Acute Intravenous Synaptamine Complex Variant KB220™ "Normalizes" Neurological Dysregulation in Patients During Protracted Abstinence From Alcohol and Opiates as Observed Using Quantitative Electroencephalographic and Genetic Analysis for Reward Polymorphisms: Part 1, Pilot Study with 2 Case Reports

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Abstract: It is well established that in both food- and drug-addicted individuals, there is dopamine resistance due to an association with the DRD2 gene A1 allele. Evidence is emerging whereby the potential of utilizing a natural, nonaddicting, safe, putative D_2 agonist may find its place in recovery from reward deficiency syndrome (RDS) in patients addicted to psychoactive chemicals. Utilizing quantitative electroencephalography (qEEG) as an imaging tool, we show the impact of Synaptamine Complex Variant KB220™ as a putative activator of the mesolimbic system. We demonstrate for the first time that its intravenous administration reduces or "normalizes" aberrant electrophysiological parameters of the reward circuitry site. For this pilot study, we report that the qEEGs of an alcoholic and a heroin abuser with existing abnormalities (ie, widespread theta and widespread alpha activity, respectively) during protracted abstinence are significantly normalized by the administration of 1 intravenous dose of Synaptamine Complex Variant KB220™. Both patients were genotyped for a number of neurotransmitter reward genes to determine to what extent they carry putative dopaminergic risk alleles that may predispose them for alcohol or heroin dependence, respectively. The genes tested included the dopamine transporter (DAT1, locus symbol SLC6A3), dopamine D_4 receptor exon 3 VNTR (DRD4), DRD2 TaqIA (rs1800497), COMT val158 met SNP (rs4680), monoamine oxidase A upstream VNTR (MAOA-uVNTR), and serotonin transporter-linked polymorphic region (5HTTLPR, locus symbol SLC6A4). We emphasize that these are case studies, and it would be unlikely for all individuals to carry all putative risk alleles. Based on previous research and our qEEG studies (parts 1 and 2 of this study), we cautiously suggest that long-term activation of dopaminergic receptors (ie, DRD2 receptors) will result in their proliferation and lead to enhanced "dopamine sensitivity" and an increased sense of happiness, particularly in carriers of the DRD2 A1 allele. This is supported by a clinical trial on Synaptamine Complex Variant KB220™ using intravenous administration in $>$ 600 alcoholic patients, resulting in significant reductions in RDS behaviors. It is also confirmed by the expanded oral study on Synaptose Complex KB220Z™, published as part 2 of this study. Future studies must await both functional magnetic resonance imaging and positron emission tomography scanning to determine the acute and chronic effects of oral KB220™ on numbers of D_2 receptors and direct interaction at the nucleus accumbens. Confirmation of these results in large, population-based, case-controlled experiments is necessary. These studies would provide important information that could ultimately lead to significant improvement in recovery for those with RDS and dopamine deficiency as a result of a multiple neurotransmitter signal transduction breakdown in the brain reward cascade.

Keywords: intravenous Synaptamine Complex Variant KB220™; reward deficiency syndrome; dopaminergic pathways; neurotransmitter genetics; reward genes; qEEG analysis

© Postgraduate Medicine, Volume 122, Issue 6, November 2010, ISSN – 0032-5481, e-ISSN – 1941-9260

Introduction

Bridging Quantitative Electroencephalographic Abnormalities, a Neuroadaptogen, and Reward-Dependence Behaviors

The focus of this article is the partial restoration of quantitative electroencephalographic (qEEG) abnormalities (brain dysregulation) with a regulatory response to a nonpharmaceutical nutraceutical (neuroadaptogen) during protracted abstinence in drug abusers (ie, alcohol and opiates). Specifically, this is the first report of a preliminary study using qEEG to indirectly observe the neurological activity of mesolimbic reward in 2 patients during protracted abstinence from alcohol and opiate abuse following an intravenous (IV) dose of the nutraceutical (neuroadaptogen) Synaptamine Complex Variant KB220™ (LifeGen, Inc., San Diego, CA), a neuronutrigenomic complex variant.

While there are only a few studies involving qEEG analysis and pharmaceuticals and none for nutraceuticals (neuroadaptogens), there are > 510 studies on the generalized effects of qEEG on brain function, especially EEG biofeedback. This area is well researched, with many studies showing long-term success rates, improved personality, and longer tenure in treatment.[1] Moreover, our laboratory initiated the first use of a non-drug nutraceutical (neuroadaptogen) to influence alcohol craving behavior in the late 1970s.[2]

A reasonable rationale for the execution of the present study, albeit small in size, seemed to be presented by an opportunity to observe the effects on qEEG abnormalities of addicted individuals during protracted abstinence[3,4] following the administration of a well-studied neuroadaptogen.[5] In this study, we used qEEG to indirectly observe the neurological activity of mesolimbic reward system. The mesolimbic reward circuitry and neuroanatomy inter-relationships, especially involving dopaminergic activity, have been thoroughly studied, and a brief exploration of this most complicated area is pertinent.

It is important to understand the relationship between drug-seeking behavior and personality traits such as novelty seeking, which can be visualized in separate striatal circuits. These traits have been linked to polymorphisms on the dopamine (DA) receptor gene (DRD2) as well as other candidate genes in the brain reward pathway. Recently, investigators in Germany[6] used diffusion tractography (a magnetic resonance imaging [MRI] technique that reveals the "strength" of white matter tracts, which is determined by factors such as number, myelination, and density of fibers) to visualize striatal circuits in healthy adults who had completed a personality trait questionnaire that is typically used in genetic studies. High self-reported novelty-seeking behavior was associated with stronger connections in a circuit from the hippocampus and the amygdale to the ventral and medial striatum, especially in the left hemisphere.

In contrast, reward dependence was associated with stronger connections in a prefrontal-cortex-striatum circuit, especially between the striatum and the medial and lateral orbitofrontal cortices, dorsolateral prefrontal cortex (PFC), and supplemental motor area. It is known that dopaminergic circuits involving the hippocampus might support the impulsivity and exploratory drive of novelty seeking by signaling when information from the environment does not coincide with expectations. In contrast, the amygdale may modify hippocampal-striatal circuits in a manner that augments emotional arousal. Reinforcement of learning by reward signals and persistence of activities associated with reward, especially social reward (which could involve drug seeking), probably involves multiple corticostriatal loops that process complex information.

Volkow et al[7] postulated that DA contributes to addiction by disrupting the frontal cortical circuits that regulate motivation, drive, and self-control and by disrupting memory circuits that increase the motivational salience of the drug and drug-associated stimuli. Dopaminergic neurotransmission in the ventral striatum may interact with limbic processing of affective stimuli, whereas dorsal striatal dopaminergic neurotransmission can affect habitual processing of emotionally salient stimuli in the PFC. Kienast et al[8] found that the magnitude of the ratio in the ventral striatum was positively correlated with blood oxygen level-dependent (BOLD) signal increases elicited by negative versus neutral pictures in the right medial frontal gyrus (BA10), right inferior parietal lobe, and left post-central gyrus. In the dorsal striatum, the ratio was positively correlated with BOLD signal activation elicited by negative versus neutral stimuli in the left post-central gyrus. The BOLD signal elicited by positive versus neutral stimuli in the superior parietal gyrus was positively correlated with the dorsal and ventral striatal ratio. The correlations of the ratio in the ventral and dorsal striatum with processing of affective stimuli in the named cortical regions support the hypothesis that dopamine transmission (DA D_2 binding) in functional divisions of the striatum modulates processing of affective stimuli in specific cortical areas. Other brain reward genes, such as the serotonin receptor gene, have been associated with the Brodmann area and alcoholism.[9]

Five-hydroxytryptamine (5-HT2A) receptor involvement in alcoholism is suggested by less 5-HT2A binding in alcohol-preferring rats, association of a 5-HT2A receptor

gene polymorphism with alcohol dependence, and reduced alcohol intake with 5-HT2A antagonists. Underwood et al[9] found that subjects (controls or alcoholics) with a family history of alcoholism (n = 23) had less 5-HT2A binding throughout the PFC than subjects without (n = 21) a family history of alcoholism ($P < 0.05$). There was an association between genotype and the total amount of (3H)-ketanserin binding in BA46, with the TT genotype having more binding (TT > TC, approximately CC). Lower 5-HT2A receptor binding in the PFC of cases with a family history of alcoholism suggests a genetic predisposition to alcoholism. Accordingly, fewer receptors in the PFC, including Brodmann regions, may result in downstream developmental effects on the brain, resulting in a predisposition to alcoholism.[9] Moreover, we are cognizant that there are no links reported on the role of "orbital/frontal cortex" during protracted abstinence, except for a recent study by Newton et al[10] showing methamphetamine-dependent volunteers with 4 days of abstinence had increased EEG power in the delta and theta bands. Within the methamphetamine-dependent group, the majority of the conventional EEGs were abnormal (64%), compared with 18% in the non–methamphetamine-using group.

Work from our laboratory[11] provides additional support for the involvement of qEEG abnormalities in both drug-seeking individuals and those with mental disorders. Utilizing a brain mapping system, we assessed EEG, spectral analysis (qEEG), evoked potentials (EPs) (auditory and visual), and P300 (cognitive evoked potential) in a total of 111 probands divided into 3 groups: controls (n = 16), psychiatrically ill (PI) without comorbid substance use disorder (SUD) (n = 34), and PI with comorbid SUD (cocaine and alcohol abuse and dependence) (n = 61) at an outpatient neuropsychiatric clinic. Significantly different brain map abnormalities were observed relative to an assessed normal population multivariate analysis of variance (MANOVA) ($P = 0.017$). Moreover, with regard to spectral analysis, analysis of variance (ANOVA) was significant at $P = 0.038$, and we found a weighted linear trend of increased abnormal total spectral analysis ($P = 0.0113$), whereby substance use was significantly worse than controls. Moreover, among the PI and PI/SD groups, significantly greater total EP brain trap abnormalities were observed when compared with a characterized normal population ($P = 0.0023$), with increasing abnormalities as a function of SUD as measured by a weighted linear trend ($P = 0.0022$). In the frontal lobe, similar findings were observed. With temporal abnormalities (AVBITA), the ANOVA was $P < 0.011$, with a weighted linear trend of $P < 0.005$, and the PI + SUD group was significantly more abnormal than PI or control subjects on a Duncan range test. With regard to EPS and AVBITA, a weighted linear trend was observed where there were increasing abnormalities with increasing SUD ($P = 0.0001$ and $P = 0.000003$, respectively). While these earlier results are interesting, they do not provide evidence for the role of "orbital/frontal cortex" and direct influence on dopaminergic transmission changes in the NAc during protracted abstinence. This information must await studies utilizing funtional MRI (fMRI) analysis. It is of interest to briefly review the encephalographic changes associated with alcohol, marijuana, opiates, and cocaine abuse (Table 1) since the 2 probands abused these substances.

Alcohol Abuse

Quantitative electroencephalographic alterations have been described extensively in alcoholics. Most EEG reports in alcoholic patients show alterations mainly within the beta and alpha bands. Patients with a more pronounced frontal hyperarousal have a worse prognosis. Decreased power in slow bands in alcohol-dependent subjects indicates chronic brain damage. Increases in beta bands indicate cortical hyperexcitability. Moreover, abnormalities in resting EEG are highly heritable traits and are associated with a predisposition to alcoholism development. Finally, studies on the effects of alcohol dependence on EEG coherence can be summarized as lower frontal alpha and slow beta coherence in alcoholics with some topographical coherence abnormality differences between males and females.[12–17]

Marijuana Abuse

Quantitative electroencephalographic studies on acute THC exposure suggest a transient dose-dependent increase in relative power of alpha waves, decrease in alpha frequency, and decrease in relative power of beta at posterior EEG recording sites. Chronic marijuana abuse causes topographic qEEG patterns showing a persistent elevation in alpha absolute power, interhemispheric coherence over the frontal cortex, and reductions of alpha mean frequency. Another qEEG finding was the elevated voltage of all non-alpha bands in marijuana users. Of special interest is the qEEG finding of widespread decrease in the relative power of delta and beta activity over the frontal cortical regions in marijuana users.[18,19]

Heroin Abuse

Quantitative electroencephalographic changes in heroin addicts in the acute withdrawal period include low-voltage background

Table 1. Quantitative Electroencephalographic Changes in Substance Abuse

Drug of Abuse	Measure	Outcome	Comment	Reference
Alcohol	qEEG and LORETA mapping	Increase in absolute and relative beta power and a decrease in alpha, delta, and theta power.	Detoxified patients compared with normal controls	Saletu et al[12]
Alcohol	EEG	Subjects with family history have reduced relative and absolute alpha power in occipital and frontal regions and increased relative beta in both regions.	Family history of alcoholism compared with no family history	Finn and Justus[13]
Alcohol	EEG	Alcoholics differ in resting EEG coherence having lower frontal alpha and slower beta coherence in males and females.	Heavy drinkers compared with light drinkers	Kaplan et al[14]
Alcohol	EEG	In alcohol-dependent subjects, higher central alpha and slow beta coherence was found, but lower parietal alpha and slow beta coherence in males.	Alcohol-dependent compared with controls	Michael et al[15]
Alcohol	EEG	Higher left-temporal alpha and slow beta coherence and higher slow beta coherence at right temporal and frontal electrode pairs in alcoholic males and females.	Alcohol-dependent compared with controls	Winterer et al[16]
Alcohol	EEG	Moderate-to-heavy heavy drinking is associated with differences in synchronization of brain activity during rest and mental rehearsal. Heavy drinkers displayed a loss of hemispheric asymmetry of EEG synchronization in the alpha and slow beta band. Moderately and heavy drinking males also showed lower fast beta band synchronization.	Comparison of moderate-to-heavy with heavy drinking	de Bruin et al[17]
Marijuana	EEG	Acute THC exposure produced transient increases in either posterior alpha power, decreases in mean alpha frequency or increase in alpha synchrony, and decreases in relative power of beta.	Acute effects of THC	Struve et al[18]
Marijuana	qEEG	Significant association between chronic marijuana use and topographic qEEG patterns of persistent alpha hyperfrontality as well as reductions in alpha mean frequency. There was also elevated voltage of all non-alpha bands in chronic marijuana users. Finally, there was a widespread decrease in the relative power of delta and beta activity over the frontal cortical regions in chronic marijuana users.	Chronic effects of THC exposure	Struve et al[19]
Heroin	qEEG	Qualitative changes were observed in $> 70\%$ of heroin addicts in early abstinence and included low-voltage background activity with diminution of alpha rhythm, an increase in beta activity, and a large amount of low-amplitude delta and theta waves in central regions. Also, frequency shifts in fast-alpha range at the frontal and central recording sites and a slowing of slow-wave alpha mean frequency at the central, temporal, and occipital sites of recording heroin abusers who used heroin for ≥ 18 months.	Acute withdrawal	Polunina and Davydov et al[20]
Heroin	qEEG	Abstinent alcoholics have an enhanced fast-beta power compared with healthy controls.	Alcoholics compared with healthy controls	Franken et al[21]
Heroin	EEG	Elevated synchrony within beta frequency during short-term heroin withdrawal may reflect a state of central nervous system activation toward reward-seeking behavior, with this being a prerequisite to relapse among opiate drug-dependent patients.	Polydrug abusers with emphasis on heroin abuse	Bauer[22]
Cocaine	EEG	Acute effects of cocaine include increase in beta activity, increase in delta waves, increase in frontal alpha waves, as well as an increase in alpha waves on EEG associated with bursts of cocaine-induced euphoria.	Human studies	Prichep et al[23] Alper[24]

(Continued)

Table 1 *(Continued)*

Drug of Abuse	Measure	Outcome	Comment	Reference
Cocaine	qEEG	During protracted abstinence from cocaine qEEG effects include long-lasting increases in alpha and beta bands together with reduced activity in delta and theta bands.	Several studies reported similar effects on withdrawal	Roemer et al[25]
Cocaine	qEEG	Cocaine produced a rapid increase in absolute theta, alpha, and beta power over the PFC, up to 25 minutes after drug administration. The increase in theta power was correlated with a positive drug high, and the increase in alpha power was correlated with anxiety. Also, an increase in delta coherence over the PFC correlated with nervous energy.	qEEG profiles in cocaine-dependent patients in response to an acute, single-blind, self-administered dose of smoked cocaine base (50 mg) versus placebo	Reid et al[26]
Cocaine	qEEG	Changes occur 5–14 days after last reported crack-cocaine use induced changes in brain function. These changes lasted up to 6 months.	Subjects with cocaine dependence have persistent changes in brain function	Venneman et al[27]
Cocaine	qEEG	qEEG techniques demonstrate an association between beta activity in the spontaneous EEG and relapse in cocaine abuse.	qEEG changes associate with relapse	Ceballos et al[28]

Abbreviations: EEG, electroencephalogram; LORETA, low-resolution electromagnetic tomography; PFC, prefrontal cortex; qEEG, quantitative electroencephalogram; THC, tetrahydrocannabinol.

activity with a diminution of alpha rhythm, an increase in beta activity, and a large amount of low-amplitude delta and theta waves in central regions. Pronounced desynchronization is characteristic for acute heroin withdrawal, but the spectral power of EEG tends to normalize almost completely after 3 months of abstinence. Consistent alterations in the EEGs of heroin addicts include a deficit in alpha activity and an excess of fast beta activity in early heroin abstinence.[20–22]

Cocaine Abuse

Acute effects of smoked crack-cocaine lead to a rapid increase in absolute theta, alpha, and beta power over the PFC, lasting up to a half hour after drug administration. The increase in theta power correlates with positive drug effects and the increase in alpha bands correlates with nervousness. Some EEG characteristics are due to neurotoxicity and others indicate a predisposition toward the development of cocaine addiction. During protracted abstinence from cocaine, qEEG effects include long-lasting increases in alpha and beta bands together with reduced activity in delta and theta bands. Quantitative electroencephalographic techniques have demonstrated associations between the amount of beta activity in spontaneous EEG and relapse in cocaine abuse.[23–28]

Evidence for the Existence of Reward Deficiency Syndrome in SUD

There have been many articles on the role of reward deficiency syndrome (RDS), a term that incorporates genetic antecedents to explain common shared genes related to common addictive behaviors.[29] We provide important information related to an effect of a novel neuronutrigenomic formula on qEEG response in protracted abstinence in polydrug abusers. The neuroadaptogen utilized in this study has been proposed (ongoing fMRI research) to alter mesolimbic neurochemistry, especially at dopaminergic pathways specific to the NAc. Dopamine is a major component in the mechanisms involving RDS and brain function.[30] It is well known that certain polymorphisms of a number of reward genes, including the DRD2 gene, play a role in the function of dopamine.[30] Reward deficiency syndrome seems to be linked to flawed dopamine functioning, especially to genetically induced low D_2 receptor (D_2R) density.[31] Moreover, RDS results from a dysfunction in the mesolimbic system of the brain, which directly links abnormal craving behavior with a defect in the DRD2 gene as well as other dopaminergic genes (D_1, D_3, D_4, and D_5, DAT1A, MAO, COMT), including many genes associated with the brain reward function (Figure 1).[32]

The role of specific candidate genes has been the subject of much debate, and to date there is no consensus of a unique gene panel for addiction. There are many candidate genes representing the neurochemical mechanisms involved in reward-dependence behaviors linked to mesolimbic circuitry. Most recently, Hodgkinson et al[33] developed a panel of markers able to extract full haplotype information for candidate genes in alcoholism, other addictions, and disorders of mood and anxiety. A total of 130 genes were haplotype-tagged and

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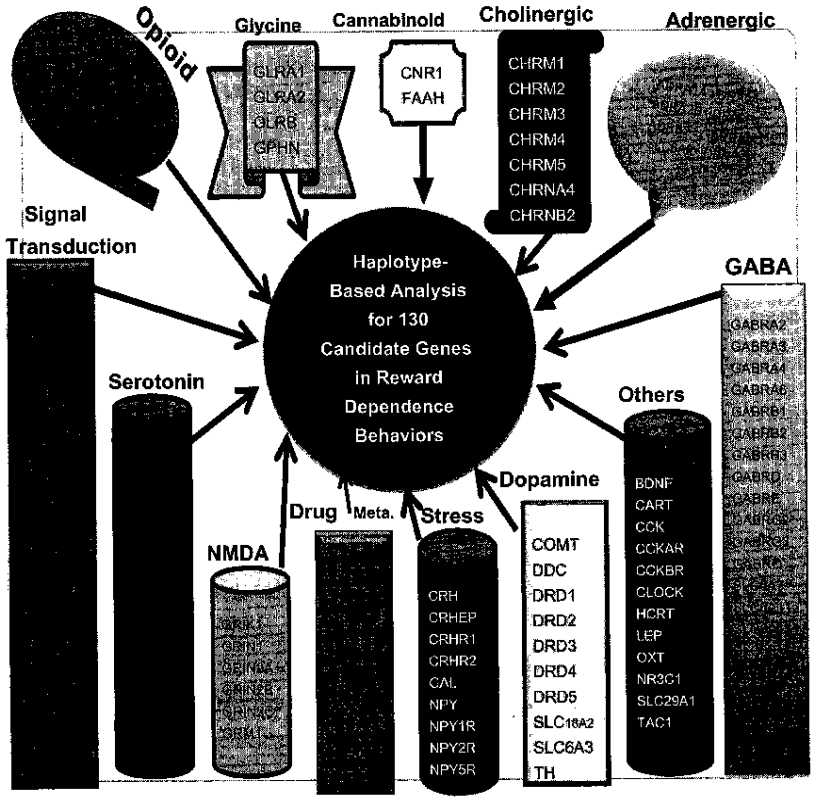
genotyped in 7 case-control populations and 51 reference populations using Illumina Golden Gate SNP genotyping technology, determining haplotype coverage. Figure 1 shows the 130 candidate genes involved.

Other array analysis work has been accomplished by Li et al,[34] who integrated 2343 reports from peer-reviewed publications between 1976 and 2006 linking genes and chromosome regions to addiction by single-gene strategies, microarray, proteomics, or genetic studies. They identified 1500 human addiction-related genes and developed the Knowledgebase for Addiction-Related Genes, the first molecular database for addiction-related genes with extensive annotations and a Web interface. Li et al[34] then performed a meta-analysis of 396 genes that were supported by ≥ 2 independent items of evidence to identify 18 molecular pathways that were statistically significantly enriched, covering both upstream signaling events and downstream effects. Five molecular pathways significantly enriched for all 4 different types of addictive drugs were identified as common pathways that may underlie shared rewarding and addictive actions, including 2 new ones, GnRH signaling pathway and gaps junction. They connected the common pathways into a hypothetical common molecular network for addiction. Interestingly, 2 final pathways emerged, which included the glutamate pathway and the dopaminergic pathway.

Dopamine is a neurotransmitter in the brain that controls feelings of well-being. This sense of well-being results from the interaction of dopamine and neurotransmitters, such as serotonin, opioids, and other brain chemicals.[30,31,35] Low serotonin levels are associated with depression. High levels of opioid peptides are associated with a sense of well-being. Blum et al[35] created the term "the brain reward cascade" to describe the complex interactions of these powerful neurotransmitters ultimately regulating the dopaminergic activity of the brain reward center of the brain.

In individuals possessing an abnormality in the DRD2 gene, the brain lacks enough D_2R sites to achieve adequate DA sensitivity and function. Carriers of the A1 variant in the DRD2 gene may have unhealthy appetites; may tend to be serious cocaine abusers; can indulge in overeating, which can lead to obesity; or at the other extreme, can be anorexic with extremely low caloric intake and/or suffer the greater consequences of chronic stress.[30,31,36,37] The addictive brains of

Figure 1. Addiction biology: haplotype-based analysis for 130 candidate genes on a single array in reward deficiency syndrome.



Modified from Hodgkinson et al.[33]

these individuals leads them to high generalized cravings and addictive behaviors. In essence, they seek substances, such as alcohol, opiates, cocaine, nicotine, and/or glucose, which are known to cause preferential release of DA at the NAc. This additional DA release is needed to activate their dopaminergic pathways and to make up for the lack of DA uptake in the NAc, the consequence of low D_2R density caused by the dopamine DRD2 gene TaqIA1 allele antecedents.[36-38]

Also, it has been found by our laboratory and others[39] that this genetic polymorphism is associated with abnormally aggressive behavior, which also stimulates the brain's use of DA. Not unlike the greater nutrient demands of extreme athletic performance, such excessive behavior exhausts nutrient availability, frustrates gene-nutrient interactions, and can lead to RDS, which results in further aberrant behavior (eg, excessive cravings and pleasure seeking).

Specifically, RDS involves DA resistance, a form of sensory deprivation[39] of the brain's reward or pleasure mechanisms. The syndrome can be manifested in relatively mild or severe forms that follow as a consequence of an individual's biochemical inability to derive reward from ordinary, everyday activities. Following the initial discovery by Blum et al,[40] there have been > 2866 PubMed articles on the DRD2 gene. This genetic variant is also associated with a spectrum of impulsive, compulsive, and addictive behaviors.[30,39-41] Thus, the RDS concept unites those disorders and may explain how simple genetic anomalies give rise to complex aberrant behavior.

In 1990, Blum et al,[40] using the TaqIA polymorphism of the dopamine D_2R gene locus (DRD2), reported a strong association between a virulent form of alcoholism and the minor allele (A1) of the DRD2 gene in this population. Other more recent studies further support an association of the A1 allelic form of the DRD2 gene with substance abuse vulnerability, including heroin and other compulsive behaviors.[40,41] This association serves as the cornerstone of the biogenetic disease model, which points us toward better diagnosis and more effective targeted treatment protocols. Reviews of this work provide evidence for the need to stimulate D_2Rs in the treatment of RDS.[42-44]

It is our belief that the real genesis of all behavior, whether so-called normal (socially acceptable) or abnormal (socially unacceptable) behavior, derives from an individual's genetic makeup at birth. This genetic predisposition, due to multiple gene combinations and polymorphisms, is expressed differently based on numerous environmental elements, including family, friends, educational and socioeconomic status, environmental contaminant exposure, and the availability of psychoactive drugs and food.[45] We believe the core of predisposition to these behaviors is a set of genes that promote a feeling of well-being via neurotransmitter interaction at the reward site of the brain, located in the mesolimbic system, leading to normal DA release. The DRD2 gene is responsible for the synthesis of D_2Rs. And, further depending on the genotype (allelic form A1 versus A2), the DRD2 gene dictates the number of these receptors at post-synaptic sites.[37,38]

A low number of D_2Rs[46-48] suggests a hypodopaminergic function in addictive disorders and attention-deficit/hyperactivity disorder (ADHD).[49,50] When there is a paucity of DA receptors, the person will be more prone to seek any substance or behavior that stimulates the dopaminergic system (a sort of "dopamine fix"). In this regard, most recently, Yan[50] reported that ethanol, given at a peak concentration within 5 to 10 minutes after intraparenteral administration, significantly increased both extracellular DA and serotonin in the NAc, supporting the role of these 2 neurotransmitters in the reinforcing properties of ethanol. Moreover, Honkanen et al[51] also found low basal DA release in alcohol accepting (AA) compared with alcohol non-accepting (ANA) rats, showing that dopamine plays a role in high alcohol preference of AA rats. One important study provides further support for the role of the DRD2 gene in alcohol intake in rats.[53] Utilizing a cDNA construct of the DRD2 gene implanted into the NAc of rats, they found that following a 4-day treatment, the D_2Rs increased to 150% above pretreatment level and alcohol drinking was reduced by 50%. After a period of 8 days without treatment, the D_2R density returned to pretreatment level, as did alcohol consumption. Twenty-four days later, second injections of the same construct caused a similar increase in density, with a 2-fold decrease in drinking.[54] The same group replicated this work in mice.[55]

Of particular interest is the recent work of Zijlstra et al[56] linking DRD2 receptor availability and cue-induced heroin craving response. It is known that opiate addiction is a chronic disorder characterized by relapse behavior, often preceded by craving and anhedonia. Chronic craving and anhedonia have been associated with low availability of D_2Rs, and cue-induced craving has been linked with endogenous DA release. Franken et al[21] found lower baseline D_2R availability in opiate-dependent subjects than controls in the left caudate nucleus. The D_2R availability in the putamen correlated negatively with years of opiate use. Opiate-dependent subjects demonstrated higher dopamine release after cue exposure in the right putamen than controls. Chronic craving and anhedonia were

positively correlated with DA release. Treatment strategies that increase D_2Rs may, therefore, be an interesting approach to prevent relapse in opiate addiction.

It is noteworthy that acute opiate administration has been shown to increase, while abstinence from chronic opiate use has been shown to decrease extracellular DA in the NAc. In contrast, extracellular DA in the PFC is not modified by acute opiate use, but is markedly increased during morphine and heroin abstinence syndrome.[57]

Reward Genes and the Addictive Brain

Dopaminergic genetic anomalies previously found to be associated with alcoholism are also found among people with other addictive, compulsive, or impulsive disorders.[39] The list is long and remarkable and comprises overeating and obesity, Tourette's syndrome, ADHD, and pathological gambling, among others. We believe these disorders are linked by a common biological substrate, a "hardwired" system in the brain (consisting of cells and signaling molecules) that provide pleasure in the process of rewarding certain behavior. In comparison, consider the "hardwired" response to how people respond positively to safety, warmth, and a full stomach. If these needs are threatened or are not being met, we experience discomfort and anxiety.

Moreover, the immune and neuroendocrine systems are intricately wired into sensing and responding to the stress and survival threats triggered by various aspects of this process. An inborn chemical imbalance that alters the intercellular signaling in the brain's reward process could supplant an individual's feeling of well-being with anxiety, anger, or a craving for a substance that can alleviate the negative emotions. Genotyping patients for a number of dopaminergic and other gene polymorphisms will be informative. It could help explain neurological deficits during protracted abstinence and potentially provide genetic therapeutic targets to curtail future substance abuse.[58]

Animal model support for the cascade theory[34] can be derived from a series of experiments carried out by Lee et al[59] on their substance-preferring (P) (ie, seek carbohydrates, alcohol, opiates, etc.) and non-preferring (NP) rat lines. They found that P rats have the following neurochemical profile:

- Lower serotonin neurons in the hypothalamus
- Higher levels of enkephalin in the hypothalamus (due to a lower release)
- More GABA neurons in the NAc
- Reduced dopamine supply at the NAc
- Reduced densities of D_2Rs in the mesolimbic areas
- Reduced salsolinol (the tetrahydroisoquinoline derivative)

This suggests a 6-part cascade sequence leading to a reduction of net dopamine release in a key reward area. This was further confirmed when McBride's group demonstrated reduced craving behavior by administering substances that increase the serotonin supply at the synapse, or by directly stimulating D_2Rs.[59,60] Specifically, D_2R agonists reduce alcohol intake in high alcohol-preferring rats, whereas D_2R antagonists increase alcohol drinking in these inbred animals, a finding related to blocking of GABA sites.[60]

It is well known that enkephalinergic pathways play a pivotal role in addictive behavior.[30,36,43,44,61] In this regard, Blum et al[61] reversed alcohol-seeking behavior in genetically preferring C57BL/6J mice with the chronic administration of an enkephalinase inhibitor. Other work by George et al[62] concluded that a relative lack of enkephalin peptides transynaptically, possibly resulting from enhanced enkephalin degradation, might contribute to increased alcohol consumption in C57BL/6J mice. Moreover, further research demonstrated that intracranial self-stimulation by rats was reduced by NAc microinjections of kelatorphan, a potent enkephalinase inhibitor, and that chronic D_2R blockade by kelatorphan was protective.[63]

Brain Hypodopaminergic Function and the Self-Healing Process

Because deficits have been found in neurotransmitter functions underlying craving behavior, and because these deficits may be alleviated by facilitated DA release consequent to the use of opiates, nicotine, alcohol, and food, the studies mentioned above indicate enkephalinase inhibition may similarly compensate for neurotransmitter imbalance (ie, opioids, thereby attenuating craving behavior). To understand generalized craving behavior due to hypodopaminergic function (an impaired reward cascade), scientists believe individuals self-heal (or self-medicate) through biochemical (illicit or non-illicit) attempts to alleviate the low dopaminergic brain activity via drug-receptor activation (eg, alcohol, heroin, cocaine, and glucose).[30–32,36,39,41,43–46,48,50,52,53] It is conjectured that this will substitute for the lack of reward and yield a temporary compensatory sense of well-being. To help explain this so-called self-healing process, it is germane that the reinforcing properties of many drugs of abuse may be mediated through activation of common neurochemical pathways, particularly with regard to the mesolimbic DA system. In predisposed genotypes, gene polymorphic

expression (and aberrant behavior) is amplified in response to chronic nutritional deficiencies. These deficiencies result from habitual dietary patterns that are continuously unable to meet the greater nutrient needs mandated by those polymorphisms (manifesting as RDS). In this regard, glucose, opiates, nicotine, cocaine, THC, and ethanol have been shown to directly or indirectly enhance release or block reuptake of DA in at least 1 of the primary terminal sites of the limbic DA neurons in the NAc. These findings suggest the importance of genotyping for polymorphisms of the dopaminergic and other reward pathways. To this purpose, a genetic positioning map has been developed.[64,65]

RDS: Human Studies

There are a number of studies using precursor amino acids and enkephalinase inhibition that have been shown to affect various aspects of RDS.[65–75] These cited and uncited human studies (18 clinical trials in total) support the utilization of a putative natural dopaminergic agonist for the prevention of relapse, as was discussed in a recent published article by Blum et al,[39] indicating the novel mechanism and proposed treatment termed "deprivation-amplification relapse therapy" (DART). In brief, the following is a list of positive outcomes related to addictive behaviors that can be derived from the series of clinical trials cited above with macronutrients (specifically, precursor amino acid loading and enkephalinase inhibition), indicating:

- Reduced alcohol and cocaine craving
- Reduced stress rates
- Reduction of leaving treatment against medical advice
- Facilitated recovery
- Reduced relapse rates
- Reduction in carbohydrate bingeing
- Loss of body weight
- Prevention of weight regain
- Reduction of glucose craving
- Enhancement of insulin sensitivity (reversal of metabolic syndrome)
- Reduction of cholesterol
- Enhancement of memory and focus
- Enhanced compliance with narcotic antagonist
- Increased energy
- Enhanced happiness

To date, while there a number of clinical trials using amino acid precursors and enkephalinase inhibition that support the beneficial effects of this putative dopaminergic agonist (eg, D_2R activation), there is a paucity of information related to the direct interaction of this proposed nutraceutical at mesolimbic reward circuitry loci. The purpose of this preliminarily case series was to evaluate the effect of acute intravenous administration of Synaptamine Complex Variant KB220™ (neuronutrigenomic complex formula) in alcohol and opiate addicts after protracted abstinence. The study was designed to determine the potential for normalizing aberrant neurological deficits by using qEEG analysis, which measures postsynaptic potentials of pyramidal cells at a 90° angle to the cranium. The qEEG measurement has been specifically correlated to Broadmann area 25, which has been positively linked to mesolimbic function of mood regulation.[76] In addition, dopaminergic genotyping was used to determine the potential genetic antecedents involved in the abnormal neurological activity of the orbital/frontal cortex of the brain during protracted abstinence from alcohol and opiate dependence. It was hypothesized that indirect putative activation of mesolimbic sites using Synaptamine Complex Variant KB220™ will normalize neurological deficits by increasing alpha activity with a concomitant increase in low beta activity. This result would provide a mechanism in part for the previous clinical observations of the reduction in aberrant craving behavior and other associated positive outcomes following Synaptamine Complex Variant KB220™ administration.[65–75]

Material and Methods

Patients

Two subjects were selected from a cohort of patients attending Bridging the Gaps, a 30- to 90-day chemical dependence rehabilitation program located in Winchester, VA. The patients were interviewed and evaluated for chemical dependence using standard diagnostic tests and questionnaires. The tests included the following: drug history questionnaire, physical examination, urine drug tests, breathalyzer, complete blood count test, and symptom severity questionnaire. The patients were determined to be substance dependent according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria. The 2 subjects signed an approved consent form (approved by the IRB at PATH Foundation NY, New York, NY; registration # IRB00002334) and agreed to volunteer for this feasibility study. For protection of the patients, the genotyping data conformed to standard HIPPA and GINA practices mandated by law.

The 2 patients were detoxified from drugs within the previous 2 months and had symptoms of craving behavior associated with protracted abstinence. The drugs of choice

for patients 1 and 2 were alcohol and opiates, respectively. Prior to the intravenous (IV) administration of Synaptamine Complex Variant KB220™, each patient underwent a baseline EEG and qEEG analysis. The administration of the IV dosage was accomplished over a 3-hour period. Immediately after completion of IV therapy, each patient underwent a post-treatment EEG and qEEG analysis. Coupled with the qEEG, each patient was subsequently clinically assessed for any behavioral symptom changes.

Synaptamine Complex Variant KB220™

The basic patented (US patent #6,132,734) formula includes amino acid precursors such as L-phenylalanine, *l*-tyrosine, L-tryptophane, 5-hydroxytryptophane, L-glutamine, a serotonin-concentrating substance chromium, an enkephalinase inhibitor D-phenylalanine, a neurotransmitter synthesis promoter, vitamin B_6, as well as methionine and leucine. The IV protocol utilized was developed by LifeStream Solutions, Inc. The quantity of each ingredient in the patented formula was accordingly tailored for each subject and was varied according to individualized assessment and responses to the neurotransmitter questionnaire. Subject 1 was given a mixture primarily targeting serotonin (additional L-tryptophan to increase serotonin synthesis in the neuron)[77] based on the response suggesting serotonergic deficits. Subject 2 was given a mixture primarily targeting endorphins (additional D-phenylalanine) based on the response suggesting opioid peptide deficits.[78]

EEG Analysis Explanation

Abundant research has established that the EEG recorded from a healthy, normally functioning human has a predictable distribution of electrical power, just like an electrocardiogram (ECG). These predictable electrical signals are regulated by the homeostasis of a complex neuroanatomical brain system that utilizes all known neurotransmitters. Just as the ECG can be used to assess heart dysfunctions, EEG analysis can be used to assess a wide variety of brain dysfunctions related to developmental, neurological, and psychiatric disorders, whether caused by structural or functional abnormalities.[76,79,80]

Quantitative EEG techniques include frequency analysis (spectral analysis), significance probability mapping, and other analytic techniques. Each can be done on spontaneous EEG in various states or in conjunction with sensory stimulation. Several types of displays are available, including topographic mapping of scalp electrical activity. Assessment of normalcy in these records must take into account age, gender, state of alertness, medications, and other factors. Substantial statistical issues are critical in these assessments and must be thoroughly understood by all users. In this study, while being cognizant of these problems and the limitations that are imposed on a 2-case study, we report the results as preliminary.[79] For example, the head maps in this article use the color red to indicate excessive activity while blue is indicative of a deficit of brain activity. Particular disorders manifest themselves in particular parts of the brain, and thus can be identified in the activity or lack thereof in these areas. In cases such as addiction, impulsivity, craving, and depression, specific patterns of brain activity have been identified.[81]

Procedure

Nineteen electrodes using an electro-cap consistent with the modified International 10/20 systems were placed on the subjects. Routine EEGs were recorded on a Cadwell Easy II EEG (Cadwell Laboratories, Kennewick, WA) using a linked-ear montage with electrodes digitally referenced to the CZ electrode, allowing for retrospective remontaging and post hoc analysis of all data. Using data gathered under technical conditions as listed above, 99.24 seconds of EEG was selected for subject 1 and 98.52 seconds for subject 2, which were subjected to quantitative analysis of the absolute power, relative power, power asymmetry, and coherence. During EEG analysis, eyes-open and eyes-closed analyses were performed to evaluate subtle state changes in the EEG frequency and amplitude. Occasionally, other wave frequencies can be masked during eyes-closed recording. By recording eyes open, one can evaluate the presence of other frequencies due to the attenuation of alpha.[45] All recordings were performed with impedances < 7 ohms with a band pass filter of 0.5 to 70 Hz. Software was used to evaluate the EEG data.

IV Protocol

The 2 patients were detoxified from drugs within the past 2 months and had symptoms of craving behavior associated with protracted abstinence.[82] Subject 1's drug of choice was alcohol and subject 2's drug of choice was opiates. Prior to the IV administration of the Synaptamine Complex Variant KB220™, each subject underwent a baseline EEG recording. Coupled with the pre- and post-treatment EEG recording, each patient was subsequently clinically assessed for any behavioral symptoms positive or negative, which is the subject of the second part of this study.

Genotyping

Details of the genotyping methods for the polymorphisms to be assayed in this project, including primer sequences and specific PCR conditions, may be found in Anchordoquy et al,[83] Haberstick and Smolen,[84] and Haberstick et al.[85] All methods are routinely performed in the IBG laboratory. While this is not an association study and interpretation must await further confirmation in a large, case-controlled study, the genotyping data are used here for illustrative purposes based on literature consensus.

The Dopamine Transporter (DAT1, locus symbol SLC6A3)

The dopamine transporter (DAT1, locus symbol SLC6A3), which maps to 5p15.3, contains a 40 base-pair variable number tandem repeat (VNTR) element consisting of 3 to 11 copies in the 3' untranslated region (UTR) of the gene according to Vandenbergh et al.[86] The assay[83] is a modification of the method of Vandenbergh et al.[86] The primer sequences were:

Forward: 5'-TGTGGTGTAGGGAACGGCCTGAG-3', and
Reverse: 5'-CTTCCTGGAGGTCACGCTCAAGG-3'.

Dopamine D_4 Receptor

The dopamine D_4 receptor, which maps to 11p15.5, contains a 48 bp VNTR polymorphism in the third exon which according to Van Tol et al,[87] consists of 2 to 11 repeats. The assay[83] is a modification of the method of Lerman et al.[88] The primer sequences were:

Forward: 5'-VIC -GCT CAT GCT GCT GCT CTA CTG GGC-3', and
Reverse: 5'-CTG CGG GTC TGC GGT GGA GTC TGG-3'.

Monoamine Oxidase A Upstream VNTR

The monoamine oxidase A upstream MAOA gene, which maps to Xp11.3–11.4, contains a 30 bp VNTR in the 5' regulatory region of the gene, which has been shown to effect expression according to Sabol et al.[89] A genotype by environment interaction has been reported for this polymorphism by Caspi et al.[90] The MAOA-uVNTR assay is a modification[85] of a published method.[89] Primer sequences were:

Forward: 5'ACAGCCTGACCG-TGGAGAAG-3', and
Reverse: 5'-GAACGTGACGCTCCATTCGGA-3'.

Serotonin Transporter-Linked Polymorphic Region (5HTTLPR)

The serotonin transporter (5HTT, locus symbol SLC6A4), which maps to 17q11.1–17q12, contains a 43 bp insertion/deletion (ins/del) polymorphism in the 5' regulatory region of the gene according to Heils et al.[91] Due to an error in sequencing, this was originally thought to be a 44 bp deletion. According to Lesch et al,[92] the long variant (L) has approximately 3 times the basal activity of the short promoter (S) with the deletion. The primer sequences were:

Forward: 5'- 6FAM - ATG CCA GCA CCT AAC CCC TAA TGT - 3', and
Reverse: 5'- GGA CCG CAA GGT GGG CGG GA - 3'.

Hu et al[93] reported that an SNP (rs25531, A/G) in the L form of 5HTTLPR may have functional significance: the more common L_A allele is associated with the reported higher basal activity, whereas the less common L_G allele has transcriptional activity no greater than the S. The SNP rs25531 is assayed by incubating the full-length PCR product with the restriction endonuclease MspI.

For all of the above VNTR and ins/del polymorphisms, PCR reactions contained approximately 20 ng of DNA, 10% DMSO, 1.8 mM MgCl2, 200 μM deoxynucleotides, with 7'-deaza-2'-deoxyGTP substituted for half of the dGTP, 400 nM forward and reverse primers, and 1 U of AmpliTaq Gold® polymerase, in a total volume of 20 μL. Amplification was performed using touchdown PCR according to Sander et al.[94] After amplification, an aliquot of PCR product was combined with loading buffer containing size standard (Genescan 1200 Liz) and analyzed with an ABI PRISM® 3130 Genetic Analyzer. Genotypes were scored by 2 investigators independently.

DRD2 TaqIA (rs1800497)

The gene encoding the D_2R maps to 11q23, and contains a polymorphic TaqI restriction endonuclease site in the 3' untranslated region of the gene. The A1 allele has been reported to reduce the amount of receptor protein according to Pohjalainen et al.[95] This SNP is done using a TaqMan (5'nuclease) assay.[84] Primer and probe sequences were:

Forward primer: 5'-GTGCAGCTCACTCCATCCT-3',
Reverse primer: 5'-GCAACACAGCCATCCTCAAAG-3',
A1 Probe: 5'- VIC-CCTGCCTTGACCAGC-NFQMGB-3', and
A2 Probe: 5'- FAM-CTGCCTCGACCAGC-NFQMGB-3'.

Catechol-O-Methyltransferase val[158]met SNP (rs4680)

The gene encoding catechol-O-methyltransferase (COMT) maps to 22q11.21, and codes for both the membrane-bound and soluble forms (according to Männistö and Kaakkola[96]) of the enzyme that metabolizes dopamine to 3-methoxy-4-hydroxyphenylethylamine (according to Akil et al[97]). An A→G mutation results in a valine to methionine substitution at codons 158/108, respectively. This amino acid substitution has been associated with a 4-fold reduction in enzymatic activity.[95] The COMT SNP is assayed with a TaqMan method.[79] Primer and probe sequences were:

Forward primer: 5'-TCGAGATCAACCCCGACTGT-3',

Reverse primer: 5'-AACGGG-TCAGGCATGCA-3',

Val probe: 5'-FAM-CCTTGTCCTTCACGCCAGCGA-NFQMGB-3', and

Met probe: 5'-VIC-ACCTTGTCCTTCATGCCAGC-GAAAT- NFQMGB-3'.

Saliva was collected by utilization of specialized collection tubes for DNA isolation. In this study, we isolated the DNA and analyzed a number of genes. The dopaminergic genes include DRD1, DRD2, DAT1, DBH, COMT, and MAO. All subjects were genotyped based on a neutral identification number and read without knowledge of the individual being genotyped. Total genomic DNA was extracted from each coded buccal sample, and aliquots were used for polymerase chain reaction analysis.

Results

Preliminary Scan

We first assessed the pre- and post-qEEG results on each patient on the same day. In doing so we noticed from preliminary pre-experimental classic brain maps of the 2 subjects that in both cases there was characteristic low level left-to-right asymmetry of the prefrontal lobe. In the post-experimental scans there was a notable difference in the brightness signifying and increased anterior later left to anterior lateral right asymmetry of these images. This suggested an indication of progression toward normal brain activity. This was achieved by the administration of 1 neurotransmitter replenishing dosage of Synaptamine Complex Variant KB220™ IV. As discussed in the methods section, the data used for the asymmetry analysis were collected using 19 electrodes placed according to the international 10/20 system.[76]

Data used for the preliminary asymmetry study were not quantified but rather modeled using artifact-free raw data. These data were only to be used as a general, nonclinical representation of potential clinical findings. Data were digitally referenced at CZ and visually artifacted. The asymmetry topographic image was averaged from 99.2 and 98.52 seconds, respectively, of routine EEG. The data served only for visual reference of the participant's brainwave asymmetry before and after the administration of the LifeStreams IV amino acid protocol (modified KB220™) conducted at Bridging the Gaps addiction recovery center in Winchester, VA. This preliminary data prompted further in detail analysis.

EEG Analysis Amino Acid Study Interpretation

Based on this preliminary scan, we decided to further analyze the qEEG data.

Subject 1: Experimental Outcome

Subject 1 was a 24-year-old single white man who was unemployed with 2 years of college. The patient was admitted to Bridging the Gaps on May 20, 2009. His diagnosis was alcohol dependence as the major drug of choice. He also abused cannabis and cocaine. He had a history of cocaine dependence, but at the time of admission to Bridging the Gaps was in full remission. He had a history of major depression. He was taking Wellbutrin from January 2007 to March 2008. The patient stated that Wellbutrin was working well until he decided to drink alcohol again. His drug history included up to 24 beers/day or 1 L of liquor/day. He also reported experimentation with OxyContin 8 to 10 times at age 22 years, including at that time LSD and mushrooms at least 4 times. The patient started to smoke cigarettes at the age of 16 years and is currently smoking 1 pack per day. At admission, the patient was not taking any medications. The patient was not detoxified by any treatment center at the time of admission or in the past. The patient was administered the oral Synaptamine Complex Variant KB220™ variant with a particular emphasis on serotonergic precursors based on results of filling out the behavioral questionnaire. The patient received 1 IV treatment as part of this study. He is still in recovery.

The following information was generated from subject 1's scan (Figure 2). For this study, the labeling "Subject 1.1" is the pretreatment assessment (Figure 2). "Subject 1.2" is the post-treatment assessment (Figure 3). A baseline EEG and subsequent quantitative assessment were performed followed by a 3-hour IV amino acid drip. Approximately 45 minutes after the IV drip, a post-treatment qEEG was performed. Figure 2 shows significant contributing frequency bands.

The statistical tables in Figure 2 show the half-test and test-retest reliability to be very strong. The z score tables provide an overview of the statistically significant standard deviations summated in the topographical illustrations. The z scores become statistically significant at ± 1.9. Each subsequent table represents reliability for each scan presentation. Baseline EEG analysis in Figure 2 shows increased widespread theta (4–8 Hz) and increased frontal beta (12–25 Hz) waves compared with the norm. Widespread increased theta activity is associated with metabolic disturbance/dysregulation and increased frontal beta activity is associated with mood disturbance, over-arousal,

Figure 2. Subject 1.1: Baseline EEG.

Reliability

	Split half	Test retest
Average	0.99	0.94
FP1	0.99	0.98
FP2	0.99	0.96
F3	0.99	0.94
F4	0.99	0.90
C3	1.00	0.98
C4	1.00	0.99
P3	0.99	0.95
P4	1.00	0.93
O1	0.99	0.90
O2	0.99	0.89
F7	0.99	0.96
F8	0.99	0.91
T3	1.00	0.92
T4	1.00	0.90
T5	0.99	0.96
T6	0.99	0.91
Fz	1.00	0.94
Cz	1.00	0.97
Pz	0.99	0.94

Z scored peak frequency

Intrahemispheric: LEFT

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
FP1 - LE	0.63	0.64	−0.56	−0.57	−1.12	1.21	−0.50	−1.90
F3 - LE	0.25	0.53	−0.53	−0.25	−1.23	1.04	−0.29	−1.48
C3 - LE	0.17	0.43	−0.20	0.31	−1.09	0.76	0.30	−1.38
P3 - LE	0.15	1.46	−1.51	0.13	−0.69	0.69	1.10	−2.03
O1 - LE	0.02	1.89	−1.37	−0.04	−0.82	0.30	1.23	−1.67
F7 - LE	0.09	0.95	−0.80	0.19	−1.99	1.17	0.19	−1.28
T3 - LE	0.28	0.52	−0.93	−0.30	−0.27	0.19	−0.21	−0.89
T5 - LE	−0.01	1.12	−1.63	−0.10	−0.56	0.45	1.06	−1.77

Intrahemispheric: RIGHT

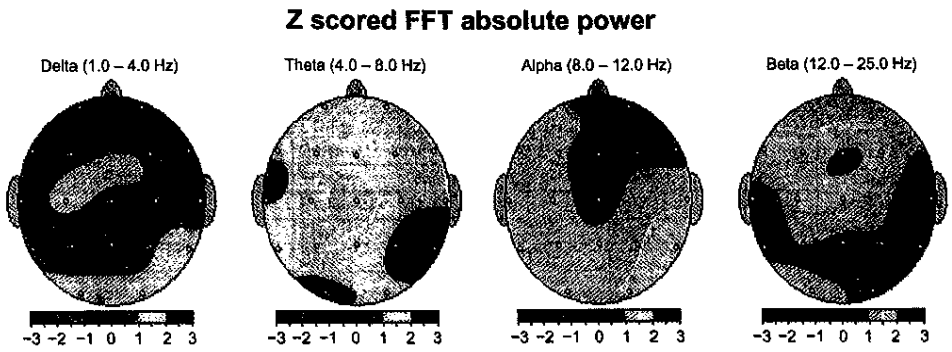
	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
FP2 - LE	0.54	0.91	−0.98	−0.46	−1.27	1.34	−0.44	−1.91
F4 - LE	−0.03	0.90	−0.74	−0.21	−1.42	1.27	−0.21	−1.79
C4 - LE	0.24	1.03	−0.89	0.07	−1.12	0.72	0.17	−1.48
P4 - LE	0.08	1.89	−1.07	0.07	−0.92	0.57	0.71	−1.77
O2 - LE	0.20	1.92	−1.29	−0.24	−0.86	0.25	0.57	−1.75
F8 - LE	0.24	1.20	−1.14	0.16	−1.69	1.19	−0.54	−1.16
T4 - LE	0.16	1.02	−1.71	−0.57	−1.04	0.12	−0.29	−1.37
T6 - LE	−0.42	2.13	−1.85	−0.35	−0.82	0.36	0.39	−2.31

Intrahemispheric: CENTER

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
Fz - LE	−0.30	0.47	−0.54	−0.14	−1.08	1.50	−0.38	−1.50
Fz - LE	0.27	0.19	−0.37	0.39	−1.34	1.98	0.37	−1.04
Cz - LE	0.14	1.36	−1.28	0.06	−0.89	0.63	0.69	−1.77

(Continued)

Figure 2. *(Continued)*



and anxiety.[83,98] These features are often associated with anxiousness, obsessive behavior, anger, and difficulty de-escalating when upset.[83,98,99] Figure 3 shows significant contributing frequency bands. Post-EEG analysis shows reduction in frontal theta (4–8 Hz) and frontal beta (12–15 Hz) activity, indicating improved functioning immediately after amino acid IV treatment. When individual frequencies are reviewed changes are noted in more detail compared with the norm. Figure 4 shows baseline EEG images, indicating single-hertz activity of theta and beta frequencies. Figure 5 shows the post amino acid EEG. Post-IV amino acid treatment decreased widespread theta waves at 4 to 7 Hz and at 15 to 16 Hz, indicating improved functioning (Figure 4). Note increased widespread theta waves from 4 to 7 Hz and increased frontal/central beta waves at 15 to 16 Hz.

Subject 2: Experimental Outcome

The EEG analysis generated from subject 2 was from a 24-year-old single white man. The patient was unemployed with 5 years of college. The patient was admitted to Bridging the Gaps on May 8, 2009. His diagnosis was opiate dependence, with heroin being the major drug of choice. He also abused alcohol, cannabis, and cocaine. He had a history of cocaine dependence and cannabis but was in sustained remission at the time of admission. The patient was also in full remission from any opiate at the time of admission. Prior diagnosis was major depression with anxiety, and he was pre-

Figure 3. Subject 1.2: Post-amino acid electroencephalography.

Reliability

	Split half	Test retest
Average	0.99	0.95
FP1	0.99	0.97
FP2	1.00	0.97
F3	1.00	0.98
F4	1.00	0.98
C3	0.99	0.97
C4	0.99	0.90
P3	1.00	0.91
P4	0.89	0.88
O1	0.99	0.92
O2	0.99	0.93
F7	0.99	0.99
F8	0.99	1.00
T3	0.99	0.98
T4	0.99	0.91
T5	0.89	0.94
T6	0.98	0.89
Fz	1.00	0.98
Cz	1.00	0.97
Pz	1.00	0.90

(Continued)

Z scored peak frequency

Intrahemispheric: LEFT

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
FP1 - LE	0.16	0.39	−0.75	−0.21	−0.95	1.60	0.44	−2.04
F3 - LE	0.08	0.45	−0.30	0.14	−1.13	1.39	0.82	−1.68
C3 - LE	−0.92	0.36	−0.01	0.56	−0.89	0.89	1.32	−1.69
P3 - LE	−0.07	1.48	−1.28	0.41	−0.32	0.79	2.04	−2.30
O1 - LE	−0.13	1.87	−1.01	0.45	−0.15	0.17	1.64	−1.21
F7 - LE	0.00	0.45	−0.71	0.47	−1.83	1.33	0.71	−1.30
T3 - LE	0.18	0.53	−0.65	−0.05	−1.18	0.26	0.65	−1.31
T5 - LE	0.21	1.16	−1.28	0.24	−0.29	0.60	1.55	−1.55

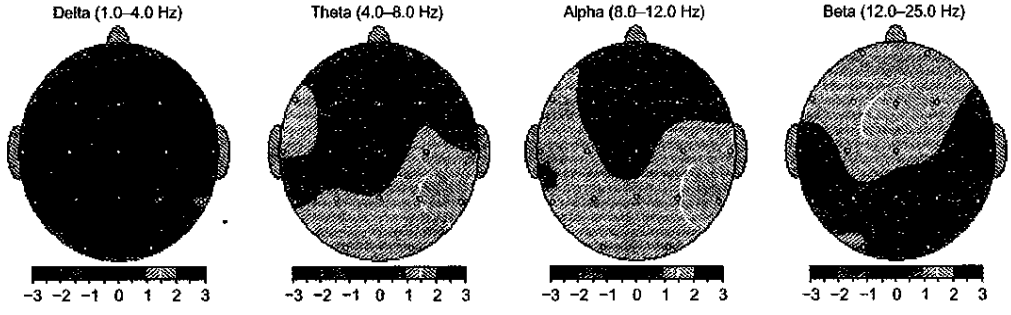
Intrahemispheric: RIGHT

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
FP2 - LE	0.12	0.73	−0.74	−0.20	−0.87	1.73	0.35	−2.02
F4 - LE	0.28	0.92	−0.42	0.14	−1.09	1.34	0.77	−1.98
C4 - LE	0.04	1.18	−0.87	0.24	−0.90	0.60	1.03	−1.84
P4 - LE	−0.63	1.98	−1.31	0.30	−0.54	0.38	1.46	−1.95
O2 - LE	0.05	1.84	−0.97	0.14	−0.13	0.16	0.83	−1.45
F8 - LE	0.87	1.37	−0.77	−0.01	−1.55	1.31	0.50	−1.51
T4 - LE	0.41	1.56	−1.59	−0.34	−0.68	0.15	0.23	−1.62
T6 - LE	−0.45	2.26	−1.49	−0.04	−0.39	0.20	0.64	−2.39

Intrahemispheric: CENTER

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
Fz - LE	−0.45	0.49	−0.29	0.33	−0.74	1.74	0.79	−1.66
Fz - LE	−0.05	0.23	−0.15	0.49	−1.00	1.09	1.21	−1.41
Cz - LE	0.04	1.41	−1.00	0.31	−0.51	0.58	1.58	−2.13

Z scored FFT absolute power



scribed Valium. His drug history included reports of using drugs in April 2009. He used OxyContin (up to 7 pills per day) at 80 mg each dose and IV heroin intermittently for 3 to 8 years. His secondary drug was cannabis. He also reported consuming 2 glasses of wine once per month on average. He currently smokes 1.5 packs of cigarettes per day. The patient stopped all previous medications on admission. This patient had undergone a number of previous detoxification treatments, including: on April 2008, standard detoxification; on January 2009, rapid detoxification followed by 30 days of sobriety; and on July 2009, rapid detoxification followed by 90 days of sobriety. The patient was administered the oral Synaptamine Complex Variant KB220™ with a particular emphasis on enkephalinase inhibition therapy (DL-phenylalanine) based on results of filling out the behavioral questionnaire. The patient received 1 IV treatment as part of this study. He is still in recovery. Figure 6 shows the subject's pretreatment assessment and Figure 7 shows the post-treatment assessment; a baseline qEEG was performed followed by a 3-hour IV amino acid drip. Approximately 45 minutes after the IV drip, a post-treatment qEEG was performed. Figure 6 shows subject 2's baseline EEG results. The patient was noted as having a relatively normal EEG with minimal z-score differences However, increased widespread alpha > 2 standard deviations from

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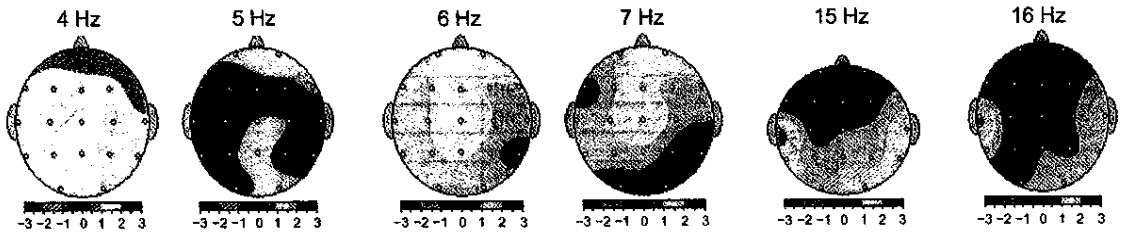
Figure 4. Subject 1.1: Baseline EEG.



normal database is noted at 11 Hz. Increased frontal alpha is most associated with depression.[83]

Subject 2 showed the most relevant change after the amino acid treatment at 11 Hz, indicating decreased widespread (11 Hz) alpha waves compared with the pretest. The previous figures represent the pre- and post-qEEG results assessed on each patient on the same day.

In subject 1 we see a typical alcohol-induced parietal/frontal abnormality[98] resulting in increased widespread theta waves, which improved with 1 IV treatment of Synaptamine Complex Variant KB220™. In subject 2, unlike subject 1, the brain scan revealed only an abnormality in the frontal region, which is related to depression, whereby there was increased widespread alpha activity.[99] This abnormality was also improved following only 1 IV treatment.

Genotyping: Addiction Risk Score

Table 2 indicates the resultant genotyping data for each patient. Based on a literature review, there are 7 risk alleles involved in the 6 candidate genes studies in this patient population. To determine severity of the 2 patients studied, we calculated the percentage of prevalence of the risk alleles and provided an arbitrary severity score based on percentage of risk alleles present. Subjects carried the following alleles: DRD2 = A1; SLC6A3 (DAT) = 10R; DRD4 = 3R or 7R; 5HTTLPR = L or L_A; MAO = 3R; and COMT = G. As depicted in Table 2, low severity (LS) was 1% to 36%, moderate severity was 37% to 50%, and high severity was 51% to 100% of subjects. Based on this model, the 2 subjects tested have at least 1 risk allele. Out of the 2 subjects, we found subject 1 to be high severity and subject 2 to be moderate severity. These scores are then converted to a fraction and represented as an ARS, whereby we found the average ARS to be: 0.64 for subject 1 and 0.43 for subject 2, respectively.

Discussion

The use of the EEG analysis shows electrophysiological signatures associated with behavioral correlates that are closely related to that of the functions assumed to be modulated by the mesolimbic pathways. The EEG analysis is measuring the postsynaptic potentials of pyramidal cells. These synaptic cells are associated with subcortical dendritic activity.[76] The findings of the EEG analysis of subject 1 show a cortical dysregulation most prominently associated with widespread diffuse theta and frontal beta waves associated with depression and mood regulation.[99] Subject 2 showed a relatively neurotypical EEG analysis with increased 11 Hz alpha waves. This feasibility study evaluated if the aforementioned dysregulations could be ameliorated by the use of an IV amino acid mixture (a modified patented Synaptamine Complex Variant KB220™).

Most importantly, the findings of the EEG analysis yielded interesting normalization across most disordered EEG spectrums and neurophysiology present in both subjects. As noted, subject 1 showed widespread diffuse theta and frontal beta waves. After administration of the IV amino acid mixture, the post-EEG analysis showed

Figure 5. Subject 1.2: Post-amino acid EEG.

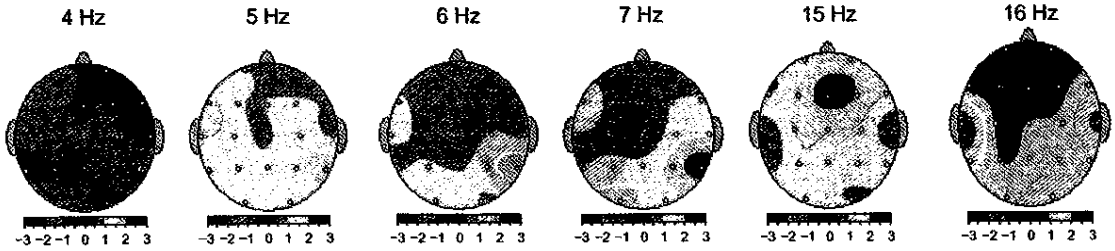


Figure 6. Subject 2.1: Baseline EEG.

Reliability

	Split half	Test retest
Average	0.98	0.97
FP1	0.96	0.95
FP2	0.97	0.95
F3	0.94	0.96
F4	0.97	0.96
C3	0.97	0.96
C4	0.97	0.98
P3	0.97	1.00
P4	0.98	1.00
O1	0.98	0.99
O2	0.99	0.98
F7	0.95	0.99
F8	0.98	0.97
T3	0.97	0.94
T4	0.98	0.93
T5	0.99	0.99
T6	0.99	0.99
Fz	0.96	0.96
Cz	0.97	0.97
Pz	1.00	1.00

Z scored peak frequency

Intrahemispheric: LEFT

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
FP1 - LE	−0.04	−0.19	2.15	−1.86	−0.38	2.28	−0.55	−0.39
F3 - LE	0.45	−0.22	2.28	−1.78	−0.22	−1.81	−0.68	−0.06
C3 - LE	0.41	−0.09	1.36	−1.50	−0.25	−0.93	−0.87	0.18
P3 - LE	0.79	0.25	1.21	2.01	−1.14	−0.87	−0.77	0.57
O1 - LE	−0.05	0.53	1.52	2.68	−1.86	−1.15	−1.68	0.58
F7 - LE	0.99	0.04	2.14	−1.37	−0.20	2.02	−0.23	0.09
T3 - LE	−0.35	−0.20	1.15	−1.42	−0.84	−1.13	−0.63	−0.13
T5 - LE	0.39	0.24	1.55	2.42	−1.09	−1.42	−0.86	0.75

Intrahemispheric: RIGHT

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
FP2 - LE	2.02	−1.02	2.32	−1.70	−0.45	2.16	−0.75	−0.57
F4 - LE	0.74	−0.42	2.26	−1.73	−0.86	−1.76	−0.54	−0.11
C4 - LE	0.22	−0.07	1.56	−1.82	−0.70	−1.21	−0.61	0.22
P4 - LE	0.73	0.24	1.33	2.10	−1.17	−0.88	−1.31	0.51
O2 - LE	0.47	0.15	1.53	2.87	−1.61	−1.29	−1.85	0.48
F8 - LE	0.18	0.03	2.36	−1.37	−0.43	1.98	−0.59	0.11
T4 - LE	1.11	0.10	1.71	−1.36	−0.77	−1.52	−0.54	0.03
T6 - LE	2.03	−1.05	1.67	2.02	−1.81	−1.64	−1.50	0.57

Intrahemispheric: CENTER

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
Fz - LE	0.65	−0.22	2.25	−1.74	−0.46	1.97	−0.64	0.16
Fz - LE	−0.00	−0.09	1.73	−1.81	−0.53	−1.54	−0.74	0.20
Cz - LE	0.71	0.07	1.14	2.02	−0.83	−0.85	−0.83	0.40

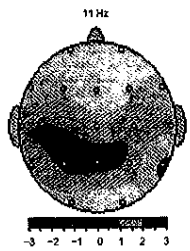


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Figure 7. Subject 2.2: Post-amino acid EEG.

Reliability

	Split half	Test retest
Average	0.98	0.93
FP1	0.99	0.86
FP2	0.99	0.87
F3	0.97	0.91
F4	0.96	0.92
C3	0.98	0.93
C4	0.97	0.93
P3	0.99	0.95
P4	0.99	0.99
O1	1.00	0.96
O2	0.98	1.00
F7	0.96	0.88
F8	0.98	0.93
T3	0.98	0.92
T4	0.98	0.91
T5	0.99	0.93
T6	0.98	0.95
Fz	0.96	0.93
Cz	0.96	0.93
Pz	0.99	0.95

Z scored peak frequency

Intrahemispheric: LEFT

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
FP1 - LE	0.70	−0.02	1.01	−1.12	0.05	−1.51	−0.26	−0.42
F3 - LE	−0.01	0.05	1.24	−0.88	0.20	−1.17	−0.11	−0.40
C3 - LE	0.38	0.27	0.76	−0.75	0.22	−0.75	−0.29	−0.42
P3 - LE	0.57	0.46	1.26	−1.91	−0.30	−1.12	−0.75	−0.00
O1 - LE	0.42	0.52	1.34	−2.44	−0.78	−1.27	−1.17	0.20
F7 - LE	0.57	0.00	1.05	−0.49	0.21	−0.94	0.35	−0.24
T3 - LE	0.38	0.07	0.69	−0.50	0.37	−0.92	0.25	−0.40
T5 - LE	0.41	0.09	1.38	−1.84	−0.20	−1.53	−0.40	0.25

Intrahemispheric: RIGHT

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
FP2 - LE	0.54	0.17	1.18	−1.07	0.13	−1.67	−0.47	−0.35
F4 - LE	0.72	0.22	1.27	−0.83	−0.09	−1.33	−0.40	−0.22
C4 - LE	0.84	0.31	1.06	−1.22	0.09	−1.02	−0.41	0.01
P4 - LE	0.39	0.56	1.52	−2.24	−0.60	−1.17	−1.27	0.24
O2 - LE	0.35	0.55	1.61	−2.72	−1.01	−1.48	−1.17	0.17
F8 - LE	−0.49	0.07	1.34	−0.54	0.26	−1.36	−0.38	−0.07
T4 - LE	1.10	0.44	1.36	−0.63	0.08	−1.25	−0.37	0.32
T6 - LE	0.48	0.45	1.65	−3.08	−1.00	−1.84	−1.53	0.17

Intrahemispheric: CENTER

	DELTA	THETA	ALPHA	BETA	HIGH BETA	BETA 1	BETA 2	BETA 3
Fz - LE	0.23	0.06	1.25	−0.82	0.25	−1.41	−0.25	−0.13
Fz - LE	0.55	0.26	1.01	−1.11	−0.01	−1.23	−0.65	−0.24
Cz - LE	0.37	0.38	1.07	−1.90	−0.35	−1.01	−0.66	−0.10

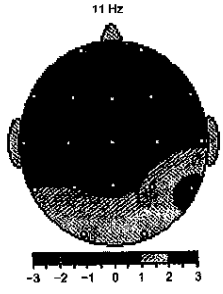

Table 2. Genotyping

Lab ID	MAOA uVNTR	5HTTLPR	5HTTLPR	SLC6A3	DRD4	DRD2	COMT	Any Risk Allele	Severity[a] ARS
CL-148754 (#1)	3R/3R	L/L	L_A/L_A	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.64 -HS
CL-148750 (#2)	4R/4R	S/L	S/L_A	10R/10R	4R/7R	A1/A2	A/A	POSITIVE	0.43-MS

[a]Percentage of severity is calculated based on 14 alleles whereby there are 7 different risk alleles. This is then converted to a fraction which represents the ARS. Severity score: low severity, 1%–36%; moderate severity, 37%–50%; high severity, 51%–100%.
Abbreviation: ARS, addiction risk score.

a significant (> 2 z score) decrease in diffuse theta and frontal beta waves showing a regulation in the frontal cortical region. Subject 2 originally showed only a < 2 z score dysregulation within the frontal 11 Hz alpha waves. The post-EEG analysis showed a regulation of the 11 Hz alpha waves, indicating the regulation of the cortical activity associated with mood depression. Both subjects showed, respectively, cortical regulation diminishing fixational tendencies and an inability to regulate mood and depression. These same functions are assumed to be associated with regulatory functions of the mesolimbic pathways. The primary confound to this study is a lack of directly correlative data associating the mesolimbic chemical pathways with the cortical post synaptic potentials. More data need to be accumulated coregistering fMRI studies with EEG analysis to correlate subcortical activation with cortical EEG activity. Other confounds include a negligible sample size and the lack of a corresponding symptom or craving questionnaire.

While there is support for a higher likelihood of treatment response and compliance using dopaminergic agonist therapy in carriers of the DRD2 A1 allele (utilizing nutrigenomic principles) compared with DRD2 A2 allele genotype by several investigators, the actual mechanism for positive clinical outcomes remains unknown.[100,101]

However, for the first time in the history of this work involving dopaminergic genetics, Laakso et al[102] have provided a clue. Accordingly, the A1 allele of the TaqI restriction fragment length polymorphism (RFLP) of the human DRD2 gene is associated with a low density of D_2Rs in the striatum. Because of the important role of D_2Rs in regulating dopamine synthesis, they aimed to examine whether subjects with the A1 allele have altered presynaptic dopamine function in the brain. They also studied the effects of 2 other DRD2 polymorphisms, C957T and –141C ins/del, which have been suggested to effect D_2R levels in the brain. The relation between the Taq IA RFLP, C957T, and –141C ins/del polymorphisms and striatal dopamine synthesis in 33 healthy Finnish volunteers was studied. They used PET scans and (18F) fluorodopa ([18F]FDOPA), a radiolabeled analog of the DA precursor L-DOPA. Heterozygous carriers of the A1 allele (A1/A2; 10 subjects) had significantly higher (18%) (18F)FDOPA uptake in the putamen than subjects without the A1 allele (A2/A2; 23 subjects). C957T and –141C ins/del polymorphisms did not significantly affect (18F)FDOPA Ki values. These results demonstrated that the A1 allele of the DRD2 gene is associated with increased striatal activity of aromatic L-amino acid decarboxylase, the final enzyme in the biosynthesis of DA and the rate-limiting enzyme for trace amine (eg, beta-phenylethylamine) synthesis. It is theorized that the finding can be explained by lower D_2R expression leading to decreased autoreceptor function, and suggests that DA and/or trace amine synthesis rate is increased in the brains of A1 allele carriers to compensate. Aromatic L-amino acid decarboxylase is the final enzyme in the biosynthesis of DA and the rate-limiting enzyme for trace amine (eg, beta-phenylethylamine). We are proposing that with an increased striatal activity of aromatic L-amino acid decarboxylase, DA synthesis should occur with a more natural and less powerful agonistic compound relative to L-DOPA. This would support the use of Synaptamine Complex Variant KB220™, a precursor amino acid and enkephalinase therapy, as a powerful DA agonist. While the acute benefits of KB220™ occur within a 1-hour timeframe, we cannot provide a clear mechanism of action. However, chronic or long-term administration of KB220™ influences dopaminergic signaling mechanisms. It is postulated that a lower DA quantity release at presynaptic neurons in the NAc should result in an upregulation of postsynaptic D_2Rs in A1 carriers, which will ultimately result in a reduction of craving behavior.

Our present findings of "normalization" of qEEG brain electrical activity abnormalities are quite interesting. Specifically, in subject 1, where we see a typical alcohol-induced parietal/frontal abnormality resulting in an increased widespread theta,[82,98,99] we found that this was improved with 1 IV treatment of KB220™. In subject 2, unlike subject 1, the brain scan revealed only an abnormality in the frontal region, which is related to depression,[82,99] whereby there was an increased widespread alpha wave activity. However, this abnormality

was also improved following only 1 IV treatment. Other qEEG studies at G&G Holistic Addiction Treatment Center (North Miami Beach, FL) from our laboratory using oral KB220™ administration revealed positive outcomes demonstrated by qEEG imaging in a randomized, triple-blind, placebo-controlled, crossover study.[103] We showed an increase of alpha and low beta activity in the parietal brain region. Using t statistics, significant differences observed between placebo and Synaptamine Complex Variant KB220™ consistently occurred in the frontal regions after week 1 and then again after week 2 of analyses ($P = 0.03$). These findings may have relevance to the potential diagnosis and treatment of drug-seeking individuals with comorbid ADHD symptoms observed in numerous RDS experiments.[104]

In earlier studies, we showed significant improvement in a number of RDS-related behaviors in > 600 patients treated with Synaptamine Complex Variant KB220™[65] as well as confirmation in an unpublished independent analysis using IV therapy for induction of recovery at Bridging the Gaps. A case for environmental pollutants/trace elements as a plausible cause of EEG abnormalities seen in both subjects could be made. For example, Ruiz Martínez et al[105] studied trace elements (zinc, copper, magnesium, iron, and lithium) by atomic absorption spectrophotometry in the plasma and erythrocytes of 120 subjects: 20 healthy controls and 100 parenteral drug addicts (69 heroin and 31 heroin + other drugs). Plasma zinc and intraerythrocytic zinc and iron were decreased, whereas plasma and intraerythrocytic copper were significantly increased in the group of drug addicts compared with the healthy controls. Moreover, a period of abstinence > 10 days was associated with lower plasma levels of zinc and lithium in subjects who had taken drugs shortly before they were examined. The presence of serological markers against hepatitis B virus and human immunodeficiency virus did not seem to influence the behavior of the trace elements in blood. There are studies showing that diesel fuel induces changes in the brain as imaged by EEG.[106] However, to date there have been no published studies involving toxic metals persistency in the brain as revealed by EEG.

However, it is more parsimonious to consider persistent EEG abnormalities in alcoholics and heroin addicts to be genetic. While there have been a number of studies to show qEEG abnormalities in addicted individuals,[107] as reviewed herein, little has been studied involving the role of genes as possible antecedents. As cited earlier, there are a number of reported articles that directly link altered frontal beta and diffuse alpha and theta wave rhythms to drug craving. In this regard, we found the following gene polymorphic alleles that are of interest. Both individuals carried the DRD2 A1 allele, suggesting a strong likelihood of a predisposition to RDS-related behaviors,[31,35] including alcohol and/or heroin abuse.

The association of the DRD2 A1 allele in alcoholism is well established.[30,36,40,108,109] The D_2R plays an important role in the reinforcing and motivating effects of ethanol. Several polymorphisms have been reported to affect receptor expression. The amount of DRD2 expressed in a given individual is the result of the expression of both alleles, each representing a distinct haplotype. Most recently, Kraschewski et al[108] found that the haplotypes I-C-G-A2 and I-C-A-A1 occurred with a higher frequency in alcoholics ($P = 0.026$, odds ratio [OR], 1.340; $P = 0.010$; OR, 1.521, respectively]. The rare haplotype I-C-A-A2 occurred less often in alcoholics ($P = 0.010$; OR, 0.507), and was also less often transmitted from parents to their affected offspring (1 vs 7). Among the subgroups, I-C-G-A2 and I-C-A-A1 had a higher frequency in Cloninger 1 alcoholics ($P = 0.083$ and $P = 0.001$; OR, 1.917, respectively) and in alcoholics with a positive family history ($P = 0.031$ and $P = 0.073$; OR, 1.478, respectively). Cloninger 2 alcoholics had a higher frequency of the rare haplotype D-T-A-A2 ($P < 0.001$; OR, 4.614) compared with controls. In patients with a positive family history, haplotype I-C-A-A2 ($P = 0.004$, OR: 0.209) and in Cloninger 1 alcoholics, haplotype I-T-A-A1 ($P = 0.045$; OR, 0.460) was often present. They confirmed the hypothesis that haplotypes, which are supposed to induce a low DRD2 expression, are associated with alcohol dependence. Furthermore, supposedly high-expressing haplotypes weakened or neutralized the action of low-expressing haplotypes.

Interestingly, we found that subject 1, who has struggled with alcoholism, not only had the DRD2 A1 allele but also carried the Val158Met polymorphism (A/G genotype), showing high COMT enzyme activity. The COMT gene is an enzyme involved in the metabolism of DA, adrenaline, and noradrenaline. The Val158Met polymorphism of the COMT gene has been previously associated with a variability of the COMT activity and alcoholism. Serý[110] found a relationship between the Val158Met polymorphism of the COMT gene and alcoholism in male subjects. We found the significant difference between male alcoholics and male controls in allele and genotype frequencies ($P < 0.007$ and $P < 0.04$, respectively). Interestingly, subject 2, who struggles with heroin as an addiction while carrying the DRD2 A1 allele, also carries the low-enzyme COMT activity genotype (A/A). This is in agreement with the work of Cao et al,[111] who did not find an association with the high G/G and heroin

addiction. No differences in genotype and allele frequencies of 108 val/met polymorphism of the COMT gene were observed between heroin-dependent subjects and normal controls (genotype-wise: chi square, 1.67; $P = 0.43$; allele-wise: chi square, 1.23; $P = 0.27$). No differences in genotype and allele frequencies of 900 ins C/del C polymorphism of the COMT gene were observed between heroin-dependent subjects and normal controls (genotype-wise: chi square, 3.73; $P = 0.16$; allele-wise: chi square, 0.76; $P = 0.38$). While there is still some controversy regarding the COMT association with heroin addiction,[112] it was also interesting that the A allele of the val/met polymorphisms (-287 A/G) found by Cao et al[113] was much higher in heroin addicts than controls.

Cook et al[114] was the first group that associated tandem repeats of the DAT gene in the literature. While there have been some inconsistencies associated with the earlier results, the evidence is mounting in favor of the view that the 10R allele of DAT is associated with high risk for ADHD in children and in adults alike. Specifically, Lee et al[115] found the nonadditive association for the 10-repeat allele was significant for hyperactivity-impulsivity (HI) symptoms, which is consistent with several others studies. However, consistent with other studies, exploratory analyses of the nonadditive association of the 9-repeat allele of DAT1 with HI and oppositional defiant disorder (ODD) symptoms also were significant.

Most recently, Biederman et al[116] evaluated a number of putative risk alleles using survival analysis and showed that by age 25 years, 76% of subjects with a DRD4 7-repeat allele were estimated to have significantly more persistent ADHD compared with 66% of subjects without the risk allele. In contrast, there were no significant associations between the course of ADHD and the DAT1 10-repeat allele ($P = 0.94$) and 5HTTLPR long allele. Their findings suggest that the DRD4 7-repeat allele is associated with a more persistent course of ADHD. This is consistent with our finding of the presence of the 7R DAT genotype in the heroin addict. Moreover, in a study by Grzywacz et al,[117] which evaluated the role of DA D_4 receptor exon 3 polymorphisms (48 bp VNTR) in the pathogenesis of alcoholism, they found significant differences in the short alleles (2-5 VNTR) frequencies between controls and patients with a history of delirium tremens and/or alcohol seizures ($P = 0.043$). A trend was also observed in the higher frequency of short alleles among individuals with an early age of alcoholism onset ($P = 0.063$). The results of this study suggest that inherited short variants of DRD4 alleles (3R) (subject 1) may play a role in pathogenesis of alcohol dependence, and carriers may have a protective effect for alcoholism risk behaviors. It is of further interest that work from Kotler et al[118] performed a study in heroin addicts, which showed that central dopaminergic pathways figure prominently in drug-mediated reinforcement, including novelty seeking, suggesting that D_2Rs are likely candidates for association with substance abuse in humans. These researchers show that the 7-repeat allele (as observed in subject 2) is significantly over-represented in the opioid-dependent cohort and confers a relative risk of 2.46.

Both subjects carried the L/L genotype of the serotonin transporter gene, which is not usually associated with alcoholism or heroin dependence.[82] Finally, the alcoholic subject carried the MAOA-uVNTR 3R allele, which has been shown to modify or increase risk of alcoholism especially in the anxiety/depressive alcoholic type (subject 1) in individuals carrying the DRD2 A1 allele. The heroin addict carried the MAOA-uVNTR 3R allele, which has not as yet been associated with heroin dependence. Specifically, low MAO activity and the neurotransmitter DA are 2 important factors in the development of alcohol dependence. Monoamine oxidase is an important enzyme associated with the metabolism of biogenic amines. Therefore, Huang et al[119] investigated whether the association between the DRD2 gene and alcoholism is affected by different polymorphisms of the MAOA gene. The genetic variant of the DRD2 gene was only associated with the anxiety, depression (ANX/DEP) ALC phenotype, and the genetic variant of the MAOA gene was associated with ALC. Subjects carrying the MAOA 3-repeat allele and genotype A1/A1 of the DRD2 were 3.48 times (95% CI, 1.47–8.25) more likely to be ANX/DEP ALC than the subjects carrying the MAOA 3-repeat allele and DRD2 A2/A2 genotype. The MAOA gene may modify the association between the DRD2 gene and ANX/DEP ALC phenotype.

Finally, alpha brainwaves are smooth, high-voltage brainwaves in the frequency range of 9 to 13 Hz. Some research suggests that alpha brainwaves are associated with a subjective state of relaxed alertness or tranquility[120,121] while other research suggests that alpha brainwaves are not associated with any particular subjective physiological state.[122] The theta rhythm state is defined as a dominance for 4 to 7 Hz brainwaves. Transient elevation of theta waves occurs during Zen meditation[123] or while entering the early stages of sleep, and is reported to be associated with vivid visualization, imagery, and dream-like states. The origin of theta waves is predominately the hippocampus,[124] although theta activity can be recorded throughout the cortex and cerebellum.[125]

It was during the late 1980s and early 1990s that Peniston and Kulkosky developed an innovative therapeutic EEG alpha-theta neurofeedback protocol for the treatment of alcoholism and prevention of its relapse.[126,127] The Peniston/Kulkosky brainwave neurofeedback therapeutic protocol combined systematic desensitization, temperature biofeedback, guided imagery, constructed visualizations, rhythmic breathing, and autogenic training incorporating alpha-theta (3–7 Hz) brainwave neurofeedback therapy.[128,129]

These investigations prompted a reexamination of EEG neurofeedback as a treatment modality for alcohol abuse. Successful outcome results included: 1) increased alpha and theta brainwave production; 2) normalized personality measures; 3) prevention of increases in beta-endorphin levels; and 4) prolonged prevention of relapse. These findings were shown to be significant for experimental subjects who were compared with traditionally treated alcoholic subjects and nonalcoholic control subjects. Subjects in several studies were chronic alcoholic male veterans, some of whom also suffered from combat-related posttraumatic stress disorder. For many subjects, pharmacological treatment was not generally beneficial. Data suggested that alpha-theta brainwave neurofeedback training appeared to have potential for decreasing alcohol craving and relapse prevention.

It is of interest that while the Peniston/Kulkosky protocol requires extensive training and there is a time lag in obtaining results, the present findings and that of the expanded study[103] (the qEEG findings with both Synaptamine Complex Varian KB220™ and Synaptose KB220Z™ showing increased alpha activity with concomitant increase low beta activity) may be quite important by possibly combinding this natural therapy with the Peniston/Kulkosky protocol to treat RDS behaviors.

Conclusion

The findings of the EEG analysis yielded interesting normalization across most disordered EEG spectrums and neurophysiology present in both subjects. As we stated earlier, more data need to be accumulated coregistering fMRI studies with EEG analysis to correlate subcortical activation with cortical EEG activity. Given the limitations, which include a negligible sample size and the lack of a corresponding symptom or craving questionnaires, we must interpret these preliminary findings with caution. Future work is warranted based on this case report. However, it is encouraging that the basic findings have been corroborated in part 2 of this research series published on Synaptose KB220Z™.[103]

To reiterate, the statistical tables show the half-test and test-retest reliability to be very strong. The z score tables provide an overview of the statistically significant standard deviations summated in the topographical illustrations. The z scores become statistically significant at ± 1.9. Findings support that the topographical illustrations are statistically significant in the pre-to-post improvement in cortical regulation and in normalization of EEG activity across the cortex after 1 dose of the KB220™ IV amino acid protocol.

This present pilot case series is supported by numerous clinical trials on KB220™ using IV administration in > 600 alcoholic patients showing significant reductions in RDS behaviors.[65] Future studies must await both fMRI and PET scanning to determine acute/chronic effects of oral KB220Z™ on numbers of D_2Rs and direct interaction at NAc. Certainly the role of KB220™ may involve positive qEEG changes that may enhance reward learning and decision making in the NAc. This is plausible since Cohen et al[130] show that the human NAc plays a key role in learning about risks by representing reward value. Thus, qEEG analyses of cross-correlations between the accumbens and simultaneous recordings of the medial frontal cortex suggest a dynamic interaction between these structures. The high spatial and temporal resolution of these recordings provides novel insights into the timing of activity in the human NAc, its functions during reward-guided learning and decision making, and its interactions with medial frontal cortex. It is of further interest that DA is critical for reward-based decision making, yet dopaminergic drugs can have opposite effects in different individuals. This apparent discrepancy can be accounted for by hypothesizing an "inverted-U" relationship, whereby the effect of DA agents depends on baseline DA system functioning. Cohen's group[131] used fMRI to test the hypothesis that genetic variation in the expression of D_2Rs in the human brain predicts opposing dopaminergic drug effects during reversal learning. Consistent with an inverted-U relationship between the DRD2 polymorphism and drug effects, cabergoline, a powerful D_2R agonist, increased neural reward responses in the medial orbitofrontal cortex, cingulate cortex, and striatum for A1+ subjects but decreased reward responses in these regions for A1– subjects. In contrast, cabergoline decreased task performance and frontostriatal connectivity in A1+ subjects but had the opposite effect in A1– subjects. Further, the drug effect on functional connectivity predicted the drug effect on feedback-guided learning. Thus,

individual variability in how dopaminergic drugs affect the brain reflects genetic disposition. These findings may help to explain the link between genetic disposition and risk for addictive disorders. This has pertinent relevance to the use of KB220™ as a D_2 agonist as evidenced from recent preliminary fMRI studies in China, whereby the acute dose directly activated the caudate accumbens brain region in protracted abstinent heroin addicts.[132]

Finally, confirmation of these results in large population-based, case-controlled experiments is necessary. Further studies may establish DA deficiency due to one's genotype as a trait marker for RDS and provide clues for reversal of the multiple neurotransmitter signal transduction breakdown in the brain reward cascade.

Acknowledgments

The authors would like to thank LifeGen, Inc., San Diego, CA, Electronic Waveform Lab, Huntington Beach, CA, Synaptamine, Inc., San Antonio, TX, and Path Foundation NY, New York, NY for their financial support during the development of this article. The authors would like to thank the internet support services of Roger L. Waite, DC, Reward Deficiency Solutions, LLC, San Diego, CA, and Quadsurf, Inc., Salt Lake City, UT.

Conflict of Interest Statement

David K. Miller, PhD discloses conflicts of interest with LifeStream Solutions, Bridging the Gaps, and LifeGen, Inc. Abdalla Bowirrat, MD, PhD discloses no conflicts of interest. Matthew Manka, PhD discloses a conflict of interest with LifeStream Solutions. Merlene Miller, MA discloses conflicts of interest with Bridging the Gaps, LifeGen, Inc., and LifeStream Solutions. Stanley Stokes, MS discloses a conflict of interest with Bridging the Gaps. Debra Manka, MHD discloses a conflict of interest with Lifestream Solutions. Cameron Allen, BA discloses a conflict of interest with C. A. Stewart, LLC. Charles Gant, MD discloses no conflicts of interest. B. William Downs, BSc discloses a conflict of interest with LifeGen, Inc. Andrew Smolen, PhD discloses no conflicts of interest. Emily Stevens, PhD discloses no conflicts of interest. Swetha Yeldandi discloses no conflicts of interest. Kenneth Blum, PhD discloses a conflict of interests with Electronic Waveform Lab, LifeGen, Inc., Path Foundation NY, and Synaptamine, Inc.

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