[Frontiers In Bioscience, Landmark, 22, 1247-1288, March 1, 2017]

Neurogenetics of acute and chronic opiate/opioid abstinence: treating symptoms and the cause

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1. ABSTRACT

This review begins with a comprehensive history of opioid dependence and treatment in the United States. The focus is an evidence-based treatment model for opioid/opiate dependent individuals. The role of reward genetic polymorphisms and the epigenetic modifications that lead to vulnerability to use and

misuse of opiates/opioid to treat pain are reviewed. The neurochemical mechanisms of acute opiate withdrawal and opiate/opioid reward mechanisms are explored with a goal of identifying specific treatment targets. Alterations in functional brain connectivity based on neurobiological mechanisms in heroin dependence and abstinence are also reviewed. A new clinical model an alternative to merely blocking acute withdrawal symptoms as identified in the DSM –5 is proposed. Genetic diagnosis at the onset of detoxification, to determine risk stratification, and identify polymorphic gene targets for pharmaceutical and nutraceutical interventions, followed by the simultaneous initiation of Medication Assisted Therapy (MAT), to enable psychological extinction, and steady pro-dopaminergic therapy with the goal of developing "dopamine homeostasis" is recommended. The objective of these interventions is to prevent future relapse by treating all "Reward Deficiency Syndrome" (RDS) behaviors and eventually make an addiction-free life possible.

2. INTRODUCTION

This manuscript begins with the history of the hardships caused by America's affliction with opioid use. This review offers a brief examination of the past and current opioid epidemic in America. In 2014, data showed that more than 16,000 lives are lost each year due to opioid-related overdoses. An estimated two million people used prescription opioids non-medically for the first time - nearly 5,500 people a day - in 2010 alone (1). However, the primary goal is to underscore the need to understand the neurobiological underpinnings of acute opiate/opioid abstinence. So that rather than treating withdrawal symptoms alone, a new model has been developed that focuses on addressing the lingering and long-term effects of these potent, dangerous substances that kill many of our young people every day. This model is the "Anti-Opiate Dopamine Restoration" (AODR).

2.1. A discussion of the opioid epidemic in America: past and present

America's first opioid epidemic resulted from the opium smoking habits of Chinese immigrants' that spread beyond their culture, aided by the extensive use of morphine in the civil war. In 1898, the German drug company, Bayer, introduced heroin into the United States as non-habit-forming medicine that would cure opium and morphine addiction (2). Over a hundred years ago, America's first drug czar, Dr. Hamilton Wright, was the subject of an op-ed in the New York Times. Wright revealed that per capita, Americans were the biggest consumers of raw opium and opium-based products, in the world. These products included morphine, heroin, laudanum, over-the-counter medicines, and patented drugs. His efforts lead to a series of Federal laws that restricted the importation, distribution, and prescription of opiates and opioids; effectively ending the first American opiate/opioid epidemic. In the 1960's, many of our military returning from Vietnam brought their heroin addiction home with them. The growing number of heroin addicts caused President Nixon to pass legislation (The Narcotic Addict Treatment Act of 1974) allowing Methadone Clinics to open across the country to combat heroin abuse (2).

The birth of America's current opiate/opioid epidemic coincides with a pharmaceutical company's launch of a powerful new and highly addictive painkiller in the late 1990's. The concept that pain should be considered a vital sign (3) was initiated in the mid-1990s by the leadership of the American Pain Society (4, 5) and mandated in January of 2001, by the Joint Commission on Accreditation of Health Care Organizations (JCAHO). An extensive educational campaign required health care professionals to assess pain level in all patients and provide treatment (6). Physicians were encouraged by pharmaceutical companies to prescribe narcotics – and they did - enough to keep every single adult in America high for a month. Opioid prescriptions nearly quadrupled in less than fifteen years. Physicians prescribed responsible, hard-working, non-drug abusing Americans opioids for pain resulting from sports injuries and minor and major surgeries. As they became addicted to Oxycodone; pain clinics and infamous "pill mills" eventuated (7). Today we find ourselves in a full blown opiate/opioid epidemic that, if not addressed, will continue to expand. It is imperative that this concern is structured appropriately. To imply America has a heroin epidemic is insincere and misrepresentative. We have included evidence that portrays a nation hooked on opioid painkillers – the most prescribed medication in America.

An opioid is defined as an artificial narcotic that is not derivative of opium. Opiates are analgesic alkaloid compounds found naturally in the opium poppy plant; Papaver somniferum. One main source fueling America's opiate/opioid epidemic that continues to expand its boundaries is the overprescribing of addictive opioid painkillers. The number of newborns in the US in neonatal intensive care units who are addicted to opiates and opioids (prescription and illicit) nearly quadrupled from 2004 through 2013 and still continues to rise (8). Every day in America, 2,500 youth (ages 12 to 17 years) abuse an opioid prescription painkiller for the first time and 46 people die from an overdose. Twice as many people die from prescription painkillers than from heroin (9). There were 47,055 lethal drug overdoses in 2014 making drug overdose as the leading cause of accidental death in the US. In 2014, opioid addiction was already an epidemic, with 18,893 deaths from overdose of prescription pain relievers, and 10,574 deaths from heroin addiction (6). Almost 50% of chronic opioid users took only

short-acting – rather than longer-acting medications – hence, increasing their risk for addiction. Anywhere from 45-75% of heroin addicts surveyed said they were first addicted to opiate/opioid painkillers then moved on to heroin (6). Quoting from the Journal of American Medical Association Psychiatry (JAMA Psychiatry), 2014; "Although the "high" produced by heroin was described as a significant factor in its selection, it was often used because it was more readily accessible and much less expensive than prescription opioids" (6). Although Americans make up only 4.6. % of the world's population, they consume over 80% of the global opioid supply, 99% of the global hydrocodone supply, as well as two-thirds of the world's illegal drugs (6).

2.2. Facts from the Centers for Disease Control and Prevention (CDC) about the Opioid Epidemic

- Starting in 1999, the amount of prescription painkillers prescribed and sold in the U.S. has almost quadrupled (an estimated 272%) to nearly 207 million in 2013 (10).
- These staggering numbers reveal that enough prescription painkillers were prescribed to medicate each American adult every four hours for an entire month (11).
- The CDC also found that there has NOT been a drastic change in the magnitude of pain that Americans report within this particular time frame (11).
- Several states report issues with for-profit, high-volume pain management clinics (so-called "pill mills") that overprescribe painkillers to those who do not require them medically to the CDC (12).
- The CDC has observed that overprescribing increases abuse and overdose fatalities (10).
- The CDC estimates that 43,982 drug-poisoning deaths occurred in 2013; 16,235 (45 people daily) drug-poisoning deaths involved opioids (prescription painkillers) alone; 8,257 (23 people daily) drug-poisoning deaths involved heroin alone, and 1,342 deaths involved both opioid analgesics and heroin (13).

In short, Americans, who have not reported additional pain during the 14-year period beginning in 1999 and ending in 2013, were prescribed by their physicians approximately 300% more prescription opiate/opioid painkillers than required. Recent studies have observed a trend of moving from the opiate/opioid painkillers that initiated the addiction, to more available and less costly heroin –, a detail that evidently designates prescription opiate/opioid painkillers as a gateway drug to heroin use (13).

Indeed, to believe we can end America's opiate/opioid epidemic by prescribing more opioids, specifically methadone, buprenorphine, and combinations with narcotic antagonists like Naloxone (Suboxone, and Zubsolv), seems counterintuitive. Based on the amassed and unbiased empirical data, it seems parsimonious that guidelines regarding addiction treatment will not stop this epidemic if we do not initially turn off the running tap. Wright's work played a major role in terminating America's first opiate/opioid epidemic. However, fragmentation of vital parts of the Harrison Narcotics Act (HNA) of 1914 as recommended by recent drug/addiction treatment policy plans can have major negative results that cannot be overlooked. To be clear, measures required to bring this opiate/opioid epidemic to its end entail more than satisfying short term treatment goals while the serious and avoidable long-term consequences of addiction are ignored.

The policy proposals for drug addiction treatment feature federal financial assistance to states that provide plans that recommend Medication-Assisted Treatment (MAT). MAT is the use of U.S. Food and Drug Administration (FDA) approved medications – most of which are opioids – for the treatment of opiate/opioid addiction. The two primary opioids used to treat opiate/opioid addiction are methadone and buprenorphine. Buprenorphine is approximately 50 times more potent than morphine. Through media reports, the published drug/addiction policy plans include a component allowing for greater availability and use of buprenorphine/naloxone combinations (Suboxone/Zubsolve) in addiction treatment. This plan lacks detail and can have unintended consequences that in practice, are likely to fail to have the desired effect.

Some of the narcotics mentioned above that have FDA approval for use in opiate/opioid addiction treatment are habit-forming, addictive just like any other opiate or opioid, potentially just as deadly and subject to the same abuse as any other prescription or illicit narcotic (14, 15). While the effects of MAT may not be optimal, they are very useful in the short-term to reduce harm but must be utilized with care. In 2010, Methadone was responsible for 31.4.% of overdose fatalities reported in thirteen states (16). Methadone has a longer half-life than heroin and can stay in the system up to 59 hours compared to heroin up to 6 hours. Users are tempted to take another dose if they cannot feel the previous dose, and the methadone accumulates to toxic levels. According to the CDC, in the United States, there were 41,502 mortalities in 2012 due to drug poisoning, which included 16,007 opioid deaths and 5,925 heroin deaths (17). The difference between the number who died from an accidental overdose of methadone, and the number who died from heroin is 643 people. Despite the empirical data that clearly shows a pattern of increased deaths directly attributed to methadone

and the slim margin between methadone deaths and heroin deaths, –deaths, we were told methadone would prevent – the FDA-approved MAT narcotic is still prescribed. Methadone is widely available and considered safe by the FDA for treating opiate/opioid addicts with opiates/opioids (16).

Deaths attributed to Buprenorphine/Subutex/Suboxone/Zubsolve are harder to track. According to the Drug Abuse Warning Network (DAWN), 21,483 emergency department visits were estimated to be were associated with nonmedical use of buprenorphine in 2011.The American Association of Poison Control Centers Annual Report indicated that 3,625 case and three deaths involving toxic exposure from buprenorphine were recorded in U.S. poison centers 2011 (18).

In America's first MAT program, albeit it was not called that in late 1800's and early 1900's, physicians prescribed heroin from their offices – promoted by the German manufacturer, Bayer, as a "cure for opium and morphine addictions" (2). From this history the lesson is that adding more opiates/opioids into an already over-served market with lax oversight mechanisms in place, no matter how well intended, has the potential to extend America's second opiate/opioid epidemic into perpetuity.

The FDA approved pharmaceutical agents either reduce cravings or suppress the pleasurable effects of drugs. While these agents have helped many patients over the years, they have not adequately prevented cravings and relapse. This fact is highlighted by the recent findings that used data from the sophisticated Comprehensive Analysis of Reported Drugs (CARD). The study revealed a significant lack; of "compliance" with many treatment medications and "abstinence" from psychoactive drug use, in both inpatient and outpatient treatment settings (19).

The short-term use of MAT, possibly, from detoxification to less than 12 months, especially, opiate substitution therapy like Methadone or Buprenorphine/Naloxone (Suboxone/Zubsolve) may have substantial benefits regarding harm reduction and preventing unwanted opiate/opioid withdrawal. Moreover, these potent narcotics can contribute to patient stability, provide an opportunity to initiate treatment, and for workforce reinstatement, and productivity. The neuropharmacology of MAT relies on the action of blocking dopamine and leads to acute prevention of use of the individuals' drug of choice due to "psychological extinction." Why use if the thrill is gone? This mechanism of action involving reduced dopamine function alone is not an efficient or cost-effective way to combat America's second opiate/opioid epidemic.

Recently, in the states of Massachusetts and Ohio, members of Congress from both the Democratic and Republican parties have developed very comprehensive bills to assist in the reduction of harm including opiate/opioid overdoses, especially to minors. These bills seem to be on the right track and should help. However, more in-depth knowledge must be provided to lawmakers by the scientific community, regarding the neuroscience of addiction medicine to create change in the current landscape. With this detailed summary of the current opiate/opioid epidemic in the United Sates in mind, and given the need to curtail the current loss of lives, this article will focus on the neurobiological mechanisms involved in acute opiate/opioid abstinence and long-term relapse prevention.

A new "anti-opiate dopamine restoration model" (AODR), is proposed. Instead of merely blocking withdrawal symptoms, for example, with clonidine in combination with buprenorphine/naloxone, the preferred modality would be, a gentile non-opioid dopaminergic agonist-like therapy initiated early in recovery (at detoxification). Our proposed AODR model is based on known mechanisms involved in both glutaminergic and dopaminergic pharmacology, that could lead to the development of "dopamine homeostasis" in the long-term to treat opiate/opioid use or misuse and to reduce future relapse.

2.3. Importance of preclinical models of addiction

The pathophysiology and etiology of addiction or Reward Deficiency Syndrome (RDS) despite a plethora of well-researched studies, especially in the pre-clinical arena, remains only partially understood. According to a recent review by Aude Belin-Rauscent and associates (20), one particular reason has to do with the gap between these pre-clinical models of addiction and the clinical criteria for the disorder as espoused by DSM-5. These authors provide an interesting and clear understanding of how after 50 years of research, the newest models may scientifically reduce the gap and provide the field with a better window into the fascinating function of the brain. While this is true, we must point out that some research, early on, did indeed help frame our understanding of acute opiate withdrawal mechanisms and, in fact, the science is still utilized as a valid treatment modality. With due respect for many others, the preclinical work of Blum's group coupled with the clinical work of Gold's group reveals how pre-clinical neuroscience can meet clinical science to assist those in recovery.

In earlier reviews, Gold *et al.* (21) encouraged the continued use of "magic bullets," including clonidine and possibly buprenorphine, to offset the "opiate drive state," incorporated into a continuing recovery model. The goals were to recover the brain's homeostasis in a

sober state and to maintain concurrently the necessary drive for novel methods to accomplish and support a pleasurable existence. Along these lines, early work of Blum and in collaboration with others (22-24), proposed the "endorphin deficiency theory" for both alcohol and opiates. Accordingly, Gold *et al.* (25-27) suggested that in addicts, endorphin deficiency (possibly genetic) could exist prior to opiate use. They also proposed that the abuse of potent exogenous endorphinomentic compounds may cause an endorphin-abnormality and that dopamine is involved in withdrawal from opiates(28). Moreover, Gold *et al.* (29) suggested the idea that endogenous peptides physiologically provided normal inhibitory tone at the locus coeruleus and during opiate withdrawal and that attenuation of this inhibitory mechanism, due to reduced endogenous peptides, leads to norepinephrine-induced hyperactivity. Other earlier work by Blum and associates revealed shared mechanisms between alcohol and opiate withdrawal (30-35), potentially through the opiate-like effects of isoquinolines, which provided the basis for understanding the role of dopamine in acute opiate abstinence (36).

Most importantly, there is protracted withdrawal during abstinence following chronic morphine dependence, which may be persistent. Kaufling and Aston–Jones (37) have provided clear evidence to reveal these adaptations involving Ventral Tegmental Area (VTA) dopamine neurons in rodent models. The adaptations involved in opiate withdrawal are linked to an altered responsiveness of mesolimbic dopaminergic neurons, a loss of dopamine cell responsively and subsequent behavioral changes. Also, Kaufling and Aston–Jones (37)) point out that GABAergic neurons in the tail of the VTA (tVTA), called the Rostromedial Tegmental Nucleus, are central to behavioral responses to opiates. They found that VTA dopamine neurons, but not tVTA GABAergic neurons, are tolerant to morphine after two weeks of withdrawal. Moreover, optogenetic stimulation of tVTA neurons inhibited VTA dopamine neurons similarly in opiate-naive and long-term withdrawn rats. Interestingly, tVTA inactivation increased VTA dopamine activity in opiate-naive rats, but not in withdrawn rats, resembling the opiate tolerance effect in dopamine cells. This work suggests that although inhibitory control of dopamine neurons by tVTA is maintained during protracted withdrawal, the capacity for disinhibitory control is impaired. Furthermore, they found that morphine withdrawal is reduced both in tVTA neural activity and tonic glutamatergic input to VTA dopamine neurons (37). This latter finding suggests that alterations in glutamate and GABA feedback motivate the evident tolerance of VTA dopamine neurons to opiates following long-lasting contact. It is important to realize that protracted abstinence from morphine, for example, leads to inhibition by tVTA, but not disinhibition. Dopamine cells following chronic opiate exposure may add to continuous negative affective states during withdrawal. Simply put, there will be less dopamine in the long-term, less well-being, and the need to induce "dopamine homeostasis."

To further comprehend the neurobiological mechanisms that predispose people to addictive behaviors, a brief review of existing pre-clinical models of addiction seems warranted. Interestingly, when scientists initiated their study of addiction during the 1930s, drug addicted persons were considered morally weak and unable to control their will, but today, with the advent of new techniques that help explore the addicted brain, and psychiatric genetics including epigenetic adaptations, these views have drastically changed. In fact, the initial finding of the association of the dopamine D2 receptor gene *(DRD2)* Taq A1 allele and severe alcoholism reversed the opinion of Americans in 1990 (38). A Gallup poll showed that before the finding, less than half of the Americans polled still believed that alcoholism was due to a lack of moral fabric, but after the genetic finding, over 56% of Americans believed that alcoholism and possibly other addictions were biologically-based.

It is well-known that abusable substances exert their reinforcing effects through activation of the mesolimbic dopamine system (39), where they "hijack" synaptic plasticity processes (40, 41) such as long-term potentiation or long-term depression (42, 43). They also trigger various between-systems, neuroadaptations (44, 45), and changes in gene transcription and function, partly mediated by epigenetic adaptations (46-52). These adaptations occur in some brain systems, including the Nucleus Accumbens (NAc) (53), amygdala (54), dorsal striatum (55-59), and prefrontal cortex (60-63), with effects on inhibitory control through glutaminergic/GABA mechanisms and stress reaction (64, 65).

Understanding relapse and drug reinstatement have been the subject of investigation since the late 1960s and early 1970's. A basic tenet is that it is believed one approach involving "psychological extinction" is indeed useful and significant in reducing relapse. This idea is based on the removal of the very thing that induces motivation to reuse and causes the reinforcing effects of the drug. It is very well accepted that by attenuating the acute impact of dopamine via some biological mechanisms, motivation to use will be reduced. These mechanisms include, but are not limited to, biosynthesis, storage, catabolism, and neuronal release, receptor blockade, blocking reabsorption through transporters, low blood–brain barrier penetration, and altering gene expression through epigenetic adaptations, among others. It is noteworthy that over many years, some relevant pre-clinical models that have attempted to understand relapse to "reward deficiency" (66), emerged and

have been reviewed in the literature (61, 64, 65, 67-79). In fact, the current FDA-approved MATs favor this approach that, although not optimal, has success in many patients (80-82).

Over the last decade, much effort has been devoted globally to the development of preclinical models that independently address psychological constructs and related clinical criteria of addiction, as defined in the Diagnostic and Statistical Manual (DSM)-5 and older versions. The following aspects of the addiction process should be considered as the focus of research. The protracted seeking responses, as observed in heroin addicts (83, 84), these impulses are controlled by stimuli in the environment (possibly epigenetic) and eventually become compulsive. After prolonged exposure to the drug (certainly beyond early withdrawal symptoms) and especially in some vulnerable individuals, addiction may be a life-long issue, due to genetic polymorphisms of reward genes (85).

Along these lines, Zou *et al.* (84) using functional Magnetic Resonance Imaging (fMRI), showed that 30 heroin-addicted subjects with three years of abstinence compared to healthy controls had weaker connections between reward processing parts of the brain and the areas associated with motor skills. Moreover, some of the subjects showed potential for healing thereby reducing the risk for relapse. This work demonstrates that the brain might heal following heroin insult, and indicates that treatment should be continued for at least three years. However, Zou *et al.* (84) did not test for genetic reward gene polymorphisms. Certainly formal genetic testing will help the clinician decide the length of potential treatments. Needless to say, treatment of heroin addiction should go beyond the typical detoxification period whereby only withdrawal symptoms are addressed. In America today, many of our adolescents, as well as adults, are sent to recovery for self-help programs with increased risk for relapse due to undiagnosed genetic polymorphism but without any further long –term neuroscience-based treatment (83).

Importantly, the next generation of pre-clinical models must focus on uncovering the pathophysiological substrates of addiction and its associated endophenotypes of vulnerability. Utilizing modern neuroimaging techniques as well as a better analysis of genetic risk including epigenetic adaptations, the opiate/opioid dependent individual will be better served by the clinical community. A coherent translational approach is required to identify the functional significance of the specific behavioral, cognitive, or genetic correlates of the vulnerability to switch from volitional drug use to compulsive drug-seeking behaviors. An approach, which integrates cognitive neuroscience and employs both animal studies and correlational approaches in humans; such as, genome-wide and candidate polymorphism analysis, is needed to help refine the unraveling of the complex etiology of addiction. If this could be accomplished, the next generation of preclinical models of all RDS behaviors will provide evidence-based support for clinical criteria and treatment.

3. POTENTIAL THERAPEUTIC TARGETS

Many potential therapeutic targets are emerging, and a brief review of some neurochemical pathways seems parsimonious. These pathways include the serotonergic, endorphinergic, glutaminergic, and dopaminergic. However, based on current knowledge, we are proposing that a gentle induction of “dopamine homeostasis,” instead of blocking dopamine, is tantamount to successful long-term treatment as well as relapse prevention in the heroin or opiate/opioid dependent person (85-87). According to Li *et al.* (87), compared with heroin non-relapsers, those who do relapse exhibit considerably higher cue-induced craving, and this brain reaction was seen mainly on fMRI of the bilateral nucleus accumbens/subcallosal cortex and cerebellum. Moreover, the difference in desire positively correlated with the initiation of cue-induced craving, as seen in the nucleus accumbens/subcallosal cortex of patients. These results indicate that in heroin-dependent persons seeking treatment, higher cue-induced cravings, and increased activation in those particular areas may be linked to reward/craving and memory recovery functions. Most importantly, these responses may predict relapse and represent important targets for the development of new treatment for heroin addiction, possibly via regulation of brain dopaminergic function, which we refer to here as the AODR model.

For the distinct role of any individual neuro-pathway to be fully appreciated, it is important to evoke the concept of the “Brain Reward Cascade” first developed by Blum & Kozlowski (88) as previously indicated by Bozarth & Wise (89). In the Bozarth and Wise article, they correctly suggest that heroin reward is dependent on a dopaminergic substrate, and the cascade intimates the various interactions of some neurotransmitters leading to NAc dopamine release. Over the years following these discoveries many reiterations have been developed and recently supported by the outstanding work of Morales’ group at the National Institute on Drug Abuse (NIDA) (90) (see Figure 1).

This review presents a snapshot of the neurogenetic and epigenetic adaptations that change neurotransmitter pathways in Opiate/Opioid Dependence. Although limited, the evidence presented here has been selected for consideration due to its relevance to the development of potential Opiate/Opioid therapeutic targets.

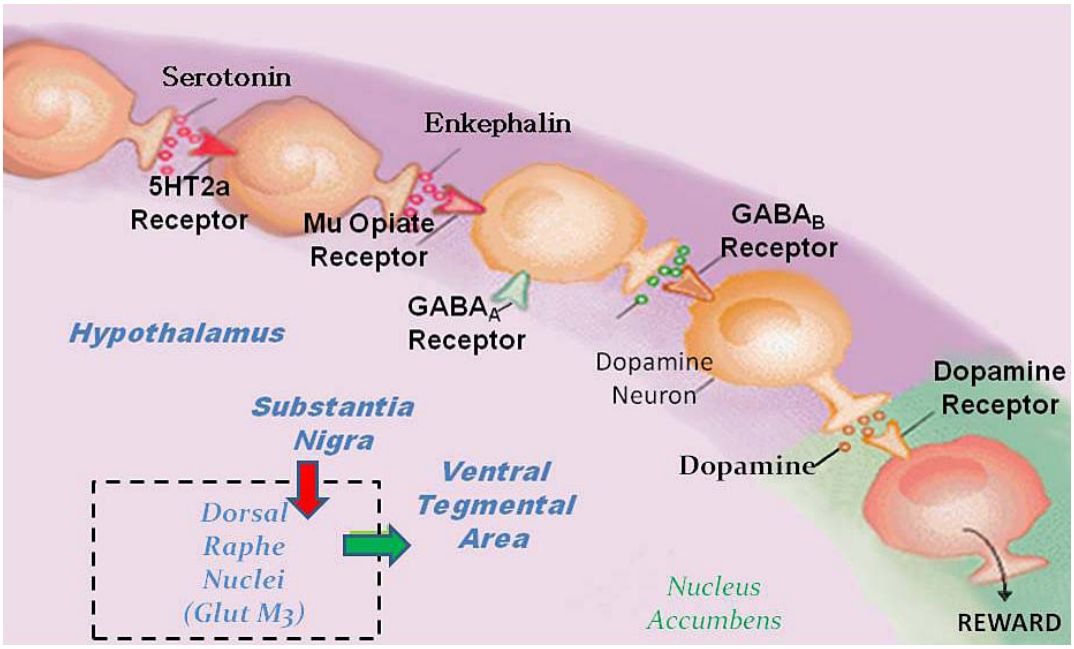

Figure 1. This is an illustration of the Brain Reward Cascade. The cascade begins with the release of serotonin, at the hypothalamus, which stimulates enkephalin. The enkephalin, then, inhibits GABA at the substantia nigra, which, in turn, regulates the amount of dopamine released at the nucleus accumbens (or "reward site"). The dopamine originates in the VTA. Various receptors (including 5HT2a receptors, μ-opiate receptors, GABAA receptors, GABAB receptors, and dopamine receptors) are utilized in the reward cascade. Recent evidence demonstrates the role of the dorsal raphe nuclei in this cascade (91). Reproduced with permission from (86).

3.1. Serotonergic system

Most recently, Müller & Homberg (91) reviewed the role of the serotonergic system, in the establishment of drug use-associated behaviors and the transition and maintenance of addiction. Their study examined the following drugs: alcohol, amphetamine, cannabis, cocaine, MDMA (ecstasy), methamphetamine, morphine/heroin, and nicotine. Interestingly, they found distinct involvement of the 5-hydroxytryptamine (5-HT), system in both the establishment of drug use behaviors and transition to addiction with considerable overlap between psychostimulant, opioidergic drugs, and alcohol. This overlap seems to be in agreement with the role of drugs of abuse in RDS and potential for genetic testing (86). Their review suggests that specific adaptations of the serotonergic system render the nervous system susceptible to the transition to compulsive drug use behaviors and often overlap with genetic risk factors for addiction. Müller & Homberg (91) highlight the fact that serotonergic neuroadaptations induced by first drug exposure pave the way for the establishment of addiction. Certainly, repeated administration of heroin intake results in both cellular sensitization and withdrawal, which can be long-lasting and devastating (92). In a recent study Wu *et al.* (93), found that of a selective 5-HT2CR agonist Lorcaserin administered during the development, the withdrawal or expression stage; suppressed heroin-induced behavioral sensitization on day nine. Moreover, the same drug also suppressed naloxone-precipitated withdrawal symptoms in heroin–treated mice.

A plethora of studies are showing that the brain neurotransmitter, serotonin (5-HT), plays a central role in the regulation of reward-related processing (94, 95). Emerging evidence suggests that there is deregulation of the serotonin system after long-term exposure to drugs of abuse (96). Dysregulated serotonin transmission has been thought to increase susceptibility to a broad range of substance abuse disorders (97). Obviously, a review of genetic polymorphisms of the serotonin system reveals a unique genetic architecture that contributes to not only the risk for addiction but also to treatment effectiveness and the potential for full recovery (98). Gao *et al.* (99) and others (100, 101) established the noteworthy link between heroin addiction and the four Single Nucleotide Polymorphisms (SNPs) of the 5-HT receptor (*HTR*) genes in a group of Han Chinese individuals. Also, Tan and colleagues (102) provided evidence of an association between heroin dependence and a VNTR polymorphism at the serotonin transporter *(5-HTT)* gene. Hungarian scientists found an association between the -521 CC vs. CT or TT genotypes (*DRD4*) and heroin dependence that was enhanced in the presence of a short (s or 14-repeat low activity) 5-HTTLPR allele (103). Other work from Yang *et al.* (104) showed that 5-HTTVNTR has a predictive effect on co-morbid Borderline Personality Disorder in female heroin-dependent

patients. Also, Cao & Hudziak (105), through a meta-analysis, showed that across multi-cultures, albeit at different risk frequencies, there is an association between 5-HTTLPR and heroin dependence. In a neuroimaging study by Lin *et al*. (106), observed that "the time to heroin relapse" is significantly higher when serotonin transporter availability is low, revealing a negative association pattern. It is established that the human 5-hydroxytryptamine (serotonin) receptor 1B, encoded by the HTR1B (*5-HT1B*) gene, is a presynaptic serotonin autoreceptor that is important in regulating serotonin synthesis and release. Cao and LaRocque (107), through a meta–analysis, reported an association between the functional SNP-161A>T (rs130058) and heroin dependence. Specifically, a study by Gau *et al.* (99) also clearly supports an association between the HTR1B (*5-HT1B*) gene polymorphism (G861C) and heroin dependence.

While a quick word search in PUBMED (12-25-2016) for "epigenetics of serotonin genes and opiates" did not reveal any studies, certainly, some studies are showing epigenetic effects on serotonergic genes (108). The HTR2A promoter has been connected to many disorders in adults and infants, including bipolar disorder, borderline personality disorder, chronic fatigue syndrome, schizophrenia, suicidality, and other neurobehavioral conditions. Along these lines, epigenetic effects have been shown to exist in placenta tissue as a way of determining the role of environment in fetal brain development. Paquette and Marsit (109) did find evidence of placental epigenetic variation of HTR2A to be associated with infant neurobehavioral outcomes, which could be a possible link to adult mental health disorders. Specifically, hypermethylation of SLC6A4 (serotonin transporter) was observed in bipolar disorder (110-112).

Certainly, opiate/opioid dependence or withdrawal symptoms are a polygenic inheritable phenomenon impacted by epigenetic communication and pleiotropy. Thus, it is improbable that this physiological state is due to any one single gene. With this stated, new gene research utilizing microarray analysis, coupled with candidate convergence including epigenetic effects, should be applied to serotonergic genes as they relate to the entire process of addiction and its recovery to enhance therapeutic targeting approaches.

3.2. Endogenous opioid peptides

In the early to mid-1970s, we learned about the presence of the opiate receptor (113) as well as the identification of brain opioid peptides (114). At that time, Blum's laboratory proposed a common mechanism theory linking opiates with alcohol through the "genotype-concept" called "endorphin deficiency," which set the stage for an enormous amount of neuroscience and genetic research (22, 24, 115). Thirty years later, we know that G-protein-coupled μ-, δ- and κ-opioid receptors, are activated by opioid peptides which have different response profiles and affinities. The endogenous neuropeptides β-endorphin, leu-enkephalin, met-enkephalin, and dynorphin physiologically activate opioid receptors. These peptides are not limited to binding with one type of opioid receptor. Individuals can be genetically predisposed to substance abuse due to defects in opioid peptide and receptor genes (116). Regarding support for the common mechanism theory, opioid receptors not only facilitate the pharmacological functions of opioids, but they also control *in vivo* outcomes of other abused drugs (117). While the human mu-opioid receptor (*MOR or OPRM1*) represents the most important target for morphine, the Delta, and Kappa receptors are similarly significant in addiction, and the genetic variants of these receptors have been studied extensively (118). Genetic polymorphisms in *OPRM1* gene have been associated with heroin dependence in Chinese samples(119) and other ethnic groups (120). Analysis of a combined effect of *OPRM1 (mu receptor)* and *OPRD1 (delta receptor)* showed that rs510769 and rs2236861 increase the risk of heroin addiction.

However, numerous studies with negative outcomes have also been described (121-123). Independently, studies by Tan *et al.* (102) and by Shi *et al.* showed an addiction-like relationship at the time of first drug use and during drug-seeking behavior was modulated and confirmed by *OPRM1* polymorphisms (124). Using a postmortem brain analysis, others (125, 126) showed down-regulation of preproenkephalin and preprodynorphin genes in all heroin users. However, the effects were exaggerated in subjects with the 118G and were most prominent for preproenkephalin in the nucleus accumbens shell. Also, the same scientists revealed that alterations in opioid neuropeptide systems might underlie enhanced opiate abuse vulnerability apparent in 118G individuals. It is well-known that μ Opioid receptors are crucial for heroin dependence, and A118G SNP of the μ opioid receptor gene (*OPRM1*) has been linked to heroin abuse. In the post-mortem study population of European Caucasians (n = 118), ≈90% of 118G allelic carriers were heroin users. The OPRM1 genotype was shown to be associated with the processing of the human striatal opioid neuropeptide system including transcription, and translation. Exclusively in the 118G heroin subjects, increased dynorphin, and enkephalin peptide concentrations were observed together with reduced opioid neuropeptide transcription. Enhanced vulnerability to opiate abuse apparent in 118G individuals may be the consequence of these alterations in peptide processing. Heroin users also had abnormal gene expression related to peptide convertase and ubiquitin/proteasome regulation (125).

Preprodynorphin, the primary endogenous ligand for the κ-opioid receptor, is a natural derivative of prodynorphin. Kreek *et al.* (127) found an association between polymorphisms of the preprodynorphin gene and opiate addiction. Wei and associates (128) reported an association of three variants of the Preprodynorphin (*PDYN*) gene and heroin dependence in Chinese subjects. Clarke *et al.* (129) also found that *PDYN* was significantly related to the risk of developing opioid dependence, primarily in females. Interestingly, according to Nikoshkov *et al.* (126), the data suggests that the dysfunction of the opioid reward system is considerably related to opiate abuse susceptibility, and that heroin consumption modifies the evident impact of genetic dopamine tone on mesolimbic PENK and tyrosine hydroxylase function.

Along similar lines, recent work from Navratilova *et al.* (130) provides interesting and relevant evidence that endogenous opioid activity in the anterior cingulate (AC) is necessary for pain relief. Understandably, aversive pain and its relief, require dopaminergic transmission in the NAc. Navratilova *et al.* (130) specifically found that the blockade of opioid signaling in the rostral AC Cortex (rACC) inhibited NAc dopamine release. In contrast, pharmacological activation of rACC opioid receptors of injured, but not pain-free, animals was sufficient to stimulate dopamine release in the NAc. Based on these and other related findings, the authors concluded that endogenous opioid signaling possibly via delta opioid receptor activation in the ACC seems to be both necessary and sufficient for the relief of pain aversiveness. Finally, the same group revealed the importance of having standard delta-opioid receptor expression in the VTA as a possibly protective mechanism against high alcohol intake in humans (131-133).

We believe this suggestion involving endogenous opioid expression seems important in protecting against heavy heroin consumption. These findings align with earlier findings by Blum *et al.* (134, 135). Here, we suggest that targeting the endorphinergic system regarding treating acute opiate/opioid abstinence and its protracted clinical outcome, and neuro-adaptations seems parsimonious. Enhancing signaling in the AC is required to prevent poor decision making since the ACC is the seat of relapse and drug reinstatement.

3.3. Cannabinoids and anandamide system

Since the discovery of endogenous Cannabinoids and their receptors, it has been researched and is now well established, that Cannabinoids functionally interact with opioid systems. The endogenous cannabinoid system is a signaling method comprised of the central cannabinoid (CB1) and the peripheral cannabinoid (CB2) receptors, as well as numerous lipid transmitters, like 2-arachidonoylglycerol and anandamide. The system is the target for natural cannabinoids: the psychoactive constituents of preparations of Cannabis *Sativa* like marijuana and hashish (136-139). Specifically, cannabinoid CB1 receptors are present in dopamine brain areas in primates and certain rat strains. Cannabinoid CB1 receptors are also located in dopamine cells of the A8, A9, and A10 mesencephalic cell groups and co-localize with dopamine D1/D2 receptors in dopamine-projecting neurons. Manipulation of dopaminergic transmission can alter the expression of CB1 receptors, as well as, the synthesis and release of anandamide. Cannabinoid CB1 receptors can switch its transduction mechanism to oppose the ongoing dopamine signaling.

Lopez-Moreno *et al*. (138, 139) have reported that the cannabinoid brain receptor type 1 (CB1) and mu-opioid receptor type 1 (MOR1) co-localize in the same presynaptic nerve terminals and signal through a common receptor-mediated G-protein neuronal system. In fact, Lopez-Moreno *et al.* (139) indicated that the cannabinoid receptor 1 (*CNR1*) gene is expressed in the central nervous system (CNS). Moreover, specific polymorphisms of the *CNR1*gene have repeatedly been found to be associated with drug addiction in general. It is known that a microsatellite polymorphism (AAT) at the cannabinoid CB1 (brain) receptor gene (CNR1) consists of 9 alleles. Comings *et al.* (140) studying CNR1 alleles found that the number of intravenous drugs consumed was considerably higher for those carrying the > or =/> or = 5 genotype, as opposed to other genotypes. These results are further support for a role for cannabinoid receptors in the modulation of dopamine and cannabinoid reward pathways. Benyamina *et al.* (140, 141) conducted a meta–analysis involving eleven articles supporting a minor implication for CNR1 AAT polymorphism in illicit substance dependence vulnerability. Unlike Comings *et al.* (140), others could not find an association with the same CNR1 polymorphism in German IV drug users (142). In 2006, Yale scientists (143) also failed to show an association with CNR1 AAT polymorphism in illicit substance dependence vulnerability following statistical correction (multiple testing errors). It is possible that with a more specific phenotype and better assessment of controls, the CNR1 AAT may associate with opiate/opioid dependence, but we must await these studies.

3.4. Glutamatergic and GABAergic systems

It is well established that opiate reinforcement is mediated by the inhibition of GABA release, thus disinhibiting dopamine neurotransmission. Humans with a dysfunctional GABAergic system may release higher amounts of dopamine, which has been considered an important early target as represented

by FDA-approved MATs (144). A plethora of research indicates that GABA receptors play an essential role in the actions of benzodiazepines, barbiturates, alcohol and morphine abuse and dependence (145-148). Glutamate is among the most abundant excitatory neurotransmitters in the brain (149). Glutamate receptors, which function in many brain areas such as the mesocorticolimbic dopamine sections, play a part in addiction. The Dorsal Raphe Nucleus (DRN)) and the VTA(150) are two of the more relevant brain reward areas where electrical stimulation produces responses, at the highest rates and lowest thresholds, meaning that they are very sensitive. For over 40 years, the DNR has been classified as a serotonergic structure and the VTA as a dopaminergic structure. Although multiple studies have examined both the DRN and VTA and their effects on reward, these studies have been focused on the serotonergic contribution to reward. As a result, these investigations have produced conflicting results, and the true role of DRN in the VTA circuitry regulation of motivated behaviors is still unknown. Contrary to the widespread idea that the major input from DRN to VTA is serotonergic, the Morales Group in Qi *et al.* (151) found that DRN neurons expressing the vesicular glutamate transporter-3 (GluT3) are the major input from DRN to VTA. Within the VTA, these DR-GlutT3 neurons mostly develop synapses on dopamine neurons. Importantly, some of these dopamine neurons as found by Qi *et al.* (152), specifically innervate the NAc. Via genetic approaches to specifically express rhodopsin in channel DR-GlutT3 neurons, it was also found that intra-VTA light stimulation of the VGLUT3 -fibers elicit AMPA-mediated excitatory currents in the dopamine neurons that innervate the NAc. Such stimulation causes dopamine release in the NAc, reinforces instrumental behaviors, and establishes conditioned place preference. Qi *et al.'s (151)* discovery of a rewarding excitatory synaptic input to the meso-accumbens' dopamine neurons by a glutamatergic projection arising selectively from neurons of the DRN that contain VGLUT3 suggested that new targets may be important to boost motivation in the RDS patient. Moreover, unpublished work from NIDA (the Morales Group) also found that GABA from the Substania Nigra, and possibly even co-localized in the same VGLUT3 neurons, induces regulation of the VGLUT3 neurons and as such, fine tunes the release of dopamine from the VTA to NAc.

De Azeredo *et al.* (152) correctly pointed out that glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the transformation of glutamate to GABA. In 2009, Levran *et al.* (153) found a significant association of *GAD1* with heroin dependence. Other work by Wu *et al.* (154) examining 15 SNPs of the *GAD1* gene using the Mass-ARRAY system among Han Chinese, found significant associations of some novel SNP and haplotypes with heroin dependence. In 2003, Lin *et al.* (155) reported a female-specific contribution of the GABA (A) receptor subunit genes to non-psychotic methamphetamine use disorder. Moreover, Loh *et al.* (156) reported that the prevalence of the rs211014 SNP in the GABAAγ2 receptor subunit gene was significantly different between heroin-dependent and the control Han Chinese group. Thus, scientists have helped to delineate the functioning of the glutaminergic system in addictive and reward deficiency behaviors RDS. Certainly, Glutaminergic input at the VTA impacts dopaminergic release in the NAc and other brain regions and is involved in protracted acute opiate abstinence adaptations and possibly neuroplasticity (157).

3.5. Dopaminergic system

The dopamine system has a crucial role in reward mechanisms, control of locomotion, cognition, emotion, and even the neuroendocrine system. A word search in PUBMED reveals 147,833 articles as of 12/25/2016. The genetic polymorphisms of numerous genes that encode dopamine receptors, dopamine transporters, and dopamine metabolic enzymes, influence the heritability of drug and many behavioral addictions (158-160). Work from many laboratories across the globe has been able to elucidate the role of this neurotransmitter in the CNS. It appears that there are nine dopamine receptors; however the most studied receptors, the D1, and D2 have been linked extensively to drug-seeking behavior (161-167).

The actual role of dopaminergic genetics initiated with the first association of the dopamine D2 receptor *(DRD2)* Taq A1 allele and severe alcoholism. This discovery by Blum *et al.* sparked the field of psychiatric genetics (38). Since that time, many candidate reward gene polymorphisms, especially in the dopamine system including dopamine receptors, dopamine transporter on addiction, and even obesity, have long been established (168). Particular studies maintained the hypothesis that genetic deviations in dopamine systems increase the addiction disorder risk by motivating diverse features of impulsivity or due to its capacity to impede the selection of less rewarding signals (158). In 1991, Bouthenet *et al.* (169) reported that the *DRD2* Messenger *Ribonucleic* Acid (mRNA) was copiously expressed in all dopaminergic terminal-enriched regions. Certainly, other work by Hou and Li (170) showed that *DRD2* ***Taq A1*** allele carriers were prone to heroin abuse. Additional work by Mehić-Basara and associates (171) revealed that polymorphisms of the DRD2 (rs1800497) were associated with some personality and environmental states as liability for subsequent heroin-seeking behavior. Along similar lines of investigation, Li *et al.* (85) observed that carriers of the *DRD2* ***TaqI*** A1 allele presented with considerably stronger cue-elicited cravings. Other work from China by Du and colleagues (172) performed a meta-analysis and suggested a possible association

between the dopamine transporter gene (*DAT)* polymorphisms DAT1 and alcoholism. Regarding a common mechanism between numerous addictive substances, many studies have shown the association between DAT1 and alcohol, nicotine, and even cocaine abuse and dependence (173). Furthermore, Ling *et al.* (173) described that polymorphisms of the *DAT* gene may function in the start of smoking and that there is a potential interactive effect between *DAT* and early smoking onset that adds to the vulnerability to nicotine addiction. Li and associates (174), followed by Lai *et al.* (175), showed that the Dopamine D_4 receptor (*DRD4*) polymorphisms were related to heroin dependence. Chen *et al.* (176) found that the *DRD4* exon III variable number of tandem repeat (VNTR) polymorphisms may be important in the development of opiate abuse. Additionally, Shao *et al.* (177) reported stronger cue-elicited cravings in heroin addicts who carried the *DRD4* VNTR long-type allele. Chen *et al.* (178) also found some evidence for an association between polymorphisms of the Catechol-*O*-methyltransferase (*COMT)* gene and opiate abuse. Vereczkei *et al.* (179) observed that ***TaqIA*** (rs1800497) and ***TaqIB*** (rs1079597) deviations were related to heroin addiction. Furthermore, -521 C/T SNP (rs1800955) of the *DRD4* gene presented no significant connection with a potential protective effect of the C allele. Following the application of the Bonferroni modification, ***TaqIB*** remained noteworthy, implying that the insignificant (A) allele of the ***TaqIB*** SNP is a genetic risk factor for heroin addiction. This finding is in agreement with the Blum *et al.* (38) finding of a significant association of ***TaqIB*** (rs1079597) with severe alcoholism. A literature review regarding the several associations of dopaminergic genes and many RDS behaviors, including opiate/opioid addiction, can be found in Blum *et al.* (180).

3.5.1. Dopamine catabolism genes

There are some catabolizing enzymes such as *COMT* and Monoamine oxidase (*MAO*) known to catabolize biogenic amines that effect substance-seeking behavior including opiates/opioids (181). Certainly, *COMT* plays a role that is essential for dopamine inactivation. The rs4860 (Val158Met) is a functional SNP on the *COMT* gene that brings about a three- to four-fold increase in enzyme activity and has been linked to drug dependence (182).

One study by Cao *et al.* (183) found a weak, but significant difference in the genotype of -287 A/G polymorphism of *COMT* gene was observed among heroin-dependent subjects and controls. In an earlier study, Vandenbergh *et al.* (184) showed an association between the high-activity COMT polymorphism and polysubstance abuse in a group of North American subjects. This finding was confirmed by Horowitz *et al.* (185), they found an excess of the Val COMT allele in heroin addicts compared to an Israeli control group. Chinese heroin dependent subjects with the TT genotype of *COMT* rs737866 variants had higher novelty-seeking scores, and an earlier age of onset of heroin use than subjects with the CT or CC genotype (186). There have been controversial negative findings with *COMT* and other dopaminergic-based genes such as *DAT1,* and even *DRD2,* in Chinese samples, as well as, other ethnic groups. These negative findings may have resulted from the inadequate assessment of controls who may have multiple RDS behaviors (187-193). Asians, for example, are known to carry the DRD2 A1 allele at 72% a very high prevalence and have multiple RDS behaviors (194), all of these behaviors, not substance abuse alone must be screened for the selection of controls.

It is well known that MAO can catalyze the oxidative deamination of various biogenic amines, including the key neurotransmitters: dopamine, norepinephrine, and serotonin (195). Of the two forms of MAO: monoamine oxidase A (*MAOA*) and B (*MAOB*), in 1987 both Fowler *et al.* (196) and Thorpe *et al.* (197) estimated, that 70% of neuronal MAOs are type A, which is expressed at the highest level in catecholaminergic neurons. Interestingly, *MAOA* is localized in brain regions that have been implicated in behavioral response to novel stimuli and addiction (198, 199). It is known that two *MAOA* polymorphisms, the EcoRiV polymorphism at position 1460 (200) and the VNTR polymorphism in the promoter region (201), are important because they influence enzyme activity and transcriptional activity, respectively. Studies by Cases *et al.* (202) and Shih *et al.* (203) reveal that a modest increase in dopamine due to *MAOA* knockout in mice results in a dramatic increase in aggressive traits. This finding is in agreement with others that show aggressive behavior in adolescents with substance use disorder that is linked to polymorphisms of both the *DRD2* and *DAT1* genes (204).

There is evidence that genetic variants in the *MAO* gene have been associated with risk for substance abuse (205, 206). Other work by Chinese scientists assessed the role of *MAO* gene polymorphisms in alcoholism in five ethnic groups in Taiwan. They found significant associations between alcohol abuse and *MAOA* alleles in Han Chinese. However, this finding was specific for Han Chinese, but not among the aboriginal groups (207-209). Jin and associates (210) determined that the *MAOA* gene polymorphisms affect the origination of smoking in a Chinese cohort, persons with the 1460T/O and three-repeat VNTR genotypes had an appreciably higher risk for nicotine addiction. Nonetheless, there is no substantial connection between the long repeat alleles of the *MAOA* promoter VNTR polymorphism and heroin dependence in Chinese men (211). One study by scientists in Sweden in alcoholics suggested that

carriers of the DRD2 A1 allele compared to DRD2 A2 allele have lower platelet MAO-B activity. This finding may represent a protective mechanism. Lower platelet MAO-A activity allows for higher availability of plasma dopamine and may after penetration through the blood brain barrier indeed stimulate more D2 receptors in the reward system in the brain, especially when the D2 receptors are 30-40% deficient due to the *DRD2 A1* allele (212). This increase in dopamine and its subsequent penetration through the blood –brain barrier, especially in dependent individuals having a higher permeability than non-dependent individuals, may have relevance even for acute heroin abstinence due to the proliferation of dopamine receptors induced by brain mechanisms.

3.6. Cytochrome P450 enzymes

Cytochrome P450 (CYP) is a superfamily of enzymes that metabolize clinical medications, toxins, endogenous molecules and abusable drugs. Narcotic metabolism by genetically polymorphic enzymes can have significant clinical implications for therapeutic failure, disease susceptibility and abuse liability (213). Many CYP enzymes belong to the highly polymorphic CYP2 drug-metabolizing family. Central functional pathways that are involved in drug-reinforced behavior and neurotoxicity may be modulated by CYP2 family enzymes (214). Certainly, many scientists have also identified valuable associations between the dosage and side effects of pharmacological treatments for substance abuse disorders, and the genetic polymorphism of the *CYP450* enzyme gene (188, 215, 216). Moreover, De Fazio *et al.* (217) suggested that heterozygous carriers of the CYP3A5(*)1 allele and of two single nucleotide polymorphisms in the P-glycoprotein gene (1236C/T and 3435C/T) showed poor adherence to methadone maintenance due to rapid clearance of methadone.

Along these lines, related to narcotic metabolism and following the early work by Gold that showed the sensitization of norepinephrine (NE) in the locus coeruleus during opiate withdrawal is blocked by clonidine, paved the way for understanding acute opiate abstinence (218). Van Bockstaele & Valentino (219) extended this work by reviewing the current literature showing how stress-related neuropeptides and endogenous opioids co-regulate the function of the locus coeruleus (LC) - NE structure, and how chronic morphine, or stress, interrupts this regulation.

4. OPIATE/OPIOID REWARD MECHANISM: A SNAPSHOT OF NEUROTRANSMITTER INTERACTIONS

Opioids are the most powerful analgesics utilized in a clinical setting; yet, their potent rewarding properties can cause several reward deficiency behaviors including addiction (220). The scientific challenge is to limit the development of tolerance, dependence, and addiction while retaining analgesic potency. It is understood that the first ascending pathways for pain are in dorsal horn and the medulla of the spinal cord, however, the regulation of, and sensitivity to pain, may exist in other neurological loci. In particular, the brain's mesolimbic structure the reward center, and several genes and related polymorphisms may influence both pain tolerance and sensitivity. It is hypothesized that these polymorphisms are related to a susceptibility to intolerance or tolerance for pain and that documentation of specific gene polymorphisms offers a particular therapeutic target to aid in pain treatment. It is suggested that pharmacogenetic assessment of specific candidate genes like mu receptors, PENK, and others will result in pharmacogenomic solutions tailored to the specific patient, with possible advancement in clinical results (221).

Moreover, based on the study results reviewed herein, we hypothesize that the subsequent coupling of these identified genes as described in this paper, as well as other genes and their polymorphisms, would allow for additional pharmacologically active substance-based pharmacogenomic mapping. The grouping will offer a map; a platform for the development of new DNA targeted regions, which will guide the selection of bioactive substances with possible anti-craving mechanisms and pain relief actions. In principle, the identification of reward gene polymorphisms and variations in additional physiologically-based endogenous opioid receptors and further signaling substrates will guarantee effective tailored clinical treatments for persons with atypical inborn pain sensitivity (see Figure 2). This information will undoubtedly serve as a way to combat the current opiate/opioid epidemic. The attending clinical team will be able to focus on short–term detoxification, and also target known genetic, and even possibly, epigenetic impairments that may be linked to the etiology of the initial cause of intensive opiate/opioid-seeking behavior. While the system, as Li *et al.* (222) indicated is very complex and involves almost 400 genes, we must, at least, attempt to address both the glutaminergic and dopaminergic systems (86). Without this strategy, short-term detoxification related to only focusing on Norepinephrine sensitization, by opiates, at the locus coeruleus, is short-sighted at best.

Both the rewarding and pain-relieving mechanisms of opioids rely on actions at opiate receptor sites, including, but not limited to, the mu opioid (*MOR*) receptor. However, systemic opioid reward entails MOP receptor activity in the midbrain VTA, which is comprised of dopaminergic neurons. VTA dopaminergic neurons are associated with several features of reward, including reward prediction

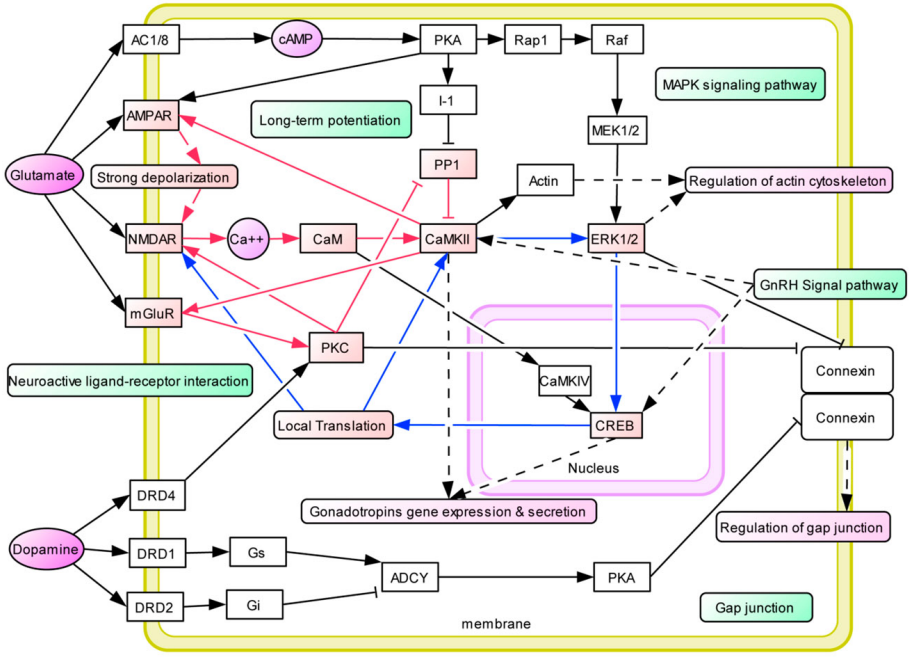

Figure 2. This is a hypothetical Common Molecular Network for Drug Addiction. Li and his associates established an addiction-gene-system that was centered on the common pathways classified in their 2008 study and protein communication data. Addiction-related genes were characterized as white boxes while neurotransmitters and secondary messengers were emphasized in purple. The common pathways are emphasized in green boxes. Associated functional modules like “regulation of cytoskeleton”, “regulation of cell cycle”, “regulation of gap junction”, and “gene expression and secretion of gonadotropins” were emphasized in carmine boxes. Many positive feedback loops were classified in this particular network. Rapid positive feedback loops were stressed in red lines, and relaxed ones were emphasized in blue lines. This detailed addiction gene map was manually constructed based on the common pathways identified showing the final common pathway being glutaminergic and dopaminergic (86, 222). Reproduced with permission from, (222)

inaccuracy, working memory, and incentive salience. It is evident that subsets of VTA neurons have diverse pharmacological characteristics and engage distinct circuits (223). It is known that both dopaminergic and non-dopaminergic circuits can contribute to VTA opioid reward. Although there is widespread acceptance of the idea that a crucial step in *MOR* reward is activation of midbrain dopamine neurons, there may be additional work required to tease out the possibility that in some cases, the involvement of dopamine may not be a necessary component of opioid reward (224-227). Certainly, we are in an early stage of truly understanding the interactive role of MOP and dopaminergic and GABAergic interactions regarding producing reinforcement, or its inhibition. We must, therefore, remain vigilant about promoting clear cut treatment and relapse prevention techniques by simply accepting the two-neuron model (glutamate and dopamine), because reward may not be solely a dopamine phenomena despite the dopamine neurons in the VTA that form a single functional group with uniform pharmacology (228, 229).

While the latter may not be the sole target for treatment success, especially within an anti-opiate dopamine restoration model, until more is understood, we should consider developing treatment methods that target the ” two-neuron model” in an attempt to at least enhance functional connectivity of the brain especially at rest (230). Li’s group showed a dysfunctional Default Mode Network (DMN) in methadone-treated patients who have higher heroin relapse risk. They found that the left inferior temporal gyrus and the right superior occipital gyrus associated with DMN had decreased functional connectivity in heroin relapsers when compared with heroin abstainers while, the right middle cingulum and the left precuneus had greater functional connectivity. Mean intensity signal, isolated from left inferior temporal gyrus of heroin-relapsers, presented a substantial negative correlation consistent with the level of heroin relapse (231).

5. DOPAMINE AND BRAIN FUNCTIONAL CONNECTIVITY

The role of dopamine in the brain at rest is an important and an emerging area of research with interest especially in Parkinsonism (231). Piray *et al*. using systematic pharmacological manipulation of dopamine D2-receptors and resting-state functional imaging in humans, found that dopamine modulates interactions between motivational and cognitive regions, as well cognitive and motor regions of the striatum. Specifically, stimulation or blockade of the

dopamine D2-receptor has opposite effects (increasing or decreasing) on the efficacy of those interactions. In fact, attribute impulsivity, in particular, was linked to the dopaminergic variation of ventral-to-dorsal striatal connectivity. Subjects with increased attribute impulsivity displayed exaggerated drug-induced increases (following stimulation) and decreased (following obstruction) of ventral-to-dorsal striatal connectivity as compared to those with little attribute impulsivity (232).

It is well known that dopamine signaling through D2 and other dopamine receptors has been associated with the regulation of reward processing, cognition, the outcomes for drug abuse, relevant aversive stimuli and is also the meaningful response to stressors (233). In fact, Peciña *et al.* discovered that a haplotype block comprised of two SNPs, rs4274224, and rs4581480, caused the hemodynamic reactions within the dorsolateral prefrontal cortex (DLPFC) during reward anticipation, and in the subgenual anterior cingulate cortices (sgACC) during continuous emotional processing. The authors suggest that these findings may contribute to susceptibility to psychopathology related to those actions, for example, the risk for mood and substance use disorders or RDS behaviors (233).

Recent evidence supports the fact that the DMN consists of a group of interconnected brain areas with correlated activity during resting state fMRI. Moreover, this activity in the DMN is associated with functional connections to the striatum and dopamine levels in this brain region (234). Specifically, it was found that a decreased dopamine state resulted in the following system alterations: lowered global and local productivity of the entire brain system, decreased regional productivity in limbic regions, decreased modularity of brain systems, and a better connection between the generally anti-correlated task-positive and default-mode systems. In support of the work, earlier studies by Sambataro *et al.* (235) evaluated a functional SNP in the *DRD2* gene, (rs1076560 G > T), which changes the 2 D2 isoform splicing in D2 short and D2 long. Within the anterior DMN, the variant GG subjects had fairly increased connectivity in the medial Prefrontal Cortex (mPFC), which was associated with striatal DAT binding. However, within the posterior DMN, GG participants had decreased connectivity in the posterior cingulate compared to T carriers. Additionally, rs1076560 genotype may indicate connectivity variances in a striatal system and these variations were associated with connectivity in mPFC and posterior cingulate in the DMN. Sambataro *et al.* (235) proposed that the hereditarily resolute D2 receptor signaling is linked with DMN connectivity and that these variations are associated with striatal function and presynaptic dopamine signaling. Moreover, regarding cognitive processing, non-carriers of the A1 allele with higher DRD2 density, display higher task-switching rates, greater prefrontal switching functioning in the inferior frontal junction region, and greater functional connectivity in the dorsal frontostriatal circuits, compared to the A1 allele of the DRD2/ANKK1-Taq A1 polymorphism, carriers (236). Also, Stelzel *et al.* carried out a DRD2 haplotype analysis and confirmed an association between high D2-density and increased switching effort (236). Accordingly, these results emphasize the importance of individual differences in striatal D2 signaling in healthy humans, leading to individual differences in changing intentionally to newly relevant behaviors.

Finally, understanding that personality traits linked to emotion processing, are, in part, heritable and genetically based, Blasi *et al.* (237) evaluated the role of the DRD2 (intronic single nucleotide polymorphism in the DRD2 (rs1076560, guanine > thymine or G > T). They found increased amygdala functioning throughout implicit processing, and higher dorsolateral Prefrontal Cortex (DLPFC) reaction during explicit processing of emotional facial stimuli in GG participants paralleled with GT. Also, the rs1076560 genotype is associated with differential relationships between amygdala/DLPFC functional connectivity and emotion control scores.

The mesolimbic dopamine network is a portion of the brain's reward circuitry. It regulates a person's reactions to rewards, like food, social exchanges, and money, and is a significant factor involved in motivational drive. Midbrain dopamine neurons prominent in the striatum are a finite part of the reward-like processes. Recent work from Ferenczi *et al.* (238) clearly demonstrated that the stimulation of midbrain dopamine neurons pushes both striatal fMRI Blood Oxygen Level–dependent (BOLD) functioning and reward-seeking behavior. Moreover, they also showed that suppressing dopamine neurons subdues functioning in the striatum, as well as other brain areas, such as the hypothalamus (238), and pushes avoidance behavior (239). They also detected striatal reactions to dopamine, as well as, the behavioral motivation to pursue dopamine neuronal stimulation and natural rewarding stimuli. Most importantly, they determined that steadily increased mPF excitability coordinates corticolimbic BOLD and electrophysiological functioning, which can, in turn, determine anhedonic behavior in individual animals (238). Interestingly, the mPFC has glutaminergic neuronal input (90)), and there is indeed a requirement to balance and optimize the fine interaction between mPFC-Glutaminergic input to striatal mid-brain dopamine, and the resultant release of dopamine at the VTA-NAc. These new findings have direct implications for the decreased functional connectivity in heroin relapsers found by Li's group (230) and our pivotal finding that KB220Z complex (discussed below) may indeed induce BOLD

activation due to a potential utilization of the mechanism of glutaminergic-dopaminergic optimization.

6. EPIGENETIC EFFECTS ON REWARD GENES CAN LEAD TO ABERRANT-SEEKING BEHAVIOR

A PUBMED word search for "epigenetics and addiction" revealed 145 articles as of 01/09/2016. However, a narrower search using "epigenetics and opiate addiction" listed only four articles (240-243). Kenney (242), pointed out that changes in gene expression are a part of addiction-related neuroplasticity, but that the methods by which, addictive drugs alter brain motivation circuits, continues to be uncertain. Moreover, MicroRNAs (miRNAs) are a group of non-coding RNA that can control the expression of many groups of protein-coding mRNA transcripts by binding to the 3' untranslated region (3' UTR) of the target transcripts and hindering their translation into the encoded protein or activating their disruption and degradation. Research has increasingly supported the involvement of miRNAs in controlling, addiction-related neuroplasticity in the brain, and in regulating the motivational characteristics of cocaine and other drugs of abuse. Along these lines, others (244) have shown that leukocytes from methadone-substituted former opiate addicts, compared with matched healthy controls, had an increased methylation of a CpG-rich island in the *OPRM1* gene which codes for μ-opioid receptors and expression impacted by global methylation site (LINE-1). Thus, higher DNA methylation was associated with chronic opioid exposure; that effect was reproduced in an independent cohort of opioid-treated patients, compared to pain patients not treated with opioids. Thus, opioids may stimulate DNA methylation. Furthermore, Doehring *et al.* (240) also found that the global DNA methylation at LINE-1 was significantly correlated with increased chronic pain. While more evidence is required, this important work suggests that opioids may be causally associated with increased genome-wide DNA methylation. Higher methylation may provide a reasonable epigenetic mechanism for opioid-induced hyperalgesia.

In other work, Abdolmaleky *et al*.(244) and others (109) suggested that the epigenetic effects on an array of genes, may result in altered gene expression across the brain reward circuitry, leading to not only risk for opiate/opioid addiction, but many RDS behaviors. The array of genes includes: *(DRD2, DRD3,* and *DRD4),* serotonin receptor *2A (HTR2A)* and *COMT, DRD1, NMDA* receptor genes *(GRIN1, GRIN2A, GRIN2B),* brain-derived neurotrophic factor (*BDNF*), and dopamine transporter *(SLC6A3).* They suggested, for example, that studies have indicated epigenetic alterations of reelin (*RELN), BDNF*, and the *DRD2* promoters that may present vulnerability to psychiatric disorders. They further point out, that the hypoactive *DRD2* alleles and the hyperactive *COMT* alleles, which damage the dopamine in the synaptic cleft, are linked to poor brain function. In an attempt to provide some clinical translational therapeutic targets, Abdolmaleky and associates (244) suggested that employing dopamine D2 receptor agonists or COMT inhibitors will be beneficial for patients with negative symptoms such as depression. These concepts have also been reported by Kato & Iwamoto (111), especially for bipolar disorders.

One interesting example from Szutorisz *et al.* (245) clearly showed that parental THC exposure was related to variations in the mRNA expression of Cannabinoid, dopamine, and glutamatergic receptor genes in the striatum, constituents of the neuronal circuitry arbitrating compulsive actions and reward sensitivity. Specifically, they showed that adolescent exposure to Δ(9)-tetrahydrocannabinol (THC) effects behavioral and neurobiological irregularities in the succeeding generation of rats as an outcome of parental germline exposure to THC. In fact, adult F1 offspring that were unexposed to THC displayed increased work effort to self-administer heroin during the period of acute heroin withdrawal, showing the long-term impact of epigenetics (245).

7. ANTI-OPIATE DOPAMINE RESTORATION MODEL: PROPOSING NOVEL CLINICAL STRATEGIES TO CHANGE THE RECOVERY LANDSCAPE

7.1. Genetic addiction risk

A flood of research studies in the field of neurogenetics followed the discovery of an association between the *DRD2* gene polymorphism and severe alcoholism. Genome-Wide Association Studies (GWAS), Whole Exome Sequencing (WES) lead to the development of Functional Genome Convergence. These developments have driven controversy nonetheless grouping these approaches with the multiple-candidate gene method has value as a very practical approach to identifying behavioral and actual, genetic allelic associations, that will eventually describe both risk and etiology.

The umbrella phrase, Reward Deficiency Syndrome was conceived of in 1996 to elucidate the common neurochemical and genetic pathways that are part of both substance and non-substance addictive, behaviors(66). Notably, the suggestion is that the actual phenotype is RDS, and deficiencies in the brain's reward cascade, either hereditary or environmentally (epigenetically) produced, are responsible for impulsive, compulsive, and addictive behaviors both substance and non-substance. Comprehension of this shared mechanism will eventually lead to improved diagnosis, treatment

and relapse-prevention. We cannot as yet proclaim that we have "hatched the behavioral addiction egg" (246), we are, however, starting to make the right inquiries. Based on numerous independent studies from around the world, it is becoming increasing clear that risk analysis of reward gene polymorphisms could provide vital information, for addiction clinicians. While many studies are investigating high and low drug metabolism, in particular for opiates like buprenorphine/naloxone with polymorphisms of the P450 system, pharmacogenetic information seems limited regarding altering clinical outcomes and the test, by itself, has questionable value. However, many strongly believe that pharmacogenetic testing is indeed relevant to clinical practice, and it will continue to be a wave of the future (247). Moreover, the development of a polygenic polymorphic test to evaluate risk for all addictive behaviors is a worthwhile endeavor, and some studies have clearly addressed this possibility for future clinical practice (248). For example, Gerra *et al.* (249) provided clear evidence that the dopaminergic system is linked to buprenorphine treatment response in heroin-addicted humans. Surprisingly, they found no difference between responders and non-responders to buprenorphine in the incidence of kappa opioid receptor (OPRK1) 36G>T SNP. Nevertheless, the incidence of dopamine transporter (DAT) gene polymorphism (SLC6A3/DAT1), allele 10, was significantly increased in "non-responder," above "responder" persons (64.9.% vs. 55.9.%). The incidence of the class of additional alleles was increased in the responder group, rather than in non-responder persons (11.0.% vs. 2.1.% respectively). These outcomes dovetail with the effort of others, presenting improved treatment results and agreement based on dopaminergic polymorphisms, where hypodopaminergic qualities facilitate an enhanced reaction throughout treatment. We theorize that carriers of the 9 allele of the *DAT1* would present an improved treatment reaction with buprenorphine because of its rapid transport function, causing a hypodopaminergic attribute. Based on these and many other studies reviewed previously, (250) we encourage further research and development of a risk stratification test for RDS behaviors (251-254). Other important work by Pearson-Fuhrhop *et al.* (255) drew data from three separate groups: 1. a discovery group of healthy adult subjects (n=273); 2. a duplication group of adults suffering from depression, (n=1,267); and 3. a group of healthy adult subjects (n=382). A genetic risk score was then produced by merging functional polymorphisms from five genes involved in synaptic dopamine availability (*DAT* and *COMT*) and dopamine receptor binding (*DRD1, DRD2, DRD3*). They found that the genetic risk score associated with depressive symptomatology and poor dopamine genetic risk scores specified decreased dopaminergic neurotransmission that anticipated increased levels of depression. The authors also simulated these results with a comparable genetic risk score based on genetic data from adults suffering from depression Based on these results, Pearson-Fuhrhop *et al.* (255) suggested that a sequence variation in multiple dopaminergic genes may influence depressive symptoms in an apparently, additive manner.

These novel advances suggest the possibility of utilizing genetic profiles to determine genetic risk for opiate/opioid dependence, especially before patients are placed on powerful pain-relieving, narcotic–like compounds.

7.2. Drug –urine testing

Substance use disorders are multi-faceted and difficult to treat with the progression of the disorder impacted by, aspects of treatment results and relapse. Assessment and quantification of these aspects are vital to decrease the disorder and increase positive outcomes. A majority of clinicians would concur that compliance with prescribed treatment medications, as well as patient abstinence from drugs of abuse throughout treatment, are significant challenges in chemical addiction programs. A 01/10/2016 PUBMED search resulted in only one article that matched the following terminology: "urine analysis and compliance to prescribed treatment medications and abstinence during *in-patient* or *out-patient* treatment" (19)**.** A briefer word search did not uncover any other articles. One article was discovered regarding non-cancer pain patients, and the authors had established that "regular urine drug testing should be a part of acute and chronic pain management whether or not the patient has any signs or symptoms of drug misuse" (256). Likewise, a 01/10/2016 PUBMED search discovered no articles that equal the following terminology: "urine analysis and abstinence to drugs of abuse during *in-patient* or *out-patient* treatment." While medications have been used and studied for over 20 years, the PLosOne article (19) is the only systematic analysis of both compliance to treatment medications and abstinence from licit and illicit drugs throughout treatment in one group investigation. However, there are arguments for, and against, standard drug urine screens regarding clinical outcome during treatment. Starrels *et al*. (257) argued that there is weak evidence to support the success of opioid treatment agreements and urine drug testing in decreasing opioid abuse by patients with chronic pain throughout treatment. In contrast, others suggest that urine drug testing is still an invaluable resource for primary care (258).

In support of continued drug urine testing during treatment, it was reported that the Comprehensive Analysis of Reported Drugs (CARD) data used in a post hoc retrospective observational study from 10,570 patients, categorized to comprise 2,919 patients given at minimum one treatment medication through 2010 and 2011. Specifically, the

initial and final urine samples (5,838 specimens) were examined; compliance with treatment medications and abstinence from drugs of abuse maintained treatment success for several patients. Paralleled with non-compliant patients, compliant patients were slightly less prone to abuse opioids, cannabinoids, and ethanol throughout treatment, though more probable to abuse benzodiazepines. Nearly 17% of the non-abstinent patients used benzodiazepines; 15% used opiates, and 10% used cocaine throughout treatment. Compliance was considerably increased in residential, compare to non-residential treatment facilities. Furthermore, in 2010, 16.9.% of the patients were abstinent initially, but not at final urine testing, whereby this diminishing abstinence declined and in fact abstinence levels increased in 2011 and this outcome was statistically substantial. Lastly, a longitudinal analysis for abstinence revealed a statistically significant upward trend of abstinence frequencies as well as a comparable, but more powerful, tendency for compliance. Interestingly, similar findings have been obtained in unpublished work, showed significant opiate abuse in compliant buprenorphine/naloxone patients. These, and other results by Jabobs *et al.* (259), provide a strong rationale to use urine drug testing as an intervention. In fact, urine drug screening may have relevance in a global arena as well, where females living in a household may provide relevant information about substance abuse in the family (260).

7.3. Gentile pro dopamine therapy: with glutaminergic-dopaminergic optimization required for long–term dopamine homeostasis

A feeling of well-being may be achieved only when dopamine is released in the nucleus accumbens at balanced “dopamine homeostatic” levels. Genetic and epigenetic abnormalities produce a dysfunction of dopamine called “dopamine resistance,” that can cause aberrant cravings. Even if we have not yet determined other potential opioid non-dopamine reward mechanisms as proposed by Fields’ group (261). Consequently, there is a necessity for a compound that can target and achieve dopamine regulation (i.e., dopamine homeostasis) is required for well-being. Further, there is a need for a non-addictive compound that can be administered to normalize brain impairments by activating the release of optimal amounts of brain dopamine at the reward site and thus, reduce excessive craving behaviors.

It is accepted that drug addiction is characterized by extensive irregularities in brain activity and neurochemistry that incorporate drug-related alterations in the concentrations of the excitatory and inhibitory neurotransmitters glutamate and gamma - aminobutyric acid (GABA), respectively. In healthy persons, these neurotransmitters activate the resting state, a default state of brain activity that is also interrupted in addiction. We are in agreement with the concept that resting state functional connectivity may have clinical relevance crucial to the development of and risk for all RDS behaviors. Studies have shown that addicted individuals tended to show decreases in the glutaminergic system compared to healthy controls (262). Moreover, select corticolimbic brain regions showing glutamatergic and/or GABAergic abnormalities have been similarly implicated in resting-state functional connectivity deficits in drug addiction (262). There are many studies showing impairments of resting state functional connectivity with alcohol, opiates, cannabis, psychostimulants, nicotine, glucose, and even some behavioral addictions, further suggesting the need to find compounds that will restore normal resting state functional connectivity (263-277).

Along these lines, it has been shown that N-Acetyl-Cysteine, compared to placebo in smokers who maintained abstinence, reported fewer cravings and higher positive effects, and concomitantly exhibited stronger rsFC between ventral striatal nodes, medial prefrontal cortex and precuneus-key default mode network nodes, and the cerebellum (264). Most recently, our laboratory proposed the combination of N-Acetyl-L-Cysteine with a well-known enkephalinase inhibitor and other pro-dopaminergic substances to combat aberrant RDS behaviors The Blum *et al.* laboratory (83) showed that a pro-dopamine complex mixture called KB220Z induced an increase in BOLD activation in caudate-accumbens-dopaminergic pathways of abstinent heroin addicts when compared to placebo 1-hour after acute administration. Also, in these abstinent heroin addicts, resting-state activity was reduced, in the putamen by KB220Z. In the second phase of this pilot study, three brain regions of interest were observed to have been significantly activated above resting-state by KB220Z compared to the placebo in all ten abstinent heroin-dependent subjects (with protracted abstinence on average of 16.9. months). Specifically, increased functional connectivity was seen 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 electroencephalography (qEEG) study results suggest a putative anti-craving/anti-relapse role of KB220Z in addiction by direct or indirect dopaminergic interaction (278-280).

Regarding support for the concept of long-term activation instead of blocking dopamine release in the NAc and other relevant brain regions like the cingulate gyrus (relapse region), Willuhn *et al*. (281) pointed out that cocaine consumption, and even non-substance-associated addictive behavior, increases as dopaminergic activity declines. Habitual cocaine exposure has been linked to a reduction in D2/D3 receptors and was also linked to decreased activation in

response to cues in the occipital cortex and cerebellum as indicated in a recent PET study by Tomasi *et al*. (282). Also, Volkow *et al*. (283) showed that stimulant–induced dopamine increases are markedly blunted in active cocaine abusers despite methylphenidate-induced changes in the ventral striatum, which were associated with intense drug craving. It is our opinion that this seemingly paradoxical response is consistent with super sensitivity, as proposed earlier with the possibility of relapse, especially in *DRD2 A1* carriers (69). In clear support for the potential for utilizing compounds that induce dopamine homeostasis in the long-term, Badgaiyan, and associates (284) recently reported, that at rest, the ligand binding potential (BP) was significantly higher in the right caudate of ADHD volunteers, suggesting reduced tonic dopamine release. During task performance, significantly lower ligand BP was observed in the same area, indicating increased phasic release. In ADHD, the tonic release of dopamine is attenuated, and the phasic release is enhanced in the right caudate. This characterization of the nature of dysregulated dopamine neurotransmission in ADHD helps to explain earlier mixed findings of reduced or increased dopaminergic activity, which may also be the case in other RDS behaviors, including risk for opiates/opioids. Certainly, it is known that carriers of the *DRD2 A1* allele have a higher chance of relapse as reported by Dahlgren *et al.* (285). Therefore, while we agree with the short-term utilization of FDA MATs to block excessive dopamine release leading to psychological extinction, we must at the same time reject long-term treatment strategies such as the use of potent D2 agonists like bromocriptine which will ultimately reduce dopamine D2 expression (286). As such, long-term even life-long treatment with gentle pro-dopamine therapy, not potent D2 agonists, may provide dopamine homeostasis. We are therefore proposing that an anti-opiate restoration strategy that can preserve dopamine activity may be a unique and effective method of relapse prevention in opiate/opioid abuse, acute abstinence, and behavioral addictions, and warrants considerably more research.

Our essential tenet is that addiction has a high genetic inheritability factor, based upon reward deficiency, a hypodopaminergic characteristic, and does not follow Mendelian inheritance (sui generis). We believe that in order to change the continued abuse of opiates/opioids by a very significant number of people in the USA, an anti-opiate dopamine restoration model AODR if adopted might have better long-term clinical outcomes (287). The studies presented in this review support following our proposed strategic treatment plan, and scientists across the globe may be inspired to evaluate our concept further.

Substantial progress can be seen in our present comprehension of several features of RDS and associated addictive behaviors including neurobiology, candidate reward and additional genes, and numerous genomic-based human and animal experiments. With the advent of neuroimaging tools, comprehension of each psychiatric disorder has improved, and vast knowledge about brain activity and behavioral functions has been acquired. Genome-wide association studies have recognized unique clusters of gene polymorphisms and may indeed find real answers by gene convergence linked to top candidate genes in the final analysis. Genome-wide studies may have failed, to date, due to poor controls, whereby these so –called controls have hidden or unscreened RDS behaviors. Perplexity in the literature has transpired because we have not accepted the right phenotype to assess, and we have not obtained disease-free controls in several of our genetically-based studies - something that is continuously problematical in behavioral genetic research.

There remains a large health concern with few treatment options permitted by the FDA and presently accessible. A new KB220 variant that can induce dopamine homeostasis a ***"Glutaminergic-Dopaminergic Optimization Complex"*** is just one part of the AODR model (see Figure 3). This model proposes that we should begin to employ genetic testing to determine risk stratification, drug urine screening for patients in both in-patient and out-patient opiate substitution programs, and provide, especially during treatment and aftercare, a methodology that will promote long-term "dopamine homeostasis."

7.4. Promising new therapies

There are other promising therapies primarily affecting cocaine abuse that could, however, have similar mechanisms, and effect opioids. We need to encourage additional research, such as the new work reported by Harraz and Snyder (288), which provides convincing evidence that Nitric Oxide-nitrosylation glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcriptional signaling mediates behavioral actions of cocaine. They propose that a new compound, CGP3466B, powerfully prevents GAPDH nitrosylation, impeding the signaling cascades and hindering both behavioral activation and the neurotoxic results of cocaine use. Also, others have used optogenetics; opsin microbial engineering and molecular-genetic models for cell-type targeting and optical strategies for guiding light through brain tissue, allowing for optical control of defined cells in living systems. Deisseroth's group (289) recently used target transcranial magnetic stimulation (rTMS) in a clinical study to help patients addicted to cocaine. In essence, they found that 69% of the rTMS-treated group of 32 cocaine dependent individuals compared with 19% of the control group in remained drug-free during the initial treatment phase, (as tracked by urine drug tests). The rTMS treated group also reported significantly less cocaine

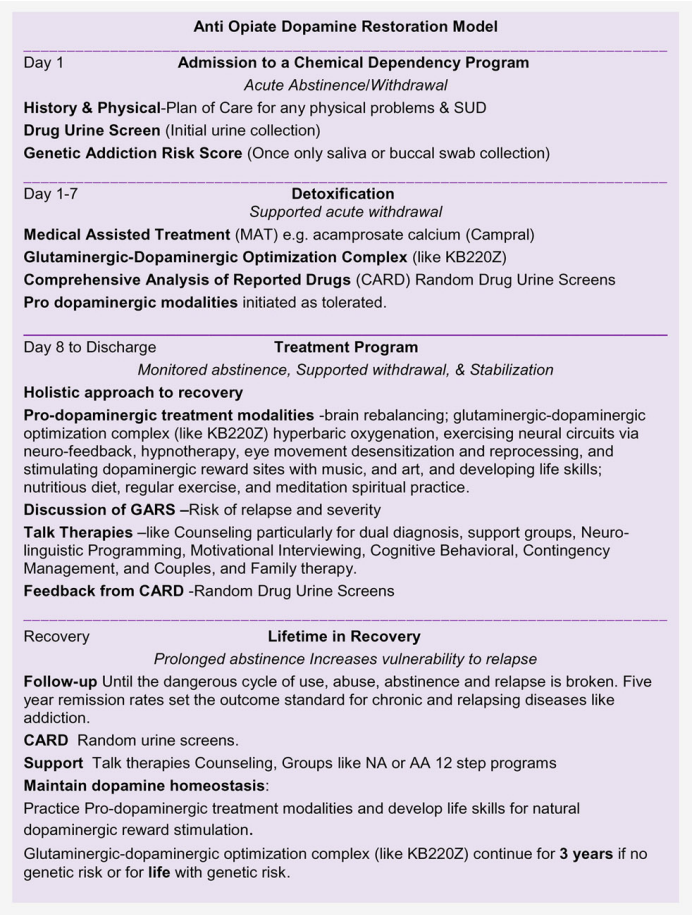

Figure 3. This is a schematic of the anti-opiate dopamine restoration model. The AODR model a suggested plan that starts when an opiate/opioid-dependent patient enters a treatment center interested in detoxification. An initial history and physical examination that includes the collection of saliva/cheek cells and urine is taken on day one. The cheek cells will be used to determine the Genetic Addiction Risk Score (GARS), and the urine will be processed for initial screening for compliance with MAT like Buprenorphine/Naloxone and absences from other licit and illicit drugs of abuse in the urine. The detoxification process will then take place over six-days. A careful tapering process that utilizes clonidine benzodiazepines and includes a glutaminergic-dopaminergic optimization complex; KB220Z and any other medications necessary to provide an easier acute opiate withdrawal is recommended. The genetic test results could be discussed with the patient following detoxification whereby the GARS test could determine risk severity and identify risk of relapse. Patients with either risk or no risk alleles can be identified. Both groups should receive short–term non-opioid MATs such as Acamprosate®, to extinguish reward from their drug of choice. Concurrently they should be treated with KB220Z to assist in the normal release of dopamine and improve resting state connectivity, cognition and reduce cravings. They should continue with routine drug urine screening, and attend self-help groups like Alcoholics and Narcotics Anonymous. Finally, in the long term, the non-genetic risk group should receive KB220Z for at least 3 years; the time considered for the brain to heal following protracted abstinence from opiate/opioid dependence, while the high genetic risk patients may need to take a glutaminergic-dopaminergic optimization complex for life, possibly customized against specific polymorphic reward genes (278)

craving. Others have proposed the utilization of TMS in refractory heroin addicts, especially by targeting the cingulate gyrus and NAc brain regions (290).

7.5. Understanding "the changed setpoint theory" of opiate withdrawal

Finally, we are cognizant of what has been termed *"the changed setpoint model"* of drug addiction (291), which is based on the altered neurobiology of the dopamine neurons in the VTA and of the Locus Coeruleus (LC) neurons during the early stages of acute withdrawal and abstinence. It is well–established that neurons of the mesolimbic reward pathways are naturally "set" to release enough dopamine in the NAc to provide a normal level of pleasure (292). Importantly, Koob & LeMoal (293) propose that opioids trigger addiction by starting a vicious cycle of altering this set point, such that the discharge of dopamine is decreased when typically enjoyable activities happen and opioids are not in the system. Likewise, an alteration in set point occurs in the LC, but in the reverse direction, such that NAc discharge rises throughout withdrawal.

In this model, both the positive (drug-liking) and negative (drug withdrawal) features of drug addiction are taken into account. A particular method that the dopamine neurons can become dysfunctional is linked to a modification of their standard resting

levels of electrical functioning and dopamine discharge (292). However, it is noteworthy that in ADHD patients and possibly in RDS, the tonic resting dopamine trait is low as discovered by Badgaiyan *et al.* (284). In this additional variant of the altered setpoint model, this resting level is the outcome of two aspects that affect the quantity of resting dopamine discharge in the NAc. Firstly, the cortical excitatory (glutamate) neurons that push the VTA dopamine neurons to discharge dopamine, and secondly, autoreceptors ("brakes" potentially GABA from the Substantia Nigra) that stop additional discharge when dopamine levels become extreme. Acute stimulation of the opioid receptors by heroin and heroin-like drugs primarily avoid these brakes and lead to a large discharge of dopamine in the NAc. Nonetheless, with frequent heroin use, the brain reacts to these consecutive great dopamine discharges by raising the amount and force of the brakes on the VTA dopamine neurons.

Eventually, the enhanced "braking," essentially unknown auto-receptors, prevent the neurons' resting dopamine discharge. When this occurs, the individual will consume even more heroin to counterbalance the decrease of normal resting dopamine discharge. When he or she ends using heroin, a state of dopamine deficiency will occur, causing withdrawal symptoms; dysphoria, pain, distress, nausea that can ultimately lead to a series of drug relapse events. One option is that the excitatory cortical pathways may create slight reactions in the VTA through the resting state, leading to decreases in dopamine. Nevertheless, when the opiate dependant individual is open to cues that generate cravings, the glutamate pathways may activate to increase dopamine and motivate a desire for a superior high. This corresponding rise in glutamate functioning will increase NAc discharge from the LC to generate a dysphoric state, prompting relapse and prolonged addiction. While this tenet seems reasonable and as proposed by Kosten & George, drugs that are antagonists to the glutaminergic system, like lamotrigine, will reduce dopamine during opiate-induced withdrawal, may not be prudent. (291), drugs that are antagonists to the glutaminergic system, like lamotrigine, will reduce dopamine during opiate-induced withdrawal, may not be prudent. In fact, in 1976, Blum *et al.* (35) using an ethanol–inhalation technique found that both L-DOPA and intracranial-injection of dopamine resulted in attenuation of ethanol-induced withdrawal convulsion scores; whereas, haloperidol, a known dopaminergic-D2 receptor blocker, was found to significantly increase convulsion scores. Moreover, using the same experimental design, they found that the acute administration of morphine, alcohol or dopamine effects a marked suppression of the convulsions created by alcohol in mice. The suppressive reaction of morphine on alcohol withdrawal in the mouse is seemingly not a result of morphine intoxication, but rather of some, particular additional contact between alcohol and morphine in the central nervous system. The assumption proposes that dopamine may serve as a modulator in the withdrawal symptoms of both alcohol and opiates/opioids based on common blocking of induction of protein into the brain RNA by cycloheximide (32, 294, 295).

Importantly Gronier *et al.* (296), proposed that activation of midbrain dopamine neurons by the systemic administration of 5-HT1A agonists does involve the inactivation of a tonic GABAergic tone, mainly in the GABAB receptors. This activation probably leads to the stimulation of a glutamatergic excitatory drive from the PFC to the VTA and an increase in glutamate release. This increased glutamate release will stimulate dopamine neurons, favorably within NMDA receptors. While there are many facets to understanding the complex nature of glutamine and dopamine interactions, the exact role of the glutaminergic drive onto VTA dopamine neurons is not understood. In fact, most recently, Baker *et al.* (107, 297) and NIDA scientists (107) reported outcomes that presented that multiplexed VTA neurotransmission may be facilitated by either the separation of dopamine and glutamate into distinctive micro-domains within a single axon or by the incorporation of glutamate and GABA into a single axon terminal. This convergence suggests actual cross–talk between glutamate and GABA in the same neuron, whereby both genetic and epigenetic factors provide the basis for the net release of VTA dopamine at, for example, the NAc.

Understanding the mechanisms involved in acute opiate/opioid abstinence provides the framework to determine therapies not just for withdrawal symptoms in the short–term, but also directed towards finding new ways to induce long-term dopamine homeostasis. The AODR model is an attempt to target both neurogenetic and epigenetic mechanisms so that dopamine balance may be maintained to achieve a normal experience of pleasure, free of addictive agents like methadone and buprenorphine (Figure 4).Lives are being lost, we must proceed, but with great caution and continue the work of addiction science until the real "magic bullets" are discovered (107, 297-301).

In the United States, 8-10% of individuals, ages ≥12 years, approximately 20-22 million persons nationwide, are addicted to alcohol or other drugs of abuse. The abuse of tobacco, alcohol, and illicit drugs in the United States causes greater than $700 billion per year in expenditures associated with crime, lost work throughput, and health care (302-305). With almost one trillion in annual productivity cost and thousands dying every day in America (25,000 last year), we must begin to understand that all addictive behaviors result from a real brain disorder. Most recently, Volkow from NIDA, Koob from NIAAA, and others (302) provided clear evidence linking addiction

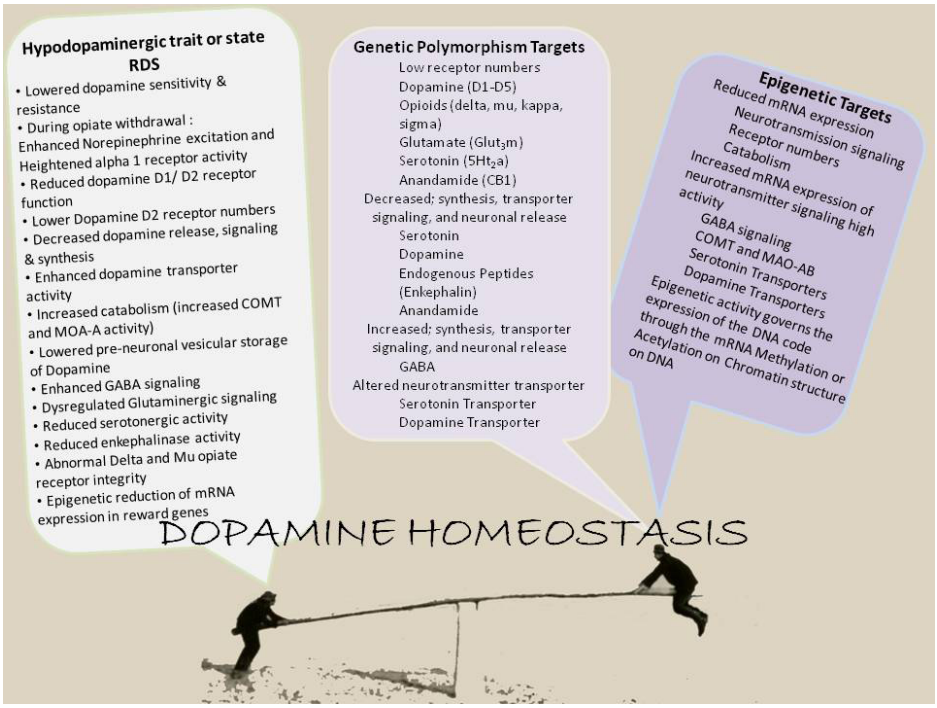

Figure 4. Therapeutic targets. Both neurogenetic and epigenetic mechanisms can be so that dopamine balance may be maintained to achieve a normal experience of pleasure.

to neurobiology. They adequately assessed evidence about the desensitization of reward circuits, which reduces the capacity to feel pleasure and the drive to persue ordinary healthy activities and undertakings. They assessed the rising power of habituated reactions and stress reactivity, which causes more cravings for alcohol and other drugs and adverse emotions when these cravings are not satiated. Also, they noted that the diminishing of brain areas involved in executive actions such as decision making, inhibitory control, and self-regulation, lead to recurrent relapse. The take home message based on the Volkow *et al.* review (302) is the suggestion that brain regions need to balance to help regulate the "normalization" of brain function. It points to a better understanding of high genetic risk and the need to fix these infractions that in some individuals are present at birth through either DNA gene polymorphisms or chromatin epigenetic alterations (environmental) passed from one generation to another (245).

Recent work from Zhang *et al*. (306) used Granger Causality Analysis to investigate directional causal influences among the brain circuits in heroin-dependent individuals (HDI)s - during opioid maintenance treatment (OMT) compared to non-opioid users. Their results revealed a weaker effective connectivity between the caudate nucleus implicated in mediating the reward circuit and other brain regions and also a weaker connectivity between the anterior cingulate cortex and medial prefrontal cortex involved in mediating inhibitory control. In contrast, HDIs-OMT exhibited stronger effective connectivity between the hippocampus and amygdala implicated in mediating learning-memory, and the anterior cingulate cortex involved in mediating inhibitory control while the putamen mediated learned habits, suggesting that the hippocampus and amygdala may propel the memory circuit to override the control circuit and drive the learned habit in HDIs-OMT. These interesting findings may provide insight into treatment targets. The authors correctly suggest that sustained neural effect of opioid dependence on methadone maintenance including hyperactivation in the memory circuit and impairment in the control circuit, support the role of the memory circuitry in relapse, and may help redefine targets for treatment. Interestingly, our findings with KB220Z showed an enhanced resting state in abstinent heroin addicts accompanied with an enhanced functionality in the control circuit (cingulate gyrus) as well as a reduced or balanced activity of the hippocampus putamen seems to help explain the delayed onset of relapse in poly-drug abusers obtained, in earlier work (84).

Finally, in alcohol dependent and abstinent subjects and rodent models surprisingly Hirth *et al* (307) found convergent evidence revealing a "hyperdopaminergic "state during three weeks alcohol abstinent in rats that seems to agree with their post-mortem human data. While this could be the fact, it would be of interest to apply the Granger

Causality Analysis as described by Zhang *et al*. (306) to provide a clearer view as to exactly which regions of the brain maybe "hypodopaminergic" compared to "hyperdopaminergic" as reported for maintained heroin addicts as well as abstinent heroin addicts in earlier studies the same group in China (83-85). Moreover, it would have been important to characterize the alcoholic cohort presented by the Hirth *et al*. study by genotyping the entire sample and then by genotype re-evaluate the results to eliminate DNA polymorphic traits. However, even until this question is resolved the best approach for targeting relapse prevention at least for opiate/opioid dependence during recovery is to balance cannabinergic- endorphinergic -glutaminergic-dopaminergic brain function by using D-Phenylalanine and N-Acetyl L-Cysteine NAC novel therapeutic ingredients as found in KB220Z.

Regarding therapies many psychiatrists treat opiate and alcohol dependent individuals with the substance Gabapentin that has been shown in some studies to reduce subsequent substance seeking (308). The effect is simply due to an attenuation of dopamine release at NAc leading to psychological extinction. The pharmacological effect of Gabapentin is due to its activation of GABA signaling. With this said, we would like to caution clinicians as to the prolonged use of Gabapentin especially in recovery, because blocking dopamine function in the long-term will induce relapse. Moreover, there is a growing concern about gabapentin misuse. In one study, Bastiaens *et al*. (309) showed that 26 percent of opiate addicted patients reported illegally obtaining, overusing, or malingering to obtain gabapentin. This effect seems to be specific for opiate addicts.

8. CONCLUSION

The steep increase in prescription opioids in the United Sates has led to a significant parallel increase in opioid and heroin misuse and fatal overdoses. Unfortunately, there has also been a drastic increase in the number of infants born with neonatal abstinence syndrome (NAS). Moreover, in the U.S., where approximately 14-22% of pregnant women receive these opioids legally, the rise in NAS may be due to prescription opioids.

This review was written in support of our proposed AODR model (Figure 3). We encourage the scientific community to, as suggested, in the DSM-5, treat acute opiate/opioid abstinence in the short-term focusing on withdrawal symptoms. Additionally, the model we are proposing is to concentrate at the same time, on treating the etiology of RDS, the long-term "hypodopaminergic" trait/state as demonstrated by reduced resting-state dopamine tone. Through required additional research, we may find new ways to enhance an optimization of glutaminergic/dopaminergic systems and induce "dopamine homeostasis" despite either a "hypo" or "hyper" dopaminergic trait/state.

9. ACKNOWLEDGEMENTS

The initial idea for this paper was inspired by a conversation between Drs. Gold, McCall and Jacobs. The original draft was developed by KB, MG, WJ and VM. Subsequent drafts were provided by MF, DB, KD ZD and RB. All authors made contributions to many aspects of this manuscript and subsequently approved the final work. Any and all concerns or questions of accuracy or integrity of any part of this intensive review has been resolved. The authors are very appreciative of Margaret A. Madigan for her expert edits of the entire manuscript and development of Figures 3 and 4

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Abbreviations: RDS: reward deficiency syndrome DSM-5: diagnostic and statistical manual-5; AODR: anti-opiate dopamine restoration; JCAHO: joint commission on accreditation of health care organizations; CDC: centers for disease control and prevention;

HNA: Harrison narcotics act; MAT: medication-assisted treatment; FDA: U.S. food and drug administration; DAWN: drug abuse warning network; VTA: ventral tegmental area; tVTA: tail of the VTA; GABA: gamma-Aminobutyric acid

DRD2: dopamine D2 receptor gene; NAc: nucleus accumbens; fMRI; functional magnetic resonance imaging; 5-HT: 5-hydroxytryptamine; MOR: mu-opioid receptor; AC: anterior cingulate; rACC: rostral AC Cortex; CB1: central cannabinoid; CB2: peripheral cannabinoid; CNS: central nervous system; CNR AAT: n-repeat microsatellite of the CNR1 gene; BOLD: Blood Oxygen Level–dependent; DRN: dorsal raphe nucleus; VGLUT3: gene encodes a vesicular glutamate transporter; SNP: single nucleotide polymorphisms; mRNA: messenger *ribonucleic* acid; COMT: catechol-*o*-methyltransferase; DAT: dopamine transporter gene; VNTR: variable number of tandem repeat; CYP: cytochrome P450; NE: norepinephrine; CL: locus coeruleus; PENK: Proenkephalin Gene ; DNA: deoxyribonucleic acid; sgACC: subgenual anterior cingulate cortices; mPFC: medial prefrontal cortex; DLPFC: dorsolateral prefrontal cortex; GAPDH: nitric oxide-nitrosylation glyceraldehyde-3-phosphate dehydrogenase; rTMS: transcranial magnetic stimulation; NMDA:N-methyl-D-aspartate receptor; THC: Δ(9)-tetrahydrocannabinol; F1: first offspring generation ; GWAS: genome-wide association studies; WES: whole exome sequencing; OPRK1: kappa opioid receptor; CARD: comprehensive analysis of reported drugs; NAC: d-phenylalanine and n-acetyl l-cysteine; NAS: neonatal abstinence syndrome.

Key Words: Review, Acute Opiate, Opioid Abstinence, Withdrawal, Anti-Opiate Dopamine Restoration Model, AODR, Functional Connectivity, Dopamine Homeostasis, Endorphinergic, Glutaminergic and Dopaminergic Reward Mechanisms.

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