Postgraduate Medicine

http://informahealthcare.com/pgm
ISSN: 0032-5481 (print), 1941-9260 (electronic)

Postgrad Med, 2015; Early Online: 1–10
 DOI: 10.1080/00325481.2015.994879

informa
healthcare

ORIGINAL RESEARCH

rsfMRI effects of KB220Z™ on neural pathways in reward circuitry of abstinent genotyped heroin addicts

Kenneth Blum[1,7,10,11,12,13,14], Yijun Liu[1,3], Wei Wang[2], Yarong Wang[2], Yi Zhang[1,3], Marlene Oscar-Berman[4], Andrew Smolen[5], Marcelo Febo[1], David Han[6], Thomas Simpatico[7], Frans J. Cronjé[8], Zsolt Demetrovics[9] & Mark S Gold[1]

[1]Department of Psychiatry & McKnight Brain Institute, University of Florida, College of Medicine, Gainesville, FL, USA, [2]Department of Radiology, Tangdu Hospital, Fourth Military Medical University, Xi'an, Shaanxi, China, [3]School of Life Science and Technology, Xidian University, Xi'an, Shaanxi 710071, China, [4]Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA, [5]Institute for Behavioral Genetics, University of Colorado, Boulder, CO, USA, [6]Department of Management Science and Statistics, University of Texas at San Antonio, San Antonio, TX, USA, [7]Department of Psychiatry, Human Integrated Services Unit, University of Vermont Center for Clinical & Translational Science, College of Medicine, Burlington, VT, USA, [8]University of Stellenbosch, Cape Town, South Africa, [9]Institute of Psychology, Eötvös Loránd University, Budapest, Hungary, [10]Dominion Diagnostics, LLC, North Kingstown, RI, USA, [11]RDSolutions, LLC, La Jolla, CA, USA, [12]Victory Nutrition, LLC, Lederoch, PA, USA, [13]Department of Clinical Neurology, Path Foundation NY, New York, NY, USA, and [14]Department of Addiction Research & Therapy, Malibu Beach Recovery Center, Malibu Beach, CA, USA

Abstract

Recently, Willuhn et al. reported that cocaine use and even non-substance-related addictive behavior increases as dopaminergic function is reduced. Chronic cocaine exposure has been associated with decreases in D2/D3 receptors and was also associated with lower activation of cues in occipital cortex and cerebellum, in a recent PET study by Volkow's et al. Therefore, treatment strategies, like dopamine agonist therapy, that might conserve dopamine function may be an interesting approach to relapse prevention in psychoactive drug and behavioral addictions. To this aim, we evaluated the effect of KB220Z™ on reward circuitry of 10 heroin addicts undergoing protracted abstinence (average 16.9 months). In a randomized placebo-controlled crossover study of KB220Z, five subjects completed a triple-blinded experiment in which the subject, the person administering the treatment, and the person evaluating the response to treatment were blinded to the treatment that any particular subject was receiving. In addition, nine subjects were genotyped utilizing the $GARS_{DX}$™ test. We preliminarily report that KB220Z induced an increase in BOLD activation in caudate-accumbens-dopaminergic pathways compared to placebo following 1-hour acute administration. Furthermore, KB220Z also reduced resting-state activity in the putamen of abstinent heroin addicts. In the second phase of this pilot study of all 10 abstinent heroin-dependent subjects, we observed that three brain regions of interest were significantly activated from resting state by KB220Z compared to placebo ($p < 0.05$). Increased functional connectivity was observed in a putative network that included the dorsal anterior cingulate, medial frontal gyrus, nucleus accumbens, posterior cingulate, occipital cortical areas, and cerebellum. These results and other quantitative electroencephalogy (qEEG) study results suggest a putative anti-craving/anti-relapse role of KB220Z in addiction by direct or indirect dopaminergic interaction. Due to small sample size, we caution definitive interpretation of these preliminary results, and confirmation with additional research and ongoing rodent and human studies of KB220Z is required.

Keywords:

Abstinent heroin addicts, brain reward circuitry, dopamine, fMRI, KB220Z

History

Published online 16 December 2014

Introduction

Previously, other authors have found resting-state functional connectivity patterns in heroin-dependent individuals that were significantly different from healthy subjects. There is evidence that such impairments in functional connectivity may negatively impact decision making and inhibitory control [1]. Moreover, in earlier studies, it was found that heroin addicts showed reduced activation in right amygdala in response to the affective pictures, when compared to normal controls [2]. Other studies showed persistent abnormalities in brain function following 1 month of heroin withdrawal in the orbitofrontal cortex (OFC) [3]. Zijlstra et al. found lower baseline dopamine D2 receptor (D2R) availability in opiate-dependent subjects than controls in the left caudate nucleus [4]. D2R availability in the putamen correlated negatively with years of opiate use. Opiate-dependent subjects demonstrated higher dopamine release after cue-exposure in the right putamen than controls. Chronic craving and anhedonia were positively correlated with altered dopamine release [4] in psychoactive drug-dependent subjects.

Correspondence: Kenneth Blum, PhD, Department of Psychiatry & McKnight Brain Institute, University of Florida, 1149 Newell Drive, Gainesville, FL 32610, USA. Tel: +1 619 890 2167.
E-mail: drd2gene@gmail.com

Understanding the true role of dopamine in escalated cocaine intake, for example, has been the subject of recent study by a number of investigators. Suto et al. provided strong evidence that attenuated dopamine actions in the core (but not the shell) of accumbens results in greater cocaine intake and shorter inter-response times – during periods of self-administration [5]. Conversely, they found that enhanced dopamine actions in the core (but not the shell) resulted in decreased intake and longer inter-response times [6]. In agreement with these findings Willuhn et al. surprisingly found that phasic dopamine decreased in ventromedial striatum (VMS) as the rate of cocaine intake increased, with the decrement in dopamine in the VMS significantly correlated with the rate of escalation [7]. Moreover, administration of the dopamine precursor L-DOPA at a dose that replenished dopamine signaling in the VMS reversed escalation. Thus, this evidence demonstrates a cause and effect relationship between diminished dopamine transmission and excessive drug use. Although this work is intriguing, it may not be in conflict with surfeit rather than deficit theories of dopamine function [8,9]. For example, Stewart suggests that the overall effect of widespread increases in phasic dopamine transmission is to precipitate drug-seeking behaviors and not to decrease it [8]. In essence, these authors correctly suggested that, during periods of abstinence, dopamine receptors become supersensitive and, in fact, relapse could even be linked to fatalities because of the higher euphoric response.

However it does seem to support dopamine-based agonistic modalities as suggested by Willuhn et al. [7]. Along these lines, Caprioli et al. correctly concluded that at present there is no FDA-approved medication for cocaine addiction [9]. However, several clinical studies have suggested that agonist-based substitution treatment (eg, prescription oral amphetamine and methylphenidate) decreases illegal cocaine use [10]. Although we do not clinically agree with the amphetamine approach (chronically leading to downregulation of D2Rs as seen with other D2 agonists as well as the potential induction of relapse [11]), it does point to the importance of dopamine agonist therapy compared to current therapies that utilize dopamine antagonistic therapy to treat cocaine escalation during protracted abstinence.

According to Volkow et al., drug addiction is characterized by a compulsive drive to take drugs despite serious negative consequences; it is a disorder that involves complex interactions between genetic and environmental variables [11,12]. Undoubtedly heroin and other addictions are a complex phenomenon of the brain, involving both affective and cognitive processes [13,14]. It has been found that in heroin-dependent individuals there is a decrease in white matter (WM) volume in the frontal area and decreased gray matter density in the bilateral prefrontal cortices and in the temporal regions compared to healthy subjects [15]. In support, other authors consistently found differences in activation pattern between heroin-dependent subjects and healthy individuals during resting-state brain activities. These differences of activation patterns were found in the OFC; hippocampal/parahippocampal region; amygdala; caudate; putamen as well as the insula and thalamus [1].

Importantly, Elman et al. proposed a map consisting of four circuits involved in drug abuse and possibly reward behaviors: (1) reward, located in the nucleus accumbens (NAc) and ventral pallidum; (2) motivation/drive, located in the OFC and subcallosal cortex; (3) memory and learning, located in the amygdala and hippocampus; and (4) control, located in the prefrontal cortex and anterior cingulate gyrus [16]. Simply stated but evidently even more complex, our current knowledge indicates that whereas aberrant craving behavior resides in the caudate accumbens brain region, loss of control and thus relapse occurs in the cingulate gyrus as determined in cocaine abusers [17]. Moreover, Thanos et al. and Rothman et al. independently suggested that dopamine agonist therapy, by either increasing D2R availability or enhancing dopamine release, respectively, could be useful therapeutic adjuncts for the treatment of cocaine and alcohol addictions [18,19], as well as for obesity, attention-deficit disorder, other reward deficiency syndrome (RDS) behaviors and depression [20]. Indicating that higher dopaminergic activity could lead to reward-seeking in the short term, whereas, it must be noted that in the long term it is dopaminergic deficiency that can prevent recovery and lead to relapse [21].

We hypothesized that the putative anti-craving/anti-relapse compound KB220Z™, as reviewed by Chen et al., may activate dopaminergic pathways as determined by *fMRI* [22]. KB220Z is a neuroadaptagen consisting of amino acid neurotransmitter precursors and enkephalinase-catechol-*O*-methyltransferase (COMT) inhibition therapy called neuroadaptagen amino acid therapy. Ongoing research on KB220Z repeatedly confirms the numerous clinical effects that ultimately result in significant benefits for victims having genetic antecedents for all addictive, compulsive and impulsive behaviors [23]. In earlier research, using quantitative electroencephalogy (qEEG) during protracted abstinence in psycho-stimulant-dependent subjects, this compound showed an increase in both α- and low β-bands in the OFC and cingulate gyrus overcoming qEEG abnormalities [24].

To this aim, we evaluated the role of KB220Z on reward circuitry in a triple-blinded, randomized, placebo-controlled crossover study of 10 heroin addicts undergoing protracted abstinence (average 16.9 months). A triple-blinded experiment is an experiment in which neither the subject, the person who administers the treatment nor the person evaluating the response to treatment knows which treatment any particular subject is receiving.

Methods

Institutional Review Board and subjects

The experimental protocol was approved by Institutional Review Board (IRB) of the Fourth Military University, China. Ten abstinent male heroin-dependent patients (HDP, right-handed, age: 33 ± 7.57 years, range: 20–44 years) were enrolled from a local inpatient treatment research facility. All subjects signed an informed consent. All HDP volunteers met DSM-IV criteria for heroin dependence. They regularly used cigarettes and denied any psychotropic agent in the 3 months before *fMRI* scan. All HDP had a confirmed diagnosis of heroin dependency with mean abstinence from heroin of 16.0 ± 7.91 months (range 3–24 months), a negative test for morphine in urine analysis (reagent box produced by China

Table I. Demographic and clinical characteristics of male Chinese (n = 10) heroin-dependent subjects.

Demographic	Heroin-dependent subject (mean/SD)
Age (years)	33 ± 7.57
Duration of abstinence from heroin (months)	16 ± 7.91

Abbreviation: SD = Standard deviation.

Carrie City International Engineering Co.), and a negative HIV blood test. None of the HDP had a history of neurological illness or injury other than drug addiction. None of the subjects were taking psychoactive prescription drugs within 1 week of the *fMRI*. None of the subjects were previously exposed to a high magnetic field (Table I).

Experimental protocol

For the first part of the experiment we are reporting data on 5 of the 10 subjects who completed the crossover study (Figure 1). The second phase analyzed data derived from all 10 subjects (Figure 2) in terms of brain regions of interest (ROIs) relative to resting-state placebo and KB220Z treatment. The experimental protocol has been reported earlier by Zhang et al. [1]. The *fMRI* research was carried out at Life Sciences Research Center, School of Life Sciences and Technology, Xidian University, Xi'an, Shaanxi, China.

Briefly, the experiment was carried out in a 3T GE (Medical Signa EXCITE) scanner. Prior to the functional run, a high-resolution structural image for each subject was acquired using three-dimensional MRI sequences with a voxel size of 1 mm^3 and with an axial Fast Spoiled Gradient Recalled (TR 500 ms, TE 7.7 ms, matrix 256 × 256, field of view 220 × 220 mm, 25 slices, 4 mm thickness, 1 mm interslice gap). A gradient echo T2*-weighted sequence with in-plane resolution of 3.75 × 3.75 mm (TE 30 ms, TR 2 s, matrix 64 × 64, field of view 240 mm, and flip angle 90°) was also acquired. A total of 150 echo-planar volumes were acquired during the resting scan, and functional image scanning lasted 5 min. Subjects were instructed to close their eyes but remain awake during the entire scanning procedure. After scanning, all of the subjects reported that they had remained awake during the full length of the scan.

Imaging data were preprocessed for motion and signal drift and were analyzed using Statistical Parametric Mapping 5 (SPM5, http://www.fil.ion.ucl.ac.uk/spm). Images were first corrected for within scan acquisition time differences between slices (slice timing correction) and then realigned to the first volume to correct for interscan head motions. On reviewing the translation and rotation of the images, head movements >1 mm or head rotations >1° were discarded. The multi-voxel pattern analysis (MVPA) method is a straightforward application of pattern classification techniques to *fMRI* datasets, where the patterns to be classified are vectors of voxel activity values (BOLD signal) [25]. In the current study, we used a novel variant of the "searchlight" (SL) approach (a form of decoding) [26] to select an appropriate set of voxels in order to define multivariate features as the input for the pattern classification analysis [27]. Following earlier reported procedures by Zhang et al., the training and test procedures were repeated 24 times [1]. Each test used feature vectors with k^{-1} different runs/subjects assigned as a training dataset and a feature vector of a different run/subject assigned as the test dataset. The classifier accuracy was measured by the proportion of runs correctly classified for the central voxel (*vi*). Thus, the mean classifier accuracy would yield *vim* by averaging all of the accuracies calculated for every fold of this k-fold cross-validation procedure. The same procedure was then repeated for the next spatial position at *vi*. The mean for the decoding accuracy of each voxel was then used to create a three-dimensional spatial map representing the decoding accuracy for each position *vi* in the whole brain and would thus represent the statistical differences between the KB220Z compared to placebo groups.

It is noteworthy that the SL procedure for the pattern analysis technique provides an indication of effect size independent of the amount of data, which in the case of the present work involves a preliminary set of test subjects. This means that the voxel activations are due to "activations" across subjects and that they are statistically significant. Thus, from the maps themselves we obtain information regarding statistically significant spatial activation patterns. Since this is a pilot result, summary tables are not necessary and we prefer not to include these summary tables for now because they may even be misleading.

KB220Z

Specifically, the patented product comprises of the following ingredients in validated, evidence-based intake levels: thiamine, 15 mg (1033% of daily value); vitamin B6, 10 mg (500%); chromium polynicotinate (as ChromeMate®) 200 μg (166%); a fixed dose combination of amino acids and herbs called Synaptose™ (1.9 g), which contains DL-phenylalanine, L-tyrosine, passion flower extract; a Metalloglycoside™ complex containing arabinogalactans, *N*-acetylglucosamine, astragalus, aloe vera, Frankincense resin, white pine bark extract, and spirulina; rhodiola (as RhodiGen™; L-glutamine; 5-hydroxytryptophan); thiamine hydrochloride; pyroxidal-5-phosphate; and pyridoxine HCl. The matching placebo powder was manufactured by Cephram, Inc. (NJ, USA). The product is safe and is exempt from IRB concerns. None of the patients took KB220Z or placebo during the 7-day period between testing.

Phase I of the *fMRI* experiment utilized a 2 × 2 design crossover comparing placebo against experimental KB220Z employing a randomized, triple-blinded, study design for 5 out of 10 HDP subjects undergoing protracted abstinence. Each of the five patients was randomly assigned to either the experimental or placebo group. The placebo was matched in cherry-flavored inert powder that was subsequently mixed with orange juice (no pulp). The KB220Z product was similarly mixed with orange juice. Each patient consumed the product (placebo or experimental) in the morning before breakfast and 1 hour prior to scanning. After 7 days the experimental and the placebo groups were switched and *fMRI* tests were repeated on each patient. The acute dose for each patient was 24 g in vehicle (before breakfast) and 1 hour prior to *fMRI* testing. In Phase II of the experiment,

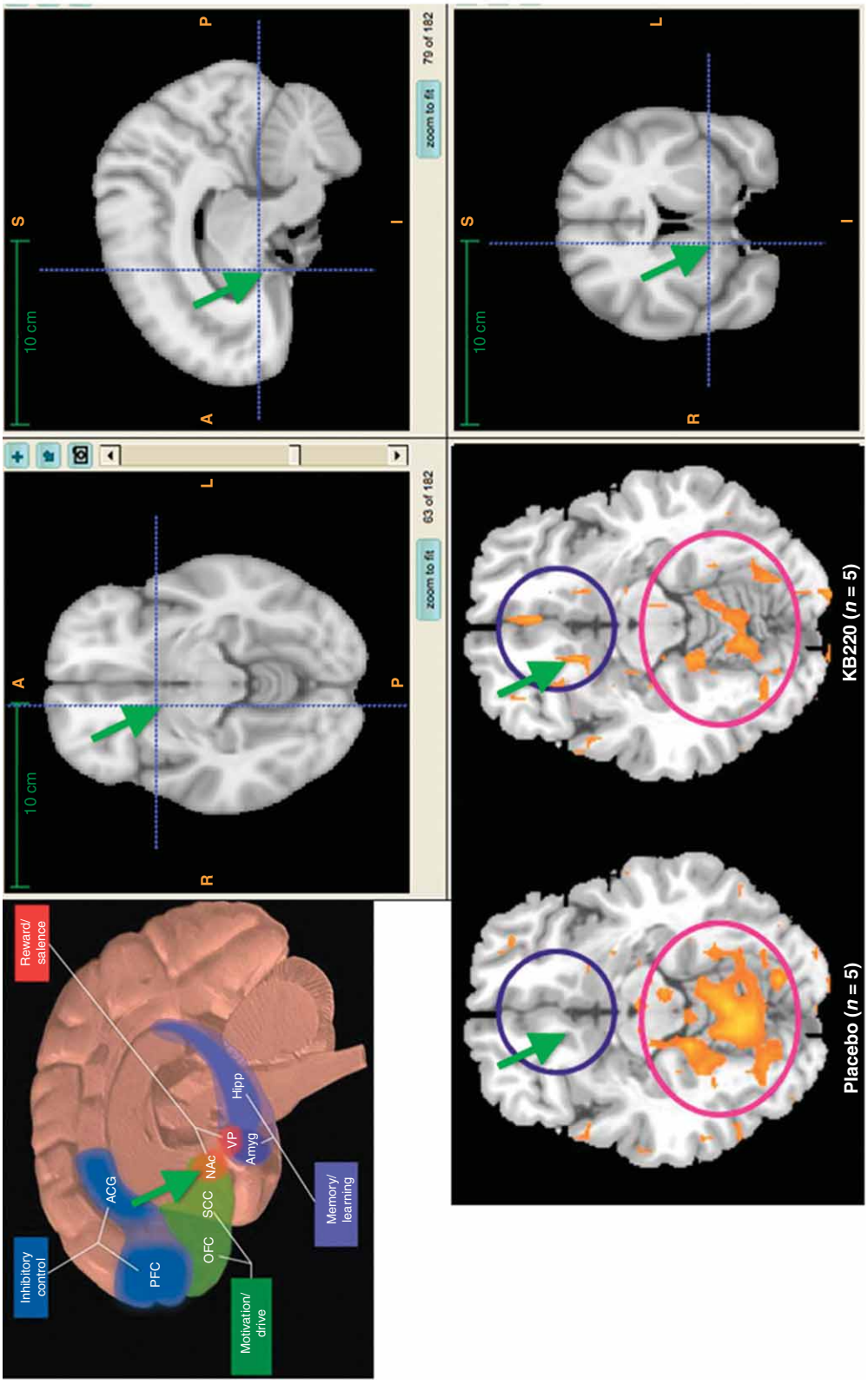

Figure 1. Location of the NAc in human central nervous system is shown. Resting *fMRI* data analysis in the heroin users ($n = 5$) before and after KB220Z (Synaptose™) and placebo is shown. Standard atlas shows the location of the NAc (surrounding area) with significant increases in *rsfMRI* response with KB220Z. A modified figure was utilized in a review article by Blum *et al.* [23].
Abbreviation: NAc = Nucleus accumbens.

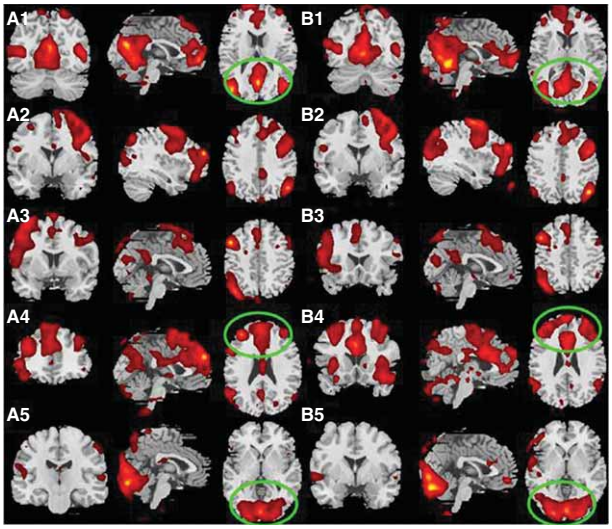

Figure 2. *rsfMRI* (first scan A) before, the baseline and *rsfMRI* (second scan B) sfter KB220Z treatment are shown; the circles defined the ROIs showing significant differences in the resting-state (*rsfMRI*) brain activity. Abbrevitation: ROIs = Regions of interest.

the remaining five subjects similarly participated in the crossover experiment. Like the first group they were tested then switched to either placebo or KB220Z and were tested again after 7 days. The data collected were then sent blinded for statistical analysis. All patients were genotyped by means of a saliva genotyping collection tube.

Genotyping

A brief description of the genotyping methods for the polymorphisms to be assayed in this project follows. All methods are routinely performed in the Institute of Behavioral Genetics laboratory at the University of Colorado, Boulder, USA. Of the 10 Chinese candidates, 1 was excluded from analysis due to poor polymerase chain reaction (PCR) amplification. Details, including primer sequences and specific PCR conditions, may be found in Anchordoquy et al. [28], Haberstick and Smolen [29] and Haberstick et al. [30]. Each patient was also genotyped for the following gene polymorphisms: monoamine oxidase A variable number tandem repeat (MAOA-VNTR), serotonin transporter-linked polymorphic region (5HTTLPR), SLC6A3, dopamine D4 receptor (DRD4), ANKKI-dopamine D2 receptor (DRD2) TaqIA (rs1800497), and the COMT val^{158}met single nucleotide polymorphism (SNP) (rs4680).

The dopamine transporter (DAT1, locus symbol SLC6A3): This maps to 5p15.3 and contains a 40 base-pair VNTR element consisting of 3–11 copies in the 3′ untranslated region (UTR) of the gene [31]. The assay by Haberstick and Smolen is a modification of the method of Vandenbergh et al. [29,31]. Primer sequences were:

Forward- 5′-TGTGGTGTAGGGAACGGCCTGAG-3′; and
Reverse- 5′-CTTCCTGGAGGTCACGCT CAAGG-3′.

The DRD4, which maps to 11p15.5, contains a 48 bp VNTR polymorphism in the third exon, which consists of 2–11 repeats [32].

The assay by Haberstick and Smolen is a modification of the method of Lerman et al. [29,33]. Primer sequences were:

Forward- 5′-VIC -GCT CAT GCT GCT GCT CTA CTG GGC-3′; and
Reverse-5′-CTG CGG GTC TGC GGT GGA GTC TGG-3′.

Monoamine Oxidase A upstream VNTR (MAOA-uVNTR): 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; the MAOA-uVNTR assay is a modification of a published method of Sabol et al. [34]. Primer sequences were:

Forward- 5′-ACAGCCTGACCG-TGGAGAAG-3′; and
Reverse- 5′-GAACGTGACGCTCCATTCGGA-3′.

5HTTLPR: The serotonin transporter (5HTT, Locus Symbol SLC6A4), which maps to 17q11.1–17q12, contains a 43 bp insertion/deletion (ins/del) polymorphism in the 5′ regulatory region of the gene [35]. Due to an error in sequencing, this was originally thought to be a 44 bp deletion. The long variant (L) has approximately three times the basal activity of the short promoter (S) with the deletion [36,37]. Primer sequences were:

Forward- 5′- 6FAM - ATG CCA GCA CCT AAC CCC TAA TGT - 3′;
Reverse- 5’- GGA CCG CAA GGT GGG CGG GA - 3′.

Hu et al. reported that a SNP (rs25531, A/G) in the long form of 5HTTLPR may have functional significance [38]. The more common L_A allele is associated with the reported higher basal activity, whereas the less common L_G allele has transcriptional activity no greater than the S-form. The SNP rs25531 is assayed by incubating the full length PCR product with the restriction endonuclease MspI.

For all of the above VNTR and ins/del polymorphisms, PCR reactions contained ~ 20 ng of DNA, 10% DMSO, 1.8 mM $MgCl_2$, 200 μM deoxynucleotides, with 7′-deaza-2′-deoxyGTP substituted for one-half of the dGTP, 400 nM forward and reverse primers and 1 unit of AmpliTaq Gold® polymerase, in a total volume of 20 μl. Amplification was performed using touchdown PCR [39]. After amplification, an aliquot of PCR product was combined with loading buffer containing size standard (Genescan 1200 Liz) and analyzed with an ABI PRISM® 3130 Genetic Analyzer. Genotypes were scored by two investigators independently.

ANKKI-DRD2 TaqIA (rs1800497): The gene encoding the dopamine D2R maps to 11q23, and contains a polymorphic TaqI restriction endonuclease site in the 3′ UTR of the gene. The A1 allele has been reported to reduce the amount of receptor protein [40]. This SNP is done using a Taqman (5′ nuclease) assay [29,30]. Primer and probe sequences were:

Forward primer- 5′-GTGCAGCTCACTCCATCCT-3′;
Reverse primer- 5′-GCAACACAGCCATCCTCAAAG-3′;

Table II. Genotyping data for each Chinese patient.

Subject	MAOAuVNTR	5HTTLPR	5HTTLPR	SLC6A3	DRD4	*DRD2*	*COMT*	Any risk allele	SEVERITY* GARS
1	4R	S/L	S/L_A	10R/10R	4R/4R	A2/A2	A/A	POSITIVE	0.30–LS
2	3R	S/S	S/S	10R/10R	2R/4R	A1/A2	GAG	POSITIVE	0.38–MS
3	4R	S/S	S/S	10R/10R	3R/4R	A1/A2	G/G	POSITIVE	0.46–MS
4	3R	S/S	S/S	10R/10R	4R/6R	A2/A2	G/G	POSITIVE	0.38–MS
5	4R	S/S	S/S	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.30–LS
6	3R	L/L	S/L_G	10R/10R	4R/4R	A1/A2	ND	POSITIVE	0.45–MS
7	4R	L/L	L_A/L_G	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.54–HS
8	4R	S/S	S/S	10R/10R	4R/5R	A2/A2	A/G	POSITIVE	0.23–LS
9	3R	S/L	S/L_A	10R/10R	2R/4R	A2/A2	A/G	POSITIVE	0.46–MS

Abbreviations: COMT = Catecholamine-*O*-methyltransferase; HS = High severity; L = long variant; LS = Low severity; MAOAuVNTR = Monoamine oxidase a upstream variable number tandem repeat; MS = Moderate severity; S = Short promoter.

A1 Probe- 5′- VIC-CCTGCCTTGACCAGC-NFQMGB-3′;
A2 Probe- 5′- FAM-CTGCCTCGACCAGC-NFQMGB-3′.

COMT val^{158}met SNP (rs4680): The gene encoding COMT maps to 22q11.21 and codes for both the membrane-bound and soluble forms of the enzyme that metabolizes dopamine to 3-methoxy-4-hydroxyphenylethylamine [41,42]. 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 fourfold reduction in enzymatic activity [42]. The COMT SNP is assayed with a Taqman method [29,30]. Primer and probe sequences were:

Forward Primer- 5′-TCGAGATCAACCCCGACTGT-3′;
Reverse Primer- 5′-AACGGG-TCAGGCATGCA-3′;
Val Probe- 5′-FAM-CCTTGTCCTTCACGCCAGCGA-NFQMGB-3′;
Met Probe- 5′-VIC-ACCTTGTCCTTCATGCCAGCGAAAT-NFQMGB-3′.

Genetic Addiction Risk Score (GARS$_{DX}$™) algorithm

In terms of genotyping data, we have determined, based on literature review, that there are seven risk alleles involved in the six candidate genes studied in this patient population. To determine severity of the nine patients studied, utilizing modified genetic variants of the $GARS_{DX}$ test (Dominion Diagnostics/IGENE), we calculated the percentage of prevalence of the risk alleles and provided an arbitrary severity score based on percentage of risk alleles present. Subjects who carry the following alleles: DRD2 = A1; SLC6A3 (DAT) = 10R; DRD4 = 3R or 7R; 5HTTLPR = L or L_A; MAO = 3R; and COMT = G. As depicted in Table II, low severity (LS) = 1–36%; moderate severity (MS) = 37–50%, and high severity (HS) = 51–100%.

Results

Based on this analysis, all nine genotyped heroin subjects tested had at least one risk allele as represented by prevalence and not frequencies: 11% (n = 1) were HS, 56% (n = 5) were MS, and 33% were LS (n = 3). Therefore, using $GARS_{DX}$, it was determined that 67% of the Chinese HDP were at moderate-to-high risk of addictive behavior (Hardy-Weinberg Equilibrium). Of particular interest was the finding that 56% of the HDP carried the DRD2 A1 allele (5/9) (see Table II). Our study revealed that all tested HDPs entering a residential treatment facility for heroin addiction carried at least one risk allele.

Work in progress involves the exploration of *fMRI* research using KB220Z compared to placebo in a number of important studies. Figure 1 shows the resulting composite MVPA maps from five subject scans. The additional five subjects were not averaged but showed similar results as evidenced from the individual subject maps. It is important to note that the comparisons between groups rely on a MVPA SL strategy that identifies consistent features across subjects in the resting-state datasets. Based on the five subjects, albeit a small sample size, the results suggest that, compared to placebo, KB220Z BOLD activates the caudate accumbens of the abstinent heroin addict tested (see Figure 1). Moreover, we found that KB220Z might reduce the known hyperexcitability in the putamen as evidenced by the lower connectivity pattern including this region after KB220Z treatment (Figure 1). In preliminary data, we have found that this complex activates brain reward pathways and compared to placebo it produces significant differences in at least three brain regions (Figure 2). The ROIs showing significant differences between placebo and KB220Z ($p < 0.05$) in resting state functional magnetic resonance imaging (*rsfMRI*) included prefrontal, ventral striatal, occipital, posterior cingulate, and cerebellar areas (n = 10). We observed an increased functional connectivity in the NAc and or surrounding striatal regions. Frontal regions showed an increased evidence of BOLD activity with KB220Z and occipital/cerebellar regions showed a reduction in BOLD activity (Figures 1 and 2). Specifically, prefrontal regions included dorsal anterior cingulate cortex (BA32) and bilateral (right and left hemispheres) portions of the superior and medial frontal gyrus (BA9). Occipital regions included bilateral middle occipital gyrus, lingual gyrus, and cuneus (BA18). Cerebellar nuclei included BOLD voxels lying bilaterally in anterior and posterior lobes.

Moreover, voxels identified as active (or showing similar resting BOLD activation features) on maps are significant across subjects within each group. ROI analyses are based on registration to a standard atlas. As indicated in the text, the results are derived from a small number of subjects and therefore the data, while significant, are preliminary at this time.

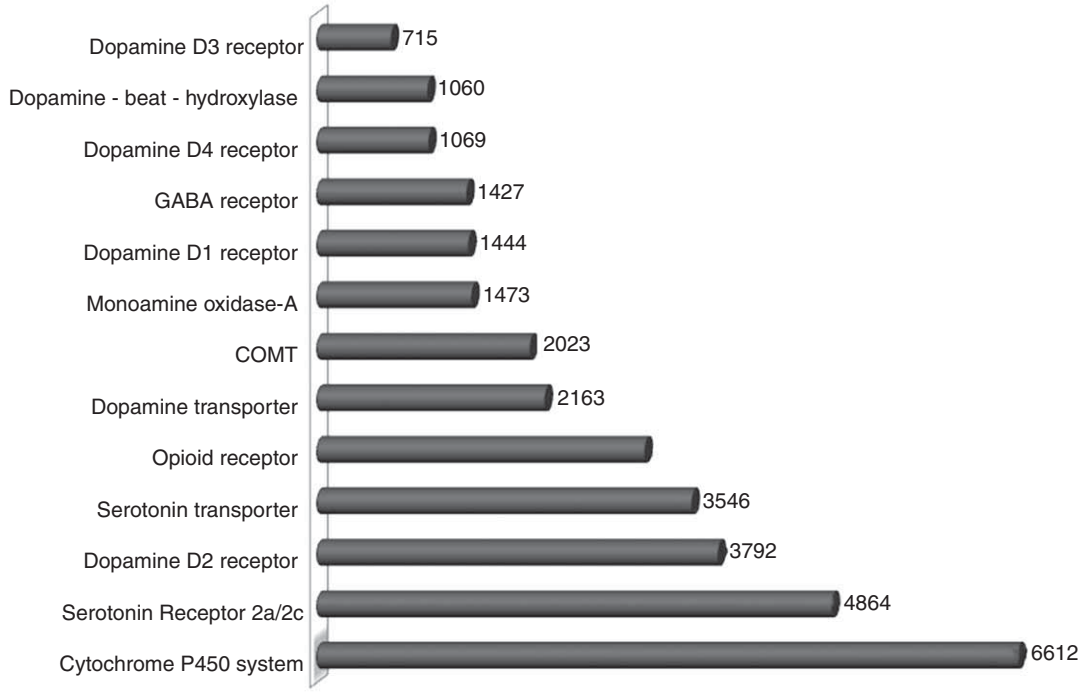

Figure 3. Reward Gene Publications as of 23 October 2014 is shown.

Discussion

Genotyping

It has been determined that when multiple RDS-associated genes are analyzed, such as the genes for serotonergic transporter (5HTTLPR), DRD2, DRD4, DAT1, COMT, and MOA, all subjects carried at least one risk allele (Figure 3). It is important to realize that over the past two decades a major difficulty has been to eliminate spurious results due to poor unscreened controls for all RDS behaviors. Figure 3 at least shows the enormous amount of work reported in PubMed in 2014. This, however, does not eliminate concerns regarding various associations but suggests that additional work in this area is warranted.

Blum et al. [20], in a paper related to the theories of "wanting" and "liking" for dopamine, discussed using Bayesian statistics showing that the DRD2 A1 allele had a predictive value of 74.4% for all RDSs. Here, the subjects studied in this investigation had multiple drug abuse relapses and were presented to inpatient residential treatment programs. The finding that 67% of these individuals had moderate-to-high $GARS_{DX}$, whereas only 33% had lower positive $GARS_{DX}$, indicates that prescreening patients prior to enrolling in a treatment program could be beneficial for relapse-risk assessment. Clinically, this will be important for understanding expectations of future success and the need for intensive treatment involving genomic solutions coupled with medical therapies, including bio-holistic therapies. It will also reduce guilt and denial when patients enter a chemical-dependency program.

The $GARS_{DX}$ experiment supports the understanding that identifying hypodopaminergic genotypes may be the best predictor of adult and adolescent drug abuse or other RDS behavior. These results are also consistent with a number of *fMRI* studies that show that the hypodopaminergic DRD2 A1 genotype leads to blunted responses that could lead to aberrant drug- and/or food-seeking behavior [43], whereas the hyperdopaminergic A2 genotype serves as a protective factor against the development of drug disorders [44].

Albeit a small sample size, a further strength of this study is that only male subjects were used: males with hypodopaminergic functioning are more likely to abuse drugs that stimulate the mesocorticolimbic system than those with normal dopaminergic functioning. In contrast, females living in a negative environment (possibly irrespective of genotype) are at increased risk of using more drugs and even more types of drugs that increase their risk of RDS [45]. With this stated, we must caution against any generalized interpretation in terms of gender.

This pilot study is in agreement with the work of Conner et al. confirming the importance of the cumulative effect of multiple genotypes coding for hypodopaminergic functioning, regardless of their genomic location, as a predictive method of drug use in males [45]. Moreover, it extends the current literature, by suggesting a simple method using genetic testing to classify risk behavior in male patients seeking inpatient residential treatment.

fMRI

Our present findings derived from this small pilot study show a clear difference between placebo and KB220Z in terms of BOLD activation of the dopaminergic pathways of the caudate accumbens area, which is encouraging. Due to the small subject number, we cannot as yet determine statistical significance using more traditional approaches; as such the experiment will continue by adding additional heroin-dependent subjects. However, it is important that we did find

statistical significance ($p < 0.05$) when we evaluated the 10 subjects at baseline compared to KB220Z treatment in three important ROIs involved in addictive behaviors for both substance and non-substance seeking. This was in large part due to the use of the MVPA approach that was able to identify similar BOLD activation patterns across subjects within each group [26]. In this pilot study, we also observed an attenuation of the resultant hyperactivity in the putamen of abstinent heroin-dependent subjects. Currently, albeit knowing that there is a lower D2R availability in the putamen of abstinent heroin-dependent subjects, we do not fully understand the mechanism by which KB220Z administration (post 1 hour) induced an attenuation of this *hypo* state. This will be the subject of additional investigation and it may involve WM synapses.

The present findings, although preliminary, speak of the ability of the KB220Z complex in modulating activity in a specific set of structures that are affected in drug dependence. Administration of KB220Z increased *rsfMRI* signal in a putative network involving dorsal anterior cingulate cortex, medial prefrontal gyrus, accumbens, and – somewhat surprisingly – the cerebellum. In addition to these regions, the posterior cingulate (retrosplenial cortex) was also significantly impacted by KB220Z. Based on the above structures, it is likely that cognition and executive functions are modulated along with rewarding experiences with KB220Z. A search through the past and recent literature, however, will show that these same structures have been implicated in dependence and drug-craving [46]. It is remarkable that in heroin-dependent subjects there is a significant decrease in functional connectivity in dorsal anterior cingulate, medial frontal cortex, and bilateral cerebellum, and subregions of the occipital gyrus [47]. One could speculate, based on the present results, that administration of KB220Z counteracts reductions in connectivity in this specific network. In addition, we find that BOLD activity in the accumbens is increased, which could provide the added effect of enhancing natural rewarding experiences (and diminishing the need for drug use). Although not directly examined here, the role of the accumbens in rewarding experiences leads us to propose that this is a likely outcome. The assessment of reward efficacy should be included in the experimental design in future neuroimaging work.

In ongoing research we are also exploring the role of KB220Z compared to placebo in cue-induced craving behavior as well as the effect of KB220Z on WM synapses in both rodents and humans. This additional experiment is important since the function and form of WM synapses becomes increasingly important in disease. White vesicular neurotransmitter release has been known to be the preserve of gray matter. It is known that synaptic style release of glutamate occurs deep in WM. As WM is increasingly well recognized as a substrate for disease, dysregulation of WM synaptic transmission will play a role in RDS and a number of impulsive/compulsive/addictive behaviors.

Interestingly, current cocaine-dependent users show reductions in WM integrity, especially in cortical regions associated with cognitive control that have been associated with inhibitory dysfunction. In a study by Bell et al. differences between the cocaine abstinent groups were observed bilaterally in the inferior longitudinal fasciculus, right anterior thalamic radiation, right ventral posterolateral nucleus of the thalamus, left superior corona radiata, superior longitudinal fasciculus bilaterally, right cingulum, and the WM of the right precentral gyrus [48]. The findings suggest that specific WM differences persist throughout abstinence, whereas others, which are spatially distinct, discriminate as a function of abstinence duration. These differences may, therefore, represent brain changes that mark recovery from addiction. Similar findings have been found in post-traumatic stress disorder-alcoholic veteran subjects from research in our laboratory [49]. Others have found that fractional anisotropy was significantly decreased in specific brain regions of the HDP ($p < 0.001$ uncorrected), including the frontal gyrus, the parietal lobule, the insula, and the corpus callosum. Thus, the presence of microstructural abnormality is found in the WM of several brain regions of HDP [50].

Based on the current literature, and our pilot findings presented herein, we are poised to further evaluate the effect of KB220Z on microstructural disruption of WM in heroin addicts revealed by diffusion tensor imaging. Certainly the coupling of BOLD activation of dopaminergic pathways in the caudate accumbens; attenuation of abnormal hyperactivity of the putamen in heroin-dependent subjects; and potential reduction of microstructural abnormalities in WM by KB220Z should ultimately lead to a novel safe dopamine agonist for prevention, tertiary treatment, and relapse attenuation in RDS victims, especially carriers of reward gene polymorphisms found in abstinent heroin addicts, having implications for both substance- and non-substance-seeking addictive behaviors.

It is known that dopamine agonist therapy has failed in treatment of addiction, because the administration of powerful D2 agonists like bromocriptine [51], apomorphine, and other D2 agonists reduce D2 receptors chronically. This, in turn, is counterintuitive and will result in reinstatement of drug-seeking behavior [23,24,52]. The parsimonious approach would be to proliferate D2 receptors and regulate electrophysiology of the brain circuitry. Our earlier qEEG studies with KB220Z show this type of regulation in abstinent psycho-stimulant abusers [24] and alcohol and heroin addicts [53]. The role of dopaminergic receptors as key in regulating a variety of RDS behaviors including cocaine and food has been further supported by work of Volkow et al. [54]. Since 1978 we have been developing the concept of nutritional genetics with special emphasis on an amino acid-based enkephalinase approach for the treatment of alcohol [55], cocaine [56], opiates [57], glucose [58], and other addictions [59], pointing out the importance of dietary supplementation and the addictive brain which has served as a basis for the current experiment.

Conclusion

Due to the pilot nature of this investigation, definitive interpretation must await larger triple-blinded, randomized, placebo-controlled crossover studies. Moreover, studies must

include cue-induced *fMRI* measurements to build an objective understanding of the mechanisms for the anti-craving actions of KB220Z, in support of 27 clinical trials confirming its effects [22,23]. Most importantly, we are repeating this experiment in animal models particularly the evaluation of functional *rsfMRI* in rats utilizing a novel segmented atlas in both non-addicted and cocaine-addicted rodents. KB220Z may have very complex effects in the reward circuitry of the brain. We have not tested the entire mechanism of action as indicated previously. However, we are suggesting that following its interaction at multiple neurotransmitter signaling pathways, there is a putative net release of dopamine [59,60] which in the long term provides benefit in attenuation of RDS behaviors.

Declaration of interest: K Blum is currently the owner of Igene LLC., Synaptamine Inc., and co-owner of RD Solutions LLC, co-owner Victory Nutrition International LLC, and he holds a number of US and foreign patents through these companies. He is currently Chief Scientific Advisor for Domininion Diagnostics, LLC. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

[1] Zhang Y, Tian J, Yuan K, et al. Distinct resting-state brain activities in heroin-dependent individuals. Brain Res 2011;1402:46–53.
[2] Xie C, Li SJ, Shao Y, Fu L, Goveas J, Ye E, et al. Identification of hyperactive intrinsic amygdala network connectivity associated with impulsivity in abstinent heroin addicts. Behav Brain Res 2011;216:639–46.
[3] Wang X, Zhou X, Liao Y, et al. Brain function of heroin addicts after withdrawal. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2011;36:733–8.
[4] Zijlstra F, Booij J, van den Brink W, et al. Striatal dopamine D2 receptor binding and dopamine release during cue-elicited craving in recently abstinent opiate-dependent males. Eur Neuropsychopharmacol 2008;18:262–70.
[5] Suto N, Ecke LE, Wise RA. Control of within-binge cocaine-seeking by dopamine and glutamate in the core of nucleus accumbens. Psychopharmacology (Berl) 2009;205:431–9.
[6] Suto N, Wise RA. Satiating effects of cocaine are controlled by dopamine actions in the nucleus accumbens core. J Neurosci 2011;31:17917–22.
[7] Willuhn I, Burgeno LM, Groblewski PA, et al. Excessive cocaine use results from decreased phasic dopamine signaling in the striatum. Nat Neurosci 2014;17:704–9.
[8] Stewart J. Review. Psychological and neural mechanisms of relapse. Philos Trans R Soc Lond B Biol Sci 2008;363:3147–58.
[9] Caprioli D, Calu D, Shaham Y. Loss of phasic dopamine: a new addiction marker? Nat Neurosci 2014;17:644–6.
[10] Grabowski J, Rhoades H, Stotts A, et al. Agonist-like or antagonist-like treatment for cocaine dependence with methadone for heroin dependence: two double-blind randomized clinical trials. Neuropsychopharmacology 2004;29:969–81.
[11] Dackis CA, Gold MS. Bromocriptine as treatment of cocaine abuse. Lancet 1985;1:1151–2.
[12] Volkow ND. Stimulant medications: how to minimize their reinforcing effects? Am J Psychiatry 2006;163(3):359–61.
[13] Volkow ND, Fowler JS, Wang G-J. The addicted human brain: insights from imaging studies. J Clin Invest 2003;111:1444–51.
[14] Schmidt A, Walter M, Gerber H, et al. Inferior frontal cortex modulation with an acute dose of heroin during cognitive control. Neuropsychopharmacology 2013;38:2231–9.
[15] Yuan Y, Zhu Z, Shi J, et al. Gray matter density negatively correlates with duration of heroin use in young lifetime heroin-dependent individuals. Brain Cogn 2009;71:223–8.
[16] Elman I, Borsook D, Volkow ND. Pain and suicidality: insights from reward and addiction neuroscience. Prog Neurobiol 2013;109:1–27.
[17] Marhe R, Luijten M, van de Wetering BJ, et al. Individual differences in anterior cingulate activation associated with attentional bias predict cocaine use after treatment. Neuropsychopharmacology 2013;38:1085–93.
[18] Thanos PK, Ramalhete RC, Michaelides M, et al. Leptin receptor deficiency is associated with upregulation of cannabinoid 1 receptors in limbic brain regions. Synapse 2008;62:637–42.
[19] Rothman RB, Blough BE, Baumann MH. Dual dopamine/serotonin releasers: potential treatment agents for stimulant addiction. Exp Clin Psychopharmacol 2008;16:458–74.
[20] Blum K, Gardner E, Oscar-Berman M, et al. "Liking" and "wanting" linked to Reward Deficiency Syndrome (RDS): hypothesizing differential responsivity in brain reward circuitry. Curr Pharm Des 2012;18:113–18.
[21] Dahlgren A, Wargelius HL, Berglund KJ, et al. Do alcohol-dependent individuals with DRD2 A1 allele have an increased risk of relapse? A pilot study. Alcohol Alcohol 2011;46:509–13.
[22] Chen TJ, Blum K, Chen AL, et al. Neurogenetics and clinical evidence for the putative activation of the brain reward circuitry by a neuroadaptagen: proposing an addiction candidate gene panel map. J Psychoactive Drugs 2011;43:108–27.
[23] Blum K, Oscar-Berman M, Stuller E, et al. Neurogenetics and nutrigenomics of neuro-nutrient therapy for reward deficiency syndrome (RDS): clinical ramifications as a function of molecular neurobiological mechanisms. J Addict Res Ther 2012;3:139.
[24] Blum K, Chen TJH, Morse S, et al. Overcoming qEEG abnormalities and reward gene deficits during protracted abstinence in male psychostimulant and polydrug abusers utilizing putative dopamine D2 agonist therapy: part 2. Postgrad Med 2010;122:214–26.
[25] Norman KA, Polyn SM, Detre GJ, et al. Beyond mind-reading: multi-voxel pattern analysis of fMRI data. Trends Cogn Sci 2006;10:424–30.
[26] Allefeld C, Haynes JD. Searchlight-based multi-voxel pattern analysis of fMRI by cross-validated MANOVA. Neuroimage 2014;89:345–57.
[27] Kriegeskorte N, Goebel R, Bandettini P. Information-based functional brain mapping. Proc Natl Acad Sci USA 2006;103:3863–8.
[28] Anchordoquy HC, McGeary C, Liu L, et al. Genotyping of three candidate genes after whole-genome preamplification of DNA collected from buccal cells. Behav Genet 2003;33:73–8.
[29] Haberstick BC, Smolen A. Genotyping of three single nucleotide polymorphisms following whole genome preamplification of DNA collected from buccal cells. Behav Genet 2004;34:541–7.
[30] Haberstick BC, Lessem JM, Hopfer CJ, et al. Monoamine oxidase A (MAOA) and antisocial behaviors in the presence of childhood and adolescent maltreatment. Am J Med Genet B Neuropsychiatr Genet 2005;135B:59–64.
[31] Vandenbergh DJ, Persico AM, Hawkins AL, et al. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays a VNTR. Genomics 1992;14:1104–6.
[32] Van Tol HH, Wu CM, Guan HC, et al. Multiple dopamine D4 receptor variants in the human population. Nature 1992;358:149–52.
[33] Lerman C, Caporaso N, Main D, et al. Depression and self-medication with nicotine: the modifying influence of the dopamine D4 receptor gene. Health Psychol 1998;17:56–62.
[34] Sabol SZ, Hu S, Hamer D. A functional polymorphism in the monoamine oxidase A gene promoter. Hum Genet 1998;103:273–9.
[35] Heils A, Teufel A, Petri S, et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621–4.
[36] Guerini FR, Beghi E, Riboldazzi G, et al. BDNF Val66Met polymorphism is associated with cognitive impairment in Italian patients with Parkinson's disease. Eur J Neurol 2009;16:1240–5.

[37] Lesch KP, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527–31.
[38] Hu X, Oroszi G, Chun J, et al. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. Alcohol Clin Exp Res 2005;29:8–16.
[39] Don RH, Cox PT, Wainwright BJ, et al. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res 1991;19:4008.
[40] Pohjalainen T, Rinne JO, Nagren K, et al. The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatry 1998;3:256–60.
[41] Mannisto PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev 1999;51:593–628.
[42] Akil M, Kolachana BS, Rothmond DA, et al. Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. J Neurosci 2003;23:2008–13.
[43] Stice E, Spoor S, Bohon C, et al. Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. Science 2008;322:449–52.
[44] Volkow ND, Wang GJ, Begleiter H, et al. High levels of dopamine D2 receptors in unaffected members of alcoholic families: possible protective factors. Arch Gen Psychiatry 2006;63:999–1008.
[45] Conner BT, Hellemann GS, Ritchie TL, et al. Genetic, personality, and environmental predictors of drug use in adolescents. J Subst Abuse Treat 2010;38:178–90.
[46] Grant S, London ED, Newlin DB, et al. Activation of memory circuits during cue-elicited cocaine craving. Proc Natl Acad Sci USA 1996;93:12040–1205.
[47] Wang Y, Zhu J, Li Q, et al. Altered fronto-striatal and fronto-cerebellar circuits in heroin-dependent individuals: a resting-state fMRI study. PLoS One 2013;8:e58098.
[48] Bell RP, Foxe JJ, Nierenberg J, et al. Assessing white matter integrity as a function of abstinence duration in former cocaine-dependent individuals. Drug Alcohol Depend 2011;114:159–68.
[49] Maksimovskiy AL, McGlinchey RE, Fortier CB, et al. White matter and cognitive changes in veterans diagnosed with alcoholism and PTSD. J Alcohol Drug Depend 2014;2:144–4.
[50] Liu H, Li L, Hao Y, et al. Disrupted white matter integrity in heroin dependence: a controlled study utilizing diffusion tensor imaging. Am J Drug Alcohol Abuse 2008;34:562–75.
[51] Ng GY, George SR. Dopamine receptor agonist reduces ethanol self-administration in the ethanol-preferring C57BL/6J inbred mouse. Eur J Pharmacol 1994;269:365–74.
[52] Blum K, Chen AL, Chen TJ, et al. 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 2008;5:24.
[53] Miller DK, Bowirrat A, Manka M, et al. Acute intravenous synaptamine complex variant KB220™ "normalizes" neurological dysregulation in patients during protracted abstinence from alcohol and opiates as observed using quantitative electroencephalographic and genetic analysis for reward polymorphisms: part 1, pilot study with 2 case reports. Postgrad Med 2010;122:188–213.
[54] Tomasi D, Wang GJ, Wang R, et al. Overlapping patterns of brain activation to food and cocaine cues in cocaine abusers: association to striatal D2/D3 receptors. Hum Brain Mapp 2014;Epub ahead of print.
[55] Blum K, Trachtenberg MC, Elliott CE, et al. Enkephalinase inhibition and precursor amino acid loading improves inpatient treatment of alcohol and polydrug abusers: double-blind placebo-controlled study of the nutritional adjunct SAAVE. Alcohol 1988;5:481–93.
[56] Trachtenberg MC, Blum K. Improvement of cocaine-induced neuromodulator deficits by the neuronutrient Tropamine. J Psychoactive Drugs 1988;20:315–31.
[57] Chen TJ, Blum K, Payte JT, et al. Narcotic antagonists in drug dependence: pilot study showing enhancement of compliance with SYN-10, amino-acid precursors and enkephalinase inhibition therapy. Med Hypotheses 2004;63:538–48.
[58] Blum K, Chen TJ, Meshkin B, et al. Reward deficiency syndrome in obesity: a preliminary cross-sectional trial with a Genotrim variant. Adv Ther 2006;23:1040–51.
[59] Blum K, Chen AL, Giordano J, et al. The addictive brain: all roads lead to dopamine. J Psychoactive Drugs 2012;44:134–43.
[60] Borsten J. Dopamine for Dinner: an easy-to- follow 4 week meal plan to help break the cycle of addiction. Borsten, Los Angeles, CA; 2014; self-published.