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Part 1: Genetic Antecedents of 'Reward Deficiency Syndrome'

Reward deficiency syndrome (RDS): a biogenic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors

Kenneth Blum and Ernest P. Noble
University Of North Texas, Denton, Texas, University Of California, Los Angeles, CA; PATH Foundation, New York, NY.

The dopaminergic system, and in particular the dopamine D2 receptor, has been profoundly implicated in reward mechanisms in the brain. Dysfunction of the D2 dopamine receptors leads to aberrant substance seeking behavior which includes but is not limited to alcohol, drug, tobacco, and food) and other related behaviors (pathological gambling, Tourette's and attention deficit hyperactivity disorder) and personality disorders (schizoid/avoidant, antisocial, novelty seeking). Over the last decade, since our initial findings (BLUM & NOBLE, 1990), it has been proposed that genetic variants of the D2 dopamine receptor gene and other "reward genes" are important common genetic determinants of the emerging concept first coined by Blum – "REWARD DEFICIENCY SYNDROME". The presenters of this conference review the results of studies concerning particular classes of biological phenotypes that may have relevance to not only alcohol dependence but to the above mentioned related addictive, compulsive and impulsive disorders. Broadly defined, these classes include brain neurotransmitter systems and neuroelectric potentials. The results of current and past studies, strongly suggest that etiology of RDS is mediated in part through sub-optimal neurotransmitter functioning, in particular a hypo-dopaminergic activity. An emerging picture of polygenic, hetergenous genetics with substantial plieotrophism. Research opportunities are offered with respect to specific candidate genes that have been cloned from these neurotransmitter systems that could be most fully utilized in both association and possibly family-based linkage studies, only if 1000's of probands are employed in the latter case. Additional evidence is submitted, suggesting that characteristics of particular neuroelectric potentials (e.g. the amplitde and the latency of the P300 components of the event-related potential) may provide the cleanest dimension of potential markers that could be used to identify children at risk for RDS. Presenters will also discuss the conflicting findings with regard to the association studies of the minor Taq1 A1 allele of the dopamine D2 receptor (DRD2) gene with alcoholism. However, we conclude that meta analyses strongly favors the positive association, and failure of association is due to failure to assess alcoholics for severity of their disorder and to screen controls for substance use and other RDS behaviors. The clinical session of the conference favorably reviews data involving the use of multiple modalities for the treatment of RDS including pharmacaceutical, nutraceutical, neuro-feedback, electrophysiological, auricular therapy and chiropractic. Further studies involving well defined animal models of RDS, such as the Lewis rat, showing hypodopaminergic limbic function, provides the field with a model to dissect the multiple genetic mechanisms involved in this complex disorder, possibly by employing Quantitative Trait Loci experiments. Finally, multiple domains of inquiry should not be viewed as "unfocused" but rather as an economical means for utilizing highly characterized samples of potential RDS probands meeting rigorous research criteria.

Reward mechanisms in normal and pathological behavior—the dopamine link as a target for therapeutic intervention

Michael A. Bozarth
Addiction Research Unit, Department Of Psychology, State University Of New York At Buffalo, NY

The problem of drug addiction is best viewed from a motivational perspective—the individual has profound motivation to continue use of a drug, while motivation for other rewards has diminished impact on the individual's behavior. This disruption of "normal" motivational priorities has been termed motivational toxicity and may be a defining feature of drug addiction. Because motivation is governed by specific brain reward pathways, the dysfunction of normal brain reward processes has been suggested to underlie drug addiction. This notion has spawned considerable interest in brain dopamine which has figured prominently in the study of brain reward mechanisms. In particular, the A_{10} dopamine system—with its cell bodies in the ventral tegmental region and its axonal projections terminating in forebrain areas such as the nucleus accumbens—may provide a common neurochemical link for diverse rewards, including reward from several drug classes and from some natural rewards. Although addiction undoubtedly involves multiple brain systems, the discovery of a common link across multiple substance abuse disorders (and indeed other mental disorders) and an understanding of how this same system participates in normal motivation provide a potential target system for therapeutic intervention and a model system for studying addiction and related mental disorders. For example, dopamine systems have been the target of pharmacological manipulations designed to treat drug addiction and of exploratory studies examining possible variations in brain dopamine function that may lead to increased susceptibility to drug addiction and other pathological behaviors. Interestingly, motivational theory could readily explain how either hypo- or hyper-dopaminergic activity might lead to a markedly enhanced reinforcing efficacy of drugs as in addiction.

The putative DRD2 reward gene in substance use disorders and its phenotypes

Ernest P. Noble
University of California, Los Angeles, CA

It has been a decade since the TaqI A D2 dopamine receptor (DRD2) minor (A1) allele was first associated with severe alcoholism. Since then, international studies both confirming and not confirming this finding were reported. However, a meta-analysis of a large number of Caucasian alcoholics (both more severe and less severe) and controls (both assessed and unassessed for substance use disorders) revealed a significantly higher frequency ($p < 10^{-7}$) and prevalence ($p < 10^{-9}$) of the DRD2 A1 allele in the alcoholics than in the controls. Further analysis showed that the more severe alcoholics had a three-fold

higher prevalence of the DRD2 A1 allele than the assessed controls ($p < 10^{-10}$), with no difference being found between the less severe alcoholics and the unassessed controls. DRD2 exonic or promoter mutations have not yet been associated with alcoholism, although two intronic variants at the TaqI B and intron 6 sites, which are in linkage disequilibrium with the TaqI A site, were associated with this disorder. Variants of the DRD2 gene have also been associated with cocaine, nicotine and opioid dependence and obesity. It is hypothesized that the DRD2 is a reinforcement or reward gene. Phenotypic differences have been associated with DRD2 variants. These include reduced D2 dopamine receptor numbers and diminished glucose metabolism in the brain of subjects who carry the DRD2 A1 allele. In addition, phenotypic differences have been found in neurocognitive and personality characteristics, stress response, and in the treatment outcome of DRD2 variants. The involvement of the DRD2 gene in substance use disorders opens up the potential of a targeted pharmacogenomic approach to the prevention and treatment of these disorders.

"Reward Deficiency Syndrome": an emerging concept

Kenneth Blum, Eric R Braverman
University of North Texas, Denton, Texas; PATH Foundation, New York, NY.

The dopaminergic system, and in particular the dopamine D2 receptor, has been implicated in reward mechanisms. The net effect of neurotransmitter interaction at the mesolimbic brain region induces "reward" when dopamine (DA) is released from the neuron at the nucleus accumbens and interacts with a dopamine D2 receptor. "The Reward Cascade" involves the release of *serotonin,* which in turn at the hypothalmus stimulates *enkephalin,* which in turn inhibits *GABA* at the substania nigra, which in turn fine tunes the amount of DA released at the nucleus accumbens or "reward Site". It is well known that under normal conditions in the "reward Site" DA works to maintain our normal drives. In fact, DA has become to be known as the *"pleasure molecule"* and/or the *"anti-stress molecule".* When DA is released into the synapse, it stimulates a number a DA receptors (D1-D5) which results in well-being and stress reduction. A consensus of the literature suggests that when there is a dysfunction in the "brain reward cascade", which could be caused by certain genetic variants (polygenic), especially in the DA system causing a hypodopaminergic trait, the brain of that person requires a DA fix to feel good. This trait leads to multiple drug seeking behavior. This is so because alcohol, cocaine, heroin, marijuana, nicotine, glucose, all cause activation and neuronal release of brain DA, which could heal the abnormal cravings. Certainly, after ten years of study, we could say with confidence, that carriers of the DA D2 receptor A1 allele have compromised D2 receptors. Therefore lack of D2 receptors cause individuals to have a high risk for multiple addictive, impulsive and compulsive behavioral propensity, such as severe alcoholism, cocaine, heroin, marijuana, nicotine, glucose bingeing, pathological gambling, sex addiction, ADHD, Tourettes Syndrome, Autism, chronic violence, posttraumatic stress disorder, schizoid/avoidant cluster, conduct disorder and anti-social behavior. In order to explain the breakdown of the "Reward Cascade"due to both multiple genes and enviromental stimuli (Plieotrophism) and resultant aberrant behaviors, we united this hypodopaminergic trait under the rubric of a "Reward Deficiency Syndrome ".

Dopaminergic genes in the context of drug abuse vulnerabilty genetics

George Uhl
Neuromolecular Branch, National Institute on Drug Abuse-Intramural Research Program, Baltimore, Md.

Increasing evidence points to roles of dopaminergic gene variation in contributing to drug abuse vulnerability genetics. In turn, evidence has shown that drug abuse vulnerability genetics contributes to about one-half of drug abuse vulnerability. Dopaminergic genes that have been studied in our group in both humans and/or mouse models include dopamine D1 receptor gene (D1), dopamine D2 receptor gene(D2), dopamine D3 receptor gene(D3), dopamine D4 receptor gene (D4), dopamine transporter gene (DAT), dopamine vesicular monoamine transport gene (VMAT2) and catecholamine–methyltransferase gene (COMT). Results of tests of contribution and roles of variants of DRD2 in drug abuse, of DRD4 in drug abuse and in personality, of DRD3 in drug abuse, of DAT in drug abuse and ADHD, of VMAT2 in Drug Abuse, amphetamine preference and narcolepsy will be summarized, and compared to results in mouse models and current evidence from human genome-wide linkage- and association–based scans for loci that contribute to alcohol, nicotine, or polysubstance abuse vulnerability. An emerging picture of polygenic, heterogenous genetics with substantial plieotrophism will be presented. Likely roles of dopaminergic gene variants will be placed on this background.

Role of dopamine in drug abuse and addiction in human subjects: results from imaging studies

Nora D. Volkow, Gene-Jack Wang, Joanna S. Fowler, Peter Thanos
Brookhaven National Laboratories, Stoneybrook, New York, NY

The ability of drugs of abuse to increase dopamine (DA) concentration in limbic brain regions including the Nucleus Accumbens has been hypothesized to underlie their reinforcing effects. We have used positron emission tomography (PET) an imaging instrument that enables us to measure the levels of receptors, transporters and enzymes non-invasively in the human brain. The putative role of dopamine in drug addiction based on our experiments is favored and can be summarized as follows: 1. In various addictions including cocaine, alcohol, heroin, marijuana, methamphetamine and even in pathological obese subjects, PET revealed a long -lasting reduction in DA D2 receptors. This lends support to the hypothesis that a common abnormality in addicted subjects is a deficit in DA D2 receptors with resultant underactivation of reward circuits in the brain which could facilitate addictive behavior as a means to temporarily compensate for this deficit. 2. The reduction in DA D2 receptors in addicted subjects was associated with decreased activity in the orbitofrontal cortex and anterior cingulate gyrus. Since these brain regions are involved with drive and with compulsive repetitive behaviors this has led us to postulate that while disruption of reward circuits is crucial, the addictive state also involves disruption of circuits that regulate motivation and drive. 3. With regard to the predisposition to addictive behaviors an analysis of the involvement of DA D2 receptors in responses to psychostimulant drugs has revealed that non addicted subjects with high levels of DA D2 receptors report unpleasant responses to psychostimulant drugs, whereas subjects with low levels report pleasant responses. 4. In rats, upregulation of DA D2 receptors

resulted in a marked reduction in alcohol drinking behavior. This suggests a protective effect of high DA D2 receptor density against drug self-administration. These data in both animals and humans, has led us to postulate that differences in the levels of DA D2 receptors is one of the variables that contributes to differences in the vulnerability to drug self-administration and abuse. Supported by DOE (OBER), NIDA and NIAAA.

Neurocognitive and personality factors associated with genetic susceptiblity to alcohol dependence

Shirley Y. Hill
Department of Psychiatry, University of Pittsburgh School Medicine, Pittsburgh, PA.

Identification of factors associated with vulnerability to alcohol and other drug dependence requires separating the characteristics resulting form alcohol and drug use from those that predate the condition. For example, long-term use of alcohol/drugs can produce neurotoxic effects leading to cognitive and mood alteration. Our research program has been designed around the study of primary alcoholics and their first-degree relatives. Comparison of adult nonalcoholic siblings of alcoholics has revealed differences in personality as measured by a well-known personality test originally designed to measure genetic differences in personality among twins (Multidimensional Personality Questionnaire). Alcoholics in comparison to their nonalcoholic siblings score higher on specific scales from this test: Negative Affectivity, Alienation, and Aggression (Hill et al 1990). Preliminary studies with polymorphisms associated with dopamine receptor genes (D2 and D4) give evidence favoring linkage between a D2 variant and Negative Affectivity, Stress Reaction and Alienation and evidence for a possible link between D4 and Stress Reaction (Hill et al 1999). These results are consistent with evidence that smokers who use nicotine to self-medicate depression are more prone to carry one variant of the D4 gene (Lerman et al 1998). One important aspect of our research has been the use of "high-risk" paradigm in which children/adolescents from families where multiple relatives are alcoholic, and who have not begun to use alcohol/drugs, are followed longitudinally at yearly intervals and assessed on a number of dimensions including the transition to use and abuse/dependence. A major focus has been on neurobiological measures that are most strongly influenced by genetic factors and less influenced by environment or culture, namely, acquisition of age-appropriate balance control and age-appropriate neurophysiological status. We have measured the event-related potential (ERP) characteristics of high and low-risk children yearly for as long as 10 years. Our focus has been predominantly on the P300 component, an ERP related to information processing. Additionally, the amplitude of the P300 component has been shown to be highly correlated within pairs of closely related individuals and uncorrelated in unrelated persons suggesting that the P300 response is heritable (Hill et al 1996). Many laboratories including our own (Hill et al 1990; Berman et al 1993) find that high-risk children from alcoholic families have reduced P300 amplitude relative to controls. A unique aspect of our research is the demonstration that high-risk children are developmentally delayed in reaching age-appropriate levels of P300 amplitude based on our follow-up of the same children over a 10 year span (Hill et al 1999). We also find that high-risk children are developmentally delayed in acquiring age-appropriate postural control (e.g., high-risk children produce more body sway in a computerized balance assessment procedure) (Hill et al 2000). These differences have led us to speculate that differences in brain structure might be found between children from alcoholic families who are at higher risk for developing alcohol problems than low-risk controls. A recent neuroimaging study has demonstrated differences in brain structures thought to be important in addiction, namely, the extended amygdala.

Reward Behaviors as a Function of Hypo-Dopaminergic Activity: Animal Models of RDS

Eliot L. Gardner
National Institute On Drug Abuse-Intramural research Program, Baltimore, Md.

Functional deficiencies in levels of the neurotransmitter dopamine (DA) in the nucleus accumbens (NAcb) of the ventral limbic forebrain in laboratory animals appear to be correlated with reward deficiencies which, in turn, appear correlated with 1) vulnerability to drug addiction or 2) active drug-seeking and drug-taking behavior. Lewis strain rats have a basal DA NAcb deficiency as compared to Fischer 344 strain rats. Lewis rats self-administer opiates, psychostimulants, and alcohol more than Fischer 344 rats. Lewis rats also cue-condition more readily to opiates and psychostimulants than Fischer 344 rats. During active voluntary intravenous self-administration of either opiates or cocaine, relative deficiencies in NAcb DA precede and predict each "hit" of self-administered drug. Relative deficiencies of NAcb DA are also seen during acute withdrawal from opiates, psychostimulants, alcohol, and marijuana /and the acute withdrawal state is known to correlate with increased vulnerability to re-initiate drug-seeking and drug-taking behavior. Also, opponent-process neural mechanisms appear to operate during repeated opiate administration such that the reward threshold of NAcb DA neurons is diminished/reflecting a functional diminution in reward "set-point" and consequent drug-seeking and drug-taking behavior. From such evidence, it is inferred that hypo-dopaminergic activity in the NAcb may constitute an animal model of a reward deficiency syndrome, with important implications for understanding the neurobiology of drug addiction and for developing clinically useful treatments for addiction

DRD2 *taq* A1 polymorphism: the point gene for polygenic psychiatric disorders

David E. Comings
City Of Hope Medical Center, Duarte, CA.

The original publication by Blum, Noble *et al.* of an apparent association between severe alcoholism and the *Taq* A1 allele of the dopamine D2 receptor (DRD2) gene was the first report of an association between a CNS gene and a behavioral trait using a SNP or an RFLP. The subsequent association studies between the DRD2 gene, alcoholism, and a range of other behaviors related to "Reward Deficiency Sydrome", positioned it as the "point gene" for a decade of struggling to understand the genetics of complex, polygenic disorders. Immediately, several negative studies challenged the association between the DRD2 gene and other behaviors. The most popular explanation for the positive and negative studies was the case control association studies which were susceptible to hidden ethnic stratification. To avoid these, family-based linkage (lod score, sib pair analysis) or association

studies (haplotype relative risk, STD) were recommended. However, polygenic disorders are due to the additive effect of many gene variants. Linkage studies lack the power to detect genes with a small effect and family-based association studies proved to be just as variable in their outcome as case control studies. Over the past 10 years we have identified a number of variables that we believe play a role in explaining the variability in studies of single genes in polygenic disorders. These are 1. the small percent of the variance attributable to each gene, 2. different disorders can share the same genes and same alleles in common, 3. different disorders can share the same genes but different alleles, 4. molecular heterosis, 5. gender, 6. childhood stress, 7. maternal and paternal age, 8. The additive effect of multiple genes, and 9. The additive effect of functionality similar sets of genes.

Dopaminergic correlates of genetic influences on vulnerability to cocaine addiction: studies in Lewis and Fischer 344 inbred rats

Kosten TA[1], Haile CN[2,1]
[1]*Division of Substance Abuse, Yale University School of Medicine, New Haven, CT;* [2]*CNS Gene Therapy Center, Thomas Jefferson Medical College, Philadelphia, PA.*

Vulnerability to initiate cocaine use and progress into addiction reflects genetic and environmental influences. Such behavioral effects may follow from differences in the mesolimbic dopamine (DA) system with its projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). The influence of genetic factors on this system is demonstrated by profound differences in biochemical characteristics between Lewis and Fischer 344 (F344) inbred rats. In NAc, Lewis rats exhibit lower levels of $G_{i\alpha 1/2}$ but not $G_{o\alpha}$ or $G_{s\alpha}$, and of the dopamine transporter (DAT) and D2 receptors, but not D1 receptors, compared to F344 rats. Chronic cocaine causes neuroadaptations in several proteins in F344 rats whereas relatively few occur for Lewis rats. These differential dynamics of mesolimbic DA protein levels may relate to strain differences in behavioral responsiveness to cocaine. In addition to strain differences in cocaine-induced locomotor and stereotypy signs, Lewis rats show greater cocaine conditioned place preference compared to F344 rats. Lewis rats more readily acquire intravenous cocaine self-administration and appear more sensitive to its reinforcing effects under maintenance conditions relative to F344 rats. This profile of low D2 and DAT levels with greater sensitivity to cocaine reinforcement is homologous to neuroimaging data in humans. Many potential cocaine addiction pharmacotherapies affect DA transmission. Yet, matching a pharmacotherapy to a dopaminergic profile has yet to be tested in humans. In Lewis rats, the D2 antagonist, eticlopride, antagonizes cocaine self-administration but has no effect in F344 rats. The effects of the D1 antagonist, SCH 23390, on cocaine self-administration do not differ between strains. In contrast, the partial D1 agonist SKF38393 acts like an agonist in Lewis rats and like an antagonist in F344 rats. These data suggest that genetic background influences vulnerability to cocaine addiction as well as the responsiveness to potential treatment agents.

Genetic dopaminergic deficits: clinical correlates to schizoid/avoidant personality, ADHD probands, pathological violence, P300 event related potential and TOVA attention test

Eric R. Braverman, Kenneth Blum, Joel F.Lubar, Judith O.Lubar and David E. Comings
Path Medical Clinics, New York, NY, University Of North Texas, Denton, Texas, University Of Tennessee, Knoxville, Southwestern Biofeedback Institute, Knoxville, TN, and City Of Hope National Medical Center, Duarte, CA.

Our laboratory performed an association study that employed the Millon Clinical Multi-Axial Inventory (MCMI–II) self–report computerized test to reveal a contribution of polymorphisms of three dopaminergic genes to schizoid/ avoidant cluster. We found the first association of two dopaminergic genes (dopamine(DA) transporter (DAT) & dopamine D2 receptor) to be associated with schizoid/avoidant behavior . In addition, starting from two ADHD probands, we genotyped 51 subjects from four generations derived from two multiply affected families. Eighty percent of all subjects carried the DAD2A1. When compared with "super controls" (N=30) a significant association was observed ($p < 0.00000001$). As the number of RDS behaviors increase in the subjects, the presence of the DRD2A1 allele also increases. We also genotyped pathologically aggressive and violent juveniles absent of P300 responses. 100% of the subjects tested showed polymorphisms of the DAT10 allele. A significant association was found for the DAT10 allele and pathological aggression when compared to literature controls ($P<.00006$). 56% of the subjects showed polymorphisms of the DRD2A1 allele. When compared to non-aggressive controls, a significant association for the DRD2A1 and pathological aggression was observed ($P < .00006$). Moreover, we found a significant prolongation of P300 latency in homozygous D2A1/A1 genotypes. P300 latency correlated with three risk factors (1) parental Substance Use Disorder (SUD), (2) chemical dependency (i.e., cocaine dependence), and (3) carbohydrate bingeing ($p<0.03$). Moreover, decreased P300 amplitude correlated with family history of alcoholism and SUD ($p<0.049$), but did not correlate with DRD2A1. A weighted linear trend revealed a significant worsening effect of evoked–potentials (EPs) in the presence of the DRD2A1 compared to the DRD2A2 and comorbid SUD ($p<0.0001$). One hundred patients were given the TOVA (testing variables of attention) and brain electroactivity mapping. When TOVA scores were summed (< 1 standard deviation above the norm), a significant ($p <0.01$) linear trend was observed, where increasing abnormal TOVA scores associated with a percentage of patients having an abnormal prolonged latency. We found significant differences (at least $p<0.05$) between the various scores and abnormal P300 latency. These data suggest that DA deficits may indeed be one genetic antecedent to "Reward Deficiency Syndrome", as related to electrophysiological markers, and may provide new insight and clinical directions for treatment in the 21St century.