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Review

AMPHETAMINE INDUCED ACTIVATION OF THE OPIOID SYSTEM: A FOCUSED REVIEW OF ANIMAL AND HUMAN STUDIES

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Stimulants are commonly prescribed as first line medications for ADHD and also used as adjunct treatment in other psychiatric conditions. While much is known about stimulants' influence on brain neurochemistry, particularly on the dopaminergic neurotransmission, there has been less research into prescription stimulants' effects on the endogenous opioid system. What we know about the mechanisms underlying the effects of stimulants on the opioid system come predominantly from animal studies and a relatively small number of studies in humans using positron emission tomography (PET) to examine the activation of the endogenous opioid system as evidenced by radioligand binding to opioid receptors. This paper is a focused review of the currently available literature on both animal and human studies examining the effects of stimulant administration on the endogenous opioid system, which suggest that stimulant administration results in increased occupancy of the opioid receptors in a widespread network of brain regions. We discuss the possible underlying mechanisms of this interaction, it's potential impact on our understanding of substance abuse and addiction, particularly as viewed through the model of behavioral sensitization, and possible clinical implications. ***Biomed Rev*** **2022; 33: 17-31**

Keywords: attention deficit hyperactivity disorder (ADHD), positron emission tomography (PET) imaging, amphetamine, opioid receptors

INTRODUCTION

Psychostimulants, including methylphenidate and amphetamine derivatives, are Food and Drug Administration (FDA) approved and commonly prescribed agents for the treatment of attention deficit hyperactivity disorder (ADHD) in both children and nadults. Despite their proven clinical efficacy in treating symptoms of inattention and impulsivity (Arnold *et al.*, 1972) concerns have been raised about their potential for abuse and misuse (Kuszenski & Segal, 2005). It is established that acute administration of amphetamines can produce euphoria while chronic use may lead to the need for escalating doses to achieve similar psychological effects (e.g. tolerance; Berman *et al.*, 2009). Abrupt discontinuation of stimulant treatment after prolonged use may be associated with withdrawal symptoms including dysphoria and anxiety; in turn there is a high probability for relapse of misuse, even after prolonged abstinence,

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triggered to re-exposure to both drug related cues or the drug itself (Heal *et al.*, 2013). Moreover, evidence from animal and a small number of human studies suggest that repeated exposure to amphetamines can induce behavioral sensitization (i.e. the propensity to elicit, after a period of time, a similar behavioral response with a lower dose of the drug; Ivanov *et al.*, 2022). As stimulants have been shown to have a strong and ubiquitous effects on dopamine release in the basal ganglia there is emerging evidence that sensitization protocols for humans can produce lasting changes in the striatum in human participants in regions associated with motivation and reward processing (Boileau *et al.*, 2006). There is also evidence showing that dopamine release associated with stimulant administration also affects the activation of the endogenous opioid system. In this paper we will discuss the existing evidence of possible biomechanisms of the interactions between the opioid and the dopaminergic systems that may underlie amphetamine induced behavioral sensitization.

Amphetamine types stimulants are a class of compounds including amphetamine, methamphetamine, 3,4-methylenedioxy-methamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDEA), methcathinone, fenetylline, ephedrine, pseudoephedrine, and methylphenidate. (Souza *et al.*, 2012). They are divided into two groups according to their effects on the central nervous system. Amphetamines and methamphetamines are considered psychostimulants and mescaline, MDMA, MDA and MDEA are considered hallucinogenic stimulants. (Cao et. al., 2016). Although the exact mechanism leading to possible behavioral sensitization in animals remains unclear, it has been shown that amphetamines affect the brain dopamine reward systems in the mesocorticolimbic pathway, and that these influences are modulated by other neurotransmitter systems including glutamatergic, y-aminobutyric acid and endogenous opioidergic systems. There is ample evidence that the dopaminergic and opioidergic systems interact in the mesolimbic brain areas (Spanagel *et al.*, 1992). Endogenous opioids act through three distinct opioid receptor subtypes referred to as μ, δ and κ. Activation of each of these receptors decreases adenylyl cyclase and increases calcium and potassium conductance (Cao *et al.*, 2016). Opioid receptors and peptides are highly expressed in brain areas involved in reward and motivation, notably the ventral striatum, putamen, caudate, frontal and cingulate cortex, hypothalamus, amygdala, and ventral tegmental area. Opioid signaling is thought to affect intrinsic reward properties and to mediate the effects of psychostimulants (Le Merrer *et al.*, 2009). The endogenous peptides that target opioid receptors include enkephalins, dynorphins and B-endorphins that are produced by proteolytic cleavage of preproenkephalin, prerpodynorphin and proopiomelanocortins.

Research in substance use disorders has been focused on the role of opioid receptors in the dorsal or ventral striatal circuitry, where the activation of opioid receptors affects pain perception, locomotion, motivation, and reward. In turn, amphetamine administration studies have shown that striatal κ opioid receptor stimulation may reduce sensitization types of behaviors and gene expression induced by a single dose of amphetamine by decreasing dopaminergic and glutamatergic transmission (Gray *et al.*,1999; Tzaferis & McGinty 2001). In contrast, amphetamine stimulation of μ and δ receptors inhibits GABA release and disinhibits dopamine neuronal firing and increases extracellular dopamine release in terminals (Gray *et al.*,1999; Tzaferis & McGinty 2001). Studies show that blockade of the μ opioid receptors results in reduced levels of dopamine and its binding of dopamine receptors, leading to reduction of the amphetamine induced sensitization (Chiu *et al.*, 2005; Lan *et al.*, 2007; Tien *et al.*, 2007). In the next section we will review in more detail, animal and human studies that provide evidence demonstrating that changes in the endogenous opioid system may contribute to the development of amphetamine reinforcement neural circuitry and consequently, behavioral sensitization.

ANIMAL MODELS OF AMPHETAMINE INDUCED BEHAVIORAL SENSITIZATION THROUGH OPIOIDERGIC SYSTEMS:

Studies related to sensitization secondary to administration of amphetamine show behavioral sensitization in different animal species including rodents (Haggkvist *et al.*, 2009) and rhesus monkeys (Jimenez-Gomes *et al.*, 2011). Repeated amphetamine administration can manifest in stereotypic behaviors such as increased locomotion (Tanimura *et al.*, 2009). For instance, sensitization protocols that used high doses of amphetamine (2.0 mg/kg) documented an initial reduction in activity in response to the administration followed by a subsequent increase in locomotor activity. (Leith & Kuczenski, 1982). Others have also used conditioned placed preference or drug self-administration to show sensitization to amphetamines. In one study, sensitization was demonstrated when amphetamine induced conditioned place preference was increased in rats that had been previously exposed to the drug. (Lett, 1989) Animals

with exposure history to externally administered amphetamine subsequently showed a higher likelihood of amphetamine self-administration. (Piazza *et al.*, 1990)

A number of animal studies point to the specific role of the endogenous opioid system in mediating and reinforcing the deleterious effects of drugs of abuse. For the purpose of this review we conducted a literature review on 2 databases (e.g. PubMED and PsychNFO) of the animal studies investigating the behavioral sensitization associated with effects of amphetamines on the opioidergic system using the following criteria: 1) publications in English, 2) original research full text reports, 3) published between 1990-2020 and 4) excluded book chapters, reviews, abstracts, meta-analysis and 5) included the search terms "endogenous opioids" "amphetamine" "animal". In result we selected 11 animal studies that represent most adequately the current state of research in the role of the opioid reward system in amphetamine use and illustrate their findings below. We decided to limit our search to studies using amphetamine since this agent is in wide therapeutic use for ADHD and has somewhat elevated potential for misuse than the alternative (e.g. methylphenidate; Kollins, 2003).

Opioid Receptor Manipulation Studies:

Receptor manipulation studies focus on determining the functions of the μ, δ and κ types of opioid receptors (OR) with amphetamine administration. Generally, systemic μ, and to a lesser extent, δ agonism is associated with positive reinforcement whereas κ agonism produces aversion, hallucination and malaise (Le Merrer *et al.*, 2009). Studies in μ-OR knockout mice have shown that these animals become less sensitive to amphetamine induced behavioral sensitization (Shen *et al.*, 2010). Specifically, male wild type and μ-OR knockout mice were sensitized to methamphetamine (0.62 mg/kg) or control saline through single dose intraperitoneal injections. Stereotyped behavioral sensitization was initiated in 12 mice from both genotypes with single daily intraperitoneal injections (2.5 and 10 mg/kg) for 7 consecutive days. The initial single dose exposure induced behavioral locomotor sensitization in the wild type mice but not in μ-OR knockout mice. Further, the repeated administration induced attenuated behavioral stereotypy in the μ-OR knockout mice, indicating that μ-OR animals were less susceptible to repeat behavioral sensitization. This evidence suggests that μ-OR subtype is involved in the development of behavioral sensitization to the psychostimulant. It is hypothesized that the μ opioid system modulates dopaminergic neurotransmission and associated behavioral responses to amphetamines via decreasing the release of y-aminobutyric acid (GABA). As μ-ORs are localized mainly on the inhibitory GABA interneurons that synapse on dopamine neurons, it is speculated that μ-ORs directly dampen the release of GABA which in turn disinhibits the dopamine neurons in the mesolimbic system resulting in increased extracellular dopamine (Di Chiara & Imperato, 1988).

Similar effects have been observed in relation to the activity of the δ-OR showing influences on amphetamine induced behaviors and neuropeptide gene expression. One report showed that antagonists to both μ and δ-OR decreased amphetamine induced behavioral activity, measured as amphetamine stimulated vertical activity and distance traveled, as well as the expression of preprodynorphin, substance P and preproenkephalin mRNA in the striatum. (Gonzalez-Nicolini *et al.*, 2003). Specifically, adult male rats that were randomized to receive one bilateral intrastriatal injection of control vehicle or one dose of the selective μ-receptor antagonists (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (e.g. CTOP) or H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2 (e.g. CTAP) or the selective δ-receptor antagonist (naltrindole or H-Tyr-Tic[CH2NH]-Phe-Phe-OH (e.g. TIPP). All animals were then administered amphetamine at dose 2.5 mg/kg. The animals that received μ-OR and δ-OR antagonist had significant decreases in vertical activity, however only the animals that received μ-OR antagonist showed reduced amphetamine induced distance traveled. Quantitative in-situ hybridization histochemistry revealed that μ-OR antagonism through CTAP blocked amphetamine induced production of preprodynorphin and substance P mRNA in the striatum. In turn, δ-OR antagonists significantly decreased the amphetamine induced mRNA expression of all three neuropeptides.

As previously mentioned, κ-OR may have a protective effect in relation to amphetamine sensitization. Adult male rats that were administered amphetamine with pretreatment of a κ-OR agonist showed a significant decrease in behavioral activity when compared to control animals (Tzaferis & McGinty, 2001). Additionally, in-situ hybridization histochemistry revealed that the κ-OR agonist decreased amphetamine induced mRNA expression of opioid neuropeptides preprodynorphin, substance P and preproenkephalin in the caudoputamen and nucleus accumbens. Acute administration of amphetamine may also selectively desensitize κ-OR in the nucleus accumbens (NAcc) as demonstrated by a study examining the effects of naltrexone on κ-OR function (Xia *et al.*, 2007). When an initial single injection of amphetamine reduced the κ-OR inhibi-

Table 1. *Summary of behavioral animal studies investigating the relationship between dopamine and opioid systems in the brain.*

Title	Authors	Study Description	Results
Naloxone blockade of amphetamine place preference conditioning	Trujillo *et al*., 1991	After determined initial preferences adult male Sprague-Dawley rats (n=141) were conditioned with amphetamine alone (1.0 mg/kg SC), naloxone alone (0.02, 0.2 or 2.0 mg/kg SC) or combinations of amphetamine plus naloxone to study possible interactions between endogenous opioids and catecholamines in reinforcement.	No preference or aversion was observed in animals that received saline in both compartments. Naloxone (0.02, 0.2 and 2.0 mg/kg) produced a dose-dependent place aversion. Naloxone, at all three doses, prevented the ability of amphetamine to produce a place preference. Despite no effects on place conditioning the lowest dose was still able to block the reinforcing effects of amphetamine.
Kappa opioid receptor Stimulation decreases Amphetamine-induced behavior and neuropeptide mRNA expression in the striatum	Tzaferis *et al*., 2001	Amphetamine-treated adult male Sprague-Dawley rats (n=18) were pretreated with U69593, a kappa agonist (0.16 or 0.32 mg/kg s.c.) and monitored for changes in behavioral activity with amphetamine administration. In-situ hybridization histochemistry was then performed to detect differences in preprodynorphin, substance P, and preproenkephalin mRNA expression after amphetamine administration to investigate the role of kappa opioid receptor stimulation on stimulant induced behavior and neuropeptide gene expression in the striatum in rat models.	Administration of the kappa agonist with amphetamine showed a significant decrease in behavioral activity and in situ hybridization histochemistry revealed that the kappa agonist significantly decreased amphetamine induced mRNA expression of opioid neuropeptides preprodynorphin, substance P and preproenkephalin.
Local μ and δ opioid receptors regulate amphetamine-induced behavior and neuropeptide mRNA in the striatum	Gonzalez-Nicolini *et al*., 2003	Adult male Sprague-Dawley rats (n=20) were administered amphetamine 2.5 mg/kg and were randomly divided into 4 groups to test the effect of μ opioid antagonist (CTAP) and δ opioid antagonist (TIPP) and their effects on amphetamine induced behavioral activity and expression of preprodynorphin, substance P and preproenkephalin mRNA. Aim was to investigate the role that μ and δ opioid receptor blockade has upon stimulant induced behavior and neuropeptide gene expression in the striatum.	Both μ and δ opioid antagonist decreased amphetamine induced vertical activity. Only μ opioid antagonist reduced amphetamine induced distance traveled. Quantitative in-situ hybridization histochemistry revealed that CTAP blocked amphetamine induced preprodynorphin and substance P mRNA.
Nociceptin inhibits acquisition of amphetamine-induced place preference and sensitization to stereotypy in rats	Kotlinska *et al*., 2003	Pre-conditioned adult male Wistar rats were administered saline or nociceptin and confined to a black compartment for 30 min. After at least 6 h, the rats received nociceptin and amphetamine and were placed in the white (drug-associated) compartment for 30 min. This conditioning period consisted of two 30-min sessions daily for 4 consecutive days. Changes in place preference were measured drug-free on day 5 to examine the ability of nociceptin to block the acquisition of amphetamine-induced place.	Repeated administration of nociceptin at increasing doses during conditioning significantly attenuated the reinforcing effect of amphetamine in conditioned place preference paradigm. Nociceptin did not change the acute effect of amphetamine-induced stereotypy but prevented the development of sensitization to stereotypy measured on the challenge day.
Acute Amphetamine Exposure Selectively Desensitizes k-opioid receptors in the nucleus accumbens	Xia *et al*., 2007	Adult male Sprague-Dawley rats were administered one single subcutaneous injection of amphetamine (2.5mg/kg) or control saline 15min after control saline or naltrexone to examine the effects of amphetamine on κ opioid receptor function in both the nucleus accumbens and the ventral tegmental area.	Single administration of amphetamine (2.5 mg/kg) reduced the κ receptor-mediated inhibition of glutamate release in the nucleus accumbens shell. This effect was blocked by dopamine receptor antagonists or the nonselective opioid antagonist, naltrexone (1 mg/kg, s.c.), indicating that an amphetamine-induced release of dynorphin is producing a long-lasting desensitization of the κ opioid receptor.

Title	Authors	Study Description	Results
Preclinical Study: The Effect of naltrexone on amphetamine induced conditioned place preference and locomotor behavior in the rat	Haggkvist *et al*., 2009a	Adult male Wistar rats received amphetamine (2mg/kg) to induce conditioned place preference (CPP) and then underwent extinguish period of 12 days when animals received saline. Reinstatement of CPP was induced by a priming dose of amphetamine (0.5 mg/kg). Interaction of naltrexone and amphetamine was evaluated using three paradigms of CPP: with naltrexone (0.3, 1.0, 3.0 mg/kg) administered either 30 minutes prior to amphetamine conditioning or 30 min before the expression or 30 minutes before the amphetamine priming.	Naltrexone had no effect on the conditioning, the expression or reinstatement induced by a priming dose of amphetamine. Naltrexone by itself did not induce place preference or place aversion. In contrast, NTX did significantly attenuate the locomotor response to a priming dose of amphetamine without affecting general locomotor behavior.
The opioid receptor antagonist naltrexone attenuates reinstatement of amphetamine drug- seeking in the rat	Haggkvist *et al*., 2009b	Adult male Wistar rats (n=14) were trained to self-administer amphetamine under fixed ratio 1 schedule (0.1mg/kg/infusion) followed by an extinction period were pre-treated with naltrexone (0, 0.3, 1.0, 3 mg/kg) before given a priming dose of amphetamine (0.5mg/kg). The aim was to study the naltrexone effects on reinstatement of self- administration of amphetamine. Lever presses for food pellets under schedule of enforcement were considered markers for the attenuation effect of naltrexone.	Single administration of amphetamine reinstated self- administration behavior marked by lever pushes, whereas naltrexone 0.3 and 1 mg/kg significantly attenuated the amphetamine induced reinstatement.
μ-Opioid Receptor Knockout Mice Are Insensitive to Methamphetamine- Induced Behavioral Sensitization	Shen *et al*., 2010	Adult male wild type and u-OR knockout mice (n=48) were divided in 6 groups. Animals in each group received a single i.p. injection of methamphetamine at doses 0.0 (e.g. control) and 0.31, 0.62, 1.25, 2.5 or 10 mg/kg. Animal locomotor activity at 30 min before and 120 min after injection was measured. Drug induced locomotor hyperactivity and stereotyped behaviors were used as markers for development of behavioral sensitization.	Repeat administration of methamphetamine (2.5 and 10mg/kg) induced behavioral locomotor sensitization in the wild type mice but not in u-receptor knockout mice. Repeated admin of methamphetamine (2.5 and 10mg/kg) induced behavioral stereotypy sensitization was attenuated in u-receptor knockout mice.
Naltrexone attenuates amphetamine-induced locomotor sensitization in the rat	Haggkvist *et al*., 2011	Adult male Wistar rats underwent sensitization by repeated administration of amphetamine (2 mg/kg) for 10 days. After 10 day drug free period, rats were administered naltrexone 3mg/kg 30 minutes prior to administration of a challenge dose of either amphetamine or saline to investigate the effect of naltrexone on the expression of locomotor sensitization and conditioned locomotor response in animals previously conditioned with amphetamine.	Following a 10 day drug free period naltrexone had no effect on acute amphetamine induced locomotor activity in animals without amphetamine conditioning vs conditioned animals Naltrexone pretreatment inhibited sensitization and blocked the conditioned locomotor response in conditioned animals when placed in the previously amphetamine paired context.
Naltrexone decreases d-amphetamine and ethanol self-administration in rhesus monkeys	Jimenez-Gomez *et al*., 2011	Rhesus monkeys (n=5) were trained to self-administer i.v. injections of either d-amphetamine or ethanol on fixed ratio of 30 10sec- timeout schedule of drug reinforcement during one 90-min session daily. Naltrexone was administered i.m. 30 min prior to start of treatment test sessions to examine naltrexone ability to modify self-administration of i.v. amphetamine.	Naltrexone at 0.3 and 1 mg/kg doses significantly decreased response rates and injections earned per session in a dose-dependent fashion.
Naltrexone modulates dopamine release following chronic but not acute amphetamine administration: a translational study	Jayaram-Lindstrom *et al*., 2017	Adult male Wistar rats received daily injections of either saline or amphetamine (2 m/ kg) for 10 consecutive days after which the animals were left untreated for another 10 days. Microdialysis surgery was performed 8 days into the drug-free period. In the following experiment the rats received an injection with naltrexone or vehicle, followed 40 min later by a saline injection for the previously saline-treated rats and i.p. amphetamine (0.5 mg/kg) for the previously amphetamine treated rats. Dialysate was collected for 180 min after the last drug administration to study the modulatory effects of naltrexone on dopamine levels after acute and chronic amphetamine exposure.	Naltrexone had no effects on the rise in striatal dopamine levels after acute injection of amphetamine. Conversely, Naltrexone significantly attenuated the dopamine release caused by reinstatement of amphetamine after chronic administration.

tion of glutamate release in the NAcc, the pretreatment with naltrexone blocked this effect of amphetamines on the κ-OR. Additional results also showed that amphetamine caused a loss of κ-OR function in the nucleus accumbens due to long lasting desensitization of the receptor. This reflects that the κ-OR modulation of glutamate release in the nucleus accumbens may be selectively blocked by progressive amphetamine exposure resulting in loss of regulatory activity on the glutamate terminals and creating a negative feedback process, in turn leading to hyperexcitability of NAcc neurons.

Amphetamine Administration and Opioid Peptide Expressions:

Endogenous opioid ligands have also been demonstrated to play an important role in reward processes. Amphetamine administration affects endogenous opioid ligand quantity and expression. As a general overview, there are three families of endogenous opioid peptides derived from proopiomelanocortin (POMC), proenkephalin (PENK) or prodynorphin (PDYN) (Trigo *et al.*, 2010). Active peptides include B-endorphin, met- and leu-enkephalin, dynorphins and eno-endorphins. These opioid ligands have different affinities for respective opioid receptors. For example, B-endorphins binds highly with μ -OR whereas met- and leu-enkephalin has a high affinity for δ-OR. Manipulation of these ligands theoretically can influence amphetamine derived sensitization behaviors. The opioid ligand nociceptin, a 17-amino acid natural ligand of the nociceptin opioid peptide receptor was studied and shown to inhibit the rewarding effects of amphetamine in a study by Kotlinska *et al.* (2003). Increasing nociceptin administration in conjunction with amphetamine exposure significantly attenuated the effects of amphetamine as measured through conditioned place preference and prevented sensitization to stereotypy behaviors in repeat exposures to amphetamine. In this study, nociceptin was administered at increasing doses (starting at 5nmol and doubled on day 2, 3, 4 of the CPP experiment to amphetamine conditioned rats, and time observed in preference place was observed on day 5. As for stereotypical behaviors, rats received injections of either nociceptin or saline prior to amphetamine or saline control group. The dose of nociceptin was increased daily during development of sensitization and stereotyped behavior through a consistent scale.

Naltrexone Administration and Behavior:

Naltrexone (NTX) is a non-selective opioid antagonist that has been used in multiple mammalian studies examining the role of opioidergic involvement in the actions of amphetamine. A consistent and replicable finding is that NTX attenuates the subjective behavioral effects of amphetamine, in both animal (Haggkvist *et al.*, 2009a, 2009b, 2011) and human studies (Jayaram-Lindström *et al.*, 2004, 2007). In two studies done by Haggkvist *et al.*, (2009a, 2009b) NTX effects on locomotor activity and amphetamine induced place preference were examined. In their 2009a study, conditioned place preference was assessed in five phases: initial preference, conditioning, expression of preference, extinction and finally reinstatement of preference. Rats were conditioned with amphetamine (2 mg/kg) to induce place preference, then subsequently received saline for 12 days to extinguish any conditioned behavior. A priming dose of amphetamine (0.5 mg/kg) was given to reinstate place preference and NTX at doses 0.3, 1.0, 3.0 mg/kg were administered either 30 minutes prior to amphetamine conditioning, 30 minutes before the expression or 30 minutes before the amphetamine priming. Study results showed that NTX did not modulate the conditioning, expression or reinstatement of amphetamine induced place preference. However, a single dose of paired NTX, most notably at the highest dose of 3.0 mg/kg, significantly attenuated the amphetamine induced locomotor response during reinstatement in animals conditioned with amphetamine (Haggkvist *et al.*, 2009a). The subsequent study from this group addressed the effect of NTX on expressions of locomotor sensitization in rats with and without a history of amphetamine conditioning. Sensitization was induced by repeated administration of amphetamine (2 mg/kg) for 10 days, followed by a 10 day drug free period. Rats were then given NTX (3 mg/kg) 30 minutes prior to administration of a challenge dose of amphetamine (0.5mg/kg) or saline. NTX was found to have no effect on locomotor activity in animals without a history of amphetamine conditioning. However, animals previously conditioned with amphetamine showed sensitized locomotor response to the amphetamine challenge following the 10 day drug free period. NTX pre-treatment also blocked the conditioned locomotor response when the preconditioned animals were placed in the previously paired amphetamine context. (Haggkvist *et al.*, 2011).

A study by Jayaram-Lindstrom 2017 *et al.* further examined these different results for NTX behavior attenuation in amphetamine acute administration vs. chronic administration models through in vivo analysis of rats with repeated administration of amphetamine. Briefly, in the acute exposure experiment, dialysate was collected through a probe into the nucleus accumbens in rats after priming with NTX (3 mg/kg)

followed by standardized dose of either saline or intraperitoneal amphetamine (0.5 or 2 mg/kg). In the chronic dialysis study, rats were conditioned to amphetamine with protocol what induced robust locomotor sensitization to amphetamine, followed by a nontreatment period of 10 days. In the microdialysis experiment that followed, the same rats were injected with NTX or vehicle, followed by either saline or amphetamine (0.5 mg/kg). Dialysate was subsequently collected. In the acute model, the microdialysis data showed that NTX did not affect amphetamine induced dopamine release in previously drug naive animals. However, following chronic exposure to amphetamine, NTX administration attenuated amphetamine induced dopamine response by 50%.

Short acting opioid antagonist naloxone has also notably attenuated place preference response in rats (Trujilo *et al.*, 1991). Naloxone (0.02, 0.2, 2.0 mg/kg) was administered to male rats after reinforcing properties of amphetamine was illustrated through repeated and reliable preference for a compartment associated with amphetamine. The study observed a dose-dependent place aversion, noting that all three doses of naloxone prevented place preference while the higher doses produced place aversions, which the study measured through distance from the preferred compartment box.

Through the process of evaluating addiction related preclinical research that involves screening with a variety of behavioral techniques, drug self-administration procedures are usually the important last step in testing potential relationships between substance administration and behavioral sequelae. One report showed that NTX attenuated the amphetamine induced self-administration behavior in rats (Haggkvist *et al.*, 2009b). Rats were initially operant task primed to press active and inactive levers through food pellet training. The setup was programmed that the animal would receive a food pellet through pressing an "active lever." There was no programmed consequence for pressing the inactive lever. Following food training, rats were trained to self-administer amphetamine under a fixed ratio schedule (0.1mg/kg/infusion) by pressing levers for amphetamine administration. After they received stable drug intake, the stimulus amphetamine was replaced with saline and the animals went through an extinction period, which was considered to be less than 10 lever presses daily for 3 consecutive days. The rats were then pre-treated with NTX (0, 0.3, 1.0, 3 mg/kg) before receiving a priming dose of amphetamine (0.5 mg/kg.) Results of this study showed that a priming dose of amphetamine after the extinction period reinstated amphetamine seeking behavior and that NTX administration significantly attenuated the reinstatement induced by amphetamine, as measured by a statistically significant decrease in the number of active lever presses. As a control, operant task behavior through food reinforced responses were also measured with NTX administration and that NTX did not affect the number of lever presses related to desired number of pellets, allowing the authors to conclude that the effect of NTX on reinstatement is solely an attenuation of amphetamine seeking behavior and not an effect explained by changes or suppression in motor behavior.

NTX effects on the self-administration of amphetamine were also studied in rhesus monkeys (Jimenez-Gomez *et al.*, 2011). Five rhesus monkeys that had previous experience in drug self-administration studies were trained to respond to intravenous injections of D-amphetamine (0.003 mg/kg/injection). Once a baseline of drug self-administration was established, as determined by three consecutive sessions with no changing trend in response, the mitigating effects of NTX at doses 0.01-1.0mg/kg were evaluated. A single dose of NTX was delivered intramuscularly 30 minutes before the beginning of an amphetamine self-administration session. This study showed that pretreatment with NTX decreased amphetamine self-administration in a dose-dependent fashion, where the higher doses of NTX (e.g. 0.3 and 1mg/kg) significantly decreased response rates and injections earned per session.

The mechanisms by which NTX attenuates the subjective effects of amphetamine in these animals are not fully elucidated, however, attenuation effects appear to be most significant in models of chronic amphetamine exposure. NTX, as an unselective opioid antagonist, may proportionally affect μ and δ- OR sensitivity or expression with chronic administration. Alternatively, the differences in NTX benefits in acute vs. chronic models may suggest that NTX affects a higher order cognitive and affective processing of the pharmacological stimulus rather than immediate opioid actions.

HUMAN STUDIES OF THE OPIOID SYSTEM WITH AMPHETAMINE CHALLENGE

As we have conducted literature search using similar criteria as described above we have identified only a few imaging studies in humans looking specifically at the endogenous opioid system response to amphetamine challenge. A total of four studies have examined the occupancy of the μ-OR after oral administration of amphetamine using [(11)C] carfentanil ligand and positron emission tomography (PET) imaging. Two of these recruited healthy volunteers while the rest included

participants with gambling and alcohol use disorders in two separate protocols. Another group used a different methodology of IV administration of amphetamine and placebo to compare μ-OR occupancy utilizing the same ligand but a different scanning schedule (Guterstam *et al.*, 2013). It should be also noted that the existing human studies have not been designed to assess for possible "sensitization" but to mainly gain a deeper understanding of the physiological relations between the known effects of amphetamines and opioid system's response.

Two reports from healthy adult volunteers used a similar protocols including PET scanning before and 3 hours after oral administration of amphetamine at the dose of 0.5mg per kg (Colasanti *et al.*, 2012; Mick *et al.*, 2014). Of note the Colasanti study also included an ultra-low amphetamine dose of 1.25 mg total dose (approximately .017 mg/kg), which produced no physiological effects. Both studies had small samples (e.g. N=12 in the Colasanti *et al.* and N= 9 in the Mick *et al.* study) and reported consistent reduction of the ligand [(11)C] carfentanil binding in several brain regions of interest (ROI) following the amphetamine administration. The importance of these two reports is that they demonstrated changes in the μ-OR occupancy presumably related to the stimulant challenge in brain regions that are known to be rich in opioid projections. Specifically, opioid neuron fibers positive for β-endorphin are found in all diencephalic structures (Dudas & Merchenthaler, 2004)), in the striatum (Gramsch *et al.*, 1979) and in the cingulate and superior and medial frontal gyri (Bernstein *et al.*, 1996). Further, enkephalin-containing cell bodies and fibers are widely distributed in the striato-pallidal regions and in the diencephalon (Gramsch *et al.*, 1979) and endomorphinergic, (e.g. endomorphin-1) fibers are highly distributed in striatal and thalamic regions (Zadina, 2002). Accordingly, these reports showed that the changes in the ligand binding to the μ-OR was decreased in the frontal cortex, putamen, caudate, thalamus, anterior cingulate, and insula in the Colasanti report and in the putamen, thalamus, frontal lobe, nucleus accumbens, anterior cingulate, cerebellum and insula cortices in the Mick *et al.* report. It should be noted that the report by Guterstam failed to document significant differences in opioid release following amphetamine vs placebo administration (Guterstam *et al.*, 2013). In this investigation, however, the researchers used a different methodology; they administered amphetamine at the dose of 0.3 mg/kg and placebo intravenously in within-group design of 10 healthy volunteers. All participants underwent 3 PET scans with [(11)C] carfentanil; one scan before any drug administration and then one scan 15 min (vs 3 hrs in the studies using PO administration) after amphetamine and placebo. These discrepant results were thought to be possibly accounted by the differences in methodology.

Others have also used oral amphetamine administration to assess both density and μ-OR occupancy in relation to the presence of either gambling or alcohol use disorders. In two separate studies researchers found significantly blunted dexamphetamine-induced opioid release in individuals with pathological gambling (PG; N=14) vs controls (N=15; Mick *et al.*, 2016) or alcohol use disorder (N=13) vs controls (N=15, Turton *et al.*, 2020). Both studies examined 10 brain ROI and documented that PG participants showed blunted opioid release in 7 of these regions including the frontal lobe, insula, ACC, caudate, putamen, accumbens and cerebellum whereas participants with alcohol use disorder had blunted response in 5 ROIs including insula, frontal lobe and putamen. In addition, the Mick *et al.* study also showed blunted amphetamine-induced euphoria and alertness in PG compared to controls and that impulsivity positively correlated with baseline MOR binding in the caudate for the PG only (Mick *et al.*, 2014). Similarly both groups found that μ-OR availability at baseline was no different between the groups. The results from these studies are broadly consistent with the assumption that a dysregulated endorphin system appears to be present in behavioral and substance addictions and of a possible relationship between μ-OR availability/biding capacity and impulsivity (the latter being a prominent feature of addiction disorders).

As discussed earlier, animal research has provided evidence for the relation between the dopaminergic and opioid systems, therefore a few possible mechanisms underlying the above findings in humans can be put forward. First, it is argued that direct displacement of endogenous opioids by amphetamine resulting in decreased ligand binding is highly unlikely as amphetamine's affinity to the opioid receptors is very low and such displacement effect can not be achieved by the doses used in the studies. Second, the authors also reject the hypotheses that amphetamine directly induces opioid release in the brain. Alternatively, it is suggested that the dopamine release associated with amphetamine administration in turn mediates the release of endogenous opioids. This last notion seems supported by two observations. One, the amphetamine-induced changes in [^{11}C] carfentanil binding tend to overlap partially with the known distribution of the dopamine transporter (DAT), which is highly expressed in the putamen, caudate, and ventral striatum and to a lesser extent in the frontal cortex, cingulate cortex, insula, and thalamus. Second, the different magnitude in dopamine release

Table 2. *Summary of human imaging studies indicating basic methods and the main findings supporting the premise that stimulants may influence the occupancyof the opioid receptors through the changes in dopamine release in wide spread brain regions.*

Title	Authors	Study Description	Results
Endogenous Opioid Release in the Human Brain Reward System Induced by Acute Amphetamine Administration	Colasanti *et al*., 2012	12 healthy male volunteers received low (0.017 mg/kg) and high (o.5 mg/kg) doses of AMP administered PO to assess radiolabled carfentanyl binding to μ-OR before and 3 hrs after amphetamine administration	Reported that high dose was associated with decreased binding in the frontal cortex, putamen, caudate, thalamus, anterior cingulate and insula presumably due to the effect of amphetamine on μ-OR occupancy.
Effects of amphetamine on the human brain opioid system - a positron emission tomography study	Guterstam *et al*., 2013	10 healthy volunteers received placebo and AMP 0.3 mg/kg administered IV to assess radiolabled carfentanyl binding to μ-OR after placebo vs amphetamine administration in a double blinded randomized protocol. Scans were obtained before and 15 min after placebo or amphetamine administration.	Reported no significant differences in prefrontal cortex, amygdala, striatum, hippocampus; failure to detect differences might be related to methodology specifically the 15 min post administration interval.
Amphetamine induced endogenous opioid release in the human brain detected with carfentanyl PET: replication in an independent cohort	Mick *et al*., 2014	9 healthy volunteers received AMP 0.5mg/kg administered PO to assess radiolabled carfentanyl binding to μ-OR before and 3hrs after amphetamine administration	Reported decreased binding in the putamen, thalamus, frontal lobe, nucleus acumbens, anterior cingulate, cerebelum, and insula presumably due to the effect of amphetamine on MOR occupancy. Replication of earlier study by Clasanti *et al*., 2012
Blunted Endogenous Opioid Release Following Oral Amphetamine Challenge in Pathological Gamblers	Mick *et al*., 2016	14 pathological gamblers and 15 healthy controls received dexAMP 0/3 mg/kg administered IV to assess radiolabled carfentanyl binding to μ-OR in 10 regions of interest before and 3hrs post amphetamine administration	Reported no differences in baseline μ-OR availability between PG and controls and reduction in binding in 7 regions of interest (e.g. frontal lobe, insula, anterior cingulate, thalamus, caudate, putamen, accumbens, cerebellum) in the PG group. Impulsivity scores positively correlated with baseline MOR binding in the caudate for the PG only.
Naltrexone modulates dopamine release following chronic but not acute amphetamine administration: a translational study	Jayaram-Lindstrom *et al*., 2017	7 healthy participants received PL+ AMP 0.3 mg/kg and NTX 50 mg +AMP 0.3 mg/kg. NTX was administered PO and AMP was administered IV. Participants underwent 3 scans – baseline, after PL+AMP and after NTX+AMP administrations PO to assess radiolabled raclopride binding to DA2R in 10 regions of interest via PET emission data obtained for 51 min after IV administration of amphetamine.	Reported attenuated subjective effects of NTX+AMP administrations. Amphetamine produced significantly decreased binding in striatal ROI compared to baseline for both PL+AMP and NTX_AMP administrations. Results suggest that the opioid system may became engaged during the chronic phase of drug use (see ref for details).
Blunted Endogenous Opioid Release Following Oral Dexamphetamine Challenge in Abstinent Alcohol- Dependent Individuals	Turton *et al*., 2020	13 participants with AD (abstinent for > 4 weeks) and 15 control received dexamphetamine 0.5mg/kg administered PO to assess radiolabled carfentanyl binding to μ-OR in 10 regions of interest before and 3hrs after amphetamine administration.	Reported no differences in baseline μ-OR availability between AD and controls and reduction in binding in 5 regions of interest (e.g. insula, frontal lobe thalamus, anterior cingulate and putamen). Results are comparable to results of blunted dexamphetamine-induced opioid release in PG, suggesting that similar dysregulation in opioid tone is common to both behavioral and substance use disorders.

Abbreviations: AD – alcohol disorder; AMP- amphetamine; DA2R – dopamine-2 receptor; μ-OR – μ-opioid receptor; NTX – Naltrexone; PG- pathological gamblers.

in the frontal vs striatal regions seems to parallel the changes in [^{11}C] carfentanil binding across these regions. This is of interest since DAT is involved with the release of other monoamines (e.g. norepinephrine) and therefore the involvement of other monoamines might be important in brain regions other than the striatum, however, the relationship between monoamine release and endogenous opioid release might not be straightforward and at this point requires further investigations.

Moreover, dysregulation between opioid and dopamine transmission is speculated to also underpin the reported blunted opioid release in individuals with PG and alcohol use disorder. In addition to animal studies suggesting that dopamine-2 and -3 receptors may play a role in regulating endorphin release (Doron *et al.*, 2006; Soderman & Unterwald, 2009), one study used a similar amphetamine challenge to compare dopamine-2/3/ receptor availability in PG vs controls indexed by [^{11}C]PHNO PET (Boileau *et al.*, 2013). This study found no marked differences in the dopamine -2 and -3 receptor levels between PG and controls, but documented possible relationships between [11C]-(+)-PHNO binding and gambling severity/impulsiveness suggesting involvement of the dopamine-3 receptor in impulsive/compulsive behaviors, similar to above cited reports (Mick *et al.*, 2016; Turton *et al.*, 2020). The previously mentioned report by Jayaram-Lindstrom *et al.* (2017) has adopted a translational approach to investigate the dopamine–opioid interactions conducting in parallel animal and human experiments to assess the effects of NTX, a μ-OR inhibitor, on dopamine release after acute vs chronic amphetamine administrations (see Animal Studies section, Naltrexone Administration and Behavior). The human experiment included 7 healthy volunteers pretreated with either NTX or placebo who subsequently received i.v. amphetamine and underwent PET scan with dopamine-2 receptor radioligand [^{11}C] raclopride. The results show that NTX attenuated the subjective effects of amphetamine and produced a significant reduction in striatal radioligand binding, indicating increased levels of endogenous dopamine, which however did not significantly differ from the placebo condition. The same effects were documented in the animal experiment, where the changes in striatal dopamine release pre to post amphetamine administration were not affected by the NTX pretreatment. In contrast, the chronic administration of NTX significantly attenuated the dopamine release caused by reinstatement of amphetamine. Also of interest is that two prior behavioral reports from the same group in human volunteers (Jayaram-Lindstrom *et al.*, 2004) and participants with amphetamine use disorder (Jayaram-Lindstrom *et al.*, 2007) showing that NTX attenuates the subjective experience associated with amphetamine taking, including cravings, but did not affect the physiological indexes like heart rate, blood pressure, skin conductance, reading speed or cortisol levels. Taken together, these data suggest that while the opioid and dopamine systems seems to interact closely they may exhibit different types of responses to the acute vs chronic phase of drug administration. These differences may in turn be further modulated by both the route of administration (oral vs i.v.) and the dose of the stimulant. It is therefore crucial to note that the recommended doses of stimulants for ADHD treatment do not produce states of euphoria (e.g. "high") and therefore stimulant administration as part of ADHD treatment in humans may engage the opioid system over time through some cumulative effects that at present are poorly understood. Further, although limited to amphetamine, which has strong effects on dopamine release, the above reported results can be extrapolated to other substances since it is well documented that all substances of abuse produce strong dopamine release in the striatum (Pierce & Kumaresan, 2006).

DISCUSSION AND FUTURE DIRECTIONS

Understanding the mechanisms underpinning the relations between stimulants and opioid receptor activity is important for several reasons. For instance, these data provide useful information about the neurophysiological processes and neuroanatomical regions purportedly involved in mediating the effects of amphetamines (and possibly other drugs of abuse) on the human brain and related psychological phenomena (i.e. euphoria related to drug taking). As mentioned above, most (if not all) drugs of abuse share the ability to stimulate dopamine release in the brain subcortical regions and it appears that these surges of dopamine release are related to the psychological experiences of euphoria (i.e. feeling high). However, some have differentiated between what is defined as the "wanting" and the "liking" phases of drug taking (Robinson & Berridge 2000, 2008). In that construct dopamine release can be linked to the process of identifying the most salient stimuli (e.g. drug abuse vs other natural stimuli) which may constitute the "wanting" (e.g. drug seeking) phase of addiction whereas a more robust engagement of the opioid system can be linked to the experience of pleasure and euphoria associated with drug taking thus constituting the "liking" phase of addiction. The above mentioned human studies offer some support of such distinction.

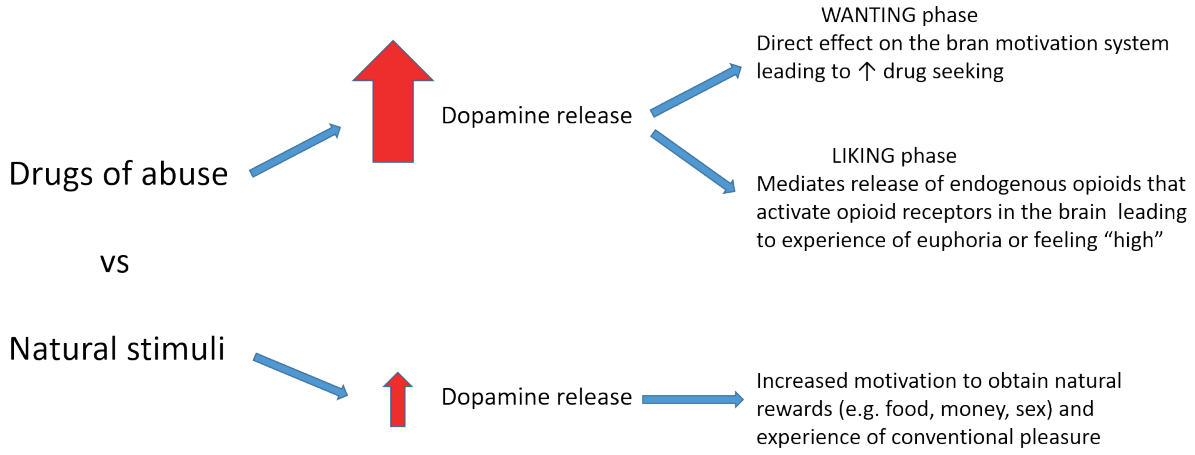

Figure 1. *Schematic presentation of the effects of drugs of abuse and natural stimuli on dopamine release in the brain limbic system. Drugs of abuse produce notably higher levels of dopamine release that in turn can directly stimulate the brain motivation system possibly leading to increased drug seeking; the purported stimulation the opioid system through increased release of endogenous opioids can produce feeling of euphoria. This model schematically illustrates the two phases of addiction (e.g. "wanting" and "liking") as proposed by Robinson & Berridge (2000, 2008).*

In addition to these physiological effects, current research provides some information on the possible brain regions implicated in the physiological relations between dopamine and opioid systems namely the hypothalamus. Some have suggested that hypothalamus may play an important role since opioid projections originating from the hypothalamus appear to modulate dopaminergic neuronal activity in the ventral tegmental area (VTA) (Bourdy & Barrot, 2012). Currently there is only one report in human subjects with alcohol use disorder (N=16) compared to controls (N=13), all of whom were scanned with dopamine-3 receptor preferring ligand [^{11}C] PHNO, which showed higher binding in alcohol-dependent patients in hypothalamus

(V_T: 16.5±4 *vs* 13.7±2.9, p=0.040), a region in which the [^{11}C] PHNO signal almost entirely reflects dopamine-3 receptor availability (Erritzoe *et al.*, 2014). As these data are preliminary further investigation of the purported role of the network involving hypothalamus and VTA and related changes in the sensitivity of dopaminergic and opioid systems is warranted.

There are also several clinically relevant implications. We already mentioned sensitization, which, as pointed out in a recent review on the effects of stimulant treatments for ADHD (Ivanov *et al.*, 2022), remains poorly understood in humans in contrast to reports from animal research (Chang *et al.*, 2019). Specifically, when there are some compelling animal data, suggesting that sensitization might be ubiquitously registered in animal experiments, the results from human longitudinal studies (that have notable limitations) have shown no evidence supporting possible sensitization in ADHD children exposed to stimulants. Moreover, recent large-scale epidemiological studies have suggested protective properties of stimulant treatment for ADHD patients in relation to substance use related outcomes (Quinn *et al.*, 2017). In turn, there is dearth of experimental studies that can reliably elucidate such discrepancies (Ivanov *et al.*, 2022). As we point out in this last publication future studies may need to consider two critical clinical aspects. One is related to possible brain based changes in the human reward system as result of exposure to an abusable substance like amphetamines. Such exposure can certainly occur via the use of amphetamines for ADHD treatment in childhood, however, there is a notable amount of data documenting that conventional ADHD pharmacological treatments with FDA approved doses of stimulants are not associated with increase prevalence of substance use disorders in later life (Chang *et al.*, 2019). What remains unknown is the possibility that certain individuals may have "elevated" or "high" risk for SUD development and that those individuals may have predisposing neurobiological states (e.g. altered responsiveness to anticipation vs reward outcomes) that may respond differently to stimulant exposure. These possibilities have been discussed in a recent paper reviewing the effects of dopamine on motivation, learning and behavioral control

in healthy adults (Webber *et al.*, 2021). The above described differences in the response of the opioid system to acute vs chronic amphetamine exposure certainly adds potentially critical new evidence suggesting that chronic amphetamine exposure might have a measurable effect on the opioid system. That aligns with the fact that ADHD treatments tend to be long term.

It is very important to acknowledge that the above discussed possibilities might be relevant only to a subset of patients with ADHD who also have additional risk factors such as familial SUD and/or comorbid conditions, especially other behavioral disorders (e.g. conduct disorder). One notable challenge is that such purported effects of amphetamine on the brain reward system might be difficult to assess through behavioral tasks or scales and be most evident on neurobiological level, which in turn can be indexed only with the use of neuroimaging. Sophisticated neuroimaging techniques that can index such changes, however, are still out of the realm of routine clinical use. It is therefore essential to design and conduct appropriate experiments that can reliably demonstrate if such neurobiological changes suggestive of brain "sensitization" may occur after chronic amphetamine exposure in high risk individuals. We should mention that the development of such an algorithm to detect "sensitization" at the neurobiological level in humans may go beyond the use of amphetamine treatment and be relevant to other pharmacological interventions such as treatments with opioids for pain management and the use of ketamine for depression.

The second clinically relevant aspect is related to impulsivity, which by itself is a risk factor for SUD development. As number of reports seem to consistently show the link between opioid system responses and impulsivity, this relationship may further suggest opportunities for alternative interventions to control impulsive behaviors. The data showing that baseline μ-OR binding positively correlates with impulsivity measures in PG suggest that low endogenous opioids may be linked to higher propensity for impulsive behaviors. While impulsivity in ADHD is often adequately treated with stimulants (e.g. amphetamines) there might be alternative options to influence opioid receptors via opioid partial agonists to alter and decrease impulsivity thus bypassing the use of dopaminergic agents with abuse potential. However, these possibilities are still far from clinical practice and should be undertaken more robustly only after there is convincing evidence that sensitization by stimulants is a true phenomenon that is relevant in the human case.

In conclusion we have reviewed and presented compelling data from animal studies showing that activation μ-OR and δ-OR seem to modulate the interactions with the dopamine system that can produce behavioral sensitization. Further, animal studies with NTX pretreatment suggests differences in acute vs chronic effects the latter being demonstrated by the influence of the pharmacological intervention on cognitive and affective processing. Although limited, preliminary data from human research suggest a robust link between dopaminergic and opioid systems in the human brain that could be relevant to understanding the "wanting" and "liking" phases of drug use and possible sensitization in individuals at high risk for SUD. Sensible next steps in these lines of research would be to combine treatment (e.g. long term exposure) and neuroimaging protocols in individuals at high SUD risk to assess brain responses to stimulant before exposure to any other abusable substances (Ivanov *et al.*, 2022). Such approach may be useful to identify reliable behavioral and biological indicators of "sensitization" in order to confirm vs rule-out sensitization effects in the human case.

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