] and Intra-dimensional/Extra-dimensional Set-Shift task [IDED]) with fMRI at baseline and study endpoint. Blood samples were collected to examine OMT polymorphisms associated with treatment response.

Results: Individuals experienced significantly greater reductions on the total score of the primary outcome measure (PG-YBOCS) from study entry (mean 22.5 ± 5.1) to endpoint (mean 9.2 ± 5.8; $p = <.001$). Subjects also demonstrated significantly greater reductions on the gambling urge and behavior subscales of the PG-YBOCS (each $p < .001$), the total score on the G-SAS ($p = <.001$), and on depression ($p = .015$), anxiety ($p = .035$), and quality of life ($p = .026$) rating scales. Mean effective dose of tolcapone was 266.7 ± 65.1 mg/day. Cognitive tasks demonstrated significant improvement ($p < .05$) on the IDED task (cognitive flexibility) and pre- and post-fMRI changes reflected prefrontal cortical improvement. Liver function tests showed no elevations during the study.

Discussion: Tolcapone significantly reduced gambling symptoms, particularly gambling urges, and this improvement was associated with improvement on the IDED task and changes on fMRI. Cognitive performance and fMRI data suggest that tolcapone appears to target prefrontal cortical functioning resulting in improved cognitive flexibility and associated reduction in gambling behavior. These findings provide support for the use of COMT inhibitors in the treatment of PG and possibly other addictive behaviors.

Disclosure: J. Grant: Part 1: Investigator-initiated research grant from Psyadon Pharmaceuticals., Part 4: Investigator-initiated research grant from Psyadon Pharmaceuticals. **B. Odlaug:** None. **S. Kim:** None.

184. The Relationship between Serum Lithium Concentrations and Clinical Response in Bipolar Depression

Caleb M. Adler*, Tyler L. Thompson, Melissa P. DelBello, Thomas J. Blom, David E Fleck, Stephen M

University of Cincinnati College of Medicine, Department of Psychiatry, Cincinnati, USA

Background: Despite recent increases in the pharmacopeia of mania, treatment options for bipolar depression remain quite limited; only two medications currently have FDA approval for this indication. Although not FDA approved, lithium remains a common treatment intervention for bipolar depression. While lithium efficacy in bipolar mania has been associated with serum concentrations of 0.8 to 1.2 mEq/L, there are little data as to the

most effective serum concentration for bipolar depression. A partial consensus has arisen around establishinga minimum serum lithium concentration of 0.8 mEq/L, but no published studies have directly studied optimal levels for these patients. For this study, we acquired behavioral data with serum lithium concentrations in a cohort of depressed bipolar patients. Based on the limited previous findings, we predicted that a minimum concentration of 0.8 mEq/L would show the largest association with symptom remission.

Methods: 60 patients (42 women) 15-54 years of age (mean $\pm$ SD $= 25 \pm 11$ years) diagnosed with bipolar disorder type I depression participated in mood ratings weekly for up to 6 weeks as part of IRB-approved protocols. Diagnoses were determined in adults and minors using the SCID or K-SADS, respectively. Remission was separately defined as Hamilton or Montgomery-Asberg Depression Rating Scale scores $\leqslant 12$, and Clinical Global Impression for Bipolar Disorder depression subscale(CGI-BPD) $\leqslant 2$. Response was defined as a 50% reduction in depression rating score from study entry. Mood ratings and serum lithium concentrations were obtained at baseline, and weeks 1, 2, 4, and 6. The relationships between measures of remission and response, and corresponding lithium concentrations were examined using a mixed linear model. Within-subject covariance in the longitudinal data was modeled using an unstructured covariance matrix. In addition to lithium level, other predictors in the model were visit, lithium level by visit interaction, gender and age- as well as baseline score in the CGI-BPD analysis. Lithium level was considered as a binary variable and modeled for intervals of 0.1 mEq/L from 0.4 to 1.1. As secondary analyses, we compared remission and response rates at 6 weeks, as well as change in CGI-BPD for all subjects over six weeks. For all analyses, significance was defined as $p \leqslant 0.5$.

Results: Overall, lithium treatment was associated with significant decrements in CGI-BPD ($p = 0.001$). Using percent change in depression rating scores, we observed a significant main effect of serum lithium concentration on remission over 0.4 to 0.9 mEq/L. P values formed a u-shaped curve with the most significant effect at 0.6 mEq/L ($p = 0.0002$). Using the CGI-BPD, we observed a significant main effect of lithium levels on remission only for concentrations of 0.5 and 0.6 mEq/L; the effect was greatest at 0.6 mEq/L ($p = 0.04$). We observed a significant main effect of serum lithium concentration on response from 0.4 to 0.7 mEq/L; effect was largest at 0.5 ($p = 0.002$). At week 6, differences in depression rating-based remission were greatest at 0.8 and 0.9 mEq/L ($p = 0.008$). Differences in CGI-BPD-based remission were not significant at any lithium concentration but were also greatest at 0.8 and 0.9 mEq/L. Differences in response rates were significant only at 0.7 mEq/L ($p = 0.01$).

Discussion: These data suggest that bipolar depression may respond best to a range of lithium concentrations approximately centered on 0.6 mEq/L- lower than the 0.8 mEq/L typically recommended. Although these findings are exploratory, the data supporting the necessity of a higher concentration is quite sparse; if patients can be effectively treated with lower doses of lithium it may increase lithium tolerability and patient compliance. Lithium remains one of the most widely available therapeutic interventions for patients with bipolar depression. Given its relative affordability as well as the limited number of medications with well-supported efficacy, improving lithium compliance has clear therapeutic implications for the treatment of bipolar depression.

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185. Lack of Tyramine Pressor Response Effect with Oral CX157, a Reversible MAOI

Daniel J. Burch*[1], Mahnaz Asgharnejad[1], William A. Gerson[2]

CeNeRx BiioPharma, Cary, USA

Background: TraditionalMAOIs are considered very effective antidepressants but have been limited due to their potential for a hypertensive reaction associated with diets high in tyramine. This effect is known as the "cheese effect"[1,2]. Newer MAOIs that are selective and reversible significantly diminish the risk for potential tyramine interactions. Objective: CX157 is a Reversible Inhibitor of Monoamine Oxidase A (RIMA) in development for depression. The objective of this study was to assess cardiovascular safety of CX157 following administration of oral tyramine.

Methods: This was a Phase 1, single-center, DB, PC, three-period study in 15 male healthy volunteers. In Period 1 the subjects' sensitivity to oral tyramine was established by determining the dose of tyramine elevating SBP $\geqslant 30$ mmHg on $\geqslant 3$ consecutive occasions (i.e., $TYR30_3$). Twelve subjects qualified for randomization in period 2 during which oral CX157 Modified Release Tablet 125 mg BID (N $= 10$) or placebo BID (N $= 2$) were administered to steady-state. In period 3, CX157 and placebo were administered with oral tyramine in fed state with daily increases in the tyramine dose of 20, 40, & 80 mg in an attempt to achieve the "$TYR30_3$".

Results: PK sampling demonstrated that robust levels of CX157 required for an antidepressant effect were achieved during the challenge period. None of the subjects reached the $TYR30_3$. CX157 was well tolerated.

Discussion: The pharmacological properties of CX157 (selectivity & reversibility) in conjunction with its novel, modified release formulation contributed to a clean tyramine safety profile. Subjects who were co-administered CX157 (125 mg BID) and tyramine up to and including 80 mg (twice the FDA threshold for a high tyramine diet), did not manifest a tyramine interaction or classic "cheese reaction" common with older MAOIs. These data provide support that CX157 can be given safely without the need for the tyramine dietary restriction of older MAOIs.

References:

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[2]MurphyDL, et al. Selective inhibition of monoamine oxidase type A: Clinical antidepressant effects and metabolic changes in man in Monoamine Oxidase Inhibitors. John Wiley and Son, Ltd, New York, NY, 1981, pp. 189-295.

Disclosure: D. Burch: Part 1: Employee, Shareholder CeneRx BioPharma, Inc Shareholder GSK, Part 2: CeNeRx BioPharma Inc, Part 3: CeNeRx BioPharma, Inc., Part 4: None, Part 5: CeNeRx BioPharma Inc. **M. Asgharnejad:** Part 1: Employee and Shareholder CeNeRx BioPharma Inc, Shareholder GSK, Part 2: CeNeRx BioPharma Inc,, Part 3: CeNeRx BioPharma Inc, Part 4: N/A, Part 5: CeNeRx BioPharma, Inc. **W. Gerson:** Part 1: Employee Comprehensive Clinical Development, Part 2: EmployeeComprehensive Clinical Development, Part 3: Comprehensive Clinical Development, Part 4: N/A, Part 5: Comprehensive Clinical Development.

186. How Useful is Intravenous Ketamine for Depression in the 'Real World'?

David Feifel*, Kai MacDonald, Michael Messer, Luis Giuffra

UCSD Medical Center, San Diego, USA

Background: Several recent clinical studies have found that a single intravenous infusion of ketamine hydrochloride, an N-methyl-D-aspartate (NMDA) antagonist, produces significant and rapid antidepressant effects in a large proportion of treatment refractory patients who receive this treatment. The published reports of these trials have generated considerable interest among clinicians prompting some to begin providing it as an off-label treatment to patients with treatment refractory depression. It is not known to what extent the highly promising effects seen in the clinical studies are translatable to 'real world' clinical settings. Two of the authors (DF & KM) recently organized a consortium for clinicians who have provided ketamine treatments to their patients in order to exchange information and share experiences about this treatment. As part of their participation in this consortium members are asked to complete a questionnaire about their use of ketamine treatments and the outcomes they have observed.

Methods: To serve as a collaborative resource for the professional exchange of information on the clinical use of ketamine in psychiatry, we have begun to assemble a nationwide network of providers who have used ketamine in a clinical (rather than research) context. These providers were identified through an internet psychopharmacology provider chat group with a large number of members and through word-of-mouth. Clinicians with experience treating depression with ketamine were invited to join an internet "Google-group" for the purpose of professional exchange of knowledge and asked to provide information about their ketamine treatment experiences via an online survey.

Results: At the time of writing we have identified 6 practitioners or practice groups across the United States who have provided ketamine treatments to treatment refractory patients and, thus far, information has been provided by 5 of these individuals or groups One practitioner reported employing only intranasal but not IV ketamine and his information is not included in the current descriptive summary. The remaining practitioners reported treating 22 unipolar and 6 bipolar patients with treatment refractory depression with at least a single infusion of ketamine. The most common dose used was 0.5 mg/kg and the treatment was offered in diverse clinical settings ranging from private office to intensively monitored hospital setting. Regarding tolerability, though practitioners noted transient perceptual disturbances in several patients, all adverse effects were rated between moderate and mild, and no severe side effects were reported. Fifty percent (11 patients) of the unipolar, but none of the bipolar patients, were deemed to have at least a 50% reduction in their depression symptoms after one ketamine infusion. Interestingly, there was a large disparity in the efficacy experienced across sites, with a couple of sites experiencing no benefit among their series of patients, while another site had mixed results and another reported mostly good results. Two of reporting sites indicated they had stopped providing ketamine treatments due to disappointment in efficacy or sustainability of the effects. Only one practitioner considered IV ketamine "a very useful tool" for treating depression and was unequivocally enthusiastic about continuing to offer the treatment.

Discussion: This small sample of clinicians who are early adopters of IV ketamine for depression report consistently good tolerability associated with the procedure but a surprising diversity in efficacy. This contrasts the consistent strong efficacy in published research reports. As this consortium was formed shortly before writing this abstract, we anticipate more clinicians will be joining over the coming months, providing a more robust database. It will be important to see if this anticipated additional data provides more clarity regarding the potential utility of IV ketamine in naturalistic clinical settings and helps identify practice or patient parameters which may account for the diversity of efficacy seen across sites.

187. Course of Improvement in Depressive Symptoms to a Single Intravenous Infusion of Ketamine vs. Add on with Riluzole: Results from a Four-Week, Double-Blind, Placebo-Controlled Study

Jose A. Franco-Chaves*, Lobna Ibrahim, Nancy DiazGranados, Nancy Brutsche, Philip Kronstein, David A. Luckenbaugh, Husseini K. Manji, Carlos A. Zarate Jr.

National Institute of Mental Health, Bethesda, USA

Background: The N-methyl-D-aspartate (NMDA) antagonist ketamine has rapid antidepressant effects in patients with treatment-resistant major depression (TRD); these effects have been reported to last one week in some patients. However, the extent and duration of this antidepressant effect over longer periods has not been well-characterized under controlled conditions. Riluzole, a glutamatergic modulator with synaptic-enhancing and antidepressant effects, could conceivably be used to promote ketamine's antidepressant effects. This study sought to determine the extent and time course of antidepressant improvement to a single ketamine infusion over four weeks, comparing the addition of riluzole versus placebo after the infusion.

Methods: Forty-two subjects (18 to 65) with TRD and a Montgomery-Asberg Depression Rating Scale (MADRS) score of 22 received a single intravenous infusion of ketamine (0.5 mg/kg). Four to six hours post-infusion, subjects were randomized to double-blind treatment with either riluzole (100-200 mg/day; n = 21) or placebo (n = 21) for four weeks. Daily ratings for depression were obtained.

Results: A significant improvement from baseline in MADRS (p < .001) scores was found in the study. The effect size of improvement with ketamine was initially large and remained moderate throughout the 28 day trial. For patients in both treatment groups who reached response criteria prior to randomization (riluzole or placebo), 29% (7/24) did not have a relapse throughout the four-week study. However, the MADRS scores between the riluzole and placebo treatment groups did not differ significantly.

Discussion: In this group of TRD patients, a single ketamine infusion had significant antidepressant effects that lasted four weeks. The combination of riluzole with ketamine treatment did not significantly alter the course of anti-depressant response to ketamine alone.

submitted listing Dr. Zarate among the inventors; he has assigned his rights on the patent to the U.S. government but will share a percentage of any royalties that may be received by the government, Part 2: None, Part 3: None, Part 4: Intramural Research Program, NIMH.

188. Changes in Suicide Risk and Depressive Symptoms with Low Dose Lithium Combined with Citalopram Compared to Citalopram with Placebo in a Group of Severely Ill Depressed Patients

Arif Khan*, Shirin R.F. Khan, Joy Hobus, James Faucett, Vishaal Mehra, Earl L. Giller, Richard L. Rudolph, Walter A. Brown

Columbia Northwest Pharmaceuticals, Bellevue, USA

Background: Although there are several retrospective analyses suggesting lithium may reduce suicide risk, no prospective data for lithium's effect in acutely suicidal patients exist. To evaluate this possibility we conducted a trial combining lithium with an antidepressant (citalopram). Secondarily, we also evaluated if the antidepressant effects of citalopram, with or without lithium, precede any anti-suicidal effects.

Methods: A proof-of-concept trial of four weeks duration using a randomized, double-blind, parallel-group method. Forty severely depressed and suicidal outpatients received citalopram (20 mgs/day) + lithium (300 mgs/day), and 40 severely depressed and suicidal outpatients received citalopram (20 mgs/day) + placebo. Primary outcome measures were the Sheehan-Suicidality Tracking Scale (S-STS) and the Montgomery-Asberg Depression Rating Scale (MADRS).

Results: No deaths occurred among the 93 patients screened for the study, either by suicide or any other means. No suicide attempts occurred during study period including during safety follow-up period. The reduction in suicide risk measures was twice as rapid and twice as large compared to the reduction in depressive symptoms among the entire sample of 80 severely depressed patients. Though no significant differences in reduction of S-STS scale scores between the two groups of depressed outpatients were observed, *post hoc* analysis showed the subgroup of depressed patients ($n = 11$) with lithium level 0.5mEq/L or higher had several indicators for better outcome on suicide risk measures. Five of these 11 patients (45%) achieved complete remission of suicidal thoughts and behaviors compared to 16/69 (23.1%) of the rest of the patients, $\chi^2(df = 1) = 3.9$, $p = 0.049$. Kaplan-Meier survival analysis showed the effect of lithium appeared between three and four weeks into the trial. Survival analysis also showed a higher probability of complete remission of suicidal symptoms for the depressed patients with therapeutic lithium levels as compared to those assigned to citalopram + placebo, $\chi^2(df = 1) = 4.22$, $p = 0.036$.

Discussion: These data support three suggestions. It is possible to conduct a prospective clinical trial among severely depressed and suicidal patients if adequate safety measures are included. Second, citalopram may have a direct therapeutic effect on suicidal thoughts and behaviors. Finally, lithium given in appropriate doses may augment any reduction of suicidal thoughts and behaviors that occurs with citalopram alone.

Disclosure: A. Khan: Part 1: This project was funded by the Northwest Clinical Research Center in collaboration with Columbia Northwest Pharmaceuticals. Arif Khan and his family are the Principle Owners of both NWCRC and CNWP. CNWP has filed a provisional patent for treatment of suicidality with lithium combination therapies., Part 4: In 2010 this project was awarded a grant from the Qualifying Therapeutic Discovery Program under the Patient Protection and Affordable Care Act for 2009 and 2010. **S. Khan:** Part 1: Shirin RF Khan is a minority owner of Columbia Northwest Pharmaceuticals and Northwest Clinical Research

Center. **J. Hobus:** Part 1: Joy Hobus is a minority owner of Columbia Northwest Pharmaceuticals. Ms. Hobus is a full time employee of Northwest Clinical Research Center. **J. Faucett:** Part 1: Mr. Faucett is a full time employee of Northwest Clinical Research Center. **V. Mehra:** Part 1: Dr. Mehra is the Principle Owner of Artemis Institute for Clinical Research. Artemis was one of the two clinical trial sites that enrolled patients for this trial. **E. Giller:** None. **R. Rudolph:** None. **W. Brown:** None.

189. Interferon-Stimulated Genes: Antiviral Response and Psychiatric Side Effects of IFN-alpha in HCV patients

Gregory Oxenkrug*, Paul Summergrad

Tufts Medical Center/Tufts University, Boston, USA

Background: Interferon (IFN)-alpha activates IFN-stimulating genes (ISG) in hepatitis C virus (HCV) patients. One of ISG, the IFN-gamma (IFNG) + 874 T/A gene, encodes the production of IFNG protein, a pro-inflammatory cytokine. Antiviral response to treatment and development of psychiatric side-effects (e.g., depression) might be mediated via IFNG protein-induced up-regulation of rate-limiting enzymes of pteridines biosynthesis (guanosine triphosphate cyclohydrolase) and tryptophan (TRY) – kynurenine (KYN) metabolism (indoleamine 2,3-gihydroxy-genase).

We studied the association between IFNG gene activation and 1). Antiviral response and 2). Development of depression in HCV patients treated with IFN-alpha.

Methods: IFNG (+ 874) T/A polymorphism and plasma concentrations of neopterin, stable by-product of IFNG-stimulated biosynthesis of pteridines, were evaluated in a retrospective study of 300 HCV patients treated with IFN-alpha in the past 3 years.

Results: 1). Antiviral response rate in HCV patients negatively correlated with neopterin plasma concentrations ($p < 0.0001$, Chi-square test). 2). Carriers of T (high producer) were more frequent while carriers of A (low producer) alleles were about two times less frequent among depressed than in not depressed HCV patients treated with IFN-alpha ($p < 0.01$, Chi-square test).

Discussion: Presence of T allele of IFNG (+ 874) gene might represent a genetic risk factor for the development of depression during IFNG-alpha treatment; neopterin concentrations might predict antiviral response to IFN-alpha treatment. Assessment of pretreatment IFNG-related laboratory markers might predict antiviral response and risk of psychiatric complications of IFN-alpha treatment of HCV patients. Acknowledgement. Study was partly supported by MH083225

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190. Longstanding Cannabis Abuse is Associated with Decreased Likelihood of Remission from Major Depression

Ihsan M. Salloum*, Jack R. Cornelius , Antoine Douaihy , Ricardo Caceda , Feng Miao, Kirisci Levent, Thase E. Michael

University of Miami Miller School of Medicine , Miami, USA

Background: Symptom remission of major depressive disorder (MDD) is generally defined as improvement in the Hamilton Rating Scales of Depression (HRSD) to a score of 7 or below. Remission rather than response (HRSD decrease by 50% from baseline) is considered the most desirable outcome of successful treatment. Cannabis is the most commonly used illegal drug among the general population and in patients with psychiatric disorders as well. While concurrent substance abuse is generally associated with poor treatment response, mostly due to medication

compliance, there are no rigorous studies that have examined the role of cannabis abuse and remission of MDD. The aim of this study was to examine whether longstanding cannabis abuse impact on rate of depression remission among patients with MDD and comorbid alcoholism.

Methods: The sample consisted of 64 subjects (50% females) who completed a 24-week randomized, double-blind, placebo-controlled, parallel-group trial evaluating the efficacy of fluoxetine (dose range 20-60 mg/day) ± naltrexone hydrochloride (dose 50 mg/day) in the treatment of MDD with comorbid alcoholism. After confirmation of eligibility, patients were randomly allocated (1:1) to receive naltrexone or placebo. Post-randomization assessments were undertaken weekly for the first 4 weeks, every two weeks for the subsequent 8 weeks, and every 4 weeks for the remaining 12 weeks of the 24-week study. The baseline assessment battery included the Psychiatric Research Interview for Substance and Mental Disorders (PRISM), the Hamilton Rating Scale for Depression (HRSD-17 & 25), the Addiction Severity Index (ASI), the Hamilton Anxiety Rating Scale (HARS), the Pittsburgh Sleep Quality Index (PSQI), and the Global Assessment of Functioning Scale (GAF) among others. We examined whether longstanding cannabis abuse, defined as 10 or more years of reported marijuana use, is related to remission from MDD defined as a clinician rating of very much improved on the Clinical Global Improvement Scale and a mean HRSD score below 7.

Results: The longstanding cannabis abuse group (n = 26) had significantly lower proportion of patients remitted (very much improved on the CGI with a mean HRSD of 5.7 (SD 2.7)) compared to the remainder of the sample (n = 38) (5.8% vs. 14.7% respectively, p = 0.002). The Odds Ratio for remission of longstanding abuse was 0.358 (p = 0.0018). The two groups were similar on age, ethnicity, and marital status, but there were more males in the longstanding abuse group (65.4% vs. 39.5%, p = 0.04). The two groups were similar on baseline alcohol dependency scale score, functioning (GAF mean score), anxiety (HARS mean score), and hours of sleep (PSQI mean score). They differed however on baseline depression (HRSD-25 mean score 19.6 (sd 4.4) vs. 22.4 (sd 5.9) respectively, P = 0.045), psychiatric severity index (ASI mean score 0.56 (sd. 0.15) vs. 0.48 (SD 0.09) respectively, p = 0.014)), and on lifetime years of cannabis abuse (mean years 20.35 (SD 6.7) vs. 0.92 (SD 1.67) respectively, p = <0.001). The longstanding cannabis abuse group also reported higher proportion of drug use days during the study (mean weakly days 0.9 (SD 1.8) vs. 0.05 (0.2) respectively, p = 0.025). The two groups did not significantly differ on average weekly alcohol drink (10.83 (SD 16.31) vs.16.48 (19.19) respectively) or on average dose of fluoxetine (mean dose 31.69 (SD 14.63) vs. 30.10 (SD 14.15) respectively).

Discussion: The results of this study indicate that those with longstanding cannabis abuse are only about one-third as likely to remit as compared to the remainder of this depressed and alcohol-dependent sample. This is despite the fact that those with longstanding cannabis abuse reported lower baseline depressive symptoms, non-significantly lower weekly alcohol use during the study and had similar average dose of fluoxetine. While this is a secondary analysis in a relatively small sample of patients with MDD and alcohol dependence, to our knowledge, this is the first rigorously controlled study focusing on the potential impact of longstanding cannabis abuse on treatment remission of major depression. Given the high frequency of cannabis abuse among patients with major depression and other psychiatric disorders, future studies are warranted to further elucidate the role of cannabis abuse as a predictor of treatment response and remission of major depressive disorder.

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R. Caceda : Part 3: Partial owner of My Healthwin llc, Part 4: Arsht Foundation. **F. Miao:** None. **K. Levent:** None. **T. Michael:** Part 1: Advisory/Consultant: Alkermes AstraZeneca Bristol-Myers Squibb Company Eli Lilly & Co. Dey Pharma, L.P. Forest Laboratories Gerson Lehman Group GlaxoSmithKline (ended 2008) Guidepoint Global H. Lundbeck A/S MedAvante, Inc. Merck and Co. Inc. (formerly Schering Plough and Organon) Neuronetics, Inc. Novartis (ended 2008) Otsuka Ortho-McNeil Pharmaceuticals (Johnson & Johnson) Pamlab, L.L.C. Pfizer (formerly Wyeth Ayerst Pharmaceuticals) PGx Health, Inc Shire US Inc. Supernus Pharmaceuticals Takeda Transcept Pharmaceuticals Grant Support: Agency for Healthcare Research and Quality Eli Lilly and Company Forest Pharmaceuticals GlaxoSmithKline (ended 7/10) National Institute of Mental Health Otsuka Pharmaceuticals Sepracor, Inc. (ended 1/09) Speakers Bureau: AstraZeneca (ended 6/30/10) Bristol-Myers Squibb Company Dey Pharmaceutical Eli Lilly & Co. (ended 6/30/09) Merck and Co. Inc., Pfizer (formerly Wyeth Ayerst Pharmaceuticals) Equity Holdings: MedAvante, Inc. Royalties: American Psychiatric Foundation Guilford Publications Herald House W.W. Norton & Company, In. Spouse's Employment: Embryon (Formerly Advogent; Embryon does business with BMS and Pfizer/Wyeth) As of: March 16, 2011, Part 2: AstraZeneca (2009), Part 3: None, Part 4: Agency for Healthcare Research and Quality Eli Lilly and Company Forest Pharmaceuticals Glaxo-SmithKline (ended 7/10) National Institute of Mental Health Otsuka Pharmaceuticals Sepracor, Inc. (ended 1/09), Part 5: N/A.

191. Comparative Efficacy and Tolerability of Quetiapine Monotherapy in Major Depression

Angelos Halaris*, John Piletz, Edwin Meresh, Steven Kimmons, James Sinacore

Loyola U Stritch School of Medicine, Maywood, USA

Background: It has long been recognized that treatment with an antidepressant agent acting through inhibition of monoamine transporters may not achieve optimal efficacy, symptom remission or a high response rate. Inhibition of D2 and 5-HT2 receptors may augment antidepressant response and possibly convert non-responders to responders. Escitalopram (ESC) has proven mono-therapeutic efficacy for major depressive disorder (MDD) and generalized anxiety disorder (GAD). Quetiapine (QTP) has received FDA approval as adjunctive therapy, but not mono-therapy, for MDD in the USA. A report has indicated efficacy of QTP XR as monotherapy for MDD (Cutler *et al.*, 2009) when compared to duloxetine. The biologically active metabolite of QTP, norquetiapine, is a potent inhibitor of norepinephrine reuptake, and, as such, it would be expected to exert antidepressant efficacy. This property is not shared by other atypical antipsychotics, at least in clinically relevant doses. Additionally, QTP has affinity for the 5-HT1 receptor that may provide antianxiety efficacy to this compound. We hypothesized that QTP XR would be efficacious as a monotherapeutic agent in MDD with associated anxiety and insomnia. Additionally, we performed a comparison against ESC from a previously conducted study in a different MDD cohort.

Methods: In two consecutive, open label studies (ISSSERX0015, LU108527) of identical design, QTP XR and ESC were used monotherapeutically to treat for 12 weeks MDD patients diagnosed with the MINI. In this analysis we have included 25 patients treated with QTP, and 23 patients treated with ESC. The average daily dose was 169 mg of QTP (range 25-300 mg) and 25 mg of ESC (range 5-40 mg). The dose was titrated gradually as tolerated. The primary efficacy endpoint was depression response; the secondary endpoint was anxiety response; the third endpoint was insomnia response. The following rating scales were administered: HAMD-17, HAMA, BDI, BAI, CGI, PSS at weeks 0, 2, 4, 8 and 12. Safety and tolerability measures included laboratory measures, and an

adverse events inventory. Treatment response was defined as at least a 50% reduction in the baseline HAMD-17 score. Remission was defined as a score of 7 or less. Insomnia was analyzed separately by extracting the three scores from the HAMD-17 scale (early, middle and late insomnia).

Results: Of the 25 patients receiving QTP 61% remitted and 78.3% were classified as responders. Of the non-remitters 44% were responders. Of the 23 patients on ESC 65% remitted and 80% were responders. Of the non-remitters 43% were responders. Both agents showed comparable statistical significance ($p < 0.01$) in score reduction at the end points in all scales. The time course of antidepressant efficacy was comparable between the two drugs based on HAMD-17 scores. The BDI scores were also comparable between the two treatments. Anxiety scores very similar for the two drugs, but QTP showed slight superiority over ESC at 12 weeks. BAI scores were significantly different between baseline and end-of-treatment for QTP. Insomnia improved with both agents when week 12 was compared to baseline. Early: P-value ESC (0.045) vs QTP (0.002); Middle: P-value ESC (0.041) vs QTP (0.003); Late: P-value ESC (0.082) vs QTP (0.0001). QTP was equally effective as ESC for early insomnia, but superior to ESC for middle ($p < 0.05$) and late insomnia ($p < 0.01$). Stress perception improved significantly with both agents. With respect to safety measures, neither treatment showed significant changes from baseline in any of the parameters studied. The QTP trial was associated more frequently with weight gain and lipid and glucose abnormalities compared to ESC; however, these changes were not statistically significant. Higher doses did not worsen lab values. Common adverse events included gastrointestinal complaints, headache, sexual dysfunction and weight gain for ESC; somnolence, drowsiness and dry mouth for QTP. No sexual dysfunction was reported with QTP.

Discussion: The data clearly indicate antidepressant and anti-anxiety efficacy of QTP XR monotherapy equivalent to that of ESC in MDD patients. QTP exhibited a slight advantage over ESC in controlling anxiety and a clear superiority in improving insomnia. The dose range of ESC was aligned with clinical practice. The dose of QTP XR at onset was 25-50 mg daily and dose titration was individualized and contingent upon patient response and tolerability. This dosing regimen differed from previously utilized guidelines. Neither drug produced adverse events akin to the metabolic syndrome. These preliminary results indicate that QTP XR monotherapy is an effective and safe treatment option for MDD

192. Brain Volume Changes in Adult Bipolar Depression Patients with Lithium Treatment

Abhishek Lunagariya, Mark Nicoletti, Giovana Zunta Soares, John Hatch, Jair Soares*

Center for Excellence on Mood Disorders, Department of Psychiatry and Behavioral sciences, UT Health Science Medical School, Houston, USA

Background: Bipolar depression is frequent cause of impairment in affected individuals. Brain mechanisms involved in it have not been clearly identified. Structural abnormalities in brain regions and their changes with treatment have been detected in previous studies. Key brain regions where such changes have been identified are hippocampus and anterior cingulate cortex. However these findings are sometimes conflicting and possibly confounded by patients being on multiple psychotropic medications, requiring further investigation.

Methods: We studied 30 DSM- IV Bipolar Depression (mean age = 31.75 years, SD = 9.68, female = 20) and 20 healthy controls (mean age = 40.64, SD = 12.87, female = 10). They were off all psychotropic medications for at least two weeks, in order to reduce or eliminate their influence on results. The Bipolar Depression patients took Lithium for 16 weeks. Lithium was started at 300 mg BID orally and titrated by 300 mg every five days to reach the therapeutic levels (0.6-1.0 m Eq/L). After four weeks, clinical non-responders (those with < 50% decrease in depression scale) were given additional Lamotigrine for remaining 12 weeks. Lamotigrine was started at 50 mg/day on week four and was increased to 200 mg/day by week six. In both Bipolar Depression and healthy control; baseline MRI was obtained at week 0(drug naïve) and follow up MRIs were obtained at week 4 and week 16. We used higher resolution 3 T MRI to obtain high precision in detecting brain volume changes. Pair wise comparisons with Bonferroni adjustment were done between the 3 time points.

Results: There is a significant increase in Left Hippocampal volumes in Bipolar Depression patients during the first four week of Lithium treatment ($p = .008$) and these changes are sustained after 16 weeks of treatment ($p = .007$). For healthy control there is no such significant Left Hippocampal volume change ($p = .885$ at four weeks, $p = .380$ at 16 weeks). There is a significant increase in Left Anterior Cingulate volumes in Bipolar Depression patients during first four weeks of treatment with Lithium ($p = .001$). However Left Anterior Cingulate volume shrinks back to baseline level after 16 weeks of treatment ($p = .025$). For healthy control there is no such significant Left Anterior Cingulate volume change ($p = .104$ at four weeks, $p = .517$ at 16 weeks).

Discussion: The beneficial effects of Lithium treatment in Bipolar Depression might be correlated to increase in Left Hippocampus volume after initiation of treatment; as noted by MRI. These increases in volume can be due to neuro-protective effects of Lithium. Increase in size of Left Anterior Cingulate in initial phase of treatment can be attributed to similar effects of Lithium. However, these increases in brain volume in Left Hippocampus and Left Anterior Cingulate are not significantly different among clinical responders and non-responders. Therefore, these neuro-protective properties of lithium might not be entirely responsible for its clinical benefits in Bipolar Depression. Other mechanisms contributing to these clinical benefits of Lithium need to be explored.

193. Efficacy of Adjunctive OPC-34712 across Multiple Outcome Measures in Major Depressive Disorder: A Phase II, Randomized, Placebo-controlled Study

Michael Thase*, Maurizio Fava, Mary Hobart, Aleksandar Skuban, Peter Zhang, Robert D. McQuade, William H. Carson, Raymond Sanchez, Robert A. Forbes

University of Pennsylvania School of Medicine, Philadelphia, USA

Background: OPC-34712 is a new D_2 dopamine and 5-HT_{1A} partial agonist and high affinity 5-HT_{2A} antagonist. This study assessed the efficacy and safety of OPC-34712 as an adjunctive to standard antidepressant therapy (ADT) in patients with major depressive disorder (MDD) who had exhibited inadequate response to one to three prior ADTs using both clinician-rated and patient-reported scales of symptoms and functional outcomes.

Methods: This was a Phase II, multicenter, randomized, double-blind, placebo-controlled trial (Study 331-08-211) comprised of three phases: a screening phase (7–28 days); a prospective phase (Phase B): an 8-week single-blind, adjunctive placebo to assess response status to ADT ($\geqslant 50\%$ reduction in the 17-item Hamilton Depression Rating Scale [HAM-D17] total score); and a randomized phase (Phase C): a 6-week double-blind, assessment of adjunctive OPC-34712 vs. placebo in patients with an inadequate

response to ADT. Randomized subjects had been in the current depressive episode >8 weeks, had a HAM-D17 total score >18 at baseline and had not responded to ADT in Phase B (<50% reduction in HAM-D17 total score). Randomization was to daily OPC-34712 (0.15 mg, n = 62; 0.50 ± 0.25 mg, n = 120; or 1.5 ± 0.5 mg, n = 121) or placebo (n = 126) adjunctive to ADT. Primary efficacy endpoint was mean change from baseline of Phase C to endpoint on the Montgomery–Åsberg Depression Rating Scale (MADRS) total score. Primary analysis objectives were to compare the efficacy of the 0.50 mg/day dose and the 1.5 mg/day dose of OPC-34712 with placebo. Secondary measures included the clinician-rated Clinical Global Improvement – Severity scale (CGI-S), patient-rated Inventory of Depressive Symptoms (IDS) and the Sheehan Disability Scale (SDS) to assess patient functioning.

Results: Among 429 randomized patients, completion rates at Week 14 were 82–85% and were similar for all treatment groups, with a low incidence of discontinuation due to adverse events. Significant improvements in mean MADRS total score, from baseline to endpoint, were observed for subjects receiving adjunctive OPC-34712 at the 1.5 mg/day dose (n = 121, mean change from baseline: –8.2) compared with placebo (n = 126, mean change from baseline: –6.1, $p = 0.03$), while subjects receiving the 0.5 mg/day (n = 120) and 0.15 mg/day (n = 62) doses did not have significant improvements in MADRS total score compared with those receiving placebo. The 1.5 mg/day dose also showed statistical superiority to placebo on secondary outcomes: the clinician-rated CGI-S (–1.1 vs. –0.7, $p = 0.006$), the patient-rated IDS (–7.5 vs. –3.6, $p = 0.002$), and the SDS measure of patient function (–1.3 vs. –0.6; $p = 0.016$). Patients receiving the 1.5 mg/day dose also showed higher response rates (34.7% [n = 41/118] vs. 19.8% [n = 25/126], $p = 0.008$) and remission rates (23.7% [n = 28/118] vs. 13.5% [n = 17/126], $p = 0.052$). Commonly reported adverse events (all doses of OPC-34712 >5%) were upper respiratory tract infection (6.9%, n = 21/303), akathisia (6.6%, n = 20/303), weight gain (6.3%, n = 19/303), and nasopharyngitis (5.0%, n = 15/303). Mean change from baseline in body weight was 1.6 kg for adjunctive OPC-34712 (1.5 mg/day) compared with 0.77 kg for adjunctive placebo.

Discussion: OPC-34712 at a dose of 1.5 mg/day was well tolerated and effective as adjunctive treatment for patients with MDD using both clinician-rated and patient-reported scales of depressive symptomatology as well as on measures of patient function.

Disclosure: M. Thase: Part 1: Dr Thase has been an advisory/consultant for Aldolor, AstraZeneca, Bristol-Myers Squibb Company, Eli Lilly & Co., Dey Pharma, L.P., Forest Laboratories (including the company formerly known as PGx), Gerson Lehman Group, GlaxoSmithKline, Guidepoint Global, H. Lundbeck A/S, MedAvante, Inc., Merck and Co. Inc. (including the companies formerly known as Organon and Schering-Plough), Neuronetics, Inc., Novartis, Ortho-McNeil Pharmaceuticals, Otsuka, Pamlab, L.L.C., Pfizer (including the company formerly known as Wyeth Ayerst Pharmaceuticals), Rexahn, Shire US Inc., Supernus Pharmaceuticals, Takeda, Thomson-Reuters, and Transcept Pharmaceuticals., Part 2: He has equity holdings in MedAvante, Inc. and receives royalties from the American Psychiatric Publishing, Inc., Guilford Publications, and Herald House W.W. Norton & Company, Inc. His spouse is an employee of Embryon, Inc. (formerly Advogent; Embryon does business with Bristol-Myers Sqibb and Pfizer/Wyeth)., Part 4: In the past 3 years, he has received grant support from the Agency for Healthcare Research and Quality, Eli Lilly and Company, Forest Laboratories, GlaxoSmithKline, National Institute of Mental Health, Otsuka, Pfizer, and PharmaNeuroboost. In the past 3 years, he has received honoraria for talks from AstraZeneca, Bristol-Myers Squibb Company, Dey Pharma, Eli Lilly & Co., Merck and Co. Inc., and Pfizer (formerly Wyeth Ayerst Pharmaceuticals). M. Fava: Part 1: He has spoken/written for Adamed, Co, Advanced Meeting Partners, American Psychiatric Association, American Society of Clinical Psychopharmacology, AstraZeneca, Belvoir Media Group, Boehringer Ingelheim GmbH, Bristol-Myers Squibb, Cephalon, Inc., CME Institute/Physicians Postgraduate Press, Inc., Eli Lilly and Company, Forest Pharmaceuticals, Inc., GlaxoSmithKline, Imedex, LLC, MGH Psychiatry Academy/Primedia, MGH Psychiatry Academy/Reed Elsevier, Novartis AG, Organon Pharmaceuticals, Pfizer Inc., PharmaStar, United BioSource, Corp., and Wyeth-Ayerst Laboratories., Part 2: Maurizio Fava has received research support from Abbott Laboratories, Alkermes, Inc., Aspect Medical Systems, AstraZeneca, BioResearch, BrainCells Inc., Bristol-Myers Squibb, Cephalon, Inc., CeNeRx BioPharma, Clinical Trials Solutions, LLC, Clintara, LLC, Covidien, Eli Lilly and Company, EnVivo Pharmaceuticals, Inc., Euthymics Bioscience, Inc., Forest Pharmaceuticals, Inc., Ganeden Biotech, Inc., GlaxoSmithKline, Icon Clinical Research, i3 Innovus/Ingenix, Johnson & Johnson Pharmaceutical Research & Development, Lichtwer Pharma GmbH, Lorex Pharmaceuticals, National Alliance for Research on Schizophrenia & Depression (NARSAD), National Center for Complementary and Alternative Medicine (NCCAM), National Institute of Drug Abuse (NIDA), National Institute of Mental Health (NIMH), Novartis AG, Organon Pharmaceuticals, PamLab, LLC., Pfizer Inc., Pharmavite® LLC, Photothera, Roche, RCT Logic, LLC, Sanofi-Aventis US LLC, Shire, Solvay Pharmaceuticals, Inc., Synthelabo, and Wyeth-Ayerst Laboratories. 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Inc., Dov Pharmaceuticals, Inc., Edgemont Pharmaceuticals, Inc., Eisai Inc., Eli Lilly and Company, ePharmaSolutions, EPIX Pharmaceuticals, Inc., Euthymics Bioscience, Inc., Fabre-Kramer Pharmaceuticals, Inc., Forest Pharmaceuticals, Inc., GenOmind, LLC, GlaxoSmithKline, Grunenthal GmbH, i3 Innovus/Ingenis, Janssen Pharmaceutica, Jazz Pharmaceuticals, Inc., Johnson & Johnson Pharmaceutical Research & Development, LLC, Knoll Pharmaceuticals Corp., Labopharm Inc., Lorex Pharmaceuticals, Lundbeck Inc., MedAvante, Inc., Merck & Co., Inc., MSI Methylation Sciences, Inc., Naurex, Inc., Neuronetics, Inc., NextWave Pharmaceuticals, Novartis AG, Nutrition 21, Orexigen Therapeutics, Inc., Organon Pharmaceuticals, Otsuka Pharmaceuticals, PamLab, LLC., Pfizer Inc., PharmaStar, Pharmavite® LLC., PharmoRx Therapeutics, Precision Human Biolaboratory, Prexa Pharmaceuticals, Inc., Puretech Ventures, PsychoGenics, Psylin Neurosciences, Inc., Rexahn Pharmaceuticals, Inc., Ridge Diagnostics, Inc., Roche, RCT Logic, LLC, Sanofi-Aventis US LLC., Sepracor Inc., Servier Laboratories, Schering-Plough Corporation, Solvay Pharmaceuticals, Inc., Somaxon Pharmaceuticals, Inc., Somerset Pharmaceuticals, Inc., Sunovion Pharmaceuticals, Supernus Pharmaceuticals, Inc., Synthelabo, Takeda Pharmaceutical Company Limited, Tal Medical, Inc., Tetragenex Pharmaceuticals, Inc., TransForm Pharmaceuticals, Inc., Transcept Pharmaceuticals, Inc., and Vanda Pharmaceuticals, Inc. He has equity holdings with Compellis and has received royalty/patent/income from Patent for Sequential Parallel Comparison Design (SPCD) and patent application for a combination of azapirones and bupropion in major depressive disorder, copyright royalties for the MGH Cognitive & Physical Functioning Questionnaire (CPFQ), Sexual Functioning Inventory (SFI), Antidepressant Treatment Response Questionnaire (ATRQ), Discontinuation-Emergent Signs & Symptoms (DESS), and SAFER. He has patents for research and licensing of SPCD with RCT Logic, Lippincott, Williams & Wilkins, and World Scientific Publishing Co. Pte.Ltd. M. Hobart: Part 5: Employee of Otsuka Pharmaceutical

194. N-methyl-D-aspartate Mechanism of Depression and a Glycine Transporter-I Inhibitor, N-Methylglycine (Sarcosine) for the Treatment of Depression

Guochuan Emil Tsai*, I-Hua Wei, Chih-Chia Huang, Kuo-Hao Huang, Hsien-Yuan Lane

Harbor-UCLA Medical Center, Torrance, USA

Background: Antidepressants, aiming at monoamine neurotransmission, exhibit limited efficacy, delayed onset of action and poor compliance. Glutamatergic neurotransmission is involved in depression. However, it is unclear how both the agonist and antagonists of N-methyl-D-aspartate (NMDA) subtype glutamate receptor can be potential treatments for depression. Our studies are to understand the difference between NMDA antagonist and agonist' actions in an animal model of depression and explore the effects of sarcosine, a glycine transporter-I inhibitor that potentiates NMDA function, on major depression.

Methods: We compared a NMDA antagonist, MK-801, and two NMDA-enhancing agents, D-serine, a full agonist on the NMDA-glycine site, and sarcosine in the forced swim test (FST) and elevated plus maze (EPM) test. We also conducted a randomized, double-blind, citalopram-controlled sarcosine trial for six weeks in patients with major depressive disorder. The main outcome measure were: Hamilton Depression Rating Scale (HAMD), Global Assessment of Function (GAF), and remission rate. Forty patients were enrolled. Clinical efficacy and side-effects were assessed biweekly. Time course of response, remission and dropout rates were also compared.

Results: D-serine, sarcosine and MK-801 all decreased the duration of immobility, similar to desipramine, in the FST. Nevertheless, there were distinctive stereotyped swimming behaviors elicited by MK-801 treatment, and the combination of D-serine and sarcosine could reverse these unique stereotyped behaviors. In EPM, MK-801 elicited behavioral effects opposite to D-serine and sarcosine and the NMDA-enhancing agents could rescue this anxiogenic effect of the antagonist. Taken together, our findings suggest depression involves complex neural substrates, in that both NMDA enhancement and inhibition can result in improvement of the behavioral manifestation of depression. In the case of anxiety behavior, however, NMDA inhibition resulted in anxiety-like behaviors but NMDA enhancement is anxiolytic.

Clinically, sarcosine produced greater improvement in HAMD scores, clinical global impression and GAF than citalopram. Superior efficacy of sarcosine over citalopram treatment was manifested by the following: (1) the effect sizes for between-group comparisons were substantial in all the measures, (2) sarcosine not only diminished severity of symptomatology but also improved global function, (3) sarcosine recipients' improvement was faster, with 40% of subjects reaching remission at the end of week 4, and 65% at week 6, (4) subjects given sarcosine were more likely to respond and to remit and less likely to drop out. Sarcosine was well tolerated without significant side-effects.

Discussion: Our findings suggest that the anti-immobility induced by MK-801 is due to the increase of locomotor activity and raises the possibility that the antidepressant property can be false-positive as seen with other psychostimulants. In addition, the contentious findings of the antidepressant-like effect of NMDA antagonists in clinical depression need to be considered cautiously given their potential neurotoxic and anticognitive effects if administered in longterm. D-serine and sarcosine increase the percentage of open arm time and entries dose-dependently. On the contrary, MK-801 does not produce behavioral responses indicative of an anxiolytic action in EPM. The dissociation between FST and EPM of MK-801, vs. agonist treatment, suggests that NMDA antagonists may not be good candidates as antidepressant. Antidepressants that potentiate 5-HT-mediated neurotransmission increase swimming behavior whereas those with primary actions through catecholamines increase climbing behavior. Our findings reveal that NMDA antagonist induces swimming behavior similar to catecholamine compounds while NMDA agonists induce swimming behavior similar to SSRIs. Compared to SSRI treatment, sarcosine therapy shows a fast, efficacious promise of remission of depression and a benign safety profile with minimal attrition. In summary, both enhancing and inhibiting NMDA function can improve depressive behavior in a rodent model and in humans. Agents modulating NMDA function may provide benefits for depressed patients who do not respond or are partially responsive to conventional monoamine-modulating antidepressants. Both our animal and human studies indicate that the GlyT-1 inhibitor sarcosine or other potentials avenues for NMDA receptor enhancement represent a novel therapeutic approach for the treatment of depression.

195. The Effects of Highly Active Antiretroviral Therapy (HAART) on Neurocognitive Functioning of Patients with HIV

Tanya Alim*, Mansoor Malik, Ziad Safadi, Anne Marie Duc, Maria Hipolito

Howard University, Washington, USA

Background: The detrimental effects of HIV on neurocognition are well known. Early stages of infection reveal subtle neurocognitive impairment on testing. Various studies have demonstrated an improvement in cognitive functioning with HAART. However the association of immune status with neurocognition is less clear in African American patients. HAART is effective in decreasing the systemic viral load in HIV infection and the impact of HAART on HIV-associated cognitive impairment in African Americans is unclear. There is an urgent need to investigate this question as African Americans are the largest group with HIV. The current study investigated the relationship of immune status as measured by the CD 4 count and viral load with the improvement in neurocognition in African American patients with HIV on HAART.

Methods: A continuous cohort of patients were studied at the Howard University Hospital HIV clinic. Thirty eight African American participants with HIV + were classified by treatment into two groups: antiretroviral naïve and HAART medicated. Participants underwent detailed clinical evaluation and completed computerized neuropsychological testing at baseline and 3 months. Cognistat: Trails A & B, Controlled Oral Word Association, WAIS-R Digit Symbol and Digit Span, Stroop, Reye Auditory Verbal Learning Test, Benton Visual Retention & PASAT) and assessment of global functioning (Karnofsky Functional Performance Scale) were obtained. CD4 count and viral load were obtained at repeated visit as well.

Results: Sample size included 38 participants: 23 males and 15 females: (N = 32) medication naïve and (N = 6) receiving HAART.

There were no significant differences in any of the socio-demographic variables between the two groups. 30 out of 38 patients had 2 cognitive domain deficits, 4 patients had no deficits; and 4 patients had 1 domain deficit. Nineteen out of 38 participants had Global Cognitive Scores 85, one standard deviation below average. HAART group obtained higher scores in six of the cognitive subtests on repeat visit. Independent t-test demonstrated no significance differences.

Cognitive improvement for a subset of HAART group (N = 9) was found however findings were not statistically significant. The verbal function and memory domains were significantly greater than the other cognitive domains in both groups: naïve and HAART. Viral load was highly negatively correlated with attention and motor skills (r = −0.41; -0.3 respectively). No effect was found of HAART's impact on CD4 counts.

Discussion: Although HAART affects the viral load in HIV +, our findings demonstrate no effect of the HAART on HIV associated dementia among African American patients. Findings suggest that improvement in viral load may improve a subset of the cognitive domains (ie. Attention and Motor skills). A larger sample size and a longitudinal study of current participants may yield additional information regarding neurocognitive improvement, functioning, adherence and ultimately prevention of HIV associated dementia in this population.

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196. Metabotropic Glutamate Receptor 4 (mGluR4) Activation reverses Acute Motor Deficits and Protects against Neurodegeneration in Animal Models of Parkinson's Disease

Danielle Graham*, Gael Hedou, Audrey Gray, Jeff Pimentel, Dongzi Yu, Nicholas Clark, Hui Tian, Mark Shearman, Christoph Wiessner, Tammy Dellovade

EMD Serono Research Institute, Billerica, USA

Background: Parkinson's disease (PD) is characterized by a progressive loss of nigro-striatal dopamine (DA) neurons leading to debilitating motor disturbances, postural abnormalities, signs of depression, and inexorable disease progression. Specifically, degeneration of the nigro-striatal dopaminergic pathway leads to an over-activation of GABA and glutamate neurotransmission. Activation of mGluR4 receptors can counteract the enhanced neurotransmission and alleviate the core motor symptoms of PD. For example, PHCCC, a non-selective mGluR4 positive allosteric modulator (PAM), can reverse acute Parkinsonian-like behaviors in an animal model of rigidity. In this study, the effects of various mGluR4 compounds including a potent and selective mGluR4 PAM (Engers9c) and an mGluR4 PAM/agonist (VU0155041) were compared in animal models of PD such as haloperidol-induced catalepsy (HIC) and neurodegeneration models.

Methods: In male Sprague Dawley (SD) rats, catalepsy was induced with haloperidol (1.5 mg/kg, IP). Sixty minutes following haloperidol, animals received a single systemic injection of vehicle (50% DMSO or saline) or a single injection of one of multiple test compounds. Catalepsy, the latency to remove at least one forepaw from an elevated bar, was assessed roughly 100 min after haloperidol. To further investigate the therapeutic potential of mGluR4 activation in PD, the symptomatic and neuroprotective properties of mGluR4 compounds were examined in the rat 6-hydroxydopamine (6OHDA) lesion model. Female, SD rats received four, 7 ug intra-striatal injections of 6OHDA (symptomatic model) or a single, unilateral, 7 ug intra-striatal injection of 6OHDA (neuroprotection model). To examine the symptomatic effects of mGluR4 activation, a single systemic injection of select mGluR4 compounds was administered immediately before rotation behavior analysis. To examine the neuroprotective properties

of mGluR4 activation, animals received 14 day repeated systemic treatment with select mGluR4 compounds. And finally, current studies are underway to examine the symptomatic properties of mGluR4 activation in a transgenic mouse model of progressive DA loss (MitoPark [MP] mice). MP mice have a DA neuron selective deletion of the mitochondrial transcription factor A protein (tfam) and display profound behavioral deficits as well as progressive age-dependent loss of DA neurons. The loss of striatal DA fibers and substantia nigra neurons parallels the motor behavior deficits. In addition, MP mice also have a significant decrease in DA neurons in the ventral tegmental area providing a unique animal model to examine changes in both motor deficits and depressive behaviors following activation of mGluR4.

Results: In the HIC study, vehicle-treated rats demonstrated significant catalepsy (180 sec). As reported previously, PHCCC significantly reversed HIC (p < .05). In addition, Engers9c and VU0155041 significantly reversed HIC (p < .05). In the symptomatic 6OHDA rat model, VU0155041 marginally influenced drug-induced rotational behavior. However, in the neuroprotection 6OHDA rat model, a 14 day systemic administration of PHCCC or VU0155041 significantly ameliorated DA fiber density loss in the striatum as compared to vehicle-treated animals (p < .05). Vehicle-treated 6OHDA animals had a 39% reduction in TH staining of the 6OHDA-lesioned striatum as compared to the non-lesioned (intact) striatum. Repeated treatment with PHCCC or VU0155041 significantly reduced DA fiber density in the striatum equally (26% reduction in TH staining as compared to intact side). The EMD Serono-derived line of MP mice show normal behavior until about 10-11 wks of age, at which time a significant age-dependent decrease in general activity is observed; acute administration with LDopa can reverse this motor deficit (p < .05). In addition, MP mice have a significant deficit in rotarod performance at 16-16 wks of age and significant levels of catalepsy at 18-20 wks of age (p < .05). Studies are currently ongoing to examine these behaviors following systemic treatment with mGluR4 compounds.

Discussion: A single systemic injection of an mGluR4 compound can significantly reverse HIC. Repeated administration of an mGluR4 compound can protect against DA loss in the 6OHDA-induced lesion model. Collectively, these data provide clear evidence that systemic treatment with mGluR4 activators can reverse and prevent the motor deficits resembling PD symptoms, providing significant evidence of the therapeutic potential of mGluR4 compounds as a treatment for PD.

Disclosure: D. Graham: Part 5: EMD Serono Research Institute. G. Hedou: Part 5: Merck Serono. A. Gray: Part 5: EMD Serono Research Institute. J. Pimentel: Part 5: EMD Serono Research Institute. D. Yu: Part 5: EMD Serono Research Institute. N. Clark: Part 5: EMD Serono Research Institute. H. Tian: Part 5: EMD Serono Research Institute. M. Shearman: Part 5: EMD Serono Research Institute. C. Wiessner: Part 5: Merck Serono. T. Dellovade: Part 5: Merck Serono.

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197. Effect of 5HT2c Receptor Blockade in a Novel Social Approach Test of Anxiety-Like Behavior during Withdrawal from Chronic Intermittent Ethanol

Catherine A. Marcinkiewcz*, Alberto J. Lopez, Nora M. McCall, Alexa M. Waters, Sheryl S. Moy, Thomas L. Kash

University of North Carolina at Chapel Hill, Chapel Hill, USA

Background: Anxiety is a core symptom of ethanol withdrawal that has been associated with relapse to drinking and ethanol dependence. Serotonin 2c (5HT2c) systems have been shown to play a key role in anxiety induced by ethanol withdrawal and may be a potential target for treatment. In this study, we used a novel social approach test to assess anxiety-like behavior in a chronic

intermittent ethanol (CIE) model and the role that 5HT2c signaling plays in this maladaptive behavior. Further, we also examined the effects of CIE on 5HT2c receptor expression in the bed nucleus of stria terminalis (BNST), a brain region implicated in anxiety.

Methods: Male DBA/2J mice aged 7-9 weeks were exposed to ethanol vapor or room air for 5 days, 16 hours a day. The alcohol dehydrogenase inhibitor pyrazole (1 mmol/kg, i.p.) was administered 30 minutes before ethanol exposure to stabilize blood ethanol concentration (BEC). Volatilized ethanol (95%) is generated by bubbling air through an air stone submerged in the ethanol solution at a flow rate sufficient to generate BECs in the range of 150-200 mg/dl in adult male DBA/2J mice throughout the exposure. Twenty-four hours after the last exposure, mice were injected with SB 242,084 (3 mg/kg, i.p.) or vehicle one hour before the social approach test, which is described in detail in Singewald et al., (2003). Briefly, mice were placed in the center of an automated 3-chambered apparatus and allowed to habituate for 10 minutes. In the social approach phase, an unfamiliar mouse was placed inside of a metal cage in one of the side chambers, while an empty cage was placed in the opposing chamber. The amount of time spent sniffing the stranger mouse is used as a measure of social approach, which is inversely related to anxiety-like behavior. We also quantified 5HT2c receptor expression using a microarray approach, and will confirm these results using immunohistochemistry and Western blotting techniques.

Results: Mice exposed to CIE displayed significantly more anxiety-like behavior in the social approach test than control mice. Initial results indicate that the 5HT2c antagonist SB 242,084 attenuates anxiety-like behavior in CIE mice, but not in control mice. We also have microarray data showing that 5HT2c receptor expression is significantly increased in the BNST of CIE mice compared to controls, and there is a trend toward an increase in 5HT2c protein expression after 1-cycle CIE based on immunoblotting experiments. Current studies are following up by measuring 5HT2c protein expression using immunohistochemistry.

Discussion: Chronic exposure to and withdrawal from ethanol can produce adaptations in neurocircuitry that govern emotional behavior, including anxiety. There is evidence to suggest that 5HT2c receptor signaling may increase excitability in a sub-class of neurons in the BNST, so ethanol-induced increases in 5HT2c signaling may mediate anxiety-like behavior following ethanol withdrawal. We have found that CIE increases 5HT2c receptor expression in the BNST, and preliminary results indicate that blockade of 5HT2c receptors reduces anxiety-like behavior in the social approach test. These results indicate that increased 5HT2c receptor signaling may underlie ethanol withdrawal-induced anxiety-like behavior, and that drugs targeting the 5HT2c receptor may be a viable treatment for anxiety associated with excessive alcohol use.

Disclosure: C. Marcinkiewcz: None. A. Lopez: None. N. McCall: None. A. Waters: None. S. Moy: None. T. Kash: None.

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198. The Neurosteroid Pregnenolone prevents Predator Stress-Induced Decreases in Open Arm Time in the Elevated Plus Maze Model for Anxiety-Like Behaviors: Potential Relevance to Novel Secondary Prevention Strategies in PTSD?

Shawn K. Acheson, Jason D. Kilts, Joshua T. Rogers, Lawrence J. Shampine, Christine E. Marx*

Duke University Medical Center, Durham, USA

Background: Secondary prevention strategies for PTSD are currently extremely limited and the need for new interventions is acute. Emerging preclinical and clinical data suggest that neurosteroids may have potential as novel pharmacological interventions. Neurosteroids modulate the stress response, and neurosteroid reductions have been associated with PTSD. These molecules may thus be logical candidates for the treatment of PTSD and other anxiety disorders. We therefore investigated the effects of pregnenolone administration prior to predator stress exposure on rat anxiety-like behaviors in the elevated plus maze.

Methods: Sprague-Dawley male rats were acclimated to IP saline injection for four consecutive days prior to experimentation. The following experiments were conducted: 1.) Elevated plus maze investigations consisting of pregnenolone 4 mg/kg or vehicle pre-treatment, followed by predator stress vs. sham exposure (n = 6-8 animals per group), and 2.) Characterization of neurosteroid levels in rat hippocampus and serum one hour post-treatment with pregnenolone 4 mg/kg or saline vehicle (n = 6 per group) utilizing gas chromatography/mass spectrometry preceded by high performance liquid chromatography.

Results: The predator stress paradigm reliably increased anxiety-like behaviors as assessed by the elevated plus maze model; specifically, vehicle-treated sham animals (non-stressed/no predator exposure) spent a significantly greater proportion of time in the open arms of the elevated plus maze compared to vehicle-treated stressed/predator-exposed animals, t(13) = 3.25, p = 0.003. Pre-treatment with pregnenolone (4 mg/kg) prevented these predator stress-induced decreases in open arm time; analysis of variance revealed a significant interaction between stress (predator stress exposure vs. sham) and treatment (pregnenolone vs. vehicle pre-treatment), F(1,23) = 4.86, p = 0.04. Animals pre-treated with pregnenolone and exposed to predator stress spent a significantly greater proportion of time in the open arms of the elevated plus maze compared to vehicle pre-treated animals exposed to predator stress, t(12) = 1.86, p = 0.04, and also demonstrated significantly decreased anxiety index scores. Treatment with pregnenolone 4 mg/kg significantly increased hippocampal (p = 0.034, n = 6 per group) and serum (p = 0.049, n = 6 per group) pregnenolone levels by approximately 10-fold. Pregnenolone levels in hippocampus (n = 12) and serum (n = 12) were significantly correlated (Pearson r = 0.98, p < 0.0001).

Discussion: Predator stress exposure significantly decreased open arm time in the elevated plus maze compared to sham-exposed animals, and pre-treatment with pregnenolone 4 mg/kg prevented predator stress-induced anxiety-like behaviors. It is thus possible that pregnenolone may represent a promising secondary prevention strategy for PTSD. Additional investigations will be required to test this hypothesis. Hippocampal and serum pregnenolone levels were highly correlated, suggesting that blood neurosteroid levels could serve as proxy or surrogate biomarkers for brain neurosteroid levels. Pregnenolone may have potential as a novel therapeutic for anxiety disorders.

Disclosure: S. Acheson: None. J. Kilts: None. J. Rogers: None. L. Shampine: None. C. Marx: Part 1: Pending patents for the use of neurosteroids and derivatives in CNS disorders and for lowering cholesterol. No patents issued; no licensing in place.

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199. Adolescent Rats are Insensitive to Fluoxetine: Role of Terminals and Receptors

Cynthia Kuhn*, Andrew Arrant

Duke University Medical Center, Durham, USA

Background: There has been considerable concern about the clinical efficacy of serotonin specific reuptake inhibitors (SSRIs) and the risk of self-injury in depressed children and adolescents. One reason that SSRIs might cause different effects in adolescents and adults could be immaturity of the neural circuits through which they regulate affective behavior. One critical component of this circuitry is the serotonergic innervation of the frontal cortex which is critical for the behavioral inhibition caused by SSRIs and the anxiety that can be a side effect of this class of drugs. In the present study, we evaluated the ability of the SSRI fluoxetine to

cause anxiety-like, inhibited behavior in adolescent and adult rats using the light/dark task after a single dose of 10 mg/kg. We also tested the ability of fluoxetine to increase synaptic serotonin in the prefrontal cortex. In addition, we evaluated the behavioral effects of model 5 HT1a and 5 HT2 agonists to explore the role of postsynaptic mechanisms in the altered behavioral response to SSRIs in adolescents.

Methods: Adolescent (PN 28) or adult (PN 70) from Charles River Laboratories were used in all experiments. Vehicle, fluoxetine (10 mg/kg), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) (0.25 or 0.5 mg/kg) or meta-Chlorophenylpiperazine(mCPP) (0.5 or 1 mg/kg) were administered to rats prior to performance of the light/dark (LD) test for anxiety (N = 8-12/group). Behavioral data were analyzed by one or two way repeated measures ANOVA with age (and dose for 8OHDPAT and mCPP) as factors. Serotonin release from the prefrontal cortex at baseline and after fluoxetine (2.5, 5, and 10 mg/kg at hourly intervals ip or 30 um through the microdialysis probe) was determined by microdialysis followed by HPLC. Density of serotonin terminals in the prefrontal cortex was determined by densitometry after immunocytochemistry using an anti-serotonin transporter antibody, fluorescent antibody visualization and quantitation with ImageJ. Microdialysis data were analyzed by three way repeated measures ANOVA with age and time as factors. Terminal density data were analyzed by 2 way repeated measures ANOVA followed by posthoc Newman Keuls. All experiments were approved by the Duke University IACUC.

Results: Fluoxetine caused a significant delay in emergence into the light and decreased time in light in adults but not adolescents. To determine whether immature response of the 5-HT terminal mediated this behavioral response, effects of fluoxetine on extracellular 5-HT in the prefrontal cortex were determined. However, fluoxetine caused a similar rise in extracellular 5-HT after either peripheral administration or direct infusion into the dialysis probe. Furthermore, the density of 5-HT terminals in the prefrontal cortex was only slightly lower in adolescents than adults. The behavioral effects of receptor agonists were probed to investigate the role of postsynaptic receptors in the fluoxetine response. 8-OHDPAT evoked a behavioral pattern that resembled fluoxetine, as the drug caused an increased latency to emerge and decreased time in light only in adults. However, the 5-HT2 agonist mCPP had similar effects in adolescent and adult rats.

Discussion: The results of this study confirm our hypothesis that fluoxetine causes less behavioral inhibition in adolescent rats. However, the mediating mechanism may reflect mainly an immature response at the 5-HT1a receptor or in the downstream neural circuits. These findings suggest that at the onset of therapy, SSRIs may not cause the behavioral inhibition/anxiety in adolescents that they cause in adults. It is possible that this lack of behavioral inhibition could contribute to impulsive self-harm in adolescents. The present data do not provide any specific predictions about clinical efficacy of SSRIs in adolescents. Supported by F31 DA032532-01A1 to AA and DA009079 to CMK.

Disclosure: C. Kuhn: None. **A. Arrant:** None.

200. Identification of a New Therapeutic Intervention that Ameliorates Behavioral Deficits in a Mouse Model of Fragile X Syndrome and Autism Spectrum Disorder

Marjelo A. Mines*, Christopher J. Yuskaitis, Margaret K. King, Eleonore Beurel, Richard S. Jope

University of Alabama at Birmingham, Birmingham, USA

Background: Recent emphasis in research has been dedicated to understanding the pathophysiological mechanisms contributing to Fragile X Syndrome (FXS) and drug interventions can provide significant therapeutic benefits. FXS is the most common hereditary form of mental retardation caused by a single genetic defect, the loss of expression of the *fragile X mental retardation 1* (*FMR1*) gene. FXS is caused by expansion of a tri-nucleotide CGG-repeat in the 5' UTR of the *fmr1* gene. This expansion appears as a weak, or fragile-like, end on the X chromosome. FXS occurs when over 200 CGG-repeats are present in the *FMR1* gene, which prompts methylation of the expanded repeats and leads to silencing of gene transcription and the ultimate loss of the fragile x mental retardation protein (FMRP). FMRP plays important roles in RNA binding and translation regulation, as well as regulation of extracellular transport and sodium-activated potassium channels. FXS results from inadequate expression of functional fragile X mental retardation protein (FMRP). FMRP may have several functions, but it is most well-established as an RNA-binding protein that regulates translation, and it is by this mechanism that FMRP is capable of affecting numerous cellular processes by selectively regulating protein levels. The multiple cellular functions regulated by FMRP suggest that multiple interventions may be required for reversing the effects of deficient FMRP. Evidence that inhibition of glycogen synthase kinase-3 (GSK3) may contribute to the therapeutic treatment of FXS is investigated here.

Methods: This study used adult, male C57Bl/6J littermates, ∼3 months of age, with or without a disruption of the *Fmr1* gene (originally kindly provided by Dr. W. Greenough, University of Illinois) and matched wild-type mice. The *Fmr1* knockout mice were generated by breeding male C57BL/6J hemizygous *Fmr1* knockout mice and female C57BL/6J heterozygous *Fmr1* knockout mice to generate male homozygous *Fmr1* knockout mice and wild-type littermates. Genotype was confirmed by PCR using the Jackson Laboratory protocol for genotyping *Fmr1* mice. For chronic lithium treatment, mice were given *ad libitum* water and saline (to prevent hyponatremia caused by lithium-induced increased excretion of sodium) and were fed pelleted chow containing 0.2% lithium carbonate. All mice were housed and treated in accordance with National Institutes of Health guidelines and procedures with mice were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. Cognitive deficits, hyperactivity, macroorchidism, and autism-like behaviors, all of which characterize FXS, were investigated in *Fmr1* knockout mice.

Results: *Fmr1* knockout mice displayed significantly greater GSK3 activity than wild-type littermates. Chronic lithium treatment was able to normalize GSK3 activity levels, making them comparable to wild-type baseline activities. *Fmr1* knockout mice also displayed hyperactivity in the open-field behavior paradigm, as compared to wild-type mice, and this hyperactivity was normalized by chronic lithium treatment. *Fmr1* knockout mice displayed impaired memory retention in the passive avoidance test and retention was improved by chronic lithium treatment. Studies of *Fmr1* knockout mice may be useful for research focusing on Autism Spectrum Disorders (ASD), because many patients with FXS also display autistic characteristics. Accordingly, examination of socialization behaviors of *Fmr1* knockout mice revealed that *Fmr1* knockout mice respond similarly to wild-type mice when introduced to 1 stimulus mouse (S1) and display no sociability deficits. When introduced to a second stimulus mouse (S2), the *Fmr1* knockout mice displayed increased social impairments, including inability to recognize the "new" mouse, spending less time in S2 chamber and decreased nose contacts with S2, indicative of impaired preference behavior. Inhibition of GSK3 with chronic lithium treatment reversed all socialization impairments in *Fmr1* knockout mice, thereby increasing sociability and restoring social preference.

Discussion: Amelioration of FXS- and ASD- associated phenotypes suggests that targeting GSK3 may serve as a plausible therapeutic avenue for treatment. Utilizing genetic mouse models, which mimic some but not all of FXS-associated deficits, our group, as well as others, have linked GSK3 and its hyperactivity in FXS, to deficits and impairments commonly seen in FXS. Collectively,

these results confirm that abnormally active GSK3 in *Fmr1* knockout mice contributes to FXS- and ASD-associated deficits and that these can be ameliorated by therapeutic lithium treatment.

Disclosure: **M. Mines:** None. **C. Yuskaitis:** None. **M. King:** None. **E. Beurel:** None. **R. Jope:** None.

201. The Intrinsic Rewarding Properties of Pramipexole in Rats: Comparison with L-DOPA, Effects on Risk-taking and Associative Learning, and Alterations Following Dorsal Striatal Dopamine Deafferentation

T. Celeste Napier*, Stephanie Tedford, Gregory Ruber, Sandra Rokosik

Center for Compulsive Behavior and Addiction, Department of Pharmacology, Rush University Medical Center, Chicago, USA

Background: D2/D3 preferring dopamine receptor agonists (e.g., pramipexole) are approved therapy for Parkinson's disease (PD) and restless leg syndrome, but are being widely explored for off-label therapies for a variety of CNS disorders, including fibromyalgia, depression, and bipolar disorder. Commiserate with this wide use, impulse control disorders (ICDs) are reported for some patients regardless of the underlying pathology. In contrast, therapy with the indirect dopamine agonist, L-DOPA, is not typically associated with ICDs (however, an addiction-like dopamine dysregulation syndrome (DDS) is observed with L-DOPA treatments). To better understand these phenomena, we developed a novel means to study risk-taking aspects of ICDs, i.e., a probability discounting task wherein intracranial self-stimulation (ICSS) is used as the positive reinforcer (Rokosik and Napier, J Neurosci Meth 198:260, 2011). As ICSS provides a means for repeated testing that is devoid of reward tolerance/satiety, we studied the effects of chronic pramipexole and L-DOPA in this task. Agonist-induced ICDs and L-DOPA-induced DDS are most frequently reported in PD. Thus, discounting was compared to motoric and rewarding effects of the drugs in a rat (Sprague Dawley) model of PD and controls.

Methods: All studies were IACUC approved. Lever pressing for various ICSS current levels provided an indication of reward magnitude. A choice task was used to ascertain each rat's preference for a large reward delivered with an uncertain probability as opposed to a small reward that was always delivered. Preference for the former, an indication of riskiness, was analyzed with rmANOVA and *post hoc* Newman Keuls. Condition place preference (CPP) was used to indicate reward-mediated associative learning. Time spent in the drug-paired side before and after conditioning was analyzed with a paired *t*-test. Motor function was evaluated using the forelimb adjustment stepping task and analyzed with rmANOVA + Newman Keuls. For appropriate protocols, some rats underwent stereotaxically guided implantation of stimulating electrodes within the lateral hypothalamus of the medial forebrain bundle and/or infused with 6-OHDA (PD-like) or its 0.2% ascorbic acid vehicle (controls) into the dorsolateral striatum. Lesions were verified with tyrosine hydroxylase immunohistochemistry.

Results: Pramipexole and L-DOPA doses used in these studies improved stepping deficits in PD-like rats. We observed that 13 days of pramipexole increased risk-taking above pretreatment baseline in both controls (n = 10) and PD-like (n = 11) rats (e.g., preference for the large reinforcer was enhanced 30-45% at the most uncertain probabilities; p < 0.05). This effect did not show tolerance or sensitization. Risk-taking subsided upon termination of treatment and was reinstated with reinitiation of pramipexole treatments (p < 0.05). Results collected to date reveal that L-DOPA did not increase risk-taking (p > 0.05). L-DOPA nor pramipexole altered ICSS current vs. lever pressing function in the range tested

with the discounting task. However, subthreshold ICSS frequencies became sufficient to support lever pressing with pramipexole treatments. Supporting the idea that pramipexole can directly mediate reward, the agonist was sufficient to induce CPP in both controls (n = 14; p = 0.04) and PD-like rats (n = 14; p = 0.01). The PD-like rats were distinguished in showing CPP at a pramipexole dose half of that needed to induce place preference in controls.

Discussion: These studies revealed that (i) pramipexole can increase risk-taking using an ICSS-mediated probability discounting task, (ii) tolerance does not develop to these effects with chronic administration, (iii) the effects are reversible, and (iv) are independent of the PD-like condition. Further, (v) pramipexole is sufficiently rewarding to support place preference, and (vi) the PD-like condition may render the individual more vulnerable to the rewarding effects of the agonist. The risk-taking profile of pramipexole was not emulated by L-DOPA. As pramipexole directly activates D2/D3 dopamine receptors, these receptors are critical in both the risk-taking and rewarding effects of the drug. The current findings identify a vulnerability of the PD-like state for the rewarding effects of pramipexole. Ongoing studies in the probability discounting task are determining if such a vulnerability is also revealed for risk-taking when lower doses of the agonist are tested. Regardless, these findings indicate that pramipexole therapy has the potential to promote ICDs regardless of the pathology being treated.

Disclosure: **T. Napier:** None. **S. Tedford:** None. **G. Ruber:** None. **S. Rokosik:** None.

202. Noradrenergic Function and Impulsivity in ASPD and Healthy Controls

Alan C. Swann*, Marijn Lijffijt, Scott D. Lane, Joel L. Steinberg, F. Gerard Moeller

UT Health Science Center, Houston, USA

Background: There is conflicting information on relationships between noradrenergic function and impulsivity. Data from animals and humans suggest that rapid-response impulsivity may by increased by noradnergic (NE) stimulation and related to increased stress-related arousal. This relationship may differ in ASPD, where stress-activated autonomic activation is apparently reduced. We administered placebo or yohimbine, which increases NE release by blocking alpha-2 receptors, in controls and matched subjects with ASPD, and compared effects on plasma MHPG (NE metabolite), plasma HVA (dopamine metabolite), blood pressure and pulse, and performance on a Continuous Performance Test designed to measure impulsivity.

Methods: Diagnosis used SCID for DSMIV-TR. There were 24 healthy controls and 12 subjects with ASPD. Procedures were carried out in the Clinical Research Unit of Memorial hermann Hospital, a component of the UT health Science Center's Center for Clinical and Translational Sciences. Subjects were administered placebo or yohimbine (0.4 mg/kg po) in randomized, counterbalanced order. Blood pressure, pulse and plasma MHPG and HVA were measured 30 minutes before (after acclimatization) and every 30 minutes after drug administration for 4 hours. The Immediate Memory Task (IMT), a continuous performance test designed to measure impulsivity and attention, was administered before and 15, 45, 75, and 105 minutes after drug administration. Data analyses used analysis of variance or Pearson correlation coefficients. Standard effect sizes (ES) were calculated as the difference divided by the pooled standard deviation. Significance of differences between correlation coefficients was estimated using the Fisher r-z transformation.

Results: At baseline, subjects with ASPD had higher systolic BP (ES = 0.85) and lower pulse rate (ES = 0.96) than controls. Effects of yohimbine were identical in the two groups for systolic

BP (ES 0.95 in controls and 1.11 in ASPD), diastolic BP (ES 0.63 and 0.56) and pulse (ES = 0.48 and 0.47). Baseline plasma HVA and MHPG did not differ between groups (ES = 0.1). However, yohimbine increased plasma MHPG more in controls (ES = 1.3) than in ASPD (ES = 0.25). Yohimbine increased impulsive IMT errors in controls and decreased them in ASPD; the individual effects were not significant but the effect of yohimbine differed between the two groups (F 1,34 = 8.2). Change in plasma MHPG correlated significantly with change in impulsive errors in controls (r = 0.794) but not in ASPD (r = −0.18); the correlations differed significantly (z = 2.75). There were no significant relationships between plasma HVA and impulsive errors.

Discussion: Autonomic effects of yohimbine were identical in controls and ASPD, although yohimbine had a significantly smaller effect on plasma MHPG in ASPD. This suggests that NE release was identical in the groups, but that metabolism of the NE differed, consistent with lower monoamine oxidase activity in ASPD. The results directly confirm previous indirect evidence that NE is related to increased rapid-response impulsivity in controls. Relationships between NE, or autonomic arousal, and impulsive behavior may differ between controls and individuals with ASPD.

Disclosure: A. Swann: Part 1: Abbott Laboratories (honorarium); Astra Zeneca (consultant); Bristol Myers Squibb (consultant/data analysis); Cephalon (DSMB); Merck (honorarium, consultant); Pfizer (DSMB); Sanofi Aventis (consultant, honorarium. **M. Lijffijt:** None. **S. Lane:** None. **J. Steinberg:** None. **F. Moeller:** None.

203. Dopamine D_4 receptors Influence "Near-Miss"-like Errors on a Rodent Slot Machine Task

Paul J. Cocker, Robert D. Rogers, Bernard Le Foll, Catharine A. Winstanley*

University of British Columbia, Vancouver, Canada

Background: Gambling is becoming more socially acceptable and opportunities to gamble are increasing. Concern is growing as to the impact these changes will have on the incidence and prevalence of pathological gambling, treatments for which are limited and often ineffective. Clinical research has demonstrated that the severity of disordered gambling correlates with the degree to which individuals display cognitive biases related to gambling outcomes. One such bias is the "near-miss" effect, in which a stimulus proximal to a win induces the sensation of "almost winning". Such near-miss stimuli, although reported to be subjectively aversive, galvanise further game play. Slot machines provide clear examples of the near-miss effect when two out of the three wheels align, and are also a highly compelling form of gambling. We have successfully developed a rat slot machine task to explore the neurobiological basis of the near-miss effect, and previously observed that the D_2-like receptor agonist quinpirole significantly increased erroneous responses, such that animals responded to near-miss and loss trials as if they would lead to a winning outcome (Winstanley et al., 2011). We therefore designed the current study to determine which of the D_2 receptor family (D_2, D_3 or D_4) was critically involved in quinpirole's effects.

Methods: 32 rats were trained to perform the slot-machine task. In this paradigm, rats had 30 minutes to earn as many sugar pellets as possible. Animals began each trial by responding on the "roll" lever which caused the first light in a three-light aperture array to begin flashing. Once the rat made a nosepoke response at the flashing aperture, the light set to either on or off. The second light in the array then began to flash, and the animal was again required to respond at the flashing aperture to set the position of the light, at which point the third aperture began to flash. Once the rat had responded at all three apertures, the collect and roll levers were extended and the rat was required to choose whether to start a new

trial or to attempt to collect reward. If all three lights had set to the on position (a "win" trial), responding on the collect lever lead to delivery of 10 sugar pellets. On any other trial type (any 2 lights on, any 1 light on, 0 lights on), responding on the collect lever lead to a 10s time-out period during which reward could not be earned. Once a stable pattern of choice was observed across the different trial types, the effects of selective D_2 (L741,626), D_3 (SB277011A), and D_4 (L745-870) antagonists were observed, as well as selective D_3 (PD128907) and D_4 (PD168077) agonists. We also determined whether any selective antagonist could block the impairment in performance caused by quinpirole.

Results: As reported previously, animals responded appropriately on the collect lever on almost 100% of win trials, and favoured the roll lever on 1-light and 0-light losses. On 2-light loss trials, rats exhibited a significant bias towards responding on the collect lever (60-70%), reminiscent of a near-miss effect. Compounds selective for the D_3 receptor did not affect task performance, and neither did the selective D_2 receptor antagonist. The selective D_4 receptor agonist increased erroneous collect responses on non-win trials in a manner similar to quinpirole. Conversely, the D_4 receptor antagonist produced small improvements in task performance. Quinpirole still increased erroneous collect responses on all loss trials in the presence of the selective D_2 and D_3 receptor antagonists. However, co-administration of the selective D_4 receptor antagonist significantly attenuated quinipirole's effects.

Discussion: These data suggest that D_4 receptors play a pivotal role in guiding choice behavior and expression of the near-miss bias in this slot machine analogue. We originally hypothesised that quinpirole administration may lead to an increase in collect responses on non-win trials due to the loss of phasic dopamine signalling at the level of the striatum or VTA, leading to erroneous reward prediction errors. However, D_4 receptors are predominantly expressed within the frontal cortex, implying that the decision to collect or roll may be driven by top-down cognitive signals pertaining to the appropriate interpretation of the pattern of lights. Ultimately, these results indicate that D_4 receptor antagonists may be useful in the treatment of compulsive slot machine play.

Disclosure: P. Cocker: None. **R. Rogers:** None. **B. Le Foll:** None. **C. Winstanley:** Part 1: Theravance.

204. Allosteric Modulators, Atypical and Constrained Agonists of the TRPV1 Ion Channel

Michael J. Iadarola*, Krisztian Kaszas, Hong-Tao Ma, Jason M. Keller, Theresa R. Lii, Steven A. Titus, Melanie Bryant, Marc Ferrer, Erika Englund, Juan Marugan, Owen B. Mcmanus, Noel Southall

NIDCR/NIH, Bethedsa, USA

Background: Despite the fact that pain is one of the main reasons patients seek medical care, new pharmacological agents for acute and chronic pain have been difficult to develop into new medicines. One approach to fill this need is to obtain a deeper understanding of the molecular basis for sensing tissue damage, the neural circuits in which such molecular sensors are embedded, and the pathophysiological environment in which they function[1]. The transient receptor potential ion channel, family V, number 1 (TRPV1), is expressed in a subpopulation of A-delta and C-fiber primary afferent nociceptors. TRPV1 is activated by capsaicin, low pH, and noxious heat and sensitized by inflammatory mediators and is a potential target for analgesic drug development[2,3]. Blocking or interfering with pain signals before they enter the CNS is viable approach to producing analgesia without central side effects. However, direct orthosteric capsaicin antagonists can produce a complete loss of sense heat pain and this lack of feedback is a burn risk[4]. We propose TRPV1 allosteric modulation

as a strategy to impose an activity-dependent constraint within the context of inflammation and tissue damage. The goals of this study are to characterize (a) the diverse agonists and (b) the allosteric modulators emerging from HTS screening.

Methods: We screened TRPV1 for agonists and positive allosteric modulators using the Molecular Libraries Probe Production Centers Network small molecule library ($>$300,000 compounds). The assay was performed on HEK 293 cells ectopically expressing TRPV1 with a no-wash calcium fluorescence assay in 1536 well format on a Hammamatsu FDSS 7000 dynamic plate reader. The assay design was a two-addition screen involving a baseline read followed by addition of test drugs from the library and then addition of an EC_{20} concentration of capsaicin. Agonists were identified by calcium increases upon first addition of compounds from the library. For allosteric modulators, an increase in the EC_{20} capsaicin signal was evidence for PAM activity. Follow up involved calcium-45 uptake, cell-based calcium imaging, electrophysiology, synthesis and testing of analogs, and computational studies of the chemical characteristics of the compounds.

Results: The TRPV1 PAM part of the screen yielded a variety of compounds that induced an increase in capsaicin-stimulated free intracellular Ca^{++} concentration. Re-screening of candidates on the FDSS 7000 identified 18 candidate PAMs. Examination of these 18 with three different approaches disclosed three compounds with PAM activity in multiple assays and one further compound was observed to positively modulate activation by low pH. Screening with rat primary cultured dorsal root ganglion neurons, which contain a sub-population that endogenously expresses TRPV1, yielded one compound with prominent PAM activity. Regarding orthosteric agonists, the primary screen revealed a very high number of compounds ($>$10,000) that were weak agonists ($\geq$20% increase in fluorescence) and progressively fewer as potency approached that of capsaicin. Two groups of agonists were selected for further study. One set (8 analogs) was based on a rigidified 1,4 benzodiazepine scaffold. The other set was based on a N-(p-tolyl)benzofuro[3,2-d]pyrimidin-4-amine backbone with analogs modified at the N-(p-tolyl) position. These were the most chemically distinct from capsaicin and initially were classified as "atypical" TRPV1 agonists, however, several orthosteric vanilloid antagonists blocked agonist activity suggesting overlap in the binding sites. The conformationally constrained agonists were approximately equipotent with capsaicin, but all agonist activity was lost when free rotation was permitted at certain sites.

Discussion: New mechanism-based compounds for treating severe to moderate pain to replace or augment opioids are being discovered, but are proving to be difficult to translate into new analgesics. The present studies suggest wide latitude in chemical structures capable of eliciting TRPV1 agonist activity, yet specific spatial requirements for high potency; conclusions also supported by the diversity of natural product agonists[5]. We identified several TRPV1 PAMs with diverse structures. The use of allosteric sites in TRPV1 for therapeutic purposes may represent a method to modulate inflammatory pain conditions. We hypothesize that a TRPV1 PAM may produce a calcium overload in active nociceptive nerve endings leading to functional inactivation and, potentially, a long duration, non-opioid analgesic action.

1. Gavva N 2008
2. Gunthorpe & Szallasi 2008
3. Patapoutian A et al 2009
4. Rowbotham M et al 2011
5. Appendino G et al 2008

Disclosure: M. Iadarola: None. **K. Kaszas:** None. **H. Ma:** None. **J. Keller:** None. **T. Lii:** None. **S. Titus:** None. **M. Bryant:** None. **M. Ferrer:** None. **E. Englund:** None. **J. Marugan:** None. **O. Mcmanus:** None. **N. Southall:** None.

205. Pharmacological and Behavioral Characterization of the Norepinephrine and Dopamine Reuptake Inhibitor EB-1020: A Novel Pharmacotherapy for ADHD

Frank I. Tarazi*, Yong Kee Choi, Krystyna Gołembiowska, Magdalena Kowalska, Frank P. Bymaster

Harvard Medical School/McLean Hospital, Belmont, USA

Background: Attention-deficit hyperactivity disorder (ADHD) is a serious neuropsychiatric disorder found in 5-10% of children and 3-4% of the adult population. Drugs used to treat ADHD activate norepinephrine (NE) and/or dopamine (DA) neurotransmission, but pharmacotherapy of ADHD is less than optimal and is typically associated with adverse events.

Methods: We have developed and characterized a new norepinephrine/dopamine reuptake inhibitor EB-1020 (1R,5S)-(+)-1-(naphthalen-2-yl)-3-azabicyclo[3.1.0]hexane HCl) as a novel agent for ADHD pharmacotherapy. We assessed EB-1020's effects on monoamine uptake, and NE and DA levels in cortex and striatum using in vivo microdialysis, as well as its effects on locomotor activity, stereotyped behaviors, and tail suspension test in intact adult rats and locomotor activity in juvenile rats lesioned with 6-hydroxydopamine (6-OHDA) as neonates.

Results: EB-1020 inhibited NE and DA reuptake in cell lines transfected with human NE and DA transporters with IC_{50} values of 6 and 38, respectively. In microdialysis studies, EB-1020 markedly increased NE and DA levels in rat prefrontal cortex and DA in striatum. Behavioral studies demonstrated that EB-1020 decreased immobility in the mouse tail-suspension test of depression, and modestly stimulated locomotor activity in adult rats only at the highest dose tested, suggesting minimal drug abuse liability. EB-1020 dose-dependently inhibited juvenile hyperactivity in the rat neonatal 6-OHDA forebrain lesion ADHD model with minimal effects on sham-lesioned control littermates.

Discussion: EB-1020 has marked effects on NE and lesser effects on DA uptake, a unique profile that may activate catecholaminergic circuitries in cortical regions involved in attention and execution of cognitive functions. Furthermore, EB-1020 was active in animal models of ADHD and depression, which may be useful for patients with combined disorders since ADHD is highly comorbid with depression. Overall, these data suggest that EB-1020 is a novel agent with improved pharmacotherapy for pediatric and adult ADHD patients, with benign safety, tolerability profiles, and a low incidence of drug abuse liability.

Disclosure: F. Tarazi: Part 4: Novartis, Forest Labs, Lundbeck, Euthymics Bioscience. **Y. Choi:** None. **K. Gołembiowska:** Part 4: Euthymics Bioscience, Inc. **M. Kowalska:** Part 4: Euthymics Bioscience, Inc. **F. Bymaster:** Part 2: Euthymics Bioscience, Inc., Part 3: Euthymics Bioscience, Inc., Part 5: Euthymics Bioscience, Inc.

206. Individual Differences in Inherent Impulsivity Track with Cocaine-Seeking Behavior and Serotonin (5-HT) 2C (5-HT$_{2C}$) Receptor mRNA Editing in the Corticoaccumbens Circuit

Noelle C. Anastasio*, Robert G. Fox, Sonja J. Stutz, F. Gerard Moeller, Ronald B. Emeson, Kathryn A. Cunningham

Center for Addiction Research, Dept Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, USA

Background: Various aspects of drug abuse, including the initiation of drug-taking, the transition from casual to compulsive drug use, the maintenance of drug-seeking behaviors as well as the penchant to reinstate drug-seeking correlate with high levels of impulsivity. Dysregulation in serotonin 2C receptor (5-HT$_{2C}$R) function within the mesocorticolimbic circuit has been implicated in the stages of the addiction cycle as well as the manifestation of the impulsive enodphenotype. Editing of 5-HT$_{2C}$R mRNA, a

mechanism that post-transcriptionally alters the coding properties of the mRNA, results in the synthesis of several receptor variants (*e.g.*, $5\text{-HT}_{2C-VNV}R$) with reduced functional properties. We tested the hypothesis that individual differences in inherent impulsivity are associated with edited isoforms that encode for low tonic/constitutive $5\text{-HT}_{2C}R$ activity in key corticolimbic nuclei.

Methods: Male rats were screened in the one-choice serial reaction time (1-CSRT) task to assess level of impulsive actions. Rats were maintained at $\sim 90\%$ free-feeding weight and trained on a 5-sec intertrial interval (ITI) and then subjected to an 8-sec ITI challenge session to more readily detect impulsive action. The upper and lower 25% of rats were identified based on premature responses on ITI8 as high impulsive (HI) or low impulsive (LI). Brain tissue was analyzed for levels of $5\text{-HT}_{2C}R$ isoform mRNA expression using high-throughput multiplexed transcript analysis. Two different cohorts of HI/LI rats were then trained to self-administer (SA) cocaine at a low dose (0.25 mg/kg/inf; 60 min/day; FR1; 14 days) or a higher dose (0.75 mg/kg/inf; 180 min/day; FR1-5; 14 days) to examine cocaine-taking or cocaine-seeking behavior, respectively. Following 14 days of stable self-administration, rats were returned to their home cage; on day 14 of forced abstinence, rats were returned to the test chambers and allowed to lever press to receive presentations of cocaine-paired stimuli (i.e., lights, pump motor) in the absence of a cocaine reinforcer (cue reactivity).

Results: Premature responses assessed on the 8-sec ITI challenge were significantly higher in HI vs. LI rats ($p < 0.001$).The frequency of expression of the most abundant edited $5\text{-HT}_{2C-VNV}R$ isoform in rats, and the fully edited $5\text{-HT}_{2C-VGV}R$ isoform were elevated in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) of HI vs. LI rats ($p < 0.05$). The frequency of expression of the $5\text{-HT}_{2C-IDI}R$ and $5\text{-HT}_{2C-VSV}R$ isoforms was elevated in the NAc while the $5\text{-HT}_{2C-IGI}R$ and $5\text{-HT}_{2C-VGI}R$ isoforms were elevated in the mPFC of HI vs. LI rats. HI rats acquired cocaine SA (10.2 ± 0.2 days) more rapidly than LI rats (13.2 ± 0.5 days; $p < 0.05$) while cocaine intake was higher in HI rats (2.12 ± 0.5 mg/kg/day) vs. LI rats (0.97 ± 0.2 mg/kg/day; $p < 0.05$). HI rats ($81.6 +/- 9.9$ active lever presses) exhibited higher cue reactivity vs.LI rats ($56.7 +/- 5.6$ active lever presses; $p < 0.05$).

Discussion: These data indicate a relationship between the expression of reduced-function $5\text{-HT}_{2C}R$ isoforms and the impulsivity endophenotype. Thus, it is possible that diminished $5\text{-HT}_{2C}R$ function in the NAc and mPFC represents a mechanistic imbalance that drives impulsivity and cue reactivity, two key neurobiologically-determined constructs that contribute to relapse.

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207. Glycosylation of Neuroactive Peptides to Enhance CNS Bioavailability

Edward Bilsky*, Torsten Falk, Scott Sherman, Jim Cormier, Lindsay St. Louis, Denise Giuvelis, Robin Polt, Glenn Stevenson

University of New England, Biddeford, USA

Background: Peptides are traditionally thought to be poor candidates for development as CNS therapeutics. Our group has been studying glycosylation of enkephalin and endorphin based peptides as a strategy for increasing the stability and blood-brain barrier penetration of the native peptide. These glycopeptides have shown broad spectrums of activity in preclinical models of acute and chronic pain while having a more favorable side effect profile compared to opioid analgesics that target the mu opioid receptor. The most recent research has made further assessments of *in vivo* pharmacology, antinociceptive efficacy and side effects of lead compounds, while also exploring potential applications in movement disorders and depression.

Methods: Rodent models (ICR mice and Sprague-Dawley rats) of neuropathic and inflammatory pain were used to assess anti-nociceptive efficacy. Measures of opioid side effects included actions on gastrointestinal transit, respiration, and addiction liability (place preference and intravenous self administration). Unilateral 6-OHDA lesions and amphetamine induced rotations were used as a model of Parkinson's disease and the mouse forced swim test was used to assess antidepressant activity. Morphine and other commonly used opioid analgesics were used as comparison controls.

Results: The enkephalin analog MMP2200 produced dose-related antinociceptive effects in neuropathic and post-surgical models of pain, with effects being mediated through delta and mu opioid receptors. The compound demonstrated less addiction liability compared to equivalent doses of morphine and fentanyl while producing side effects profiles different than (tolerance/dependence, muscular rigidity) or similar to (GI transit and respiration) morphine and fentanyl. MMP2200 reduced immobility time in the forced swim test and reduced amphetamine-induced rotations in hemi-parkinsonian rats; this effect was fully blocked by naloxone, an opioid receptor antagonist. MMP-2200 blocked apomorphine-induced hyper-kinesia in reserpine treated animals. Preliminary data with the endorphin-based glycopeptides indicated that glycosylation did not negatively impact receptor affinity and antinociceptive potency.

Discussion: Taken with our earlier findings, glycosylation of enkephalin and endorphin based peptides increases stability and CNS penetrability, in some cases making them more potent than systemic morphine. The lead enkephalin glycopeptide MMP-2200 has been extensively characterized in various preclinical efficacy and side effect assays in rodents, demonstrating broad efficacy and generally more favorable side effect profiles than morphine. MMP-2200, could also have utility as an anti-dyskinetic, rather than a prokinetic, agent in Parkinson's disease.

Disclosure: E. Bilsky: Part 2: Co-founder of Biousian Biosystems Inc. **T. Falk:** None. **. Sherman:** None. **J. Cormier:** None. **L. St. Louis:** None. **D. Giuvelis:** None. **R. Polt:** Part 3: Co-Founder of Biousian Biosystems Inc. **G. Stevenson:** None.

208. ^{1}H-MRS Analysis of Neurochemical Profiles after Acute Ethanol in Adult Mice Lacking Adenylyl Cyclase Isoforms 1 and 8

Matthew P. Galloway*, Farhad Ghoddoussi, Nadeem Sawaf, H. Michael Marsh, Alana C. Conti

Wayne State Univ Sch Med Psychiatry, Anesthesiology, Neurosurgery, Detroit, USA

Background: Double knock out (DKO) mice with a genetic deficit in the calcium-dependent adenylyl cyclase (AC) isoforms 1 and 8 show increased sensitivity to the neurodegenerative and sedative effects of ethanol as well as decreased phosphorylation of synapsins I & II, eukaryotic elongation factor-2, and dynamin after acute challenge. Additionally, DKO mice exhibit pro-depressant behavioral phenotypes, impaired LTP and LTD, neurotransmitter vesicle recycling, decreased responses to acute and chronic pain, and decreased behavioral and electrophysiological responses to repeated morphine. Given the role of glutamate (GLU) systems in the altered DKO physiology, we used proton magnetic resonance spectroscopy (^{1}H-MRS) to determine neurochemical profiles after acute ethanol treatment of DKO and wild type (WT) mice.

Methods: Adult male AC1/8 DKO and WT mice were sacrificed 45 min after treatment with either saline or ethanol (4g/kg IP, $n = 8\text{-}10$ per group). Brains were rapidly removed, 2 mm coronal slices obtained and quickly frozen on solid CO_2; 1.5 mm diameter punches containing a region of interest were taken from the frozen slice. Intact tissues, in a defined buffer with TSP, were analyzed

with magic angle spinning ^{1}H-MRS on an 11.7T Bruker magnet (Avance) system. Absolute quantities of 20 neurochemicals were determined with a customized LCModel and normalized to tissue weight ($\sim$ 2-3 mg). Neurochemical profiles were also determined in a second cohort of untreated DKO and WT mice. Significant differences ($P < 0.05$) were assessed with a t-test for each neurochemical compared to saline controls.

Results: In the anterior cingulate cortex (ACC) and nucleus accumbens (NAc) of untreated DKO, glycerophosphorylcholine (GPC) levels increased (60-70%) significantly compared to WT. After acute ethanol, glutamine and lactate increased (30%-50%) significantly in the ACC regardless of genotype (compared to respective saline controls); the GLN effect was also observed in NAc of WT, but not DKO, mice. In DKO ACC, ethanol significantly increased the GLN/GLU ratio and decreased GABA (-10%) significantly. Finally, N-acetylaspartyl glutamate (NAAG) decreased (-20%) in the NAc of both genotypes.

Discussion: Increased GPC in the DKO is consistent with perturbed membrane turnover, possibly reflecting previous observations of disrupted vesicle recycling or the absence of membrane bound AC1/8 protein. Since GPC is derived from PLA-2 mediated hydrolysis of membrane PtDCho to form inflammatory prostaglandins and leukotrienes, the GPC elevation may represent a pro-inflammatory status in the DKO. Ethanol-induced elevations of cortical GLN and LAC is consistent with enhanced GLU turnover and disordered energy homeostasis, a potential prelude to neurotoxicity. However, a similar response in the DKO cortetx suggests that these responses in the cortex do not underlie DKO vulnerabilities to ethanol. However, increased GLN/GLU combined with decreased GABA in the DKO may reflect a milieu contributing to potential neurotoxicity. Ethanol mediated decreases in NAAG, a glutamate autoreceptor agonist, represents a novel response of the accumbal GLU systetm. On going studies of other brain regions as well as the effects of the μ opioid agonist fentanyl will provide further insight into the unique DKO phenotype. Support: Joe Young Sr Research Fund in Psychiatry (MPG), Fund for Medical Research in Anesthesiology (MPG, FG, HMM), VAMC and K01-AA017683 NIH (ACC).

Disclosure: M. Galloway: Part 1: My spouse is district manager of pharmaceutical sales for Glaxo Smith Kline (GSK)., Part 2: 2011-GSK (Spouse's employer). **F. Ghoddoussi:** None. **N. Sawaf:** None. **H. Marsh:** None. **A. Conti:** None.

209. Synergistic Reduction of Cocaine Actions in Mice Treated with Cocaine Hydrolase and Anti-Cocaine Antibodies or Vaccine

Yang Gao*, Xiaoyun Shen, Frank Orson, Berma Kinsey, Tom Kosten, Marilyn E. Carroll, Stephen Brimijoin

Mayo Clinic, Rochester, USA

Background: We are investigating potential treatments for cocaine addiction by intercepting drug molecules in the circulation and preventing access to reward centers in the brain. Current experiments focus on cocaine-binding antibodies and cocaine-hydrolyzing enzyme (CocH) derived from human plasma butyrylcholinesterase (BChE). Each agent previously proved effective in reducing cocaine-stimulated locomotor activity in mice and rats, and gene transfer of CocH was recently shown to prevent cocaine-induced reinstatement of cocaine seeking behavior in rats for six months. Our current objective was to determine if cocaine antibody and cocaine hydrolyzing enzyme work efficiently in concert to provide enhanced therapeutic effects while reducing cocaine toxicity.

Methods: CocH (a mutant BChE: A199S/S287G/A328W/Y332G) was expressed in Cos cells and purified as previously described (Gao *et al*, Chem Biol Interact 175: 83-87, 2008). Cocaine vaccine was based on conjugates of norcocaine with keyhole limpet hemocya-

nin (100 μg/mouse with booster dose at 3 weeks). Titrated antibody-rich antisera from vaccinated animals were given in i.p. doses of 12 mg/kg (cocaine-binding IgG). Cocaine hydrolase activity and plasma / tissue levels of ^{3}H-cocaine were determined by radiometric assays. Histopathology was carried out with hematoxylin-eosin stains on cryostat sections of fresh frozen liver.

Results: In a functional observational battery (FOB) with measures of spontaneous locomotor activity, grip strength, gait, and other signs, BALB/c mice exhibited no direct toxicity from CocH (up to 3 mg/kg iv or ip), or antibody, or vaccine. CocH at 1 mg/kg reduced drug-induced locomotor activity to baseline after moderate cocaine doses (40, 60 mg/kg, i.p.). Antibody alone or cocaine vaccine had smaller effects. At toxic cocaine doses (100 or 120 mg/kg) enzyme, antibody, and vaccine each prevented lethality but had little effect on the cocaine-induced hyperlocomotion. When given in combination, however (e.g., enzyme + vaccine) they again reduced locomotor activity to baseline. Assays of liver-derived ALT activity in plasma showed increases from near-zero resting values to 17,000 ± 5000 U/ml in unprotected mice 24 hours after high dose cocaine (100 mg/kg). ALT levels were slightly reduced by antibody pre-treatment (9000 ± 3000 U/ml) or enzyme (7500 U/ml) and were reduced almost to baseline (346 ± 200 U/ml, p < 0.01) by antibody and enzyme combined. Grip strength was fully preserved after dual pre-treatment. Livers sampled after euthanasia showed severe damage including centrilobular necrosis except in mice given dual treatments. Livers from animals given vaccine in addition to enzyme were indistinguishable from those of control mice receiving no drug.

Discussion: We conclude that the BChE-based cocaine hydrolase has little intrinsic toxicity but can greatly reduce cocaine's behavioral and liver toxicity. The favorable effects are substantially enhanced in the presence of anti-cocaine antibodies.It is likely that specific antibodies and hydrolase enzymes can act in concert to provide highly effective interception of cocaine molecules before they reach brain centers. Combined treatments based on this concept appear worth exploring for human application. Testing effects on drug-seeking behavior in operant models is a logical next step.

Disclosure: Y. Gao: Part 2: Salary from Mayo Clinic. **X. Shen:** Part 2: Salary from my home institution. **F. Orson:** Part 2: Veterans Affairs Medical Center salary. **B. Kinsey:** Part 2: Baylor College of Medicine. **T. Kosten:** Part 2: Baylor College of Medicine. **M. Carroll:** Part 2: University of Minnesota. **S. Brimijoin:** Part 2: Mayo Clinic and Mayo Foundation.

210. Effect of Adolescent vs. Adult Delta-9-THC Exposure on Neurocognitive Function in Rats

Loren H Parsons*

The Scripps Research Institute, La Jolla, USA

Background: Heavy cannabis use by adults is associated with neurocognitive deficits including impairments of attention, inhibitory control, impulsive behavior and memory that are thought to result from altered prefrontal cortical and hippocampal function. Endogenous cannaboind signaling plays a substantial role in neural development during adolescence, and accordingly exogenous cannabinoid exposure during this developmental period may produce more pronounced and enduring effects than results from exposure during adulthood. The purpose of this study was to compare the effects of Δ9-THC exposure given during periadolescence versus adulthood on subsequent neurocognitive function.

Methods: Δ9-THC Dosing: Male Wistar rats were given 3.0 mg/kg Δ9-THC (i.p.) 2x/day for 21 consecutive days. Adolescent dosing occurred during PND 35-56 and adult dosing during PND 75-96 (n = 10/grp). Age matched control groups received identical dosing

with VEH (n = 10/grp). Antagonist-precipitated withdrawal was evaluated using the CB₁ antagonist SR141716A (3 mg/kg, i.p.) on the final day of drug/VEH dosing. Non-spatial recognition memory testing (object substitution) was conducted on the 14th day after Δ9-THC exposure. Spatial recognition memory testing (object-in-place) was performed on the 15th day after Δ9-THC exposure. 5-Choice Serial Reaction Time Task (5-CSRTT) training began 28 days after Δ9-THC exposure and proceeded until a baseline criterion of 80% accuracy under a 0.5 sec stimulus and 5 sec ITI was achieved. Subsequent challenge tests employed within-session ITI randomization to increase task difficulty.

Results: Administration of 3 mg/kg Δ9-THC resulted in peak plasma levels of 160 ± 21 ng/ml Δ9-THC in adolescent rats and 172 ± 24 ng/ml Δ9-THC in adult rats, with no significant differences between age groups. Antagonist-precipitated withdrawal: In Δ9-THC-exposed rats SR141716A administration induced significant levels of withdrawal-related behaviors including wet dog shakes, paw tremor, teeth chattering and ptosis. This same treatment did not produce any of these behaviors in Δ9-THC -naïve rats. The severity of precipiated withdrawal was significantly more pronounced in rats given drug exposure during periadolescence vs. adulthood (p < 0.01 – 0.05, depending on behavioral measure). Non-spatial recognition memory testing (object substitution): Significant impairment of non-spatial object recognition was evident in Δ9-THC-exposed animals regardless of age group (p < 0.05 vs. VEH for each age), and there was no significant effect of drug exposure age. Spatial recognition memory testing (object-in-place): Significant impairments in spatial recognition memory were evident in rats given Δ9-THC exposure during adulthood (p < 0.05 vs. VEH), though no Δ9-THC effect was evident in animals given drug exposure during periadolescence. 5-Choice Serial Reaction Time Task (5-CSRTT): Although all groups achieved stable baseline performance on this task, the time required for task acquisition was signigicantly greater in animals previously given Δ9-THC exposure (p < 0.05 vs. VEH), and this was more pronounced in animals given Δ9-THC exposure in periadolescence vs. adulthood (p < 0.05). Once acquired, baseline task performance did not differ between Δ9-THC exposed and drug naïve groups, regardless of age. However, Δ9-THC exposed animals displayed greater deficits in accuracy and greater increases in premature and perseverative responding during the within-session ITI challenge tests (p < 0.05 vs VEH for each measure), with a trend toward greater performance deficits in animals given Δ9-THC exposure during periadolescence vs. adulthood.

Discussion: Consistent with prior literature the present findings demonstrate that Δ9-THC exposure induces deficits in both spatial and non-spatial working memory. The age-related difference in disrupted spatial vs. non-spatial working memory suggests a relative protection against Δ9-THC-induced impairment of hippocampal but not cortical function during adolescence. We also find that Δ9-THC exposure impairs the acquisition of the 5-CSRTT and increases impulsive and perseverative behavior under conditions of increased cognitive load. The somewhat greater impairments in 5-CSRTT performance following adolescent vs. adult drug exposure suggests that adolescent Δ9-THC exposure may produce greater cortical disruption than does Δ9-THC exposure during adulthood.

Disclosure: L. Parsons: None.

211. Sex Differences in Fear Conditioning in Posttraumatic Stress Disorder

Sabra Inslicht*, Thomas Metzler, Mohammed Milad, Suzanne Pineles, Scott Orr, Charles Marmar, Thomas Neylan

University of California, San Francisco, San Francisco, USA

Background: Epidemiological data suggest that women are twice as likely as men to develop Posttraumatic Stress Disorder (PTSD). Yet, there is a striking lack of research on the biological mechanisms that may account for increased rates of PTSD in women. Several have argued that PTSD is associated with individual differences in the acquisition and extinction of conditioned fear such that certain individuals may be more likely to associate a conditioned stimulus (CS) to an unconditioned stimulus (US) and have more difficulty in extinguishing the association. Yet, much of our understanding of fear conditioning in PTSD is from research conducted in males or from studies that failed to examine the impact of gender. Surprisingly, studies of healthy humans have suggested that women are more likely to have reduced acquisition or no differences in fear conditioning compared to men. However, this has not been examined in men and women with PTSD. We examined sex differences in the acquisition of conditioned fear in men and women with full or subsyndromal PTSD.

Methods: Twenty-seven adult participants (men, $n = 9$; women, $n = 18$; ages 20-61 years) with full and subsyndromal PTSD completed a laboratory fear conditioning task to test the effect of sex differences on fear conditioning. Participants were interviewed on DSM-IV criteria for PTSD using the Clinician Administered PTSD Scale (CAPS). PTSD status (full and subsyndromal) was determined by a score > 40 and meeting 2 out of 3 of B, C, and D symptom clusters related to trauma. Participants were then trained in a classical aversive conditioning paradigm adapted from Orr and colleagues (2000). During the baseline habituation phase participants were shown computer-generated colored circles (CS). During the acquisition phase colored circles were paired (CS+) or unpaired (CS-) with a mild electrical stimulus (US). Skin conductance levels were assessed throughout the task. Repeated measures ANOVAs were conducted to determine sex differences in responses to the CS+ versus CS-trials within each phase.

Results: Results indicated that while there were no baseline sex differences during the habituation phase, $p = .99$, during the acquisition phase, there was a significant stimulus by sex interaction, $F(1, 78) = 7.64$, $p < .01$. Women showed greater conditioned skin conductance responses (CS+ trials compared to CS- trials) than did men (Differential scores: $M = .56 \mu S$, $SD = .52$ for women and $M = .14 \mu S$, $SD = .19$ for men), suggesting greater acquisition of conditioned fear in female participants with PTSD.

Discussion: Increased acquisition of fear in women may be one possible mechanism involved in differential risk for PTSD. Characterizing these mechanisms may clarify the pathophysiology of PTSD, and account for sex related differences in both vulnerability to trauma and phenomenology of posttraumatic symptomatology.

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212. Influence of Selective Serotonin Reuptake Inhibitors on Fear Extinction

Kelimer Lebron-Milad*, Alina Tsareva, Roger K Pitman, Mohammed R Milad

Mass General Hospital and Harvard Medical School, Charlestown, USA

Background: A common treatment for PTSD as well as others psychiatric disorders are selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine. We recently demonstrated that subjects with PTSD exhibit a deficit in fear extinction. Whether SSRIs improve symptoms of this disorder by promoting the extinction of fear associated with traumatic events has not been

examined. In this study, we began to investigate the role of SSRIs in modulating extinction learning and recall.

Methods: Male rats were exposed to a 3-days fear conditioning and extinction protocol for behavioral analysis. On Day 1, rats were conditioned with 7 tone-footshock trials. On Day 2 the rats were injected with either fluoxetine HCl (10mk/kg in 0.5 mL) or vehicle half an hour prior to 20 tone-no footshock extinction learning trials. On Day 3, additional 15 tone-no footshock extinction trials were presented to measure extinction recall.

Results: Fluoxetine rats extinguished fear responses at the same rate compared to vehicle rats, suggesting no effect of fluoxetine acute administration on extinction learning and extinction recall. However, fluoxetine rats exhibited higher freezing during extinction learning and extinction recall compared to vehicles rats, suggesting an effect of the drug on fear expression.

Discussion: Our results are consistent with observation that some symptoms of psychiatric and mood disorders are temporarily exacerbated at the initiation of SSRI treatment. Further studies in our lab are currently investigating the effects of chronic fluoxetine administration on extinction learning and extinction recall and the influence of sex on the effects of fluoxetine during extinction learning and recall.

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213. Sleep in PTSD vs. Controls - Evidence for Possible Gender Dimorphism

Anne Richards, Thomas J. Metzler, Maryann Lenoci, Leslie Ruoff, Thomas C. Neylan*

University of California, San Francisco, San Francisco, USA

Background: Although subjective sleep disturbance and non-restorative sleep are characteristic symptoms of posttraumatic stress disorder (PTSD), the underlying neurophysiology of these symptoms is poorly understood. Visually scored polysomnography has not consistently demonstrated objective differences in sleep in PTSD compared to healthy subjects. Emerging research using quantitative analysis of the electroencephalogram (EEG) indicates that delta sleep, the type of sleep considered most associated with the homeostatic recovery function, may be decreased in PTSD. Because such research has been conducted primarily in male subjects, information about objective sleep disturbances in female subjects with PTSD is scant.

Methods: A cross-sectional 2x2 design (PTSD/Control x Male/Female) included medication-free medically healthy subjects. The sample was comprised of 42 individuals with current chronic PTSD (52% female; M age = 30.81, SD = 6.55) and 45 age and sex-matched controls without PTSD (51% female; M age = 30.04, SD = 8.07), ranging in age from 20 to 50 years. Ambulatory polysomnography performed over 2 nights in the sleep lab was utilized to measure sleep. Only data from the second night were utilized for analyses, to eliminate first night adaptation effects. The sleep of female subjects was assessed during the follicular phase of the menstrual cycle. Sleep was visually scored using standard methods. Power spectral analysis was conducted using PassPlus software. Energy in the delta band (1-4 Hz), reflecting total power in the delta band through the course of the night, was calculated by Fast Fourier transformation. Epochs visually scored as wake were excluded from the analyses. An inverse square-root transformation was utilized to normalize the delta variable prior to statistical analysis. Two-way ANOVA was used to compare total sleep time (TST) in men and women with PTSD and sex and age-matched controls. Two-way ANOVA was also used to compare energy in the delta band in men and women with PTSD and sex- and age-matched controls.

Results: Male sex was associated with shorter mean TST (F = 12.04, p < 0.001). PTSD status was not a significant predictor of TST, nor was a sex by group interaction detected. There was a significant main effect of PTSD for decreased delta sleep (F = 5.04, p = 0.02) and a trend main effect of male gender for decreased delta sleep (F = 3.24, p = 0.08). Furthermore, there was a statistically significant sex by group interaction, which was driven by the significant relationship between PTSD and decreased delta sleep in men (F = 4.42, p = 0.039). Consistent with our lab's prior research, decreased delta sleep in PTSD is a finding that is mainly found in male subjects.

Discussion: Although existing research has provided conflicting findings about the effects of PTSD on visually scored sleep EEG, decreased delta sleep is emerging as a consistent feature of PTSD in men. Further research is necessary to understand how sleep may differ in men and women with PTSD and to understand the implications of such differences with regards to risk, pathophysiology, comorbid medical illness, treatment and outcomes in PTSD.

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214. Fetal Programming of Sex Differences in Stress Response Circuitry, Endocrine and ANS Deficits in Adulthood: Implications for Understanding Sex Differences in Comorbidity of Depression and CVD Risk

Jill M. Goldstein*, Laura M. Holsen, Brandon Abbs, Sara Cherkerzian, Anne Klibanski, Mady Hornig, Richard Sloan, Stephen Buka

Brigham and Women's Hospital, Boston, USA

Background: Comorbidity of major depressive disorder (MDD) and cardiovascular disease risk (CVD), with a prevalence of ∼20%, will be the number one cause of disability worldwide by 2020, and is significantly higher in women than men. We are investigating shared pathophysiology beginning in fetal brain development that implicates a prenatal stress model. Preeclampsia and growth restriction, implicated in MDD and CVD, are associated with maternal immune activation and fetal disruption of the development of the HPA axis. Here we hypothesized that these fetal risk factors significantly predict higher comorbidity in women than men. Further, their impact will be expressed in adulthood as sex-specific stress response circuitry, and endocrine and autonomic nervous system (ANS) deficits that mediate the association between fetal risk and MDD and CVD risk in women.

Methods: In a 40-year cohort study with mothers followed through pregnancy and sera stored at NIH, 295 fetally-exposed and unexposed adult siblings were recruited. Pro- and anti-inflammatory prenatal assays included IL-1β, IL-6, TNF-α, IL-10, IL-8. 60 of these adult offspring were scanned using fMRI tasks of the stress response circuitry (∼half exposed/half unexposed; 26 with recurrent DSM-IV MDD in remission). Subjects viewed images with negative valence/high arousal vs. neutral valence/low arousal stimuli. 3T Siemens MR scanner was used and SPM8 for analyses of blood oxygen-level dependent (BOLD) signal changes in hippocampus (HIPP), amygdala, anterior hypothalamus (aHYP), orbitofrontal and medial prefrontal cortices (OFC; mPFC), anterior cingulate cortex (ACC), and periacqueductal gray (PAG). Hormones, heart rate, and high frequency R-R interval variability (HF-RRV)) were collected during scanning timed to stress response (e.g., DHEAS, cortisol, E², progesterone, and testosterone). General linear models were used to relate adult hormone and HF-RRV and

prenatal cytokines to BOLD signal changes in fetal-exposed vs. unexposed subjects, MDD and sex.

Results: Fetal exposure was significantly associated with low HF-RRV in response to stress 40 years later ($F = 6.06$, $P = 0.01$), which was 3 times greater among MDD vs. non-MDD. Fetal-exposed females had a significantly higher risk for comorbidity of MDD and low HF-RRV than males ($RR = 1.38$, $p < 0.01$), which was significantly related to TNF-α in maternal sera of exposed vs. unexposed comorbid women ($t = 2.53$, $p < .01$). In response to stress, there were significantly greater BOLD changes in aHYP ($p < .01$; $ES = 1.0$), HIPP ($p < .04$; $ES = .67$), and ACC ($p < .05$; $ES = .74$) and hypoactivity in OFC ($ES = .48$) in fetal-exposed women (the latter of which was the only significant finding in exposed men). Fetal-exposed female MDD cases showed the greatest *hyperactivity* in aHYP ($ES = 1.5$) and *hypoactivity* in ACC, OFC, and HIPP ($ESs = .80$; 1.15; $.62$), suggesting hyperarousal and lack of cortical and HIPP control in fetal-exposed MDD women. Key stress response brain regions were significantly associated with loss of parasympathetic cardiac regulation. *Higher* TNF-α/IL-10 and IL-6 were significantly related to *lower* BOLD changes in HIPP and ACC, which co-occurred with disruptions in HPA and HPG hormones (i.e, lower DHEAS, E^2, and testosterone, and higher progesterone and cortisol in response to stress).

Discussion: Findings suggest shared fetal risk factors for the comorbidity of MDD and CVD risk in women. They may, in part, act through a disruption of the maternal immune response resulting in sex-specific effects on offspring's HPA circuitry, expressed in adulthood as brain activity, endocrine and ANS deficits. Thus, the *fetal* programming of the stress response circuitry may be important for understanding vulnerability to MDD and CVD risk, particularly in women.

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215. Sleep Abnormalities in Women with Bipolar Disorder may be Mediated through Abnormal Response to Light Exposure and Bedtime Cortisol

Erika F.H. Saunders*, Julio Fernandez-Mendoza, Masoud Kamali, Scott Langenecker, Edward Bixler, Alexandros Vgontzas, Melvin McInnis, Alan Gelenberg

Penn State College of Medicine Hershey, USA

Background: Sleep disturbances in bipolar disorder (BP) are not only hallmarks of mood episodes, but also impact symptom presentation, overall quality of life, and lifetime depressive episodes when present between episodes. Circadian preference describes sleep time and activity time (eg. people with an early circadian preference go to bed early and awake early) which changes predictably throughout the life span. In BP, manic and mixed episodes are more prevalent in the spring and fall when daily length of light exposure is changing the most. Perhaps through a mechanism involving light exposure, individuals with BP with extreme circadian preference may be more vulnerable to relapse. Circadian preference and sleep patterns differ by sex and may be influenced by hormonal factors. Abnormalities in cortisol levels or the diurnal variation of cortisol secretion may be biomarkers for altered HPA axis in insomnia and mood disorders. Here we investigate the subjective report of sleep and circadian preference in BP women and men, and their association with light exposure, manic episodes, and awakening and bedtime cortisol levels.

Methods: Subjects with BP ($N = 103$: Women = 71, Men = 32) were enrolled in the Prechter Longitudinal study of BP, an observa-

tional, longitudinal study to evaluate biological, genetic, environmental, personality and cognitive factors that impact BP. Subjects were administered the Diagnostic Interview for Genetics Studies, the Munich Chronotype Questionnaire (MCQ), the Hamilton Depression Rating Scale (HDRS), the Young Mania Rating Scale (YMRS), the Pittsburgh Sleep Quality Index (PSQI), and the Epworth Sleepiness Scale. Scores for all mood scales were calculated without sleep items to reduce redundancy. From the MCQ, freeday and workday sleep duration, midsleep time and weekly amount of exposed light were calculated. Freeday midsleep time was used to measure circadian preference. For a subset of subjects ($N = 35$: Women = 20, Men = 15), salivary cortisol was measured 15 minutes before bedtime and 15 minutes after awakening for three days. Correlations, t-tests, and linear regression were used to determine the relationship between variables. All regression equations controlled for mood state and age.

Results: Sex differences were found in self-reported sleep measures. Women with BP reported sleeping one hour more than men with BP on workdays (W = 9:25 h, M = 8:24 h; p = .029), but a half-hour less than men on freedays (W = 8:18 h, M = 8:51 h; p = .279). Sleep quality and daytime sleepiness were worse for BP women than BP men (PSQI W = 10.0, M = 8.0; p = .010; Epworth W = 8.2, M = 5.9; p = .018). As expected, there was a strong correlation between older age and earlier circadian preference for both BP men and BP women (Pearson correlation, W = -.350, p = .001; M = -.328, p = .036). There was no difference between BP men and BP women in use of sleeping medications (p = .240). Circadian preference did not differ between BP men and BP women (p = .766), and was not associated with sleep duration in men or women (beta W = .137, p = .261; M = .287, p = .105). In women with BP, the amount of light exposure was positively associated with later circadian preference (beta = .361, p = .003) and with the number of manic episodes over a lifetime (beta = .417, p = 3.9×10^{-4}). Mean awakening and bedtime cortisol levels did not differ between men and women with BP, but circadian preference was strongly correlated with bedtime cortisol level in men but not women (Pearson W:-.165, p = .244; M:.683, p = .002).

Discussion: Our finding that women with BP report more sleep disturbances than men may be due to increased sensitivity to the zeitgeber of light and an uncoupling of sleep phase and circadian variation of bedtime cortisol. In this sample, greater light exposure caused a shift to evening circadian preference in women with BP, whereas exposure to light advances sleep phase in the general population. This abnormal response to light exposure may mediate the risk for developing manias, and may be driven by abnormalities in HPA axis. In men with BP, the positive association with bedtime cortisol levels and circadian preference suggests that in individuals with phase-delayed sleep, diurnal cortisol rhythm may be shifted as well. Women did not show this same pattern, which may indicate an uncoupling of the HPA axis and sleep phase in women with BP. Limitations include the use of subjective measurements of sleep, use of two timepoints to measure salivary cortisol and an unbalanced sample with regard to sex. We propose further investigation into sex-mediated differences in biological and environmental dimensions of BP.

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216. Impulsivity as a Consequence or a Predictor of Cocaine use in Male and Female Smoked Cocaine Users

Stephanie Collins Reed*, Richard W. Foltin, Eric Rubin, Suzette M. Evans

Columbia University College of Physicians & Surgeons, New York, USA

Background: Impulsivity has been shown to be greater in cocaine users than non-drug users, however it has been questioned whether impulsivity is a consequence or a predictor of cocaine use. The current study examined whether 1) smoked cocaine increased various behavioral measures of impulsivity, i.e., consequence and 2) impulsivity self-report and behavioral performance correlated with cocaine self-administration, i.e., predictor.

Methods: Smoked cocaine users (11 men and 7 women) were admitted as inpatients on 2 separate occasions for 4 days each, spaced 3-4 weeks apart; women were tested during the follicular phase of the menstrual cycle. During one admission, participants received repeated doses of smoked cocaine (0, 12, 25, and 50 mg) in separate sessions. A battery of behavioral measures of impulsivity and risk-taking consisting of the Immediate Memory Task (IMT), the GoStop Task, the Delay Discounting Task (DDT) and the Balloon Analog Risk Task (BART) was completed at baseline and after the last dose of cocaine each session. During the other admission, participants had the opportunity to self-administer up to 5 doses of smoked cocaine (0, 6, 12, and 25 mg) in separate sessions. The order of the 2 admissions was counterbalanced.

Results: Overall, smoked cocaine decreased impulsive performance on the IMT and GoStop tasks and risk-taking on the BART, but not the DDT. With respect to sex differences, although there were no differences in impulsivity self-report scores between males and females, males (1) showed greater impulsivity and risk-taking, as measured by behavioral tasks, at baseline and (2) showed a greater decrease in behavioral impulsivity and risk-taking performance after smoked cocaine administration; but (3) self-administered less cocaine, compared to females. Additionally, impulsivity self-report scores and behavioral performance did not correlate with the number of cocaine doses self-administered in either group.

Discussion: Overall, although greater impulsivity in cocaine users in general may be related to the continued use of cocaine, in the current study, greater impulsivity did not predict more cocaine taking within a brief session among long-term cocaine users. Additionally, the reduction in impulsivity by cocaine suggests that impulsivity may not be a consequence of cocaine use and, conversely, cocaine may aid in controlling impulsivity in cocaine users. Supported by NIDA grants K01DA022282 and R01DA009114, and NCRR grant UL1RR024156.

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217. Social and Environmental Enrichment alter the Effects of Marijuana on Cocaine Reward in Male and Female Adolescent Rats

Sari Izenwasser*, Amy K. Starosciak, Amanda Tristan, Shervin Liddie, Jannifer Matos

University of Miami Miller School of Medicine, Miami, USA

Background: Marijuana is one of the most widely used illegal drugs, with adolescents being particularly vulnerable to its use and abuse. Use of marijuana during adolescence increases the likelihood of subsequent use of other substances such as cocaine. It is not known whether housing environment can impact this effect. This study was done to investigate whether social and environmental conditions alter the effects of tetrahydrocannabinol (THC), the active ingredient in marijuana, on cocaine reward in adolescent male and female rats.

Methods: On postnatal day (PND) 23, rats were housed in either an isolated impoverished condition (alone with no toys, II) or social enriched (3/cage with toys, SE3). Starting on PND 29, 3 mg/kg THC was injected daily for 5 days and locomotor activity was measured for one hour beginning 15 min after injection. On PND 37, cocaine conditioned place preference (CPP) began with a 30 min pretest, during which the rats could roam freely on both sides of the test chamber. For the next three days, saline was paired with the preferred side and cocaine was paired with the non-preferred side during 30 min conditioning sessions. On PND 41, a post-test was done to determine preference. Data will be presented as post-pre time in cocaine-paired side, where a value of 0 indicates no change in behavior.

Results: On the first day of testing, locomotor activity was significantly higher in isolated male and female rats injected with vehicle than in enriched rats. However, the II females became habituated to the test environment and activity decreased across the 5 days of testing. In contrast, the SE3 females and the males in both housing conditions did not habituate across days. In all groups of rats injected with THC, activity was decreased compared to vehicle on each of the 5 days. In isolated females but not males, THC increased the potency of cocaine reward. In enriched males, there was a large increase in cocaine reward after pretreatment with THC compared to vehicle, but this was not evident in females.

Discussion: The effects of THC on locomotor activity differ in male and female adolescent rats depending upon housing conditions. In addition, cocaine reward is differentially altered in male and female adolescents exposed to THC depending upon social and environmental conditions. These data suggest that drug prevention and treatment strategies need to be specific to adolescent males or females, and need to take into account the different environments in which teenagers might live.

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218. The Role of Orexin in Adverse Menopause-Associated "Hot Flash" and Anxiety Symptoms

Philip Johnson*, Lauren Federici, Stephanie Fitz, Todd Skaar, Janet Carpenter, Anantha Shekhar

Indiana University School of Medicine, Indianapolis, USA

Background: Menopause is a condition in which estrogen levels are severely depleted which leads to a cluster of adverse menopausal symptoms such as cutaneous vasomotor/sudomotor "hot flashes", anxiety, sleep disturbances, and appetite changes (Freeman et al., 2005; Seritan et al., 2010). Currently, estrogen replacement therapy is the first line treatment for menopausal symptoms. However, it is no longer acceptable because of shifts in its risk (e.g., cancer)-benefit ratio uncovered by the Women's Health Initiative study (Rossouw et al., 2002). Therefore, there is a need for non-hormonal therapies to reduce the incidence of adverse menopausal-related symptoms. Unfortunately, the scientific understanding of menopausal symptoms is limited and the few non-hormonal therapies that exist are much less effective than estrogen replacement and have adverse side effects (Nelson et al., 2006). Although the understanding of the neural circuits involved in menopausal symptomology is not clear, it is commonly accepted that the hypothalamus plays a critical role (Miller and Li, 2004). For instance, menopausal symptoms are clearly induced by estrogen withdrawal and, within the brain, estrogen receptors are highly expressed and fairly concentrated in the hypothalamus (Laflamme et al., 1998). Furthermore, the hypothalamus plays a critical role in setting the thermoneutral zone (Guyton, 1976), and

postmenopausal women have a reduced thermoneutral zone (i.e., lower thresholds of ambient temp increases to elicit hot flashes) (Freedman and Krell, 1999). In 1998, a neuropeptide called orexin (ORX: also known as hypocretin) was found to be exclusively synthesized in the perifornical hypothalamus (De Lecea et al., 1998 and Sakurai et al., 1998) that has long been known to play a critical role in wake-promotion, thermoregulation, and anxiety (Ferguson and Samson, 2003; Sakurai, 2007), which are all components of menopausal symptoms. Recently our lab determined that a hyperactive ORX system is linked to an animal model of panic disorder and in patients with panic symptoms (Johnson et al., 2010, Nature Medicine). Similarly, in female rats ORX expression in the hypothalamus is highest when estrogen levels are low (Porkka-Heiskanen et al., 2004).

Methods: In order to test this hypothesis, we removed the ovaries (ovariectomy: OVEX) in female rats to model an acute menopausal state then surgically implanted radiotelemetry probes to measure core body and tail temp. Next, we created a novel model of menopause-induced "hot flashes" utilizing peripheral vasodilators that reliably result in tail-flushing response in female rats, utilizing either an acute CO_2 inhalation-induced or yohimbine-induced vasodilation.

Results: We were able to demonstrate that submaximal doses of these compounds were able to induce profound and significantly greater tail flushes in OVEX rats compared to SHAM rats. Utilizing these and other accepted models of anxiety and autonomic responses, we determined that pretreating OVEX rats with a systemic injection of a centrally active ORX 1 receptor antagonist: 1) blocked OVEX-induced anxiety behavior; and 2) blocked an exacerbated "hot flash"-associated increase in tail flushes and temperature following a clinically relevant "hot flash" provocation stimulus.

Discussion: These results clearly demonstrate preclinical evidence that orexin antagonists may be very effective therapies for post-menopausal hot flashes. This is consistent with clinical data demonstrating that compared to reproductive female controls, menopausal women had 300% higher ORX levels in their cerebrospinal fluid and these levels were restored to control levels following estrogen replacement (El-Sedeek et al., 2010). Therefore, loss of normal inhibitory control by estrogens of the ORX system may lead to menopausal-related symptoms, and ORX antagonists could constitute a potential novel treatment strategy for adverse menopausal symptoms.

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219. The Impact of Placebo on IL-18 and its Relation to Analgesic Expectation and Central μ-Opioid Receptor Activation

Alan Prossin*, Steven Zalcman, Alisa Koch, Phillip Campbell, Jon-Kar Zubieta

Department of Psychiatry, University of Michigan, Ann Arbor, USA

Background: Existing evidence implicates IL-1 family pro-inflammatory cytokines (i.e. IL-1ß, IL-18) in their association with negative affective states (i.e. pain, depression). In animal models and humans, peripheral injection of IL-1ß has been identified as having a hypernociceptive effects and as being associated with negative affective state. These effects are blocked by peripheral injection of an IL-1ß antagonist, IL-1 receptor antagonist (IL-1ra) in animal models. Such effects (i.e. IL-1ß's hypernociception and its reversal by IL-1ra) have been shown to result in part through effects on endogenous opioid neurotransmission in animal models. Another IL-1 family cytokine, namely IL-18, is structurally similat to IL-1ß and induces pro-nociceptive cytokines, including IL-1ß. However, few studies have investigated IL-18 for its nociceptive (or negative affective) effects. Placebos have been shown to exert powerful effects on mood and pain, albeit the interface between placebo-induced neurobiological responses and inflammatory mechanisms is poorly understood, and barely examined either in animal models or in humans.

Methods: We applied a standardized sustained pain model within a PET neuroimaging paradigm using the μ-opioid receptor selective radiotracer [¹¹C]Carfentanil (CFN), to determine the relation between plasma IL-18 concentration (as measured by standard ELISA techniques) and endogenous opioid neurotransmitter responses to a pain-stress challenge in the presence or absence of placebo administration (isotonic saline iv with expectations of analgesic efficacy). Following PET scanning subjects rated their pain experience using the McGill Pain Questionnaire and measures of pain intensity.

Results: We observed a significant placebo induced reduction in IL-18 plasma levels ($p < 0.001$). Also, placebo-induced reductions on IL-18 plasma levels were significantly correlated with the expected magnitude of placebo effect, as subjectively rated by the volunteers ($r = 0.485$, $p < 0.012$). and placebo-induced activation of μ-opioid neurotransmission bilaterally in the nucleus accumbens (R: $xyz = -9,11, -6$; $Z_{1,65} = 6.04$; $p_{corr} = 0.000$; L: $xyz = 15,10, -12$; $Z_{1,65} = 4.55$; $p_{uncorr} = 0.000$), and unilaterally (i.e. left) in the amygdala ($xyz = 17,0, -27$; $Z_{1,65} = 5.77$; $p_{corr} = 0.000$), hippocampus ($xyz = 25, -7, -21$; $Z_{1,65} = 4.31$; $p_{uncorr} = 0.000$), subgenual cingulate ($xyz = 7,35, -10$; $Z_{1,65} = 4.03$; $p_{uncorr} = 0.000$), and the medial thalamus ($xyz = 4, -10,2$; $Z_{1,65} = 4.62$; $p_{uncorr} = 0.000$).

Discussion: These findings are consistent with an effect of placebo administration on pro-inflammatory cytokines in humans in the context of expectations of analgesia, and relationships between those effects and a neurotransmitter system, the endogenous opioid and μ-opioid receptors, involved in pain, stress and mood regulation.

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220. A Frontoparietal Cortical Network in Humans that is Preferentially Responsive to Approaching Faces

Daphne J. Holt*, Garth Coombs, Brittany S. Cassidy, Xiaomin Yue, Scott L. Rauch, Shahin Nasr, Roger B.H. Tootell

Massachusetts General Hospital, Boston, USA

Background: Functional neuroimaging studies of social cognition and behavior have focused on regions of the medial temporal and prefrontal lobes that are known to be involved in emotional learning, memory and regulation. However, emerging evidence suggests that differential responses to socially–salient information can occur at early, perceptual stages of information processing. For example, studies in non-human primates have identified a network of polymodal regions that responds preferentially to objects and movements near the body. This 'near-space network' is proposed to serve the purpose of protecting the body surface from harm, as well as facilitating contact with rewarding stimuli (e.g., food or potential mates). In the monkey brain, several of these near-space preferring regions, the ventral intraparietal area (VIP) and ventral premotor cortex (PMv), contain 'approach-selective' neurons. Approach-

selective cells respond more to stimuli that are close to or approaching the body surface, compared to stimuli that are further or withdrawing from the body surface. Several fMRI studies have identified candidate human homologues of monkey VIP and PMv. For instance, an area surrounding the dorsal intraparietal sulcus (DIPS) described by Orban and colleagues may be homologous to monkey VIP given its location and polymodal sensory responses. It is not yet known, however, whether responses of the human DIPS or PMv show approach-selective biases.

Methods: Here we tested whether DIPS and PMv show larger responses to objects (human faces, cars, spheres) that are *approaching* (i.e., symmetrically expanding, appearing to 'loom' towards and about to collide with the subject), compared to the otherwise-identical objects *withdrawing* from the subject (symmetrically contracting), using functional MRI (fMRI) in 22 healthy subjects. Anatomical region-of-interest and cortical surface based fMRI analyses were conducted using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). We also measured the spontaneous functional coupling of the regions of this network using resting-state BOLD connectivity methods. Finally, we tested for associations between neural activity within this network and indices of social behavior, such as sociability and the typical interpersonal distance maintained by the subjects (interpersonal space size).

Results: Significantly larger responses to approaching compared to withdrawing face stimuli (a 'looming' bias) were found in DIPS and PMv, as well as in inferior temporal and occipital gyri, middle occipital gyrus, precuneus and middle cingulate gyrus. This bias was not seen in responses to cars or simple spheres. Functional connectivity analyses revealed significant correlations between spontaneous BOLD activity of DIPS and PMv, as well as significant anti-(inverse) correlations between activity of these looming-driven regions and the 'default network' (medial prefrontal, posterior cingulate, lateral temporal and parietal cortices). Importantly, activity in DIPS and PMv were more strongly correlated with activity of face-responsive areas of the inferior temporal cortex (IT) (e.g., the fusiform face area, FFA), rather than to activity of object- and place-responsive areas of IT (e.g., the parahippocampal place area, PPA). Moreover, the strength of the functional connectivity between FFA and DIPS or PMv predicted the magnitude of looming-related activation of DIPS or PMv to faces. Lastly, we found the following associations with social behaviors: 1) The percentage of time that subjects preferred to spend with others predicted higher looming-related responses in DIPS (bilaterally) and PMv (left hemisphere) to faces, higher resting-state BOLD activity correlations between DIPS and other looming-responsive regions, and lower correlations between DIPS and regions of the default network. 2) The size of the interpersonal space of the subjects predicted lower correlations between DIPS and other looming-responsive regions, and higher correlations between DIPS and regions of the default network.

Discussion: These data demonstrate the existence of a frontoparietal network in the human brain that responds preferentially to face stimuli that approach the body. Resting-state functional connectivity analyses indicate that connections between this network and face-selective inferior temporal cortex may contribute to the face selectivity of these responses. Taken together with links found between social behavioral traits and the function of these regions, these findings suggest that this network may play an important modulatory role during social interactions.

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221. Midbrain Dopamine Synthesis modulates Sustained BOLD and Transient Gamma Oscillatory Neural Response to Facial Emotional Dynamics

Mbemba Jabbi*, Tiffany Nash, Philip kohn, Angela Ianni, Dani Rubinstein, Tom Holroyd, Frederick Carver, Jonathan Shane Kippenhan, Richard Coppola, Karen Faith Berman

National Institute of Mental Health, Section on Integrative Neuroimaging, CBDB, GCAP, Bethesda, USA

Background: Successful processing of emotional signals is critical for survival in a complex social environment. The midbrain dopamine (DA) system is implicated in mediating the reactivity of the organism to salient environmental stimuli including emotional events at different timescales (Grace 1991; Schultz 2008; Bromberg-Martin *et al.*, 2010), but the role of the DA system in modulating the transient (in milliseconds) distributed neural representation of human emotional cognition is unknown. We investigated this open question by testing the relationship of midbrain presynaptic DA (directly measured with PET) to "sustained" and "transient" neural responses to salient emotional facial expressions (measured with fMRI and magnetoencephalography [MEG], respectively).

Methods: Sixteen healthy participants (mean age 34, four females) underwent 90-minute, 16 mCi 18F-fluoroDOPA (FDOPA) PET scans. A voxel-wise Patlak method, with a cerebellar reference region, was used to determine FDOPA Ki, a measure of presynaptic DA stores and synthesis. A midbrain volume of interest was manually delineated on each individual's structural MRI and coregistered to his or her FDOPA scan in native space for extraction of mean midbrain Ki values.

These 16 participants also underwent 3T fMRI and MEG studies while viewing videos of 10 different dynamic emotional (fearful or happy) or neutral facial expressions. MEG was acquired using a CTF 275 MEG system with a whole-head array of 275 radial 1st order gradiometer/SQUID channels.

Global facial movement parameters for each video were assessed to determine the average timecourse of the 10 emotionally salient expressions. fMRI data (dynamic emotional expressions > dynamic neutral expressions) were analyzed using SPM5. MEG data were analyzed with synthetic aperture magnetometry, a voxelwise beamformer method for estimation of source power. The midbrain FDOPA Ki values were used as regressors of the fMRI and the MEG data as follows: we first mapped the correlation between the "sustained" emotional dynamics-related BOLD response and midbrain FDOPA Ki at p < 0.005, and then used these regions as our search areas for the subsequent, more time-resolved correlational analysis between FDOPA and MEG oscillatory power related to 'fear > neutral' and 'happy > neutral' dynamics at p < 0.001. We focused on the gamma (30-50 Hz) frequency band because of its role in emotional processing (Luo *et al* 2009) and in routing cortical information flow (Fries 2009).

Results: Analysis of global facial movement showed that expressions of fear and happiness reached their peak between 400-700 milliseconds on average. FDOPA-BOLD correlations were observed in a distributed network including occipital, superior temporal sulcus (STS), supplementary motor area, amygdala, hippocampus, striatum, insula, and medial prefrontal, orbitofrontal, subgenual and posterior cingulate cortices. Within this FDOPA-BOLD correlation map, we found FDOPA-gamma band activity (GBA) correlation patterns that were regionally and temporally valence-specific: observing fearful expressions led to FDOPA-predicted GBA at 400-600 ms in occipital and STS regions, followed by FDOPA-predicted GBA at 600-800 ms in the amygdala, hippocampus, cingulate and prefrontal cortex; observing happy expressions led to FDOPA-predicted GBA at 300-500 ms in the STS and anterior cingulate cortex, followed by FDOPA-predicted GBA at 400-600 ms in supplementary motor cortex. Interestingly, these correlations between FDOPA and fear- and happiness-evoked GBA

predominantly occurred during the 400-700 ms time window when emotional facial movement reached its peak.

Discussion: Midbrain DA synthesis levels predicted sustained BOLD as well as transient, 200 ms, GBA responses to environmentally valid, dynamic emotional cues in sensorimotor and frontolimbic regions known to code perceptual, mnemonic and experiential aspects of salient emotional signals (Dolan 2002). These data provide novel evidence that midbrain dopaminergic tone relates not only to the regional distribution of the neural response to emotional dynamics, but also to the time course over which this neural response evolves. The temporal coincidence of the peak emotional expressions and the time windows within which measures of presynaptic midbrain DA synthesis predicted transient gamma oscillatory response to the fearful and happy facial expressions, further supports a role for the midbrain DA system in mediating the time-resolved neural coding of emotional salience. Perturbations of such a system may underlie neuropsychiatric disorders in which impairments of emotion cognition is prevalent (Laviolette 2007; Ulhaas & Singer 2010).

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222. Dual Receptor Antagonism: How Monoamines May Modulate Brain Activity

Jacob Geday*, Albert Gjedde

Aarhus University, Aarhus, Denmark

Background: Monoamines are served by a multitude of receptor types of opposing actions, mediating activation or inhibition[1]. The receptors often reside on the same neurons, and the co-location raises the issue of how the same monoamine release, at the same cells and at the same time, can elicit a combined inhibitory and excitatory response. However, for monoamines, the relations between inhibitory and excitatory receptors are such that when one receptor type is of high affinity, the other is of low affinity. This concept of dual receptor antagonism (DRA) has distinct functional consequences: Thus, an increase of monoaminergic signaling to a given area of the brain elicits one kind of response when the baseline monoamine concentration is low (i.e., below a certain threshold), but the opposite response when the baseline concentration is high (i.e., above a certain threshold).

Methods: To test the concept, we raised brain extracellular serotonin by means of a clomipramine challenge in normal control subjects,[2] and brain extracellular dopamine in STN-deep-brain-stimulated Parkinson's disease patients by activating the STN stimulator.[3] We measured regional cerebral blood flow (rCBF) by means of positron emission tomography by injection and circulation of labeled water for 1 minute. We compared the resulting changes of rCBF as index of brain activity with the individual emotional reactivity of the subjects by standardized ratings of responsiveness to images of pleasant or unpleasant emotive content.[4,5] We determined the significance or otherwise of the changes of rCBF with statistical parametric mapping.

Results: As predicted by the concept of dual receptor antagonism, we found that subjects of low emotional impact who had a narrow range of reactivity to unpleasant and pleasant images had increased brain activity induced by the drug challenge or the electrical stimulation, as imaged by rCBF in the inferior medial prefrontal cortex, a site previously demonstrated to be implicated in emotional perception, while subjects of high emotional impact had a decrease,[4,5] despite similar increases of purported monoaminergic neurotransmission.

Discussion: The DRA concept introduces an intrinsic mechanism of functionally homeostatic feedback concerning the baseline concentration of monoamines. This concept may explain phenomena such as the switch between default and attention modes of brain operation and habituation, as well as the emotional impact differences among individuals. We conclude that DRA reveals a general mechanism of modulation of cerebral activity that may apply to all neuromodulators for which opposing receptors of different affinity co-habit the same neurons. References: 1) Geday J; Thesis 2009. 2) Geday J et al Hum Brain Mapp. 2009. 3) Smith DF, Geday J, Brain Res. 2001. 4) Geday J, Gjedde A, Synapse. 2009. 5) Gjedde A, Geday J, PLoSOne. 2009

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223. Cognitive Deficits Produced by Impaired Glutamate Release from Astrocytes: Potential Implications of Diminished System x_c- Activity to Addiction and Schizophrenia

Victoria Lutgen, Aric Madayag, Peter Dietrich, Krista Qualmann, David A. Baker*

Marquette University, Milwaukee, USA

Background: Reduced cystine-glutamate exchange by system x_c-, which is primarily expressed on astrocytes, has been implicated in addictive disorders and schizophrenia. Interestingly, both disorders are associated with significant cognitive impairments including deficits in reversal learning that may impact disease severity and treatment efficacy. In support, studies have demonstrated that repeated administration of cocaine to rhesus monkeys or methamphetamine to rats produces a selective deficit involving reversal learning. Similarly, deficits in reversal learning are evident in schizophrenia, and can be modeled using the attentional set shifting paradigm. To explore the potential link between reduced cystine-glutamate exchange and impaired cognition evident in these diseased states, we examined the impact of system x_c- blockade during adulthood or in utero on reversal learning in rodents using the set-shifting paradigm. Additionally, we examined whether system xc- activity contributes to reversal learning deficits observed in the methylazoxymethanol (MAM) neurodevelopmental model of schizophrenia.

Methods: Drug Administration:in some experiments, adult male Sprague Dawley rats received the system x_c- inhibitor sulfasalazine (0 or 8 mg/kg, IP) prior to behavioral testing using the attentional set shifting paradigm. In the remaining experiments, pregnant Sprague Dawley rats received either the system xc- inhibitor sulfasalazine (0 or 16 mg/kg IP) or methylazoxymethanol (MAM; 0 or 22 mg/kg, IP) on gestational day 17. Male adult offsprings were then examined for changes in cognitive performance using attentional set shifting in the presence or absence of the cysteine prodrug, N-acetyl cysteine (60 mg/kg, IP) or were used to determine the status of system x_c-. Attentional Set Shifting: Rats were trained and tested on a four-arm cross maze (60 X 20 X 12 inches). Following training to habituate the rats to receiving and consuming food in the maze, rats underwent three days of testing. On day 1, rats were trained to enter the arm containing a visual cue to obtain a food reinforcer. On day 2, rats were reinforced upon entering the arm of the maze opposite to their turn bias expressed during training. The visual cue was placed with equal frequency randomly in each arm. On the third day, rats were reinforced when turning into the arm that represented their turning bias.

Results: Acute administration of sulfasalazine to adult male rats produced a deficit in reversal learning (ANOVA: $F_{3,44} = 6.77$, $p < .05$; Tukey *post hoc*, $p < .05$) similar to what has been reported in schizophrenia and following repeated administration of psychomotor stimulants. In addition, sulfasalazine administration in utero produced similar deficits in reversal learning when offspring were tested as adults (t-test: $t_3 = 2.39$, $p < .05$), suggesting a link between astrocytic glutamate release and neurodevelopment underlying reversal learning. In support, rats receiving MAM in utero,

commonly used as a neurodevelopmental model of schizophrenia, exhibited markers consistent with altered cystine-glutamate exchange including abnormal ^{14}C-cystine uptake ($t_3 = 2.62$, p < .05) and blunted glutathione levels ($t_3 = 2.35$, p < .05) in the medial prefrontal cortex. Further, deficits in reversal learning observed in these rats were blocked by acute administration of the cysteine prodrug N-acetylcysteine (ANOVA: $F_{3,37} = 3.5$, p < .05; Tukey *post hoc*, p < .05).
Discussion: These data indicated that reduced cystine-glutamate exchange is sufficient to produce cognitive deficits evident as decreased capacity to engage in reversal learning. Further, altered cystine-glutamate exchange in utero, either through direct inhibition or as linked with the MAM neurodevelopmental model of schizophrenia, establish a role for cystine-glutamate exchange by system x_c- in neurodevelopment underlying reversal learning. Taken together, these findings implicate glutamate release from astrocytes with impaired cognition similar to what has been reported in addiction and schizophrenia.
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224. Cholinergic Pathways and Cognition in Patients with Schizophrenia

Tarek K. Rajji*, Tiffany W. Chow, Aristotle N. Voineskos, Kira A. Links, Dielle Miranda, David C. Mamo, Zahinoor Ismail, Bruce G. Pollock, Benoit H. Mulsant

University of Toronto Toronto, Canada

Background: Cognitive deficits are core features in schizophrenia. Disruption in cholinergic neurotransmission has been associated with frontal lobe executive dysfunction in animal and human studies. The objective of this study was to evaluate the impact of compromised cholinergic pathways on frontal executive versus non-executive cognitive functions of patients with schizophrenia.
Methods: 62 patients with schizophrenia and 62 age- and sex-matched non-psychiatric control subjects ("controls") were assessed and compared using clinical measures; cognitive measures of global cognition, executive function, memory, and processing speed; and an MRI-based visual rating scale that assesses damage strategically localized within the cholinergic pathways.
Results: 11 of the 62 patients with schizophrenia were identified with severe damage to the cholinergic pathways. While all patients were impaired compared to controls, these patients with severe damage to the cholinergic pathways were specifically more impaired on executive functions but not on other cognitive functions compared to patients with milder damage.
Discussion: Compromised cholinergic pathways appear to specifically aggravate executive but not memory or other dysfunctions observed in schizophrenia. If replicated, these findings could lead to the identification of a subgroup of patients with schizophrenia who could specifically benefit from interventions enhancing cholinergic neurotransmission.
Disclosure: T. Rajji: None. **T. Chow:** Part 1: Bristol-Myers Squibb Lunbeck Canada Roche, Part 2: None, Part 3: None, Part 4: Lunbeck Canada, Part 5: None. **A. Voineskos:** None. **K. Links:** None. **D. Miranda:** None. **D. Mamo:** Part 4: Pfizer. **Z. Ismail:** Part 1: Lundbeck, Janssen and Pfizer, Part 2: None., Part 3: None., Part 4: Pfizer, Part 5: None. **B. Pollock:** Part 1: Dr. Pollock, within the past two years, was a faculty member of the Lundbeck International Neuroscience Foundation (LINF) (last meeting was April 2010). **B. Mulsant:** Part 4: Bristol Myers Squibb and Wyeth are providing Dr. Mulsant with medications for an NIMH-funded clinical trials, Part 5: Not applicable.

225. Effective Connectivity of AKT1-Mediated Dopaminergic Working Memory Networks and its Relationship to the Pharmacogenetics of Cognition in Schizophrenia

Hao Yang Tan*, Anthony G. Chen, Bhaskar Kolachana, Jose A. Apud, Venkatta S. Mattay, Joseph H. Callicott, Qiang Chen, Daniel R. Weinberger

NIMH, Bethesda, USA

Background: Working memory (WM) integrates and manipulates information across time, engaging a network of prefrontal, parietal and sub-cortical brain regions. While cortical dopamine (DA) enhances signal-to-noise tuning at the neuronal level, less is known about the genetic control of DA-associated signaling mechanisms and their impact in distributed brain networks during WM. Here, we examined functional genetic variants in COMT, DRD2 and AKT1 impacting brain DA bioavailability, D2 receptor function and its downstream signal transduction, and studied their influence across prefrontal-subcortical-parietal networks during segregated WM maintenance and manipulation events.
Methods: We first examined 46 healthy subjects as they engaged an event-related numerical WM task in a GE 3T MRI scanner. The maintenance task events involved arrays of 2 or 4 numbers, while the manipulation events involved performing arithmetic operations on 1 or 2 of the maintained array of numbers. Subjects were genotyped for putatively functional polymorphisms in AKT1 rs1130233 (Tan et al 2008), DRD2 rs1076560 (Bertolino et al 2009) and COMT Val158Met (Egan et al 2001). Dynamic causal models (DCM; Friston et al 2003) of effective connectivity in prefrontal-parietal-striatal regions of interest in these task phases were examined. In translating these D2-AKT1 effects to cognition in schizophrenia, we tested for DRD2 and AKT1 pharmacogenetic effects on antipsychotic dose in a sample of schizophrenia patients (n = 111).
Results: Using dynamic causal modeling (DCM) of effective connectivity in fMRI (n = 46 healthy controls), we found that COMT-Met individuals with putatively greater cortical DA bioavailability showed relatively stronger prefrontal-to-parietal and prefrontal-to-striatal connectivity during respective WM maintenance and manipulation processes (p < 0.05). On the other hand, functional polymorphisms in DRD2 and AKT1 selectively impacted prefrontal-to-striatal connectivity (minor allele-carriers with relatively reduced effective connectivity, p < 0.05) during WM manipulation events. Regional prefrontal activation also showed interactions between COMT and AKT1 (p < 0.005 uncorrected), and between DRD2 and AKT1 (p < 0.001 uncorrected) functional variants, consistent with the role DRD2-AKT1 coupling may play in the prefrontal-subcortical control of information gating and updating during WM manipulation. To the extent that DRD2-AKT1 might impact cognition in schizophrenia and is a target for antipsychotic drug action, we also found that the same DRD2 and AKT1 variation predicted dose-response relationships between antipsychotic drug treatment and illness-related IQ change (n = 111 patients, p < 0.05).
Discussion: We suggest that genetic differences in dopaminergic neural signaling impact communication and information processing in prefrontal brain systems that may be relevant to individual variation in schizophrenia treatment and cognitive outcome.
Disclosure: H. Tan: Part 3: HT. **A. Chen:** Part 3: HT. **B. Kolachana:** Part 3: HT. **J. Apud:** Part 3: HT. **V. Mattay:** Part 3: HT. **J. Callicott:** Part 3: HT. **Q. Chen:** Part 3: HT. **D. Weinberger:** Part 3: HT.

226. Somatosensory Timing deficits in Schizophrenia

Peter Teale*, Bryce Pasko, Dan Collins, Don C. Rojas, Martin Reite

UCDenver, Aurora, USA

Background: Schizophrenia is often accompanied by disturbances in motor behavior as well as disturbances in cognitive function

thought to result from abnormalities in the brain's timing mechanisms. Virtually all behavior has a motor component, and proper regulation and timing of motor behavior is most often dependent upon accurate registration of somatosensory input. This study utilizes an electrophysiological metric, the steady state evoked response (SSR), to quantify the accuracy of timing of the neocortical response to rapidly presented tactile somatosensory stimuli in patients with schizophrenia compared to control subjects. We find this response to be significantly disturbed in patients with schizophrenia.

Methods: We studied 13 patients who met DSM-IVTR criteria for schizophrenia. We compared their data with that of 18 healthy comparison subjects. The project was approved by the Colorado Multiple Institutional Review Board, and after thorough explanation, all subjects signed informed consent. We recorded 248 channel MEG evoked fields while the tips of their index fingers were stimulated with 500 msec trains of 25 Hz tactile stimulation delivered by a piezoelectrically operated Braille cell. Averaged field data from each hemisphere were band-passed from 20 to 30 Hz and then modeled with a single moving dipolar source. The location and strength parameters of the best fitting dipoles were then averaged and source space projection methods were used to estimate the time course of the source strengths in each individual trial. These estimates were then convolved with complex Morlet's wavelets in 1 Hz increments from 20 to 70 Hz. The modulus of the mean across trials of this convolution divided by the modulus of the convolution for each trial results in the so-called phase locking factor (PLF), a number whose value ranges from near zero for random phase trials, to one for completely synchronized trials. We extracted the mean PLF's and the mean source strengths in two time windows: the transient region from 20 to 150 ms post stimulus onset, and the SSR region from 200 to 500 ms post stimulus onset.

Results: A 2 x 2 mixed design ANOVA (group by hemisphere) was evaluated separately for source strength and PLF for the two time windows. Transient responses: For source strength, a significant main effect of diagnosis ($F(1,29) = 8.89$, $p = .006$) indicated that the control group had higher source strengths than the schizophrenia group. For PLF the schizophrenia group had significantly reduced values relative to controls, $F(1, 29) = 12.61$, $p = .001$. No other significant effects were observed. Steady-state responses: For source strength, a significant main effect of diagnosis ($F(1,29) = 8.79$, $p = .006$) indicated that the control group had higher source strengths than the schizophrenia group. The hemisphere and diagnosis by hemisphere interaction were non-significant. For PLF the schizophrenia group had significantly reduced values relative to controls, $F(1, 29) = 10.51$, $p = .003$. There was also a significant main effect of hemisphere, $F(1,29) = 5.34$, $p < .03$, indicating higher phase-locking in the right hemisphere.

Discussion: Somatosensory steady state evoked responses in schizophrenic subjects demonstrated both decreased amplitudes and poorer phase locking in both left and right hemispheres to contralateral 25 Hz tactile stimulation. Previous studies have also reported similar amplitude and phase control abnormalities in the SSR in both auditory and visual domains. The timing of optimal stimulus presentation to elicit the SSR varies by sensory system, often optimal at about 40 Hz (in the gamma range) in the auditory system, and in the high alpha and low beta range in visual studies. In our study thestimulation rate, 25 Hz, was selected as the optimal rate based on previous research. In all cases however, there are deficits in phase control of the resulting cortical response. The MEG SSR to tactile somatosensory stimuli is thought to reflect activation of layer 3 pyramidal cells in primary sensory cortex, thus these findings, as in other sensory domains, are suggestive of impaired GABAergic inhibitory interneuronal control of the timing of layer 3 pyramidal cell activity in primary sensory cortex. It has not yet been determined on the basis of these recordings alone whether the timing deficit is intrinsic to neocortex, or might reflect as well impairment of cerebellar and/or thalamic contributions to neocortical pyramidal timing mechanisms. These findings reinforce the notion that abnormalities in the brain's timing mechanisms are a central component of the schizophrenia syndrome.

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227. Anterior Cingulate Cortex Controls Stopping during Self-Limiting Behavior

Benjamin Y. Hayden*, John M. Pearson, Michael L. Platt

University of Rochester, Rochester, USA

Background: Many diseases impair our ability to abandon a maladaptive behavior pattern and switch to a more beneficial one. Indeed, difficulty in giving up stubborn habits is a defining feature of addiction and obsessive compulsive disorder, and also characterized pessimistic thought patterns in depression. The neural processes that help us stop unrewarding actions and switch to other ones remain almost wholly obscure. One feature that unites these diseases is dysregulation of the anterior cingulate cortex (ACC). Based on physiology and anatomy, we hypothesize that ACC integrates reward information and computes the optimal time to stop and switch to a new behavioral pattern, and that it sends this signal to premotor and motor structures.

Methods: We trained rhesus macaques to perform a laboratory version of a naturalistic choice task based foraging theory's patch-leaving probem. The optimal strategy in this task is to repeat a specific action for several turns and then stop and switch to a new strategy when reward intake rate declines beyond a certain point. We recorded extracellular neural responses using intracranial electrodes in the anterior cingulate sulcus while the macaques performed this task.

Results: Subjects' stopping behavior was nearly optimal. We found that ACC neurons carry a phasic spiking response that rises with time in patch. Stopping and switching occurred when neural activity reached a specific threshold, suggesting that this threshold process is a neural correlate of the stopping decision. Trials on which macaques maladaptively continued to choose the same patch, despite declining or no payoff, were associated with slower accumulation to this threshold, suggesting that maladaptive persistence reflects dysregulation of the computational processes in (or upstream to) ACC. Additionally, exogenous changes in task structure that affected optimal and observed stopping times caused a change in the threshold value itself, suggesting the existence of a regulatory control mechanism, possibly located in the basal ganglia.

Discussion: Although many studies have highlighted the importance of ACC for diseases associated with inability breaking out of fixed behavioral and mental patterns, its specific contribution has remained unknown. Our results suggest that it mediates self-generated stopping, and does so through a rise and threshold process. Our results also identify changes to threshold as a possible regulatory mechanism in stopping. Future studies will investigate the source of these control signals. Interestingly, these mechanisms are used for other cognitive skills, including perceptual decision-making and saccade countermanding, supporting the idea that the brain makes use of a limited suite of mechanisms to accomplish many cognitive tasks. Ultimately, these results endorse the use of formalism derived from control theory in understanding brain processes.

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228. The Glial Modulator Propentofylline impairs Reinstatement in a Rat Model of Cocaine Abuse

Kathryn J. Reissner*, Peter W. Kalivas

Medical University of South Carolina, Charleston, USA

Background: Accumulating evidence indicates that activation of glial cells is a cellular feature associated with drug abuse. Noncontingent administration of cocaine leads to upregulation of markers for glial activation, including GFAP and vimentin, 3 weeks after one week of daily cocaine administration. Moreover, glial high affinity glutamate transporters GLT-1/EAAT2 and GLAST/EAAT1 are also chronically downregulated following cocaine self-administration and extinction training. Because these observations occur several weeks following withdrawal, the findings collectively suggest that glial reactivity may represent a long-lasting cellular adaptation following cessation of cocaine use, and may thus contribute to mechanisms of relapse. As an initial step to test this hypothesis, we have employed the glial modulator propentofylline in a rat reinstatement model of cocaine abuse. Propentofylline is a xanthine derivative with numerous cellular functions, including adenosine uptake inhibition and phosphodiesterase inhibition. Propentofylline has also been shown to reverse markers for reactive gliosis following nerve injury, including changes in expression of GFAP, GLAST, and GLT-1.

Methods: Following intrajugular catheterization and recovery from surgery, male Sprague-Dawley rats were trained to self-administer cocaine on an FR1 schedule during 2 h sessions each day. A lever press resulted in a cocaine infusion (0.2 mg per infusion), as well as presentation of light and tone drug-paired cues. Criteria for self-administration was a minimum of 10 sessions receiving 10 infusions or more. Following self-administration, rats entered an extinction training phase for 2 weeks, during which a lever press no longer resulted in drug or drug-paired cues. During the last 6 days of extinction, rats received an injection of propentofylline (10 mg/kg, i.p.) or saline 30 min prior to the extinction session. One final injection was also given 30 min prior to a cue-prime reinstatement test. In a separate series of experiments, one injection of propentofylline (10 mg/kg or 25 mg/kg, i.p.) or saline was given 30 min prior to a reinstatement test only.

Results: Animals that received chronic propentofylline treatment during the last 6 days of extinction and prior to a reinstatement test reinstated significantly less than animals receiving saline only, as measured by number of active lever presses (experiment 1). However, acute propentofylline given once prior to a reinstatement test was without effect at 10 mg/kg or 25 mg/kg (experiment 2). Animals receiving chronic propentofylline or saline (experiment 1) were allowed to re-enter extinction training following reinstatement without continued treatment, for a second test one week later. Number of active lever presses was not significantly different between animals receiving propentofylline or saline in the second reinstatement test, indicating that continued treatment with propentofylline is necessary for an enduring effect on reinstatement.

Discussion: Results presented here indicate that chronic administration of the glial modulator propentofylline can impair drug seeking behavior. These findings indicate that reactive gliosis may represent a credible target for pharmacotherapy for cocaine abuse. Ongoing studies are designed to measure changes in protein expression for glial markers known to be affected by cocaine abuse which are also identified targets for propentofylline, including GFAP, GLT-1, and GLAST.

Disclosure: K. Reissner: None. **P. Kalivas:** None.

229. Dopamine Release in the Nucleus Accumbens Shell is Modulated by Conditioned Odor Cues that have been Associated with Access to or the Absence of Alcohol: A Bidirectional Mechanism Underlying the Anticipation/Craving of Alcohol Reward

Gerald A. Deehan, Zheng-Ming Ding, Sheketha R. Hauser, William A. Truitt, William J. McBride, Zachary A. Rodd*

Indiana University: School of Medicine, Indianapolis, USA

Background: Conditioned drug cues have been shown to regulate of the anticipation/craving of drugs of abuse and are cited as a key component in drug relapse. There are two distinct types of conditioned cues. Excitatory conditioned cues (CS+) are associated with the availability of a reinforcer, while inhibitory conditioned cues (CS-) are associated with the unavailability of a reinforcer. Presentation of a CS+ can elicit or enhance the expression of drug-seeking, while the presentation of a CS- can inhibit or prevent the expression of drug-seeking. Data generated by our laboratory has indicated that exposure to a CS+ or a CS- in a non-drug paired environment (eliminating the expression of drug self-administration behaviors and environment cues) resulted in distinct patterns of activation throughout the brain. This indicated that effects of a CS+ and CS- may be mediated through different neurocircuits, and possibly different neurotransmitter systems. Specifically, presentation of a CS+, but not a CS-, resulted in increase cfos+ neurons within the posterior VTA. Additionally, neuronal activity was differentially altered in the nucleus accumbens shell (AcbSh) by presentation of a CS+ or CS-. Dopaminergic activity within the mesolimbic system, specifically the AcbSh, is a key mediator of the rewarding properties of alcohol seeking, craving and relapse. The current study sought to determine the dopaminergic response of the AcbSh in response to odors that had been previously associated with access to alcohol (CS$^+$), absence of alcohol (CS$^-$), or had no association with alcohol access (CS^o). Given the neuronal activity data, we hypothesized that presentation of a CS+ would result in increased DA levels in the AcbSh, while presentation of a CS- should be DA-independent and/or produce the opposite effect on the mesolimbic DA system observed following CS+ presentation.

Methods: Three groups of Naïve alcohol-preferring (P) rats were trained and tested in a two lever operant paradigm where alcohol (15% vol/vol) was available on one lever and water was available on the second lever. Groups were counterbalanced for odor stimulus presentation. When the CS$^+$ was present in the operant chamber, rats could respond for alcohol. When the CS$^-$ was present, alcohol was not available. The CS^o was presented in neutral environment and had no predictive relationship with alcohol access. Following odor cue discrimination testing, all rats were randomly divided into three groups (CS$^+$, CS$^-$, or CS^o exposure) and underwent microdialysis during odor stimulus presentation to assess the effect of the three odor cues on DA release in the AcbSh in a fourth, non-drug paired environment. Previous experiments have indicated that this experimental protocol is sufficient to alter the expression of EtOH-seeking. Three baseline samples (8 min samples) were collected before the odor stimulus was presented.

Results: The data show that throughout testing the CS^o group did not significantly differ from their baseline level of DA release. Exposure to the CS- resulted in an immediate reduction in DA in the AcbSh (33% reduction) which was observed for 2 sample periods. Presentation of the CS+ in the non-drug paired environment resulted in an increase in DA (65%) during the 2nd and 3rd sample periods following cue exposure. DA levels returned to baseline levels in subsequent samples.

Discussion: The data indicate that CS+ and CS- have divergent effects of DA levels within the AcbSh, and this DA affect may mediate drug expectation/anticipation. In addition, the data provide convergent evidence of distinct neurocircuits regulating the effects of CS+ and CS- (CS+ increased DA, CS- reduced DA in the AcbSh). The paradigm employed above could provide information for the development of pharmacotherapeutics for the treatment of drug addiction, suggesting a goal of enhancing the CS- neurocircuit while inhibiting the CS+ neurocircuit.

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