

# Overcoming qEEG Abnormalities and Reward Gene Deficits During Protracted Abstinence in Male Psychostimulant and Polydrug Abusers Utilizing Putative Dopamine D<sub>2</sub> Agonist Therapy: Part 2

Kenneth Blum, PhD<sup>1-5</sup>  
 Thomas J. H. Chen, PhD<sup>6</sup>  
 Siobhan Morse, MHA<sup>7</sup>  
 John Giordano, MAC,  
 PhD(Hon)<sup>2,3</sup>  
 Amanda Lih Chaun Chen, PhD<sup>7</sup>  
 James Thompson, PhD<sup>8</sup>  
 Cameron Allen, BA<sup>9</sup>  
 Andrew Smolen, PhD<sup>10</sup>  
 Joel Lubar, PhD<sup>11</sup>  
 Eric Stice, PhD<sup>12</sup>  
 B. William Downs, BSc<sup>4</sup>  
 Roger L. Waite, DC<sup>4</sup>  
 Margaret A. Madigan, BSN<sup>4</sup>  
 Mallory Kerner<sup>5</sup>  
 Frank Fornari, PhD<sup>13</sup>  
 Eric R. Braverman, MD<sup>5,14</sup>

<sup>1</sup>Department of Psychiatry and McKnight Brain Institute, University of Florida College of Medicine, Gainesville, FL; <sup>2</sup>National Institute of Holistic Studies, North Miami Beach, FL; <sup>3</sup>Department of Holistic Medicine, G&C Holistic Addiction Treatment Center, North Miami Beach, FL; <sup>4</sup>Department of Nutrigenomics, LifeGen Inc., San Diego, CA; <sup>5</sup>Department of Clinical Neurology, PATH Foundation NY, New York, NY; <sup>6</sup>Department of Occupational, Safety and Health, Chang Jung Christian University, Taiwan, ROC; <sup>7</sup>Engineering and Management of Advanced Technology, Chang Jung Christian University, Taiwan, ROC; <sup>8</sup>Brain Enhancement Solutions and Technologies, Orlando, FL and New York, NY; <sup>9</sup>Synaptic Connections Research Institute, and Welcome Home Vets, Charleston, SC; <sup>10</sup>Institute for Behavioral Genetics, University of Colorado, Boulder, CO; <sup>11</sup>Department of Psychology, University of Tennessee, Knoxville, TN; <sup>12</sup>Oregon Research Institute, Eugene, OR; <sup>13</sup>Dominium Diagnostics Inc., Kingstown, RI; <sup>14</sup>Department of Neurological Surgery, Weill Cornell College of Medicine, New York, NY

Correspondence:  
 Kenneth Blum, PhD,  
 700 West E St. #380,  
 San Diego, CA 92101.  
 Tel: 619-890-2167  
 E-mail: d-d2gene@aol.com

## Abstract

**Background:** It is well established that in both food- and drug-addicted individuals there is "dopamine resistance" associated with the DRD2 gene A1 allele. Based on earlier studies, evidence is emerging wherein the potential of utilizing a natural, nonaddicting, safe, putative D<sub>2</sub> agonist may play a significant role in the recovery of individuals with reward deficiency syndrome, including those addicted to psychoactive chemicals. **Findings:** Positive outcomes demonstrated by quantitative electroencephalographic (qEEG) imaging in a randomized, triple-blind, placebo-controlled, crossover study involving oral Synaptose Complex KB220Z™ showed an increase of alpha waves and low beta wave activity in the parietal brain region. Using t-statistics, significant differences observed between placebo and Synaptose Complex KB220Z™ consistently occurred in the frontal regions after week 1 and then again after week 2 of analyses ( $P=0.03$ ). This is the first report to demonstrate involvement of the prefrontal cortex in the qEEG response to a natural putative D<sub>2</sub> agonist (Synaptose Complex KB220Z™), especially evident in dopamine D<sub>2</sub> A1 allele subjects. Independently, we have further supported this finding with an additional study of 3 serious polydrug abusers undergoing protracted abstinence who carried the DRD2 A1 allele. Significant qEEG differences were found between those who received 1 dose of placebo compared with those who were administered Synaptose Complex KB220Z™. Synaptose Complex KB220Z™ induced positive regulation of the dysregulated electrical activity of the brain in these addicts. The results are indicative of a phase change from low amplitude or low power in the brain to a more regulated state by increasing an average of 6.169 mV<sup>2</sup> across the prefrontal cortical region. In the first experiment we found that while 50% of the subjects carried the DRD2 A1 allele, 100% carried  $\geq 1$  risk allele. Specifically, based on the proposed addiction risk score for these 14 subjects, 72% had moderate-to-severe addiction risk. Similar findings were obtained by repeating the experiment in 3 additional currently abstinent polydrug abusers carrying the DRD2 A1 allele. **Conclusion:** This seminal work will provide important information that may ultimately lead to significant improvement in the recovery of individuals with psychostimulant and polydrug abuse problems, specifically those with genetically induced dopamine deficiency. Based on this small sample size, we are proposing that with necessary large populations supporting these initial results, and possibly even additional candidate genes and single nucleotide polymorphisms, we may eventually have the clinical ability to classify severity according to genotype and possession of risk alleles, along with offering a safe, nonaddicting, natural dopaminergic receptor agonist that potentially upregulates instead of downregulates dopaminergic receptors, preferably the D<sub>2</sub> subtype.

**Keywords:** drug addiction; reward deficiency syndrome; Synaptose Complex KB220Z™

## Introduction

Nora Volkow, director of the National Institute on Drug Abuse, recently reported that the estimated societal cost of drug abuse in 2002 was \$1.81 billion. Of that figure, \$107 billion in costs were associated with drug-related crime.<sup>1</sup> However, according to research

conducted in the United Kingdom, every dollar spent on addiction treatment results in a 4- to 7-dollar reduction in community spending on drug-related crimes. Moreover, approximately 1 in 68 people (1.47% or 4 million people) in the United States are addicted to illicit drugs.<sup>1</sup> It is well known that addiction to psychostimulants is very difficult to treat, specifically cocaine, which has the lowest rate of abstinence compared with alcohol and other drugs.<sup>2</sup> The World Health Organization reported a 16% prevalence of cocaine use in the United States, which is a higher prevalence than in any other country.<sup>3</sup>

There have been a number of studies showing persistent quantitative electroencephalographic (qEEG) abnormalities in crack-cocaine users in general<sup>4,5</sup> and at 6 months of drug abstinence.<sup>6</sup> For example, for a group of 90 subjects recovering from polysubstance abuse (median, 90 days abstinent) who preferentially used cocaine, qEEG analysis showed significant decreases from normal in both absolute and relative delta power, and decreased theta power in both absolute and relative power. Significantly increased relative (but not absolute) alpha and beta power was found. Asymmetry of frontal delta, theta, and alpha power differed from normal with right power greater than left power. Globally, reduced interhemispheric coherence was found in delta and theta bands and frontally in beta bands.<sup>5</sup> However, to our knowledge, there are no published research papers showing "normalization" of qEEG abnormalities following acute administration of any biological/pharmaceutical agent except cocaine.<sup>7</sup> Moreover, there are only 2 PubMed-indexed studies on the relation of genes and qEEG,<sup>8,9</sup> neither of which relates to reward-dependence behaviors.

Since the discovery of the double helix, explorations of brain function in terms of both physiology and behavioral traits have resulted in a plethora of studies linking these activities to neurotransmitter functions with a genetic basis. The mechanisms underlining gene expression and the potential impairments due to polygenic inheritance (and therefore, predisposition to addiction and self-destructive behaviors) have been amply identified. Earlier studies by Noble et al<sup>10</sup> showed that the prevalence of the DRD2 A1 allele in cocaine-dependent (CD) subjects (n = 53) was 50.9%. It was significantly higher than either the 16% prevalence ( $P < 10^{-4}$ ) in non-substance abusing controls (n = 100) or the 30.9% prevalence ( $P < 10^{-3}$ ) in population controls (n = 2650), wherein substance abusers were not excluded. Logistic regression analysis of CD subjects identified potent routes of cocaine use and the interaction of early deviant behaviors and parental alcoholism as significant risk factors associated with

the A1 allele. The cumulative number of these 3 risk factors in CD subjects was positively and significantly ( $P < 10^{-3}$ ) related to A1 allelic prevalence. The data showing a strong association of the minor allele (A1) of the DRD2 gene with CD suggests that a gene located on the q22-q23 region of chromosome 11 confers susceptibility to this drug disorder as a subtype of reward deficiency syndrome (RDS).<sup>10,11</sup>

Due to the paucity of information related to persistent qEEG cortical brain abnormalities and reward gene polymorphisms (eg, DRD2), we genotyped psychostimulant abusers (Table 1) for a number of reward gene polymorphisms undergoing protracted abstinence for > a 6-month period, and studied a potential association between qEEG cortical brain abnormalities and these known candidate polymorphisms. Perhaps more importantly, we also evaluated the acute effect of a putative, natural D<sub>2</sub> agonist known from earlier studies to have multiple positive clinical antistimulant and antipsychotic properties<sup>12-21</sup> on persistent qEEG cortical brain abnormalities.

Further impetus comes from the companion paper on intravenous Synaptamine Complex Variant KB220™ published in the same issue in this journal,<sup>15</sup> whereby we detailed a large body of evidence from both preclinical and clinical literature illustrating drug-induced neuroadaptations in the frontal cortical brain region. The coupling of the results utilizing Synaptamine Complex Variant KB220™ and the existing qEEG literature (especially in psychostimulant abuse) provides strong rationale for examining the frontal cortex.

**Table 1.** Demographics of All Subjects for Both Experiments Combined

Parameters	Median	SD	(Minimum, Maximum)	(N = 14)
Age	33.5	± 9.11	(19, 48)	14
Clean time (months)	1	± 24.43	(6, 101)	14
White				12
Hispanic				2
Men				4
Primary substance: cocaine only				3
Primary substance: crack-cocaine				4
Primary substance: cocaine + other <sup>a</sup>				7

<sup>a</sup>In the first experiment (N = 10) patients used cocaine as the drug of choice but some also abused opiates. In the second experiment (N = 4) patients used cocaine as the drug of choice but some also abused opiates.

## Methods

Synaptose Complex KB220Z™ is a combination of neurotransmitter precursor amino acids, tryptophan, concentrating trace metal, metalosaccharides, and natural modulators promoting enkephalinase inhibition and COMT inhibition, and is a putative D<sub>2</sub> receptor (D<sub>2</sub>R) agonist. The difference between Synaptose Complex KB220Z™ and Synaptamine Complex Variant KB220™, as utilized in the intravenous study by Miller et al,<sup>15</sup> is that due to the absence of solubility issues, we were able to add *Rhodiola rosea* (a COMT inhibitor), botanicals, and metalosaccharides (for their potential biological benefits) to the oral formula. In earlier studies we have shown that KB220™ variants do not have abuse liability and are safe.<sup>11–14,19,20</sup> This qEEG experiment utilized a 2 × 2 design comparing placebo with experimental Synaptose Complex KB220Z™ in 2 randomized, triple-blind studies involving 14 psychostimulant abusers undergoing protracted abstinence. Fifty percent had been using other psychoactive drugs as well. Specifically, in the first experiment, 10 subjects used cocaine as the drug of choice, and in some cases also abused opiates. The second experiment involved 4 subjects with cocaine as the drug of choice, who also abused alcohol, marijuana, and opiates.

For the oral Synaptose Complex KB220Z™ experiment, the clinical trial was a randomized, triple-blind, placebo-controlled investigation. This qEEG study consisted of 10 serious psychostimulant abusers (eg, cocaine, methylphenidate, and/or amphetamines). Each patient was randomly assigned to either the experimental or placebo group. A saliva sample from each patient was collected in a genotyping collection tube for subsequent genotyping. The placebo was matched in cherry-flavored inert powder that was subsequently mixed with pulp-free orange juice. The Synaptose Complex KB220Z™ product was similarly mixed with orange juice. Each patient consumed the product (placebo or Synaptose Complex KB220Z™) after at least 8 hours of fasting, including no caffeine (eg, in the morning before breakfast), and 1 hour prior to scanning. After 7 days, the experimental and placebo groups were switched, and qEEG tests were repeated on each patient. The acute dose for each patient was 24 g in vehicle (before breakfast), 1 hour prior to qEEG testing. The amount of active ingredients was 7.54 g.

Table 1 shows the demographics of the overall sample, including gender, race, age, and length of abstinence. In this study there were a total of 14 individuals. There were 14 men and 0 women, with a median age for the men of 33.5 years ± 9.11 standard deviation (SD). Twelve subjects were white and 2 subjects were Hispanic (Table 1). The average number of months abstinent for the entire sample was 11 ± 24.43 (SD) months. There were 3 pure-cocaine only

addicts, 4 crack-cocaine addicts, and 7 cocaine-plus-other-drugs-of-abuse (eg, alcohol, opiates, and/or marijuana) addicts. We were able to capture only 9 of 10 subjects for qEEG analysis in the first experiment; 1 subject was dropped due to computer corruption of the data.

For the second experiment, we selected 4 polydrug abusers undergoing protracted abstinence and carrying the DRD2 A1 allele. We used the same 2 × 2 design used the first experiment. For the second confirmatory experiment there were 4 men and 0 women. All were white. In the second study, 1 subject dropped out also due to computer corruption of the data. The product is safe, and was exempt from institutional review board requirements. None of the patients took Synaptose Complex KB220Z™, placebo, or any other amino acid compound during the 7-day washout period between testing. This was a crossover experiment, whereby each patient was switched to either placebo or Synaptose Complex KB220Z™ accordingly and subsequently tested. The data collected were then sent blinded for statistical analysis.

## Genotyping

A brief description of the genotyping methods for the polymorphisms to be assayed in this project is as follows. All methods are routinely performed in the Institute for Behavioral Genetics laboratory. Details, including primer sequences and specific polymerase chain reaction conditions, may be found in Anchordoquy et al,<sup>21</sup> Haberstick et al,<sup>23</sup> and Haberstick and Smolen.<sup>24</sup> Each patient was also genotyped for the following gene polymorphisms: MAOA-VNTR, 5HTTLPR, SLC6A3, DRD4, ANKK1-DRD2 TaqIA (rs1800497), and the COMT val<sup>158</sup>met SNP (rs4680).

### Dopamine Transporter DAT1

The dopamine transporter DAT1 (locus symbol SLC6A3), which maps to 5p15.3, contains a 40-base-pair (bp) variable number tandem repeat (VNTR) element consisting of 3 to 11 copies in the 3' untranslated region (UTR) of the gene.<sup>25</sup> The assay<sup>22</sup> is a modification of the method of Vandenberg et al.<sup>23</sup> Primer sequences were:

Forward: 5'-TGTGGTGTAGGGAACGGCCTGAG-3', and

Reverse: 5'-CTTCCTGGAGGTCACGCTCAAGG-3'.

### Dopamine D<sub>4</sub> Receptor

The dopamine D<sub>4</sub> receptor (DRD4), which maps to 11p15.5, contains a 48-bp VNTR polymorphism in the third exon,<sup>26</sup> consisting of 2 to 11 repeats. The assay<sup>22</sup> is

a modification of the method of Lerman et al.<sup>27</sup> Primer sequences were:

Forward- 5'-VIC-GCTCATGCTGCTGCTCTACTG GGC-3', and

Reverse-5'-CTG CCG GTC TGC GGT GGA GTC TGG-3'.

#### Monoamine Oxidase A Upstream VNTR

The 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 affect expression.<sup>28</sup> A genotype by environment interaction has been reported for this polymorphism.<sup>29</sup> The MAOA-uVNTR assay is a modification of a published method.<sup>28</sup> Primer sequences were:

Forward- 5'-ACAGCC1GACCG-1GGAGAAG-3', and  
Reverse- 5'-GAACGTGACGC1CCAT1CGGA-3'.

#### Serotonin Transporter-Linked Polymorphic Region

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.<sup>30</sup> Due to an error in sequencing, this was originally thought to be a 44-bp deletion. The long variant (L) has approximately 3 times the basal activity of the short promoter (S) with the deletion.<sup>31</sup> Primer sequences were:

Forward- 5'-6FAM-ATGCCAGCACCTAACCCCTA-ATGT-3', and

Reverse- 5'-GGACCGCAAGCTGGCCGG GA-3'.

Hu et al<sup>32</sup> reported that an SNP (rs25531, A/G) in the L form of 5HTTLPR may have functional significance. The more common L<sub>A</sub> allele is associated with the reported higher basal activity, whereas the less common L<sub>C</sub> allele has transcriptional activity no greater than the S. The SNP rs25531 is assayed by incubating the full-length polymerase chain reaction 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 MgCl<sub>2</sub>, 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<sup>®</sup> polymerase, in a total volume of 20 μL. Amplification was performed using touchdown PCR.<sup>33</sup> After amplification, an aliquot of PCR product was combined with loading buffer containing size standard (Genescan 1200 Liz) and analyzed with an ABI PRISM<sup>®</sup> 3130 Genetic Analyzer. Genotypes were scored by 2 investigators independently.

#### ANKKI-DRD2 TaqIA (rs1800497)

The gene encoding the dopamine D<sub>2</sub>R 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.<sup>34</sup> This SNP is done using a Taqman (5'Nuclease) assay.<sup>23</sup> 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'.

#### COMT val<sup>158</sup>met SNP (rs4680)

The gene encoding COMT maps to 22q11.21 and codes for both the membrane-bound and soluble forms<sup>35</sup> of the enzyme that metabolizes dopamine to 3-methoxy-4-hydroxyphenylethylamine.<sup>36</sup> 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.<sup>36</sup> The COMT SNP is assayed with a Taqman<sup>™</sup> method. Primer and probe sequences were:

Forward primer- 5'-TCGAGATCAACCCCGACTGT-3',

Reverse primer- 5'-AACGGG-TCAGCCATGCA-3',

Val probe- 5'-FAM-CCCTGTCCTTCAAGCCAGCGA-NFQMGB-3', and

Met probe- 5'-VIC-ACCTTGCTTCATGCCAGC-GAAAT-NFQMGB-3'.

#### Addiction Risk Score

In terms of genotyping data, we have determined (based on literature review) that there are 7 risk alleles involved in the 6 candidate genes studied 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 unweighted percentage of risk alleles present. The risk score was calculated based on subjects who carried the following alleles: DRD2 = A1; SLC6A3 (DAT) = 10R; DRD4 = 3R or 7R; 5HTTLPR = L or L<sub>A</sub>; MAO = 3R; and COMT = G. As depicted in Table 2, low severity (LS) = 1% to 36%, moderate severity (MS) = 37% to 50%, and high severity (HS) = 51% to 100%.

#### Procedure

Nineteen electrodes using an electro-cap consistent with the International 10/20 systems were placed on specific

brain foci. Routine electroencephalogram (EEG) was recorded on a Cadwell Easy II using a linked-ear montage and with electrodes digitally referenced to the Cz electrode, allowing for retrospective montage analysis of all data. Using data gathered under technical conditions as listed above, 99.24 seconds of EEG were selected and subjected to quantitative analysis of absolute power, relative power, power asymmetry, and coherence. These measurements are logarithmically transformed and referenced to age-adjusted population norms.

### EEG Analysis Explanation

Abundant research has established that an EEG recorded from a healthy, normally functioning human has a predictable distribution of electrical power, just as the electrocardiogram (ECG) does. These predictable electrical signals, distinctive for each brain region, 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, the EEG can assess a wide variety of brain dysfunctions related to developmental, neurologi-

cal, and psychiatric disorders, whether caused by structural or functional abnormalities, which is called EEG analysis.

### Synaptose Complex KB220Z™

The following ingredients constitute the variant utilized in this study: thiamine HCL USP; pyridoxine HCL USP; pridoxal-5-phosphate chromium; 10% elemental as-patented O-coordinated Cr polynicotinate; phenylalanine (DL); tyrosine (L) USP; rhodifolol rosea extract 3% powder; passiflora incarnata extract 3% vitexin powder; 5HTP; L glutamine; and metallosaccharide complex.

## Results

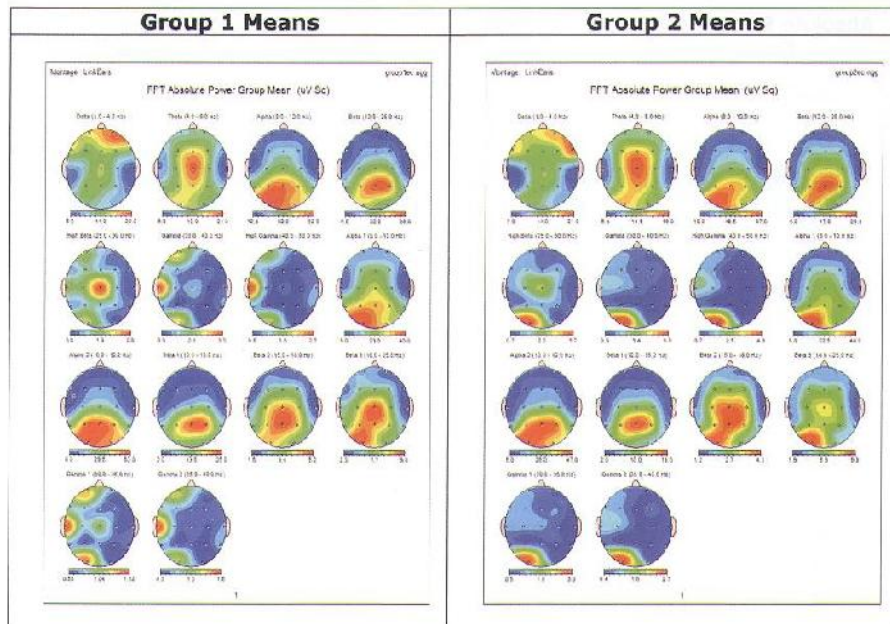
### Oral qEEG Experiments

We tested an acute dose of oral Synaptose Complex KB220Z™ on neural reward circuitry during protracted abstinence following psychostimulant dependence in 10 subjects associated with G&G Holistic Addiction Treatment Center of North Miami Beach, FL. These subjects were diagnosed as having severe psychostimulant dependence and have been in recovery for  $\geq 2$  years.

**Table 2.** Group Absolute Power Means

Site	Synaptose Complex KB220Z™ Group						Placebo Group						
	FFT Absolute Power ( $\mu V Sq$ )						FFT Absolute Power ( $\mu V Sq$ )						
	Theta	Alpha	Beta 1	Beta 2	Beta	High Beta	Theta	Alpha	Beta 1	Beta 2	Beta	High Beta	
	4.0–8.0	8.0–12.0	12.0–15.0	15.0–18.0	12.0–25.0	25.0–30.0	4.0–8.0	8.0–12.0	12.0–15.0	15.0–18.0	12.0–25.0	25.0–30.0	
FP1	10.16	21.42	2.77	2.04	8.64	1.67	FP1	9.54	17.45	2.83	1.79	8.16	1.21
FP7	10.77	21.47	2.85	1.88	7.92	1.09	FP2	9.45	16.90	2.77	1.63	7.65	1.00
F3	14.34	30.71	4.19	2.71	11.51	1.52	F3	13.52	25.28	4.51	2.59	2.17	1.54
F4	14.22	30.49	4.86	2.87	12.45	1.28	F4	12.98	25.21	4.57	2.61	2.12	1.43
C3	13.58	40.61	6.86	3.85	17.28	1.82	C3	17.86	32.44	7.61	3.40	6.98	1.79
C4	12.86	35.47	10.64	3.70	20.00	1.33	C4	12.23	35.33	8.58	3.29	7.33	1.30
P3	14.64	83.19	18.15	4.42	30.66	1.43	P3	13.35	65.31	13.70	3.37	23.69	1.50
P4	13.47	72.46	22.37	4.29	33.79	1.26	P4	12.60	65.01	13.85	3.32	22.89	1.17
O1	16.07	91.30	14.17	4.24	26.71	1.93	O1	14.85	86.30	9.74	3.86	23.38	3.66
O2	13.61	75.75	12.36	3.49	21.69	1.13	O2	12.29	61.58	7.24	2.57	15.0	1.48
F7	8.89	17.97	2.42	1.70	7.28	1.21	F7	8.30	14.53	2.48	1.66	7.60	1.32
F8	8.23	15.35	2.85	1.66	7.17	0.85	F8	8.4	13.28	2.36	1.45	6.52	0.97
T3	7.58	20.06	3.21	2.29	10.08	1.78	T3	6.8	14.56	2.74	1.68	7.77	1.25
T4	5.04	12.87	2.95	1.52	6.85	0.83	T4	5.19	10.60	2.21	1.22	5.41	0.72
T5	11.82	59.77	9.39	3.11	18.34	1.18	T5	9.11	35.70	5.88	2.34	12.74	1.38
I6	9.99	45.61	0.76	2.87	17.83	1.07	T6	8.00	43.30	5.29	1.98	11.04	0.98
F2	17.34	36.51	4.74	3.04	12.86	1.42	F2	15.64	29.37	4.85	2.68	12.70	1.33
CZ	20.06	53.23	10.34	4.69	24.02	2.78	CZ	17.88	44.55	9.57	3.91	21.05	2.52
PZ	17.06	84.91	24.04	5.11	37.02	1.47	PZ	15.70	74.30	17.54	4.02	28.08	1.37

Figure 1. Graphical representations of group differences before and after intervention.



As part of the inclusion criteria, each patient had a urine analysis to determine the absence or presence of any psychoactive drugs (illicit). None of the subjects tested showed a positive drug test, and were therefore admitted to the study. The following qEEG outcomes have been interpreted by one of us (JT) and are shown herein. Table 2 and Figure 1 illustrate the group absolute means (FFT absolute power) between Synaptose Complex KB220Z™ and placebo.

The patients did not have any psychoactive drug and/or amino acid therapy for  $\geq 6$  months prior to testing. The qEEG results are shown in the images in Figure 1, showing the graphical representations of group mean differences before and after intervention, whereby Group 1 was administered Synaptose Complex KB220Z™ and Group 2 was administered placebo. A comparison of the FFT absolute power ( $\mu\text{V Sq}$ ) of alpha (8–12 Hz) demonstrated higher activity in the Synaptose Complex KB220Z™ group compared with the placebo group. Similarly, observing the FFT absolute power ( $\mu\text{V Sq}$ ) of low beta (12–15 Hz) shows that the activity is considerably larger in the Synaptose Complex KB220Z™ group compared with the placebo group. Moreover, Table 1 highlights the regions with the largest differences, demonstrating the majority of difference in the posterior regions.

When we analyzed the absolute power differences ( $\mu\text{V Sq}$ ) between groups (eyes closed), we found an alpha (8–12 Hz) activity difference of 25  $\mu\text{Vs}$  between groups in the parietal

regions. This increase was further isolated to be most prominent in the alpha 2 (10–12 Hz) and beta 1 (12–15 Hz) bands. Unfortunately, this noted difference between groups did not reach statistical significance due to large within-group variance, which suggests the need for an increase in sample size to validate results (Figure 2).

Finally, there was a consistent effect of Synaptose Complex KB220Z™ on frontal regions when compared with placebo. Figure 3 illustrates the *P* values for group 1 versus group 2 for a between-group analysis of week 1 (Figure 3A) and week 2 (Figure 3B), whereby group comparisons utilizing *t* tests were performed. The FFT power ratio independent *t* test results were significant at the 0.05 level in both analyses. In spite of a small sample size (week 1: Synaptose Complex KB220Z™ group, *n* = 5; placebo group, *n* = 5; crossover in week 2), when the between weeks were compared independently, the findings reached significance at *P* < 0.03.

### Second Confirmatory Experiment

After extensive evaluation of the data, a few significant values were presented. The findings showed total amplitude of 6.169 mV increase throughout the 19-channel cortical post-synaptic potential recording. Due to the nature of functional EEG testing, it is important to look at the total amplitude across all 19 channels, and also look at individual analysis to evaluate if the amino acids regulated each subject as needed

Figure 2. Between-group absolute differences.

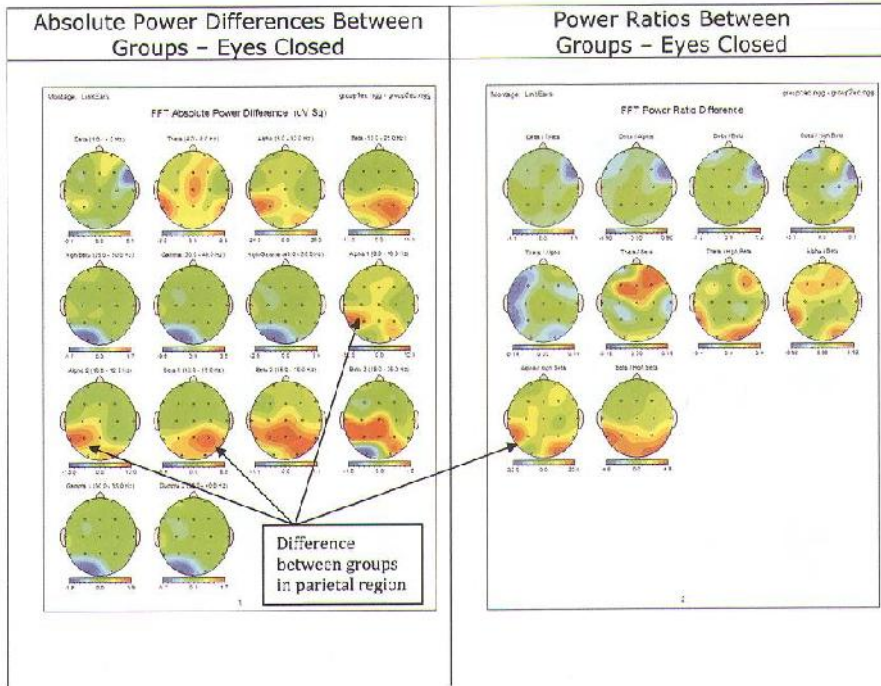


Figure 3A-B. Comparison t tests.

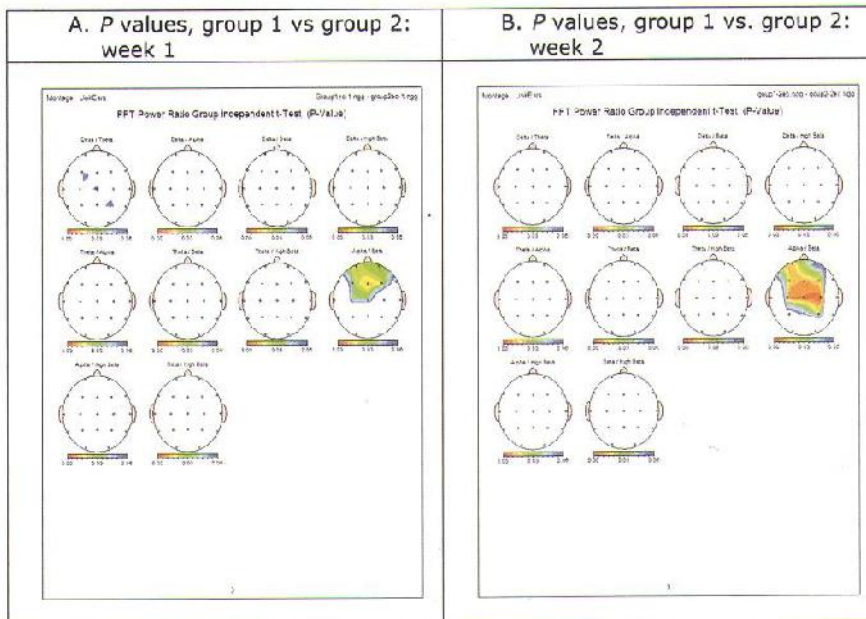
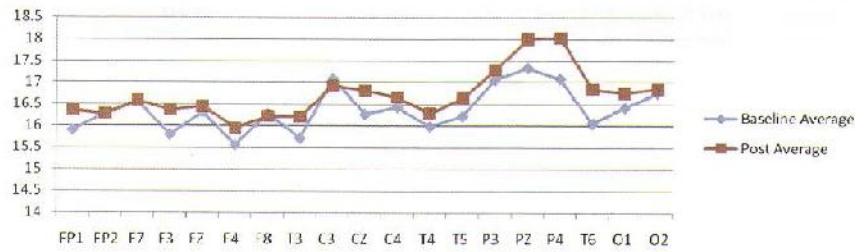


Figure 4. qEEG amplitude and frequencies across all channels.



(Figure 4). The data of these 3 subjects support the findings showing that in each case, the electrophysiology balanced according to the specific needs, as expressed by the increase in left-to-right asymmetry. Each of the subjects showed tendencies toward depression, impulsivity, anxiety, and compulsion. The sites noted for compulsion and anxiety are often associated with a relational tendency toward addictive cravings. The electrophysiology of depressive tendencies is regulated by an increased left-to-right asymmetry. The impulsivity seems to be modulated as seen in the regulation of alpha at site CZ, also known as the vertex. The anterior cingulate gyrus, which is associated with craving tendencies, seemed to be modulated by an increased alpha and a decreased high beta.

Overall, the study shows a regulation that is not diagnosis dependent. It appears that the Synaptose Complex KB220Z™ neuroadaptagen complex is an effective method for quickly increasing regulation in the neuroelectrophysiology of addicted individuals.

After extensive analysis, we found that there was an increase in amplitude of all frequencies across all 19 channels recorded (Figure 4). The chart shows that the KB220Z™ amino acid complex calmed the high amplitude sites over the posterior cingulate gyrus and to the right, causing a more balanced left-to-right asymmetry (Figure 5).

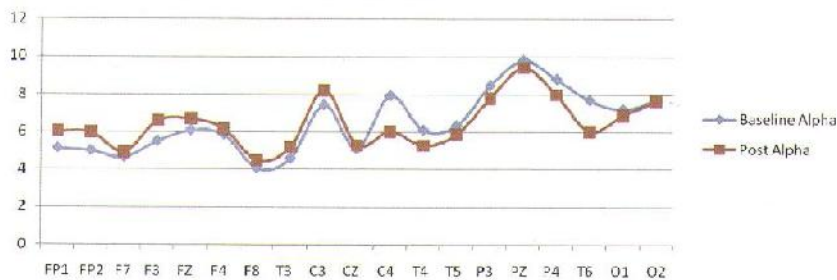
Looking more closely at the alpha and beta properties (Figure 5), we see a generalized switch in asymmetry across all sites most pronounced over C3, CZ, C4, F3, FZ, and F4, which is commonly associated with impulsivity, anxiety, compulsion, and cravings. These results are found to be reliable, with an average test-retest score of 7. In any case, where total regulation did not occur, the EEG analysis showed a trend toward regulation.

Further explorations of the previous findings were pursued on 3 subjects. The following graphs represent a trend toward brain amplitude normalization. These findings represent an increase in amplitude of the left prefrontal cortex. This region has been associated with a diminished neurophysiological correlate of anxiety.

### Genotyping: Addiction Risk Score

Based on this model (Table 3), all 14 subjects tested have  $\geq 1$  risk allele. Of the 14 subjects, we found 36% (5) HS; 36% (5) MS; and 29% (4) LS. These scores are then converted to a fraction and represented as an ARS whereby we found the average ARS to be: 0.325 LS; 0.472 MS, and 0.584 HS, respectively. Therefore, using this addiction risk score, we found that 72% of the patients were at moderate-to-high risk for addictive behavior. Most

Figure 5. Synaptose Complex KB220Z™ regulation of posterior cingulate gyrus symmetry.





**Table 3. Resultant Genotyping Data for Each Subject**

Subjects	MAOA uVNTR	5HTTLPR (biallelic)	5HTTLPR (triallelic)	SLC6A3	DRD4	DRD2	COMT	Any Risk Allele	Severity* Addiction Risk Score
1	3R/4R	S/L	S/L <sub>G</sub>	9R/10R	4R/4R	A1/A2	G/G	+	0.43-M5
2	3R/3R	S/L	S/L <sub>A</sub>	10R/10R	4R/7R	A2/A2	G/G	+	0.64-HS
3	3R/3R	L/L	L <sub>A</sub> /L <sub>G</sub>	9R/9R	3R/4R	A1/A2	A/G	+	0.57-HS
4	4R/4R	S/L	S/L <sub>A</sub>	10R/10R	3R/7R	A2/A2	G/G	+	0.57-HS
5	4R/4R	L/L	L <sub>A</sub> /L <sub>A</sub>	10R/10R	4R/7R	A2/A2	A/G	+	0.57-HS
6	3R/3R	S/S	S/S	9R/10R	4R/7R	A2/A2	A/G	+	0.36-L5
7	4R/4R	S/L	S/L <sub>G</sub>	10R/10R	4R/4R	A1/A1	A/A	+	0.36-L5
8	4R/4R	S/L	S/L <sub>A</sub>	9R/10R	3R/4R	A2/A2	A/A	+	0.29-L5
9	3R/3R	L/L	L <sub>A</sub> /L <sub>A</sub>	9R/9R	4R/7R	A2/A2	A/G	+	0.57-HS
10	4R/4R	L/L	L <sub>A</sub> /L <sub>A</sub>	9R/10R	4R/4R	A2/A2	G/G	+	0.50-M5
11	3R/3R	S/L	S/L <sub>A</sub>	9R/10R	4R/4R	A1/A2	G/G	+	0.50-M5
12	4R/4R	L/L	L <sub>A</sub> /L <sub>A</sub>	9R/10R	4R/4R	A1/A2	A/G	+	0.50-M5
13	4R/4R	S/L	S/L <sub>A</sub>	10R/10R	4R/4R	A1/A2	A/G	+	0.43-M5
14	4R/4R	S/S	S/S	9R/10R	4R/4R	A1/A2	G/G	+	0.29-L5

\*Severity score: 1%–6% = low severity; 37%–50% = moderate severity; 51%–100% = high severity. 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.

importantly, we found that 50% of the subjects carried the DRD2 A1 allele (7/14).

**Discussion**

To date, there are numerous clinical trials showing various recovery benefits from RDS behaviors using Synaptose Complex KB220Z™.<sup>11–21</sup> However, prior to the imaging studies as represented in this report, the mechanism of action has been elusive. The results of these studies support an interaction of Synaptose Complex KB220Z™ and mesolimbic activation leading to “normalization” of abnormal dopaminergic function in anticipation of patients carrying a number of reward gene polymorphisms. Although Synaptose Complex KB220Z™ appears to be a natural, nonaddicting D<sub>2</sub> agonist, cautious interpretation must await future functional magnetic resonance imaging (fMRI) and positron-emission tomography (PET) scan analysis to determine chronic induction of D<sub>2</sub>/D<sub>3</sub> receptors, especially in DRD2 A1 allele carriers and direct interaction at D<sub>2</sub>R in the NAc.

Most interesting is our finding that in a randomized, triple blind, placebo-controlled study, psychostimulant protracted abstinent abusers showed typical parietal region abnormalities in the qEEG, as seen in subjects with *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* diagnosis of depression, anxiety

disorders, and inattention. We are confident that in the small number of subjects tested there was a consistent difference observed between oral Synaptose Complex KB220Z™ compared with placebo. In fact, the Synaptose Complex KB220Z™ group revealed an increase in alpha and low beta activity in the parietal regions of the brain compared with placebo.

Since the SDs are high with this small number, we could not achieve a statistical difference when we tested the entire Group 1 with Group 2 over the 2-week study. However, between-group differences in frontal brain regions did reach significance when we compared the differences in week 1 with a crossover in week 2. Moreover, we did not find any within-group differences week to week. The fact that Synaptose Complex KB220Z™ induced an increase in both alpha and low beta activity seems to mimic the protocol used in neurofeedback to treat alcoholics. The EEG maps of drug-dependent patients differ significantly from those of normal controls and patients with other mental disorders. Decreased power in slow bands in these patients may be an indicator of brain atrophy and chronic brain damage, while an increase in the higher beta band may be related to various factors, such as medication use, family history of alcoholism and drug dependence, and/or hallucinations, suggesting a state of cortical hyperexcitability.<sup>15</sup>

In the second experiment, 3 subjects were analyzed who were in a state of protracted abstinence from multiple drugs

(eg, cocaine, alcohol, opiates, and marijuana) and carried the DRD2 A1 allele genotype. The results yielded roughly the same conclusion that the dysfunctions appeared to be regulated from the pre- to post-measurements. Research has shown that normal brain functioning for an individual, not under task will yield an even distribution of 7 to 11 Hz of activity throughout the cortical readings. The duration of the effects are unknown and should be explored further. Some limitations to the study include a small sample size and duration of protracted abstinence.

The overall data in the second experiment show an increased regulation in left-to-right asymmetry across all 19 channels. There was a discrepancy in the total mV<sup>2</sup>, also known as relative power between the entire population of the first study and that of the second. On further examination, the results of the EEG analysis showed that 2 of the subjects had an electrophysiological signature for alcohol abuse, whereas 1 subject showed an electrophysiological signature for amphetamine abuse. After this identification, individual analysis showed a 17-mV increase, which is a z score of 7 for the subject with a signature consistent with amphetamine abuse, and is in line with the original findings. The 2 subjects with signatures for alcoholism indicated an increase of 7 mVs in the slow wave activity and a decrease in fast wave beta activity. When looking at the individual signatures of the subjects, the raw data show a z score of 5.7. This adequately explains the difference between the 2 experiment groups.

We are confident that the alpha and beta increases following Synaptose Complex KB220Z™ have physiological relevance. This is reasonable because we found an increase in mVs to be between 15 and 25 mVs, which approaches the electrical activity of this brain region in nonaddicted individuals. This change occurs after only 1 dose and within 1 hour following Synaptose Complex KB220Z™ administration. We are in the progress of investigating long-term effects using PET scans to determine the number of D<sub>2</sub>/D<sub>3</sub> receptors.

The limitations of this study should be considered when interpreting the findings. This investigation was exploratory, meaning that replication of these findings require confirmatory studies using a larger sample size as well as fMRI and PET evaluations.

## Conclusion

This is the first study to report the results of the qEEG analyses supportive of the concept that beneficial effects are observed with Synaptose Complex KB220Z™ in the oral

form. The study indicates that this natural agent activates the parietal and frontal regions of the brain and increases both alpha and low beta activity. This effect suggests that Synaptose Complex KB220Z™ is “normalizing” brain abnormalities associated with drug dependence (alcohol, opiates, and psychostimulants) by acting as a dopaminergic receptor agonist during protracted abstinence in polydrug abusers. As anticipated, in agreement with other studies showing enhanced treatment response in only A1 versus A2 carriers,<sup>37–39</sup> the greatest effect occurred in those individuals possessing a higher percentage of risk alleles.

We cautiously suggest that long-term activation of dopaminergic receptors (ie, DRD2 receptors) will proliferate D<sub>2</sub> receptors, leading to enhanced “dopamine sensitivity” and an increased sense of happiness even in carriers of the DRD2 A1 allele.<sup>40</sup> This is supported by numerous clinical trials and awaits PET scanning to determine chronic effects of Synaptose Complex KB220Z™ on numbers of D<sub>2</sub>Rs. Positive outcome will provide important information that could ultimately lead to significant improvement in the recovery of individuals with RDS who have dopamine deficiency.<sup>40–44</sup>

Moreover, in future experiments we plan to evaluate the potential of Synaptose Complex KB220Z™ to correct the blunted response in the striatum and orbitofrontal cortex to intake of palatable food and imagined intake (respectively) that is associated with the DRD2 A1 allele in humans, as reported by Stice et al.<sup>45</sup> Certainly, overcoming the blunted response to palatable food would reduce overeating and ultimately induce weight loss and an enhanced sense of happiness. This is a worthy goal since obesity affects > 100 million people in the United States alone (with millions more worldwide), and should impact standard of care worldwide. Earlier studies have evoked a unique mechanism linking obesity to dopaminergic genes and glucose metabolism and receptor sensitivity.<sup>46</sup>

Increasing the microvolts within the analyzed subject population is associated with a mild cortical regulation and is assumed, based on the current research, to decrease the symptomatology of addictive tendencies, including impulsivity, obsessive tendencies, depression, and anxiety. Close clinical observation should be employed to confirm these findings. Increasing the amplitude or microvolts of the EEG is thought to be associated with an increase in postsynaptic potentials. The neurological dynamics of synaptic function suggest that higher amplitude within therapeutic values, as occurred in the current experimental population, is associated with an increase in functional potential of self-

regulation across associated correlative sites. It is widely believed that an increase in the amplitude within therapeutic values is associated with an increased cognitive potential, including self-regulation, short-term memory, working memory, and limbic functions.<sup>15</sup>

We are proposing that, based on this small sample size, with necessary large-population studies to confirm these initial results, and possible use of additional candidate genes and SNPs, we may be on track to having the clinical ability to classify risk severity according to genotype and possession of risk alleles. For a brief explanation of the relationship between these studied alleles and RDS behaviors, the authors suggest perusal of the companion paper by Miller et al.<sup>12</sup>

The risk allele analysis is based on numerous studies that associate these known alleles with many RDS behaviors including drug and alcohol abuse. A recent study by Conner et al<sup>17</sup> in adolescents provided evidence for the development of a genetic addiction risk test by assessing a number of neurotransmitter candidate genes. Like our proposed test, Conner et al<sup>17</sup> assessed both dopaminergic genes (ANKK1/TaqI A, DRD2 C957T, DRD47R, COMT val<sup>158</sup>/met substitution, and SLC6A3 R) and a GABA gene (GABRB3), and found that hypodopaminergic functioning predicted drug use in men; however, in women, a deleterious environment was the salient predictor. We assessed the same dopaminergic genes but included the MAOA-A gene as well as the serotonin transporter (5HTTLPR) gene. However, we did not genotype for the GABRB3 polymorphism. Most interestingly, we were also successful in obtaining stratification data on our smaller sample size. For the present study we have not as yet analyzed our data for recursive partitioning, a type of exploratory decision tree analysis found to be an ideal method when searching for candidate genes and haplotypes.<sup>18</sup> Unlike the study by Conner et al,<sup>17</sup> we identified the 10R, not the 9R, as the DAT1 risk allele. 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 the DAT1 gene is associated with high risk for attention-deficit/hyperactivity disorder in children and in adults alike. A more detailed discussion of these risk alleles is presented in Miller et al.<sup>15</sup>

A further strength of this study is that we only used male subjects. De Courten-Myers<sup>16</sup> noted that one of the difficulties in replicating single-gene associations with drug use disorder is sex-based differences in neurochemistry and neuroanatomy. Moreover, Conner et al<sup>17</sup> suggested that males with hypodopaminergic functioning are more likely to abuse drugs that stimulate the mesocortical-limbic system than those with normal dopaminergic functioning. In contrast, females living in

a negative environment are at increased risk (possibly not due to their genotypes) for using more drugs and even more types of drugs, which increases their risk for substance use disorder.

This work provides a potential neuromechanism of action to help explain for the first time the observed clinical antidrug-seeking benefits published in 18 trials (open-label, placebo-controlled, double-blind, triple-blind, randomized, placebo-matched) utilizing variants of the Synaptamine Complex Variam™ [SAAVE, tropamine, phenac, SG8839, LG839, KB220™ and KB220Z™]. Finally, this is the only known agent in the nutraceutical industrial space that on an acute basis “normalizes” persistent qEEG abnormalities in protracted abstinence in male psychostimulant and polydrug abusers and warrants intensive neuroimaging investigation involving both fMRI and PET.

Moreover, we are cognizant that increases in dopamine in the synapse may not be specific to the D<sub>2</sub>Rs but could also interact with the other dopamine receptors (D<sub>1</sub>, D<sub>3</sub>, D<sub>4</sub>, and D<sub>5</sub>). However, in preliminary work in Chinese heroin addicts using fMRI, we did find that Synaptose Complex KB220Z™, in a triple-blind, placebo-controlled study, induced direct caudate-accumbens dopaminergic pathway activation. Once again, PET scan analysis is underway.<sup>22</sup> We believe that typical D<sub>2</sub> agonists, such as quinpirole and bromocryptine, are more powerful than Synaptose Complex KB220Z™, and cause downregulation of the D<sub>2</sub> density.<sup>21,22</sup> Furthermore, by adding the metallosaccharide to Synaptose Complex KB220Z™, we have increased the potential benefits to recovering addicts who possess an impaired immune response.<sup>24</sup>

Although the exact mechanism of action is not known, the coupling of gene testing<sup>24,25</sup> along with a safe, nonaddicting, natural putative dopaminergic receptor agonist that upregulates instead of downregulates (eg, quinpirole, bromocryptine), dopaminergic receptors, preferably the D<sub>2</sub> subtype, could ultimately assist in the recovery process of subjects with RDS.

## Conflict of Interest Statement

Kenneth Blum, PhD discloses conflicts of interest with LifeGen, Inc., Electronic Waveform Lab, Synaptamine, Inc., and Path Foundation NY. Thomas J. H. Chen, PhD discloses no conflicts of interest. Siobhan Morse, MHA and John Giordano, MAC, PhD(Hon) disclose a conflict of interest with G&G Holistic Addiction Treatment Center. Amanda Lih Chaun Chen, PhD discloses no conflicts of interest. James Thompson, PhD discloses a conflict of interest with DJ Technologies, Inc. Cameron Allen, BA discloses a conflict of interest with C. A. Stewart, LLC. Andrew Smolen, PhD, Joel Lubar, PhD, and Eric Stice, PhD disclose no conflicts of interest. B. William Downs, BSc, Roger

L. Waite, DC, and Margaret A. Madigan, BSN disclose conflicts of interest with LifeGen, Inc. Mallory Kerner discloses no conflicts of interest. Frank Fornari, PhD discloses a conflict of interest with LifeGen, Inc. Eric R. Braverman, MD discloses no conflicts of interest.

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