Psychology Research and Behavior Management

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REVIEW

Chronic Methylphenidate Effects on Brain Gene Expression: An Exploratory Review

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Abstract: Methylphenidate (MP) is a psychostimulant commonly prescribed for individuals with attention deficit hyperactivity disorder (ADHD) but it is also taken with and without a prescription for performance enhancement. Prior research has characterized the effects of MP on behavior, cognition, and neurochemistry. This exploratory review covers the uses of MP and examined the effects of MP on gene expression in the brain following exposure. Overall, MP causes a wide-spread potentiation of genes, in a region-specific manner; consequently, inducing neuronal alterations, such as synaptic plasticity and transmission, resulting in observed behaviors and affects. Monoamine neurotransmitters and post-synaptic density protein genes generally had a potentiating effect in gene expression after exposure to MP.

Keywords: addiction, substance abuse, methylphenidate, gene expression, monoamine neurotransmitters postsynaptic density proteins, reward deficiency syndrome

Introduction

History and Uses of MP

Methylphenidate (MP), also marketed as Ritalin®, is the treatment of choice of attention deficit hyperactivity disorder (ADHD). MP was first synthesized in 1944, then subsequently patented in 1954; however, it was not until 2002 when the FDA approved it for the treatment of both ADHD and narcolepsy.[1]

MP Legally Prescribed for a Number of Medical Conditions

ADHD. MP is prescribed for individuals diagnosed with ADHD, which is a neurobehavioral disorder associated with lack of attention, hyperactivity, and impulsivity, according to the National Institute of Mental Health.[2–6] While it is an important prescription medication for ADHD, it is also misused, abused, prescribed off-label, and taken for performance enhancement.[7] According to the CDC, it is estimated that, as of 2016, 6.1 million children in the US were diagnosed with ADHD, which is 9.4% of all children.[8] One study used functional magnetic resonance imaging (fMRI) to isolate the regions of activity in the brains of individuals with ADHD, through neurophysiological tests.[9] Results showed an increase in activity, specifically in the frontostriatal regions of the brain.[9] The frontostriatal regions of the brain are the areas of the brain associated with inhibitory control, including motor, limbic, and cognitive control, along with attention and focus control.[6,10] ADHD can be diagnosed from childhood to adulthood; yet diagnosis in adults is more closely accessed to limit abuse.[9] Continually, ADHD patients using MP may experience adverse side-effects, although usually well tolerated. These include, but are not limited to, insomnia, nervousness, anorexia, and cardiovascular complications such as hypertension.[11]

Narcolepsy. Narcolepsy is a neurological sleep disorder affecting about 0.05% of people, generally with onset before the age of 25 in the US.[12] Narcolepsy is characterized by excessive sleepiness, "…irresistible sleep attacks, cataplexy (sudden bilateral loss of muscle tone), hypnagogic hallucination, and sleep paralysis".[6,13] There are two types, type one

Received: 7 November 2023
Accepted: 5 February 2024
Published: 15 February 2024

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and two narcolepsy, (Na-1) and (Na-2), respectively.[12] Moreover, it has been found that MP has improved the conditions for individuals living with narcolepsy.[14] Overall, MP, through its effects on catecholamine release, results in changes in heart rate and constriction and improvements in REM sleep in narcolepsy patients.[15]

MP non-prescribed and misuse. MP is considered a narcotic, recognized as a class II controlled substance and, thus, has a high potential for individuals developing physical dependence.[16] Previous studies have indicated that MP is a relatively safe treatment when taken within intended use and prescription. However, one study included that 8.9% of participants of a national US surveyadmitted to having a MP prescription.[17] Moreover, MP is abused specifically for its cognitive effects rather than its intended use. And, in terms of unsolicited use, chronic safety has not yet been established.

While addictive behaviors substance and non-substance is a multi-faceted disorder impacted by both genetic DNA antecedents and epigenetics, one important area of research involving brain function reveals that in-part these unwanted addictive-like behaviors may be a function "of neuroplasticity" and it has been shown that compulsive drug-seeking behavior/developing addictions can be directly associated with increased neuroplasticity.[18,19] Drugs, like MP, have also previously been studied and linked to potentiating the expression of genes and proteins associated with neuronal plasticity.[20] This increase in the neuroplasticity can alter the synapses to respond and adapt to the drug use and increase reward seeking. Therefore, adolescents, for example, college students, are neurologically at higher risk of abusing and forming addictions due to their age.[19]

As of 2018 in the US, nearly 16 million adults had been found to have used prescription stimulants, such as MP.[21] Other contributing factors to the susceptibility of abuse include individuals seeking a specific outcome onset from using MP. This may include individuals looking to lose weight. MP's adverse effects can cause weight loss and loss of appetite.[6] Other individuals susceptible to abusing psychostimulants would include those aiming for performance enhancement, as suggested by the NIH drug facts.[4,6,7] It was determined as of 2017, nearly 30% of college students, who were included in a survey, admitted to illicit use.[22] MP increases attention span and focus, resulting in many college students looking to abuse the drug for their academic purposes.[6,7,19,23,24] This misuse has not only increased, but with it so has the number of ADHD diagnoses and therefore the prescription of MP.[17]

MP abuse has dramatically increased since 1990, according to the Drug Enforcement Administration (DEA), with the National Survey on Drug Use and Health (NSDUH) recording 3.5 million people; equivalent to 1.3% of the population, of 12 years and older using MP for non-medical purposes as of 2015.[25] Another study, looking at the efficacy of MP, determined the variables that could increase the abuse through analyzing other papers and brain imaging.[26] The dose, pharmacokinetics, the individual, and the context of the MP were pinpointed for their contributions to the increase in the potential abuse.

Illicit users of MP generally are not using MP for its intended purpose. Rather there are multiple reasons why individuals may illicitly abuse MP. First most, although MP may counteract symptoms of ADHD, it has an opposing "amphetamine-like" effect on those without ADHD.[1,6,16] Yang et al found the effect of acute MP to have similar behavioral effects to amphetamines, MDMA/ecstasy, on female mice, and cross-sensitization did occur between the two drugs.[27] Another study conducted by Yuan et al used MP exposure in female rats to study the changes in neurodevelopment due to cross sensitization when exposed to a dose of amphetamine based on changes in environment and differences in age. This study concluded that the intensity of the cross sensitivity of MP and amphetamine is most significantly altered by environmental changes and adolescent exposure.[19] This suggests the increased risk of developing drug dependency in adolescence due to early exposure to MP.[19]

Generally, users are looking for the intoxicating "high" that can be associated with taking MP. By releasing and increasing synaptic dopamine, intravenous users have reported mirror-like effects to that of cocaine, by the binding of MP with dopaminergic pathways.[1]

Methods

This narrative review utilized various search engines, including: PubMed, google scholar and the University at Buffalo's Library database, to obtain articles. These studies were all peer-reviewed published research dating from 1998 to the present. Studies were required to (i) be peer-reviewed publications, (ii) have a case-control design, and (iii) have performed valid statistical analysis tests and indicated significance. Keywords and phrases included: Methylphenidate, chronic exposure, gene expression, neurotransmitter genes, or postsynaptic density genes. Specific genes examined

Table 1 Monoamine Receptor Genes Affected by MP

Gene / Subunit	Association	Brain Region	Result with MP	References
Grik2	Mutations associated with the motor and higher order cognitive function deficits	Dorsolateral, ventromedial caudate putamen and in the pre-frontal cortex	Long-term exposures cause ↑	[28]
Htr7	Association with impulsivity behaviors	Nucleus accumbens, pre-frontal cortex	Basal behavioral impulsivity ↓	[29]
ADRA2A	Associated with inattentiveness	Pre-frontal cortex, locus coeruleus, amygdala, hippocampus, septum	↑ of inattentive and hyperactive-impulsive symptoms	[30]
GABA	Association with impulsivity behaviors and inhibitory control	Pre-frontal cortex, medial pre-frontal cortex.	↑ age-dependent response responsivity and ↓ GABA levels in adulthood, impulsivity	[31]

Notes: ↑ upregulated/potentiated expression. ↓ downregulated expression. ∅ no detection or change.

included Htr7, Grik2, Zif268/Egr1, Homer1a (Homer scaffold protein 1a), and the other genes (shown in Tables 1–3) were based on the extent of the prior research found and related to neurotransmitter monoamine genes or postsynaptic density genes. In addition, specific transporters, receptors, and neurotransmitters such as dopaminergic, norepinephrine, and serotonergic systems. This review focused on the effects of MP on gene expression and associated neurologic processes such as neurotransmitter function.

Mechanism of Action

The mechanism of action of MP is to inhibit the transporters of different neurotransmitters, such as dopamine (DAT), norepinephrine (NET), and serotonergic (SERT) reuptake transporters (see Figure 1). Therefore, inhibiting the transporters blocks the reuptake, which in turn increases the synaptic concentrations of the neurotransmitters.[44–46] This is occurring in the striatum, with prior research identifying that the striatum is the area of the highest MP uptake.[11] Additionally, Manza et al identified that the type of receptor, dopamine 1 receptors (DRD1) vs dopamine 2 receptors (DRD2), affected the effect of MP; compared to DRD2, DRD1 had increased brain activity and excitation.[47] By this, MP is acting as a stimulant to increase the synaptic neurotransmitters, to provide the analgesic and/or therapeutic effects of the psychostimulant.

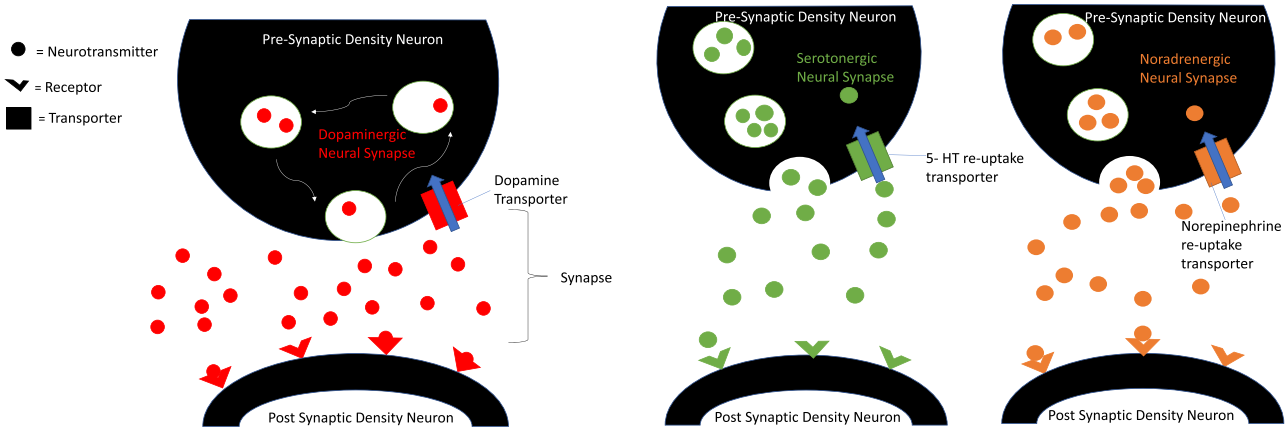

Figure 1 MP pharmacological mechanism of action. A schematic depiction showing presynaptic neurons releasing neurotransmitters into the synapse, the post-synaptic neuron and receptors, and resulting in the elicitation of a response. MP inhibits the reuptake of these transporters on the pre-synaptic neuron, causing the synaptic concentrations of these neurotransmitters to be increased. The image on the far left, in red, shows the effect of MP increasing dopamine in the synaptic cleft. The middle image, in green, shows the same process that occurs with serotonin increasing in the synapse, and the far right, in orange, shows norepinephrine increasing in concentration in the synapse due to MP inhibiting the reuptake transporters.

Table 2 Common Post Synaptic Density Genes Affected by MP

Gene / Subunit	Association	Brain Region	Result with MP	References
AMPA	Regulating synaptic transmission at excitatory synapses	Basal ganglia	High dose MP suppressed their receptor-mediated excitatory synaptic currents (EPSCs) via pre-synaptic mechanism	[32]
NMDA	Regulating synaptic transmission at excitatory synapses	Basal ganglia	Low dose MP ↑ ⇑ receptor-mediated EPSCs significantly via post-synaptic mechanism; ↑ ⇓ by high dose MP via pre-synaptic mechanism	[32]
Homer1a	Associated with neural in corticocortical circuits and synaptic plasticity	Hippocampus, PFC, striatum	↑ cognitive function	[33,34]
Zif268/Egr1	Associated with neural plasticity in corticocortical circuits and acts as a transcriptional factor	PFC, striatum	↑ Long-term gene blunting	[33,34]
Shank2	Associated with remodeling and dendritic spine formation, related the AMPAR and NMDAR functioning	Striatum, hippocampus	↑ Cognitive functioning and structure altered	[35]

Notes: ↑ upregulated/potentiated expression. ↓ downregulated expression. ∅ no detection or change.

Table 3 Summary Results for MP on Gene Expression

Gene(s) Regulated	MP Dosage	Acute or Chronic	General Result	Reference
Neurotransmitter receptor: Grik2, Htr7, (Adr) α1b, GABAA, etc. PSD family: Homer1, Shaknk2, and MAGUK Other: Homer2, Homer3, PSD-95, Htr2a, Drd1, Drd2	0 or 2 mg/kg I.P. once daily	Subchronic; 16 days	Neurotransmitter receptors ↑ PSD family ↑ Other: ∅	[29]
Grik2, Htr7	2 mg/kg/day	Chronic	↑ of both	[28]
Zif268/ Egr1, c-fos, and Homer 1a with agonists: CP94253, 5-HT1B	MP and FLX 5 mg/kg	Acute	5-HT1B agonist: ∅ Homer 1a, ↑ zif268 and c-Fos	[36]
Htr-7, Grik2CAMKII, C-FOS	3 mg/kg MP or vehicle	Acute effects vs Chronic; 14 days	↑ of Htr7, Grik2 Gene expression; MPH ↓ expression of DA D1/D2 receptors, CAMKII and c-FOS	[35]
Zif268 and Homer 1a with D1R antagonist: SCH-23390	2 μg/kg or 10 μg/kg I.P.	10 days	SCH-23390: ↓ both	[37]
ADRA2A	0.5, 0.65 mg/kg	Acute	∅ for Arp2 mRNA or protein expression	[30]
IRSp53, Cdc42, Arp2 (genes associated with neuroplasticity)	2.0 mg/kg MP, twice daily for 15 days	Chronic; 15 days	IRSp53, Arc, IPSp53, Cdc42, Arp2 expression ↑ Arc and IRSp53 were ↓ in the cerebellum	[20]
Potassium channels (TASK-1, TASK-5), intracellular junctions, connexin30, neurotransmitter receptors (adrenergic alpha 1B, kainate 2, serotonin 7, GABA-A); Homer 1, MAGUK MPP3, Shank2	2 mg/kg	Sub-chronic; 16 days	↑ suggesting the therapeutic effects on behavioral plasticity but potentiating genes involved with synaptic plasticity and dendritic formation of dendritic spines	[38]

(*Continued*)

Table 3 (Continued).

Gene(s) Regulated	MP Dosage	Acute or Chronic	General Result	Reference
Homer 1a, Homer 2a/b, Homer 1b/c	2 mg/kg oral	Sub-chronic; 14 days	Homer 1a and Homer 2a/b ↑ in PFC, ∅ Homer 1b/c	[39]
Zif268/ Egr1, Homer1a	6 daily injections of 5 mg/kg; 5 mg/kg FLX; or MP+FLX for 14 days after cocaine challenge (25 mg/kg)	Chronic MP, acute cocaine administration; 14 days	MP alone- modest gene building, FLX alone – no effect Combined group – had pronounced potentiation of MP-induced blunting for both genes	[33]
Zif268/ Egr1, c-fos	5 mg/kg FLX, 2–5 mg/kg MP	Acute, 7 days	↑ both in the striatum and cortex (in 23 sectors)	[40]
Zif268/ Egr1, c-fos	5 mg/kg FLX, 2–5 mg/kg MP	Acute, 5 days	↑ striatum and cortex in the sensorimotor parts; regional specificity	[41]
Zif268/Egr1, Homer1a	0.5, 2, 5, 10 mg/kg MP, 0.02% ascorbic acid, 1 mL/kg, i.p.	Acute	↑ expression of both Homer 1a and Zif 268 in the cortex and striatum	[42]
NMDAR- and AMPAR-EPSCs	0.5mg/kg MP, 10 mg/kg MP	7 days	Low-dose MPH (0.5 mg/kg) ⇑ NMDAR-mediated excitatory synaptic current (EPSCs) via adrenergic receptor activation. High-dose MP ⇓ both NMDAR- and AMPAR-EPSCs	[32]
Glutamate subunits (NR1, NR2A, NRB2)	1 mg/kg IP	Acute	MP ↓ NR1 and NR2B, but not NR2A subunits, in juvenile prefrontal cortex. MP also ↓ NMDAR-EPSCs but increased AMPAR-mediated short-term plasticity. • MP ↑ the probability of LTP induction, but had a small effect on LTD.	[43]

Notes: ↑ upregulated/potentiated expression. ↓ downregulated expression. ∅ no detection or change.

MP Effects on Monoamine Neurotransmitters

MP is commonly known for having the highest affinity for dopamine.[48] The noradrenergic system is associated with the body's "fight or flight" response, preparing the body for a stressor, but also involved in attention and working memory. Noradrenaline, or better known as norepinephrine, follows a similar pattern to that of dopamine, however binding to three potential receptors; including alpha1, alpha2, and beta receptors.[49] When MP inhibits the reuptake, the noradrenaline concentration in the synapse or extracellular area increases. For an individual with ADHD, this would help increase focus and attention span.[1,24,45] However, norepinephrine is associated with our fight or flight, sympathetic response; therefore, this increase in norepinephrine concentrations can also contribute to increased stress or anxiety.[50,51] This can be considered an adverse side-effect and is found to be elevated in ADHD individuals; studies have proven a correlation of norepinephrine on oxidative stress.[50]

As for increased serotonin concentrations, this has been suggested to promote calming effects by reducing locomotor activity, as seen in dopamine transporter knockout (KO) mice. Again, these mice have increased dopaminergic tone, making them more active compared to normal, untreated mice, to reflect the brain activity of cognitive disorders, such as ADHD.[52] As seen in an analysis of the DAT knockout mice, there lacks evidence that MP directly increases brain

serotonin, but rather the, "the DAT-KO mice clearly does confirm the important role of serotonin in modulating DA's regulation of locomotor activity".[48] This emphasizes there is a relationship between MP and serotonergic transmission but indirectly through the regulation of dopamine where the dopamine transporter is blocked leading to a synaptic surge in dopamine levels and tone to reflect cognitive diseases and their associated behaviors.[53–55]

Effects of MP on Behavior

The impulsivity, hyperactivity, and lack of attention that is associated with ADHD is attenuated by MP.[3,56] These behaviors such as impulsiveness, hyperactivity causing distractedness, and lack of concentration, will be reduced and the patient's ability to pay attention will be improved; these behaviors are measurable or identifiable symptoms as seen through changes in mood such as depression, euphoria, or agitation, as well as physical symptoms such as anxiety-related, dizziness, drowsiness, restlessness, staring, etc.[1,6,56] In contrast, individuals using non-prescribed MP will experience the opposite, amphetamine-like, heightened effects, depending on the route of administration, and an increased risk for misuse and abuse.[1,16] Since MP causes synaptic dopamine levels to increase, it would therefore be in excess in a non-ADHD individual, causing intense emotions like enhancements in cognition, euphoria, or reward-seeking.[24] This is considered substance abuse and has been studied in relation to negative emotions such as depression or anxiety as well.[57] Blum et al reviewed how negative emotions lead to the abuse and dependence of alcohol consumption.[57] Another study by Gill et al also found that, when adolescent rats were isolated and chronically treated with MP, they exhibited negative emotions.[58] Hence, when exposed to ethanol, they were found to have had a greater consumption rate; this is further demonstrating the effect MP can have on substance use and dependence.[58]

MP Animal Models

Animal models can vary on the dose, duration of use, age, sex, species, and strain. Rodents are widely used to examine MP effects on behavior. For example, research assessing acute vs chronic exposure of MP in male rats looked at multiple different variables, including three different strains of rats and doses.[34] The effects in the acute study with three different doses showed a dose-dependent relationship. The chronic portion identified a similar dose-dependent relationship and activity differences for the different strains of rats.[34] This same study also looked at the age in terms of adolescence vs adults. Adolescence was defined as "… the period of development involving numerous neuroplasticities throughout the central nervous system (CNS)".[34] Dose-dependent and sex-dependent rat studies are also commonly used in MP studies.[59–61] The majority of studies included in this review used male rats (Table 2); and, according to the 2016 National Health Interview Survey (NHIS), boys are more than two times more likely to have ADHD than girls.[62]

ADHD phenotype rats. In order to be able to study the short- and long-term effects of MP, multiple rat models have been developed. Not only are rats or mice used to further understand the effects of MP, but a strain of rat was specifically bred to mimic the impulsivity and hyperactivity that would be seen in individuals with ADHD, spontaneously hypertensive rats (SHR).[63,64] SHR rats are the most popular rat model of ADHD.[65] In one study, using a variable interval of 30 seconds and a conjoint variable interval of 60 seconds, the delay of reinforcement was found to be higher in the SHR rats compared to control rats. This was mostly comprised of short interresponse times, suggesting the SHR rats have a more pronounced delay.[66] Using these SHR rats allows researchers to study the effects of MP on ADHD-like behaviors. Not all studies studying MP use SHR rats if they are simply looking at the neuropharmacology and behavior effects of MP. For example, several studies examine the effects of MP on age-dependent effects in the striatum of healthy rats with the goal of understanding MP exposure effects.[22,59–61,67–75] Therefore, untreated, healthy rats are used to understand the effect MP would have other than and in addition to individuals with ADHD.

Results

Effects of MP on Neurotransmitter Genes and Receptors

The majority of the genes that are affected by MP specifically involve genes involved in signal transduction, transport, transcription, and neural transmission.[29] The following sections review the effects of MP on various categories of gene expression.

Monoamine Neurotransmitter Receptor Genes. Monoamine neurotransmitters, more commonly known as classical neurotransmitters, include dopamine, norepinephrine, serotonin, and epinephrine, among many other known molecules and pathways[76] (see Table 1). MP inhibits the dopamine transport receptors, which then causes the extracellular synaptic dopamine levels to increase. This increase in dopamine contributes to a reduction in ADHD-like behavior, including behaviors such as hyperactivity, impulsivity, and lack of concentration.[24,45,77] Since methylphenidate causes an excess of dopamine in the synaptic synapses, if a person who does not have ADHD takes this medication, their dopamine is produced in excess, causing unwanted hyperactive-like induced behaviors.[24]

Other known neurotransmitter genes and subunits have been identified and further studied in combination with MP (Grik2, Htr7, Adrα1b, ADRA2A, GabRγ1, and the GABRβ3 subunits) (See Table 1). Glutamate ionotropic receptor kainate type subunit 2 (Grik2) acts as an excitatory neurotransmitter receptor and has been recognized in prior studies for mutations associated with motor and mental coordination and functional deficits, similar to that of individuals with ADHD.[78] As for Serotonin receptor 7 (Htr7), gene expression is correlated to MP effects on serotonergic receptors. Htr7 is a well-known subunit studied with MP, associated with reducing impulsivity.[28,29] Next, looking at the adrenergic genes and their involvement in attention and inattentiveness has previously indicated that, when MP is introduced, there are improvements in attention and behavioral impulsivity due to MP effects on the adrenergic α2A receptor gene (ADRA2A). A clinical study with a twice daily dose of at least 0.3 mg/kg MP assessed MP effects on ADHD symptoms.[30] This study referred to prior studies indicating that blocking this adrenergic α2A receptor affected MP's affect along with increased ADHD-correlated symptoms.[30] Lastly, low levels of gamma-amino butyric acid (GABA) is related to impulsivity and inhibitory control in ADHD individuals.[31] Patients with ADHD have also been found to have lowered GABA levels; therefore, MP increases the GABA levels.[6,79] However, if GABA levels remain above normal, as a result, dopamine would be reduced; Puts et al determined that, in the striatum, altered levels of GABA would then affect other neurotransmitter functions, like that of dopamine and norepinephrine.[79] Therefore, the dose of MP should be monitored to balance these opposing forces to increase GABA without reaching the point of reducing dopamine in patients with ADHD. The GABAergic system is hence associated with reduced impulsivity and improves inhibitory control when exposed to MP. The earlier mentioned study by Solleveld et al further highlighted an age-dependent relationship in which MP exposure starting in adolescents affected the GABA+ levels more and longer compared to exposure starting in adulthood; GABA+ was water-scaled and the plus is indicating that relationship.[31]

Serotonin

This study uses a sub chronic MP exposure (16 days, of a 0 or 2 mg/kg IP), a genome wide sequencing, and real-time reverse transcription polymerase chain reaction (RT-PCR) to analyze gene expression.[29] Grik2, Hrt7, adrenergic receptor alpha 1b (Adrα1b), $GABA_A$, gamma-aminobutyric acid receptor γ1 subunit (GabRγ1), and $GABA_A$ receptor β3 subunit transcriptors were all upregulated, significantly (see Table 2). However, for the adults striata, which had been chronically exposed, only Grik2 and Htr7 were upregulated.[29] This duration of the chronic MP exposure continued for 1 month; which again is a significant time frame considering the lifespan of the rat. Notably, the genes, Grik2 and Htr7 were similarly upregulated in both the adolescent and the adult rat striatal regions. This upregulation was detected in the striatal complex and could be due to the chronic MP exposure increasing the striatal availability of dopamine after the expression of ionotropic receptors and G-protein coupled receptors (Grik2, GabRγ1, gamma-aminobutyric acid receptor beta 3 subunit (GabRβ3).[29] Again, the striatal complex was focused on for its previous indication of induced alterations in genes as seen in other, earlier studies.[29] See Figure 2 for a summary of these results.

With respect to Htr7 and the Grik2 neurotransmitter receptor, rats exposed to chronic MP during their adolescence resulted in significant upregulated expression for both Grik2 and Htr7, compared the control group.[28,35] As for the adult rats, the prior exposure in adolescence causes the protein levels to be again potentiated. Htr7 has been previously associated with regulating behavioral impulsivity in individuals with ADHD, which was evaluated using the antagonist of Htr7, SB269970, to measure if the chronic effects of MP would be affected.[28] According to their analyses, Grik2 was expressed in the dorsolateral, ventromedial caudate putamen, and in the pre-frontal cortex; while the Htr7 showed only significance in the nucleus accumbens (Acb) and pre-frontal cortex.[28] These results are indicating region specificity for these genes (in the forebrain). Rats at postnatal days 30 to 34 were administered 2 mg/kg/day or saline; two of eight rats would be used for an ex vivo assessment, whereas four, two MP and two saline, would be tested for impulsivity after about 3 weeks.[28] An "intolerance-to-delay" test was performed to test motivation

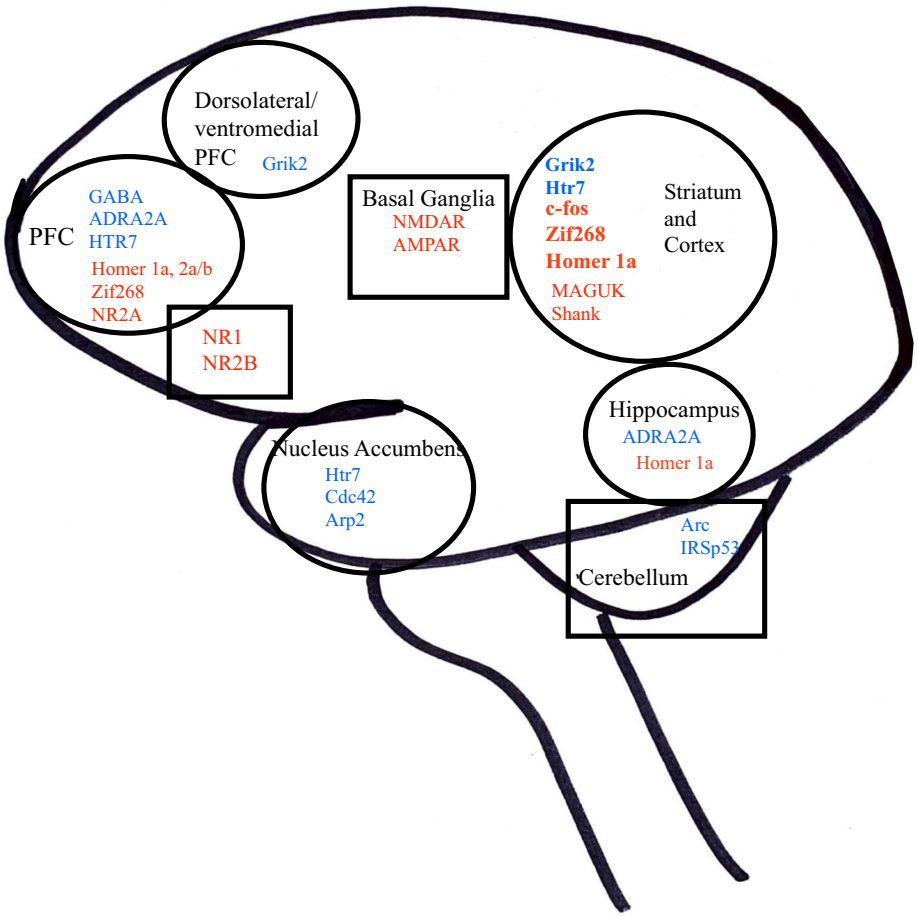

Figure 2 MP on gene expression, both region and gene specific: circle = upregulated, square = downregulated; black = region of the brain, blue = monoamine neurotransmitter genes and receptors, red = postsynaptic density protein genes.

and working memory. To measure this, on the three highest days, the rats were given a saline control or the SB269970, Htr7 antagonist, at 3 mg/kg/day.[28] The antagonist alone did not have an effect on the control; while on MP it causes significantly lower choices in the intolerance to delay portion of the study. In the naive rats, the antagonists successfully increased impulsivity as expected; preventing and resisting the effects MP has on Htr7.[28] As introduced above, the results also indicated the regional-specific upregulation due to chronic MP exposure.

MP effects on the serotonin receptor subtype, 5-HT1B, on gene regulation, were previously examined using the selective serotonin 5-HT1B receptor agonist, CP94253, on the common genes Zif268/Egr1, c-fos, and Homer1a.[36] The agonist's role is to potentiate the serotonin reuptake subunit, which will then affect the striatal availability and serotonin uptake. In combination with MP, this agonist and gene in particular were examined with specific interest in whether it would have an effect on combination exposures, such as the MP and fluoxetine (FLX), a serotonin uptake inhibitor (SSRI), together contributing to its heightened effects. To do this, male rats were injected with the agonist, 5-HT1B (CP94253) or a vehicle; 15 minutes later they received another injection of either the vehicle, MP alone, or MP with FLX combination injection of 5 mg/kg.[36] FLX alone was not tested on the genes due to previous studies indicating no effect.[36] The 5-HT1B agonist was not detected to have an affect on the Homer1a, through MP induced gene regulation; however, it did potentiate rostral, middle, and caudal striatal Zif268/Egr1, c-Fos, and locomotor activity.[36] For locomotor activity, it was measured in an open field for 40 minutes; again emphasizing the MP induced gene expression. Changes in locomotor activity, although not a direct relationship, can rather be a causal one to changes in gene expression. As previously discussed, individuals without ADHD taking MP would have heightened effects. In this case, these rats had increased locomotion as a result of hyperactivity due to the MP exposure. As for the analysis of the brains, first in situ hybridization histochemistry, a process of removing and preserving the rat brain, and an

autoradiogram, measuring the areas of gene expression in the right and left hemispheres.[36] Generally, this research study looked to find the mechanisms involved that allow for the MP to potentiate gene expression in combination with SSRIs.

Dopamine

MP is most well recognized for its effect on dopamine in the brain.[80] Rats given MP had increased DAT protein levels in the nucleus accumbens[81]. Another study used Positron Emission Tomography (PET) showed that, after 60 minutes, the basal ganglia showed more than half of the DAT blocked.[82] Therefore, MP affects dopamine by inhibiting dopamine transporters by binding to the transporter.[45,77]

Chronic (daily doses for 14 days) MP resulted in upregulation of Htr7 and Grik2 gene expression in rats, into adulthood.[35] MP decreased expression of dopamine DRD1/DRD2 receptors, calcium/calmodulin-dependent protein kinase (CAMKII) and c-FOS in the prefrontal cortex, dorsal and ventral striatum.[35] The sub chronic, 13 days, use played a crucial role on behavioral sensitization based on the age of the rats. Their data suggest, in juvenile rats, in motivation, behavior, and habit formation, that the monoamine neurotransmitters are linked to these striatal genes and subsequent upregulation.[35]

D1 dopamine receptors were blocked by their antagonist, SCH-23390, in order to review any effect the receptors had on gene expression using phMRI, pharmacological magnetic resonance imaging.[37] Following Intrastriatal injection of SCH-23390 for15 minutes, the MP in rats after 10 days showed that SCH-23390, in blocking the D1 dopamine receptors, downregulated both Zif268/Egr1 and Homer1a mRNA expression in both the striatum and cortex, dose-dependently.[37] In terms of regional specificity, Homer1a that was found to be significantly downregulated, occurred only in the nucleus accumbens.[37] When the D1 receptor is inhibited, putatively, dopamine could accumulate in the synapse. Therefore, these and other studies provide evidence that Zif268/Egr1 and Homer1a induce a negative relationship to dopamine in the striatum and cortex.

Adrenoreceptors

The adrenergic alpha 2A receptor (ADRA2A) and the presence of the G allele have been associated to the lack of attention in individuals with ADHD.[30] One study examined the effects of MP in humans. MP was administered for 3 months to 89 children, for 1 month. PCR analysis showed the relationship between MP on the noradrenergic.[30] The G allele is a significant polymorphism of ADRA2A, but commonly serotonin's rate-limiting enzyme tryptophan hydroxylase gene (TPH2).[83] According to this study, there was significance found between the MP treatment and the response of G allele at the ADRA2A polymorphism.[30] The MP effects on this gene are correlated with reduced absentmindedness and increased attention in these ADHD children.

Additional Regional Specifically

Moreover, another study by Quansah et al used chronic exposure on adolescent rats to focus the neurological effects, with a dose of 2.0 mg/kg, twice daily for 15 days.[20] To analyze the changes in gene expression, this study used both quantitative RT-PCR, to detect genes, as well as Western blot analysis, to detect proteins. These techniques suggested regional specificity and, in order to understand the effect on neuroplasticity, specific genes associated with both neuroplasticity and protein expression.[20] There was increased expression of activity-regulated cytoskeletal gene (Arc) and scaffolding protein insulin receptor tyrosine kinase substrate p53 (IRSp53) in the striatum and increased expression of the actin-related protein 2 (Arp2), and cell division control protein 42 (Cdc42) in the nucleus accumbens due to MP.[20] MP decreased the Arc and IRSp53 expression in the cerebellum.[20] This is significant because it suggests there are different effects of MP in the striatum, cerebral areas, compared to the cerebellum. The chronic MP exposure indicated changes in neuroplasticity; therefore, having to do with ADHD symptoms, use, and abuse.

Effects of MP on Postsynaptic Density Proteins Gene Expression

Regulating the traffic and targeting of glutamate receptors at the synapse, post synaptic density (PSD) proteins are involved in neurologically-related development.[84] For gene regulation, PSD genes are involved in the long-term synaptic plasticity in the striatum and contain more than 1,000 different proteins, inevitably playing a pivotal role in synaptic

transmission as well.[29,85] MP studies indicate a general upregulation of the PSD proteins, due to an increase in neurotransmitters within the synapses.[29,86] Furthermore, within the PSD family there are two important families of proteins. These include the membrane-associated guanylate kinase (MAGUK) and the Shank families (see Table 2).

Adriani et al looked at striatal gene expression at both the sexual and endocrine related parameters in male rats. This group narrowed their focus down to three groups of genes in particular. For group one, the genes were involved in neural and glial development, while group two genes suggested to be involved in the myelination of axons, and group three were associated with mature processes.[38] From the three groups, it was determined that MP acted to potentiate the synaptic plasticity; however, there was an age-dependent relationship. More specifically, they used a 2 mg/kg, I.P., (intraperitoneal injection), MP injection, with sub-chronic treatment of 14 days, which indicated upregulation of the genes involved with reward-related, striatal synaptic plasticity. The third group involved TASK-1 and TASK-5, potassium channel proteins, as well as gap junction proteins, neurotransmitters, and other supporting proteins.[38] These specific genes, ARA1B, Kainate2, Htr7, GABA-A, Homer, MAGUK, and Shank2, were discussed by Adriani et al for their known functions due to previous research.[29] The explanation for the upregulation could be due to their involvement in serotonergic or temporal processing, as suggested by this research.[38] Other neurotransmitter receptors affected were involved in the developmental synaptic plasticity and for their potential involvement in the formation of dendritic spines. However, overall, these studies by Adriani and his research team, suggested the therapeutic effects of MP on behavioral plasticity.[29,38]

Homer, Shank2, MAGUK

Reward-related behavior and striatal gene expression found PSD family proteins, including Homer1, Shank2, and "MAGUK p55 subfamily member 3" (Mpp3) mRNA, were all upregulated. The upregulation that is noted was seen in the adolescent rats and was noted to be due to their involvement in synaptic transmission, and the portion of this study focusing on the PSD family associated genes is reviewed here.[29] The genes that were upregulated tended to all be genes that encode for different proteins, neural communication, and processes.[29] Homer1a and c-fos were increased only by acute MP treatment; this just confirms the regions of specificity where the effects were observed. Furthermore, the serotonin 7 receptors (Htr7), shown to be connected to impulsivity, which again is seen in individuals with ADHD, providing the correlated upregulation by MP, would reduce the impulsivity in individuals with ADHD.[29] Further information about the Homer 1a and 1b genes is that they affect the density and size of dendritic spines and synapses; thereby associated with neural transmission.[29] Homer 1a only experienced an increase in its expression during chronic exposure, into adulthood; whereas Homer 1b had an opposing effect, only increasing during adolescence. Of the genes that were upregulated, they demonstrate that adolescent exposure to MP affects both striatal gene expression and neuroplasticity.[29] The neuroplasticity is referring to the brain's ability to reroute brain function to undamaged brain areas or changes to the brain structure of a region as a protective response.

Homer Genes

Hong et al suggested Homer genes are a scaffolding protein family associated with neuropsychiatric disorders such as ADHD.[39] Spontaneously hypertensive rats (SHR) were given an oral administration of 2 mg/kg MP, twice a day for 14 days with reverse transcription PCR to analyze gene expression in the pre-frontal cortex. Homer 1a and Homer 2a/b, but not Homer 1b/c, expressed significantly lower levels in the PFC of SHR compared with controls. MP exposure decreased the locomotor activity and non-selective attention of SHR, and it upregulated the expression of Homer 1 and 2a/b, but not Homer 1b/c.[39] In addition, this study used other behavioral tests to measure activity levels and differences in horizontal and vertical activity and the frequency of which the rats would lean on the maze walls.[39] These findings would suggest the rat's activity vs attention, respectively. It was observed, that, with even sub-chronic exposure (14 days), compared to the control (where rat's activity was measured before MP administration), MP slowed activity in the rats. This suggested that Homer 1a and 2a/b are associated with ADHD, specifically correlated to hyperactivity and lack of attention.

Homer1a and Zif268/Egr1 Genes

Beverley et al showed the potentiated gene regulation by MP with fluoxetine (FLX) treatment. They looked at behavior and long-term gene blunting of Zif268/Egr1 and Homer 1a, due to their involvement in neuronal plasticity. With six daily

injections of MP, fluoxetine (FLX), [serotonin reuptake inhibitor (SSRI) antidepressant] or the combination MP+FLX induced gene regulation. Zif268/Egr1 expression showed the greatest effect due to MP.[33] The combined group did show pronounced upregulation of MP-induced blunting for both genes; which concluded that SSRIs in combination with MP may increase the risk of developing addiction.[33] The MP+FLX group experienced heightened striatal gene regulation.[33,41,42] Zif268/Egr1 is a transcriptional factor and Homer1a is a synaptic plasticity factor according to Yano et al[42]. Results indicated that, in adult rats, acute exposure of MP increased both Homer1a and Zif268/Egr1 expression.[40] They were upregulated in a dose-dependent manner and occurred in the cortex and the striatum (see Table 3). Furthermore, Cotterly et al similarly suggested acute administration of MP demonstrated a significantly stronger gene induction of both the Zif 268/Egr1 and Homer 1a genes. The Zif268/Egr1 and Homer1a genes were mapped through the autoradiograms, with 23 striatal sectors and 22 cortical areas where Zif268/ Egr1 and the homer 2a genes were expressed.[40] Overall, their results suggest MP affects Zif268/Egr1 and Homer 1a, transcriptional and synaptic plasticity in specific striatal circuits.[40]

Glutamate

The association of the functioning roles of glutamate have been studied in combination with stimulants, such as MP. MP treatment effects on AMPARs (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) and NMDARs (N-methyl-D-aspartate receptor), have been examined. Glutamate subunits, including N-methyl-D-aspartate receptor subunit (NR2B) and (NR2A) increased the strength of the synapses, through the long-term potentiation (LTP).[43] The LTP allows for increased communication between neurons due to strengthening the synapse. According to Urban et al, acute exposure of MP mediated synaptic plasticity, reduced the NMDA receptor subunits, NMDA receptor subunit 1 (NR1) and NMDA receptor subunit 2B (NR2B) in adolescent PFC, and increased LTP induction.[43] Furthermore, MP downregulated NR1 and NR2B, as well as the NMDAR-EPSCs; what this means is that, when there are changes in these receptors, the NMDA EPSCs and AMPAR become downregulated; however, they are involved in adolescent neuroplasticity and brain functioning.[43] Glutamate, through the downregulation of AMPA and NMDA, affects the regulation of neuroplasticity[43] (see Figure 2).

MP was also tested as different doses on glutamatergic signaling. One study by Cheng's laboratory focused on the impact of different doses of MP on glutamatergic transmission in the PFC, finding a dose-dependent relationship.[32] While a low-dose allowed for upregulation of the NMDAR-medicated excitatory post-synaptic currents (EPSCs), a higher dose of MP had the opposite effect, downregulating the EPSC of NMDAR and AMPAR.[32] Further development of this study looked at if the effects from the MP were pre- or postsynaptic mechanisms. It was detected to be that low-dose of MP was via a postsynaptic mechanism, whereas the high-dose MP was considered to have resulted in either pre or post-synaptic mechanisms depending on glutamate and it's receptors.[32] Through these results suggesting the dose-dependent relationship, it is demonstrating the involvement of MP on glutamate pathways and the different effects by dose.[32]

Discussion

MP use and misuse is very prevalent, and it is important for us to understand how MP affects gene expression. These effects on gene expression can specifically be useful in understanding the long-term impact of use and its role in treatments. Understanding that role could help discover individual differences in the genome (ie, specific gene polymorphisms). Heading into the future of medicine, this would allow for a personalized approach and further precision in prescribed use of MP.

Altogether, the reviewed studies describe how MP exposure duration, dose, age, genes, and environment play an important role on gene expression. The neuroplasticity can be altered or regulated for therapeutic effects and treatments. These data emphasize the importance of further research and in the monitoring of chronic use of MP. This research can thus further our understanding of MP use and abuse in public health.

Conclusion

MP treatment caused genes to be potentiated and caused an upregulation either via a direct or indirect pathway. As seen above and summarized in Table 3, of the genes discussed, the majority are associated with ADHD (reviewed in Tables 1 and 2).

Research Implications

Review of the effects of MP on gene expression literature showed a strong effect and specific interest on the monoamine neurotransmitter genes and post synaptic density genes. Respectively, the Htr7 and Grik2 genes and zif268/Egr1, c-fos, and homer1a were most commonly upregulated by MP. This suggests these genes may be related and allows for future research to further explore these altered gene expressions focusing on mRNA transcription processes. For example, first looking at Htr7 and Grik2, these two genes are frequently studied together. With MP exposure, the gene levels of expression and observed attitudes and behavior(s) are suggested to be related.[24,45,77,87]

Htr7 is specifically associated with impulsivity and neuronal modification. Leo et al, who determined this, completed the study in-vitro; therefore, further research should be done to investigate the effects of MP on Htr7 and in ADHD patients.[28] Another very critical area to be determined is the status of methylation across these two genes in ADHD patients. Additionally, in blocking the Htr7, there were also noted improvements in depression, which could have been due to an enhanced dopamine release at the accumbens.[28] Due to these noted changes in depression status, Htr7 expression and affect can be then analyzed when the patient is exposed to SSRIs or other antidepressants like bupropion, alone and in combination with MP, as these antidepressants are frequently prescribed for depression.

From the related genes that are either studied together or similarly respond to MP, learning how to target these, especially together, could amplify the intended effect on the patient and improve the efficacy of the drug. However, we beg the question as to whether the widespread prescribing of MP to children and even adults diagnosed with ADHD should go unnoticed or be even more controlled. The advent of these exploratory concepts concerning MP induced alterations of gene expression provide the impetus for the clinical community to be more cautious.

Further Investigation

With over 700 genes upregulated by MP, 1,000 PSD proteins, and due to the number of genes and receptors in the brain that are affected by MP, more studies are required to look at the specific genes.[38,85] Different ranges of doses of MP, duration of exposure, or studies looking at particular genes may also be beneficial to investigate further. For example, due to the large number of genes whose expression can be altered by MP use, studies are not able to assess all of the potential effects; rather more studies need to look at specific genes to standardize the information known about each of the genes and the effect MP has on them.

In previous research studies, there is a large discrepancy in acute vs chronic exposure; therefore, repeating experiments to compare their results to different lengths of MP use will be beneficial for developing a more standardized model for the duration of exposures and their subsequent correlation of the rat to human lifespan. In addition to these potential research fields of study, potentially researchers could look to measuring the degree to which specific genes or gene families are altered. This may be determined through using methods such as RT-PCR as a way of quantifying the gene expression when in comparison to a control or other comparable data followed by statistical analysis.

Furthermore, these results could be used to assess the efficacy of MP across different gene polymorphisms in humans. For example, it is known that the DRD2 Taq1 A1 allele is significantly associated with cocaine dependence (CD). The prevalence of the A1 allele in CD subjects was 50.9%.[88] It was significantly higher than the 16.0% prevalence in non-substance abuse controls.[88] Logistic regression analysis of CD subjects identified potent routes of cocaine use and the interaction of early deviant behaviors and parental alcoholism as significant risk factors associated with the A1 allele. The cumulative number of these three risk factors in CD subjects was positively and significantly related to A1 allelic prevalence.[88] The data showing a strong association of the minor alleles (A1 and B1) of the DRD2 with cocaine dependence suggest that a gene, located on the q22–q23 region of chromosome 11, confers susceptibility to psychostimulant use disorder (PUD).[88] As well, as seen with the gene, ADRA2A, MP can cause reduced absent-mindedness and increase attention in individuals with ADHD; with this understanding, more studies should look at the direct correlations this has with humans, both with and without ADHD.[83] It is important to note that the majority of these studies use rat or mice subjects due to their lifespan and ability to study the brain that would not be ethical on human subjects.

Lastly, in regards to addiction and misuse potential, MP when taken by non-ADHD individuals, showed heightened cognitive effects such as improved attention and ability to focus on a given task[6,7,19,23,24] Due to MP affecting the brain's

neuroplasticity, not only does this impact addiction susceptibility, but the population affected.[18,19] This understanding leaves room for more research on the populations affected long-term, further understanding the reasons and significance for MP mis-use.

Abbreviations

ADHD, Attention Deficit Hyperactivity Disorder; MP, Methylphenidate; Htr7, serotonin 7 gene; Grik2, Glutamate Ionotropic Receptor Kainate Type Subunit 2; Zif268/ Egr-1, zinc finger protein 268/early growth response protein 1; c-fos, protein, proto-oncogene; I.V, intravenous injection; I.P., intraperitoneal; SC., subcutaneous injection; DA, dopamine, DAT, dopamine transporter; D1 dopamine 1, DRD1, dopamine receptor D1; D2, dopamine 2; DRD2, dopamine receptor D2; NET, norepinephrine transporter; SERT, serotonin reuptake transporter; MAGUK, membrane-associated guanylate kinase; PSD proteins, post-synaptic density proteins; PET, positron emission tomography; fMRI, functional magnetic resonance; GABA, gamma-aminobutyric acid; ADRA2A, adrenoreceptor alpha 2A; EPSCs, excitatory postsynaptic currents; RT-PCR, real-time reverse transcription polymerase chain reaction; KO, knock-out; NMDA, N-methyl-D-aspartate; NR1, NMDA receptor subunit 1; NR2A, NMDA receptor subunit 2A; NRB2, NMDA receptor subunit @B; Homer 1, Homer scaffold protein 1; Homer 1a, Homer scaffold protein 1a; Homer 2a/b, Homer scaffold protein 2 a/b; Homer 1b/c, Homer scaffold protein 1 b/c; TASK-1, TWIK-related acid-sensitive potassium channel 1; TASK-5, TWIK-related acid-sensitive potassium channel 5; connexin30, gap junction protein; Adrα1b, adrenergic alpha 1B; Kainate 2, GABA-A, gamma-aminobutyric acid type A; MPP3, membrane protein, palmitoylated 3; Shank2, SH3 And Multiple Ankyrin Repeat Domains 2; H3 receptors antagonist, Histamine H3 receptor antagonist; Ciproxifan, selective H3-receptor antagonist; IRSp53, scaffolding protein insulin receptor tyrosine kinase substrate p53; Cdc42, cell division control protein 42; Arp2, Actin Related Protein 2 complex; CAMKII, Calcium/calmodulin-dependent protein kinase II; CP94253, selective serotonin 5-HT1A receptor agonist; 5-HT1B, 5-hydroxytrptamine receptor 1B; PSD-95, post-synaptic density protein 95, HTR2A, serotonin receptor 2A; SCH-23390, selective D1 dopamine receptor antagonist; AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor). GABA+, gamma-aminobutyric acid +; water-scaled; PFC, pre-frontal cortex; Acb, nucleus accumbens; fluoxetine, FLX; NMDAR, N-methyl-D-aspartate receptor; CNS, central nervous system; *GabRγ1*, gamma-aminobutyric acid receptor γ1 subunit; *GabRβ3*, gamma-aminobutyric acid receptor beta 3 subunit; 5-HT1B, serotonin receptor subtype; SB269970, Htr7 antagonist; CAMKII, calcium/calmodulin-dependent protein kinase; TPH2, tryptophan hydroxylase gene; CD, cocaine dependence; PUD, psychostimulant use disorder.

Data Availability

All data is available for this manuscript. Please contact corresponding author for this manuscript if needed.

Consent for Publication

All authors consent to publication of this manuscript. The corresponding author is submitting on behalf of all authors.

Author Contributions

All authors contributed to data analysis, drafting, or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Funding

Partial funding was provided by the University at Buffalo's Experiential Learning Network to SK.

Disclosure

Dr Kenneth Blum reports a patent 10,894,024 licensed to SYNAPATAMINE. The authors report no other conflicts of interest in this work.

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