Neuropsychopharmacology

HOT TOPICS

# Antidepressant potential of metabotropic glutamate receptor $mGlu_2$ and $mGlu_3$ negative allosteric modulators

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The rapid and robust antidepressant efficacy of ketamine has broken the decades-long impasse in developing improved pharmacological approaches for the treatment of depression. However, adverse effects and other limitations have created a difficult path to ketamine's widespread clinical utility, stimulating intense efforts to develop alternative treatments that act through similar biological mechanisms. In animal models, ketamine disinhibits the prefrontal cortex (PFC), promoting activity of excitatory synapses subjected to damage or atrophy during chronic stress [1]. Two alternative antidepressant targets are metabotropic glutamate (mGlu) receptors $mGlu_2$ and $mGlu_3$, related receptors that commonly couple with $G_{i/o}$ protein signaling and attenuate synaptic transmission [2]. $mGlu_2$ and $mGlu_3$ are localized at presynaptic terminals throughout the central nervous system, however, $mGlu_3$ is also expressed flanking postsynaptic sites and on astrocytes. Non-selective $mGlu_{2/3}$ antagonists enhance glutamatergic transmission in the PFC and, consistent with that mechanism, exert rapid antidepressant-like effects in several preclinical models [3].

The roles of the individual mGlu receptor subtypes in modulating PFC transmission and inducing antidepressant-like effects remain unclear. Most $mGlu_{2/3}$ ligands do not discriminate between receptors, but in recent years, highly selective and systemically active negative allosteric modulators (NAMs) for both $mGlu_2$ and $mGlu_3$ have been developed [4, 5]. Now, using these compounds, several exciting discoveries advance our understanding of how $mGlu_2$ and $mGlu_3$ regulate PFC transmission and related behaviors. In the PFC, $mGlu_2$ modulates presynaptic glutamate release probability, whereas postsynaptic $mGlu_3$ regulates the internalization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in pyramidal cells [6]. The latter plasticity is impaired by acute stress and $mGlu_3$ NAMs can restore normal physiology and motivation. Studies using optogenetics have revealed that $mGlu_2$ and $mGlu_3$ function differentially across distinct long-range excitatory inputs to the PFC. These findings suggest NAMs for either $mGlu_2$ or $mGlu_3$ may preferentially alter amygdalo–PFC transmission without affecting inputs from the hippocampus. Furthermore, unlike ketamine and other experimental antidepressants that suppress interneuron activity, $mGlu_2$ and $mGlu_3$ do not directly modulate PFC inhibitory transmission. Taken together, these circuit-specific findings suggest that $mGlu_2$ and $mGlu_3$ NAMs may provide a means to redirect limbic system afferents to the PFC while sparing the local microcircuitry from gross disruption. This approach could be superior for patients with likely deficits in interneuron function, such as those with comorbid psychotic or cognitive symptoms. To that point, ketamine induces psychotomimetic effects, while minimal evidence suggests similar liability for $mGlu_2$ or $mGlu_3$ NAMs.

In addition to these mechanistic lines of research, recent studies have shown that selective inhibition of $mGlu_3$, but not $mGlu_2$, decreases immobility in the tail suspension test, a preclinical assay for antidepressant-like activity [5]. At face value, this finding may dampen enthusiasm for the translation of $mGlu_2$ NAMs as novel antidepressants, but tests of behavioral despair are biased to identify monoaminergic mechanisms. Further studies in etiologically relevant animal models are therefore warranted to assess the efficacy of $mGlu_2$ and $mGlu_3$ NAMs in treating anhedonia. Exciting data presented at recent meetings demonstrate that both $mGlu_2$ and $mGlu_3$ NAMs rapidly reverse deficits in sucrose preference induced by chronic stress, suggesting that $mGlu_2$ and $mGlu_3$ NAMs may provide a means to confer faster symptom relief compared with available antidepressants. With new selective compounds and sophisticated genetic models, it will be possible to systematically test this hypothesis and fully evaluate the roles for both receptor subtypes.

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## ADDITIONAL INFORMATION

**Competing interests:** PJC is an inventor on patents that protect different classes of metabotropic glutamate allosteric modulators and MEJ declares no competing interests.

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# Tinkering with THC-to-CBD ratios in Marijuana

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The composition of marijuana remains largely unregulated, even though consumption is rising in parallel with rising evidence of harm [1]. The marijuana plant produces over 100 different cannabinoids, including the structurally distinct principals, $\Delta^9$-tetrahydrocannabinol (THC) and cannabidiol (CBD). Over two decades, THC concentrations in retail marijuana rose dramatically, while CBD levels declined, with THC:CBD ratios now 8 times greater than before [2]. U.S. federal guidelines have not been established for THC content or THC:CBD ratios in retail marijuana. The Food and Drug Administration has approved low-dose THC (initial oral dose 0.04 mg/kg b.i.d.) for treating nausea/vomiting associated with cancer chemotherapy for non-responders, for treating AIDS-associated anorexia/weight loss, and CBD (initial oral dose 2.5 mg/kg, b.i.d.) to treat rare, severe forms of epilepsy. Smokable marijuana (~20% THC; ~0.9 mg/kg) delivers THC at ~20 times the FDA-approved initial dose of oral THC and is now obtainable at THC:CBD ratios varying from 1:1 to 80:1. THC doses and ratios are germane to establishing safety standards, as THC and CBD engender markedly different or even antagonistic molecular, pharmacological and neuropsychiatric effects [3, 4]. Limiting its therapeutic potential, THC in marijuana acutely elicits psychosis, anxiolysis, intoxication, and cognitive impairment. With early initiation and prolonged use, marijuana is addictive and is "likely to increase the risk of developing schizophrenia and other psychoses; the higher the use the greater the risk" [5]. No comparable evidence implicates CBD in engendering euphoria, psychosis, cognitive impairment, anxiety, or addiction. High concentrations of THC and high ratios of THC:CBD in marijuana are associated with more robust euphoria, anxiety, and psychotic symptoms in otherwise normal people. Conversely, CBD mitigates the effects of THC by attenuating anxiety, cognitive deficits or psychosis: (a) in heavy marijuana users consuming a product with high CBD:THC ratios; (b) in marijuana users administered CBD or, (c) in research subjects given CBD combined with THC [4, 6, 7]. CBD diminishes the adverse effects of THC by poorly understood processes [3]. One of many possible targets is DCC, which guides formation of frontal cortical dopamine circuits during adolescence and is associated genetically with major psychiatric disorders. In rhesus monkeys treated repeatedly with THC, our pilot data showed upregulation of *dcc* mRNA in various brain regions, but if administered CBD combined with THC (CBD:THC ratio 3:1), *dcc* was not elevated [8]. If confirmed with a larger "n", does THC in marijuana dysregulate *dcc* expression in human frontal cortex? Does dysregulation alter adolescent brain dopamine circuit formation, thereby contributing to psychosis in susceptible early onset, heavy marijuana users? Our preliminary research is one of many tantalizing leads that warrant comparisons of the pharmacological and pathological consequences of high/low THC doses, high/low THC:CBD ratios and whether CBD can attenuate the effects of a range of THC doses, especially after long-term use. Except for cannabidiol-specific products, most retail marijuana strains contain immoderately high concentrations of THC and scant CBD levels. Accumulating research documents the pitfalls of an unregulated industry producing psychoactive compounds, while operating without a foundation of informed science.

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# Sex differences in schizophrenia: estrogen and mitochondria

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Schizophrenia (SCZ) is a neuropsychiatric disorder which displays sex differences in its clinical manifestations, including women presenting with later age at onset, less severe course, and better response to antipsychotics in comparison with men. Estrogen is a steroid hormone which, in addition to its role in females' reproduction and sex characteristics, contributes to neuroprotection within the central nervous system.

In this context, there is compelling evidence that estrogen has a protective effect in females against the development of neuropsychiatric disorders, particularly SCZ. Estrogen is reported to influence several features of SCZ pathophysiology and to promote the observed sex-difference in the clinical outcomes between males and females. The molecular mechanisms by which estrogen affects SCZ are still largely unknown, but there is evidence that estrogen plays a role in synaptic plasticity, neurogenesis, and neurotransmission, as well as in modulating reactive oxygen species (ROS) burden in the brain [1]. Notably, each of these neurobiological mechanisms is disturbed in the brain of SCZ patients.

More recently, it has been reported that estrogen also modulates mitochondrial function [2]. Mitochondria are the main source of energy for most cellular activities. In the brain, mitochondria are also implicated in many neuronal processes reported to be involved in SCZ pathophysiology, and mitochondrial dysfunction is emerging as a risk factor for the disorder [3]. Over 1000 mitochondrial genes are encoded by the nuclear genome, and also, mitochondria contain their own small genome (mtDNA), which is exclusively maternally inherited. Interestingly, studies have revealed a stronger maternal inheritance of SCZ compared to paternal [4].

Mitochondria have sex-specific features, exhibiting distinct effects in males versus females, and these effects have strong links to neuroprotection. Briefly, studies in a variety of animal models have shown that females' mitochondria have increased biogenesis and oxidative capacity, and greater antioxidant defense with (consequently) reduced generation of ROS, thus less release of mitochondrial apoptotic factors [5]. There is evidence that estrogen might be a key player in this mitochondrial sexual dimorphism. Several nuclear-encoded mitochondrial genes show specific DNA sequences that are targets of estrogen, called estrogen response elements (ERE). The strongest evidence is the finding that estrogen receptor beta binds to estrogen EREs in mtDNA, directly regulating its gene transcription. Moreover, with respect to direct biochemical connection for the role of estrogen in mitochondrial function, treatment of cells with estrogen protects against electron transport chain inhibitors [2]. However, the actions of estrogen on mitochondrial functioning that may underlie this hormone's sex-specific effects on brain development and behavior in schizophrenia remain uninvestigated, and are an important target of research in animal models for the disease.

Overall, it appears that estrogen plays a role in SCZ pathophysiology and in protecting females against a more severe course of the disease, probably (but not exclusively) through its regulation of the mitochondrial system. A better understanding of the influence of estrogen on mitochondrial function in the context of SCZ will reveal new insights regarding the pathophysiology of the disease in males versus females. This new perspective will generate novel targets for drug discovery with potential to improve many of the SCZ clinical outcomes, as well as developing future preventive and therapeutic strategies.

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# Vaccines to treat opioid use disorders and to reduce opioid overdoses

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As the incidence of opioid-related fatal overdoses continues to rise in the United States, new therapeutic strategies for opioid use disorders (OUD) are needed. One proposed solution is active immunization with anti-opioid conjugate vaccines, which selectively reduce the effects of their target opioid through production of drug-specific antibodies. As opposed to pharmacotherapies (e.g., methadone, buprenorphine, and naltrexone) targeting opioid receptors in the brain, opioid-specific antibodies operate through a pharmacokinetic mechanism by sequestering the target opioid in serum and reducing its distribution to the brain. Anti-opioid vaccines could provide safe and cost-effective interventions that offer several advantages over current small molecule medications: long-lasting protection that reduces the burden of compliance; no abuse liability or risk of diversion; and due to their selectivity, vaccines do not interfere with endogenous opioids, or with nontarget opioids prescribed for pain management or for treatment of OUD. To improve clinical outcome, vaccines could be used in combination with other medications for OUD.

Extensive preclinical studies have identified a series of lead vaccines selectively targeting heroin, oxycodone, hydrocodone, or fentanyl (e.g., [1–3]). Anti-opioid vaccines effectively reduce distribution of the target opioid to the brain, and reduce opioid-induced behavior, including drug self-administration, in mice, rats, or nonhuman primates. Notably, vaccine efficacy in reducing opioid distribution to the brain depends on the target opioid, its dose, and route of exposure [2], highlighting the need to consider variables such as patterns of drug use in study design of clinical trials. Supporting a role for vaccines in overdose prevention, vaccination reduces opioid-induced respiratory depression and bradycardia, two significant factors in overdose-related fatalities [4]. Additionally, anti-opioid vaccines do not interfere with naloxone reversal of opioid toxicity [4] and improve survival following a lethal heroin dose [5].

Clinical evaluation of first-generation nicotine and cocaine vaccines has shown that only a subset of immunized subjects produced levels of drug-specific antibodies sufficient for efficacy. Therefore, it is critical to optimize vaccine formulations to maximize efficacy, to understand the immunological mechanisms underlying effective immune responses, and to identify biomarkers predictive of individual variability. Multiple studies have focused on vaccine design, including optimization of hapten and linker chemistry, choice of carrier protein and adjuvant, and development of novel carriers and delivery platforms. Effective formulations of vaccines against heroin have included the TLR9 agonist CpG [5] and liposomes containing the TLR4 agonist monophosphoryl lipid A [3]. The efficacy of an oxycodone vaccine was enhanced by shifting IgG subclass distribution through inhibition of interleukin-4 signaling, both indicating a pharmacological target for vaccine development, and granting insight into mechanisms underlying vaccine efficacy [6]. Additionally, the pre-immunization frequency of hapten-specific B cell population subsets correlated with vaccine efficacy, suggesting that subjects likely to generate clinically effective opioid-specific antibody responses could be identified prior to vaccination [7]. Accelerating the translation of vaccines for OUD will benefit from rational design of more effective vaccine components, development of clinically viable formulations, and biomarkers supporting patient stratification.

Mounting preclinical data provide proof of selectivity and efficacy for anti-opioid vaccines, demonstrating their potential to treat OUD and reduce incidence of opioid overdoses. Testing these vaccines in clinical trials is warranted.

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# Regulation of raphe serotonin neurons by serotonin 1A and 2B receptors

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Several lines of evidence implicate serotonin (5-hydroxytryptamine, 5-HT) in the etiology of mood disorders, including major depressive disorders. Serotonergic neurons have long been recognized as key contributors to the regulation of mood and anxiety as the main target of serotonin selective reuptake inhibitor (SSRI) antidepressants. The therapeutic effects of SSRIs are initially triggered by blockade of the serotonin transporter SERT increasing local extracellular serotonin. Serotonin neurotransmission is tightly regulated by autoreceptors (serotonin receptors expressed by serotonin neurons) known to act through negative feedback inhibition at the cell bodies (5-$HT_{1A}$ receptors) of the raphe nuclei or at the axon terminals (5-$HT_{1B}$ receptors). Beneficial SSRI effects rely on long-term adaptations that are, at least partially, ascribed to a selective desensitization of somatodendritic 5-$HT_{1A}$ autoreceptors [1].

A positive regulation of serotonergic neurons by 5-$HT_{2B}$ receptors has been detected in mice. Local agonist-stimulation of 5-$HT_{2B}$ receptors in dorsal raphe nuclei increased extracellular serotonin suggesting a functional role of this receptor within serotonergic neurons [2]. Expression of 5-$HT_{2B}$ receptors has been detected in subset of serotonergic neurons albeit at low levels [3]. Both acute and long-term behavioral and neurogenic effects of SSRIs are abolished in mice knockout for 5-$HT_{2B}$ receptor gene, (*Htr2b*$^{-/-}$) or after exposure to selective 5-$HT_{2B}$-receptor antagonists. Conversely, chronic stimulation of 5-$HT_{2B}$ receptors by selective agonists mimicked chronic SSRI actions on behavior and hippocampal neurogenesis, which were abolished in *Htr2b*$^{-/-}$ mice [3]. Comparable lack of SSRI effects was recently reported in mice knockout for 5-$HT_{2B}$ receptors only in serotonergic neurons (*Htr2b*$^{5\text{-}HTKO}$ mice) in which dorsal raphe serotonin neurons displayed a reduced firing frequency, and a stronger hypothermic effect following 5-$HT_{1A}$-autoreceptor stimulation [4]. Cell autonomous effects were confirmed by the increased excitability of serotonergic neurons observed upon raphe-selective 5-$HT_{2B}$-receptor overexpression. Correlative findings have been described in humans, in which expression of 5-$HT_{2B}$ receptors can be found in brain stem and a loss-of-function polymorphism of 5-$HT_{2B}$ receptors has been associated with serotonin-dependent phenotypes, including increased impulsivity and suicidality [5].

Serotonin released within raphe nuclei is known to induce feedback inhibition of serotonergic neuron firing activity by stimulating dendritic 5-$HT_{1A}$ negative autoreceptors. Unlike soma and terminals, the dendritic serotonin release is independent of action potentials, relies on L-type Ca2+ channels, can be induced by NMDA, and displays distinct sensitivity to the SSRI antidepressants [6]. Dendritic serotonin release, and hence 5-$HT_{1A}$ receptor-mediated autoinhibition, is thus engaged by excitatory glutamatergic inputs to the dorsal raphe, via locally triggered calcium influx, rather than by neuronal firing. The unique control of dendritic serotonin release has important implications for the antidepressant action of SSRIs. The lack of 5-$HT_{2B}$ receptor in serotonergic neurons is associated with a higher 5-$HT_{1A}$-autoreceptor reactivity and thus a lower activity of these neurons [4]. The excess of inhibitory control exerted by 5-$HT_{1A}$ receptors in *Htr2b*$^{5\text{-}HTKO}$ mice may thus explain the lack of response to chronic SSRI in these mice.

The serotonergic tone of raphe neurons and thus the SSRI therapeutic effects likely results from the opposite control exerted by 5-$HT_{1A}$ and 5-$HT_{2B}$ receptors via a mechanism that remains to be described.

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# Dynamic network targeting for closed-loop deep brain stimulation

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Deep brain stimulation (DBS) has gained wide use in movement disorders and remains an area of active research in psychiatric disorders. Recent clinical trial setbacks may reflect clinicians' trial-and-error approach to selecting stimulation parameters [1]. Newer studies focus on "closed-loop" approaches, where DBS settings are adjusted based on an objective, brain-based readout. Those studies divide roughly into "anatomical" approaches based on targeting stimulation to specific white matter (WM) bundles and "physiologic" approaches that focus on changing pathologic signatures in brain electrical activity. We argue that success in psychiatric applications may require a synthesis of both approaches: individualized anatomically guided electrode placement coupled with biomarker-responsive targeting of network dynamics.

The anatomical approach optimizes clinical response by tailoring electrode placement relative to individual brain anatomy. In subcallosal cingulate (SCC) DBS for depression, an open-label study prospectively targeted the intersection of four different tracts in peri-SCC WM using individualized probabilistic tractography. Out of 11, 9 patients responded, a substantial improvement over the same group's prior results [2]. The same approach is now being expanded beyond SCC WM to optimize DBS placement in the ventral striatum/ventral capsule and medial forebrain bundle [1].

In comparison, the physiology-based closed-loop DBS approach is making strides in neurological disorders. Clinical outcomes improve in Parkinson disease when DBS is targeted to suppress specific cortical electrical oscillations or is locked to the phase of those oscillations [3]. A similar oscillatory feature was successfully used as a control signal in responsive DBS for Tourette syndrome [4]. Closed-loop DBS-like stimulation has also enhanced human memory. Recordings from sites across the brain can predict periods of poor memory encoding, and lateral temporal stimulation at those timepoints rescues memory performance [5]. If similar biomarkers can be identified for psychiatric symptoms, an analogous responsive stimulation approach should be possible in mental disorders. Preliminary evidence suggests that such biomarkers can be identified through a focus on cross-diagnostic domains of function, and that those markers can in turn be used for closed-loop control of psychiatrically relevant functions such as emotion regulation [6].

Psychiatric disorders likely involve dysfunction across multi-scale neural networks [1, 7], and effective DBS appears to require modulation of multiple circuits [2]. These results suggest the potential power of a multinode, network approach to sensing and stimulating in DBS. Delivering stimulation in response to features on multiple time scales (for instance, both amplitude and phase) may increase symptom relief while reducing side effects [3]. In conditions with distributed pathology, recording from and stimulating multiple areas simultaneously may better control network interactions. The ability to sense multiple network nodes might allow a better assessment of stimulation's effects on network connectivity/activity. Conversely, network activity might best be modulated by multisite stimulation. DBS and related technologies are believed to act by de-synchronizing brain networks, but this depends on stimulation efficiently propagating within those networks. In cases where single-site stimulation fails to

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adequately drive "downstream" nodes, a second stimulating site could enhance the network disruption. Fusing the anatomic and physiologic approaches into a dynamic, network-targeted approach to closed-loop DBS may be the next horizon for personalized treatment in severe psychiatric disorders.

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# NMDA-receptor independent actions of ketamine: a new chapter in a story that's not so old

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It has been nearly two decades since ketamine was introduced as a rapid acting antidepressant with good clinical efficacy in subjects who failed to remit in response to more conventional therapies [1]. Given it's potential for abuse and a litany of deleterious consequences in long-term ketamine users, serious questions remain about the utility of ketamine therapy. Yet, for many with intractable depression, ketamine provides considerable benefits. Hence the question: is there some substance that has the actions of ketamine, without being ketamine. To answer this, we must determine how ketamine works as an antidepressant.

Ketamine carries the epithet, "NMDAR antagonist." However, high-throughput screens have shown that most drugs in common use have multiple targets, and ketamine is no exception. Furthermore, many NMDAR antagonists have entered clinical trials for depression, but none have displayed the rapid, robust and long-acting antidepressant effects of ketamine [2]. (*R*)-Ketamine has a fourfold decrease in affinity for the NMDAR compared to its (*S*)-enantiomer yet shows stronger and longer-lasting antidepressant effects in preclinical models of depression, strengthening the hypothesis that a NMDAR-independent mechanism may be responsible for much of ketamine's antidepressant action [1]. A metabolite derived from (*R*)-ketamine also displays antidepressant effects in murine models of depression independent of the NMDAR [1]. These findings are tempered by the apparent antidepressant efficacy of esketamine in clinical trials. To address these apparent contradictions and to parse the molecular sites of ketamine action, we have turned to a simple cellular system with a straightforward biological reporter for antidepressant action.

Using this model system, we recently identified one NMDAR-independent mechanism that may contribute to ketamine's antidepressant effects. Every antidepressant examined thus far translocates $G\alpha_s$ from lipid rafts to the non-raft membrane regions, where it enjoys a more facile and productive relationship with adenylyl cyclase, increasing cAMP production. $G\alpha_s$ translocation can be assayed directly, by cellular fractionation, or indirectly by determining mobility of a fluorescent $G\alpha_s$ with fluorescence recovery after photobleaching (FRAP) and/or by measuring augmented cAMP production [3–5]. While most antidepressants require a 3-day incubation with cells to achieve this effect, a 15-min treatment of ketamine was sufficient to translocate $G\alpha_s$ from lipid rafts to non-raft regions. This "antidepressant biosignature" also included increased FRAP and elevated cAMP. Associated downstream subcellular events consistent with elevated cAMP; phosphorylation of cAMP related proteins and expression of BDNF were also evoked by 15-min ketamine treatment. Ketamine produced similar results between 1 and 10 μM. The former reflects plasma concentrations in patients and the latter, tissue concentrations in rodent studies. The increase in cAMP was maintained after near complete elimination of the NMDAR within the cells.

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This leaves us in the position of revisiting questions about both the mode and site of ketamine action. GABA and glutamate systems have been implicated in ketamine action [1]. Perhaps ketamine exerts both pre- and post-synaptic effects and modulates multiple signaling systems in neurons and glia. Given the short half-life of ketamine, perhaps circuits are modulated in a long-term manner. Possibly, akin to antidepressants [6], ketamine or a metabolite associates with a membrane compartment where, sheltered from degradation, it enjoys a longer course of action.

Certainly, it is unsatisfying to end any document, even one so cursory as this, with more questions than answers. Nonetheless, it is the pursuit of those questions that will evoke progress that provides relief to those who suffer from depression. A kernel of hope may reside in the discovery of a single biologic hallmark, $G\alpha_s$ translocation, that provides commonality to ketamine and traditional antidepressants. Developing novel compounds that target the translocation of $G\alpha_s$ from lipid rafts and others we have learned from ketamine, may usher in a new era of rapid acting, safer and more effective therapy.

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# Lipid signalling in the mesolimbic dopamine pathway

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Nutrient-sensing mechanisms provide a means whereby information about the metabolic state of the organism can be relayed to brain circuits controlling motivation. Mesolimbic dopamine (DA) neurons play a key role in mobilizing behaviour and are targeted by peripheral hormones controlling appetite and energy expenditure, such as leptin, ghrelin and GLP-1. Emerging findings suggest that neurons responding to endocrine signals can also detect fatty acids (FA). FA are essential building blocks for nerve cells but they can also have important signalling, metabolic and neuroimmune actions. Several lines of evidence implicate FA metabolism and partitioning in the brain as a nutrient sensor regulating energy homeostasis.

The type and quantity of fat consumed can be highly variable, leading to changes in the composition and amount of circulating and central FA. Dietary lipids can also affect the generation of FA synthesized and released by neural cells [1]. The biological impact of FA depends on their chemical structure. The monounsaturated FA oleate and the saturated FA palmitate are the most abundant long-chain FA in circulation and can exert different actions in the brain. Independent of changes in body weight, prolonged intake of saturated dietary fat (palm oil; enriched in palmitate) dampens mesolimbic DA function in rats while a monounsaturated high-fat diet (olive oil; containing mostly oleate) is protective [2]. Furthermore, excessive intake of saturated fat leading to obesity elicits anxiodepressive behaviour in a manner relying on neuroinflammatory responses in the nucleus accumbens (NAc) [3].

Oleate has central actions to supress feeding and neurotransmission, actions that may be mediated by cellular FA transport and metabolism. In turn, blocking FA hydrolysis from circulating triacylglycerol in the NAc has been shown to increase feeding and weight gain [4]. FA intracellular metabolism is mediated by carrier proteins including FA transporters (brain isoforms CD36, FATP1 and 4). Inside the cell FA are bound to FA binding proteins (brain isoforms FABP3, 5 and 7) that control uptake and transport of FA to different organelles. FA are activated into Acyl CoA which can be either esterified into complex lipids or oxidized by the mitochondria. DA neurons were found to express FATP1, FATP4 and FABP3, to incorporate long-chain FA and esterify FA into lipid droplets localized to the soma and processes [5]. Administration of oleate, but not palmitate, into the VTA inhibited food intake. Intra-VTA oleate also supressed the rewarding effects of sucrose and DA neuronal firing, effects prevented by blocking intracellular FA transport [5]. Therefore, DA neurons not only have the machinery for FA handling, but can alter their activity in response to FA and

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metabolize and store fat. A critical goal of future research is to determine how dietary lifestyle, overconsumption and obesity development alters the DA neuron lipidome, intracellular FA metabolism and neuronal function. Indeed, human obesity is linked to increased brain FA uptake [6], a consequence which may underlie the psychiatric and neurodegenerative risks associated with obesity.

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# Amyloid oligomer interactions and polymorphisms: disease-relevant distinct assembly of α-synuclein and tau

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Neurodegenerative diseases are complex diseases characterized by set of disease-specific clinical symptoms with one or more characteristic protein aggregates. However, there has been considerable overlap between multiple diseases, both in terms of clinical manifestations and protein accumulation [1]. The co-occurrence of α-synuclein and tau protein pathologies in several brain diseases implies a toxic relationship and has become an active area of research. Two of the major challenges faced in the neurodegeneration field are the formation and biological relevance of amyloid polymorphisms (strains) and the toxic interactions between amyloidogenic proteins [2]. Previously, we demonstrated that, in addition to the meta-stable oligomeric α-synuclein, tau oligomers are also present in Parkinson's disease and Dementia with Lewy bodies [3].

In our recent study, we presented novel evidence supporting the role of α-synuclein potentiating the harmful effects of tau [4]. We demonstrated that tau aggregates induced by pre-formed α-synuclein oligomers evade larger aggregate or fibril formation, thus, prolonging their oligomeric state compared to the self-aggregated tau. We evaluated the seeding propensity of tau oligomers prepared with or without pre-formed α-synuclein oligomers by exogenously adding them in three different cell models: YFP-tau expressing CV-1 cells, differentiated human neuroblastoma cell line SH-SY5Y and primary cortical neurons from embryos of Htau mouse, a transgenic tauopathy mouse model. Pre-formed α-synuclein oligomers induced tau aggregates were more potent in altering cell morphology and increasing cell death in CV-1 and SH-SY5Y cells. Moreover, these aggregates caused significant dendritic spine retraction in primary neurons when compared to the self-aggregated tau, indicating the differences in their seeding properties and toxic effects. Therefore, to gain more insight into the toxic interaction between α-synuclein and tau, we isolated complexes of oligomeric α-synuclein and tau from post-mortem Parkinson's disease (PD) brain tissues, and tau oligomers from brain tissues of progressive supranuclear palsy (PSP), a pure tauopathy without any documented α-synuclein pathology. Upon administration of these brain-derived aggregates into Htau mice, α-synuclein-tau complexes accelerated endogenous tau aggregation, caused memory deficits and spread disease pathologies as compared to pure tau oligomers.

Our study demonstrates the combined deleterious effects of α-synuclein and tau, suggesting a toxic mechanism of interaction. The ability of oligomeric α-synuclein to induce tau aggregation also points to the mechanism of cross-seeding, which has been observed among several amyloidogenic proteins, including Aβ in multiple neurodegenerative diseases [5]. This is in accordance with our previous observation, where oligomeric aggregates of Aβ, PrP, α-synuclein and TDP-43 proteins are shown to co-localize in AD pathology [6]. In conclusion, our study represents the first step to elucidate the toxic interplay between α-synuclein and tau altering the aggregation profiles and nature of amyloid deposits, possibly, resulting in the formation of unique aggregates that can cause specific loss of functions of important proteins and impairment of cellular machineries. Insights into the pathogenic interaction between α-synuclein and tau will lead to further investigation of their upstream or downstream interacting proteins that may also

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have potential roles in disease pathologies. This will lay the groundwork for more successful therapeutic interventions by targeting multiple candidate molecules, such as α-synuclein and tau in diseases.

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# Ovarian hormones, genes, and the brain: the case of estradiol and the brain-derived neurotrophic factor (BDNF) gene

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The need for novel therapeutics has fueled the burgeoning impetus to uncover biological mechanisms that contribute to sex and individual differences in the manifestations of neuropsychiatric disorders. Investigating interactions between sex hormones and genetic makeup holds promise for this quest.

Animal studies have clearly established that sex hormones impact brain organization and function at critical developmental periods, including gestation and puberty; the neuromodulatory effects of ovarian steroids are well documented across the lifespan. However, little is known about the genesis of individual differences in cognitive and behavioral response to these hormones in humans. For example, why do some women develop postpartum depression while others do not, even in the face of the same hormonal events? Because ovarian hormones are important transcriptional regulators, their actions on the brain may vary according to individual differences in genetic make-up, suggesting an important research direction that could provide information regarding this and similar clinical questions.

The potential importance of such investigations is supported by preclinical studies in female transgenic mice harboring the uniquely human *BDNF* Val$^{66}$Met variant. The *BDNF* gene is of particular interest for investigating gene–hormone interaction because estradiol induces *BDNF* expression that mediates hippocampal function [1]. Likely as a consequence, in female $BDNF_{Met}$ knock-in mice, the estrus cycle critically interacts with the Val$^{66}$Met variant to modulate anxiety-related behaviors [2] as well as hippocampally dependent function and behavior [3].

Building on these preclinical experiments, we used two different but complementary neuroimaging modalities, the blood-oxygen-level depndent functional magnetic resonance imaging and positron emission tomography regional cerebral–blood flow techniques, to measure working memory-dependent brain function in healthy, regularly menstruating women during a 6-month hormone-manipulation protocol with three hormone conditions: ovarian suppression induced by the gonadotropin-releasing hormone agonist leuprolide acetate (Lupron), Lupron + estradiol replacement, and Lupron + progesterone replacement. We found a genotype–hormone interaction in the hippocampus, a region that is typically not recruited and is often even deactivated during working memory: in women carrying the $BDNF_{Met}$ variant, the hippocampus was atypically activated (i.e., abnormally recruited), but only in the presence of estradiol [4]. The results were consistent between both imaging platforms, providing important confirmatory data. Our findings demonstrate an estrogen sensitivity in the context of the Met variant in women, and thus provide an important translational step by demonstrating that the *BDNF* genotype–ovarian steroid interaction impacts neural function.

These studies offer evidence that harboring a genetic predisposition regulated in part by sex hormones in the brain, such as the $BDNF_{Met}$ allele, may have clinical implications [5]. Additionally, a recent preclinical study showing $BDNF_{Met}$ variant-specific elimination of hippocampal function during peri-adolescence [6] suggests the importance of future studies examining gene–hormone interactions during this critical period of brain development and hormonal change.

Delineating how the interplay between genes and sex hormones influences the brain has important relevance for women's mental health, for understanding individual differences

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in hormonal effects on brain and behavior, and for revealing mechanisms that confer sex-related differential risk for neuropsychiatric disorders. Moreover, the potential importance of such gene–hormone interactions suggests a general strategy for further exploration that may inform individualized treatments.

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# Sex differences in the incidence of antidepressant-induced mania (AIM) in bipolar disorders

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Bipolar disorder has similar prevalence between the sexes, but evidence suggests that men and women experience the disorder differently. Disorder onset, comorbidities, and treatment diverge significantly between men and women. For example, depressive episodes seem to predominate bipolar illness episodes in women, whereas in men, mania is more frequent [1]. There are also sex differences in management of medication side effects and comorbidities [2], and women with bipolar disorder report poorer sleep quality, which predicts more intense mood symptoms [3].

An oft-cited but poorly understood phenomenon in bipolar disorder is antidepressant-induced mania (AIM) or antidepressant emergent manic symptoms (AEMS). AIM describes the observation that hypomanic and manic symptoms can emerge when bipolar disorder patients use antidepressants, particularly when they are not taking a concurrent mood stabilizing medication. However, most bipolar disorder patients do not develop AIM when exposed to antidepressants, and the current data suggest that the risk factors for AIM in men and women may differ [4]. Clinicians routinely screen for AIM when treating bipolar depression with antidepressants, but little is known about which demographic and clinical variables increase risk for AIM, and even less is known about gender-specific risk factors.

In our retrospective study of 416 patients with bipolar disorders, we found that women were more likely to receive antidepressants than men [5]. This is in agreement with data from Karanti et al. [6] who showed that women with bipolar disorder were more likely to be prescribed antidepressants than men, which was independent of illness severity or other clinical factors. Strikingly, in our sample, female sex was the only variable that emerged from regression modeling as a statistically significant risk factor for AIM. In another recent study, Scott et al. [4] identified a number of factors that convey differential risk for AIM between men and women. They reported that male AIM patients were more likely to have an alcohol or substance use disorder, a history of suicide attempt(s), and a greater number of depressive episodes per year. Female AIM patients were more likely to have a history of thyroid disorder, family history of bipolar disorder type I, or a depressive episode at the onset of bipolar illness.

These early findings of differences between men and women with bipolar disorder, and how this may affect the incidence of AIM, are compelling and merit further study. Tools for identifying patients at highest risk would be very helpful, particularly since the incidence of AIM is low (generally estimated to be ~10–20% of bipolar disorder patients who take antidepressants) but the dangers of mania are significant. It is unclear whether the observed treatment and outcome discrepancies between men and women with bipolar disorder are due primarily to physiological differences between sexes (e.g., hormonal pathways, sex-specific neuronal circuitry), or as described in Karanti et al [6], have less basis in physiology and more to do with disparities in how physicians treat women and men (Fig. 1).

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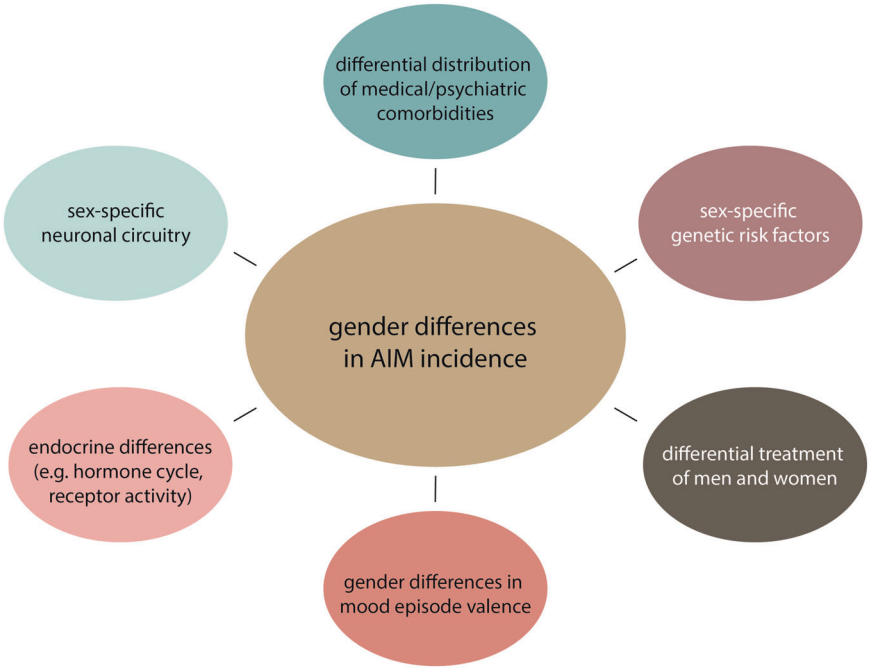


**Fig. 1** Potential factors that contribute to gender differences in antidepressant-induced mania

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# Guidance cues: linking drug use in adolescence with psychiatric disorders

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Adolescent onset of drug use is associated with an enduring elevation in the risk of progressing from recreational use to addiction. Unfortunately, adolescent experimentation with drugs of abuse remains common, with more than half of initiates under the age of 18 years old. This peak age for drug initiation coincides with a critical developmental period for

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reward-relevant substrates. Most notably, mesocorticolimbic dopamine circuitry is uniquely sensitive to disruption by drugs in adolescence, resulting in lasting behavioral changes linked to addiction vulnerability [1].

Our recent work in rodents highlights a novel role for guidance cues in (a) the adolescent establishment of mesocorticolimbic dopamine connectivity and cognitive processing and (b) the enduring effects of drugs of abuse on these events. First, we discovered that dopamine axons still grow to the prefrontal cortex (PFC) in adolescence [2]. This is the first concrete demonstration of long-distance axon growth in late postnatal development, which entails targeting decisions by dopamine axons en route to their final postsynaptic partners. Axon targeting relies on the interaction of extracellular guidance cues and their receptors. We find that the guidance cue Netrin-1 and its receptor DCC dictate dopamine axon targeting in adolescence. Specifically, mesolimbic dopamine axons have high levels of DCC and recognize the nucleus accumbens as their final target in adolescence. In contrast, mesocortical dopamine axons have little or no DCC and therefore fail to recognize this region as their final target and grow to the PFC instead. Reduced DCC expression in mesolimbic dopamine axons induces targeting errors in the nucleus accumbens and their ectopic growth to the PFC. By segregating dopamine innervation to cortical or non-cortical regions, DCC receptors organize PFC structure and function, including cognitive behaviors that are altered in addiction [2].

Second, we demonstrated that repeated non-contingent exposure to amphetamine in adolescence, at a dose resembling human recreational use, downregulates DCC expression in dopamine neurons [3]. This perfectly positions DCC signaling to mediate the enduring neuroanatomical and behavioral consequences of adolescent drug use. Indeed, amphetamine in early adolescence leads to an increase in the span of dopamine innervation to the PFC. However, it also leads to disorganized synaptic contacts and reduced dopamine turnover in adulthood [4, 5]. These alterations, in turn, produce deficits in behavioral inhibition and exaggerated salience attribution to drug-paired contexts; two behaviors associated with addiction susceptibility [4, 5].

Our findings indicate that DCC signaling wires the adolescent PFC and contributes to amphetamine-induced susceptibility to addiction. Interestingly, amphetamine downregulates DCC via micro-RNAs, which are important biomarkers and mediators of psychiatric conditions [3]. Furthermore, variations in DCC expression occur in humans and lead to altered mesocorticolimbic connectivity [6]. Our work therefore contributes a novel perspective to the ongoing efforts of developing prevention and treatment strategies for addiction. The DCC pathway represents a promising site for targeted intervention during adolescence both to counteract detrimental effects of early drug use and promote healthy brain development.

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# The ventromedial prefrontal cortex: a putative locus for trait inattention

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Efforts to identify structural brain correlates of attention-deficit/hyperactivity disorder (ADHD) have yielded inconsistent results, with no common structural biomarker emerging across studies. Methodological factors may obscure underlying brain–behavior

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relations in the study of ADHD symptomatology, including the use of categorical diagnoses [1], relying on behavioral ratings from a single informant, and small sample sizes. Indeed, empirically based assessment of psychopathology has revealed aspects of dimensionality with regard to many psychiatric conditions, including ADHD [2]. Further, recent reports suggest that information from multiple informants increases the validity of ADHD assessment [3]. Population-based neuroimaging studies provide the opportunity to more rigorously characterize the neurobiological underpinnings of ADHD symptomatology by using dimensional, multi-informant behavioral ratings, and by examining convergence across multiple assessment modalities—including genetic and neurocognitive domains.

Studying a population-based sample of adolescents ($N = 1538$; 785 females) ($M = 14.53$ years old, SD = 0.41), we investigated the relationship between dimensional measures of ADHD symptomatology, brain structure, and reaction time variability (RTV)—an objective index of attentional lapses [4]. Parent ratings of ADHD symptoms, adolescent self-reports of ADHD symptoms, and RTV were each negatively associated with gray matter volume (GMV) in an overlapping region of the ventromedial prefrontal cortex (vmPFC). To our knowledge, this study represents the largest voxel-based morphometry (VBM) study to date on adolescent ADHD symptomatology. Despite modest correlations between multi-informant behavioral ratings ($r = 0.36$–$0.66$), we observed striking convergence in the vmPFC with regard to the anatomical correlates of each behavioral measure. Similarly, while RTV was weakly correlated with behavioral ratings of ADHD symptomatology ($r = 0.11$–$0.14$), there was considerable overlap with regard to anatomical correlates. Given convergence across dimensional, multi-informant behavioral ratings, and a measure of neurocognitive functioning that has been previously tied to ADHD, our findings indicate that vmPFC structure is a brain-based marker for inattention in adolescents.

Utilizing gene expression data collected as part of the Allen Human Brain Atlas [5], we found that the statistical map representing the relationship between GMV and ADHD symptomatology was differentially correlated with patterns of *DRD1* and *DRD2* gene expression. Our results appear consistent with models of cognitive dysfunction that postulate relative imbalance between D1 and D2 systems; however, it is important to emphasize that these gene expression analyses were meant to be hypothesis-generating in nature. More comprehensive studies of gene expression represent a plausible direction for future research.

The National Institute of Mental Health has placed growing emphasis on the need to, "identify and validate biomarkers and novel treatment targets relevant to the prevention, treatment, and recovery of psychiatric disorders," as evidenced by the new Computational Psychiatry Program (https://www.nimh.nih.gov/about/organization/dtr/adult-psychopathology-and-psychosocial-interventions-research-branch/computational-psychiatry-program.shtml). An exciting avenue of our research has been to examine the extent to which vmPFC structure during adolescence predicts subsequent symptom trajectories into adulthood. Using data from the 5-year follow-up wave of the IMAGEN study, we found that adolescent vmPFC volume predicts adult ADHD symptomatology while controlling for baseline symptomatology [6]. Intriguingly, these latter results suggest that early structural development of the vmPFC may be consequential for the subsequent expression of hyperactive/inattentive symptoms in adulthood.

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# Are lithium effects dependent on genetic/epigenetic architecture?

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Although lithium (Li) is considered the gold-standard for treatment of bipolar disorder (BD), the mechanisms by which it exerts its mood stabilizing effects remain unclear. Studies have long suggested that, acting via inhibition of glycogen synthase kinase-3 (GSK3β), Li induces the transcription and expression of neurotrophic proteins linked to the activation of cell survival signaling cascades. This activation is thought to occur, at least in part, through the phosphatidylinositol 3-kinase (PI3K) pathway, as Li exposure increases PI3K/Akt1 anti-apoptotic activity and inhibition of PI3K blocks the neurotrophic effects of Li. Our recent study evaluating Li-induced gene expression alterations in lymphoblastoid cell lines (LCL) from BD patients and controls supports a role for PI3K/Akt and anti-apoptotic pathways in Li mechanisms of action [1]. While treatment in LCLs from controls did not lead to any significant differences, Li altered the expression of 236 genes in cells from patients. As hypothesized, those genes, which included some related to the PI3K pathway, were significantly enriched for signaling pathways related to inhibition of apoptosis and promotion of resilience mechanisms.

Such apparent beneficial effects of Li have raised important discussions regarding its potential widespread use in the population. However, while associations have been reported between Li content in drinking water and psychiatric outcomes, its prophylactic use in preventing BD has not been confirmed [2]. Accordingly, our findings on the more pronounced changes in patients compared to controls suggest that the effects of Li are highly dependent on the disease genetic architecture, and cell survival mechanisms may not be induced in all subjects. This is supported by evidence of a genetic and epigenetic basis for Li responsiveness, as suggested by studies showing that particular genetic variants and/or methylation patterns may interfere with treatment outcome in patients [3, 4].

Accordingly, SNPs in or near the *AKT* gene have been associated with Akt differential activity [5], which strongly supports the hypothesis that genomic variability may underlie PI3K/Akt pathway-mediated responses. Moreover, inhibition of Gsk3 by direct activation of Akt or by Li has been shown to reduce DNA methylation. In this same vein, decreased global methylation has been observed in BD patients who respond to Li, strongly suggesting that Li responsiveness goes beyond the effects of genetic variants alone. Considering that polymorphisms and methylation status can work together to control gene expression, these studies point toward possible genetic/epigenetic regulation of the PI3K/Akt pathway and perhaps of Li mechanisms of action.

Stratification of BD patients according to their response to Li treatment has been considered an important strategy to define more homogeneous phenotypes for genetic studies. In fact, cellular features of induced pluripotent stem cell-derived neurons from BD patients, such as neuronal excitability and spiking activity, have been recently shown to discriminate between Li responders and non-responders [6], suggesting that further investigation of findings in regard to specific effects of Li based on a person's genetic and epigenetic architecture is warranted.

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# Unique treatment potential of cannabidiol for the prevention of relapse to drug use

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Cannabidiol (CBD), the main non-psychoactive and non-addictive constituent of *Cannabis sativa*, has long received interest as a therapeutic for numerous psychiatric and neurologic disorders (e.g., ref. [1]). Recently, investigations on the scope of CBD's medical benefits have encompassed its potential to treat substance use disorders. Both clinical and preclinical data are overall promising in this regard. However, presently available data are scattered across several classes of abused drugs (i.e., tetrahydrocannabinol, nicotine, opiates, alcohol) and several stages of the addiction cycle (i.e., self-administration, withdrawal, drug seeking, abstinence). Moreover, both positive and negative findings have been obtained such that scope and nature of CBD's effects on addictive behavior remained to be more clearly established [2].

Among the studies of CBD's "anti-addiction" potential, a major positive finding reported by Hurd and colleagues [3] was that CBD attenuates cue-induced heroin reinstatement with effects that outlasted treatment by two weeks. Intrigued by these findings, we focused our efforts specifically on the anti-relapse potential of CBD and identified distinct leads in the literature suggestive of potential for several indications critical for relapse prevention. Many general behavioral effects of CBD, including anxiolytic, anti-stress, anti-depressant, and anti-compulsive actions (e.g., ref. [1]), are predictive of therapeutic benefit also for drug seeking and relapse. Moreover, CBD interacts with signaling mechanisms within the brain circuitry that regulates anxiety, drug desire associated with drug-related cues/contexts, and effects of stress on drug seeking. Lastly, CBD has both neuroprotective and proneurogenic actions [4]. The former includes attenuation of ethanol-induced neurodegeneration, an intoxication-induced deficit contributing to impaired impulse control in alcoholics. The proneurogenic effects of CBD have been implicated in the drug's anti-anxiety actions, and are predictive of anti-craving effects given emerging evidence implicating neurogenesis as an important factor in inhibiting drug seeking.

We therefore predicted that CBD may be suitable for targeting several relapse-promoting factors: craving associated with drug cue exposure, susceptibility to stress, heightened anxiety, and impaired impulse control. Testing this prediction in animal models of drug seeking (reinstatement), anxiety (elevated plus maze), and impulsivity (delay discounting), using rats with alcohol or cocaine self-administration histories, we found that CBD attenuated context-induced and stress-induced reinstatement of drug seeking without producing tolerance, sedative effects, or interfering with normal motivation. Following treatment termination, the attenuation of both context and stress-induced reinstatement remained unabated for the duration of the experiments (up to ≈5 months). CBD also reduced experimental anxiety in rats with alcohol and cocaine histories, and prevented the development of high impulsivity in rats with a dependence-inducing alcohol intoxication history [5].

These findings reveal a profile of potential benefits of CBD in relapse prevention that is unique in several respects: (1) Effects relevant for multiple vulnerability states that are often experienced concurrently by drug addicts and likely interact to exacerbate relapse risk. Therefore, the concurrent amelioration of these states by CBD is likely to be more effective in preventing relapse than treatment drug effects that target only a single state. (2) Long-lasting protective effects that far outlast treatment. Identification of mechanisms underlying these effects may lead improved understanding of neuroplasticity responsible for chronic susceptibility to relapse, as well as the development of more effective "anti-relapse" medications. (3) Efficacy across multiple drugs of abuse, that include not only cocaine and alcohol [5] but also opiates [3], including tentative anti-craving effects in early phase clinical trials [6]. Since co-abuse of opiates and cocaine with alcohol is common, the reported anti-reinstatement actions of CBD across three major classes of abused drugs further add to the putative treatment drug promise of this phytocannabinoid.

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# Positive-allosteric modulation of the 5-$HT_{2C}$ receptor: implications for neuropsychopharmacology and neurotherapeutics

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Serotonin (5-hydroxytryptamine; 5-HT) was identified in the mammalian brain in the 1950s. We now recognize brain 5-HT to actualize flexible and complex actions directed by binding to at least 14 5-HT receptors as well as the serotonin reuptake transporter. The 5-$HT_{2C}$ receptor (5-$HT_{2C}$R) is a G protein-coupled receptor (GPCR) that has been implicated in psychiatric and neurological disorders, including anxiety, depression, schizophrenia, and substance use disorders as well as obesity. The orthosteric 5-$HT_{2C}$R site, at which the endogenous agonist binds, has been a primary target for ligand discovery for obesity based upon the role of 5-$HT_{2C}$R in the control of feeding, energy and glucose homeostasis, yielding the first-in-class 5-$HT_{2C}$R agonist lorcaserin (Belviq®) approved by the FDA for weight loss. While 5-$HT_{2C}$R antagonism potentially mediates the side effect of some antipsychotics (e.g., clozapine) to increase weight gain, the development of 5-$HT_{2C}$R antagonists has been pursued for the treatment of anxiety disorders and depression. Importantly, selective agonists and antagonists have advanced knowledge of 5-$HT_{2C}$R neuropsychopharmacology.

Binding of 5-HT to the 5-$HT_{2C}$R results in a conformational change that catalyzes the diffusion of multiple second messenger effectors and G protein-dependent signaling. An allosteric modulator is a ligand that binds to a spatially distinct allosteric site(s) and alters the receptor conformation to modulate its interaction with other ligands and/or signal transduction molecules [1]. Higher sequence divergence for allosteric sites is expected across receptor subtypes relative to the highly conserved orthosteric domain. Such allosteric modulation is saturable (comes to a finite magnitude when the allosteric site is fully occupied) and probe-dependent (varies dependent upon the orthosteric ligand) with the prospects for separate control of affinity and efficacy. A positive- (PAM) or negative-allosteric modulator (NAM) can enhance or inhibit the functional response to an orthosteric agonist, while silent allosteric ligands are suggested to compete with PAMs or NAMs at the allosteric binding site. Additionally, allosteric ligands can be antagonists or agonists with, or without, PAM or NAM activity (e.g., ago-PAMs, ligands that potentiate agonists and display intrinsic efficacy).

Small molecule 5-$HT_{2C}$R PAMs would not act as an orthosteric agonists (as does lorcaserin), but instead act at distinct allosteric site(s) on the 5-$HT_{2C}$R and potentiate signaling of an orthosteric ligand (Fig. 1). The first identified 5-$HT_{2C}$R PAM was the fatty acid oleamide [2], which lacked selectivity against other GPCRs. In 2003, chemical library screening resulted in the discovery of an analogue of the antibiotic lincomycin, PNU-69176E, as a selective 5-$HT_{2C}$R PAM [3]. In 2012, Zhou and colleagues optimized the synthetic route to generate PNU-69176E and its diastereomer [4],

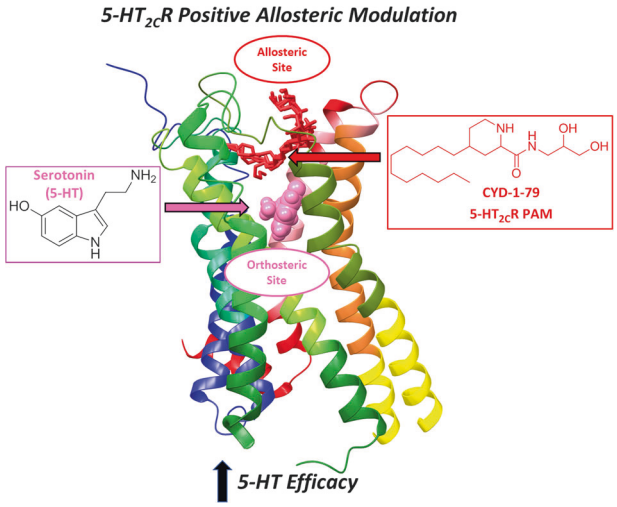


**Fig. 1** The orthosteric and allosteric sites on the 5-$HT_{2C}$ receptor are distinct. Serotonin binds to the orthosteric site to generate downstream signaling. The 5-$HT_{2C}$R PAM CYD-1-79 (*cis*-4-alkylpiperidine-2-carboxamide series) increases 5-HT efficacy through actions at the allosteric site [5]

and we have recently designed, synthesized and characterized a series of new chemical entities as selective 5-$HT_{2C}$R PAMs [5]. Several molecules potentiated 5-$HT_{2C}$R-evoked signaling in vitro without displaying 5-$HT_{2C}$R efficacy or altering 5-$HT_{2A}$R signaling. CYD-1-79 exhibited a favorable overall pharmacokinetic profile, potentiated 5-$HT_{2C}$R-mediated behaviors, and attenuated impulsive action and sensitivity to cocaine-associated cues in a preclinical self-administration model. This series of 5-$HT_{2C}$R PAMs is complemented by the discovery of a 5-$HT_{2C}$R PAM, which suppressed food intake in rodents [6]. Thus, the chemical space for discovery of novel 5-$HT_{2C}$R neuroprobes and therapeutics is now expanding to include allosteric 5-$HT_{2C}$R modulators, providing the opportunity to explore cellular mechanisms of action, functional selectivity, neurochemical mechanisms, and neural sites of action for 5-$HT_{2C}$R to control behavior.

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# Motion mapping in humans as a biomarker for psychiatric disorders

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Measuring movement has been a cornerstone of studying behavior in animals in controlled studies.

Movement data have served as a primary window into behavior, and by proxy, social function, mood, and cognition [1]. In humans, variations in locomotor activity have served as markers of a range of psychiatric syndromes including major depression, bipolar disorder, anxiety, catatonia, and substance use disorders [2]. Reports of sensor-based motion measurement in humans date back to the 1950s [2], and motion-based models of human psychopathology have been key to developing animal models of psychopathology [1]. A vast literature has documented how these animal models have facilitated development of motion-based signatures of antidepressant, anxiolytic, and other drug effects and guided drug development [3]. However, the limitations of technology thus far have meant that motion-mapping in humans has remained largely restricted to experimental settings.

Phenotyping of naturalistic human behavior continues to depend on self-report or observer-report measures, with sampling often at intervals of hours to days. Newer technologies, supported by advances in wireless connectivity, more compact and reliable sensors and devices, and higher computing power can now sample movement at intervals of seconds, and can facilitate measurement of human motion in the natural living environment in ways previously not possible [4]. Such technologies are setting the stage for movement to become a major new phenotypic biomarker in neuropsychiatry.

A wide array of validated technologies can quantify motion. These range from active sensors that move along with the body (e.g., accelerometers, gyroscopes), passive "line of sight" technologies (e.g., cameras, thermal sensors, and lasers), passive technologies that can detect motion through walls (e.g., radio wave sensors) and positioning technologies (e.g., GPS, satellite imaging) [5]. It is now possible to track individual contractions of facial muscles (used in computer vision and analysis of micro emotions), complex motions such as smoking or drinking coffee [6] or even the movement of an individual across a global geographic field. Work in this new emerging field necessitates collaborative teams with a range of expertise that includes hardware development, signal processing, big data processing and analytics, machine learning, data visualization, and clinical implementation.

Early work in this field has demonstrated feasibility of translating this intensive sensor-based approach to clinical research. As an example, our group has demonstrated that by measuring how low frequency radio signals bounce off the human body and objects in a defined space (up to 800 sq.ft.), it is possible to elicit gait speed, gait patterns and spatial location. Using clinical correlation, we have established that these data can serve as markers of apathy, pacing, and disrupted circadian rhythms in patients with dementia [5].

A marker of the maturing of this field is recent NIH investment into major collaborative initiatives such as the MD2K initiative [7], which supports the acceleration of translating motion sensor data into validated biomarkers of behavior. While the need for privacy and security around sensor data is well recognized, innovative solutions around secure data storage and transfer will be crucial to this approach gaining widespread acceptance and use [4].

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# Treating cue-reactivity with brain stimulation: a new (transdiagnostic) approach

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The struggle between internal self-control and external temptation from environmental cues is a tale as old as written history, yet as relevant today as any time in the past. Just as Homer wrote about Odysseus and the seductive Siren Songs (800 B.C.), or Tintorreto painted Adam's temptation in the Garden of Eden (1551 A.D.), twenty-first century depictions of life frequently highlight the struggle to maintain focus despite proverbial "apples" that interrupt our journey. For most individuals, the occasional surrender to a tempting cue will not impair their ability to fulfill daily and longer term responsibilities. For other individuals, however, elevated reactivity to positive or negative cues causes a disabling cascade of events ultimately impeding long-term goals. Elevated cue-reactivity is also a prominent feature of alcohol and substance-use disorder, posttraumatic stress disorder (PTSD), and obsessive behavior disorders, such as eating and gambling.

In these populations, salient cues evoke elevated activity in a consistent network of neural regions: the ventral medial prefrontal cortex (MPFC), anterior cingulate cortex (ACC), and insula. This network may be thought of as a "transdiagnostic neural biomarker" for cue-reactivity. In substance-abuse literature, meta-analyses have demonstrated that these regions are reliably activated by drug cues and may predict relapse [1, 2]. In a recent study by our group, 156 substance dependent individuals performed a drug cue-exposure task tailored to their drug of choice (55 cocaine, 53 alcohol, 48 nicotine) [3]. Multivariate k-means clustering revealed three distinct clusters of elevated activity when the participants were viewing the drug cues vs. neutral non-drug cues: the MPFC/ACC, the left inferior frontal gyrus/insula, and the right premotor cortex.

From a therapeutic perspective, novel non-invasive brain stimulation treatment protocols are being designed to target the MPFC–ACC–Insula circuit directly [4]. In the cue-reactivity study described above, cortical projection analysis revealed that the frontal pole (FP) was the cortical location closest to the maximal number of significant cue-reactivity clusters. A recent sham-controlled study in 49 individuals demonstrated that continuous theta burst stimulation (TBS)—a particularly potent and efficient form of transcranial magnetic stimulation (TMS)—directed to the left FP decreases drug cue-reactivity among heavy alcohol users and cocaine users [5]. This protocol also decreases functional connectivity in this MPFC/ACC/Insula network [6].

FP TMS is also being used to improve cue-reactivity in PTSD and obsessive behavioral disorders. Dr Rebecca Price and colleagues at the University of Pittsburg, e.g., are currently evaluating FP TBS, as a tool to decrease compulsive behaviors in obsessive compulsive disorder, many of which are cue-evoked (NCT #03265015). The use of this MPFC–ACC–Insula network as a framework for modulating cue-reactivity is just beginning. Although there will be several challenges associated with developing TMS strategies to modulate this network (e.g., reaching these deep targets, disease-tailored protocols), the MPFC–ACC–Insula network appears to be a fruitful and transdiagnostic neural biomarker to explore for next generation brain stimulation protocols.

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# Decoding the role of the microbiome on amygdala function and social behaviour

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We are living in a microbial world with our bodies having as many microbial cells as human cells. Growing evidence implicates these microbes, known collectively as the microbiome, as key regulators of brain function and behaviour [1]. One of the key findings from across many species is that the microbiome affects social behaviour [2]. We have shown that germ-free (GF) mice, which grow up in a sterile environment and thus have no bacteria in or on their bodies, are less sociable than normal mice[2]. Moreover, the amygdala, a brain region important for social behaviour, is particularly sensitive to changes in microbiome composition [3] and GF mice have widespread changes in amygdala neuronal morphology and function [4].

Ongoing research is trying to determine the molecular mechanisms underpinning such effects. Initially, we exploited unbiased genome-wide transcriptional profiling to determine gene expression in the amygdala of male GF mice. We found differential gene expression, exon usage and RNA-editing in GF mice (Fig. 1). We noticed upregulation of several immediate early response genes such as *Fos*, *Fosb*, *Egr2* or *Nr4a1* in association with increased cAMP response element-binding protein (CREB) signalling in GF mice [5]. In addition, we found differential expression and recoding of several genes implicated in a variety of neuronal processes such as neurotransmission, neuronal plasticity, metabolism and morphology. These data strongly suggest altered baseline neuronal activity in the amygdala of GF animals, which may underpin the social deficits. However, what happens under a social stimulus remained known.

To this end we recently described dynamic regulation of several previously undescribed pathways in response to social stimulation. These include regulation of RNA-processing non-coding RNAs that are crucially involved in splicing regulation. Moreover, social stimulus evoked an increase in transcripts of genes involved in neuronal activity, which includes induction of several well established immediate early genes such as *Fos* or *Arc*, the MAP-K pathway and neurotrophic signalling via *Bdnf*. Moreover, we find upregulation of complement components, which have lately been established to be necessary for synaptic rearrangements and plasticity upon neuronal activity [6].

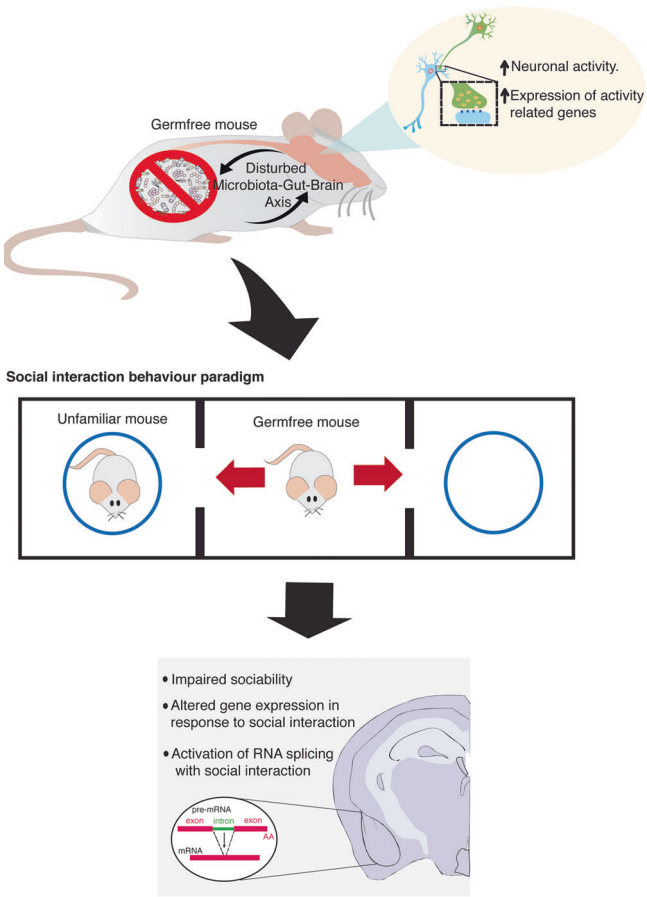


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**Fig. 1** Altered social behaviour-induced changes in the amygdala. Under baseline activity the amygdala of germ-free mice is an activated state. Transcriptomic analysis demonstrated an upregulation of several immediate early response genes such as *Fos*, *Fosb*, *Egr2* or *Nr4a1* in association with increased CREB signalling in GF mice (see [5] for full details). Moreover, when a germ-free mouse is introduced to a social stimulus the normal transcriptional pathway recruitment is absent but instead genes involved in alternative splicing are enriched (see [6] for full details of genes affected)

However, GF mice displayed a strikingly different pattern of amygdala gene activity in response to social interaction [6] (Fig. 1). In particular, the dynamic, stimulus-dependent transcriptional regulation seen in controls was attenuated and replaced by a marked increase in expression of splicing factors and alternative exon usage. This reveals a potential molecular basis for how the host microbiome is crucial for a normal behavioural response during social interaction. Moreover, social behaviour was correlated with the amygdala gene-expression response. These results reveal one of the key steps leading from absence of bacteria during brain development to a phenotype associated with reduced sociability in adulthood in mice. These data thus enhance our understanding of the link between the microbiome and brain health and neurodevelopmental disorders such as autism spectrum disorders. Future studies will be needed to determine what are the exact microbial signals that regulate alternative splicing events in the amygdala and whether they can be harnessed for therapeutic benefit.

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# Novel models of drug relapse and craving after voluntary abstinence

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Relapse to drug use during abstinence is a core feature of addiction. Since the 1980s, this clinical scenario has been studied using animal models where relapse is assessed after experimenter-imposed cessation of drug self-administration by either extinction of the drug-reinforced responding or homecage forced abstinence [1]. However, despite strides toward understanding neuronal mechanisms of relapse in these models, treatment options remain largely unchanged. One potential reason for this state-of-affairs is that animal models of addiction rarely incorporate voluntary aspects of human abstinence, which often occurs due to the availability of alternative nondrug rewards (e.g., employment and supportive social environment). This is exemplified in contingency management where nondrug rewards (monetary vouchers), given in exchange for being drug-free, can maintain abstinence for many months. However, when contingency management discontinues, most drug users relapse.

Based on these considerations, we introduced a contingency management-based relapse model, where we achieve long-lasting voluntary abstinence prior to the relapse tests by giving rats mutually exclusive choices between a drug and palatable food [2]. In the initial study, we trained male rats to self-administer the food and methamphetamine in established addiction models—escalation and DSM-IV-based—and found that rats will voluntarily abstain from drug self-administration for at least 3 weeks and then show incubation of methamphetamine craving (time-dependent increases in drug seeking during abstinence) [2]. Subsequently, we

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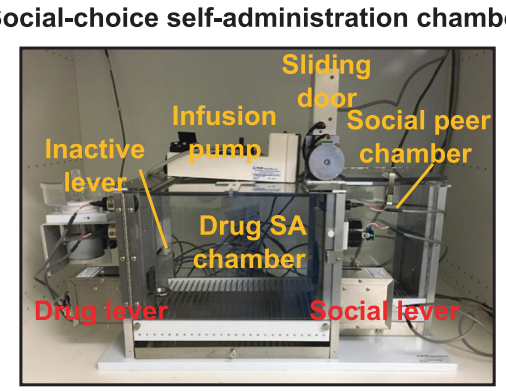


**Fig. 1** Custom-made social-choice self-administration apparatus. For instructions on how to build the apparatus, see [6], and technical advice is available upon request from the authors

demonstrated the generality of food-choice voluntary abstinence to female rats and to heroin, and that surprisingly voluntary abstinence prevents incubation of heroin craving [3]. In mechanistic studies, we found a role of dorsomedial striatum neuronal ensembles in incubation of methamphetamine craving after voluntary abstinence [4], and a role of glutamatergic projections from the anterior insular cortex to central amygdala in relapse after voluntary abstinence [5].

However, the use of palatable food as the nondrug reward may limit the model's clinical translation. This is because for most humans, the rewards that compete with drugs are primarily social (family and employment). Based on this consideration, we recently introduced a newer voluntary abstinence model that involves choices between a drug and operant access to social interaction [6] (Fig. 1). We found that rats trained in established addiction models—escalation, DSM-IV-based, and intermittent access—will voluntarily abstain when given mutually exclusive choices between methamphetamine or heroin versus social interaction. This effect was independent of their 'addiction score', persisted through 4 weeks of forced abstinence, and could only be reversed by delay or punishment of the social reward. We also found that social-choice-induced voluntary abstinence prevents the emergence of incubation of methamphetamine craving, even 1 month after cessation of the social choice. This protective effect was associated with activation (assessed by the activity marker Fos) of inhibitory central amygdala PKCδ-expressing neurons and decreased neuronal activity in the anterior insular cortex [6].

In conclusion, we introduced two novel models of choice-based voluntary abstinence and demonstrated the profound protective effects of positive social interaction on drug addiction and relapse in rat models. Our findings support wider implementation of social-based behavioral treatments, which include not only the established community reinforcement approach, but also social-based psychotherapies and family-based social support systems to provide social support before and during drug-seeking episodes.

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# An emerging epigenetic framework of systemic and central mechanisms underlying stress-related disorders

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Advances in translational neuroscience are pointing to a new paradigm for conceptualizing diagnosis and treatment of major morbidities, such as major depressive disorders (MDD), diabetes, and dementia [1]. Here we explore the emerging framework that focuses on the epigenetic actions of metabolic mediators on regulation of gene expression in brain regions controlling cognition and emotion as an approach to examine the systemic, as well as neural bases, of stress-related CNS disorders.

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Central to this epigenetic framework linking neural and systemic functions is the concept of allostasis (adaptation) and allostatic load (pathophysiology) [1]. This concept emphasizes that endogenous mediators for adaptation contribute to pathologies, including MDD, when activated persistently or dysregulated under circumstances of toxic stress and associated health-damaging behaviors [1]. There is an increasing scientific consensus to support a connection between systemic illness and MDD, a disorder still primarily seen as brain based. Recently, we reported decreased plasma levels of acetyl-L-carnitine (LAC) in two cohorts of patients suffering with MDD [2]. The LAC deficiency was greater with stronger severity, earlier disease onset and treatment-resistant-depression, which was associated with childhood trauma [2]. In rodent models, targeting the deficiency in LAC by supplementation of exogenous LAC leads to antidepressant-like responses after a few days of administration, while standard antidepressants require multiple weeks [3–5]. LAC is an endogenous molecule with a plethora of biological functions important for brain plasticity and systemic metabolism relevant to stress and pathophysiology of MDD [3]. In rodent models with sustained activation of neural mineralocorticoid receptors (MR), supplementation of LAC increased acetylation of histone markers of activate gene transcription leading to increased expression of an inhibitor of spontaneous glutamate release, mGluR2, and the neurotrophin, BDNF [3, 4]. Normalizing glutamate overflow and BDNF expression ameliorated decreased dendritic branching in the hippocampus and connected brain areas important for the pathophysiology of depression [1, 3–6].

Within this framework, it is important to emphasize that, at least in rodent models, low LAC serves as a biomarker of insulin resistance (IR), which was also ameliorated by supplementation with LAC [4]. IR is a modifiable inflammatory state that is often observed in patients suffering from MDD [1]. Furthermore biomarkers of allostatic load involving imbalance in systemic stress-related physiology, and also including heightened release of proinflammatory cytokines and hypercortisolemia [1], are inversely correlated with hippocampal volume in patients with affective dysregulation [1].

These studies provide a foundation for future research of the mechanisms of LAC action related to the pathophysiological role of systemic physiology in a variety of stress-related CNS disorders, including MDD and interrelated disorders, such as Alzheimer's disease. This framework can lead to identify therapeutic targets for development of mechanism-based treatment strategies tailored to biologically defined patient populations [5].

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