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Review Article

Molecular neurological correlates of endorphinergic/dopaminergic mechanisms in reward circuitry linked to endorphinergic deficiency syndrome (EDS)

Kenneth Blum[a,*], David Baron[a], Thomas McLaughlin[b], Mark S. Gold[c]

[a] Graduate School of Biomedical Sciences, Western University Health Sciences, Pomona, CA, United States of America
[b] Center for Psychiatric Medicine, Lawrence, MA, United States of America
[c] Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, United States of America

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ABSTRACT

The consensus of the current literature strongly supports the concept that brain neurotransmitters, and second messengers involved in the net release of dopamine in the mesolimbic region, especially the Nucleus Accumbens (NAc), is directly linked to motivation, anti-stress, incentive salience (wanting), and well-being. The role of dopamine in terms of alcohol withdrawal symptomology, cocaine craving behavior, dopamine –condensation products (TIQs), and more recently, the genetic aspects of drug-seeking and pro-dopamine regulation, provide compelling evidence of the relevant molecular neurological correlates of dopaminergic /endorphinergic mechanisms in reward circuitry due to genetic polymorphisms and epigenetic insults. In the face of an Americans opioid epidemic, the clinical consensus is to treat Opioid Use Disorder (OUD) with life-long opioid substitution therapy. However, the authors suggest a paradigm shift involving novel modalities like targeting the endorphinergic system linked to dopamine release at the NAc, in terms of the induction of required "dopamine homeostasis." Utilizing the known genetic - environmental interaction theorem P = G +E, the authors provide a clear rationale for the adoption of genetic risk testing coupled with endorphinergic/dopamine regulation to address dysfunction across the brain reward circuitry. The goal of altering resting-state, functional connectivity may require a gentle "neurotransmitter fix" vis enkephalinase inhibition to overcome or combat - self-induction of acute dopamine release via psychoactive substance misuse resulting in chronic dopamine down-regulation. As subsets of reward deficiency, we are poised to provide novel, genetically guided therapy for endorphinergic, opioidergic, and dopaminergic deficiencies and related syndromes, utilizing "Precision Addiction Management.

1. Introduction

There are several neurotransmitters involved in the processing of reward and punishment. These pathways involve at least six major neurotransmitters and many second messengers, linked to the mesolimbic and Pre-Frontal Cortex (PFC). One function is to regulate the final pathway of "wanting," causing net neuronal dopamine release [1]. Fig. 1 provides a schematic representation of the Brain Reward Cascade (BRC) showing the interaction of serotonergic, cannabinoidergic, opioidergic, GABAergic, glutaminergic, and dopaminergic systems related to net dopamine release at the Nucleus Accumbens (NAc). In this article, the authors highlight dopamine with the understanding that healthy processing of an initial action potential in the brain requires the integrity of the entire neurotransmitter complex of the brain reward circuitry. The cascading interactions result in the balanced release of dopamine at not only the NAc but across many brain regions. These regions are involved in motivation, cognition (memory), pleasure, stress reduction, decision-making, recall, drug reinstatement, cravings, and well-being. The result is to provide *Homo sapiens* with a healthy happiness set –point as well as resting-state functional connectivity [2].

Fig. 1 illustrates the interaction of at least six major neurotransmitter pathways involved in the Brain Reward Cascade (BRC). In the hypothalamus, environmental stimulation causes the release of serotonin, which in turn via, for example, 5HT-2a receptors activate (the green, equal sign) the subsequent release of opioid peptides also in the hypothalamus. Then, in turn, the opioid peptides have two distinct effects, possibly via two different opioid receptors. A) inhibits (the red hash sign) through the mu-opioid receptor (possibly via enkephalin) and projecting to the Substania Nigra to GABAA neurons. B) stimulates (the green, equal sign) Cannabinoid neurons (e.g., Anandamide and 2-

* Corresponding author.
E-mail address: drd2gene@gmail.com (K. Blum).

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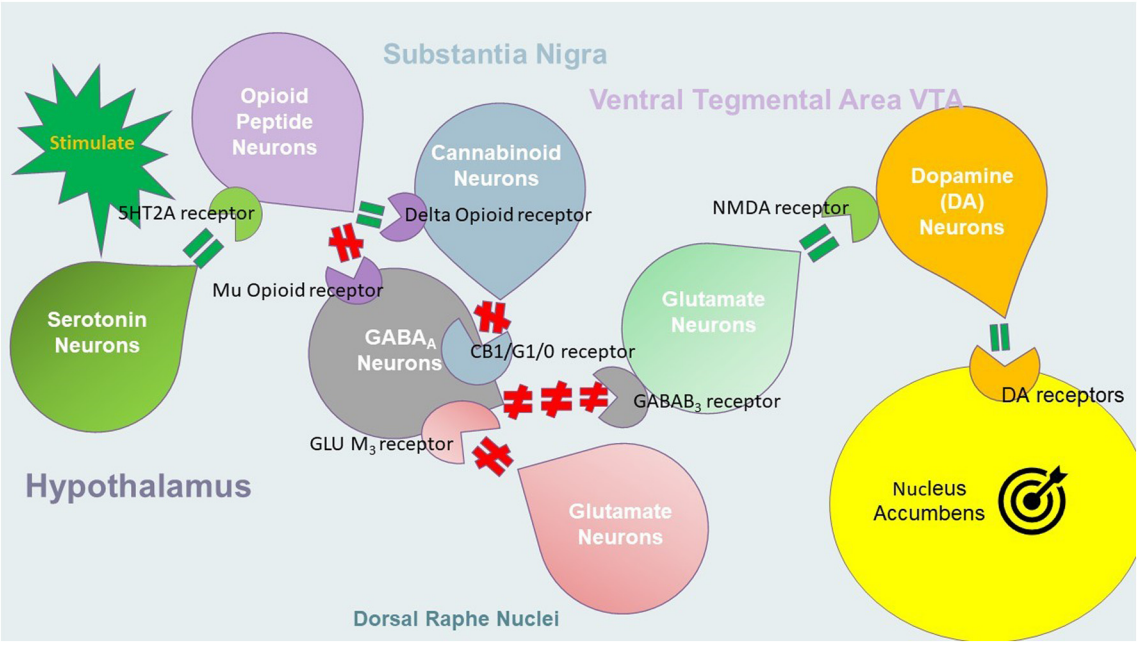

Fig. 1. Fig. 1 illustrates the interaction of at least six major neurotransmitter pathways involved in the Brain Reward Cascade (BRC). In the hypothalamus, environmental stimulation causes the release of serotonin, which in turn via, for example, 5HT-2a receptors activate (the green, equal sign) the subsequent release of opioid peptides also in the hypothalamus. Then, in turn, the opioid peptides have two distinct effects, possibly via two different opioid receptors. A) inhibits (the red hash sign) through the mu-opioid receptor (possibly via enkephalin) and projecting to the Substania Nigra to GABAA neurons. B) stimulates (the green, equal sign) Cannabinoid neurons (e.g., Anandamide and 2-archydonoglcerol) through Beta –Endorphin linked delta receptors, which in turn inhibit GABAA neurons at the substania nigra. Cannabinoids primarily 2-archydonoglcerol, when activated, can also indirectly disinhibit (the red hash sign) GABAA neurons in the Substania Nigra through activation of G1/0 coupled to CB1 receptors. Similarly, Glutamate neurons located in the Dorsal Raphe Nuclei (DRN) can indirectly disinhibit GABAA neurons in the Substania Nigra through activation of GLU M3 receptors (the red hash sign). GABAA neurons, when stimulated, will, in turn, powerfully (the red hash signs) inhibit Ventral Tegmental Area (VTA) glutaminergic drive via GABAB 3 neurons. Finally, Glutamate neurons in the VTA will project to dopamine neurons through NMDA receptors (the green, equal sign) to preferentially release dopamine at the NAc shown as a bullseye indicating euphoria, (Blum with permission Activates 〓; Blocks ≠. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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2. Neurological dopaminergic function from a historical perspective

Dopamine (DA 3,4-dihydroxyphenethylamine) named after the precursor levodopa, L-DOPA, is formed in the brain and kidneys. Dopamine is a catecholamine from the phenethylamine family. The brain possesses several distinct dopaminergic pathways, including those involving motor control, the release of various hormones, and neurotransmission.

Popular culture and the media often depict dopamine as the main chemical of pleasure; however, based on animal research, the current opinion is that dopamine instead confers motivational salience [1,2]. Dopamine plays a principal role in behavioral motivation. Dopamine release indicates motivational prominence, the desirability or aversiveness of an outcome, and effects the behavior toward or away from achieving that outcome [3]. The dopamine level in the brain increases with anticipation of most types of reward, and many known addictive drugs, like opioids, acutely increase dopamine release or block its reuptake into neurons following release. However, chronically, the reverse is accurate, and, in fact, in these cases, dopamine function is downregulated [4–7]. Relevant, informative reviews in by Uhl, Koob, and Cable [6], and Elman, et al. [5] support of these concepts.

Dopamine was first synthesized by George Berger and James Ewens at Wellcome Laboratories in London, England [8] in 1910 long before 1957 when Kathleen Montagu located it in the human brain. Dopamine's function as a neurotransmitter was recognized one year later by Arvid Carlsson and Nils-Ake Hillarp. Carlsson, Paul Greengard, and Eric Kandel received the 2000 Nobel Prize in Physiology or Medicine for discoveries concerning nervous system signal transduction of dopamine and its involvement in learning and memory. In humans, dopamine binds to and activates cell surface receptors. Dopamine has a high binding affinity for dopamine receptor subtypes D1 to D5 and human trace amine-associated receptor 1 (hTAAR1) [9,10]. Dopamine also functions as metabotropic, G protein-coupled (guanine nucleotide-binding protein). When dopamine is bound to the receptors, they exert cellular responses via a complex second messenger system [11]; that detects molecules outside the cell and activates internal signal transduction pathways.

3. Dopamine and reward (a snapshot)

Rewarding events activate substantia nigra and VTA dopamine neurons, as seen on neuronal signal recordings from animal brains [12]. These reward-responsive dopamine neurons are crucial for reward-related cognition and serve as the central component of the reward system. Dopamine function is different in each axonal projection. For example, shell projections from the VTA and substantia nigra assign

incentive salience ("want") to cues for rewarding stimuli, while projections from the orbitofrontal cortex of the VTA evaluate different behavioral goals according to incentive importance. Projections from the amygdala and hippocampal VTA mediate the consolidation of reward-related memories, and both the VTA–NAc core and substantia nigra– dorsal striatum pathways are involved in learning motor responses that facilitate the acquisition of rewarding stimuli. Additionally, some activity within the VTA dopaminergic projections appears to be associated with reward prediction [13–15].

While dopamine is central to "wanting", it has also associated with the appetitive or approach behavioral responses to rewarding stimuli reflected in the consummatory behavioral response, and many animal experiments reveal that dopamine does not equate to hedonic "liking" or pleasure. There is a controversy regarding the involvement of dopamine in pleasure and reward that has led to confusion about how to separate motivation from pleasure as well as "wanting" versus "liking." Sousa et al. [16] provided evidence that the brain system appears to have evolved differently in humans than in apes. They reported that animal studies are not the same as clinical information described by self-reports in humans. Their findings also suggest that relying entirely on extrapolations from rodent and non-human primate studies is not supported by the data. When it comes to the concepts of pleasure, dopamine, and reinforcement, human experience compared to animal data is most important.

Nevertheless, animal data has value, and the use of animal models must be encouraged, together with a caution against the interpretation of results, without leaping to conclusions that may be explained by data from follow-up human experiments [16,17]. A clinical study in January 2019 assessed the effect of the dopamine precursor (L-DOPA), antagonist (risperidone), and placebo on reward responses to music. Ferreri et al. [18] measured changes in electrodermal activity to assess the degree of pleasure experienced during musical chills, as well as subjective ratings. They found that the manipulation of dopamine neurotransmission regulates pleasure cognition (specifically, the hedonic impact of music) bidirectionally in human subjects. The authors suggest that increased dopamine neurotransmission acts as a sine qua non-condition for pleasurable hedonic reactions to music in humans [18].

4. Dopamine and addictive behavior

Addiction is a disordered brain reward system acquired through epigenetic mechanisms and chronic transcriptional mRNA expression. Chronic exposure to addictive stimulus results in uncontrollable engagement with rewarding stimuli regardless of adverse consequences. Natural stimuli like food and exercise, as well as high-thrill behaviors, like gaming and gambling, and substances, like opioids, all induce neuroplasticity [5,19–21].

Recognition of the role of DNA polymorphisms, especially as it is related to dopaminergic function, is of equal importance [22,23]. Treating tertiary drug-induced chronic symptomology is short-sighted at best when compared to the consideration of newer epidemiological targets for long-term prophylaxis linked to early interventions. For example, Delta FosB (ΔFosB), a gene transcription factor, is a central mediator in the epigenetic development of all drug and non-drug addictions [24–27]. Based on an intensive literature review, ΔFosB modulates compulsivity during the acquisition of addiction [28,29]. The overexpression of ΔFosB in the D1 type medium spiny neurons of the nucleus accumbens regulates drug self-administration and reward sensitization, directly, through positive reinforcement while attenuating sensitivity to aversion [30].

5. Identification of neurotransmitters and their function

In 1921, an Austrian scientist named Otto Loewi discovered the first important neurotransmitter acetylcholine (ACH) and other primary neurotransmitters, such as serotonin, glutamine, enkephalins, and dopamine followed. Their neurological functions discovered in the 20th century, with the opioid-like receptors identified as late as the mid- 70s [31] while the potential role of serotonin in alcohol intake first discovered by Myers group identified the late 60s was one of the seminal findings embraced by the new field of neuroscience [32]. Blum reported on the effects of dopamine and other catecholamines on neuromuscular transmission in 1969. This finding provided some early evidence for the role of dopamine and tremors in Parkinson's disease [33].

6. Endorphinergic correlates of reward processing

Endorphins were discovered in 1974 by two independent groups of investigators. Hughes & Kosterlitz isolated enkephalins from pig brain [34], and Eric Simon discovered opioid receptors in vertebral brains [35]. Endorphins are endogenous opioid neuropeptides produced by the central nervous system (CNS) and the pituitary glands of both animals and *Homo sapiens*. Importantly, these peptides are classified into three categories— enkephalins, endorphins, and dynorphins—derived from three distinct precursor molecules (Pro -enkephalin A, Pro-Endorphin, and Pro-dynorphin or Pro- enkephalin B). The functional and active opioid peptides are generated from brain born precursors by enzymes named peptidases. These peptidases cleave (cut) specific precursor molecules into smaller molecules. Endorphins such as Beta-endorphin (containing 91 amino-acids) and smaller molecules such as leu-enkephalin and met-enkephalin that consist of only five amino-acids (pentapeptides). While there are alpha, delta, sigma and gamma type endorphins with selective functions in the brain and peripheral tissues derived from larger known peptides, like POMC [285 amino –acids], there are also other ligands known as Dynorphins; Dynorphin A, B, and α/β neoendorphin, derived from the larger molecule synthesized in the brain. The Dynorphins are primary ligands for the Kappa opioid receptor, but they do have a weak affinity for other opioid receptors as well, including delta and mu [36]. The class of Endorphins includes three compounds—alpha endorphins, beta-endorphins, and gamma endorphins—which preferentially bind to opioid receptors [37]. Inhibition of pain signaling is the Endorphins' primarily function. They also produce a feeling of euphoria very similar to those produced by other opioids via dopamine release [38]. Various human activities trigger the production of endorphins. Examples are laughter known to stimulate endorphin production increased resistance to pain [39], and β-endorphin released, during vigorous aerobic exercise [40,41]. Indeed, many pleasurable activities, including eating food and chocolate, which contain tetrahydroisoquinoline (TIQ), and sex, orgasm, yoga, meditation, and listening to music, cause endorphin release [41]. Notably, studies have also demonstrated that human and diverse animal tissues are capable of producing morphine, which is not a peptide [42].

Enkephalins in the brain are in high concentrations, and this polypeptide is linked to brain functioning during a response to stress, especially in the hippocampus and prefrontal cortex. Stressors impact neuropeptides, and their action in metabotropic in specific brain regions [43]. Finally, enkephalin receptors are G-protein-coupled, including delta-opioid and mu receptors. Other endogenous opioid ligands such as dynorphins bind to kappa receptors, and endorphins per se bind to mu receptors [44].

Within the mesolimbic reward system, enkephalins inhibit the release of the neurotransmitter GABA when they bind to the μ-receptor and increase the production and release of dopamine [45].

This brief review of the identification of the neurotransmitters and their function in the brain reward circuitry, emphasizes that genetic risk for all addictive behaviors substance and non-substance, posed by faulty neurotransmission attributable to genetic polymorphisms and termed Reward Deficiency Syndrome (RDS) [10,23,46]. Table 1 lists the addictive behaviors that have identified as RDS behaviors.

Table 1
Reward Deficiency Syndrome behaviors.

Addictive behaviors		Impulsive behaviors		Obsessive compulsive behaviors	Personality disorders
Substance-related	Non-substance related	Spectrum disorders	Disruptive impulsive		
Alcohol	Thrill-seeking (novelty)	Attention-deficit Hyperactivity	Anti-social	Body Dysmorphic	Paranoid
Cannabis	Sexual Sadism	Tourette and Tic Syndrome	Conduct	Hoarding	Schizoid
Opioids	Sexual Masochism	Autism	Intermittent Explosive	Trichotillomania (hair-pulling)	Borderline
Sedatives/Hypnotics	Hypersexual		Oppositional Defiant	Excoriation (skin-picking)	Schizotypal
Stimulants	Gambling		Exhibitionistic	Non-suicidal self-Injury	Histrionic
Tobacco	Internet gaming				Narcissistic
Glucose					Avoidant
Food					Dependent

7. Unlocking neurological underpinnings and emphasizing treating the root cause of addiction instead of prolonging addiction for a lifetime

"Recovery" and "The 12-Step Program and Fellowship" became household names in the early 1980s [47]. The American Society of Addiction Medicine (ASAM) introduced a new definition of "addiction" as a brain disorder in 2011 [48], acceptance of this idea is now well-established and has had a positive impact on basic research [49]. Blum's work with Ernest P. Noble, the discovery of the first gene to associate with severe alcoholism, lead to the current field of "Psychiatric Genetics" [50]. Now in the 21st-century addiction science through the era of genomic medicine is poised to begin to comprehend the true nature of this brain disorder, "Reward Deficiency Syndrome" [10]. As a result of these many years of research and reflection, there are several examples of progress briefly reviewed here. These areas include an understanding of:

- the neurochemical mechanisms involved in the addiction process including withdrawal symptomatology,
- the physiological basis for brain neurotransmission, and the neurochemical mechanisms for synaptic function,
- the mechanisms of release, catabolism, and storage of neurotransmitters in pre and postsynaptic loci,
- the role of long-term potentiation in drug self- administration and sensitization,
- the "Brain Reward Cascade" and its role in the happiness set –point and in craving behavior and relapse,
- and the role of neurogenetics in all aspects of drug-seeking and process addictions.

Has current research caught up with this progress? Regarding this question, it seems reasonable based on well-known physiological mechanisms that the addiction medicine community should not "toss the baby out with the bathwater [51]." The brain disorder known as RDS is very complicated. The difficulty several Genome-Wide Association Studies (GWAS) studies have in finding large, significant associations with various genes may be due to the disorder being polygenic, and most importantly, the flawed utilization of seemingly inappropriate controls. For consideration, is the proposition that; the actual phenotype of "addiction" includes several subtypes of RDS [see Table 1], including for example serotonergic, cannabinoidergic, endorphinergic, opioidergic, glutaminergic, and dopaminergic deficiency syndromes.

Assuming that the RDS phenotype proposition is correct, a rigorous screening of controls for RDS subtypes should be carried out before any genetic analysis, candidate, or GWAS. The concern is that having the disease as part of the controls will only lead to spurious and useless results [52]. This experimental question may take years to dissect.

8. The Genetic Addiction Risk Score (GARS) test

Turning our attention to the clinical management of these polymorphic risk alleles, whether neurochemically hyperdopaminergic or hypodopaminergic induction of neurotransmitter homeostasis, seems a reasonable treatment goal. Patients (especially when young) mandated to attend a treatment center (due to court, family, and friend intervention) may be in denial regarding their ongoing behavioral issues. The non-invasive Genetic Addiction Risk Score (GARS) [53] is a polymorphic genetic panel that enables stratification of genetic risk in an individual. In essence, it is a "mirror to brain reward function" that can reduce guessing, and assist in the resolution of denial, guilt, and shame, by demonstrating a genetic predisposition to drug use. Also, there are other clinical benefits of GARS testing. These benefits include medical monitoring for the pharmacogenetic responses of a drug, metabolic issues related to drug delivery, tailored customized treatment (Pro-dopamine regulation with the nutraceutical, KB220); and medical necessity for the type of clinical care, which involves the identification of risk for RDS behaviors, and pharmacogenomic treatment targeting gene polymorphisms. Another substantial benefit is that family curiosity concerning the coupling of GARS and pro-dopamine regulation, enhanced family willingness to participate in the patient's recovery plan [54]. Adding the genetic test together with drug urine testing in order to evaluate compliance to FDA approved treatment medications, such as Suboxone and methadone and the use of the Comprehensive Analysis of Reported Drugs (CARD) to document abstinence from illicit, psychoactive and addicting drugs, could be a valuable part of the treatment plan for Substance Use Disorder (SUD).

Specifically, developing an organized treatment approach involving the above material, concerning, for example, Opioid Deficiency Syndrome (ODS), seems most productive. Firstly, comprehension of the BRC (Fig. 1) facilitates the proposition that the net release of dopamine at the NAc requires interactive neurotransmitter function at the mesolimbic reward system of the brain. Any interference with this process will result in a lack of prerequisite neurotransmitter interaction from the hypothalamus and proceeding to raphe nuclei the substania nigra and, then, to the VTA and NAc. A disturbance within the BRC may subsequently result in an attenuation of dopamine release causing dopamine deficiency and be the result of either genetic [53] or epigenetic insult [55], linking methylation of dopamine D2 receptors to a lifetime of addictive behaviors. Relevant, informative reviews in by Uhl, Koob, and Cable [6], and Elman, et al. [5] support of these concepts.

This proposal can be illustrated by briefly pinpointing the various BRC disturbances caused by polymorphisms associated with select risk alleles measured in the GARS test. These genetic polymorphisms, therefore, promote RDS. A literature search involving case-control studies reveals a significant association between these gene functions and risk alleles (see Table 2).

The authors argue that appropriately addressing neurotransmitter deficiencies (opioid /dopaminergic) in the field if adopted, would eliminate many unwanted clinical sequelae and also reduce confusion regarding the role of genetics in addiction.

Based on previous literature results, while not representative of all association studies known to date, this sampling of case-control studies displays significant associations between the risk for alcohol and drug

Table 2
Gene functions with the risk allele used in the (GARS) test panel.

Gene	Allele	Prime function
Dopamine D1 receptor	48G	Regulation of Dopamine Release in Accumbens
Dopamine D2 receptor (ANKKI/DRD2)	Taq I A1	Controls Synthesis of Dopamine D2 Receptors
Dopamine D3 receptor (DRD3)	C	Carriers sensitive to cocaine; opioids, alcohol, and nicotine
Dopamine D4 receptor (DRD4)	7R	Pre-disposed to Novelty Seeking and ADHD
Dopamine transporter (DAT1)	9R	Fast transport of synaptic Dopamine back into pre-neuron leading to Hypodopaminergic trait.
Serotonin transporter (HTTLPR)	S	Fast transport of serotonin back into the neuron
Mu-opiate receptor (OPRM1)	G	Predisposes to heroin addiction and pain sensitivity
GABA –B_3 receptor (GABAR3)	181.	Predisposes to anxiety disorders
Mono-amine –oxidase A (MAO-uVNTR)	3R	Fast catabolism of mitochondria Dopamine
Catecholamine –Methyltransferase (COMT-vall58met)	G	Val substitution leads to fast catabolism of synaptic Dopamine leading to RDS

Table 3
The number of studied in PUBMED for each ingredient of KB220, as of January 12th, 2020.

KB220 ingredients	Number studies PUBMED
d-phenylalanine	683
l-phenylalanine	88,912
l-Tyrosine	239,717
5-hydroxytryptophan	87
Chromium salts	38,948
L-Glutamine	42,930
N-Acetylcysteine	20,687
Rhodiola Rosa	907
Passionflower	769
Nicotinamide adenine dinucleotide	65,234
COQ10	4820
Thiamin	18,127
Pyridoxine –Phosphate (B6)	20,182
Thirteen ingredients total	542,003

addiction. Several meta-analyses consisting of as many as 110,241 cases and 122,525 controls reveal significant associations between alcohol and drug addiction risk alleles.

In terms of understanding the neurological mechanisms involved in enkephalinergic and opioidergic deficiency, the role of both endogenous opioids and opioid type receptors deserves attention. For example, Margolis et al. [56] point out that the VTA plays a central role in both motivation and reinforcement. Known Kappa and mu-opioid receptor (KOP-R and MOP-R) agonists microinjected into the VTA induce powerful and opposing motivational actions. Glutamate transmission within the VTA contributes to these motivational effects. Mu opioid receptor (MOR) agonists not only produce pain relief but have reward effects that link to mesolimbic dopaminergic pathways [56,57].

Moreover, Margolis et al. [58] found that the delta-opioid receptor (DOR) activity in the VTA attenuates alcohol drinking in rats. This effect, as expected, was blocked by the GABA(A) antagonist bicuculline possibly, because reduced inhibitory control of GABA enables the augmented release of dopamine at the NAc. Similarly, Mitchell et al. [59] showed that, as expected, acute alcohol drinking even in heavy drinkers induced opioid release in the NAc and orbitofrontal cortex (OFC), both reward areas of the brain. On the other hand, chronic alcohol consumption should result in opposite effects, including the downregulation of endorphin synthesis and subsequent endorphin deficiency [60]. Hjelmstad et al. [61] have shown that opioids induce attenuation of GABAergic input onto VTA neurons, thereby inducing neuronal dopamine release at the NAc. As a result, endogenous or exogenous opioids can activate VTA neurons, including DA neurons, as observed with optogenetics. Using the selective mu-opioid receptor radioligand [^{11}C] Carfentanil, Mitchell et al., [62] also found that following acute alcohol consumption, subjects with the COMT Val158 allele have a greater opioid release in the right NAc but less release in medial OFC suggesting genetic regulation of dopamine via endorphinergic release at the reward site. In terms of pain relief, Navratilova et al. [63] found that pharmacological activation of right anterior cingulate gyrus (ACG) opioid receptors of injured, but not pain-free, animals, stimulated dopamine release in the NAc and produced condition place preference. Thus, endorphin signaling in the ACG, an area of decision –making and relapse, is important for analgesia.

Another area of research involves the Lateral Habenula (LHb) neurons known to correlate with aversive states such as pain, opioid abstinence, rodent models of depression, and failure to receive a predicted reward. Margolis and Fields [64] found that the opioid agonist DAMGO paradoxically inhibits both glutamate and GABA release in rodents. They suggest that the behavioral effects of MOR activation depend on intrinsic LHb neuronal activity, and the balance of activity in glutamate and GABA inputs to predict pain response. Research from opioid psychopharmacological experiments in pre-clinical animal models, provide several induced behavioral phenotypes observed in human OUD including vulnerability to stressors [65], decreased motivation for natural reward [66], reduced learning and memory tasks [67], social withdrawal [68], withdrawal symptoms [69] anxiety, and depression-like behaviors [70].

Also, the excitability of glutamatergic neurons projecting to the accumbens can regulate opioid use and seeking [71]. Specifically, chronic morphine treatment augments firing frequency in glutamatergic afferents from the amygdala, but not the PFC. To better understand the role of glutaminergic activity in OUD, studies have revealed that there are two primary subtypes of medium spiny neurons (MSNs) in the NAc that express both D1 and D2 –dopamine receptors [72]. Careful analysis of these and other studies [73,74], reveal that plasticity in excitatory transmission within NAc onto D1-MSNs connects with enhanced opioid seeking [60]. In contrast, similar plasticity and signaling at D2-MSNs trigger extinction-related behaviors [75]. Therefore, it follows that within the NAc shell, increased glutamate release may occur at D1-MSN, whereas attenuated glutamate release may occur at D2-MSN synapses after chronic morphine [76].

Moreover, morphine withdrawal enhances c-Fos mRNA expression in paraventricular thalamic (PVT) projections to medial NAc-shell [77] specific to D2-MSNs. However, in MOR - null transgenic mouse, reinstatement of MOR in striatal D1 MSNs restores opioid (morphine) induced loss of Conditioned Place Preference [78]. Based on these and other research [78], activation of the glutaminergic drive at the VTA via administration of N-Acetylcysteine, seems a focal target to help regulate dopamine release in RDS [79,80]. Since endorphinergic deficiency impacts RDS, the potential use of enkephalinase inhibitors to raise endorphins at the mesolimbic and PFC brain regions seems reasonable. In genetically bred alcohol-loving C57J mice, having a low level of brain methionine enkephalin and compared to DBA mice, enkephalinase inhibition with D-phenylalanine resulted in enhanced levels of endorphins in pituitary and striatum with concomitantly reduced alcohol consumption [80].

Table 4
The evidentiary basis of each ingredient from basic and clinical studies.

Ingredient *Major neurotransmitter* Neurological Effect	Seminal evolutionary finding	Major clinical benefit in KB220	References
D-phenalalanine *Opioid Peptides (enkephalins)* Inhibits the carboxylase enzyme enkephalinase to prevent opioid peptide catabolism	Shown to inhibit the degradation of enkephalins. Enkephalinase inhibitors play a role in human "endorphin deficiency diseases" (i.e., convulsive disorders; opioid withdrawal)[1] Methionine- and leucine-enkephalin produce mild and transient analgesic effects, presumably because of enzymatic degradation. D-phenylalanine reduced significantly the analgesic dose of electro-acupuncture.[2] Combined treatment with D-amino acids and electro-acupuncture produces greater analgesia than either treatment alone; naloxone reverses these effects.[3]	[4]This is the first report of *d*-phenylalanine alteration in alcohol intake in mice with a genetic predisposition, also showing an increase in brain enkephalins. [5] A negative correlation between the amount of ethanol (10%) consumed and endogenous levels of brain [Met]enkephalin in C57BL/6 J (alcohol-preferring) and DBA/2 J (alcohol-non-preferring) inbred mice strains. Higher levels of brain enkephalin low alcohol intake.	[1]Ehrenpreis S. D-phenylalanine and other enkephalinase inhibitors as pharmacological agents: implications for some important therapeutic application. Acupunct Electrother Res. 1982;7(2–3):157–72; [2]Bodnar RJ, Lattner M, Wallace MM. Antagonism of stress-induced analgesia by D-phenylalanine, an anti-enkephalinase. Pharmacol Biochem Behav. 1980;13(6):829–33; [3]Cheng RS, Pomeranz B. A combined treatment with D-amino acids and electroacupuncture produces greater analgesia than either treatment alone; naloxone reverses these effects. Pain. 1980;8(2):231–6. [4]Blum K, Briggs AH, Trachtenberg MC, Delallo L, Wallace JE. Enkephalinase inhibition: regulation of ethanol intake in genetically predisposed mice. Alcohol. 1987;4(6):449–56. [5]Blum K, Elston SF, DeLallo L, Briggs AH, Wallace JE. Ethanol acceptance as a function of genotype amounts of brain [Met]enkephalin. Proc Natl Acad Sci U S A. 1983;80(21):6510–2.
L- Phenylalanine *Synthesis of dopamine* Precursor aminoacid to dopamine	The intake of protein containing *l*-phenylalanine at approximately 20% increase brain dopamine levels[6] L-phenylalanine is converted to L-tyrosine in the liver. L-tyrosine can cross the blood-brain barrier, where it is then used for catecholamine synthesis[7].	[8]Both L-DOPA (downstream l-Phenylalanine -converted) and intracranially injected dopamine resulted in attenuation of ethanol-induced withdrawal convulsion scores, whereas, haloperidol, a known dopaminergic blocker was found to increase convulsion scores significantly.	[6]Wurtman RJ, Fernstrom JD. Control of brain monoamine synthesis by diet and plasma amino acids. Am J Clin Nutr. 1975;28(6):638–47; [7]Kuhar, M. J., Couceyro, P. R., & Lambert, P. D. In Siegel GJ, Agranoff BW, Albers RW, et al., editors. Biosynthesis of Catecholamines. In Basic Neurochemistry: Molecular, Cellular, and Medical Aspects. (6th ed.). Philadelphia Lippincott-Raven; 1999. [8] Blum K, Eubanks JD, Wallace JE, Schwertner HA. Suppression of ethanol withdrawal by dopamine. Experientia. 1976; 15;32(4):493–5.
L-Tyrosine *Rate limiting step in the synthesis of dopamine.* The rates at which monoaminergic neurons in rat brains synthesize their neurotransmitters depend on the availability of the amino acid precursors such as tyrosine (for dopamine and norepinephrine).	[9]Single injections of l-tyrosine increase brain tyrosine levels and accelerate brain catechol synthesis, while injections of a competing neutral amino acid (e.g., leucine, tryptophan) reduce brain tyrosine and its rate of conversion to dopa; [10] Depletion of tyrosine via Phe/Tyr[−] decreases phasic dopamine transmission, providing insight into the mechanism by which this method modifies dopamine-dependent behaviors in humans; [11]The tyrosine-protein kinase FYN has an important role in synaptic plasticity, learning, and memory; [12]It is accepted that the dopaminergic nigrostriatal system, which projects from the substantia nigra to the dorsal striatum, has a crucial role in the habitual and compulsive nature of alcohol addiction. [13]The FYN signaling cascade in the dorsal-medial-striatum (DMS) promotes synaptic and structural plasticity, which, in turn, induce and maintain excessive alcohol consumption. [15]In agreement with dopamine agonist therapy is the work of Maguire et al. showing the interactive role of D1 and D2 receptor activation in affecting synaptic GABAA receptors within the NAc altering GABA's inhibition of medium spiny neurons subsequently	[14]Blum's laboratory extensively published on the concept of dopamine agonist therapy (l-tyrosine) to treat iatrogenic opioids and even prevent RDS behaviors. [15] In agreement with dopamine agonist therapy is the work of Maguire et al., showing the interactive role of D1 and D2 receptor activation in affecting synaptic GABAA receptors within the NAc altering GABA's inhibition of medium spiny neurons subsequently influencing behavioral responses to cocaine. [16] Czoty and Nader showed the differences between a low-dopamine agonist compared to a high-dopamine agonist in terms of blocking food-cocaine choice in socially housed male cynomolgus monkeys primarily favoring the low agonist compared to the high agonist.	Fernstrom JD. Effects on the diet on brain neurotransmitters. Metabolism. 1977;26(2):207–23.[9] Shnitko TA et al. Acute phenylalanine/tyrosine depletion of phasic dopamine in the rat brain. Psychopharmacology (Berl). 2016;233(11):2045–2054.[10] Wise RA. Roles for nigrostriatal—not just mesocorticolimbic—dopamine in reward and addiction. Trends Neurosci. 2009;32:517–524.[11] Everitt BJ, Robbins TW. From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. Neurosci Biobehav Rev. 2013;37:1946–1954.[12] Wang J et al. Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse. J Neurosci. 2010;30:10187–10,198.[13] Blum K, Oscar-Berman M, Dinubile N, Giordano J, Braverman ER, Truesdell CE, Barh D, Badgaiyan R. Coupling genetic addiction risk score (GARS) with electrotherapy: fighting iatrogenic opioid dependence. J Addict Res Ther. 2013;4(163):100016[14] Maguire EP, Macpherson T, Swinny JD, Dixon CI, Herd MB, Belelli D, Stephens DN, King SL, Lambert JJ. Tonic inhibition of accumbal spiny neurons by extrasynaptic α4βδ GABAA receptors modulates the actions of psychostimulants. J Neurosci. 2014;34(3):823–838[15]. Czoty PW, Nader MA. Effects of dopamine D2/D3 receptor ligands on food-cocaine choice in socially housed male cynomolgus monkeys. J Pharmacol Exp Ther. 2013;344(2):329–338[16].

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Ingredient *Major neurotransmitter* Neurological Effect	Seminal evolutionary finding	Major clinical benefit in KB220	References
	influencing behavioral responses to cocaine.		
L − 5-Hydroxytryptophane *Downstream serotonin precursor synthesis* One known effect of serotonin is memory recall at the hippocampus, and 5-HTP converts to serotonin.	[17] Adjunctive administration of 5-hydroxytryptophan (5-HTP) safely elevates 5-HT_{Ext} beyond the SERT inhibitory effect in humans. [18]Serotonergic innervation of the rat hippocampus plays a major role in the regulation of the excitability of the hippocampus and in behavioral functions associated with this structure. [19] Alleviation of the symptoms of alcohol withdrawal in 20 patients suffering from alcohol addictions starting a detoxification therapy with D-phenylalanine, L-glutamine, and L-5-hydroxytripto-phan occurred 40 days post withdrawal. During the therapy, a significant decrease in SCL-90-R psychiatric symptoms scores and a significant increase in CD4 lymphocyte count was observed in the investigation group. The administration of D-phenylalanine, L-glutamine, and L-5-hydroxy-triptophan alleviates the withdrawal symptoms	[20]Blum's group found in a double-blind evaluation of KB220 in alcoholics and drug abusers compared to control group (a) had a lower BUD (building up to drink) score, 1 vs. 2; (b) required no PRN benzodiazepines, 0% vs. 94%; (c) ceased tremoring at 72 h, as compared to 96 h; and (d) had no severe depression on the MMPI, in contrast to 24% of control group. [21] In order to explore the initiation of detoxification of addictive patients to opiates/opioids (along with some other anti-withdrawal agents), Blum's group developed a protocol to be utilized in treatment centers, particularly with heavily dependent opiate/opioid subjects. Out of 17 subjects, only three received Buprenorphine/Naloxone (Bup/Nx) along with KB220Z allowing for the remaining 82% to be maintained on KB220Z up until 214 days post detoxification.	Jacobsen JPR, Krystal AD, Krishnan KRR, Caron MG. Adjunctive 5-Hydroxytryptophan Slow-Release for Treatment-Resistant Depression: Clinical and Preclinical Rationale. Trends Pharmacol Sci. 2016;37(11):933–944[17] Levkovitz Y, Richter-Levin G, Segal M. Effect of 5-hydroxytryptophan on behavior and hippocampal physiology in young and old rats. Neurobiol Aging. 1994;15(5):635–41[18]. Jukić T, Rojc B, Boben-Bardutzky D, Hafner M, Ihan A. The use of a food supplementation with D-phenylalanine, L-glutamine and L-5-hydroxytriptophan in the alleviation of alcohol withdrawal symptoms. Coll Antropol. 2011 Dec;35(4):1225–30[19]. Blum K, Trachtenberg MC, Ramsay JC. Improvement of inpatient treatment of the alcoholic as a function of neurotransmitter restoration: a pilot study. Int J Addict. 1988;23(9):991–8[20]. Blum K, Whitney D, Fried L, Febo M, Waite RL, Braverman ER, Dushaj K, Li M, Giordano J, Demetrovics Z, Badgaiyan RD. Hypothesizing that a Pro-Dopaminergic Regulator (KB220z™ Liquid Variant) can Induce "Dopamine Homeostasis" and Provide Adjunctive Detoxification Benefits in Opiate/Opioid Dependence. *Clin Med Rev Case Rep*. 2016;3(8):125. doi: 10.23937/2378-3656/1410125.[21]
Chromium salts *Increases serotonin synthesis by causing liver tryptophane to enter the brain.* Chromium directly enhances insulin and serotonergic (5HT) activity and may also have downstream effects on dopaminergic (DA) signaling – all three neurotransmitter (s) share a common protein kinase pathway involved in the central control of food intake and energy homeostasis, and 5HT and DA receptors are linked via functional heterocomplexes	[22]A study by Piotrowska et al. demonstrates the antidepressant-like activity of chromium in the mouse Forced Swim Test (FST) and indicates the major role of the AMPA receptor and participation of NMDA glutamatergic and 5-HT(1) and 5-HT(2A/C) serotonin receptors in this activity. [23]In 110 (Chr =70: Placebo = 40) with atypical depression and high carbohydrate craving, the CrPic group showed significant improvements from baseline compared with the placebo group on 4 HAM-D-29 items: appetite increase, increased eating, carbohydrate craving, and diurnal variation of feelings.	[24]In Kaates et al. study Chr Picolinate significantly improved body composition[24]. [25]In Chen et al., the utilization of CHR picolinate resulted in a significant reduction of body fat ($p < .04$), body weight ($p < 0.o17$), and percent change in weight ($P < .04$). The effect of CHr is a function of the DRD2 genotype. [26] In this long –term study, the product PhenCal containing CHR picolinate, it was found that craving for sugar was significantly reduced compared to a vitamin placebo. The effect was most prominent in families of alcoholism and drug abuse.	Piotrowska A, Młyniec K Siwek A, Dybała M, Opoka W, Poleszak E, Nowak G. Antidepressant-like effect of chromium chloride in the mouse forced swim test: involvement of glutamatergic and serotonergic receptors. Pharmacol Rep. 2008;60(6):991–5[22]. Docherty JP, Sack DA, Finch M, Komorowski JR. A double-blind, placebo-controlled, exploratory trial of chromium picolinate in atypical depression: effect on carbohydrate craving. J Psychiatr Pract. 2005;11(5):302–14[23]. Kaats, G.R., Blum, K., Fisher, J.A., Adelman, J.A. and Wood, R. Effects of chromium picolinate supplementation on body composition: a randomized, double-blind placebo-controlled study. Current Therapeutic Research 57(10),747–756, 1996[25]. Blum, K. Cull, JG. Chen TJH, Garcia-Swan Susan, Holder, JM, Wood, R. Braverman, ER, Buci, LR., & Trachtenberg, MC. Clinical evidence For Effectiveness of Phencal™ in maintaining weight loss in an open-label, controlled, 2-year study. Current Therapeutic Research. 58 (10) 1997[26].
L-Glutamine *Glutamine is the precursor to GABA* γ-Aminobutyric acid (GABA) and glutamate (Glu), major neurotransmitters in the brain, are recycled through glutamine (Gln)27.	[19]Alleviation of the symptoms of alcohol withdrawal in 20 patients suffering from alcohol addictions starting a detoxification therapy with D-phenylalanine, L-glutamine, and L-5-hydroxytriptophan occurred 40 days post withdrawal. During the therapy, a significant decrease in SCL-90-R psychiatric symptoms scores and a significant increase in CD4 lymphocyte count was observed in the investigation group. The administration of D-phenylalanine, L-glutamine, and L-5-hydroxytriptophan N alleviates the withdrawal symptoms.	[29]Neuroadaptations in glutamatergic and GABAergic signaling systems following chronic alcohol exposure play a prominent role in mediating a variety of dependence and withdrawal-related sequelae. [30]Chronic alcohol exposure decreases extrasynaptic $GABA_A$-mediated tonic current recorded from neurons in the hippocampus and cortex, and this corresponds with a decrease in extrasynaptic $GABA_A$ receptors containing the δ subunit in the hippocampus. L-Glutamine administration seems	Jukić T, Rojc B, Boben-Bardutzky D, Hafner M, Ihan A. The use of a food supplementation with D-phenylalanine, L-glutamine and L-5-hydroxytriptophan in the alleviation of alcohol withdrawal symptoms. Coll Antropol. 2011 Dec;35(4):1225–30[19]. Sanaei Nezhad F, Anton A, Michou E, Jung J, Parkes LM, Williams SR. Quantification of GABA, glutamate and glutamine in a single measurement at 3 T using GABA-edited MEGA-PRESS. *NMR Biomed*. 2018 Jan;31(1):e3847. doi: https://doi.org/10.1002/nbm.3847. Epub 2017 Nov 12. PMID: 29130590; PMCID: PMC5765428[27]. Tsai G. Glutamatergic neurotransmission in alcoholism. J Biomed Sci. 1998;5(5):309–320[28]. Lovinger DM, Roberto M (2013) Synaptic effects

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Ingredient *Major neurotransmitter* Neurological Effect	Seminal evolutionary finding	Major clinical benefit in KB220	References
	[28]Neurotransmission at the excitatory synapses through the release of glutamate is now implicated in several neuropsychiatric disorders, including drug addiction.	warranted. [31,32]L-Glutamine treatment reduced the desire for alcohol in heavy drinkers. [33]Tissue content of glutamate was significantly lower in both mPFC and NAc, whereas tissue content of glutamine was higher in mPFC but unchanged in NAc in the EW (ethanol withdrawal) group compared to control group. The tissue content of DA was significantly lower in both mPFC and NAc, whereas tissue content of serotonin was unchanged in both mPFC and NAc. [34] Blum's group proposed Reward Deficiency Solution System (RDSS) that includes: Genetic Addiction Risk Score (GARS), Comprehensive Analysis of Reported Drugs (CARD); and a glutaminergic-dopaminergic optimization complex (Kb220Z). Continued investigation of this novel strategy may lead to a better-targeted approach in the long-term, causing dopamine regulation by balancing the glutaminergic-dopaminergic pathways.	induced by alcohol. Curr Top Behav Neurosci 13:31–86[29]. Fleming RL, Acheson SK, Moore SD, et al. (2011) GABA transport modulates the ethanol sensitivity of tonic inhibition in the rat dentate gyrus. Alcohol; 45:577–583[30]. Rogers ll, Pelton RB. Glutamine in the treatment of alcoholism; a preliminary report. Q j stud alcohol. 1957;18(4):581–7[31]. Roach MK, Williams RJ. Impaired and inadequate glucose metabolism in the brain as an underlying cause of alcoholism–a hypothesis. *Proc Natl Acad Sci U S A*. 1966;56(2):566–71[32]. Das SC, Althobaiti YS, Alshehri FS, Sari Y. Binge ethanol withdrawal: Effects on post-withdrawal ethanol intake, glutamate-glutamine cycle, and monoamine tissue content in P rat model. *Behav Brain Res*. 2016 Apr 15;303:120–5. doi: https://doi.org/10.1016/j.bbr.2016.01.052. Epub 2016 Jan 25. PMID: 26821293; PMCID: PMC4779422[33]. Blum K, Febo M, Fried L, Li M, Dushaj K, Braverman ER, McLaughlin T, Steinberg B, Badgaiyan RD. Hypothesizing That Neuropharmacological and Neuroimaging Studies of Glutaminergic-Dopaminergic Optimization Complex (KB220Z) Are Associated With "Dopamine Homeostasis" in Reward Deficiency Syndrome (RDS). *Subst Use Misuse*. 2017;52(4):535–547.[34]
N -acetylcysteine (NAC) *N-acetylcysteine has shown promise for attenuating both dopamine and glutamate dysregulation.* [35]N-acetylcysteine is effective in reducing craving in substance use disorders, especially for the treatment of cocaine and cannabis use among young people, in addition to preventing relapse in already abstinent individuals.	[36]Fernandes et al. included 5 studies in a systematic review and meta-analysis of double-blind, placebo-controlled trials using *N*-acetylcysteine for depressive symptoms regardless of the main psychiatric condition. Pooled analysis with data from a total of 574 participants (*N*-acetylcysteine: 291, placebo: 283), *N*-acetylcysteine was shown to significantly ameliorate depressive symptoms and improve functionality compared to placebo. [37]Four clinical studies showed NAC's capacity to reduce craving, desire to use cocaine, cocaine-cue viewing-time, and cocaine-related spending. Studies in animal models also support this reinstatement prevention application of NAC. NAC reverses the disruption of glutamate homeostasis caused by long-term cocaine use restoring the function of cystine-glutamate exchanger in glial cells and reversing the downregulated GLT-1 receptor.	[38]NAC also reduced ethanol-seeking behavior (−77%) evaluated as extinction responding in a single extinction session. NAC was able to reduce reacquisition in rats that were abstinent for 17 days, while NAC did not affect ethanol relapse in rats previously exposed to six extinction sessions. Overall, these results demonstrate that NAC limits motivation, seeking behavior and reacquisition in rats, making it a potential new treatment for the maintenance of abstinence. [39]Dependent on dorsolateral striatum (aDLS) dopamine-dependent mechanisms, cue-controlled heroin seeking was disrupted by *N*-acetylcysteine. Comparison with previous data on cocaine suggests that the development of drug-seeking habits and the alteration of corticostriatal glutamate homeostasis, which is restored by *N*-acetylcysteine, are quantitatively similar between heroin and cocaine. [40]The proband is a female with a history of drug abuse and alcoholism. She experienced a car accident under the influence and voluntarily entered treatment. Following an assessment, she was genotyped using the GARS, which divulged a polymorphic matched neuro-nutrient with a KB220Z base. The proband had success in recovery from Substance Use Disorder (SUD) and improvement in socialization, family, economic	Ooi SL, Green R, Pak SC. N-Acetylcysteine for the Treatment of Psychiatric Disorders: A Review of Current Evidence. *Biomed Res Int*. 2018 Oct 22;2018:2469486. doi: https://doi.org/10.1155/2018/2469486. PMID: 30426004; PMCID: PMC6217900[35]. Fernandes B. S., Dean O. M., Dodd S., Malhi G. S., Berk M. N-Acetylcysteine in Depressive Symptoms and Functionality. *Journal of Clinical Psychiatry*. 2016:e457–e466.[36] Nocito Echevarria MA, Andrade Reis T, Ruffo Capatti G, Siciliano Soares V, da Silveira DX, Fidalgo TM. *N*-acetylcysteine for treating cocaine addiction - A systematic review. Psychiatry Res. 2017;251:197–203[37]. Lebourgeois S, González-Marín MC, Jeanblanc J, Naassila M, Vilpoux C. Effect of *N*-acetylcysteine on motivation, seeking and relapse to ethanol self-administration. Addict Biol. 2018;23(2):643–652[38]. Hodebourg R, Murray JE, Fouyssac M, Puaud M, Everitt BJ, Belin D. Heroin seeking becomes dependent on dorsal striatal dopaminergic mechanisms and can be decreased by N-acetylcysteine. *Eur J Neurosci*. 2019 Aug;50(3):2036–2044. doi: 10.1111/ejn.13894. Epub 2018 Mar 30. PMID: 29514413; PMCID: PMC6767855.[39] Fried et al. "Hypodopaminergia and "Precision Behavioral Management" (PBM): It is a Generational Family Affair," Current Pharmaceutical Biotechnology (2020) 21: 1. https://doi.org/10.2174/1389201021666191210112108[40]

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Ingredient *Major neurotransmitter* Neurological Effect	Seminal evolutionary finding	Major clinical benefit in KB220	References
		status, well-being, and attenuation of Major Depression. Proband tested negative over the first two months in treatment and a recent screening. Following approximately two months into the program, probands' parents also decided to take the GARS and started taking the recommended variants. The proband's father (a binge drinker) and mother (no SUD) both showed improvement in various behavioral issues. Finally, the proband's biological children were also GARS tested, showing a high risk for SUD.	
Rhodiola Rosa *Raise the activity of serotonin and dopamine* Putative inhibition of COMT and MAOA activity. *Rhodiola rosea* (*R. rosea*) is a botanical adaptogen with putative anti-stress and antidepressant properties.	[41]Following Rhodiola rosea treatment in a nicotine withdrawal model a significant increase of 5-HT content, with a significant increase of serotonin receptor 1A, suggesting an involvement of serotonin in beneficial effects of R. rosea on suffering produced by nicotine withdrawal. [42]Clinical assessment of R. rosea L. rhizome extracts in humans with various depressive syndromes is based upon results from two randomized, double-blind, placebo-controlled trials of 146 subjects with major depressive disorder and seven open-label studies totaling 714 individuals with stress-induced mild depression (diagnosed as asthenic syndrome or psychoneurosis). Overall, the results of these studies suggest a possible antidepressant action for R. rosea extract in adult humans.	[43]In chronic mild stress-induced depressed rats whereby there is a reduced cellular level of 5-HT, treatment with low –dose of Rhodiola Rosea was found to normalize these serotonin hippocampus levels. [44]Salidroside, a major compound isolated from Rhodiola rosea L., possesses potent anti-oxidative stress properties and protects against DA neuronal death. Results suggest that salidroside protects SN4741 cells and primary cortical neurons from 6-OHDA-induced neurotoxicity by attenuating ER stress. [45] The main difference with older studied variants, and the latest variant, is the inclusion of a proprietary form of Rhodiola rosea, a known catechol-*O*-methyl-transferase inhibitor (COMT) to potentially enhance the activity of presynaptic released dopamine.	Mannucci C, Navarra M Calzavara E, Caputi AP, Calapai G.P Serotonin involvement in Rhodiola rosea attenuation of nicotine withdrawal signs in rats. Phytomedicine. 2012;19(12):1117–24[41]. Amsterdam JD, Panossian AG. Rhodiola rosea L. as a putative botanical antidepressant. Phytomedicine. 2016;23(7):770–83[42]. Chen QG, Zeng YS, Qu ZQ, Tang JY, Qin YJ, Chung P, Wong R, Hägg U. The effects of Rhodiola rosea extract on 5-HT level, cell proliferation, and quantity of neurons at cerebral hippocampus of depressive rats. Phytomedicine. 2009 Sep;16(9):830–8. doi: https://doi.org/10.1016/j.phymed.2009.03.011[43]. Tao K, Wang B, Feng D, Zhang W, Lu F, Lai J, Huang L, Nie T, Yang Q. Salidroside Protects Against 6-Hydroxydopamine-Induced Cytotoxicity by Attenuating ER Stress. Neurosci Bull. 2016;32(1):61–9[44]. Blum K, Chen TJ, Meshkin B, Waite RL, Downs BW, Blum SH, Mengucci JF, Arcuri V, Braverman ER, Palomo T. Manipulation of catechol-*O*-methyltransferase (COMT) activity to influence the attenuation of substance seeking behavior, a subtype of Reward Deficiency Syndrome (RDS), is dependent upon gene polymorphisms: a hypothesis. Med Hypotheses. 2007;69(5):1054–60[45].
Passionflower *Active ingredients stimulate GABA activity* [46]*Passiflora incarnata* L. (Passifloraceae) is important in herbal medicine for treating anxiety or nervousness. The numerous pharmacological effects of Passiflora incarnata are mediated via modulation of the GABA system, including affinity to GABA(A) and GABA(B)receptors, and effects on GABA uptake.	[46]Passiflora incarnata L. shows benefits with Generalized Anxiety Disorder (GAD), symptoms of opiate withdrawal, insomnia, neuralgia, convulsion, spasmodic asthma, ADHD, palpitations, cardiac rhythm abnormalities, hypertension, sexual dysfunction, and menopause.	[47]Jawna-Zboińska et al. observed reduced anxiety and dose-dependent improvement of memory in rats given passionflower compared to the control group. In addition, hippocampal glutamic acid and cortical serotonin content were depleted, with increased levels of metabolites and increased turnover. These results partially confirmed the proposed mechanism of action of *P. incarnata* involving GABAA receptors. [48]A total of 65 opiates addicts were assigned randomly to treatment with passiflora extract plus clonidine tablet or clonidine tablet plus placebo drop during a 14-day double-blind clinical trial. The severity of the opiate withdrawal syndrome was measured on days 0, 1, 2, 3, 4, 7, and 14 using the Short Opiate Withdrawal Scale (SOWS). Both protocols were equally effective in treating the physical symptoms of withdrawal syndromes. However, the passiflora plus clonidine group showed a significant superiority over	Appel K, Rose T, Fiebich B, Kammler T, Hoffmann C, Weiss G. Modulation of the γ-aminobutyric acid (GABA) system by Passiflora incarnata L. Phytother Res. 2011 Jun;25(6):838–43[46]. Jawna-Zboińska K, Blecharz-Klin K, Joniec-Maciejak I, Wawer A, Pyrzanowska J, Piechal A, Mirowska-Guzel D, Widy-Tyszkiewicz E· Passiflora incarnata L. Improves Spatial Memory, Reduces Stress, and Affects Neurotransmission in Rats. Phytother Res. 2016;30(5):781–9[47]. Akhondzadeh S, Kashani L, Mobaseri M, Hosseini SH, Nikzad S, Khani M. Passionflower in the treatment of opiates withdrawal: a double-blind, randomized controlled trial. J Clin Pharm Ther. 2001;26(5):369–73[48]. Dhawan K, Kumar S, Sharma A. Reversal of cannabinoids (delta9-THC) by the benzoflavone moiety from methanol extract of Passiflora incarnata Linneaus in mice: a possible therapy for cannabinoid addiction. J Pharm Pharmacol.2002;54(6):875–81[49]. Blum, K., Trachtenberg, M.C., Elliott, C.E., Dingler, M.L., Sexton, R.L., Samuels, A.I. and Cataldie, L. Enkephalinase inhibition and precursor amino acid loading improves inpatient treatment of alcohol and polydrug abusers: Double-blind placebo-controlled study of the

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		clonidine alone in the management of mental symptoms. These results suggested that passiflora extract may be an effective adjuvant agent in the management of opiate withdrawal. [49]The benzoflavone moiety of P. incarnata, when administered concurrently with delta9-THC, prevented the development of tolerance and dependence of cannabinoids in mice. Even an acute administration of the benzoflavone moiety (20 mg kg one, p.o.) significantly blocked the expression of withdrawal effects in delta9-THC-dependent mice. [50]Blum et al. investigated the effects of KB220 in inpatient, chemically dependent subjects to evaluate the role of neurotransmitters in facilitating recovery and adjustment to a detoxified, sober state, and anxiety. In a double-blind, placebo-controlled, randomized study of 62 alcoholics and polydrug abusers, KB220 patients had a significantly reduced stress response as measured by the skin conductance level (SCL) and significantly improved Physical Scores and BESS Scores (behavioral, emotional, social and spiritual). After detoxification, there was a six-fold decrease in AMA rates when comparing KB220 vs. placebo groups. In this inpatient treatment, experience KB220 facilitated the rate of recovery and allowed patients to respond more fully and more quickly to the behavioral goals of the program, for example, as measured by the BESS Score.	nutritional adjunct SAAVE Alcohol 5:481–493, 1988[50].
Nicotinamide adenine dinucleotide (NADH) *NAD has been studied for its effect on the uptake of serotonin, norepinephrine, dopamine, and GABA by synaptic vesicles.* [50] It has been shown that NAD concentrations do not alter norepinephrine and dopamine uptake while the serotonin uptake is increased, and there is a decrease GABA uptake.	[51]Chronic NAM administered throughout extinction dose-dependently attenuated cue-primed reinstatement in male rats, but not female rats. In contrast, acute NAD given once before reinstatement had no effect on reinstatement.	[52]The nicotinamide adenine dinucleotide (NAD^+)/reduced NAD^+ (NADH) and $NADP^+$/reduced $NADP^+$ (NADPH) redox couples are essential for maintaining cellular redox homeostasis and for modulating numerous biological events, including cellular metabolism. Deficiency or imbalance of these two redox couples has been associated with many pathological disorders. [40,52] Xaio et al. suggested using pharmacological interventions or nutrient-based bioactive NAD^+ precursors as therapeutic interventions for metabolic diseases and RDS.	Kuchmerovskaia TM, Klimenko AP, Parkhomets PK, Donchenko GV. Effect of NAD on uptake and release of some mediators by synaptic vesicles of the rat's brain. Ukr Biokhim Zh (1978). 1993;65(3):66–70[50]. Witt EA, Reissner KJ. The effects of nicotinamide on reinstatement to cocaine seeking in male and female Sprague Dawley rats. Psychopharmacology (Berl). 2019 Dec 7. doi: https://doi.org/10.1007/s00213-019-05404-y[51]. Xiao W, Wang RS, Handy DE, Loscalzo J. NAD(H) and NADP(H) Redox Couples and Cellular Energy Metabolism. Antioxid Redox Signal. 2018;28(3):251–272[52]. Fried et al. "Hypodopaminergia and "Precision Behavioral Management" (PBM): It is a Generational Family Affair," Current Pharmaceutical Biotechnology (2020) 21: 1. https://doi.org/10.2174/1389201021666191210112108[40]
COQ10 *Coenzyme Q10 (CoQ10) is a nutrient that occurs naturally in the body. CoQ10 is also in many foods we eat. CoQ10 acts as an antioxidant, which protects cells from damage and plays an important part in the metabolism.* Coenzyme Q10 (CoQ10) is a natural compound, is involved in the mitochondrial electron transfer chain	[53]Previous studies have shown that depression is accompanied by the induction of inflammatory and oxidative stress pathways and amelioration of antioxidant status. Andalib et al. revealed in a rat depression model targeting mitochondria energy and loss of ATP (causing decline in cognitive function and other psychiatric diseases) that chronic treatment with	[54]There is evidence that CoQ10 may have benefits in reducing oxidative stress in this cohort. [55]Low-resolution electromagnetic tomography (LORETA) was used to evaluate the effects of KB220z on a 72-year-old male with ADHD at baseline and one hour following administration. The resultant z-scores averaged across Eyes Closed, Eyes Open, and Working Memory	Andalib S, Mashhadi-Mousapour M, Bijani S, Hosseini MJ. Coenzyme Q_{10} Alleviated Behavioral Dysfunction and Bioenergetic Function in an Animal Model of Depression. Neurochem Res. 2019;44(5):1182–1191[53]. Verlaet AAJ, Breynaert A, Ceulemans B, De Bruyne T, Fransen E, Pieters L, Savelkoul HFJ, Hermans N. Oxidative stress and immune aberrancies in attention-deficit/hyperactivity disorder (ADHD): a case-control comparison. Eur Child Adolesc Psychiatry. 2019;28(5):719–729[54].

(*continued on next page*)

Table 4 (*continued*)

Ingredient *Major neurotransmitter* Neurological Effect	Seminal evolutionary finding	Major clinical benefit in KB220	References
(ETC), and plays an important pattern in adenosine triphosphate (ATP) production.	CoQ_{10} could reverse the depressive-like behavior and bioenergetic effects.	conditions, increased for each frequency band, in the anterior, dorsal, and posterior cingulate regions, as well as the right dorsolateral prefrontal cortex during Working Memory, with KB220z. These scores are consistent with other human and animal neuroimaging studies that demonstrated increased connectivity volumes in reward circuitry and may offer a new approach to ADHD treatment.	Steinberg B, Blum K, McLaughlin T, Lubar J, Febo M, Braverman ER, Badgaiyan RD. Low-Resolution Electromagnetic Tomography (LORETA) of changed Brain Function Provoked by Pro-Dopamine Regulator (KB220z) in one Adult ADHD case. Open J Clin Med Case Rep. 2016;2(11). pii: 1121[55].
Thiamin B1 *A factor confounded with prolonged heavy alcohol consumption is poor nutrition, and many alcoholics are thiamine deficient.* [56]Data support the emerging theory that subclinical Thiamin Deficiency during chronic heavy alcohol consumption is critical for the development of significant cognitive impairment associated with alcohol-related brain damage (ARBD).	[57]Alcohol consumption has been shown to cause transcription-mediated inhibition of THRT-1 and − 2 expression, decreases in Thiamin Transporter Protein (TDP), reduced liver storage and decreased intestinal absorption	[58] In an experiment, one group of rats was fed a well- balanced diet; another group was fed a diet deficient in vitamin B1. The B1 complex deficient rat chose alcohol over water. The addition of rich yeast-based B1 caused a significant attenuation of alcohol intake.	Vedder, L. C., Hall, J. M., Jabrouin, K. R., & Savage, L. M. (2015). Interactions between chronic ethanol consumption and thiamine deficiency on neural plasticity, spatial memory, and cognitive flexibility. *Alcoholism, clinical and experimental research*, *39*(11), 2143–2153. doi:https://doi.org/10.1111/acer.12859 [56] Hoyumpa AM. Mechanisms of thiamin deficiency in chronic alcoholism, *Am J Clin Nutr*, 1980, 33; 2750–61[57]. Segovia-Riquelme N, Hederra A, Anex M, Barnier O, Figuerola-Camps I, Campos-Hoppe I, Jara N, Mardones J. Nutritional and genetic factors that influence the appetite for alcohol drinking Arch Biol Med Exp (Santiago). 1969;3(Suppl 3):89–96[58].
Pyridoxine –Phosphate B6 *The primary effect of B6 is its catalytic activity in the synthesis of dopamine* [59]Prenatal and postnatal vitamin B-6 undernutrition produces a loss of dopamine (DA) in the corpus striatum of the developing rat brain. The activity of dopa decarboxylase holoenzyme was found to be significantly lower in the corpus striatum of rats fed suboptimal B-6 diets. Measurements of the major metabolites of DA, homovanillic acid (HVA), and 3,4-dihydroxyphenylacetic acid (DOPAC) in rat corpus striatum showed a significant decrease in HVA level in B-6 restricted rats compared to B-6 sufficient groups.	[60]B6 is vital for the synthesis of many neurotransmitters, including GABA, serotonin, dopamine, noradrenaline, histamine, glycine, and D-serine, indicating that vitamin B6 supplementation may enhance many neurotransmitter systems. Thus, vitamin B6 supplementation can treat the impaired neurotransmitter systems in a given patient, even if the actual impaired neurotransmitter systems are not defined in that patient.	[61]These results suggest that pyridoxine promotes hippocampal functions by increasing serotonin and tyrosine hydroxylase immunoreactivity in the hippocampus. This positive effect may be associated with CRIP1a and CB1 cannabinoid receptor function.	Guilarte TR. Effect of vitamin B-6 nutrition on the levels of dopamine, dopamine metabolites, dopa decarboxylase activity, tyrosine, and GABA in the developing rat corpus striatum. Neurochem Res. 1989;14(6):571–8[59]. Sato K. Why is vitamin B6 effective in alleviating the symptoms of autism? Med Hypotheses. 2018;115:103–106[60]. Jung HY, Kim DW, Nam SM, Kim JW, Chung JY, Won MH, Seong JK, Yoon YS, Yoo DY, Hwang IK. Pyridoxine improves hippocampal cognitive function via increases of serotonin turnover and tyrosine hydroxylase, and its association with CB1 cannabinoid receptor-interacting protein and the CB1 cannabinoid receptor pathway. Biochim Biophys Acta Gen Subj. 2017;1861(12):3142–3153[61].

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9. A novel therapeutic approach, "Precision Addiction Management"

The neurological correlates responsible for RDS identified by the GARS test are used to customized pro- dopamine regulation based on the test results. The customize pro-dopamine regulator is a neutraceutical consisting of enkephalinase inhibition, precursor amino-acids, and catabolic inhibition with the research ID code KB220. During the fifty-year development of KB220 nutraceutical variants, at least 42 published studies; included animal models of addiction, human clinical trials, and double and triple blinded placebo-controlled, and neuroimaging studies [79]. The number of studies about each ingredient of KB220 found in PubMed on 12th January 2020, are listed in Table 3.

Table 4 is a review of the evidentiary basis of each ingredient, including referenced pertinent basic and clinical studies. Some early basic research by Blum et al. that spurred the initial development of the of KB220 and some clinical experiments are included, however, most of the studies are independent.

9.1. Future studies

Additional research is encouraged. One such proposal is to study the polymorphic trait of Dopamine D2 receptor Taq A1 allele known to encode for approximately 30% fewer DRD2 receptors and evaluate pre -and post 30-day treatment with KB220 variants. The proposition is that post KB220; there will be an increase in DRD2 receptors measured by mRNA expression.

While we have already shown that the pro-dopaminergic agent KB220Z produced enhanced volume and functional connectivity between the reward and the cognitive centers of naïve rodent brains [81] as well as similar enhanced functional connectivity and volume between cognitive and reward centers of abstinent heroin-dependent probands [82] we are encouraging the neuroscience community to conduct a series of other placebo-controlled crossover studies to help establish KB220 as a dopaminergic agonist.

In terms of future studies related to providing data to show that KB220 has dopaminergic homeostatic or psychopharmacological effects, we propose the following: 1. Micro-dialysis studies to show the effect of acute administration of KB220 cause the release of DA from

NAC [83]; 2. Show that if the administration of KB220 reduces the availability of D2 and D3 receptors, this reduction could indicate enhanced dopamine receptor occupancy when compared to placebo [84]. 3. Self- administration experiments with KB220 compared to placebo including Acquisition of KB220 self-administration; Extinction and tests for reinstatement; Stress-induced reinstatement; Intracranial administration of KB220 on stress-induced reinstatement [85]. 4. Localization of the mRNA for the dopamine D2 receptor in the rat brain by in situ hybridization histochemistry following acute and chronic administration compared to placebo [86] and 5. Utilizing PET to monitor probands carrying DRD2 A1 allele, compared to DRD2 A2 allele, pre and post chronic (at least 30 days) KB220 treatment, for a potential increase in DRD2 receptor number [87].

10. Summary

It is well-known that exogenous opioids produced Dopamine Deficiency Syndrome (DDS), a subset of RDS, as well as anhedonia and exogenous opioid seeking and use. A foremost therapeutic issue, currently, is the drive of many clinicians to increase the use of MAT, especially buprenorphine to treat DDS and ODS as a life-time replacement therapeutic. We have argued that this type of treatment, which substitutes one narcotic for another narcotic, is seemingly counter-intuitive, and may reduce societal harm but will also perpetuate the addiction to opioids.

Therefore, in this article, we are proposing “Precision Addiction Management” following required research; front-line treatment should consist of GARS testing to direct precision polymorphic neurotransmitter restoration.

Author contribution

All authors contributed equally.

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Declaration of Competing Interest

Dr. Kenneth Blum, through his companies Synaptamine Inc. and Igene LLC, provided worldwide exclusivity to Geneus Health LLC for, patents related to KB220. Patents related to GARS have been asssigned to Geneus Health, LLC.

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