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Putative targeting of Dopamine D2 receptor function in Reward Deficiency Syndrome (RDS) by Synaptamine Complex™ Variant (KB220): Clinical trial showing anti-anxiety effects

Research Article

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Summary

Since 1990, researchers have proposed that genetic variants of dopaminergic genes and other "reward genes" are important common determinants of Reward Deficiency Syndrome (RDS). RDS refers to the breakdown of a cascade of neurotransmitters in the brain in which one reaction triggers another — the reward cascade — and resultant aberrant conduct. Association studies have amassed strong evidence implicating the D (2) dopamine receptor (DRD2) gene in harmful conditions such as alcoholism, and the DRD2 gene also has been found to be involved in other substance use disorders including cocaine, nicotine, and opioid dependence, as well as obesity. Brain dopamine has been implicated as the so-called "anti-stress molecule." The present study investigated anti-anxiety effects of Synaptamine Complex [KB220], a dopaminergic activator, in a randomized double-blind placebo controlled study in alcoholics and in polydrug abusers attending an in-patient chemical dependency program. In this randomized double-blind placebo controlled study of 62 alcoholic and polydrug abusers, we utilized skin conductance level (SCL) to evaluate stress responses. Patients receiving Synaptamine Complex [KB220] had a significantly reduced stress response as measured by SCL, compared to patients receiving placebo. Two factor ANOVA yielded significant differences as a function of Time ($p<0.001$), and Treatment ($p<0.025$) as well as a Time–by-Treatment interaction ($p< 0.01$). The results of this study suggest that the Synaptamine Complex™ [KB220] may improve treatment response in an in-patient treatment setting by reducing stress related behaviors and warrants further investigation.

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I. Introduction

Since 1990, association studies have amassed strong evidence implicating the D (2) dopamine receptor (DRD2) gene in the etiology of alcoholism (Blum et al. 1990; Blum et al. 2000). The DRD2 gene also has been found to be involved in other substance use disorders including cocaine, nicotine, and opioid dependence, as well as in obesity. These many disorders often are considered to have a common characteristic: Reward Deficiency Syndrome (RDS) (Blum et al. 1996; Chen et al. 2007). RDS refers to the breakdown of a cascade of neurotransmitters in the brain in which one reaction triggers another — the reward cascade — and resultant aberrant conduct.

The relation of dopaminergic neurotransmission to various stress reactions also has been known for many years. The current understanding is that multiple genes interacting with dopaminergic pathways are emerging as potential therapeutic targets, especially in the treatment of addictions. Thus, because brain dopamine has been implicated as the so called "anti-stress molecule," (Bowirrat and Oscar-Berman 2005) we sought to investigate anti-anxiety effects of Synaptamine Complex [KB220], a dopaminergic activator, in a randomized double-blind placebo controlled study of two groups of patients with RDS. One group consisted of alcoholics, and the other group consisted of polydrug abusers. We reasoned that since nearly 400 different genes may work in concert to impact dopamine and glutamine release in addictions, (Li et al. 2008). Our findings potentially could provide insight regarding the involvement of dopamine in the addiction process, which in turn could lead to therapies based on influencing dopaminergic signaling (Blum et al. 2007).

A. Dopamine D2 receptor neuro-genetics and autoreceptor function

One of the major pitfalls in the approach to dampen the dopamiergic system to induce a form of drug extinction, we believe is counter-productive and may lead to mood changes including suicide ideation. Recently, our laboratory suggested that activation of dopamine receptors in the long term will induce a potential "normalization" of an aberrant genetically induced reduced dopamine D2 receptor density (Downs et al. 2009).

Interestingly, members of our laboratory have promoted the long–term utilization of dopaminergic agonist therapy to reduce craving behavior of all substances including glucose. This was based on the understanding that carriers of the DRD2 Taq A1 A1 allele have compromised DAD2 receptor density (Downs et al. 2009). In this regard, positron emission tomography (PET) studies have revealed significant inter-individual variation in dopamine D2 receptor density in vivo in human striatum. Low D2 receptor binding in vivo has been found to associate with alcohol/substance dependence. It has been suggested that the A1 allele of the human D2 receptor gene might be associated to a specific type of alcoholism and possibly to a reduced D2 receptor density in vitro. (Pohjalainen et al. (1998) have determined D2 dopamine receptor-binding density (Bmax), affinity (Kd) and availability (Bmax/Kd) in 54 healthy Finnish volunteers using PET and [11C] raclopride in order to determine whether the A1 allele is associated with a baseline difference in D2 receptor characteristics in vivo.

A statistically significant reduction in D2 receptor availability reflecting an alteration in receptor density was observed in the A1/A2 genotype group compared to the A2/A2 group. There was no difference in apparent Kd between the two groups. In conclusion, the association between the A1 allele and low D2 receptor availability in healthy subjects suggested that the A1 allele of the TaqIA polymorphism might be in linkage disequilibrium with a mutation in the promoter/regulatory gene element that affects dopamine D2 receptor expression. This study provides an in vivo neurobiological correlate to the A1 allele in healthy volunteers ((Pohjalainen et al. 1998). This important work supported the work of Noble et al. (1991), and others (Comings 1999) also showing low D2 receptor density in A1 allele carriers.

The understanding of why D2 receptor density was lower in A1 allele carriers provided impetus to suggest that raising D2 receptor density may reduce aberrant craving behavior providing a homeostatic state toward normalization (Blum et al. 2008a). This concept was first supported by Boundy et al (1995) involving studies with radiolabeled agonists. This seminal work has revealed that both agonists induce up-regulation of D2 dopamine receptors in cells transfected to express D2 receptors. Their results suggest that the increase in agonist binding after brief exposure to an antagonist is due to interactions of the receptor with one or more G proteins that are not coupled to inhibition of adenylyl cyclase, whereas the increase in agonist binding at later time points is associated with the antagonist-induced up-regulation.

Thus, agonistic therapy results in a proliferation of Dopamine D2 receptors over time if provided in a slow fashion (Boundy et al. 1996). This takes on therapeutic importance when one considers the use of the complex Synaptamine™ claimed to be a dopaminergic agonist used to treat RDS behaviors including addiction to drugs and alcohol (Blum et al. 1987). 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 to DRD2 A2 allele genotype by several investigators the actual mechanism for positive clinical outcomes remained a mystery (Chen et al. 2007; Chen et al. 2007; Blum et al. 2008b; Blum et al. 2008c; Blum et al. 2008d).

However, for the first time in the history of this work involving dopaminergic genetics Laakso et al (2005) has provided a clue. Accordingly, the A1 allele of the **TaqI** restriction fragment length polymorphism (RFLP) of the human dopamine D2 receptor gene (DRD2) is associated with a low density of D2 dopamine receptors in the striatum. Because of the important role of D2 autoreceptors 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 two other DRD2 polymorphisms, C957 T and--141C Ins/Del, which have been suggested to affect D2 receptor levels in brain. The relation between the Taq IA RFLP, C957 T and --141C Ins/Del polymorphisms and striatal dopamine synthesis in 33 healthy Finnish volunteers was studied using PET scans and [18F] fluorodopa ([18F] FDOPA), a radiolabelled analog of the dopamine 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). C957 T 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 dopamine and the rate-limiting enzyme for trace amine (e.g., beta-phenylethylamine) synthesis.

The finding can be explained by lower D2 receptor expression leading to decreased autoreceptor function, and suggests that dopamine and/or trace amine synthesis rate is increased in the brains of A1 allele carriers to compensate. We are proposing that with an increased striatal activity of aromatic L-amino acid decarboxylase, the final enzyme in the biosynthesis of dopamine and the rate-limiting enzyme for trace amine (e.g. beta-phenylethylamine), dopamine synthesis should occur with a more natural and less powerful agonistic compound relative to L-Dopa. This would support the use of Synaptamine complex, a precursor amino acid and enkephalinase inhibition –therapy, as a powerful dopamine agonist. It is postulated that a lower dopamine quanta release at presynatic neurons in the nucleus accumbens (NAc) should result in an up-regulation of D2 receptors in A1 carriers, which will ultimately result in a reduction of craving behavior.

B. Underlying psychiatric mechanisms of stress

Stress will stimulate dopamine transmission in both the medial prefrontal cortex and the NAc (Abercrombie et al. 1989). It appears, however, that the NAc dopamine response to stress is modulated by a dopamine-sensitive mechanism in prefrontal cortex such that increased dopamine transmission in this cortical region acts to dampen the NAc dopamine response to a variety of stimuli including stress (Abercrombie et al. 1989; Deutch et al. 1990; Doherty and Gratton 1996). There is evidence implicating also prefrontal cortex glutamate- (GLUT-) containing neurons some of which are known to project to NAc and to the ventral tegmental area (VTA) where the mesocorticolimbic dopamine system originates (Vezina et al. 1991; Carr and Sesack 2000; Carr et al. 1999).

In addition to stimulating dopamine transmission, stress will also increase prefrontal cortex and NAc levels of GLUT (Sesack and Pickel 1992) and there is evidence indicating that the NAc dopamine response to stress is modulated locally by a GLUT-sensitive mechanism (Moghaddam 1993; Keefe et al. 1993; Saulskaya and Marsden 1995; Wheeler et al. 1995). It has been reported that the NAc dopamine stress response is potentiated by local NMDA receptor blockade (Doherty and Gratton 1997). In that study the researchers reported evidence that the local action of GLUT on the NAc dopamine stress response is mediated by NMDA receptors located on NAc output neurons that project to the VTA. Part of this output system comprises GABA neurons that project to the VTA either directly or indirectly via the ventral pallidum (Kalivas and Churchill 1993; Walaas and Fonnum 1989; Yim and Mogenson 1980). In the VTA, GABA is known to hyperpolarize dopamine cells, inhibiting their activity by a direct $GABA_B$ receptor-mediated action (Chen et al. 2005; Erhardt et al. 2002). The activity of VTA dopamine cells is also regulated by GABA acting at $GABA_A$ receptors although here the evidence indicates both a direct inhibitory action as well as a predominant indirect disinhibitory action presumably mediated presynaptically by $GABA_A$ receptors on non-dopamine interneurons (Churchill et al. 1992; Johnson and North 1992; Kalivas et al. 1990; Klitenick et al. 1992; O'Brien and White 1987; Sugita et al. 1992; Westerink et al. 1996). Local VTA $GABA_A$ and $GABA_B$ receptor activation has been shown previously to modulate dopamine transmission in NAc and VTA. However, to our knowledge, similar information has not been obtained for the NAc dopamine response to stress.

Recent results indicate that the NAc dopamine stress response is regulated by GABA afferents to VTA dopamine cells and that this action is differentially mediated by $GABA_A$ and $GABA_B$ receptors. The data suggest that the relevant $GABA_B$ receptors are located on dopamine neurons, whereas the $GABA_A$ receptors are located on GABA inter-neurons and perhaps also on dopamine cells. The finding related to stress reduction by Synapatamine™ in polysubstance abusers as seen in the current study is consistent with the idea that the corticofugal GLUT input to NAc indirectly regulates stress-induced dopamine release in this region through the GABA feedback pathway to VTA.

Moreover, in the past decade, it has also become clear that vulnerability for substance use disorders is influenced by complex interactions between genetic and environmental determinants (Xi and Stein 1998; Barr et al. 2004; Bau et al. 2000; Caspi et al. 2003; Gunzerath and Goldman 2003; Madrid et al. 2001). Interestingly, impulsive behaviors often increase under conditions of heightened arousal or stress (Barr et al. 2004). Associations between stress and substance abuse have also been well documented (Morgan et al. 2002; Swann 2003; Gilbert et al. 2004; Karlsgodt et al. 2003; Laudet et al. 2004; Ota et al. 2004). Recent preclinical findings suggest that the dopamine system may be an important vulnerability substrate in this relation (Sinha 2001; Soderpalm et al. 2003; Cadoni et al. 2003; Matuszewich and Yamamoto 2004). Nevertheless, the exact nature of stress-induced alterations on dopamine neurotransmission, the

conditions under which these alterations occur, and the ability to generalize the preclinical findings to humans remains to be determined.

Since the findings of Blum et al (1990) associating the dopamine D2 receptor gene polymorphisms and severe alcoholism, many studies have also associated a number of DRD2 gene polymorphisms with various forms of stress both acute and chronic (Voisey et al. 2009).

In the present study, we analyzed in a double-blinded placebo controlled randomized fashion, the stress relieving effects of Synaptamine Complex™[KB220](LifeGen, Inc. San Diego,CA), a novel nutraceutical with putative dopaminergic activation properties, in patients attending an inpatient treatment facility. This nutraceutical was designed to mimic the natural release of VTA dopamine from the NAc, resulting in a reduction of substance seeking behavior based on dopaminergic genetics (Saal et al. 2003; Tidey and Miczek 1997; Noble et al. 1993; Uhl et al. 1993).

II. Methods

A. Group selection and dosage regimen

Inclusion/exclusion

~~In terms of exclusion of this study all pregnant female patients were excluded from the study. Additionally any patient suffering from a prediagnosis of liver damage including any patient having abnormal liver enzyme levels as measure by the CBC/SMAC -24 analysis were also excluded.~~

In this IRB-approved (IRB Registration # IRB00002334) prospective randomized, double-blinded placebo-controlled study, four groups of consenting adult patients signed ~~a~~ consent forms approved by the IRB.~~)~~ ~~were enrolled.~~ Inclusion into this study consisted of all patients that were assessed using the Minnesota Multiphasic Personality Inventory (MMPI) and blood analyses (CBC/SMAC-24) carried out upon admission. These were all serious polysubstance abusers with a history of multiple failures based on a one-hour structured interview by the attending physician. No further blood or urine tests were conducted. The groups were: substance A (alcoholics) with KB220 (AI), substance A (alcoholics) with placebo (AP), substance B (polydrug abusers) with KB220 (BI), and substance B (polydrug abusers) with placebo (BP). Substance B users typically had used or abused at least three and up to 13 substances on a regular basis. Exclusion criteria included pregnant women, as well as any patients suffering from a prediagnosis of liver damage (i.e., anyone having abnormal liver enzyme levels as measure by the CBC/SMAC -24 analysis). Sixty-two patients were randomly divided into appropriate treatment arms and without foreknowledge of diagnosis by the hospital pharmacist.

Four individuals left the program immediately after detoxification within the first six days. Eight more individuals left the program, one at staff request, before completing treatment. For the repeated measure analyses, four patients were dropped because of missing data.

Thus, the base set consisted of 50 individuals, divided into two groups: 28 on the intervention KB220 (AI or BI) and 22 on placebo at the initiation of the study (AP or BP). The substance A subset consisted of 25 individuals, 15 and 10 each in the KB220 and placebo subgroups, respectively who abused alcohol.

The substance B subset consisted of 25 individuals who were abusing several drugs including alcohol, cocaine, barbiturates, tranquilizers, amphetamines, hallucinogens, and marijuana. This subset was similarly divided so that 13 used the intervention KB220, and 12 were provided with placebo. The patient demographics are shown in **Table 1**.

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Table 1: Patient Characteristics

Characteristic	Substance A (Alcohol)		Substance B (Polydrug)	
	Intervention	Placebo	Intervention	Placebo
Mean Age	39.5 + 16.3	36.2 + 16.6	29.6 + 12.8	30.3 + 14.7
Gender	3F (20%) 12M (80%)	2F (20%) 8M (80%	4F (31%) 9M (69%	4F (33%) 8M (67%)
Race	2B (13%) 13W (87%	2B (20%) 8W (80%)	13W (100%)	1B (8%) 11W (92%)
BAL (mg%)	0.135	0.056	0.016	0.012
Substance A	15	10	13	12
Total Substance A	25			
Total Substance B	25			
Total Intervention	28			
Total Placebo	22			

B. Blinded randomized procedure

Age, weight, sex, race, and entry blood alcohol level (BAL) were tested as possible covariates for the dependent measures. BAL upon entry is shown for each of the groups, and despite great differences in BAL, it proved not to be a significant covariate by statistical analysis. None were found to be significantly different, substantiating the fact that, in terms of these measures, the groups are equivalent. No one in the Chemical Dependent Unit, physicians, nurses and subjects, or the data collector, knew which individuals were receiving KB220, and which were receiving the methylcellulose placebo. Each patient received a research number and the data was collected by a research assistant working at the clinic. All the information was secured and was not available to any

investigator in the unit. At the termination of the study the file was immediately submitted to the independent paid statistical person for subsequent analysis. KB the director of the study did not see the data until the analysis was complete thereby maintaining complete blindness.

The KB220 capsules and the placebo capsules were identical in appearance and the patients were randomized to receive either placebo or KB220.

Two KB220 or placebo capsules were given three times daily for 21 days to the first 50 patients enrolled. These subjects were observed for an additional seven days without KB220 or placebo. The purpose of the last seven days (without KB220 or placebo) was to verify that use of KB220 did not produce dependency.

C. Skin Conductance Level (SCL)

The electrical properties of the skin have been widely utilized in the assessment of emotional response. This technique has proven quite reliable as a measure of stress levels in the patient (for example, extent of anxiety or anger). As such, this is an indirect measure of stress levels in the patient. The SCL, the inverse of the galvanic skin resistance (GSR), monitors absolute skin conductance level as measured in micromhos (Edelberg 1972). A correlation exists between orienting and anxiety responses, which by sympathetic activation results in increase in skin conductance. Thus, a decrease in conductance is associated with a decrease in autonomic arousal (Luthe 1969; Martin and Venables 1967).

To make these measurements, an Autogen 3000 (Autogenic Systems) was attached to the middle three fingers of the dominant hand of each patient, and a reading obtained. Measurements were carried out approximately 16 times for each patient who completed treatment. Readings were taken on a non-scheduled basis, including weekends, between 5:00 and 6:00 p.m.

D. Ingredients in Synaptamine™ Complex [KB220]

The following list outlines the selected ingredients per capsule and their expected effects:

D-phenylalanine--230 mg: This amino acid inhibits enkephalinase A and increases the availability of enkephalin in the brain, thereby decreasing craving and depression (Ehrenpreis 1983; Fischer et al. 1975). The LD_{50} of D-phenylalanine in rodents is 5,452 mg/kg.

L-phenylalanine--230 mg: This isomer of phenylalanine tends to increase levels of dopamine, which is closely associated with the brain reward system. A second effect is to increase norepinephrine levels, which leads to a decrease in depression (Yamanaka and Kono 1974). The LD_{50} of L-phenylalanine in rodents is 5,287 mg/kg.

L-Tyrosine –83.3 mg: The substrate for the rate limiting enzyme Tyrosine hudroxylase to synthesize dopamine in the neuron (Yamanaka and Kono 1974). A daily dosage for a clinical test supported in the literature is about 100 mg/kg for an adult which amounts to about 6.8 Grams at 150 lbs. (Gelenberg et al 2008). The usual dosage amounts to 500-1500 mg per day (dose suggested by most manufacturers; usually an equivalent to 1-3 capsules of pure tyrosine). It is not recommended to exceed 12000 mg (12 g) per day. In fact, too high doses result in reduced levels of dopamine (Chinevere et al 2002). Tyrosine may decrease the absorption of other amino acids in high or chronic doses. It decreases absorption of l-dopa.

L-tryptophan--25 mg: This precursor to the neurotransmitter serotonin plays a role in reducing craving and improving the quality of sleep (Yamanaka and Kono 1974). The LD_{50} of L-tryptophan in rodents is 1,600 mg/kg.

L-glutamine--25 mg: Its effect is to increase brain GABA levels, thereby reducing both craving and anxiety (Rogers and Pelton 1957). The LD_{50} of L-glutamine in the mouse is 7,000 mg/kg.The amount of L-Glutamine is small to allow for adequate dopamine neuronal release.

Pyridoxal 5'-phosphate--5 mg: This activated form of vitamin B-6 is a cofactor in the production of aminergic neurotransmitters, and enhances the gastrointestinal absorption of amino acids (Virk et al. 1999).

Each participant utilized 6 capsules per day in equal divided doses per day. The capsules were manufactured by Marlyn Natural Products Corporation (Phoenix,Arizona)

~~D~~E. Statistical analyses

Effects of the Synaptamine Complex intervention (KB220) treatment were analyzed for substance A and B groups alone and in combination. In addition to highlighting differences in response of these populations, this approach addressed the question of commonality of the neurobiological mechanisms (George 1991; Blum et al. 1978). In addition, combining the samples increased the statistical power of the analyses.

Student t-tests were used to compare the mean scores of the KB220 vs. placebo data. The cumulative effects of treatment regimen were seen by analyzing the sum of all prior recordings as windowed on days three [1-3], 10 [1-10], 21 [1-21], and 28 [1-28]. The consequence of this approach is that the discrete trend differences were emphasized. By this method, each recorded measure was treated as a separate entity or "case." This method is statistically more liberal in its analysis than the repeated measure analysis described below. When many t-tests are used, the alpha criterion for any test is no longer 0.05 and a smaller value should be employed to avoid Type 1 errors, i.e., imputing significance inappropriately. To compensate for this potential error, the t-test significance values were adjusted using the Bonferroni protocol, which yields an alpha protection level for SCL of 0.0042.

Repeated measures analyses of variance (ANOVA) were carried out for the first 21 days, the period in which all patients received KB220. We utilized a statistical analysis involving repeated measures to allow for statistical dependency of the subject response over time and to test for differences between groups. The ANOVA compensates for successive case measures by incrementing the value needed for significance. Two-factor analysis of variance was used to examine the skin conductance level (SCL), BESS Score as well as the blood pressure. By this approach the group status at each of the analysis days is viewed as if frozen in time. Time factors were analyzed starting on day 5 when the dropout rate achieved a plateau (8 patients left during the first 5 days) and the sample variance was more stable. As the analysis program used is very sensitive to missing observations within subjects, values for post day five missing data were substituted by the group average for that day. Analyses were limited to days 5, 7, 9, 11, 13, and 17, since the sample size did not support extensive within group degree of freedom contrasts.

All ANOVAs were calculated on a VAX 8650 computer using the BMDP Bio-Medical Statistical Analysis Programs. For this statistical analysis a total of 50 patients were evaluated. The data are reported in **Figure 1, 2**.

III. Results

In analyzing skin conductance levels (SCL) measures analyses were performed for between-group comparisons, and repeated measures analyses were applied to evaluate within-group effects. **Table 2** illustrates the differences observed between KB220 and placebo groups on the SCL stress measurement test at 3, 10, 21, and 28 days of the program. For the substance A subjects, there was a clear trend to progressively higher scores for both the KB220 and placebo groups with a peak at day 21. Then, with KB220 discontinued, the values declined. SCL was consistently lower in the KB220 group, with the greatest difference between the groups at day 10. Using the stringent alpha acceptance value of 0.0042, significance was not reached. The pattern for the substance B groups showed progressive decline in SCL for both the KB220 and placebo sets, but a substantial and significant difference between them was evident at day 10 and proceeded to increase with time. These data indicated that the use of KB220 results in clear improvement for the substance B group early in the program.

When the data from all the patients were combined, significant differences were evident beginning at day 10 and continuing through day 28, despite discontinuance of KB220 at day 21. The KB220 effect was evident by a decreasing difference between the groups at day 28 as compared with day 21.

A two-factor ANOVA (Time and Group) yielded significant main effects of Time ($p < 0.001$), and Group ($p < 0.025$) as well as a Group-by-Time interaction ($p < 0.0I$). Over the 21-day test period there were significant differences among the four groups. Further, for each group there was a significant change over the treatment period. Single factor ANOVAs within each group over time showed significant time-dependent effects for all but the AP group. The progressive time-dependent effects for the other three groups were examined using multiple paired t-tests with the alpha acceptance level adjusted to 0.0042.

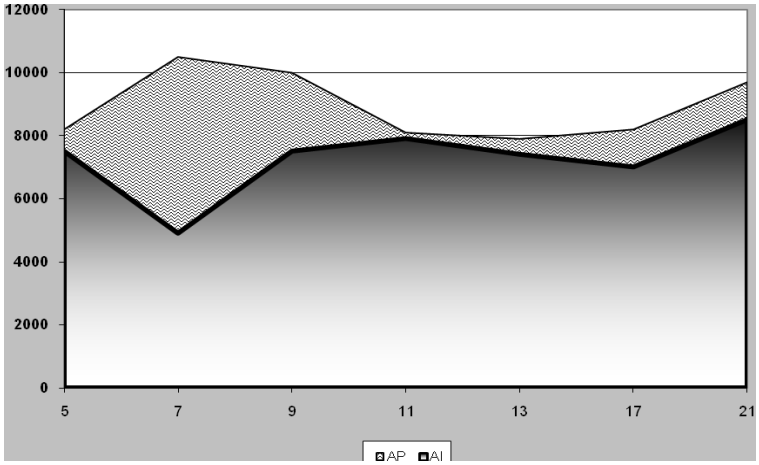
Figure 1 illustrates a significant difference ($p < 0.025$) between the AI and AP/BP groups on day 7. While the AP/BP groups demonstrated higher SCL measures, the AI/BI group had markedly lower levels. Further, as early as day 7 there was a significant decrease in SCL within the AI/BI group alone. This day is significantly different from all other days. In contrast with the AI/BI group, the AP/BP group had no time-dependent within-group significant differences. Thus, early in the program, in what appears to be a detoxification related event, the AI/BI patients showed reduced stress responses. The profile of time-dependent changes in the substance B subset SCL is mirrored in the two groups. Thus, all patients appear to respond similarly to environmental, program-dependent stimuli. With this stringent form of analysis, no differences emerged between groups. Examining the total AI/BI vs. total AP/BP population (Fig. 2), we found that the intervention group (AI/BI) had consistently lower SCL scores. Between-group differences approach significance overall ($p < 0.08$), especially for the first 11 days ($p < 0.06$).

Finally, no evidence of dependency in these subjects was observed. This was assessed by evaluating craving behavior (self-reported analog scale) through the Physical and Behavioral Emotional Stress Scores (BESS Score) filled out by the attending staff following the 21-day period whereby each patient no longer received the experimental product.

Table 2: illustrates the differences observed between KB220 and placebo groups on the SCL stress measurement test.

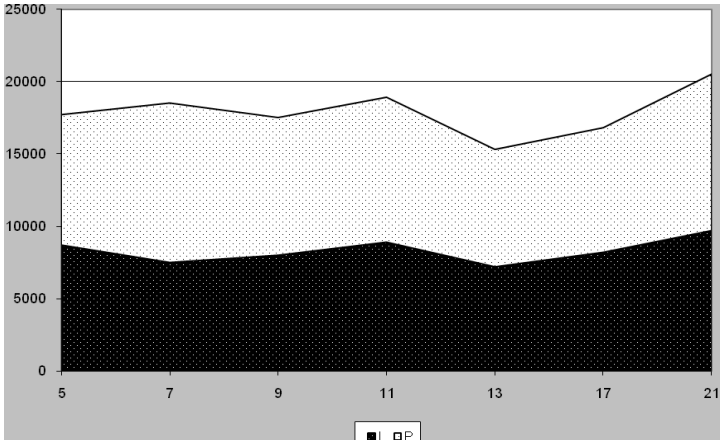
Treatment Group	**Day**			
	3	**10**	**21**	**28**
Substance A	NS	NS	NS	NS
AI	5923 + 478	6798 + 396	7500 + 312	7340 +262
AP	7551 + 150	9013 + 948	9152+ 722	8434 +595
Substance B	NS	P<0.001	P<0.001	P<0.001
BI	9470 + 911	9048 + 386	8727 +285	8693 + 257
BP	14280 + 162	12738 + 870	11686 + 519	11295 + 447
Total	NS	P<0.001	P<0.001	P<0.001
AI/BI	8051 + 645	7954 + 291	8007 +217	7902 + 186
AP/BP	10661 + 118	10827 + 640	10506 + 429	9987 + 365

NS = not significant
Alpha acceptance value – $p < 0.0042$



AP n = 12, AI n=15
Day 7, between groups at $p < 0.025$, within groups at $p < 0.025$

Figure 1: The SCL value for the total (substance A and substance B groups together) KB220 and placebo groups. After the effect of the first seven days, the striking finding is that the curves for the two groups mirror one another, though the KB220 groups are lower. This may indicate a commonality in response to the dynamics of the treatment program, or it may indicate characteristic changes in the population with recovery groups. In contrast, distinctive changes occur within groups. By day nine, the polydrug-KB220 group shows a marked time-dependent significant improvement. At day 13 a second significant change occurs with respect to days 11, 17 and 21. In contrast to the AP group, the BP group showed a significant change, which occurred later at day 13, and continued through day 21.



Intervention (I) n = 28, Placebo (P) n = 22

Figure 2: The SCL value for the total (substance A and substance A and B groups together) KB220 and placebo groups. After the effect of the first seven days, the striking finding is that the curves for the two groups mirror one another, though the KB220 groups are lower. This may indicate a commonality in response to the dynamics of the treatment program or it may indicate characteristic changes in the population with recovery groups. In contrast, distinctive changes occur within groups. By day nine the BI group shows a marked time-dependent significant improvement. At day 13 a second significant change occurs with respect to days 11, 17, and 21. In contrast to the AP group, the BP group showed a significant change, which occurred later, at day 13, and continued through day 21.

IV. Discussion

Data were presented for two groups of substance abusers in a 28-day inpatient treatment setting. The SCL, a measure of autonomic function and a known correlate of anxiety level, revealed significant improvement for the substance B group at day 10 and for the combined groups (Total). The 10-day period is known for peak post-detoxification problems associated with inpatient hospitalization (i.e., higher rates of premature patient departure). For both substance A and B groups, the KB220 groups had lower (improved) scores. These values were statistically significant using the repeated measures but just missed significance for the substance A group when the strict alpha acceptance value was imposed. The significant improvement for the substance B and total drug groups continues throughout the remainder of treatment.

Thus, these data suggest that KB220 is a particularly useful adjunct to reduce stress (e.g., as associated with anxiety and craving). It is noteworthy that in each individual situation tested the score was consistently lower for the KB220 patients than for those receiving placebo. Seven days after KB220 and placebo were eliminated from the program (measurements through day 28) the SCL response for all groups declined with the greatest decline coming in the substance A group.

Autogen 3000 SCL effects were measured using a single factor ANOVA with a Student-Newman-Keuls test for post hoc analysis of any significant ANOVA. Statistically significant differences were obtained for the different groups on day 7 (for substance A) and day 11 (for substance B). In addition, the substance B groups showed parallel curves with the KB220 patients exhibiting less stress. These findings suggest that KB220 reduces response to the situational-induced stresses evident throughout the program and particularly prevalent with the detoxification-transfer-group assimilation events occurring from days 7 to 10. Two clinical observations lend support to this conclusion. First, the patients' disciplinary problems are diminished and, second, the number of calls for the physicians is decreased.

We believe that the results of this experiment provide significant evidence that KB220 is a strong anti-anxiety agent and can provide clinical benefits as a stress reducing agent.

There are a number of limitations to this study:

1. One important caveat of this study especially in an in-patient setting, is that modest amounts of certain amino-acids might be in doses that are sufficient to permit subjects to discriminate between an inert placebo and a compound with subtle but still discernable properties. Expectations, attitudes of staff, prior information about the study, rumors in the treatment center, and so forth may have operated to sensitize placebo control and KB220 subjects to try to guess the condition (KB220 or control) to which they had been assigned. Thus once subjects categorized themselves as KB220 or placebo, and then it is possible that they behaved accordingly.

2. Another important caveat is that since these patients were not evaluated for generalized anxiety disorder using such standardized scales such as GAD (DSM-IV), and a Hamilton Anxiety Rating Scale (HAM-A), Clinical Global Impression of Severity of Illness (CGI-S) Scale; and the Montgomery-Asberg Depression Rating Scale (MADRS) one cannot generalize these findings to GAD per se and may be the subject of additional investigation.

3. Since the actual work of this study was done some years ago, newer and more powerful statistical preferred methods have emerged to handle missing data and dropout such as full information likelihood imputation.

4. Another caveat of this current study involves the lack of some type of growth curve models that first test whether change over time in the outcomes differs across experimental conditions taken the form of time-by-condition interaction.

5. We must caution any interpretation of this study, albeit double blind randomized –placebo controlled because of the small sample size.

We focused on specific days because it is well known that in most 30-day in-patient treatment facilities, day 7 is stressful because that is the one-week anniversary of group entry following drug and alcohol detoxification.

Figures 3, **4**, **5**, and **6** illustrate the brain's reward circuitry and associated neurotransmitters. We are confident that delivery of Synaptamine Complex™ due to precursor amines and enkephalinase inhibition should result in both the enhanced synthesis and release of dopamine. In this regard we are cognizant that the brain is designed to provide homeostatic mechanisms in dopamine synthesis and release. In fact, it is known that the cystosolic concentration of dopamine is quite low, and the concentration of l-dopa is extremely low (Feldman et al. 1997). It has been calculated by others at steady state, cytosolic dopamine = 2.65µM, and the concentration of l-dopa is 0.36 µM. Interestingly, it has been calculated that 27.3 µM. of cytosolic dopamine are manufactured from tyrosine per hour .81 µM /hr of dopamine are put into the vesicles by the monoamine transporter and 80.1 µM are put back into the cytosol from extracellular space by DATs. Moreover, 26.5 µM/hr of dopamine is catabolized in the cytosol (Reed et al. 2009). It is important to realize that only about 10% of the cellular tyrosine input goes to dopamine synthesis, with the remainder going to the tyrosine pool (80%) or being catabolized (10%) as seen experimentally (Bongiovanni et al. 2006). Importantly cellular tyrosine itself has a steady state concentration of 126 µM (Reed et al. 2009; Bongiovanni et al. 2005). It is known that even when taken with meals, brain tyrosine levels can double (Fernstrom and Fernstrom 1994). Therefore, by administering to a subject between 350 mg-750mg per day of tyrosine (dependent on DRD2 gene polymorphisms), especially in carriers of the DRD2 A1 whereby L-aromatic decarboxylase activity is significantly above normal, the resultant synthesis of cytosolic dopamine is likely (Laakso et al. 2005).

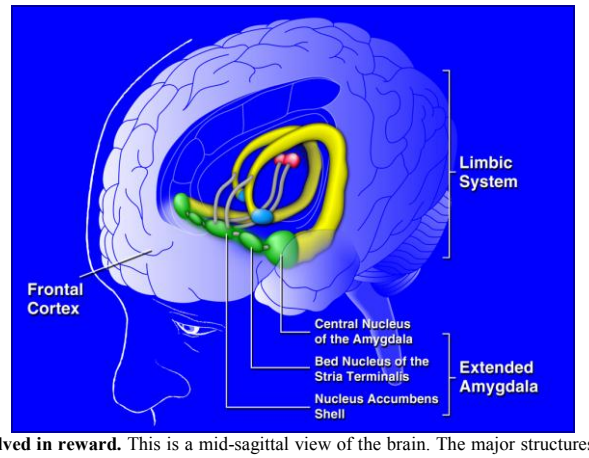


Figure 3. Brain circuitry involved in reward. This is a mid-sagittal view of the brain. The major structures of the reward pathway are the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the prefrontal cortex. When one is engaged in something pleasurable, this circuitry of the brain is activated; causing the experience of pleasure and thus making it more likely that one will repeat a pleasurable activity. The NAc plays a central role in the reward circuit. It functions mainly through the action of two essential neurotransmitters: dopamine, which promotes desire, and serotonin, whose effects include satiety and inhibition. Nonhuman animal studies have shown that drugs can increase the production of dopamine in the NAc, but reduce serotonin. The NAc maintains close relations with the VTA, a primitive part of the brain that synthesizes dopamine. VTA axons send dopamine to the NAc. The VTA is also influenced by endorphins whose receptors are targeted by opiate drugs such as heroin and morphine. The substantia nigra also synthesizes dopamine and projects dopamine to the basal ganglia. Dopamine release is inhibited by the neurotransmitter GABA. Another structure involved in pleasure mechanisms is the prefrontal cortex, which plays a role in planning and motivating action. The prefrontal cortex is a relay in the reward circuit and also is modulated by dopamine.

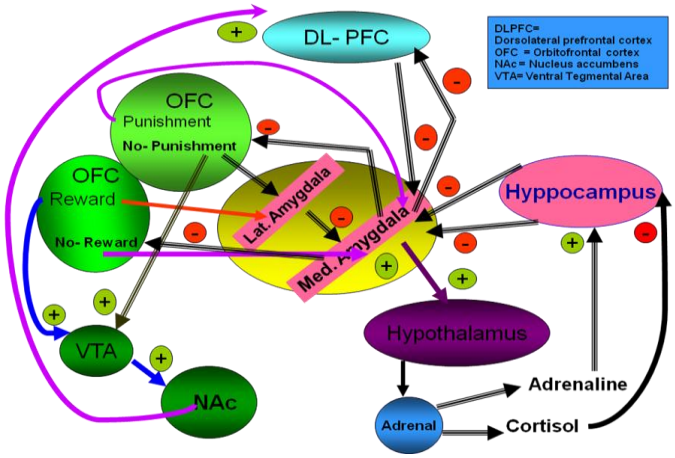


Figure 4. Different areas involved in reward circuits. This Figure illustrates how neurons from different regions of the brain communicate with each other to influence the effects of positive and negative reinforcements. The main areas involved in positive reinforcement (reward) are colored with Green: NAc - nucleus accumbens, VTA – ventral tegmental area, OFC – orbitofrontal cortex. The lateral amygdala nucleus (yellow) is involved in pleasure and receives signals from the OFC and also sends inhibitory signals to the medial amygdala. The amygdala (yellow) is a center of many activities; it receives negative signals (blockade) and it functions as a fear and panic center to enhance the action of reinforcements. Negative signals can arrive from the dorsolateral prefrontal cortex, the hippocampus, and the lateral amygdala to stabilize the medial amygdala. Positive signals are transmitted from the medial amygdala to the hypothalamus (orange). The hypothalamus triggers the adrenal gland (blue) to release adrenaline and cortisol. Adrenaline triggers the hippocampus (pink), and cortisol inhibits the hippocampus by its negative signal. The dorsolateral prefrontal cortex (DLPFC) (blue) oversees information about rewards and punishment. It inhibits emotional fear actions including those from the medial amygdala, and it receives positive stimulation from the NAc**.**

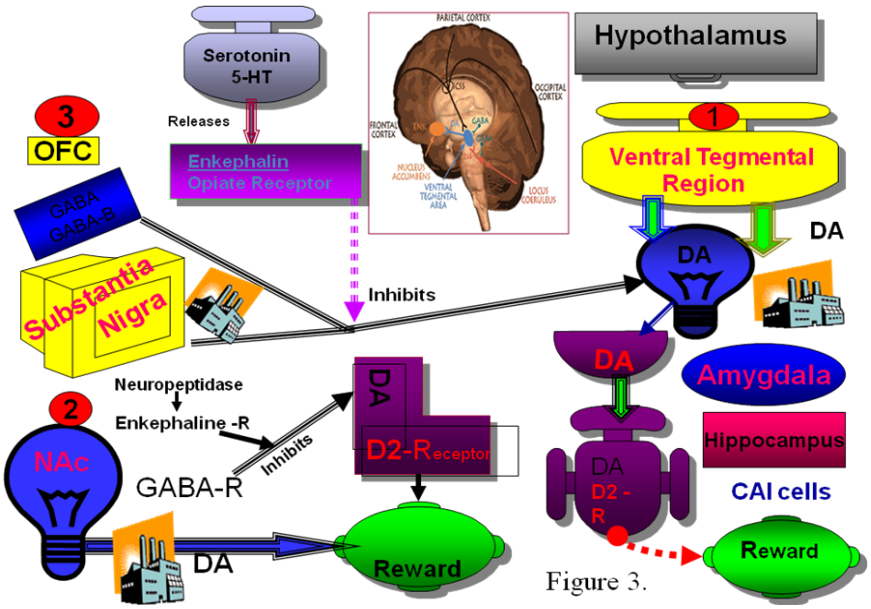


Figure 5. Brain reward pathway: Regions and neurotransmitters involved. Figure 5 illustrates different neurotransmitters and brain regions involved in reward circuitry. On the top, the hypothalamus (gray) is involved; serotonin is released triggering the enkephalin and opiate receptors that inhibit the action of GABA B receptors. This action triggers the release of dopamine from the ventral tegmental area (yellow-right). Dopamine triggers the amygdala (gray), thereby affecting the hippocampus (pink). The end result of dopamine release is a pleasurable sensation (Reward-green). On the left side of the figure the interventions of neuropeptidases and enkephalins cause inhibition to the GABA receptor, and the result is production of dopamine in the nucleus accumbens (NAc-blue). Who occupy the D2 –Receptor that leads to the manifestation of Reward also within the Nucleus Accumbens (Blue). However, at least two other neurotransmitters are also influenced by drug abuse and addiction: GABA and glutamate. The figure illustrate two of the brain-signaling pathways targeted by drugs of abuse transmit dopamine and serotonin. The mean regions involved are NAc – nucleus accumbens and Ventral Tegmental area in addition to the substantia nigra.

Dopamine and the D2 receptor gene has been correlated with susceptibility to depressive symptoms during stressful life events (Hill et al. 1999), more severe stress or stress disorders (Comings et al. 1996; Comings et al. 2000). Moreover, our research and that of others throughout the years have provided evidence that pharmacogenetic and/or nutrigenetic testing prior to administration of any agent to treat psychiatric based disorders should significantly improve treatment outcomes (Lawford et al. 1995; Blum et al. 2006).

In fact, Kirsch et al (2006) in a double-blind crossover study with 24 participants found an increase of reward system activation from placebo to bromocriptine only in subjects carrying the A1 allele. This work supports the work of Noble's group (Lawford et al. 1995). Furthermore, only A1 carrier showed an increase of performance under bromocriptine. The results are interpreted as reflecting a specific sensitivity for dopamine agonists in persons carrying an A1 allele and may complement actual data and theories of the development of addiction disorders postulating a higher genetic risk for substance abuse and proneness to stress in carrier of the A1 allele. This work is in agreement with the work cited earlier concerning treatment compliance (Chen et al. 2007; Blum et al. 2008b).

Finally, stress is a well-known risk factor in the development of addiction and in addiction relapse vulnerability. A series of population-based and epidemiological studies have identified specific stressors and individual-level variables that are predictive of substance use and abuse. Preclinical research also shows that stress exposure enhances drug self-administration and reinstates drug seeking in drug-experienced animals.

The deleterious effects of early life stress, child maltreatment, and accumulated adversity on alterations in the corticotropin releasing factor and hypothalamic-pituitary-adrenal axis (CRF/HPA), the extrahypothalamic CRF, the autonomic arousal, and the central noradrenergic systems are considered important. Noradrenergic activation

is tantamount to ones extent of the severity of stressful events (Comings et al. 1996). The effects of these alterations on the corticostriatal-limbic motivational, learning, and adaptation systems that include mesolimbic dopamine, glutamate, and gamma-amino-butyric acid (GABA) pathways are all associated with the underlying pathophysiology linked with stress-related risk of addiction (Suemaru et al. 1985).

Furthermore, the corticotropin-releasing factor (CRF)-like peptides, which include the mammalian peptides CRF, urocortin 1, urocortin 2, and urocortin 3, play an important role in orchestrating behavioral and physiological responses that may increase an organism's chance of survival when confronted with internal or external stressors. There is, however, evidence that a chronic overactivity of brain CRF systems under basal conditions may play a role in the etiology and maintenance of psychiatric disorders such as depression and anxiety disorders. Bruijnzeel and Gold (2005) suggest evidence of a role for CRF-like peptides in acute and protracted drug abstinence syndromes and relapse to drug-taking behavior. They suggest that there is a high comorbidity between stress-associated psychiatric disorders and drug dependence.

Interestingly, in one study, stress was assessed in 36 inpatient treatment-engaged cocaine dependent individuals and 36 demographically matched healthy control participants using the Perceived Stress Scale and repeated morning salivary cortisol levels over three consecutive days. The Rey Auditory Verbal Learning Test was conducted to measure verbal learning, memory, and executive function. Prospective assessment of cocaine use outcomes during 90 days following discharge from inpatient treatment was also conducted. Fox et al. (2009) found that cocaine dependent patients showed higher levels of distress compared to controls in Perceived Stress Scale scores and cortisol levels. They also demonstrated a significantly reduced learning curve, and fewer correct responses and more errors on recognition. Elevated cortisol was significantly associated with worse Rey Auditory Verbal Learning test performance in cocaine dependent patients. Poor memory scores, but not distress measures, were significantly associated with greater cocaine use after inpatient treatment. The authors suggest that their findings are the first to demonstrate that learning and memory deficits in cocaine dependent individuals are associated with enhanced cortisol and with cocaine use outcomes after inpatient treatment. The findings are consistent with recent addiction models suggesting that chronic cocaine-related neuroadaptations affect learning and memory function, which in turn, influence drug use outcomes.

Moreover, relapse to drug taking induced by exposure to cues associated with drugs of abuse is a major challenge to the treatment of drug addiction. Previous studies indicate that drug seeking can be inhibited by disrupting the reconsolidation of a drug-related memory. Stress plays an important role in modulating different stages of memory including reconsolidation. Wang et al. (2008). Determined the role of glucocorticoid receptors in the basolateral amygdala (BLA) in modulating the effects of stress on reconsolidation of this memory. The disruptive effect of stress on reconsolidation of morphine related memory was prevented by inhibition of corticosterone synthesis with metyrapone or basolateral amygdala, but not central amygdala, injections of the glucocorticoid antagonist RU38486 [(11,17)-11-[4-(dimethylamino) phenyl]-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one]. Finally, the effect of stress on drug related memory reconsolidation was mimicked by systemic injections of corticosterone or injections of RU28362 [11,17-dihydroxy-6-methyl-17-(1-propynyl)androsta-1,4,6-triene-3-one] (a glucocorticoid receptor agonist) into basolateral amygdala, but not the central amygdala. These results show that stress blocks reconsolidation of a drug-related memory, and this effect is mediated by activation of glucocorticoid receptors in the basolateral amygdala. These findings may have important clinical implications especially in inpatients undergoing treatment. In fact, it may have profound influence on the well-known treatment phenomena called AMA rate (Withdrawal Against Medical Advice). It is of note that stress may induce AMA rates by virtue of disrupting reconciliation of drug-related memory. In other studies our laboratory has shown the significant decrease in AMA rate in "in-patient" cocaine dependent patients administered a Synaptamine™ variant (KB220) (Blum et al. 1998; Blum et al. 1988). Thus, one proposed mechanism is that KB220 prevented AMA rate because of its putative anti-stress property, thereby allowing for normalized reconciliation drug-related memory to occur.

Stress associated effects with dopamine D2 receptor polymorphisms have been intensely studied. Gilbert et al. (2009), found that Nicotine Replacement Therapy (NRT) reduced personality traits related to a negative affect (NA). The NA was found to a greater extent in DRD2 A1 carriers than in A2A2 individuals during the first two weeks of treatment (when on the 21-mg patch); however, A1 carriers experienced a renewal of NA symptoms when switched to the 7-mg patch and when off the patch, while A2A2 individuals continued to benefit from NRT. Other work by Coming's group (Madrid et al. 2001) found a significant interaction between DRD2 genotype and stress score as a predictor of MAST score in alcoholics. Additionally, this difference was found to be largely accounted by the HSI occupational/economic stress score, which interacted significantly with DRD2 genotype as a predictor of MAST score. This stress score was the only one of four that showed levels of stress as high as HSI scores in a US population. The MAST scores of A2A2 genotype participants were found to be nearly identical in low stress and high stress participants, whereas the MAST scores of A1A2 participants increased modestly with stress, and that of A1A1 participants increased markedly with stress.

There have been controversies as to the actual DRD2 gene polymorphim being located in the 3' untranslated region of the gene. However, in a recent article in

Scientific American entitled "The Price of Silent Mutations" by Chamary and Hurst (2009) the authors suggested that small changes to DNA that were once considered innocuous enough to be ignored are proving to be important in human diseases, evolution, and biotechnology. Interestingly, this article discusses the role of silence in the DNA code. It turns out that mutations sitting outside of the gene regulatory intron may be very important for how a gene can code for translation to a protein. Over the years, there have been studies, which tied the 3' untranslated region to mRNA activity. Chamary and Hurst pointed out that in the dopamine D2 gene, which encodes a cell-surface receptor that detects the presence of the neurotransmitter dopamine, one silent mutation causes the mRNA to be degraded more rapid than normal. As a result, less of the encoded protein is made, and this may affect certain disease states. This suggests, in turn, that the DRD2 Taq A1 allele association in the 3' region by Grandy and our subsequent association studies are due to synonymous mutations (silent), which affect mRNA stability and thus synthesis of the receptor. Mutations such as -957T have now been shown to be linked to Taq A1 allele (Duan et al 2003). These results call into question some assumptions made about synonymous variation in molecular population genetics and gene-mapping studies of diseases with complex inheritance, like stress and RDS, and indicate that synonymous variation can have effects of potential pathophysiological and pharmacogenetic importance.

Accordingly, these findings support the hypothesis that DRD2 genotype-phenotype associations depend on the magnitude of stress exposure, and they lend support to the view that variability in DRD2 study outcomes may in part be explained by this gene-environment interaction.

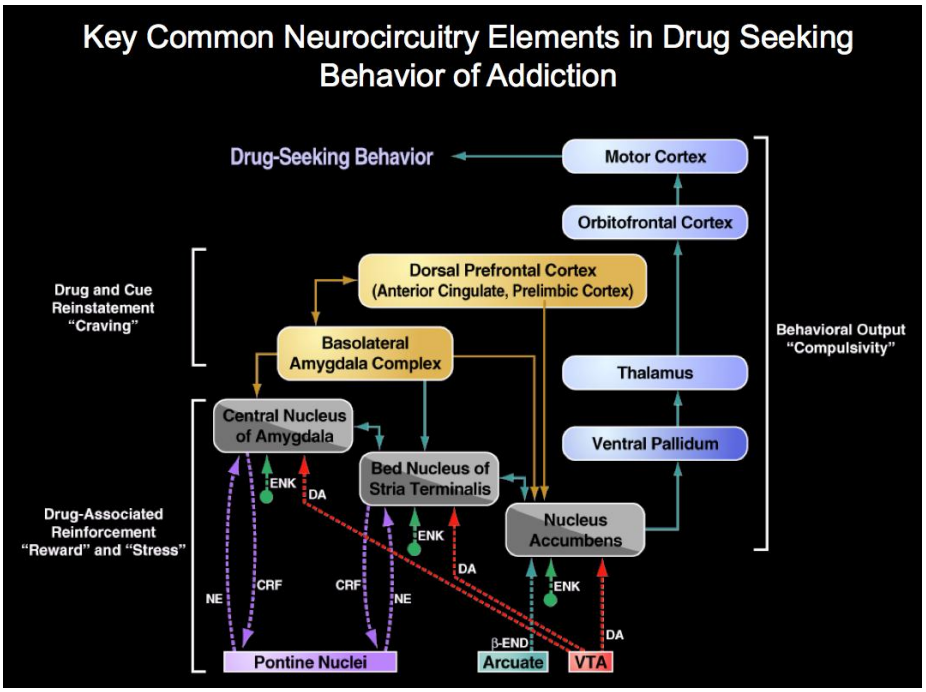


Figure 6. Summary of neurocircuitry involved in addictions. This figure shows three potential circuits active during addiction and drug abuse. These are: (1) Reward Circuit, consisting of the nucleus accumbens and extended amygdala (bed nucleus of the stria terminalis and central nucleus of the amygdala); (2) "Craving" Circuit, consisting of the dorsal prefrontal cortex, and basolateral amygdala; and (3) "Compulsivity" Circuit, consisting of a loop connections involving the ventral striatum, ventral pallidum, medial thalamic, and orbitofrontal cortex. The circuitry is interconnected and interactive.

V. Conclusions

The results of the present study support the view that the Synaptamine Complex™ [KB220] may improve treatment response in an inpatient treatment setting by reducing stress related behaviors. We believe that such treatment warrants further investigation, especially as it relates to work of Stice et al (2004,2008) (with respect to food addiction), Gold's group (relating to dysfunctional connectivity patterns in chronic heroin users and regulation of stress), (Liu et al, 2009) and Wang et al. (2004). (For Substance Use Disorder). Nutrigenetic and pharmacogentic testing (Meshkin and Blum 2007) are tantamount to improved outcomes (stress related) particularly in individuals having compromised dopaminergic (Duan et al. 2003) and/or serotonergic genetics (Geller and Blum 1970; Bruening et al. 2006). Interestingly the heritabilities of symptoms of posttraumatic stress disorder (PTSD), anxiety, depression, and the shared genetic component of these symptoms among family members exposed to the 1988 Spitak earthquake in Armenia were evaluated by Goenjian et al. (2008). Two hundred members of 12 multigenerational families exposed to the Spitak earthquake were studied using a battery that assessed earthquake exposure and symptoms of PTSD, anxiety, and depression. The heritabilities found in this multigenerational family study indicate that the genetic make-up of some individuals renders them substantially more vulnerable than others to develop symptoms of PTSD, anxiety, and depression.

While there are many research gaps in furthering our understanding of the association between stress and addiction (Koob 2008), the present preliminary findings provide a novel non-pharmacological alternative (nutritional) that in our opinion following confirmation in larger perspective studies could significantly influence new prevention and treatment strategies to address vulnerability to addiction especially when coupled with genetic testing.

Future studies from our laboratory will address stress-reliving effects of Synaptamine™ in chemical dependence rehabilitation treatment in relation to dopaminergic and serotonergic genetics.

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Conflict of interest

Kenneth Blum, Roger L. Waite, and William Downs, are Officers in LifeGen, Inc. San Diego, CA. LifeGen, Inc. has the worldwide distribution rights of the Synaptamine Complex [KB220™}.

References

Abercrombie ED, Keefe KA, DiFrischia DS et al. **(1989)** Differential effects of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial prefrontal cortex, **J. Neurochem.** 52, 1655-1658.

Barr CS, Newman TK, Schwandt M et al. **(2004)** Sexual dichotomy of an interaction between early adversity and the serotonin transporter gene promoter variant in rhesus macaques. **Proc. Natl. Acad. Sci.** 101, 12358-12363.

Bau CH, Almeida S, Hutz MH. **(2000)** The TaqI A1 allele of the dopamine D2 receptor gene and alcoholism in Brazil: association and interaction with stress and harm avoidance on severity prediction. **Am. J. Med. Genet**. 96, 302-306.

Blum, K. Allison, D., Trachtenberg, M.C., Williams, R.W. and Loeblich, L.A. **(1998)** Reduction of both drug hunger and withdrawal against advice rate of cocaine abusers in a 30 day inpatient treatment program by the neuronutrient Tropamine' **Curr.Ther. Res.** 43,1204 1214.

Blum K, Braverman ER, Holder JM et al. **(2000)** Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. **J Psychoactive Drugs**.32 Suppl:i-iv, 1-112.

Blum K, Briggs AH, Trachtenberg MC et al**. (1987)** Enkephalinase inhibition: regulation of ethanol intake in genetically predisposed mice. **Alcohol**. 4, 449-56.

Blum K, Chen AL, Chen TJ, et al. **(2008a)** Activation instead of blocking mesolimbic dopaminergic reward circuitry is a preferred modality in the long term treatment of reward deficiency syndrome (RDS): a commentary.**Theor Biol Med Model.** 15,24-39

Blum K, Chen ALC, Chen TJH, Rhoades P, Prihoda T, Downs BW, Baghi D, Bagch M,, Blum SH, Williams L, Braverman ER, Kerner M, Waite RL, Quirk B, White L**. (2008d)** Dopamine D2 Receptor **Taq A1** allele predicts treatment compliance of LG839 in a subset analysis of a pilot study in the Netherlands**. Gene Therapy & Mol Biol.** 12, 129-140.

Blum K, Chen AL, Chen TJ, Rhoades P, Prihoda TJ, Downs BW, Waite RL, Williams L, Braverman ER, Braverman D, Arcuri V, Kerner M, Blum SH, Palomo T**. (2008b)** LG839: anti-obesity effects and polymorphic gene correlates of reward deficiency syndrome. **Adv Ther**. 25, 894-913.

Blum K, Chen TJH, Downs BW, et al. **(2007)** Synaptamine (SG8839),TM An Amino-Acid Enkephalinase Inhibition Nutraceutical Improves Recovery of Alcoholics, A Subtype of Reward Deficiency Syndrome(RDS). **Trends in Applied Sciences Research** 2 , 132-13.

Blum K, Chen TJH, Williams L, Chen ALC, Downs BW, Waite RL, Huntington T, Sims S, Prihoda t, Rhoads P, Reinking J, Braverman D, Kerner M, Blum SH, Quirk B, Braverman ER. **(2008c)** A short term pilot open label study to evaluate efficacy and safety of LG839, a customized DNA directed nutraceutical in obesity: Exploring Nutrigenomics. **Gene Therapy & Mol Biol.** 12, 371-382.

Blum K, Hamilton MG, Hirst M, Wallace JE. **(1978)** Putative role of isoquinoline alkaloids in alcoholism: a link to opiates. **Alcohol Clin Exp Res.** 2, 113-120.

Blum K, Meshkin B, Downs BW. **(2006)** DNA based customized nutraceutical "gene therapy" utilizing a genoscore: a hypothesized paradigm shift of a novel approach to the diagnosis, stratification, prognosis and treatment of inflammatory processes in the human. **Med Hypotheses.** 66, 1008-1018.

Blum K, Noble EP, Sheridan PJ et al. (**1990**) Allelic association of human dopamine D2 receptor gene in alcoholism. **JAMA** 263, 2055-2060.

Blum K, Sheridan PJ, Wood RC, Braverman ER, Chen TJ, Cull JG, Comings DE. **(1996)** The D2 dopamine receptor gene as a determinant of reward deficiency syndrome. **J. R Soc Med**.1996 89,396-400.

Blum K, Trachtenberg MC, Ramsay JC. **(1988)** Improvement of inpatient treatment of the alcoholic as a function of neurotransmitter restoration: a pilot study. **Int J Addict.** 23,991-998.

Bongiovanni R, Kirkbride B, Walmire P, Jaskiw E. **(2005)** Tyrosine administration does not affect desipromine -induced dopamine levels as measured in vivo in prefrontal cortex. **Brain Res.** 1054, 203-206.

Bongiovanni R, Young D, Newbould E, Jaskiw E. **(2006)** Increased striatal dopamine synthesis is associated with decreased tissue levels of tyrosine. **Brain Res.** 115, 26-36.

Boundy VA, Lu L, Molinoff PB. (1996) Differential coupling of rat D2 dopamine receptor isoforms expressed in Spodoptera frugiperda insect cells.**J Pharmacol Exp Ther.** 276,784-94.

Boundy VA, Pacheco MA, Guan W, Molinoff PB. **(1995)** Agonists and antagonists differentially regulate the high affinity state of the D2L receptor in human embryonic kidney 293 cells. **Mol Pharmacol.** 48,956-64.

Bowirrat A, Oscar-Berman M. **(2005)** Relationship between dopaminergic neurotransmission, alcoholism, and Reward Deficiency syndrome. **Am J Med Genet B Neuropsychiatr Genet.** 132B, 29-37Bruening S, Oh E, Hetzenauer A, Escobar-Alvarez S, Westphalen RI, Hemmings HC Jr, Singewald N, Shippenberg T, Toth M. **(2006)** The anxiety-like phenotype of 5-HT receptor null mice is associated with genetic background-specific perturbations in the prefrontal cortex GABA-glutamate system.**J Neurochem.** 99,892-899.

Bruijnzeel AW, Gold MS. (**2005**) The role of corticotropin-releasing factor-like peptides in cannabis, nicotine, and alcohol dependence. **Brain Res Brain Res Rev.** 49,505-528.

Cadoni C, Solinas M, Valentini V et al. **(2003)** Selective psychostimulant sensitization by food restriction: differential changes in accumbens shell and core dopamine. **Eur. J. Neurosci.** 18, 2326-2334.

Carr DB, O'Donnell PJP, Card JP. et al**. (1999)** Dopamine terminals in the rat prefrontal cortex synapse on pyramidal cells that project to the nucleus accumbens**, J. Neurosci**. 19, 11049-11060

Carr DB, Sesack SR. **(2000)** Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesocortical and mesoaccumbens neurons, **J. Neurosci.** 20, 3864-3873.

Caspi A, Sugden K, Moffitt TE et al. **(2003)** Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. **Science** 301, 386-389.

Chamary JV and Hurst D. **(2009**) The Price of Silent Mutations**. Scientific American** 300,46-53

Chen TJ, Blum K, Waite RL et al**. (2007)** Gene \Narcotic Attenuation Program attenuates substance use disorder, a clinical subtype of reward deficiency syndrome. **Adv Ther.** 24, 402-414.

Chen TJ, Blum K, Waite RL, Meshkin B, Schoolfield J, Downs BW, Braverman EE, Arcuri V, Varshavskiy M, Blum SH, Mengucci J, Reuben C, Palomo T**. (2007)** Gene \Narcotic Attenuation Program attenuates substance use disorder, a clinical subtype of reward deficiency syndrome. **Adv Ther.** 24, 402-414.

Chen TJH, Blum K, Kaats G, Braverman ER, Eisenberg A, Sherman M, Davis K, Comings DE, Wood R, Pullin D, Arcuri V, Varshavskiy M, Mengucci JF, Blum SH, Downs BW, Meshkin B, Waite RL, Williams L, Schoolfield J, White L. **(2007)** Chromium Picolinate (CrP) a putative anti-obesity nutrient induces changes in body composition as a function of Taq1 dopamine D2 receptor polymorphisms in a randomized double-blind placebo controlled study. **Gene Ther Mol Biol** 11**,** 161-170.

Chen Y, Phillips K, Minton G, Sher E.(**2005)** GABAB receptor modulators potentiate baclofen-induced depression of dopamine neuron activity in the rat ventral tegmental area. **Br. J. Pharmacol.** 144, 926-932.

Churchill L, Dilts RP, Kalivas PW. (**1992)** Autoradiographic localization of gamma-aminobutyric acid-A receptors within the ventral tegmental area. **Neurochem. Res.** 17, 101-106.

Comings DE. **(1999)** Molecular heterosis as the explanation for the controversy about the effect of the DRD2 gene on dopamine D2 receptor density. **Mol Psychiatry.** 4,213-215.

Comings DE, Blum K. **(2000)** Reward deficiency syndrome: genetic aspects of behavioral disorders. **Prog Brain Res.** 126, 325-341.

Comings DE, Muhleman D, Gysin R. (**1996)** Dopamine D2 receptor (DRD2) gene and susceptibility to posttrpaumatic stress disorder: a study and replication. **Biol Psychiatry.** 401, 368-372.

Deutch AY, Clark WA, Roth RH. **(1990)** Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. **Brain Res**. 521, 311-315.

Doherty MD, Gratton A. **(1996)** Medial prefrontal cortical D1 receptor modulation of the meso-accumbens dopamine response to stress: an electrochemical study in freely-behaving rats, **Brain Res.** 715, 86-97.

Doherty MD, Gratton A**. (1997)** NMDA receptors in nucleus accumbens modulate stress-induced dopamine release in nucleus accumbens and ventral tegmental area. **Synapse** 26, 225-234.

Downs BW, Chen AL, Chen TJ, Waite RL, et al. **(2009)** Nutrigenomic targeting of carbohydrate craving behavior: Can we manage obesity and aberrant craving behaviors with neurochemical pathway manipulation by Immunological Compatible Substances (nutrients) using a Genetic Positioning System (GPS) Map? **Med Hypotheses.** Published May 16.

Duan J, Wainwright MS, Comeron JM,et al. **(2003)** Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. **Hum Mol Genet.** 12,205-216

Edelberg R. Electrical activity of the skin: Its measurement and uses in psychophysiology. In: Greenfield, N.S.; Sternback, R. A. eds. **(1972) Handbook of psychophysiology.** 367-418. [Publisher - Steinkopff; issue: Vol. 7(1), 1997]

Ehrenpreis S. **(1983)** D-phenylalanine and other enkephalinase inhibitors as pharmacological agents: implications for some important therapeutic applications. **Subs. Alcohol Action Misuse.** 3, 231-239.

Erhardt S, Mathé JM, Cergui K, Engberg G, Svensson TH. **(2002)** GABAB receptor-mediated modulation of the firing pattern of ventral tegmental area dopamine neurons in vivo. **Naunyn-Schmiedeberg's Arch. Pharmacol.** 365, 173-180.

Feldman R, Meyer J, Quenzer L **(1997) Principles of Neuroharmacology**. Sundrland, MA.: Sinauer Associates, Inc..

Fernstrom J, Fernstrom M. **(1994)** Dietary effects on tyrosine availability and catecholamine synthesis in the central nervous system: possible relevance to the control of protein intake. **Proc. Nutr.** 53, 419-429.

Fischer E, Heller B, Nachon M et al.**(1975)** Therapy of depression by phenylalanine . Preliminary note. **Arzneimittel-forschung.** 25, 132.

Fox HC, Jackson ED, Sinha R. **(2009)** Elevated cortisol and learning and memory deficits in cocaine dependent individuals: Relationship to relapse outcomes.**Psychoneuroendocrinology**. 2009 (in press).

Geller I., Blum K. **(1970)** The effects of 5-HTP on para-Chlorophenylalanine (p-CPA) attenuation of "conflict" behavior. **Eur J Pharmacol.** 9,319-324

George FR. **(1991)** Is there a common biological basis for reinforcement from alcohol and other drugs? J **Addict Dis.** 10, 127-139.

Gilbert D, McClernon J, Rabinovich N, et al. **(2004)**Effects of quitting smoking on EEG activation and attention last for more than 31 days and are more severe with stress, dependence, DRD2 A1 allele, and **depressive** traits. **Nicotine Tob. Res.** 6, 249-267.

Gilbert DG, Zuo Y, Rabinovich NE, Riise H, Needham R, Huggenvik JI. **(2009)** Neurotransmission-related genetic polymorphisms, negative affectivity traits, and gender predict tobacco abstinence symptoms across 44 days with and without nicotine patch. **J Abnorm Psychol.** 118,322-334.

Gunzerath L, Goldman D. G. **(2003)** A NIAAA workshop on gene-environment interactions. **Alcohol., Clin. Exp. Res.** 27, 540-562.

Hill SY, Zezza N, Wipprecht G et al.**(1999)** Personality traits and dopamine receptors (D2 and D4): linkage studies in families of alcoholics. **Am J Med Genet.** 15, 634-641.

Johnson SW, North RA. **(1992)** Two types of neurones in the rat ventral tegmental area and their synaptic inputs **.J. Physiol. (London)** 450, 455-468.

Kalivas PW, Churchill L,. **(1993)** Klitenick MA. GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. **Neuroscience.** 57**,** 1047-1060.

Kalivas PW, Duffy P, Eberhardt H. **(1990)** Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. J**. Pharmacol. Exp. Ther**. 253, 858-866.

Karlsgodt KH, Lukas SE, Elman I. (**2003)** Psychosocial stress and the duration of cocaine use in non-treatment seeking individuals with cocaine dependence. **Am. J. Drug Alcohol Abuse** 29, 539-551.

Keefe KA, Zved AF, Zigmond MJ, Abercrombie ED.**(1993)** Stress-induced dopamine release in the neostriatum: evaluation of the role of action potentials in nigrostriatal dopamine neurons or local initiation by endogenous excitatory amino acids, **J. Neurochem.** 61, 1943-1952.

Kirsch P, Reuter M, et al. **(2006)** Imaging gene-substance interactions: the effect of the DRD2 TaqIA polymorphism and the dopamine agonist bromocriptine on the brain activation during the anticipation of reward. **Neurosci Lett.** 25,196-20.

Klitenick MA, DeWitte P, Kalivas PW. **(1992)** Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. **J. Neurosci**. 12,2623-2632.

Koob GF. **(2008)** Brain stress systems in the amygdala and addiction. Brain Res. 2009 (in pressStice E, Spoor S, Bohon C, Small DM. Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. **Science.** 322, 449-452.

Laakso A, Pohjalainen T, Bergman J,et al. **(2005)** Links the A1 allele of the human D2 dopamine receptor gene is associated with increased activity of striatal L-amino acid decarboxylase in healthy subjects. **Pharmacogenet Genomics.** 15,387-391.

Laudet AB, Magura S, Vogel HS et al. Perceived reasons for substance misuse among persons with a psychiatric disorder, Am. J. Orthopsychiatr.2004; 74 : 365-375.

Lawford BR, Young RM, Rowell JA et al. **(1995)** Bromocriptine in the treatment of alcoholics with the D2 dopamine receptor A1 allele. **Nat Med.** 1, 1337-341.

Li CY, Mao X, Wei L. **(2008)** Genes and (common) pathways underlying drug addiction. **PLoS Comput Biol** 4(1),e2.

Luthe W. **(1969) Autogenic therapy**. New York and Stratton. 1-V11

Madrid GA, MacMurray J, Lee JW, Anderson BA, Comings DE. **(2001)** Stress as a mediating factor in the association between the DRD2 TaqI polymorphism and alcoholism.**Alcohol**. 23,117-122.

Martin I, Venables PA. (**1967) A manual of psychophysiological methods**. New York: American Elsevier

Matuszewich L, Yamamoto BK.**(2004)** Chronic stress augments the long-term and acute effects of methamphetamine. **Neuroscience** 124, 637-646.

Meshkin B, Blum K. **(2007)** Folate Nutrigenetics: A Convergence of Dietary Folate Metabolism, FolicAcid Supplementation, and Folate Antagonist Pharmacogenetics **Drug Metabolism Letters** 1, 55-60.

Moghaddam B. **(1993)** Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. **J. Neurochem**. 60, 1650-1657.

Morgan D,Grant KA, Gage HD et al. (**2002)** Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. **Nat. Neurosci.** 5, 169-174.

Noble EP, Blum K, Khalsa ME et al. **(1993)** Allelic association of the D2 dopamine receptor gene with cocaine dependence. **Drug Alcohol Depend.** 33, 271-85.

Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ. **(1991)** Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. **Arch Gen Psychiatry.** 48, 648-654.

O'Brien PD, White FJ. (**1987)** Inhibition on non-dopamine cells in the ventral tegmental area by benzodiazepines: relationship to dopamine cell activity. **Eur. J. Pharmacol.** 142, 343-354.

Ota A, Yasuda N, Okamoto Y. et al.**(2004)** Relationship of job stress with nicotine dependence of smokers-a cross-sectional study of female nurses in a general hospital, **J. Occup. Health.** 46, 220-224.

Pohjalainen T, Rinne JO, Någren K, Lehikoinen P, Anttila K, Syvälahti EK, Hietala J**. (1998)** The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers, M**ol Psychiatry.** 3,256-60.

Reed M, Best J, Nijhout H. **(2009)** Passive and active stabilization of dopamine in the striatum. **Biosciences Hypotheses** (in press).

Rogers IL, Pelton RB. **(1957)** Glutamine in the treatment of alcoholism: a preliminary note. **Q. J. Stud. Alcohol** 18,581-58.

Saal D, Dong Y, Bonci A et al. **(2003)** Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. **Neuron** 37 , 577-582.

Saulskaya N, Marsden CA. (**1995)** Conditioned dopamine release: dependence upon N-methyl-d-aspartate receptors. **Neuroscience** 67, 57-63.

Sesack SR, Pickel VM. **(1992)** Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine

neurons in the ventral tegmental area. **J. Comp. Neurol.** 320, 145-160.

Sinha R. **(2001)** How does stress increase risk of drug abuse and relapse? **Psychopharmacology (Berlin**). 158, 343-359.

Soderpalm A, Nikolayev L, de Wit H. **(2003)** Effects of stress on responses to methamphetamine in humans, **Psychopharmacology (Berlin)** 170,188-199.

Stice E, Fisher M, Lowe MR, (**2004**) Are Dietary Restraint Scales Valid Measures of Acute Dietary Restriction? Unobtrusive Observational Data Suggest Not - Psychological Assessment. 16(1), 51-59.

Suemaru S, Hashimoto K, Ota Z. **(1985)** Brain corticotropin-releasing factor (CRF) and catecholamine responses in acutely stressed rats. **Endocrinol Jpn**. 32,709-718.

Sugita S, Johnson SW, North RA. **(1992)** Synaptic inputs to GABAA and GABAB receptors originate from discrete afferent neurons. **Neurosci. Lett.** 434, 207-211.

Swann AC. **(2003)** Neuroreceptor mechanisms of aggression and its treatment. **J. Clin.Psychiatry** 64**,** (Suppl. 4) 26-35.

Tidey JW and Miczek KA. **(1997)** Acquisition of cocaine self-administration after social stress: role of accumbens dopamine. **Psychopharmacology (Berlin)** 130, 203-212.

Uhl G, Blum K, Noble EP et al. **(1993)** Substance abuse vulnerability and D2 receptor genes. **Trends Neurosci.** 16, 83-8.

Vezina P, Blanc G, Glowinski J et al. **(1991)** Opposed behavioral outputs of increased dopamine transmission in prefrontocortical and subcortical areas: a role for cortical D1 receptor. **Eur. J. Neurosci**. 3, 1001-1007.

Virk RS, Dunton NJ,.Young JC, et al. **(1999)** Effect of vitamin B-6 supplementation on fuels, catecholamines, and amino acids during exercise in men. **Med Sci Sports Exerc.** 31, 400-408.

Voisey J, Swagell CD, Hughes IP, Morris CP, van Daal A, Noble EP, Kann B, Heslop KA, Young RM, Lawford BR. **(2009)** The DRD2 gene 957C>T polymorphism is associated with posttraumatic stress disorder in war veterans. **Depress Anxiety**. 26, 28-33.

Walaas I, Fonnum F. **(1989)** Biochemical evidence for gamma-aminobutyrate containing fibers from the nucleus accumbens to the substantia nigra and ventral tegmental area in the rat. **Neuroscience.** 5, 325-336.

Wang GJ, Volkow ND, Thanos PK, Fowler JS. **(2004)** Similarity between obesity and drug addiction as assessed by neurofunctional imaging: a concept review. **J Addict Dis.** 23,39-53.

Wang XY, Zhao M, Ghitza UE, Li YQ, Lu L. **(2008)** Stress impairs reconsolidation of drug memory via glucocorticoid receptors in the basolateral amygdala. **J Neurosci.** 28,5602-5610.

Westerink BHC, Kwint HF, deVries JV. **(1996)** The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain.**J. Neurosci.** 16, 2605-2611.

Wheeler D, Boutelle MD, Fillenz M.**(1995)** The role of N-methyl-d-aspartate receptors in the regulation of physiologically released dopamine,.**Neuroscience.** 65, 767-774.

Xi ZX, Stein EA. **(1998)** Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptors-an in vivo electrochemical study. **Brain Res.** 798, 156-165.

Yamanaka Y, Kono S. **(1974)** Brain serotonin turnover in alcoholic mice. **J Phramacol.** 24, 247-252.

Yim CY, Mogenson GJ. (**1980)** Effect of picrotoxin and nipecotic acid on inhibitory response of dopaminergic neurons in the ventral tegmental area to stimulation of the nucleus accumbens. **Brain Res.** 199, 466-472.