Investigating the effects of methylphenidate on cue salience and behavioral flexibility: Role of D2 receptors

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ABSTRACT

INVESTIGATING THE EFFECTS OF METHYLPHENIDATE ON CUE SALIENCE AND BEHAVIORAL FLEXIBILITY: ROLE OF D2 RECEPTORS

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Department of Psychology
Northern Illinois University, 2019
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Healthy individuals worldwide are using cognitive enhancing drugs in an attempt to remain competitive in society for education, careers, and athletics. Commonly, these individuals are using prescription stimulants, such as methylphenidate (MPH), due to their known acute neuroenhancing effects. While these neuroenhancing effects have been established using a variety of learning, memory, and attention tasks in humans and rodents, little research has investigated the effects of MPH use on specific components of these tasks, such as cue salience. One process that is important for learning the relationship between a cue and a reward is salience, or the heightened perception and desirability of cues associated with the reward. Salience toward cues can facilitate learning but may also be implicated in increasing the likelihood of addiction and relapse. Cue salience is also related to dopamine D2 receptor function, which can be influenced by MPH administration. In rats, salience toward cues can be quantified using the Pavlovian Conditioned Approach (PCA) Index. Heightened salience towards cues may also make shifting one’s attention away from one learned cue to a novel cue more challenging. Many of MPH’s cognitive enhancing effects are attributed to enhancement of working memory; previous research shows that MPH enhances behavioral flexibility in an attentional set-shifting task in ADHD-diagnosed individuals. It is unknown whether prior exposure to MPH alters attentional set-shifting performance in healthy adults. The current study
hypothesized that daily administration of MPH in healthy adult rats would increase cue salience, leading to a subsequent impairment of attentional set-shifting and the hypothesis that cue salience and attentional set-shifting would be mediated by $D_2$ receptor function. Daily administration of MPH tended to decrease lever pressing and PCA Index Scores but had no effect on $D_2$ receptor immunoreactivity. Overall, there was no effect of MPH or raclopride alone on the attentional set-shift, but raclopride administration had different effects in rats previously exposed to MPH. Raclopride significantly increased trials needed to reach criterion during the set-shift in rats that were previously administered MPH, suggesting that MPH has lasting effects on $D_2$ circuitry that do not manifest changes in executive functioning unless challenged with a $D_2$ receptor antagonist. The lasting changes are now hypothesized to be an up-regulation of $D_2$ receptors due to our finding that MPH tends to decrease PCA Index Scores. Thus, when administered a $D_2$ receptor antagonist, rats with upregulated $D_2$ receptors may have dopaminergic function that is beyond an optimal performing range. These data suggest that cue salience may be a mechanism underlying the use of MPH for cognitive enhancement, which may also have implications for association mechanisms in addiction.
INVESTIGATING THE EFFECTS OF METHYLPHENIDATE ON CUE SALIENCE AND
BEHAVIORAL FLEXIBILITY: ROLE OF D2 RECEPTORS

BY

MERCEDES MCWATERS
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FOR THE DEGREE
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Leslie Matuszewich
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CHAPTER 1

INTRODUCTION

Healthy individuals worldwide are using cognitive enhancing drugs in an attempt to remain competitive in society for education, careers, and athletics. Commonly, these individuals are using prescription stimulants, such as methylphenidate (MPH), due to their known acute neuroenhancing effects (Aron, Dowson, Sahakian, & Robbins, 2003; Berridge et al., 2006; for review, see Smith & Farah, 2011; Vreco, 2013). While these neuroenhancing effects have been established using a variety of learning, memory, and attention tasks in humans and rodents, little research has investigated the effects of MPH use on the formation of associations with rewards, which may have implications for motivated and addiction-related behaviors. One process that is important for learning the relationship between a cue and a reward is salience, or the heightened perception and desirability of cues associated with the reward. Salience toward cues can facilitate learning and thus may be a mechanism by which MPH can enhance learning. Further, this enhancement of learning may have implications specifically for strengthening association learning between rewards and reward-related cues in the environment.

In rats, salience toward cues can be quantified using the Pavlovian Conditioned Approach (PCA) Index in autoshaping (Meyer et al., 2012). The PCA Index Score assesses a rat’s propensity to attribute incentive salience toward cues associated with reward; rats that have high PCA Index Scores (sign trackers) are highly cue-oriented as the cue is rewarding in its own right
following conditioning. Low PCA Index Scores (goal trackers) are indicative of goal orientation, whereby the cue can signal or be associated with the oncoming reward, but the cue never acquires incentive salience. MPH may alter the propensity to attribute incentive salience toward a reward-associated cue, which in turn can alter learning processes. Therefore, Experiment 1 sought to investigate the effects of daily MPH administration on cue salience, as measured by PCA Index Scores. We hypothesized (H1a) that daily administration of MPH prior to each autoshaping session would increase PCA Index Scores as compared to rats receiving vehicle injections. This hypothesis was predicated on previous literature that suggests that dopaminergic transmission, which is enhanced by acute MPH, mediates associative learning (Puig & Miller, 2012; for review, see Berridge & Robinson, 1998).

MPH may impact cue salience through its effects on dopaminergic transmission within reward-related brain regions. Specifically, prefrontal and striatal D₂ receptors are implicated in acquisition and strength of cue associations in humans and animals (Fraser et al., 2016; Heinz et al., 2004; Weber et al., 2016). Low levels of D₂ receptors are correlated with increased cravings in humans and animals (Flagel et al., 2007; Heinz et al., 2004; Volkow et al., 2002). To assess for potential changes in D₂ receptors in the prefrontal cortex and striatum following MPH exposure, western blots were conducted to quantify D₂ receptor numbers following behavioral testing in Experiment 1. We hypothesized (H1b) that MPH’s effects on PCA Index Scores would be D₂ receptor-dependent, with exposure to MPH decreasing D₂ receptor quantities in the prefrontal cortex and striatum. It was also hypothesized that the MPH-induced decrease in D₂ receptors in these regions would be correlated with higher PCA Index Scores.

Heightened salience towards cues also may make shifting one’s attention away from a previously learned cue to a novel cue more challenging. Many of MPH’s cognitive enhancing
effects are attributed to improvement of working memory and previous research shows that MPH enhances behavioral flexibility, a specific subcomponent within working memory (Linssen, Vuurman, Sambeth, & Riedel, 2012). Attentional set-shifting is a task designed to measure behavioral flexibility whereby an organism is trained to learn a strategy based on a rule and then adapt when the rule is suddenly changed. The organism must be flexible and inhibit the previously learned rule in order to adequately respond to the change in environmental contingencies. Within attentional set-shifting paradigms, the number of trials needed to learn the new rule can be assessed alongside the number of errors the organism makes during the set-shift. MPH improved performance on set-shifting for ADHD-diagnosed individuals, as evidenced by fewer trials to reach criterion and fewer errors (Cao et al., 2012; Mehta, Goodyer, & Sahakian, 2004). However, it was unknown whether MPH enhances attentional set-shifting in healthy adults, who use it as a cognitive enhancer. Therefore, Experiment 2 assessed whether prior exposure to MPH influences behavioral flexibility using the attentional set-shifting task and whether the effects on attentional set-shifting could be explained by incentive salience.

We hypothesized (H2a) that daily administration of MPH during autoshaping would increase trials needed to reach criterion during the attentional set-shift as compared to rats with daily administration of saline. This hypothesis was built upon H1a, suggesting that rats would have heightened salience toward the lever, and therefore we predicted that the shift to response would be more challenging, with rats making a greater number of preservative errors and requiring more trials to meet criteria. However, MPH was hypothesized to decrease regressive errors, such that once the task was learned, the new association would be stronger than the old strategy. We also hypothesized that PCA Index Scores would account for a significant portion of the variance during the attentional set-shift, as the heightened incentive salience attributed to the
cue would make the response shift more challenging, thus increasing trials to reach criterion and preservative errors.

A recent body of evidence suggests that both acquisition of cue association and attentional set-shifting are dopamine-dependent (Flagel et al., 2011; Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006). Thus, a subset of animals injected either with MPH or saline during autoshaping were administered a D₂ antagonist, raclopride, prior to the set-shift to investigate the role of D₂ transmission in the attentional set-shift task. We hypothesized (H2b) that administration of a D₂ antagonist prior to the set-shift would decrease trials needed to reach criterion in the rats previously administered MPH during autoshaping. The decrease in trials to criterion following D₂ antagonist administration in rats previously administered MPH would likely be accompanied by a decrease in preservative and regressive errors. Consistent with previous literature, we hypothesized (H2c) that the D₂ antagonist would increase trials needed to reach criterion in rats previously administered vehicle in autoshaping. Taken together, we predicted that MPH administration would increase PCA Index Scores, thus making the set-shifting more challenging, but D₂ antagonism would help reverse those effects.

Despite high use of MPH as a cognitive enhancer, there is little research on the potential influence of recreational MPH use on cue salience, which may have implications for drug addiction and/or relapse behaviors. By testing the above hypotheses, we hoped to establish the role of cue salience in working memory processes (attentional set-shifting), determine the influence of MPH on these variables, and assess the role of D₂ receptors in these processes. These findings contribute to our understanding of the mechanisms underlying the use of MPH for cognitive enhancement and associative learning.
Cognitive Enhancement

Worldwide, healthy individuals seek to buy and use prescription stimulants to enhance some element of their cognition or performance, despite potential negative repercussions of their actions (Bagot & Kaminer, 2014; de Jongh, Bolt, Schermer, & Olivier, 2008; Franke, Bagusat, Rust, Engel, & Lieb, 2014; Frati et al., 2015; Husain & Mehta, 2011). Pharmacological cognitive enhancement is the use of pharmacological substances with the intent of improving one’s cognitive abilities. Cognitive enhancement is also referred to as cosmetic neurology, academic performance enhancement, use of nootropics, academic doping, or neuroenhancement (Franke et al., 2014; Zonneveld, Dijstelbloem, & Ringoir, 2008). Frequently, the goal is to enhance cognitive abilities such as working memory, memory consolidation, attention, vigilance, wakefulness, and/or speed of processing (Franke et al., 2017). For example, cognitive enhancers are utilized by students and academics during times of high cognitive demand (e.g. exams, completing a dissertation; Burgard, Fuller, Becker, Ferrell, & Dinglasan-Panlilio, 2013; Franke et al., 2017; Hanson et al., 2013) but are also used by the elderly to delay cognitive decline, surgeons to counteract fatigue, athletes to increase alertness and reaction time, and members of the military to remain awake and alert for long periods of time (Franke et al., 2013; Husain & Mehta, 2011). The misuse of these drugs by athletes has led to the recognition of cognitive enhancers, such as stimulants, by the World and U.S. Anti-Doping Agencies; therefore, these substances are banned from sport competitions (Docherty, 2008). Cognitive enhancement being viewed as a form of cheating is not limited to sports; there is concern that illicit use is also a form of cheating in academia (Lucke, Bell, Partridge, & Hall, 2011). Whatever their reason, healthy individuals use pharmacological agents to achieve effects that are anticipated due to the drugs’ pharmacodynamic properties.
In recent years, the illicit use of cognitive enhancers has increased and is now a medical and public health concern (Arria & DuPont, 2010; Banjo, Nadler, & Reiner, 2010; Cakic, 2009; Rose, 2002; Swanson, Wigal, & Volkow, 2011; Zonneveld, Dijstelbloem, & Ringoir, 2008). For example, a cross-sectional study of 15 countries including the United States suggests that cognitive enhancement use has increased from 4.9% in 2015 to 13.7% in 2017 using the Global Drug Survey (Maier, Ferris, & Winstock, 2018). Lifetime prevalence rates of nonmedical stimulant use in the United States are estimated between 8.9% and 55% (DeSantis, Noar, & Webb, 2009; Rabiner et al., 2009; Smith & Farah, 2011). Han, Jones, Blanco, and Compton (2017) used a regression model on National Surveys on Drug Use and Health (NSDUH) data to show that the prevalence of nonmedical stimulant use in 2013-2014 was higher than in 2003-2004. In the United States, the increased use of drugs for cognitive enhancement has been attributed in part to the publication of Smart Drugs and Nutrients (Canterbury & Lloyd, 1994). In this book, Dean and Morgenthaler provided the public with information on over 75 drugs and nutrients that would enhance memory and intelligence based on current neuroscience research at the time, thus likely sparking a higher level of public interest in cognitive enhancement. A significant portion of healthy individuals in years since have illegally acquired and used prescription stimulants to achieve desired cognitive enhancing effects; this is especially common in academia (for review, see Franke, Bagusat, Rust, Engel, & Lieb, 2014). The rise of cognitive enhancer use may be due to individuals viewing procurement of stimulants as easy and stigma-free and use of stimulants as harmless and morally acceptable (in a sample of college students; DeSantis, Webb, & Noar, 2008).

Cognitive enhancers can be categorized into four broad groups. The first group of cognitive enhancers includes prescription drugs used to treat Attention Deficit Hyperactivity
Disorder (ADHD), such as psychostimulants (methylphenidate/Ritalin® or amphetamine/Adderall®). The second group of cognitive enhancers includes prescription drugs used to treat sleep disorders, such as modafinil/Vigil®. The third group consists of substances that can be purchased over the counter without a prescription, such as coffee or other caffeinated beverages like energy drinks, caffeine tablets, and Ginkgo biloba. These products have the most widespread use due to their easy accessibility. The fourth group consists of acetylcholinesterase inhibitors, which are commonly used to alleviate cognitive deficits seen in individuals with dementia. While these pharmacological cognitive enhancers have similar properties, they target different neural systems and therefore yield varied cognitive effects. Some cognitive enhancers target “online” cognitive processes, such as executive function/working memory and attention. Others target more “offline” processes like memory consolidation.

One of the most commonly prescribed medications used for cognitive enhancement is methylphenidate (MPH; Smith & Farah, 2011). There is an abundance of research on the acute effects of MPH in individuals diagnosed with ADHD, but there is relatively little research on the acute effects of MPH in normal individuals. This lack of research is surprising given the fairly high percentage of healthy individuals using MPH within their lifetime, without a clinical diagnosis (for review, see Smith & Farah, 2011). It is assumed that MPH will have beneficial and cognitive enhancing effects in healthy individuals as it does for individuals diagnosed with ADHD, but this may not be true as neural functioning requires an optimal level (Batistela, Bueno, Vaz, & Galduróz, 2016; Berridge et al., 2006), which may or may not be the same for healthy individuals compared to individuals diagnosed with ADHD.
Prevalence

Psychostimulants such as MPH and amphetamine are the most commonly used illicit cognitive enhancers, particularly in academic settings (Smith & Farah, 2011). MPH, or Ritalin®, is one of the most commonly prescribed psychostimulant treatments for ADHD (Zito et al., 2000), and is approved by the U.S. Food and Drug Administration (FDA) for treatment of ADHD and narcolepsy (Challman & Lipsky, 2000; U.S. Food and Drug Administration, 2016). Amphetamine (AMPH), or Adderall®, is also approved to treat ADHD and is often prescribed as a mixed AMPH salt, dextroamphetamine. These psychostimulants are commonly prescribed to clinical populations because they are highly effective at enhancing attention and reducing hyperactivity symptoms (Berridge et al., 2006; Faraone & Buitelaar, 2010; Mehta et al., 2000; Rapoport et al., 1980; Vaidya et al., 1998).

Due to their cognitive effects, these psychostimulants are also some of the most commonly used cognitive enhancers in college students. Use of these drugs is thought to improve the same cognitive and attentional systems in healthy individuals as they target in those with ADHD. It is challenging to assess the prevalence of illicit use of these substances because prescription stimulants are FDA Schedule II controlled substances and illegal users may not readily admit to their use. However, estimates in subpopulations can be acquired through surveys (although still prone to self-report biases). In a review, Smith and Farah (2011) reported that across various convenience samples of colleges in the United States, lifetime prevalence rates of nonmedical stimulant use ranged from 8.9% (Rabiner et al., 2009) to 55% (DeSantis, Noar, & Webb, 2009). More recently, in Germany, Dietz and colleagues (2013) employed a randomized response technique to assess cognitive enhancer use (they found a 12-month prevalence rate around 20%) and suggest that direct survey techniques underestimate prevalence.
rates. Interestingly, illicit use is higher in students at colleges with more competitive admission standards (Franke et al., 2011; Hanson et al., 2013; McCabe, Knight, Teter, & Wechsler, 2005). For example, McCabe and colleagues (2005) found that the prevalence of non-medical use of prescription stimulants across a sample of 119 four-year colleges in the United States was a function of competitive admission criteria; the majority of colleges (80%) sampled who had a past-year prevalence rate of 10% or higher were characterized as having highly competitive admissions standards.

Researchers have attempted to estimate prevalence rates in more creative ways to reduce self-report bias in surveys. Hanson and colleagues (2013) monitored status messages on social media (i.e., Twitter) around United States colleges and found over 213,000 tweets from 132,000 public users that mentioned Adderall. A significant percentage (12.9%) of these tweets about Adderall also included an academic motive, such as Adderall’s use as a study aid or to help with finals, indicating that Adderall is commonly used among college students. These tweets were more common on weekdays than weekends and spikes in the number of tweets occurred in December and May, when final exams typically occur. While this data set does not provide prevalence rates of psychostimulant use, it does provide a non-intrusive sampling method of the population using Adderall. Another group of researchers measured amphetamine and ritalinic acid, metabolites of amphetamine and MPH, respectively, in college campus wastewater; levels of amphetamine increased during high periods of academic stress, such as midterm and finals weeks, but ritalinic acid showed no obvious changes (Burgard, Fuller, Becker, Ferrell, & Dinglasan-Panlilio, 2013). This data is consistent with college student survey data that shows that the illicit use of amphetamine is more prevalent than the illicit use of MPH (Teter, McCabe, LaGrange, Cranford, & Boyd, 2006). Although MPH metabolites did not fluctuate across the
academic year (i.e., no changes corresponding with periods of high stress), the measured concentrations of the two metabolites were similar. Also, metabolites of MPH gradually rose across the first semester and leveled out the second semester, potentially indicating more continual MPH use, rather than periodic use during periods of high academic stress (Burgard et al., 2013). Overall, self-report, social media, and wastewater data suggest that the use of MPH as a cognitive enhancer is on the rise and warrants continued study of the effects of its recreational use.

**Methylphenidate: Mechanism of Action**

* Dopamine, dopamine transporters, and norepinephrine transporters. MPH is a central nervous system psychostimulant that affects cognitive and behavioral processes via its action in the brain. MPH is classified as a psychostimulant because its mechanism of action and structure are very similar to those of other stimulants, such as amphetamine and cocaine, in that it acts on the central nervous system to stimulate arousal and alertness (Santosh & Taylor, 2000). In humans and rats, MPH binds to both dopamine (DA) and norepinephrine (NE) transporters, although it has greater affinity for the DA transporter (Gatley, Pan, Chen, Chaturvedi, & Ding, 1996; Kuczenski & Segal, 1997). When administered in clinically therapeutic doses, MPH is estimated to block over half of dopamine transporters (DAT) in the brain (Volkow, Fowler, Wang, Ding, & Gatley, 2002; Volkow et al., 1998). Occupying DAT results in an increase in extracellular DA levels (Koda et al., 2010; Kuczenski & Segal, 1997; Volkow, Wang, Fowler, & Ding, 2005; Volkow et al., 2001). MPH also has a high binding affinity for norepinephrine transporters (NET), which results in an increase in extracellular NE similar to increases in DA (Koda et al., 2010; Kuczenski & Segal, 1997). MPH has a very weak affinity for serotonin transporters, producing low or no increases in extracellular serotonin at clinically effective doses
(Koda et al., 2010; Kuczenski & Segal, 1997). The minimal effects of MPH’s actions on serotonin transporters may contribute to a reduction in hyperactivity, but it is not thought to affect neurocognition (Gainetdinov et al., 1999; Pan et al., 1994).

_In vivo_ microdialysis studies in rodents show that the MPH-induced increases in DA and NE are region-specific, targeting brain regions important for motor function, cognition, and learning. Although named for the neurotransmitter that binds with the greatest affinity, the catecholamine transporters are capable of binding other catecholamines as well. For example, NET can reuptake DA as well as NE (Buck & Amara, 1994; Morón, Brockington, Wise, Rocha, & Hope, 2002; Yamamoto & Novotney, 1998). Psychostimulant drugs, such as MPH, that bind to both DAT and NET will increase particular neurotransmitters based on the distribution of their reuptake sites throughout the brain. For example, MPH-induced increases in DA and NE are largely due to its actions at NET in the medial PFC (mPFC), which has a high concentration of NET and a relatively low concentration of DAT (Morón et al., 2002; Yamamoto & Novotney, 1998). The striatum, on the other hand, has a very high concentration of DAT and a low concentration of NET; therefore, when MPH is administered, increases in striatal catecholamines are observed due to its actions on DAT primarily and NET to a lesser extent (Coulter, Happe, Bergman, & Murrin, 1995; Donnan et al., 1989; Easton, Marshall, Marsden, & Fone, 2009; Volkow, Fowler, Wang, Ding, & Gatley, 2002; for review, see Madras, Miller, & Fischman, 2005). Thus, MPH can result in extracellular increases in DA by blocking both DAT and NET, based on the distribution of these transporters in the PFC and striatum.

The distribution and density of DAT modulate basal levels of DA, which have been suggested to relate the symptoms of ADHD. The greater DAT availability, the more extracellular DA is removed from the synapse and hence lower basal levels of extracellular DA
levels. There is clear evidence that low dopaminergic function in the PFC and striatum correlates with symptoms of ADHD (for review, see Arnsten, 2009), but it is not well accepted that these low DA levels stem directly from abnormally high DAT availability. In the striatum, higher DAT levels were positively correlated with symptoms of inattention in humans (Cheon et al., 2003; Tomasi et al., 2009). However, other studies showed no changes (van Dyck et al., 2002) or decreases in striatal DAT availability (Hesse, Ballaschke, Barthel, von Cramon, & Sabri, 2006; Volkow et al., 2009) in individuals with ADHD. In one study, individuals diagnosed with ADHD who had never taken medication for ADHD had lower DAT availability in the caudate, nucleus accumbens, and hypothalamus as compared to non-diagnosed individuals (Volkow et al., 2009). A potential explanation for the opposing directionality seen in the research is that DAT is up-regulated or down-regulated as a result of some other dysfunction that alters DA levels; low extracellular DA activity could then result in the downregulation of DAT expression (Volkow et al., 2007). Because the noradrenergic system can modulate DA release, others have postulated that decreased extracellular DA seen in ADHD is an artifact of noradrenergic dysfunction, rather than increased DAT (Biederman & Spencer, 1999; Bymaster et al., 2002; Viggiano, Ruocco, Arcieri, & Sadile, 2004). Regardless of the mechanism driving imbalanced DA, therapeutic drugs known to alleviate the associated symptomatology target catecholamine transporters and therefore, DA and NE pathways.

**Dopamine receptors.** MPH’s increase in extracellular dopamine influences downstream pathways through DA receptors. DA receptors can be classified into two main families: 1) the D₁ family, which contains D₁ and D₅ receptors, and 2) the D₂ family, which contains D₂, D₃, and D₄ receptors (Vallone, Picetti, & Borrelli, 2000). Both families of DA receptors have different functionality due to the differences in their downstream mechanisms (for review, see Missale,
Nash, Robinson, Jaber, & Caron, 1998). When stimulated, DA D₁ receptors increase adenylyl cyclase activity, which in turn upregulates cyclic adenosine monophosphate (cAMP). DA D₂ receptors, when stimulated, result in the opposite effect: decreased adenylyl cyclase activity and downregulated cAMP. Both D₁ and D₂ receptors alongside their downstream effects play important roles in regulating cognition and behavior, as discussed next.

MPH has been shown to activate the cAMP pathway within the PFC, likely as a downstream result of stimulation of D₁ receptors (Pascoli et al., 2005). Stimulation of cAMP can both strengthen connectivity between neurons with shared characteristics and weaken connections of “nonpreferred inputs”, thus reducing “noise” and enhancing behavioral performance (Gamo, Phil, Wang, & Arnsten, 2010). Arnsten and Dudley (2005) have demonstrated a critical role for D₁ receptors in MPH’s behavior-enhancing effects. First, they demonstrated that rats receiving 1.0 or 2.0 mg/kg oral MPH exhibited improved performance on a delayed alternation t-maze task compared to their control trials. When a D₁ antagonist, SCH23390, was co-administered with MPH, the improved delayed alternation performance was no longer evident, providing evidence that D₁ receptors mediated MPH’s enhancing effects on working memory. The role of D₁ receptors in MPH’s enhancing effects is corroborated by primate research showing that administration of SCH23390 also blocked enhanced delayed alternation performance following individualized optimal doses of MPH (ranging from 0.1 to 2.0 mg/kg; Gamo, Phil, Wang, & Arnsten, 2010). Together, these studies suggest that MPH-induced stimulation of D₁ receptors can enhance performance on working memory tasks.

MPH activates D₁ receptors indirectly by increasing extracellular levels of DA and thus increasing the likelihood that DA will bind to a D₁ receptor. Activation of D₁ receptors also activates protein kinase A and protein kinase C second messenger signaling pathways, which can
upregulate or downregulate Ca\textsuperscript{2+} currents via L-type channels, respectively (Young & Yang, 2004). These Ca\textsuperscript{2+} currents are important for PFC spatiotemporal signal amplification, integration, and plasticity (Faber, 2010; Yang, Seamans, & Gorelova, 1999); disorganized signaling is associated with disruptions of PFC-mediated behaviors (Kabir, Lee, & Rajadhyaksha, 2016). Activation of DA D\textsubscript{1} receptors increases neuronal excitability by enhancing N-methyl-D-aspartate (NMDA) input, mimicking the excitatory effects of glutamate in the PFC and enhancing sodium currents (Gorelova & Yang, 2000; Wang & O’Donnell, 2001). This downstream effect further facilitates DA release. Thus, MPH increases extracellular release through binding to the catecholamine transporters and through its downstream effects on D\textsubscript{1} receptors, which can affect behavior.

D\textsubscript{2} receptors also play an important, but different, role in MPH-induced DA release. Volz and colleagues (2008) demonstrated that MPH administration increased DA release in the rat striatum, which could be attenuated by a co-administration of a D\textsubscript{2} receptor antagonist. MPH increased striatal potassium-stimulated DA release and decreased vesicular monoamine transporter-2 (VMAT-2) membrane-associated vesicle immunoreactivity in the striatum. Both actions contribute to increased DA release, with a decrease of membrane-associated VMAT-2 resulting in decreases in the sequestering of DA inside of vesicles. Eticlopride, a D\textsubscript{2} receptor antagonist, blocked these effects of MPH, suggesting that MPH-induced DA release is mediated by D\textsubscript{2} receptors.

D\textsubscript{2} receptors have various functions corresponding with presynaptic or postsynaptic localization and are found at high densities in the striatum, PFC, and nucleus accumbens (Beaulieu & Gainetdinov, 2011; Missale et al., 1998; Vincent, Khan, & Benes, 1993). Presynaptic D\textsubscript{2} autoreceptors are G-coupled inhibitory receptors that use a negative feedback
mechanism to regulate DA transmission, including DA synthesis, release, and reuptake. Activation of D$_2$ autoreceptors decreases DA synthesis, DA neuron excitability, and the release of DA (for review, see Ford, 2014), thus decreasing the excitability of dopaminergic PFC afferents throughout the brain (Trantham-Davidson, Neely, Lavin, & Seamans, 2004). D$_2$ receptors are also found postsynaptically on non-dopaminergic neurons receiving DA afferents, such as glutamate or acetylcholine neurons, which can then subsequently stimulate or inhibit DA release depending on neuron type. For example, postsynaptic D$_2$ receptor agonism on glutamatergic synapses and the subsequent DA release inhibit glutamate release in striatal terminals; the opposite effect is observed with D$_2$ receptor antagonism. Thus, the disinhibitory effects of D$_2$ antagonism can stimulate glutamate release and thereby stimulate further DA release in striatal terminals (Bamford et al., 2004). In contrast, simulation of D$_2$ receptors on GABAergic neurons has been shown to decrease GABA release (Chiu, Puente, Grandes, & Castillo, 2010; Seamans, Gorelova, Durstewitz, & Yang, 2001). Thus, the effects of D$_2$ receptor stimulation are complex, potentially leading to presynaptic inhibition of DA release and/or postsynaptic excitation of DA release.

Psychostimulants increase extracellular levels of DA/NE that bind to catecholamine receptors, which regulate neuronal excitability and second messenger systems. Based on our current understanding of cognitive processes, these pharmacological actions, particularly within the PFC, suggest that psychostimulants may be useful as cognitive enhancers. However, MPH affects both D$_1$ and D$_2$ receptors and the opposing effects of D$_1$ and D$_2$ receptor activation may contribute to the variability in MPH’s effects. For example, activation of these receptors can differentially regulate the tuning and inhibition of cortical networks, which could have profound impacts on working memory (Trantham-Davidson, Neely, Lavin, & Seamans, 2004). It is not
well understood how the two receptor subtypes work together to affect behavior or the relative contributions of presynaptic and postsynaptic D$_2$ receptors. By increasing extracellular DA, MPH likely activates both subtypes, which could inhibit and promote further DA transmission. As much of the early research focused on D$_1$ receptors, further research is needed to understand the role of D$_2$ receptors in working memory.

**Methylphenidate’s Effects on Working Memory: Spatial Tasks**

Cognitive enhancers improve performance in a variety of tasks, likely because of their effects on executive functioning. Executive functions, such as attention and inhibition, are high-level cognitive processes responsible for our thoughts and behaviors (Alvarez & Emory, 2006). These upper level cognitive processes organize and execute lower level functions, which yield organized cognition and goal-directed behaviors. Research on various types of executive functioning has been rapidly growing over the past 20 years, with much of the focus on sustained and selective attention, inhibition, and working memory (for review, see Alvarez & Emory, 2006). Working memory tasks have been most commonly researched, although performance on these tasks includes measures of the other constructs as well (e.g., attention), which will be discussed below.

Many of the psychostimulants’ cognitive enhancing effects are attributed to their improvement of working memory, which involves the temporary storage and manipulation of information (Baddeley, 1983). The cognitive enhancing effects of MPH have been demonstrated in a variety of working memory tasks in humans and animals (Elliott et al., 1997; Marquand et al., 2011; Mehta et al., 2000; Ramasubbu, Singh, Zhu, & Dunn, 2012; for review, see Linssen, Sambeth, Vuurman, & Riedel, 2014). For example, healthy males given either a 20 or 40 mg
oral dose of MPH performed better on a spatial working memory search task and the Tower of London executive functioning task (Elliott et al., 1997). The spatial working memory test required participants to search through colored boxes for tokens that were organized in a spatial array and measured the number of errors participants made during the search. The Tower of London task is composed of colored disks that are organized among three towers and the participant must move them one at a time to match a goal state (see Phillips, Wynn, Gilhooly, Della Sala, & Logie, 1999). Individuals given MPH made fewer errors on the spatial working memory search task and had increased accuracy on the Tower of London task compared to individuals given placebo (Elliot et al., 1997). This effect was corroborated in healthy males who were administered an oral 40 mg dose of MPH and had improved performance on a computerized spatial working memory task as measured by fewer errors than a placebo control (Mehta et al., 2000). However, the enhanced performance on this active working memory task was highest in participants with the lowest baseline working memory capacity.

The animal research on the neuroenhancing effects of MPH on working memory parallels the human literature. Oral 1.0- 2.0 mg/kg MPH significantly improved performance on a delayed alternation task in healthy rats as measured by percentage of trials correct (Arnsten & Dudley, 2005; Berridge et al., 2006). Optimal performance of the delayed alternation task requires spatial working memory, sustained attention, and response inhibition. In this task, animals are required to choose a different (or alternating) arm in a t- or y-maze in order to receive rewards on a delayed schedule; thus, a strategy of returning to a previously baited arm would not be fruitful. Similarly, MPH enhanced performance on a delayed response task in Rhesus macaques (Gamo, Wang, & Arnsten, 2010). In this task, macaques had to retain a memory of the location where a food reward had been previously placed by the experimenter;
after the macaque watched the experimenter place the food into one of six wells, the wells were covered with an opaque screen for a delay period of 10-40 seconds, thus testing spatial working memory. Chronic administration of oral MPH has also been shown to improve rats’ spatial learning and memory in a radial arm maze win-shift task (Zhu, Weedon, & Dow-Edwards, 2007). In this spatial working memory task, rats had to correctly retrieve food from eight baited arms without re-entering arms that food had already been retrieved from. Rats met criterion when they entered at least seven of the baited arms without repeating arms for four out of five trials. MPH increased the number of correct choices before the first error and decreased time to complete the first eight entries across the first seven days of testing but had no effect on days needed to reach criterion. Together, this research demonstrates that administration of MPH can improve performance on spatial working memory tests, suggesting that MPH may have similar beneficial effects for performance on other executive function tasks. This effect also extends into research on the effects of MPH on non-spatial working memory tasks, specifically in regards to reaction time, memory consolidation, and attentional set-shifting.

Methylphenidate’s Effects on Working Memory: Non-Spatial Tasks

Methylphenidate has been shown to enhance cognitive function in non-spatial working memory tasks as well, such as memory scanning speed, memory consolidation, and reaction time. In a systematic review and meta-analysis, Repantis, Schlattmann, Laisney, and Heuser (2010) found that acute doses of MPH statistically enhanced performance on memory tasks compared to placebo. For example, 10-20 mg of MPH has been shown to improve the performance of healthy adults on digit span test scores, a non-spatial working memory task requiring participants to memorize increasingly longer sequences of digits (Agay, Yechiam,

MPH has also been shown to enhance attention and reaction time/processing speed. Cooper et al. (2005) investigated the dose-dependent effects of MPH in healthy adults on behavioral performance in a working memory task. Looking at low, medium, and high therapeutic doses (5 mg, 15 mg, and 45 mg), MPH dose-dependently decreased reaction time during the Continuous Performance Test. The Continuous Performance Test assesses sustained attention, a construct within working memory, by asking participants to respond when a specific combination of letters and/or digits appears while viewing a continuous series of letters and digits (for a review of this task, see Riccio, Reynolds, Lowe, & Moore, 2002). MPH in healthy adults has also been shown to decrease response time variability and stop-signal reaction time following 30 mg MPH compared to placebo, but no effect was observed in response speed, indicating that MPH does not simply increase overall motor speed (Nandam et al., 2011). This MPH-induced improvement in performance parallels what has been observed previously in samples of ADHD-diagnosed individuals. MPH increased accuracy and decreased reaction times in adolescents diagnosed with Attention Deficit Disorder (ADD) in the Continuous Performance Test and the Sternberg memory task (Klorman, Brumaghim, Fitzpatrick, & Borgstedt, 1991; Klorman, Brumaghim, Fitzpatrick, & Borgstedt, 1992). Finke and colleagues (2010) found that 40 mg MPH enhanced performance on a Theory of Visual Attention (TVA) task. The TVA task assesses perceptual processing speed as a measure of attention as stimuli
receiving the greatest attention are processed quickest and encoded first, thus competing with other stimuli for visual short-term memory storage space. Improved performance on TVA after MPH suggests that MPH enhances processing speed; as the task is not dependent on response time, higher processing speed was not simply an artifact of heightened arousal. In conjunction with the Cooper and colleagues (2005) data, this line of evidence suggests that therapeutic doses of MPH enhance performance on attention, reaction time, and processing speed.

It is known that optimal cognition is dependent on moderate levels of neural transmission. Thus, importantly, low and high doses of psychostimulants may result in transmission below or above the optimal range, respectively. Research has shown that enhancement of working memory processing speed by MPH was most prominent in individuals with lower baseline working memory within the same task (Finke et al., 2010). Similarly, healthy males who were administered an oral 40 mg dose of MPH had improved performance on a computerized spatial working memory task; however, the enhanced performance was highest in participants with the lowest baseline working memory capacity (Mehta et al., 2000). The findings from these healthy individuals supports the body of literature suggesting that MPH effects on performance are greater in ADHD-diagnosed individuals with lower baseline performance than controls. However, MPH has also been shown to enhance performance in individuals with higher baseline performance to a greater extent than individuals with lower baseline performance. For example, 20 mg of MPH in healthy students facilitated reward versus punishment learning in individuals with high baseline working memory capacity but impaired this learning in individuals with low working memory capacity (van der Schaaf, Fallon, ter Huurne, Buitelaar, & Cools, 2013). With many healthy individuals using MPH as a cognitive
enhancer, it is important to investigate the effects of MPH in individuals with normal baseline activity, as the effects could differ from a clinical population.

Together, the data from human studies supports the enhancing effects of MPH on behavioral performance, both in normal and clinical populations. Importantly, research has recently begun to investigate the neurobiological mechanisms driving these behavioral enhancements. The enhanced behavioral performance following MPH administration is paralleled by electrophysiological assessments of working memory as measured by event related potentials (ERPs) while participants are completing tasks (Saliasi, Geerligs, Lorist, & Maurits, 2013). Components of electrophysiological recording provide additional information on short-term memory retention and the time to evaluate stimuli and make a task-related decision (Duncan-Johnson, 1981; for review, see Picton, 1992). For example, the P3 latency is indicative of the time to evaluate stimuli and make a task-related decision and background amplitude of P3 assesses short-term memory retention. Cooper and colleagues (2005) found that as the MPH dose increased, the target P3 latency decreased, indicating that MPH dose-dependently accelerated updating working memory in response to a task-related stimulus on the Continuous Performance Test. Decreased P3 latencies also corresponded with enhanced performance on the Continuous Performance Test, as measured by faster reaction times and fewer omission errors (Cooper et al., 2005; Saliasi, Geerlings, Lorist, & Maurits, 2013). Additionally, individuals given MPH showed an increase in background P3 amplitude, indicative of enhanced updating of working memory during the task, and increased reaction time and accuracy during the task, indicative of enhanced task performance coinciding with faster background P3 updating and lower P3 target latencies (Cooper et al., 2005). Although relatively few researchers have tried to elucidate the neurobiological mechanisms underlying MPH’s enhancement of behavior using
electrophysiology, many other researchers have provided strong evidence for catecholamine systems driving improvements in working memory.

**Salience.** Methylphenidate’s non-spatial enhancing effects have extended into research on enhancing salience. Salience, or the importance/prominence of a stimulus, can be based on the perception of the properties of a stimulus. For instance, we perceive many stimuli in our day-to-day lives, but only certain stimuli “grab” our attention because they are salient. Importantly, salience extends into motivation; salient stimuli that grab our attention and are desired will be more highly motivating and will therefore make us work harder to acquire them as compared to other stimuli (for review, see Berridge & Robinson, 1998). MPH may enhance performance on tasks by enhancing salience. Volkow and colleagues (2004) found that MPH increased saliency of a mathematical task. In this task, participants were given 20 mg of MPH before a mathematical task or before passively viewing scenery cards (the control measure) and DA was measured using positron emission tomography during the task. MPH increased DA (but only when given before the mathematical task, not the scenery cards) and increased self-reported ratings of the task being interesting, exciting, motivating, and less tiresome. Thus, researchers theorized that MPH heightened the saliency of the task by stimulating relevant neurons, thus making it more interesting and driving improved performance on the task.

**Behavioral flexibility.** Within the last couple of decades, researchers have begun to investigate behavioral flexibility as a high-level, prefrontal cortex-dependent cognitive process that assesses the ability of an organism to adjust behavior in response to a change in the environment (Dias, Robbins, & Roberts, 1996). Behavioral flexibility is crucial for proper executive functioning and has been implicated in clinical disorders characterized by cognitive deficits (Floresco, Zhang, & Enomoto, 2009; Seeger et al., 2004). In humans and rodents,
attentional set-shifting is a behavioral task that requires the organism to switch between strategies in order to receive rewards and thus is a well-accepted behavioral measure of behavioral flexibility (Heisler et al., 2015).

Previous research shows that MPH enhances behavioral flexibility in an attentional set-shifting task in ADHD-diagnosed individuals. ADHD-diagnosed boys receiving MPH successfully passed more stages on the attentional set-shifting task and had higher passing rates on the extradimensional set-shift within the Cambridge Neuropsychological Test Automated Battery than ADHD-diagnosed boys receiving placebo (on average, 18.21 mg; Mehta, Goodyer, & Sahakian, 2004). This MPH-enhancing effect has been corroborated in a rat genetic model of ADHD, the spontaneously hypertensive rat (SHR) strain. SHRs exhibited deficits in the ability to set-shift compared to the control Wistar-Kyoto rats, as measured by increased trials needed to reach criterion and increased errors on the task (Cao, Yu, Wang, Wang, Yang, & Lei, 2012; Kantak et al., 2008).

It is important to note that in these studies MPH enhances performance in individuals with lower baseline task performance. For example, humans diagnosed with ADHD tend to exhibit deficits in attentional set-shifting compared to their control counterparts (Chamberlain et al., 2011; Kado et al., 2005). Similarly, in the rodent model, SHRs had poorer performance on the digging bowl attentional set-shifting paradigm than control Wistar-Kyoto or Sprague-Dawley strains, as measured by an increase in the number of trials needed to achieve six correct consecutive trials (Cao et al., 2012; Kantak et al., 2008). Chronic treatment with therapeutic doses of MPH ameliorated the behavioral deficit seen in SHRs, decreasing the number of trials needed to reach criterion in both studies (Cao et al., 2012; Kantak et al., 2008). Interestingly, one study found that SHRs actually performed better than Wistars on a set-shift (Chess et al.,
Researchers argue that hypoactive DA in the SHR rats may result in failure to store or retrieve an initial discrimination (poor retention), explaining why the SHR rats may perform better during the set-shift as measured by trials to reach criterion. Together, this data suggests that it may be difficult to extrapolate these findings from ADHD-diagnosed humans and animal models to healthy controls because the groups differ in baseline levels of attentional set-shifting performance (Cao et al., 2012; Chamberlain et al., 2011; Kado et al., 2005; Kantak et al., 2008).

Fortunately, there is a small amount of research that has investigated the effects of MPH on attentional set-shifting in healthy individuals. One study has demonstrated that MPH enhances attentional set-shifting in healthy individuals, supporting its use as a cognitive enhancer. Linssen and colleagues (2012) found that 20 and 40 mg of MPH improved cross modal set-shifting in healthy young males as measured by having faster responses than controls and a higher number of correct trials. Thus, MPH may increase speed of processing and responding, but not at the expense of correctness. However, MPH did not enhance healthy males’ performance compared to the same subjects receiving placebo on the attentional set-shifting task within the Cambridge Neuropsychological Test Automated Battery, as measured by number of errors made (Elliott et al., 1997). With only two known studies investigating the effects of MPH on attentional set-shifting in healthy human adults and each finding different effects, more research is needed to investigate a potential mechanism for understanding how MPH may have neuroenhancing effects in working memory tasks.

Assessing Behavioral Flexibility with Attentional Set-Shifting

Evolutionarily, animals require the ability to adapt their behavior to the environment for survival. Within the last couple of decades, researchers have begun to investigate this...
adaptation, commonly referred to as behavioral or cognitive flexibility, which is a high-level cognitive process that allows an organism to adjust behavior in response to a change in the environment (Dias, Robbins, & Roberts, 1996). Behavioral flexibility may be viewed as a three-part cognitive process that requires the organism to: 1) attend to certain aspects of the environment and selectively respond to these stimuli; 2) filter out other aspects of the environment that are irrelevant; and 3) adapt to a changing environment through attending to relevant, new stimuli while filtering out the previously important stimuli (for review, see Audet & Lefebvre, 2017). This flexibility is important when the new environmental information will aid the organism more than the previously learned information. Deficits in aspects of behavioral flexibility are characteristic of many psychiatric disorders, such as schizophrenia, Alzheimer’s Disease, and Autism (Floresco, Zhang, & Enomoto, 2009; Seeger et al., 2004). For example, individuals with schizophrenia experience impairments in tests of executive functioning that require behavioral flexibility, such as an attentional set-shifting test. In the attentional set-shifting test, an individual must ignore previously irrelevant stimuli to learn a new rule; however, an individual diagnosed with schizophrenia has difficulty shifting attention from one stimulus dimension to another. In the real world, this cognitive deficit does not allow individuals to accurately update their behaviors and adapt to their environments in order to complete tasks in a situation where the behavior must change in order to achieve the goal/complete the task.

In humans, behavioral flexibility is commonly measured using the Wisconsin Card Sorting Test, which requires subjects to learn a card-sorting task and then switch to a new rule for sorting (Grant & Berg, 1948). This is a set-shifting task because the subjects must alter their behavioral response to new sets of environmental contingencies/rules (Monchi, Petrides, Petre, Worsley, & Dagher, 2001). The Wisconsin Card Sorting Test was initially developed to evaluate
cognition and strategy use in healthy humans but has since been used in laboratory and clinical settings as a tool for assessing cognitive deficits. The current version of the Wisconsin Card Sorting Test consists of four stimulus cards and 64 response cards (Grant & Berg, 1948). The four stimulus cards are one red triangle, two green stars, three yellow crosses, and four blue circles. Each response card has one of four shapes (star, circle, triangle, plus sign/cross), which are depicted in one of four colors (red, green, yellow, blue), and contains one, two, three, or four of that colored shape on the card. Procedurally, the response cards could be sorted/categorized based on color, number, or shape below the four stimulus cards. At the start of the experiment, participants are asked to put their response cards into four groups below the stimulus cards and given feedback as to whether they are “right” or “wrong.” In one series, the initial correct sorting category would be one dimension such as color; as soon as the participant correctly sorts a predetermined number of consecutive correct responses on color, the correct sorting category would be switched to a new dimension, such as number, without a verbal cue from the experimenter other than a change in the feedback to the participant. The correct sorting category then switches for a total of six times based on the sorting dimensions (i.e., color, number, form, number, color, form).

The rodent analog of the human Wisconsin Card Sorting Test is the attentional set-shifting paradigm. Attentional set-shifting is a behavioral task that requires the rodent to learn a new strategy while inhibiting knowledge of a previously learned strategy in order to receive rewards. Historically, rodents performed attentional set-shifting by digging for rewards in bowls (sometimes termed the digging task); these bowls could have a variety of cues (e.g., sight, smell, texture) to make the set-shift possible (Birrell & Brown, 2000; McLean, Beck, Woolley, & Neill, 2008; Tanaka, Young, Gresack, Geyer, & Risbrough, 2011; see Figure 1). In this paradigm, rats
are first habituated to the testing box and are trained to dig quickly and reliably in two sawdust-filled bowls that are baited with a palatable reward (e.g., piece of nut) located beneath the sawdust (McLean et al., 2008; Tanaka et al., 2011). After learning how to dig, a divider is placed between the bowls in the testing box and rats are trained on a simple discrimination (e.g., digging medium or odor). For discrimination training, only one bowl is baited with a reward and rats must learn to discriminate based on the digging medium (e.g., wood shavings versus cat litter) or odor (rose versus white flower). After the rat correctly discriminates for six consecutive trials, a second dimension is introduced while the rat is still rewarded for discriminating the originally learned dimension. For example, if a rat undergoes discrimination training based on digging medium, it must learn to only dig in the wood shavings bowl for reward and ignore the cat litter bowl. In the first phase of testing, odors are added to the bowls, but they are irrelevant and not predictive of reward. During the second phase of testing, the rewarded dimension shifts; the rat must ignore the previously relevant dimension (digging medium) and learn to discriminate based on a different dimension (odor).

Figure 1. Attentional set-shifting using digging bowls (Tanaka et al., 2011).
Another test of behavioral flexibility is the cross-maze task, which requires a rodent to learn a rule in a cross maze and then unexpectedly shift to a different rule with a different modality, such as direction or texture of floor (Ragozzino, Detrick, & Kesner, 1999; see Figure 2). For example, a rodent can be trained to always turn toward a visual cue within the cross maze, and after reaching a certain number of trials correctly for that rule, the rule becomes irrelevant and the rodent must learn a new rule, such as always turn to the right (switching from a visual modality to a directional modality). Although both the digging task and cross-maze task have been utilized in the rodent set-shifting literature, there are limitations to these paradigms. Generally, they require intensive hours of training per animal. Also, these tasks are manually conducted and there is risk of experimenter error, which may be particularly detrimental to a data set if a dimension is incorrectly baited (Brady & Floresco, 2015).

![Cross-maze task](image)

**Figure 2.** Attentional set-shifting using a cross maze (Ghods-Sharifi, Haluk, & Floresco, 2008).

A newer attentional set-shifting paradigm is conducted in operant chambers, where rodents must initially learn to discriminate a light cue to press a lever in order to receive a reward
and then must ignore that rule and only respond to the lever on one side for a reward (Brady & Floresco, 2015). By using operant chambers, stimulus presentation is automatic, thus reducing human experimenter error. The pre-programmed computer software executes set-shifting rules consistently and precisely records rats’ behavioral responses. In this paradigm, rats are habituated to the operant chamber and trained to press a lever quickly and reliably in order to receive a palatable reward (e.g., banana flavored sucrose pellet), which is dispensed in a magazine within the operant chamber near the levers (Brady & Floresco, 2015). After learning how to press one lever on a fixed ratio one (FR1) schedule whereby one lever press yields one sucrose pellet, the rat must learn to perform the same task on the opposite lever (see Figure 3). Following training on both levers, the organism must learn to press the levers in an alternating pattern (e.g., left, right, left, right) on a FR1 schedule. When the rat successfully completes this phase of training to criterion (i.e., predetermined number of reinforced lever trials with correct responding), it begins visual cue discrimination learning. In visual cue discrimination learning, the levers within the operant chambers are paired with lights (e.g., the levers are backlit) and the rat is rewarded for pressing only the lever that is backlit, even though both levers are extended into the chamber. After the rat correctly discriminates based on cue, the rat advances to the set-shift, whereby the rat switches from discriminating based on the light cue to only responding to one lever (left or right) regardless of the light cue, which will still be randomly paired with the left or right lever on any given trial. Thus, the rewarded dimension shifts—the rat must ignore the previously relevant dimension (light cue) and learn to discriminate based on the new dimension (responding to one side). The rat must learn to ignore the previously relevant stimulus dimension because the backlight will now be randomly paired with the left or right lever on any given trial.
Figure 3. Attentional set-shifting using an operant chamber (Brady & Floresco, 2015).

Attentional set-shifting can involve extradimensional and/or intradimensional set-shifting. In an intradimensional shift, the organism must learn a new rule set within the same dimension as that learned during the initial discrimination (i.e., requiring the organism to respond to a different smell such as vanilla to cinnamon or a different visual cue such as stripes to no stripes). In an extradimensional shift, the organism must learn a new rule set in a different dimension than what was initially reinforced (i.e., switching from a smell to a visual cue or visual cue to digging medium). As will be discussed below, the distinction between intra- and
extradimensional set-shifting is important as different brain regions subserve these two processes.

Similar to many other executive functioning tasks, the PFC has been implicated in mediating attentional set-shifting performance (Crofts et al., 2001; Floresco, 2013; Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006; Hamilton & Brigman, 2015; Phillips, Ahn, & Floresco, 2004; Ragozzino, 2007; Ragozzino, Detrick, & Kesner, 1999; Rogers, Andrews, Grasby, Brooks, & Robbins, 2000). In humans, individuals with clinical disorders associated with impaired PFC functioning, such as schizophrenia, often show deficits in the Wisconsin Card Sorting Test (Everett, Lavoie, Gagnon, & Gosselin, 2001). Individuals diagnosed with schizophrenia often show marked impairments in both intra and extradimensional set-shifting (Jazbec et al., 2007). Similarly, patients with dorsolateral PFC damage show deficits in the Wisconsin Card Sorting Test, demonstrating difficulty with switching to a new rule and making preservative or random/never-reinforced errors (Barceló & Knight, 2002; Milner, 1963). Further, in examining the dorsolateral PFC of non-clinical participants, researchers have measured cerebral blood flow during the Wisconsin Card Sorting Test with positron emission tomography. Rogers and colleagues (2000) found that the dorsolateral PFC of participants was activated when engaging in an extradimensional set-shift, but not during intradimensional set-shift. Humans with localized excisions of the frontal lobe show deficits in extradimensional set-shifting, but not intradimensional set-shifting, compared to age-matched controls (Owen, Roberts, Polkey, Sahakian, & Robbins, 1991). Together, the human data demonstrates that the PFC plays a crucial role in extradimensional set-shifting.

The importance of the PFC in extradimensional set-shifting has also been supported by causal evidence in animal research. Bilateral lesions of the medial frontal cortex with ibotenic
acid impaired an extradimensional set-shift in rats (Birrell & Brown, 2000) and excitotoxic PFC lesions impaired the extradimensional set-shift in marmosets (Dias, Robbins, & Roberts, 1996). The rat medial PFC is functionally homologous to the human/primate dorsolateral PFC; both are implicated in similar processes, such as working memory and goal-directed behaviors (Seamans, Lapish, & Durstewitz, 2008; Yang, Shi, Wang, Peng, & Li, 2014). Further refinement of PFC lesion location found that inactivation of rodent infralimbic and prelimbic PFC regions with 2% tetracaine impaired rats’ cross modal/extradimensional set-shifting (Ragozzino, Detrick, & Kesner, 1999). While lesions of prelimbic or infralimbic cortices (mPFC) in rats impaired extradimensional shifts, lesions of the orbitofrontal cortex did not (Ghods-Sharifi, Haluk, & Floresco, 2008; Ragozzino, 2007). Lesions of the orbitofrontal cortex do not disrupt the ability to acquire, maintain, or shift strategies in the bowl-digging attentional set-shifting paradigm (McAlonan & Brown, 2003). Therefore, the rodent research supports the human studies demonstrating that attentional set-shifting depends upon prefrontal cortices, although there are some subregion differences between species.

Aside from using intradimensional versus extradimensional set-shifts to explore cognitive functioning, researchers also use the number and types of errors made during the set-shift to make cognitive processing inferences. During the set-shift, subjects are likely to make a few errors; therefore, it is important to parse out different types of errors. The different types of errors represent cognitive processing difficulties in the ability to shift to a new strategy versus maintain a new strategy. These errors can be classified into three categories: 1) preservative errors, responding correctly based on a previously used strategy instead of shifted strategy before the new/shifted strategy is acquired; 2) regressive errors, responding correctly based on the previously required strategy instead of the shifted strategy after the shifted strategy is acquired;
and 3) never-reinforced errors, responding on a lever that is not reinforced for either discrimination test. Both the mPFC and striatum play crucial roles in attentional set-shifting and each region has dissociable roles in the types of errors made during a set-shift (Floresco, Ghods-Sharifi, Vexelman, & Magyar, 2006; Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006; Haluk & Floresco, 2009). Inactivation of the infralimbic and prelimbic PFC in rats with 2% tetracaine led to an increase in trials to criterion and preservative errors, with no effect on regressive errors on the cross-maze set-shifting task (Ragozzino, Detrick, & Kesner, 1999) or a strategy-switching task (a modified four quadrant cheeseboard apparatus; Ragozzino, Wilcox, Raso, & Kesner, 1999). In contrast, inactivation of the dorsomedial striatum in rats with 2% tetracaine led to an increase in regressive errors, with no effect on preservative errors in the cross-maze set-shifting task (Ragozzino, Ragozzino, Mizumori, & Kesner, 2002). These results indicate that the mPFC and striatum are crucial for the ability to learn a new strategy and then maintain a new strategy, respectively.

As in executive functioning tasks described earlier, research suggests that proper DA transmission, particularly within the mPFC and striatum, is essential for attentional set-shifting. In a human study, Nagano-Saito et al. (2008) found that depleting DA through drinking an amino acid-deficient solution eliminated functional connectivity between prefrontal and striatal regions assessed with functional magnetic resonance imaging. This reduced connectivity was correlated with poorer performance on a computerized version of the Wisconsin Card Sorting Test as measured by reaction time during the set-shift, although there were no differences in preservative or non-preservative errors. To assess the role of the D₂ receptor specifically, 400 mg of a D₂ antagonist, sulpiride, was given orally to healthy volunteers who then were tested on the Cambridge Neuropsychological Test Automated Battery version of an attentional set-shifting
task (Mehta, Manes, Magnolfi, Sahakian, & Robbins, 2004). This assessment contains an intra-and extradimensional set-shift task. The participants who received sulpiride tended to perform worse on the first extradimensional set-shift session compared to participants receiving placebo. Sulpiride had no effect on intradimensional set-shifting, indicating that D₂ receptor function is crucial for the more challenging task of switching rules between dimensions. In rats, D₂ receptor antagonism also impairs set-shifting (Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006). Floresco, Magyar, Ghods-Sharifi, Vexelman, and Tse (2006) demonstrated that a microinfusion of a D₂ receptor antagonist, eticlopride, into the medial PFC significantly impaired extradimensional set-shifting as measured by increased trials needed to reach criterion and increased preservative errors. This data suggests a role of D₂ receptor function for extradimensional attentional set-shifting in supporting the learning of new strategies.

**Cue Salience and the Pavlovian Conditioned Approach**

While the neuroenhancing effects of MPH have been established using a variety of learning and memory tasks in humans and rodents as previously discussed, little research has investigated the effects of MPH use on the formation of associations with rewards. MPH may work to enhance performance on the majority of tasks previously referenced because they incorporate rewards. For example, in rodents, MPH may enhance performance on the radial arm maze win-shift task by increasing the desirability of the food reward or by enhancing cognition or both (Zhu, Weedon, & Dow-Edwards, 2007). The outcome measures for many of the executive functioning tasks can be influenced by multiple sub processes, including reward processes. It is important to consider how MPH affects reward and association learning—specifically, the association between cues and rewards. The strength of the reward association
may facilitate performance on executive functioning tasks, with heightened importance of a reward cue enhancing cue-related behavioral performance. Therefore, cues associated with rewards are capable of incentivizing and motivating behavior (for review, see Robinson, Yager, Cogan, & Saunders, 2014).

One process that is important for learning the relationship between a cue (a stimulus that becomes associated with a reward) and a reward is salience. Salience is the heightened perception and desirability of cues associated with the reward (Berridge, Robinson, & Aldridge, 2009). Although few laboratory cognitive tasks consider this process, the study of drug addiction has focused extensively on salience due to the persistence in the association between cues and rewards, even years after cessation of the reward. In the study of drug addiction, when an addict encounters a cue (e.g., person, object, or place) that was previously associated with the rewarding properties of drug use, an addict with increased cue salience will have increased craving for that drug and engage in drug-seeking behaviors. This salience toward cues is likely acquired through Pavlovian conditioning whereby after repeated pairing with the rewarding properties of a drug, a cue can acquire incentive motivational properties, thus increasing an organism’s “wanting” of the reward (Berridge, Robinson, & Aldridge, 2009). Over the past few decades, researchers have sought to explain why some addicts, even after years of discontinued drug use, are prone to relapse or reinstatement of drug-seeking behaviors and drug use. One potential explanation for this vulnerability to relapse is heightened salience toward cues (Flagel, Akil, & Robinson, 2009). Cues can become “motivational magnets” (Berridge, Robinson, & Aldridge, 2009) that attract addicts toward them and subsequent drug use-related behaviors. Thus, salience toward cues may contribute to changes in executive functioning processes, which can then alter behaviors, such as maintaining or reinstating drug-seeking behaviors even during
abstinence (Everitt & Robbins, 2000; Kruzich, Congleton, & See, 2001). Individual variation in cue salience may explain differences observed in reward and non-drug executive function tasks as well.

The Pavlovian Conditioned Approach (PCA) is a measure designed to assess an individual rat’s “attribution of incentive salience to predictive cues” (Fitzpatrick et al., 2013). Using an automated operant chamber paradigm, rats can be conditioned to associate a lever (conditioned stimulus) with a sucrose pellet (unconditioned stimulus), although no response on the lever is needed for a reward. As the lever and the magazine (where the sucrose pellet is administered) are located in separate physical locations of the operant chamber, the time that the rat spends at each location can be assessed. Because no response on the lever is required for the reward to be delivered, some rats will not form a strong association between the lever and reward, but others will form this association and therefore will engage in lever pressing behaviors. Meyer and colleagues (2012) have distributed rats into three different categories depending upon their behaviors: 1) rats that have behaviors predominantly directed toward the lever are “sign trackers”; 2) rats that exhibit behaviors predominantly directed at the magazine are “goal trackers”; and 3) rats whose behaviors do not fit either of these phenotypes are “intermediates.” These phenotypes have been characterized following five days of autoshaping testing with the average of the last two days determining the individual rat’s phenotype. If a rat develops into a sign tracking phenotype, the rat has high attribution of incentive salience toward the lever; therefore, the lever itself can have rewarding properties (Fitzpatrick et al., 2013; Flagel et al., 2011; Robinson & Flagel, 2009). Notably, goal trackers still will learn the predictive value of the lever (as signaling an oncoming reward), but they do not acquire incentive salience toward
the lever. Thus, goal trackers are less likely to engage in lever pressing behaviors and will instead orient toward the magazine.

Heightened salience in sign trackers versus goal trackers is also supported by research in paradigms other than autoshaping. Sign trackers are more likely than goal trackers to work for reward-associated cues, measured using progressive ratio paradigms, and are more likely to reinstate reward-seeking behaviors toward cues following extinction (Saunders & Robinson, 2011; Versaggi, King, & Meyer, 2016; Yager & Robinson, 2010, 2013). For example, Yager and Robinson (2010) found that sign trackers display more robust reinstatement to a food cue following extinction than goal trackers. This effect persists in research with drug rewards as well; interestingly, animals classified as sign trackers or goal trackers to a food cue will maintain these behaviors in response to drug rewards. Sign trackers (classified with a food cue) show greater reinstatement to a nicotine-associated cue than goal trackers (Versaggi, King, & Meyer, 2016) and sign trackers have higher reinstatement of drug-seeking behavior to a cocaine-related cue than goal trackers (Saunders & Robinson, 2010, 2011). Sign trackers also exhibit more motivation to work for rewards as evidenced by achieving higher progressive ratio breakpoints/work harder for cocaine than goal trackers (Saunders & Robinson, 2011). The degree of salience sign trackers attribute to reward-related cues may have implications for future behaviors involving natural or drug rewards.

**Neural Circuitry and Neuropharmacology**

The mesolimbic dopaminergic circuit is highly implicated in reward processes (for review, see Arias-Carrión, Stamelou, Murillo-Rodríguez, Menéndez-González, & Pöppel, 2010). Broadly, the mesolimbic pathway commences with dopaminergic cell bodies in the ventral
tegmental area (VTA) that project to the nucleus accumbens, predominantly the shell (for review, see Salgado & Kaplitt, 2015). Both the VTA and nucleus accumbens are implicated in reward and pleasure in humans and animals (for review, see Arias-Carrión et al., 2010). Rats will bar press for electrical stimulation of the VTA; this motivated behavior is indicative of pleasure (Phillips, Blaha, & Fibiger, 1989). VTA stimulation also increases DA transmission in nucleus accumbens (Fiorino, Coury, Fibiger, & Phillips, 1993). Microinjections of amphetamine into the nucleus accumbens, which increases extracellular DA, increased conditioned lever pressing (Peciña & Berridge, 2013). Subsequently, the nucleus accumbens projects to various brain regions including the amygdala, thalamus, ventral pallidum, and VTA, which are all implicated in mediating goal-directed behavior (for review, see Kalivas & Volkow, 2005).

Dopaminergic neurons from the VTA also project to the prefrontal cortex, of which activation of the anterior cingulate (one subregion of the dorsomedial PFC) and ventral orbital cortices is necessary during motivationally salient events. Inactivation of the dorsomedial PFC (anterior cingulate, prelimbic cortex, or infralimbic cortex, separately) in rats prevents cue-induced reinstatement of cocaine-seeking behavior (McLaughlin & See, 2003). In human research, cue-induced craving of various substances has also been highly correlated with changes in metabolic and dopaminergic activity of prefrontal cortices (Milella et al., 2016; Wang et al., 1999; Wexler et al., 2001; Yuan et al., 2017). For example, cocaine abusers undergoing a cocaine-theme interview (versus a neutral theme interview) had metabolic activation of the orbitofrontal cortex, as measured with positron emission tomography (Wang et al., 1999). Wexler et al., (2001) found that cocaine addicts watching cocaine-cue tapes, but not control happy or sad tapes (used as control from drug-cue), had robust activation of the anterior cingulate as measured by functional magnetic resonance imaging compared to control healthy
subjects. These cocaine addicts also showed less activation of the frontal lobes overall while watching cocaine-cue tapes than healthy subjects, which indicates that cues are highly specific to the anterior cingulate, which was only activated in addicts viewing cocaine cues and not addicts viewing control tapes or healthy subjects viewing any tape.

Using diffusion sensor imaging and probabilistic tractography based on magnetic resonance imaging data in humans, Yuan and colleagues (2017) demonstrated that nicotine-dependent cigarette smokers have increased activation of the dorsolateral PFC and caudate during craving (induced by viewing smoking-related images), but as a control measure, this activation did not occur when viewing a neutral cue. Further, they had an increase in negative coupling, as a result of increased structural connectivity/integrity, between the dorsolateral PFC and caudate, indicating that the increase in dorsolateral PFC activity was associated with reduced caudate activity during the smoking cue. The coupling strength between the dorsolateral PFC and caudate was associated with higher craving (a self-reported craving score that followed the MRI scan). Milella et al. (2016) used high-resolution positron emission tomography to find that the magnitude of craving in individuals with cocaine dependence was negatively correlated with \([18F]\)fallypride \((D_2/D_3 \text{ receptor})\) binding potential in the presence of cocaine-related cues in the medial orbitofrontal cortex, anterior cingulate, dorsolateral PFC, and striatum. Together, this line of research purports a strong role for the mPFC and striatum in reward and cue associations.

Dopamine receptors within the mesolimbic system play an integral role in regulating the formation of incentive salience. In rats, systemic administration of a non-specific DA antagonist, flupenthixol, one hour prior to the start of each autoshaping session (for seven days) attenuated performance of conditioned responding, which was either orientation toward the lever or the magazine. Sign tracking behavior was not acquired even after cessation of drug, whereas goal
tracking behavior was acquired, indicating that learning the conditioned stimulus-unconditioned stimulus (CS-US) relationship was not affected (Flagel et al., 2011). It has also been demonstrated that maintenance, and not just acquisition, of incentive salience is DA-dependent. Microinjections of flupenthixol into the nucleus accumbens reduced lever pressing behavior in rats previously categorized as sign trackers but had no effect on number of magazine entries for goal trackers (Saunders & Robinson, 2012). The elimination of sign tracking behavior occurred on the first trial following flupenthixol administration, indicating that sign tracking behavior was immediately impaired and not that new learning was occurring across trials. Sign trackers have greater DAT expression in the ventral striatum than goal trackers, indicating faster DA uptake in the nucleus accumbens (Singer et al., 2016). The study also showed that an amphetamine injection into the nucleus accumbens facilitates sign tracking behavior, but not goal tracking behavior, thus potentially enhancing the attribution of incentive salience toward the lever. These results suggest that DA in the nucleus accumbens is crucial for sign tracking conditioned responses, although no known evidence exists to suggest that DA agonism can facilitate the acquisition of incentive salience.

Because flupenthixol is a non-specific DA antagonist, research on the importance of DA in sign and goal tracking has also investigated specific DA receptor subtypes. Fraser, Haight, Gardner, and Flagel (2016) found that systemic injections of the D$_2$/D$_3$ agonists, 7-OH-DPAT pramipexole, or the antagonist, raclopride, attenuated a previously acquired PCA behavioral phenotype. All three drugs attenuated lever pressing behavior in sign trackers as well as magazine entries in goal trackers, but had no effect on lever pressing in goal trackers or magazine entries in sign trackers, likely because these behaviors were already low in their respective phenotypes. The fact that agonists and antagonists yielded the same effect was
somewhat surprising but the authors speculate that the net effects of these drugs at pre- and post-
synaptic receptor sites could explain the results. The effect of the dopaminergic drugs on goal
tracking behavior was also surprising, given the specificity of the previous findings of
dopaminergic drugs only impacting sign tracking behavior (Flagel et al., 2011; Saunders &
act on pre- and/or post-synaptic terminals, which could then activate or inhibit autoreceptors and
postsynaptic receptors and lead to the same net effect on behavior. The attenuation of sign and
goal tracking behavior was not evident when rats were administered a selective D3 antagonist,
SB-277011A, suggesting the critical role of D2 transmission (as compared to the non-selective
D2/D3 antagonists). Systemic administration of a selective D2 antagonist, eticlopride, impaired
performance of both sign and goal trackers, decreasing lever pressing and magazine entries
respectively (Lopez, Karlsson, & O’Donnell, 2015). Sign trackers also responded to a D2
agonist, quinpirole hydrochloride, as it decreased lever pressing in sign trackers but had no effect
on magazine entries in goal trackers. These results indicate D2 receptor modulation may differ
between the two behavioral phenotypes, with cue salience in sign trackers seemingly more
impacted by D2 agonism and both sign and goal tracking impacted by D2 antagonism (Lopez,
Karlsson, & O’Donnell, 2015). Further research investigating pre- and post-synaptic D2
receptors in sign and goal tracking behavior is needed to understand the role of D2 receptors in
cue salience versus association learning.

There are differences in the expression and distribution of DA receptors in the brains of
sign and goal trackers. Sign trackers have greater expression of D1 receptor mRNA in the
nucleus accumbens compared to goal trackers, whereas goal trackers have greater expression of
D2 receptor mRNA in the nucleus accumbens, DAT in the ventral tegmental area, and tyrosine
hydroxylase in the ventral tegmental area (Flagel, Watson, Robinson, & Akil, 2007). Low levels of D₂ receptors have been associated with increased self-reported alcohol craving in alcoholics (Heinz et al., 2004; Volkow et al., 2002). Therefore, the human data and animal research on sign trackers suggest that lower levels of D₂ receptors contribute to greater incentive salience, and subsequently, craving and drug-seeking behaviors (Flagel et al., 2011; Flagel et al., 2007; Lopez, Karlsson, & O’Donnell, 2015; Saunders & Robinson, 2012). Low levels of D₂ receptors have also been associated with self-reported drug liking in response to MPH in non-abusing subjects (Volkow et al., 1999).

Together, this data purports that sign tracker and goal tracker phenotypes are DA-dependent and useful in studying the importance of cue salience. One major limitation to this line of research is the dichotomy in sign versus goal tracking. These categories are determined based on PCA Index score, which is a continuous variable, thus eliminating all variability seen within a particular phenotype. By eliminating this variability, researchers may be drawing inferences about a phenotype that is actually composed of organisms with slightly different behavioral profiles. For instance, a rat that has a high number of lever presses and no magazine entries is likely categorized as a sign tracker; a rat that has a high number of lever presses as well as a few magazine entries is also likely categorized as a sign tracker, although their behaviors differ. Alternatively, another approach would be to use PCA Index Score itself as a continuous measure. This may allow for a more accurate representation of each rat’s behavioral profile and be more reflective of approach behavior as a continuum rather than a dichotomous phenotype.
CHAPTER 2

CURRENT EXPERIMENTS

The goal of the current experiments was to investigate the effects of daily MPH administration on PCA Index Scores (Experiment 1) and determine whether PCA Index Scores mediate behavioral flexibility as measured by attentional set-shifting (Experiment 2). MPH exposure may alter cue salience but may only have subtle alterations that can be detected by using PCA Index Score, rather than using dichotomous sign and goal tracking categorizations. Moreover, if a rat has heightened salience toward a cue (higher PCA Index Score), requiring the rat to shift from responding to that cue to responding to a different dimension in an attentional set-shifting paradigm will likely be more challenging than a rat that did not acquire a strong salience to that cue (lower PCA Index Score). Similarly, a rat that learns incentive salience during autoshaping may also be more likely to learn incentive salience toward the initial cue dimension learned in the attentional set-shifting paradigm.

Experiment 1 Hypotheses

Experiment 1 of the current study examined the effects of daily MPH administration on the development of PCA Index Scores as a measure of cue salience. We hypothesized that daily administration of MPH prior to autoshaping would increase cue salience/PCA Index Scores.
compared to rats receiving vehicle injections. MPH has been shown to enhance learning in a healthy sample (Arnsten & Dudley, 2005; Berridge et al., 2006; Linssen, Vuurman, Sambeth, & Riedel, 2012) and may also facilitate learning in autoshaping that could raise PCA Index scores by enhancing salience (Volkow et al., 2004). Further, Experiment 1 sought to determine if daily MPH administration altered the density of D2 receptors in the mPFC and striatum. We hypothesized that MPH exposure would increase D2 receptors due to an increase in available extracellular DA in both the mPFC and striatum, regions of high dopaminergic efference, which would be similar to what has been previously observed in rats categorized as sign trackers (Flagel et al., 2007). The function and density of D2 receptors have been implicated with cue salience and cravings such that sign trackers have lower levels of D2 receptors than goal trackers and lower levels of D2 receptors have been implicated in increased cravings/cue salience (Flagel et al. 2011; Flagel, Watson, Robinson, & Akil, 2007; Saunders & Robinson, 2012). Therefore, we also hypothesized that D2 receptors would be correlated with PCA Index Scores. See Figure 4 for hypotheses.

**EXPERIMENT 1 HYPOTHESES:**

<table>
<thead>
<tr>
<th>Daily saline</th>
<th>PCA Index</th>
<th>D2 receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily MPH</td>
<td>↑ PCA Index</td>
<td>↓ D2 receptor</td>
</tr>
</tbody>
</table>

Figure 4. Experiment 1 hypotheses.

**Experiment 2 Hypotheses**
Experiment 2 further tested whether alterations of D\textsubscript{2} receptors during MPH administration would hinder a rat’s performance on the attentional set-shifting paradigm, as D\textsubscript{2} transmission is important for set-shifting. We hypothesized that daily administration of MPH prior to autoshaping would increase trials needed to reach criterion during the attentional set-shift as compared to rats with daily administration of saline. This hypothesis was built upon Experiment 1, suggesting that MPH exposed rats would have heightened salience toward the lever and therefore the shift to response would be more challenging with MPH rats making a greater number of preservative errors and requiring more trials to meet criteria. Further, by blocking D\textsubscript{2} receptors prior to the attentional set-shift with the D\textsubscript{2} antagonist raclopride, we investigated the role of these receptors in control rats and in rats with prior MPH administration. We hypothesized that control rats exposed to saline during autoshaping and then injected with raclopride for the attentional set-shift would require more trials to reach criterion as compared to controls. Previous literature has shown that D\textsubscript{2} antagonists impair attentional set-shifting in healthy humans and animals, albeit the animal evidence is based on intracerebral drug infusions (Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006; Mehta, Manes, Magnolfi, Sahakian, & Robbins, 2004). In contrast, in the rats exposed to MPH during autoshaping, we hypothesized that the D\textsubscript{2} antagonist would decrease trials needed to reach criterion. See Figure 5 for hypotheses.

**EXPERIMENT 2 HYPOTHESES:**

![Figure 5. Experiment 2 hypotheses.](image-url)
Despite high use of MPH as a cognitive enhancer, there is little research on the potential influence of recreational MPH use on cue salience, which may have implications for understanding the development of drug addiction and/or relapse behaviors as well as performance on executive functioning tasks, which are all DA-dependent. By testing the above hypotheses, we hoped to establish the role of cue salience in working memory processes such as attentional set-shifting, to determine the influence of MPH on these variables, and assess the role of D₂ receptors in these processes. These findings furthered our understanding of the mechanisms underlying the use of MPH for cognitive enhancement and associative learning. To date, no research has investigated the role of cue salience in working memory and specifically attentional set-shifting. Together, findings from Experiments 1 and 2 provide insight on the effects and one potential mechanism driving the use of MPH as a cognitive enhancer.
CHAPTER 3

METHODS

Test Subjects and Procedures

Animals and Housing

Sixty-two adult male Sprague-Dawley rats were housed in pairs on a 12:12h light:dark cycle (lights on at 6:00h and off at 18:00h) in plastic cages with aspen chip bedding. All rats received unrestricted access to food and water in a temperature-controlled room (22 ± 2 °C) throughout the experiments. All procedures were in adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition; National Research Council, 2011) and approved by the local institutional animal care and use committee.

Drugs

Methylphenidate HCl (0.5 mg/kg; Sigma Laboratories, St. Louis, MO, USA) was dissolved in 0.9% saline. The 0.5 mg/kg dose was chosen to yield similar blood plasma levels as the targeted range in human dosing and has been shown to yield enhanced working memory in rats (Andrzejewski et al., 2014; Aoyama, Kotaki, & Iga, 1990; Berridge et al., 2006; Devilbiss & Berridge, 2008; preliminary data in our lab). Generally, research on the dose effects of MPH shows that MPH has cognitive enhancing effects when administered in low to moderate doses, whereas impairments on performance may occur with higher doses. Control rats were injected with an intraperitoneal (i.p.) injection of 0.9% saline.
Raclopride tartrate (0.2 mg/kg), a D<sub>2</sub>/D<sub>3</sub> antagonist (Sigma Laboratories, St. Louis, MO, USA) was dissolved in 0.9% saline. The 30-minute pre-exposure time was selected because previous research has reported behavioral effects following 30 minute pretreatment time and the 0.2 mg/kg dose has been shown to change behavior, without affecting locomotion (Cheng & Liao, 2007; Hillegaart & Ahlenius, 1987; Marrow, Overton, & Clark, 1993; Smith & Smith, 2010). Control rats received an intraperitoneal (i.p.) injection of 0.9% saline.

**General Testing Procedures**

**Experiment 1.** Five days prior to the start of habituation and training, rats were handled daily for ~5 minutes per day. All rats received pretraining and autoshaping in the operant chamber; however, rats were split into two drug exposure groups. Rats either received an i.p. injection of MPH or saline each day of autoshaping. Body weights were recorded daily to control for physiological changes associated with drug manipulation. Following completion of autoshaping, rats were decapitated and brains collected for analysis of D<sub>2</sub> receptors.

**Experiment 2.** Five days prior to the start of habituation and training, rats were handled daily for ~5 minutes per day. All rats received pretraining and autoshaping in the operant chamber; however, rats were split into two drug exposure groups. Rats either received i.p. injections of MPH or saline each day of autoshaping. Body weights were recorded daily to control for physiological changes associated with drug manipulation. Following completion of autoshaping, rats underwent attentional set-shifting training and cue discrimination. Rats then received an injection of saline or a D<sub>2</sub> receptor antagonist, raclopride, prior to the set-shift response discrimination test. See Figure 6 for experimental design.
Figure 6. Experimental groups and design.

**Behavioral Testing**

**Habituation to banana pellets.** One day prior to training in operant chambers, rats were habituated to approximately 25 banana flavored pellets (45 mg; BioServ Product #F0059) in their home cage.

**Habituation to the operant chamber.** The day following banana pellet habituation, rats were placed in an operant chamber (24.1 cm x 20.5 cm x 29.2 cm) and banana pellets were delivered at a variable interval (VI) schedule of 30 seconds for a total of 25 pellets. Pellet delivery was not contingent upon the rat performing any behavior. This single day of pretaining was conducted using MedAssociates hardware and software (Med-PC, MedAssociates, Inc., St. Albans, VT, USA).

**Autoshaping.** To assess the degree to which rats attribute salience toward a cue, rats were assessed for goal and sign tracking behavior using the Pavlovian Conditioned Approach (PCA). PCA test sessions were conducted over the next five days. In a test session trial, a lever is introduced into the chamber for 8 seconds. A banana pellet is then delivered at a variable interval.
90-second schedule after the removal of the lever. The lever (“sign”) is near but separate from the food receptacle (“goal”) and the time the rat spends at both locations was recorded automatically. The rat did not have to respond to receive the banana pellet: the measure was simply the location of the rat after the sign (lever) that the food will be delivered soon was presented. Each autoshaping/PCA test session consisted of 25 trials (methods taken from Flagel et al. [2007]).

Twenty minutes prior to each of the five days of autoshaping, half of the rats received a 0.5 mg/kg i.p. injection of MPH and half received an i.p. injection of 0.9% saline. The 20-minute pre-exposure time was selected based on previous studies that show that approximately 20 minutes pre-exposure time is needed to reach peak DA release and behavioral effects (Arnsten & Dudley, 2005; Berridge et al., 2006; Devilbiss & Berridge, 2008; Gerasimov et al., 2000).

**Attentional set-shifting: Training.** To assess behavioral flexibility, an operant chamber paradigm was modeled after previous research (see Figure 7; Brady & Floresco, 2015). To pretrain the rats and acclimate them to the operant chambers, rats were placed on a fixed ratio one schedule daily until the rat reached a criterion of 50 lever presses (and therefore 50 banana pellets) within 30 minutes. This criterion must be met for first the right or left lever and then the other lever, independently. For the next phase of training, rats were trained to press the lever within 10 seconds of the lever’s insertion into the chambers. On these training days, the house light illuminated every 20 seconds, one lever was inserted into the chamber, and the rat had 10 seconds to respond and receive a banana pellet before the lever was retracted. These training sessions were composed of 90 trials and rats had to reach criteria of responding to at least 85 of the 90 trials in a single day (less than five omissions) to move on to the next phase. The last phase of training was conducted to assess the rats’ side preference/bias. During this session, both levers were inserted into the chamber and the first lever press was rewarded with a banana
pellet. For the next trials, lever pressing was only rewarded on the side opposite of the initial response. Once the rat chose the opposite lever, a new trial began and the rat had to press both the left and right lever per trial for a total of seven trials. The first lever response of each trial counted toward the rat’s “side bias”; the lever the rat chose first four or more times over the seven trials determined that animal’s side bias (i.e., left or right).

Figure 7. Attentional set-shifting (Brady & Floresco, 2015).

**Attentional set-shifting: Testing.** After the three phases of pretraining were complete, the visual cue discrimination test began. Finally, to assess the rat’s ability to shift strategies, the rat (after meeting criterion discussed below) was tested the next day in the response discrimination test.

For the visual cue discrimination test session, every 20 seconds one of the lever lights was illuminated and 3 seconds later the house light turned on and both levers were inserted into the chamber. Pressing the illuminated lever yielded delivery of a banana pellet, retraction of
levers, and the house light turning off after 4 seconds. An incorrect response (pressing the un-illuminated lever) or failure to response to either lever yielded no reward and an immediate retraction of levers/house light turning off. Failure to respond to either lever counted as an omission. During this test, trials were paired such that both the left and right lever were illuminated once in each pair (randomly ordered). Trials continued until the rat received a minimum of 30 trials with 10 correct consecutive responses or until the rat received 120 trials (omission trials were not counted in the criterion). If a rat did not achieve criterion on the first day of discrimination testing, it received a second day with the same procedures (eight rats needed a second day of cue discrimination testing; five of which were in the saline condition and three of which were in the MPH condition). Preliminary data on this paradigm in our lab suggested that only about 16% of rats needed a second day of testing; the majority met criteria on the first day of testing, so our current 19% is not abnormal. If a rat did not achieve criterion by the second day of testing, it was omitted from the study (only one MPH condition rat had to be eliminated from the current experiment).

For the response discrimination test, which is the attentional set-shift test, the same procedures as the visual cue discrimination test were used. However, rats were rewarded for pressing the lever opposite of their side bias, regardless of lever illumination. Thus, every 20 seconds one of the lever lights was illuminated and 3 seconds later the house light came on and both levers were inserted into the chamber. Pressing the lever on the correct side (e.g., always left because their response bias was the right side lever) yielded delivery of a banana pellet, retraction of levers, and the house light turning off after 4 seconds. The same criteria as indicated for visual cue discrimination were used for the response discrimination test. For the current experiment, only three rats (two MPH with raclopride and one MPH with saline) needed a
second day of set-shift testing. Dependent measures were summed for both days of testing in these rats, with the exception of response latency, which was averaged.

Thirty minutes prior to the start of response discrimination testing, half of the rats received an injection of vehicle and half received a 0.2 mg/kg i.p. injection of raclopride in their housing room. Trials continued as described above.

**Histology**

Following completion of behavioral testing in Experiment 1, rats were briefly anesthetized with isoflurane in combination with oxygen and then rapidly decapitated. Brains were frozen on dry ice and stored in a -80º freezer until analysis.

**Synaptosomal fractionation protocol.** After crude dissection of brain regions of interest (mPFC and striatum), tissue from one hemisphere was homogenized in ice-cold 0.32M sucrose (500 µL for mPFC and 1.0 mL for striatum) and centrifuged at 800 x g for 24 min at 4ºC. The pellet contained insoluble material and was discarded. The supernatant was then centrifuged at 22,000 x g for 17 min at 4ºC. The pellet from this second spin contained the synaptosomes (i.e., the nerve terminal) and was resuspended in ice-cold Millipore water (100 µL for PFC, 200 µL for striatum) by pipetting up-and-down. Bradford assays were done on each sample to obtain total protein content before running Western blots.

**Western blot protocol.** Samples were diluted in Novex 4X LDS Buffer (3 parts sample:1 part LDS) and 30 µg was loaded in each lane. NuPAGE Novex 4-12% Bis-Tris gels were used for gel electrophoresis at 150V for 90 min. Proteins were then transferred onto a PVDF membrane for 2 hours at 28V, blocked for 1 hour at room temperature in 5% non-fat milk in 0.5% TBS-T, and then incubated with primary antibody (anti-D2R; 1:500, ab85367, Abcam,
Cambridge, MA, USA) overnight with shaking (70 rpm) at 4°C.

The following day, membranes were washed 3x for 5 minutes each in 0.5% TBS-T and incubated with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgG, 1:2500, Santa Cruz cat#SC2004) in 5% non-fat milk in 0.5% TBS-T for 1 hour at room temperature. Membranes were then washed 3x for 5 minutes each in 0.5% TBS-T and incubated in HyGlo chemiluminescence for 2 minutes before imaging with a FujiFilm LAS-4000 camera. Band density was quantified using MultiGauge V3.1 software.

Membranes were “stripped” because of the proximity of the D2R band to the β-actin band. Briefly, the membranes were incubated with 1X Reblot Plus Mild Antibody Striping Solution (Fisher Scientific, cat# 2502MI), washed 3x for 5 minutes each with 0.5% TBS-T, and blocked for 1 hour at room temperature in 5% non-fat milk. Membranes were then incubated with β-actin (1:3000, Millipore cat#MAB1501) overnight with rocking at 4°C. The following day, membranes were washed 3x for 5 min each before incubating with horseradish peroxidase-conjugated secondary antibody (m-IgGκ BP-HRP, 1:2500, Santa Cruz cat#SC516102) for 1 hr at room temperature. Membranes were washed 3x for 5 minutes each and then imaged using the FujiFilm LAS-4000 as described above. After obtaining band density for β-actin, D2R bands were normalized to β-actin bands.

Statistical Analyses

All statistical tests were conducted using SPSS25 (IBM, Inc.) statistical software and significance was determined using an alpha level of .05. Separate t tests were used to compare MPH and saline treated rats on PCA Index Scores and D2 receptors in the mPFC and striatum. PCA Index was computed using a formula as described in Meyer et al. (2012). Correlational
analyses were run to investigate the relationship between PCA Index Scores and D₂ receptors in the mPFC and striatum.

To assess various measures within the attentional set-shifting paradigm in Experiment 2, a 2 (saline versus MPH during autoshaping) x 2 (saline versus D₂ antagonist during set-shift) ANCOVA was used for each measure. The dependent variables for the attentional set-shifting paradigm were trials to reach criterion for both days of discrimination testing and the following three types of errors to criterion during the shift in discrimination testing: 1) number of preservative errors (responding correctly based on previously used strategy instead of shifted strategy before the shifted strategy is acquired); 2) regressive errors (responding correctly based on the previously required strategy instead of the shifted strategy after the shifted strategy is acquired; shifted strategy is acquired once a rats makes less than five preservative errors in a block of eight trials); and 3) never-reinforced errors (responding on a lever that is not reinforced for either discrimination test; e.g., pressing the right lever when the left lever is illuminated during response discrimination when the rat is expected to respond on only the left lever). To assess whether PCA Index Score accounted for significant variance on attentional set-shifting measures, PCA Index Score was entered as a covariate in the analysis and was removed when non-significant (this occurred for all analyses). When Levene’s Test or Mauchly’s Test was violated, a Greenhouse-Geisser correction was used and degrees of freedom were rounded to the nearest whole number.
CHAPTER 4

RESULTS

Experiment 1

Experiment 1 examined the effects of daily MPH administration on the development of sign and goal tracking behavior as a measure of incentive salience. Daily administration of MPH prior to autoshaping was predicted to increase cue salience/PCA Index Scores compared to rats receiving vehicle injections. Further, Experiment 1 tested whether daily MPH administration altered the density of D_2 receptors in the mPFC and striatum. We hypothesized that MPH would increase D_2 receptors due to an increase in available extracellular DA in both the mPFC and striatum and be correlated with PCA Index Scores.

Body Weights

A 2 (MPH condition) x 5 (days of drug injection) repeated-measures ANOVA was used to analyze body weights in rats during MPH administration. Overall, there was no difference in body weight between rats administered MPH and saline across the five drug administration days (F(1,60)= 0.001, p=.97; see Figure 8). There was also no day by MPH condition interaction (F(4,62)= 1.04, p=.31).
Figure 8. Body weight across five days of autoshaping.

**MPH Administration During Autoshaping**

Rats receiving MPH administration (n=31; $M=-.04, SD=.57$) prior to each autoshaping session did not have significantly different PCA Index Scores as compared to control rats receiving saline (n=31; $M=.21, SD=.62$; $t(60)=1.61, p=.11, d=.41$; see Figure 9). To investigate the influence of MPH on the development of PCA Index across the five days of autoshaping, a 2 (MPH condition) x 5 (days of autoshaping) repeated measures ANOVA was conducted (see Figure 10). Overall, rats receiving MPH had statistically similar PCA Index Scores to rats receiving saline; however, there was a trend toward MPH reducing PCA Index Scores ($F(1,60)=3.41, p=.07, \eta^2=.054$). There was no significant MPH condition x day interaction ($F(2,111)=0.70, p=.49$). Mauchly’s test of sphericity was violated for this analysis, thus a Greenhouse-Geisser correction was used and degrees of freedom reported were rounded to the nearest whole number. Separate $t$ tests for each day of autoshaping revealed that rats receiving MPH had similar PCA Indices on all five days of autoshaping compared to rats receiving saline,
with a trend toward MPH reducing PCA Scores on Days 2 and 5, though Levene’s Test for Equality of Variance was violated on Day 2 and thus was corrected with statistics for equal variances not assumed (see Table 1 for statistics).

Figure 9. Average PCA Index Score for Days four and five of autoshaping.
Table 1. T-test analysis for the effect of MPH condition on PCA Scores for each day of autoshaping.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of MPH</td>
<td>$t(60) = 1.45, p = .15$</td>
<td>$t(60) = 1.85, p = .07$</td>
<td>$t(60) = 1.60, p = .12$</td>
<td>$t(60) = 1.48, p = .14$</td>
<td>$t(60) = 1.70, p = .10$</td>
</tr>
</tbody>
</table>

MPH Administration and D$_2$ Receptor Immunoreactivity

Independent $t$ tests revealed no significant effect of MPH in synaptosomal D$_2$ receptor content in either the mPFC ($t(7) = -0.11, p = .91$) or striatum ($t(15) = -1.63, p = .13$; see Figure 11). See Figure 12 for photomicrographs of the Western Blots for D$_2$ receptor immunoreactivity and...
actin bands. There was no significant correlation between PCA Index Score and D₂ immunoreactivity in either the mPFC (r(9) = -0.39, p=.31) or striatum (r(17) = 0.23, p=.37).

Figure 11. Synaptosomal D₂ content in the PFC and STR compared to saline controls.

Figure 12. Western blots of A) actin bands for normalization in mPFC; B) D₂ bands in mPFC; C) actin bands for normalization in striatum; D) D₂ bands in striatum. Saline rats are indicated as “S” and MPH rats are indicated as “M.”
Experiment 2

Experiment 2 examined the effects of prior MPH administration during autoshaping on performance during an attentional set-shifting paradigm. It was predicted that MPH exposed rats would have heightened salience toward the lever and therefore the shift to response would be more challenging, with MPH rats making a greater number of preservative errors and requiring more trials to meet criteria during the set-shift. Further, Experiment 2 tested the role of D₂ transmission on attentional set-shifting by administering the D₂ antagonist raclopride prior to the set-shift. We hypothesized that control rats exposed to saline during autoshaping and then injected with raclopride for the attentional set-shift would have hindered performance. In contrast, in the rats exposed to MPH during autoshaping, we hypothesized that the D₂ antagonist would decrease trials needed to reach criterion, hypothesized to be the result of MPH-induced long-lasting changes in D₂ circuitry.

Attentional Set-Shifting Training

A 2 (MPH condition) x 2 (days of fixed ratio 1[FR1] training) repeated measures ANOVA was used to analyze contingent lever press learning. Overall between the two days of FR1, there was no difference in lever presses between rats administered MPH and saline ($F(1,40)=1.95, p=.17$; see Figure 9). However, there was a day by MPH condition interaction ($F(1,40)=5.614, p=.02$), with MPH rats having fewer lever presses on Day 2 than saline controls ($F(1,40)=4.72, p=.04, \eta^2=.12$), although they had similar lever presses on Day 1 ($F(1,40)=0.07, p=.80$).
A 2 (MPH condition) x 5 (days of alternating lever training) repeated-measures ANOVA was used to analyze training leading up to discrimination tests. Overall, rats previously administered MPH had fewer lever presses than saline controls ($F(1,40)= 4.51, p=.04, \eta^2=.11$; see Figure 13). There was also a day by MPH condition interaction ($F(4,88)= 2.99, p=.05, \eta^2=.03$), with MPH rats pressing the lever fewer times on Day 1 ($F(1,40)= 5.61, p=.02, \eta^2=.14$) but then achieving the same number of lever presses as saline controls by Day 2 ($F(1,40)= 1.91, p=.17$) and through Day 5 ($F(1,40)= .343, p=.56$).

![Figure 13](image.png)

Figure 13. Training rats that reward is contingent upon lever pressing (both left and right lever). *p < .05 compared to saline rats.

**Cue Discrimination Test**

All 40 rats for Experiment 2 met criteria throughout training and thus received the cue discrimination test. However, one rat in the MPH condition failed to meet criterion for the cue
discrimination test and thus was excluded from the analysis. Rats that received MPH (n=19) or saline (n=20) required an equivalent number of trials to achieve a streak of 10 correct trials on the cue discrimination test ($t(39)=0.69, p=.49$; see Figure 14). There was no significant difference between drug groups for the number of correct ($t(39)=0.70, p=.49$) or incorrect responses ($t(39)=1.11, p=.23$).

Figure 14. Performance on the cue discrimination test. To meet criterion, rats had to achieve 10 consecutive correct responses of responding to the lever with the cue light.

**Response Set-Shift**

**Reminder trials.** On the day of set-shift testing, rats were administered raclopride 20 minutes prior to testing. Prior to the set-shift, rats received 20 reminder trials of the cue discrimination test, thus raclopride was on board during the 20 reminder trials and during the set-shift. There was no effect of MPH condition on the number of correct responses during the reminder trials ($F(1,37)=0.78, p=.38$), but there was a significant effect of raclopride condition
(\(F(1,37)=14.57, p<0.01, \eta^2=0.27\)), with raclopride decreasing the number of correct responses made (Figure 15).

**Set-shift.** Analysis of the number of trials needed to reach the criterion of a streak of 10 correct responses showed a significant MPH condition x Raclopride condition interaction during the set-shift (\(F(1,37)=5.187, p=0.03, \eta^2=0.12\); see Figure 16). Post hoc analyses revealed that this significant interaction was driven by the greater number of trials needed to reach criterion in the MPH rats that received raclopride during the set-shift. PCA Index Score was not a significant covariate and therefore was removed from the statistical analysis (\(F(1,37)=0.20, p=0.66\)). There was no main effect of MPH condition (\(F(1,37)=0.86, p=0.36\)) or raclopride condition (\(F(1,37)=0.88, p=0.35\)) on trials needed to reach criterion.

![Figure 15. Number of correct responses made during the 20 cue reminder trials prior to the set-shift. *, p<0.05 compared to SAL-SAL.](image-url)

*Figure 15. Number of correct responses made during the 20 cue reminder trials prior to the set-shift. *, p<0.05 compared to SAL-SAL.*
Figure 16. Number of trials needed to meet criterion during the set-shift. To meet criterion, rats had to achieve 10 consecutive correct responses of responding to one side only, regardless of cue light.

Although there was a significant MPH condition x raclopride condition interaction for trials needed to reach criterion, there was not a significant interaction for correct responses ($F(1,37)=1.741, p=.20$), preservative errors ($F(1,37)=0.68, p=.42$), regressive errors ($F(1,37)=1.06, p=.31$), never-reinforced errors ($F(1,37)=2.66, p=.11$), or number of omissions ($F(1,37)=0.814, p=.37$; see Figure 17). The MPH condition x raclopride condition interaction observed in trials needed to reach criterion parallels a trending MPH condition x raclopride condition interaction observed in incorrect responses ($F(1,37)=3.28, p=.08, \eta^2=.08$) made during the set-shift. See Table 2 for all main effects and interactions during the set-shift.
Figure 17. Responses and omissions during the set-shift. Correct responses were recorded when a rat pressed the lever on the correct side, regardless of cue light. Incorrect responses were recorded when the rat pressed the lever on the incorrect side. Omissions were counted when a rat failed to press the lever in a given trial.
Table 2. Summary of the ANOVA statistics for Experiment 2, including the main effects of MPH condition and Raclopride condition, interaction between MPH condition and Raclopride condition, and the effect of PCA Index Score as a covariate.

<table>
<thead>
<tr>
<th></th>
<th>Main Effect of MPH Condition</th>
<th>Main Effect of Raclopride Condition</th>
<th>MPH Condition x Raclopride Condition Interaction</th>
<th>PCA Index Score as a Covariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct Responses during Cue Reminder Trials</td>
<td>$F(1,37)=0.78, p=.38$</td>
<td>$F(1,37)=14.57, p&lt;.01^*$</td>
<td>$F(1,37)=.78, p=.38$</td>
<td>$F(1,37)=0.44, p=.51$</td>
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<tr>
<td>Trials to Reach Criterion</td>
<td>$F(1,37)=0.86, p=.36$</td>
<td>$F(1,37)=0.88, p=.35$</td>
<td>$F(1,37)=5.187, p=.03^*$</td>
<td>$F(1,37)=0.20, p=.66$</td>
</tr>
<tr>
<td>Correct Responses</td>
<td>$F(1,37)=0.24, p=.63$</td>
<td>$F(1,37)=0.01, p=.94$</td>
<td>$F(1,37)=1.741, p=.20$</td>
<td>$F(1,37)=1.95, p=.17$</td>
</tr>
<tr>
<td>Incorrect Responses</td>
<td>$F(1,37)=0.44, p=.51$</td>
<td>$F(1,37)=0.6, p=.80$</td>
<td>$F(1,37)=3.28, p=.08$</td>
<td>$F(1,37)=1.477, p=.23$</td>
</tr>
<tr>
<td>Omissions</td>
<td>$F(1,37)=1.22, p=.30$</td>
<td>$F(1,37)=1.36, p=.25$</td>
<td>$F(1,37)=0.814, p=.37$</td>
<td>$F(1,37)=2.00, p=.17$</td>
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<tr>
<td>Average Response Latency</td>
<td>$F(1,37)=1.33, p=.26$</td>
<td>$F(1,37)=2.837, p=.10$</td>
<td>$F(1,37)=0.25, p=.62$</td>
<td>$F(1,37)=0.80, p=.38$</td>
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<td>Preservative Errors</td>
<td>$F(1,37)=0.56, p=.46$</td>
<td>$F(1,37)=1.27, p=.27$</td>
<td>$F(1,37)=0.68, p=.42$</td>
<td>$F(1,37)=2.015, p=.16$</td>
</tr>
<tr>
<td>Regressive Errors</td>
<td>$F(1,37)=2.46, p=.13$</td>
<td>$F(1,37)=0.97, p=.33$</td>
<td>$F(1,37)=1.06, p=.31$</td>
<td>$F(1,37)=0.54, p=.47$</td>
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<td>Never-Reinforced Errors</td>
<td>$F(1,37)=0.83, p=.37$</td>
<td>$F(1,37)=1.85, p=.18$</td>
<td>$F(1,37)=2.66, p=.11$</td>
<td>$F(1,37)=0.05, p=.82$</td>
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</table>
Exploratory Analyses

Effect of MPH on Goal and Sign Tracking Categorization

As commonly seen in previous literature, rats’ PCA Index Scores are typically translated into categorizations: goal tracker, intermediate, or sign tracker. Although we proposed to assess the influence of MPH condition on PCA Index Scores as a continuous variable, the influence of MPH condition on goal and sign tracking categorizations was analyzed to parallel previous literature that assesses this behavior (Fitzpatrick et al., 2013; Meyer et al., 2012). A Pearson chi-square analysis revealed that there was a similar distribution of goal trackers, intermediates, and sign trackers between MPH and saline conditions ($\chi^2 (2, N=62) = 4.58, p=.10$; see Table 3 and Figure 18 for distribution).

Table 3. Distribution of goal trackers, intermediates, and sign trackers in saline and MPH rats.

<table>
<thead>
<tr>
<th></th>
<th>Goal Tracker</th>
<th>Intermediate</th>
<th>Sign Tracker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>MPH</td>
<td>8</td>
<td>16</td>
<td>7</td>
</tr>
</tbody>
</table>
Analysis of PCA Index subcomponents revealed that MPH rats had fewer lever presses on the first four days of autoshaping (Day 1: $t(44)= 3.21, p<.01$; Day 2: $t(42)= 3.06, p<.01$, Day 3: $t(60)= 2.28, p=.03$; Day 4: $t(50)= 2.30, p=.03$), with a trending effect on Day 5 ($t(60)= 2.30, p=.06$). This effect was paralleled by an increase in lever latency on the first four days of autoshaping (Day 1: $t(51)= -0.30, p<.01$; Day 2: $t(44)= -3.03, p<.01$, Day 3: $t(60)= -2.30, p=.03$; Day 4: $t(60)= -2.19, p=.03$; Day 5: $t(60)= -1.64, p=.11$). Interestingly, there were no differences in magazine entries during the CS period (when lever is out; Day 1: $t(60)= 1.06, p=.29$; Day 2: $t(60)= 0.50, p=.62$, Day 3: $t(60)= -0.40, p=.69$; Day 4: $t(60)= -0.59, p=.56$; Day 5: $t(60)= -0.97, p=.34$) or latency to enter...
the magazine (Day 1: \(t(60) = -1.19, p = .24\); Day 2: \(t(60) = -0.69, p = .50\); Day 3: \(t(60) = 0.53, p = .60\); Day 4: \(t(60) = 0.81, p = .42\); Day 5: \(t(60) = 1.87, p = .07\)). See Table 4 for PCA subcomponents.

**Goal and Sign Tracking Categorization with \(D_2\) Immunoreactivity**

Exploratory analyses on goal trackers, intermediates, and sign trackers revealed no significant correlation between the categorical characterization and \(D_2\) immunoreactivity in the mPFC (\(r(9) = -.36, p = .34\)) or striatum (\(r(17) = .31, p = .23\)). When assessing \(D_2\) immunoreactivity separately within each category, MPH did not affect \(D_2\) immunoreactivity in the mPFC (\(t(1) = 0.97, p = .51\)) or striatum (\(t(4) = -0.93, p = .41\)) of goal trackers, though this analysis is limited by small sample sizes (three for the mPFC and six for the striatum). In sign trackers, MPH also did not affect \(D_2\) immunoreactivity in the mPFC (\(t(2) = -3.04, p = .09\)) or striatum (\(t(5) = -1.07, p = .33\)); this analysis is also limited by small sample sizes (four for the mPFC and seven for the striatum). Intermediates were not able to be assessed separately as there were no saline rats in the intermediate group assessed for \(D_2\) immunoreactivity.

**Goal and Sign Tracking Categorization with Attentional Set-Shifting**

During the set-shift, goal and sign tracking categorization was also not a significant covariate for the dependent measures, including trials needed to reach criterion (\(F(1,36) = 0.19, p = .67\)). However, because goal and sign trackers have opposing response profiles and in order to compare to prior reports, it was important to look at the effects of MPH and raclopride during the set-shift on each group separately. Interestingly, only sign trackers (\(n = 14\)) were affected by drug manipulations. In sign trackers, a main effect of raclopride indicated that raclopride significantly increased the number of trials needed to reach criterion (\(F(1,10) = 13.73, p = .004, \eta^2 = .36\)).
<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
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<th>Day 3</th>
<th>Day 4</th>
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<td></td>
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<td>SAL</td>
<td>MPH</td>
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<td></td>
<td>±</td>
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<td><strong>Magazine Entries During CS period</strong></td>
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<tr>
<td><strong>PCA Score:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(Response Bias + Latency Score + Probability Difference)/3</td>
<td>-.07</td>
<td>-.17</td>
<td>-.14</td>
<td>-.31</td>
<td>-.04</td>
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<tr>
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<td>± .05</td>
<td>± .04</td>
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</tbody>
</table>
Further, there was a significant MPH condition by raclopride condition in sign trackers \((F(1,10)=10.98, p=.008, \eta^2=.29)\), with rats that previously received MPH during autoshaping and saline during the set-shift requiring a fewer number of trials needed to reach criterion (Figure 19). This effect was paralleled by a trending MPH condition by raclopride condition interaction on the number of preservative errors made in sign trackers \((F(1,10)=4.14, p=.07, \eta^2=.19)\). Surprisingly, sign trackers that had received MPH also had a reduced number of never-reinforced errors made during the set-shift \((F(1,10)=5.97, p=.04, \eta^2=.28)\).

![Figure 19. Interaction between MPH condition and raclopride condition in sign trackers.](image)

On the other hand, goal trackers did not exhibit any effects with drug manipulation. For example, there was no main effect of MPH \((F(1,3)=0.32, p=.61)\) or raclopride condition \((F(1,3)=0.20, p=.69)\) and no MPH condition by raclopride condition interaction \((F(1,3)=1.585, p=.30)\) on trials needed to reach criterion. However, this lack of findings in goal trackers is not conclusive as there were only seven goal trackers in Experiment 2.
Intermediates (n= 20) also did not exhibit any effects with drug manipulation. For example, there was no main effect of MPH ($F(1,16)=1.35, p=.26$) or raclopride condition ($F(1,16)=0.06, p=.82$) and no MPH condition by raclopride condition interaction ($F(1,16)=1.02, p=.33$) on trials needed to reach criterion.
CHAPTER 5

DISCUSSION

MPH is one of the most commonly used psychostimulants as a cognitive enhancer. While much of the research investigating MPH’s effectiveness at enhancing behavioral performance including executive functioning has been conducted in a clinical ADHD population or translational rodent models, MPH’s effects in the healthy, cognitive enhancement-using population are not as clear despite its continued use. The current study assessed whether daily administration of MPH in healthy adult rats increased cue salience, leading to a subsequent impairment of attentional set-shifting and whether both cue salience and attentional set-shifting are mediated by D2 receptor function.

Experiment 1 found that MPH tended to reduce PCA Index Scores, which are a measure of incentive salience that is highly related to drug addiction and relapse behavior. Notably, this effect was most prominent in sign trackers, or rats with high incentive salience and propensity for addiction liability. Further, Experiment 2 found that in sign trackers prior MPH use improved performance on the attentional set-shift test as compared to saline controls and raclopride groups, likely related to the reduced salience seen in this group in Experiment 1. Raclopride, a D2 receptor antagonist, impaired performance on the set-shift, but only in rats previously exposed to MPH, indicating that MPH administration had long-term effects on D2 circuitry that are evident when D2 receptors are further inhibited with an antagonist. Overall, the
current study suggests that the use of MPH as a cognitive enhancer in healthy individuals may have implications for alterations of dopaminergic circuitry that impacts learning and memory, potentially through reduced cue saliency, which may have lasting effects on the brain and executive functioning performance.

**Experiment 1: Influence of MPH on Incentive Salience**

Experiment 1 examined the potential effects of daily MPH administration on the development of sign and goal tracking behavior as a measure of incentive salience. It was hypothesized that daily administration of MPH prior to autoshaping would increase incentive salience/PCA Index Scores compared to rats receiving vehicle injections. Surprisingly, daily administration of MPH prior to autoshaping tended to decrease PCA Index Scores compared to rats receiving vehicle injections. Further, Experiment 1 sought to determine if daily MPH administration altered the density of D2 receptors in the mPFC and striatum. We hypothesized that MPH exposure would increase D2 receptors due to an increase in available extracellular DA in both the mPFC and striatum, which would be similar to what has been observed previously in rats categorized as sign trackers (Flagel et al., 2007). We also hypothesized that D2 receptors would be correlated with PCA Index Scores. However, MPH condition did not alter D2 immunoreactivity in the mPFC or striatum nor was D2 immunoreactivity correlated with PCA Index Scores.

**Methylphenidate Administration During Autoshaping**

This is the first study to examine the effect of MPH on PCA Index Scores, but administration of other dopaminergic drugs has been shown to alter a rat’s PCA Index Score
(Flagel et al., 2011; Fraser, Haight, Gardner, & Flagel, 2016; Saunders & Robinson, 2012). For example, systemic administration of a non-specific DA antagonist, flupenthixol, one hour prior to the start of each autoshaping session (for seven days) attenuated performance of conditioned responding in sign trackers and goal trackers (Flagel et al., 2011). Further, MPH has been shown to enhance learning in healthy human and rodent samples in various tasks that involve cues or discrimination learning, such as the delayed alternation task (Arnsten & Dudley, 2005; Berridge et al., 2006), visual signal detection testing/sustained attention (Berridge et al., 2006), and set-shifting and stop signal tasks (Linssen, Vuurman, Sambeth, & Riedel, 2012). Research has not yet investigated the behavioral mechanisms underlying the improvement in learning, such as salience toward cues, which may yield enhanced task performance. In healthy adults, MPH administration increased the saliency of a mathematical task, as measured by ratings of interest, excitement, and motivation (Volkow et al., 2004). These findings suggest that MPH may enhance learning through a salience-related mechanism, but no research has established if MPH heightens salience toward cues in learning paradigms. Thus, it was hypothesized that MPH prior to each day of autoshaping would increase PCA Index Scores as acquisition of incentive salience (sign tracking behavior) could be indicative of both enhanced learning and incentive salience. Surprisingly, MPH did not alter PCA Index Scores as compared to saline rats, although there was a trend to decrease lever pressing and incentive salience.

One difference in the current study compared to prior research is the use of a light cue with the lever cue (Meyer et al., 2012). In replicating previous autoshaping procedures, our lab has found that the light + lever consistently produces a disproportionate number of sign trackers, only a handful of intermediates, and very few goal trackers (Bond, McWaters, Anderson, & Matuszewich, 2017). For the current study, the light cue was eliminated for autoshaping,
allowing rats to form a conditioned response to an unlit lever. The exploratory analysis showed a more normal distribution of goal trackers, intermediates, and sign trackers (see Table 3 and Figure 18). Consistent with previous research, we found that rats in both the saline and MPH condition developed conditioned responses to either the lever (sign tracker), magazine (goal tracker), or to both the lever and the magazine somewhat equally (intermediate). Because the distribution of rats into goal and sign tracking categorizations was similar between MPH and saline rats, we purport that MPH did not alter learning a conditioned response during autoshaping. Notably, by eliminating the light cue, the data in the current experiment much more closely parallels the even distribution of sign trackers, goal trackers, and intermediates seen within the literature (Meyer et al., 2012), rather than our previously unevenly distributed data (Bond, McWaters, Anderson, & Matuszewich, 2017). Therefore, we can speculate that this data set without the lever light can still be used in comparison to the established PCA and autoshaping literature.

Interestingly, when analyzing Figure 18 it can be noted that the rats in the MPH condition have a more disperse distribution of PCA Index Scores than rats in the saline condition. This greater variance in PCA Index Scores within the MPH rats can be interpreted in several ways. MPH may interfere with forming any strong association at all, either between the lever and reward (sign tracker) or the magazine and reward (goal tracker) and therefore may result in more PCA Index Scores closer to 0, rather than scores indicative of strong conditioned responding closer to 1 or -1, respectively. This theory is supported by the increased number of intermediates seen in the MPH condition compared to the saline condition. The more clustered distribution of PCA Index Scores in the saline group indicates that the rats form clearer categorical groups based on behavior. For example, there is a more normal distribution of scores around the mean
for sign trackers, goal trackers, and intermediates. Alternatively, MPH may only be working to shift rats down along the PCA Index Score axis (i.e., lower PCA Index Scores). Based on the analyses for the subcomponents of PCA Index Scores, it appears that MPH consistently lowered the number of lever presses on all five days of autoshaping. Because of the shift in variance, the decreased lever responding, and the trend toward decreased PCA Index Scores seen in MPH rats, MPH may actually be working to decrease incentive salience, with no effect on learning a conditioned response. The current experimental design did not allow for assessment of PCA Index Scores prior to MPH administration; thus, we cannot conclude how MPH may be affecting goal and sign trackers differently (i.e., we would hypothesize that MPH administration only reduces PCA Index Scores for sign trackers). Future research is needed to explore this potential effect. Explicitly, allowing rats to form a goal, intermediate, or sign tracker phenotype and then administering MPH to reassess conditioned responding may provide more insight as to how MPH shifts rats along the PCA Index Score axis during performance of a previously acquired conditioned response, versus acquisition of a conditioned response in our current design.

MPH use in adults has been shown to decrease appetite, which may impact performance on the current study as it is contingent upon food-motivated behavior. Clinically, MPH has been shown to reduce appetite and cravings in adult humans (Davis et al., 2012) and decrease body weight during weeks of therapeutic doses in adult rats as compared to saline controls (Thanos et al., 2015). However, it is not likely that appetite was suppressed during our five days of MPH administration as there was no change in body weight (Figure 8), indicating that rats maintained similar eating behavior in their home cage as compared to saline controls. Further, both MPH and saline rats consumed all 25 banana pellets during each day of autoshaping, indicating similar appetite/desire to consume 25 pellets. Though appetite was likely not affected by MPH
administration, it is an important consideration because appetite states do play a role in the development of incentive salience (for review, see Berridge, 2012). While appetite is not the only contributing factor to motivated performance, it is an important contributor as demonstrated through studies that used food deprivation to increase the incentive salience associated with rewards. For example, rats exhibited preference for flavors paired with saccharin (low caloric value) or sucrose (high caloric value) over flavors paired with water, but when food deprived, however, only the preference for flavors paired with high caloric sucrose was enhanced (Fedorchak & Bolles, 1987). Thus, food deprivation increases the incentive salience of rewards with high caloric value and their associated cues, but not rewards of low caloric value or their associated cues, regardless of taste preferences (Fedorchak & Bolles, 1987; for review, see Lockie & Andrews, 2013).

The effect of food deprivation on incentive salience may be more prominent in sign trackers. Yager and Robinson (2010) found that fasting increases the incentive value of food cues in sign trackers and goal trackers as measured by significant reinstatement responding, but this effect was much more robust in sign trackers. The heightened sensitivity of sign trackers to food deprivation may help explain our finding that the effect of MPH reducing PCA Index Scores was most prominent in sign trackers, if MPH was truly reducing incentive salience and not simply decreasing appetite. Moreover, MPH rats did not have a lower number of magazine entries as compared to saline controls, suggesting a similar level of motivation during autoshaping (see Table 4). Magazine entries in this autoshaping paradigm can be indicative of approach behavior toward an expected reward (Flagel, Watson, Robinson, & Akil, 2007; Meyer et al., 2012); thus, similar magazine entries are indicative of similar reward-seeking behavior. Thus, although MPH is known to decrease appetite, rats receiving MPH in the current
experiments did not exhibit decreased appetite, displayed by similar reward seeking behavior and food consumption as compared to control rats.

In the current study, MPH had no effect on magazine entries or the average latency to enter the magazine but decreased the number of lever presses and increased average latency to press the lever (see Table 4). This decrease in lever pressing behavior may be indicative of reduced impulsivity or cue reactivity. One domain of symptomatology seen in individuals diagnosed with ADHD is hyperactivity/impulsivity, which is characterized by an inability to remain still or feelings of restlessness in adults, difficulty waiting, and/or talking excessively (American Psychiatric Association, 2013). Although no research has investigated goal and sign tracking profiles in SHRs, a rodent model for ADHD (Aparicio, Hennigan, Mulligan, & Alonso-Alvarez, 2019; for review, see Sagvolden, 2000), it can be hypothesized that their impulsive phenotype would yield high PCA Index Scores due to higher lever pressing. Therefore, in the current study’s autoshaping paradigm, we may not have found robust effects of MPH on PCA Index Scores because it would only have normalizing effects for rodents with high baseline impulsivity or hyperactivity, such as SHRs. Further, sign tracking, or high PCA Index Scores, may be indicative of impulsive behaviors. Previous data from our lab has documented a disproportionately high number of sign trackers and very few intermediates and goal trackers (Bond, McWaters, Anderson, & Matuszewich, 2017), thus our colony’s Sprague-Dawley rats may have high baseline levels of impulsivity or cue reactivity, which accounts for our observed effect of MPH reducing lever pressing and tending to reduce PCA Index Scores. Supportive of this suggestion, Flagel and colleagues (2010) selectively bred rats that had high or low reactivity to a novel environment, which can model a behavioral phenotype seen in humans with impulsive traits of addiction liability. A greater proportion of high-responder rats became sign trackers
while low-responder rats tended to become goal trackers. It has been theorized that sign tracking behavior may be indicative of impulsivity, lack of behavioral inhibitory control, novelty seeking, or cue reactivity (Beckmann, Marusich, Gipson, & Bardo, 2010; Flagel et al., 2010; Lovic, Saunders, Yager, & Robinson, 2011; Saunders & Robinson, 2013; Tomie, Aguado, Pohorecky, & Benjamin, 1998). As such, MPH’s reduction of lever pressing behavior and trend toward reducing PCA Index Scores in our healthy rats may indicate that MPH would have more robust effects in SHRs, as MPH has been previously shown to reduce impulsivity and improve response inhibition in models of ADHD (Aron, Dowson, Sahakian, & Robbins, 2003; Slezak & Anderson, 2011).

Impulsivity to press the lever in an ADHD or healthy rodent model may be dependent on the duration of cue presentation. It is possible that more robust effects of MPH on PCA Index Scores may have been observed by altering the length of time that the lever is extended into the chamber during autoshaping. In the current autoshaping paradigm, the lever was extended into the chamber for 8 seconds as described by other studies (Fitzpatrick & Morrow, 2016; Flagel, Watson, Robinson, & Akil, 2007; Meyer et al., 2012). However, 8 seconds may have been too long to detect robust group differences in PCA Index Scores, seeing as a highly motivated (or impulsive) rat is likely to press the lever in this paradigm quickly upon extension into the chamber. In a prior study, Meyer and colleagues (2012) reported that sign trackers generally have an average lever latency of fewer than 4 seconds by days 4 and 5 of autoshaping, with goal trackers having an average lever latency around 8 seconds as they are unlikely to press the lever at all.

In the current experiment, we observed that MPH not only decreased the number of lever presses but also increased the average latency to lever press across the first four days of
It is important to note, however, that these findings do not mean learning is impaired because, as previously stated, MPH rats entered the magazine just as quickly as saline controls following lever retraction, indicating similar association learning. This pattern of behavior suggests that MPH may be reducing impulsivity to lever press in healthy rats and, based on the PCA Index Score equation, decreasing incentive salience. By altering the duration of time the lever is extended into the chamber, future research may be able to tease apart the optimal duration for observing robust differences in lever pressing/PCA behavior and further investigate MPH’s effects on timing discriminability in associative learning. A shorter lever duration (i.e., 4 seconds) would make a stricter cutoff for rats’ pressing behavior, such that only the rats that are truly sign trackers (average around 4 seconds) will press the lever and have that data point calculated into their PCA Index Score; rats who are not as motivated to press the lever quickly will not meet the sign tracking criteria. In one study, rats receiving MPH were able to accurately ‘hit’ on a cued target lever during a sustained attention task after various signal lengths ranging from 0.25 to 2.0 seconds, but with no significant improvement over controls at 2.5 seconds (Berridge et al., 2006). Therefore, it is possible that by reducing lever duration, MPH rats would have significantly lower PCA Index Scores than saline controls.

Many of MPH’s effects have been studied in individuals diagnosed with ADHD or animal models of ADHD. By using a normal sample, we modeled the rising and abundant group of healthy individuals who use MPH recreationally without a clinical diagnosis (Han, Jones, Blanco, & Compton, 2017; Smith & Farrah, 2011). Thus, another plausible explanation for the lack of MPH effects could be the endogenous differences in dopaminergic systems between normal, healthy individuals and individuals with or animal models of ADHD. As our healthy rats may not have basal functioning similar to that of an ADHD model, MPH may not have the
ability to facilitate performance as expected because dopaminergic transmission already falls within the optimal range. Following the frequently hypothesized inverted-U shaped curve between performance and dopaminergic function (Aron, Dowson, Sahakian, & Robbins, 2003; Berridge et al., 2006; Brozoski, Brown, Rosvold, & Goldman, 1979; Cools & D’Esposito, 2011; Floresco, 2013; Goldman-Rakic, 1992, 1995; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007), MPH’s DA agonistic effects could push rats with low baseline functioning to the right and into peak performance, but not augment rats already performing at peak levels. To date, no research has investigated goal and sign tracking response profiles in animal models of ADHD and no comparable research has been conducted in individuals diagnosed with ADHD. It would be interesting to evaluate baseline differences in incentive salience in a rodent model, such as SHRs, and then assess whether MPH enhances or decreases PCA Index Scores. Similar studies could be done in children or adults diagnosed with ADHD as researchers have adapted a similar lever pressing and food reward paradigm to assess sign tracking and goal tracking in children (Joyner, Gearhardt, & Flagel, 2018). Because MPH is being used to treat individuals with a clinical diagnosis and by healthy individuals using MPH as a cognitive enhancer, it is important for future research to consider the impact of MPH in both populations.

**Methylphenidate Administration and D₂ Receptors**

MPH alters the dopaminergic system by increasing extracellular DA levels, which could downregulate D₂ receptors. Therefore, we hypothesized that daily exposure to MPH would decrease D₂ receptors to yield the anticipated increase in cue salience. We found that MPH did not alter D₂ receptor immunoreactivity in either the mPFC or striatum. As no published literature has investigated the effect of repeated exposure to MPH on D₂ receptors in the mPFC,
it is not surprising that we observed no effect. However, it was surprising to find no effect of MPH on D₂ immunoreactivity in the striatum given that previous literature shows that 60 mg oral MPH reduces D₂ receptor availability in the striatum of healthy adult males using positron emission tomography (Clatworthy et al., 2009). Prior research also showed changes in D₂ receptor availability following MPH administration in adult rats, but this was dependent on baseline impulsivity and availability of D₂ receptors. For example, rats with high impulsivity had lower D₂ receptor availability and chronic MPH administration reduced impulsivity and increased D₂ receptor availability. On the other hand, MPH increased impulsivity in rats with low baseline impulsivity (Caprioli et al., 2015). If MPH’s effects on D₂ receptor availability are dependent on baseline D₂ receptor availability, the potential directionality of long-lasting changes in D₂ circuitry following MPH administration may also depend on baseline D₂ receptor levels. Unfortunately, western blots are limited to collecting the tissue at one time point, which does not allow for assessing whether a within-subject change in D₂ receptors occurred following five days of MPH administration. It is possible that we may have seen increases in D₂ receptor immunoreactivity in a rodent model of ADHD or highly impulsive rodents with lower baseline D₂ receptor availability prior to MPH administration.

Previous research has shown that dopaminergic biological markers, such as DAT expression or the expression and distribution of DA receptors, differ in rats based on their propensity for sign or goal tracking (Singer et al., 2016). Goal trackers have greater expression of D₂ receptor mRNA in the nucleus accumbens, DAT in the ventral tegmental area, and tyrosine hydroxylase in the ventral tegmental area (Flagel, Watson, Robinson, & Akil, 2007). Similarly, in humans, low levels of D₂ receptors have been associated with increased self-reported alcohol craving in alcoholics and drug liking in response to MPH in non-abusing subjects (Heinz et al.,
Together, the human and animal research suggest that lower levels of D_2 receptors contribute to greater incentive salience and subsequently craving and drug-seeking behaviors seen in animals and humans with heightened cue salience (Flagel et al., 2007; Flagel et al., 2011; Lopez, Karlsson, & O’Donnell, 2015; Saunders & Robinson, 2012). In the current study, PCA Index Scores or its subcomponent measures were not correlated with D_2 receptors in either the mPFC or striatum. This was further supported by the exploratory analyses comparing D_2 receptors with the categorization of rats by their sign tracker, goal tracker, or intermediate classification, similar to previous literature. There were no significant correlations detected overall or within goal and sign trackers, suggesting that D_2 receptors in the mPFC or striatum are not critical to PCA Index Scores or the reduced lever pressing seen with daily MPH administration. Other brain regions, such as the nucleus accumbens, or another neural system, such as norepinephrine, may be more important in cue saliency. In fact, Gillis and Morrison (2019) recently found that sign and goal trackers have distinct dopamine release profiles in the nucleus accumbens and these profiles have different implications for resistance to extinction in sign trackers that may be mediated by cue-evoked activity. Future research should investigate the impact of MPH administration on nucleus accumbens dopaminergic activity, as this may serve a distinct role in reward-related behavior and incentive salience.

While the cue and craving literature suggested that D_2 receptors are a good target for understanding incentive salience seen in sign trackers, there are likely other DA receptors functioning for sign and goal trackers. As mentioned above, nucleus accumbens DA is a key target, especially for the development of a sign tracker phenotype but not the goal tracker phenotype (Flagel et al., 2011; Saunders & Robinson, 2012; Singer et al., 2016). Microinjections
of flupenthixol into the nucleus accumbens reduced lever pressing behavior in rats previously categorized as sign trackers, but had no effect on number of magazine entries for goal trackers (Saunders & Robinson, 2012). Inversely, an amphetamine injection into the nucleus accumbens, thus increasing nucleus accumbens DA, facilitated sign tracking behavior but not goal tracking behavior (Singer et al., 2016). Further, DA receptor subtypes play different roles in the acquisition of goal and sign tracking phenotypes. For example, acquisition of sign tracking was prevented by both the D₁ receptor antagonist, SCH39166, and the D₂ receptor antagonist, eticlopride, whereas only SCH39166 blocked the acquisition of goal tracking (Roughley & Killcross, 2019). This finding is supported by Macpherson and Hikida (2018), who demonstrated that blockade of D₁ medium spiny neurons in the nucleus accumbens reduced conditioned responding in both sign trackers and goal trackers. Receptors are dynamic, changing their availability depending upon the extracellular milieu. Thus, the timing between MPH administration and D₂ receptors may be critical to understanding the role of D₂ receptors on PCA Index Scores in MPH treated rats. In the current study, brains were collected two days following the last day of autoshaping/MPH administration. This timing was explicitly chosen to ensure MPH was not metabolically active in the brain but close enough to the behavior to draw comparisons. However, it may be useful to examine D₂ receptor immunoreactivity with MPH on board, at the same time interval as measured for the behavior, to investigate the active effects of the drug, rather than the long-lasting effects that we wanted to observe in the attentional set-shifting behavior in Experiment 2. Assessing nucleus accumbens dopaminergic activity with MPH on board may also provide useful information on how MPH impacts this region of the brain’s reward circuit and could be paralleled to established changes in DA transmission in the
nucleus accumbens during autoshaping (Dalley et al., 2005; Gillis & Morrison, 2019; Lopez, Karlsson, & O’Donnell, 2015).

**Experiment 2: Influence of MPH and Raclopride on Attentional Set-Shifting**

Executive function tasks have been the focus of much research due to MPH’s use in enhancing ADHD-diagnosed children’s likelihood of success in school as well as MPH’s use as a cognitive enhancer (Abikoff et al., 2004; for review, see Franke, Bagusat, Rust, Engel, & Lieb, 2014; Van der Oord, Prins, Oosterlaan, & Emmelkamp, 2008). We expected that MPH would enhance cue salience and that this enhancement would render the attentional set-shift more challenging. Thus, Experiment 2 measured the potential influence of prior MPH exposure on attentional set-shifting. It was hypothesized that daily administration of MPH prior to autoshaping would increase trials needed to reach criterion during the attentional set-shift as compared to rats with daily administration of saline and that this effect would be mediated by PCA Index Scores. Unexpectedly, previous MPH administration did not have long-lasting effects that influenced performance on the attentional set-shift, and further, PCA Index Scores failed to mediate performance with or without prior treatment with MPH. Experiment 2 also tested whether D2 receptor binding was critical for attentional set-shifting by administering raclopride prior to the set-shift. It was hypothesized that raclopride administration during the set-shift would impair performance, consistent with other D2 antagonist literature (Floresco, Magyar, Ghods-Sharifi, Vexelman, & Tse, 2006; Mehta, Manes, Magnolfi, Sahakian, & Robbins, 2004). While raclopride administration did not hinder performance on the attentional set-shift as predicted, there was an interaction between MPH and raclopride; rats previously exposed to MPH required a greater number of trials during the set-shift after an injection of
raclopride compared to an injection of vehicle. This finding suggests that the use of MPH in healthy individuals may have long-lasting effects on dopaminergic circuitry that impact later instances of drug exposure and executive functioning.

In the current study, MPH rats that received raclopride required a greater number of trials to reach criterion compared to saline rats. This finding was paralleled by a trending interaction in the number of incorrect responses made during the set-shift. The exact mechanism driving the D2 antagonist disruption of set-shift in the rats exposed to MPH previously is unknown, but we can speculate that MPH may have lasting effects on D2-related circuitry. As MPH rats in Experiment 1 did not have alterations of D2 immunoreactivity in the mPFC or striatum, the behavioral effect may be the result of alterations in another brain region affected by MPH and important for set-shifting. For example, although the cerebellum is not often a direct target of clinical MPH administration, magnetic resonance spectroscopy has shown that acute administration of MPH increases cerebellar creatine, indicative of increased cellular metabolism (Inci Kenar, Unal, Kiroglu, & Herken, 2017). Similarly, Volkow and colleagues (1997) demonstrated that MPH increased cerebellar metabolism in healthy adults using positron emission tomography. Independent of MPH, cerebellar functioning has also been implicated in executive functioning tasks. During a visual discrimination learning test, healthy adults showed activation of the cerebellum as well as various regions of the frontal cortex, as measured with positron emission tomography (Rogers et al., 2000). In mice, 95% depletion of Purkinje cells, which are the only afferent projections from the cortex of the cerebellum, resulted in impairments on an attentional set-shifting task (Dickson, Cairns, Goldowitz, & Mittleman, 2017). Therefore, MPH administration may not only target the mPFC and striatum, but other
regions such as the cerebellum, which may be critically involved in alterations of D₂ receptors after MPH exposure and explain the interaction seen with raclopride during the set-shift.

Even though there were no differences in D₂ receptors between MPH and saline treated rats, there is a possibility that the interaction observed could still be attributed to alterations of D₂ receptors in the mPFC and striatum. Methodological issues associated with western blot analyses could negate smaller changes, especially in the mPFC. Large mPFC tissues were dissected, which included different subregions of the mPFC including the prelimbic and infralimbic cortices that are implicated in various types of set-shifting and learning, but also the anterior cingulate cortex, which is not implicated in extradimensional attentional set-shifting. For example, lesions of prelimbic or infralimbic cortices in rats impair extradimensional shifts while lesions of the anterior cingulate cortex and orbitofrontal cortex do not affect extradimensional shifts, but instead may be more important for intradimensional learning/discrimination or reversal learning (Ghods-Sharifi, Haluk, & Floresco, 2008; McAlonan & Brown, 2003; Ng, Noblejas, Rodefer, Smith, & Poremba, 2007; Ragozzino, 2007; Ragozzino, Detrick, & Kesner, 1999). One study further suggests that the prelimbic and infralimbic PFC may have separate roles in a strategy-switching task, with the infralimbic PFC mediating the correct strategy in the strategy selection during the strategy-switch and prelimbic PFC mediating the maintenance of the correct strategy after switching (Oualian & Gisquet-Verrier, 2010).

Because the western blot analyses required larger tissues (i.e., amount of protein), our dissections of the mPFC included infralimbic and prelimbic cortices as planned, but also anterior cingulate cortex. Potentially, with a more refined dissection of the infralimbic and prelimbic PFC, future research could detect differences in D₂ immunoreactivity following MPH administration.
As there were no main effects of MPH on either autoshaping or D₂ circuitry in the mPFC or striatum, it is not surprising that prior exposure to MPH did not alter performance on the set-shift. The results may have been different if MPH was on board during the set-shift. Previous research indicates that acute MPH enhances set-shifting performance in an ADHD population and SHRs (Cao, Yu, Wang, Wang, Yang, & Lei, 2012; Kantak et al., 2008) as well as in healthy individuals (Elliott et al., 1997). Although there is some inconsistency in these effects on healthy humans, as another paper demonstrated that even with MPH on board, there was no change in performance during a set-shift (Linssen et al., 2012). It should be noted, however, that MPH may have had lasting effects on D₂ circuitry to explain the interaction observed between MPH and raclopride during the set-shift.

**Raclopride Administration During Attentional Set-Shifting**

Despite prior research showing that D₂ antagonism impairs performance on set-shifting paradigms, administration of 0.2 mg/kg raclopride 30 minutes prior to the set-shift did not alter performance. The discrepancy between our findings and other reports may be due to route of administration and therefore the selectivity of neural targets. In a prior study, Floresco, Magyar, Ghods-Sharifi, Vexelman, and Tse (2006) infused a D₂ antagonist, eticlopride, directly into the PFC of rats during a set-shifting task and found marked impairments in performance as measured by increased trials needed to reach criterion and increased preservative errors. However, microinfusions of eticlopride into the nucleus accumbens did not affect attentional set-shifting performance (Haluk & Floresco, 2009), suggesting that the role of D₂ receptors in set-shifting may be brain region specific. In the current study, raclopride was systemically administered via an intraperitoneal injection, which allows for non-selective antagonistic effects of raclopride at
D2 receptors in many brain regions. In a human study, healthy volunteers’ ability to perform an attentional set-shift in the CANTAB battery was only moderately impaired by systemic administration of sulpiride, a comparable D2 antagonist (Mehta et al., 2004). Thus, our systemic administration yielding no main effect of raclopride is not surprising given the result of the only other known systemic D2 administration study. The effects of raclopride in different brain regions could yield opposing behavioral effects and suggest that future research should focus on microinjections, rather than systemic administration.

D2 receptors are widely distributed throughout the brain with D2 autoreceptors concentrated in dopaminergic cell body regions, the substantia nigra and ventral tegmental area. Postsynaptic D2 receptors are also found in these regions, as well as terminal regions such as the dorsal striatum and the nucleus accumbens (Meador-Woodruff, Damask, & Watson, 1994; Meador-Woodruff et al., 1989). Systemic D2/D3 receptor agonists and antagonists can activate or inhibit autoreceptors and/or postsynaptic receptors, potentially leading to the same net effect on behavior across differently affected brain regions (for review, see Ford, 2014). The combination of the pre- and postsynaptic D2 receptor blockade with raclopride may not alter attentional set-shifting as predicted because antagonizing D2 autoreceptors yields an increase in extracellular DA, which could compete with raclopride at postsynaptic D2 binding sites (Fraser, Haight, Gardner, & Flagel, 2016). Ironically, the increase in dopamine along with D2 receptor antagonism could increase D1 receptor stimulation (Anzalone et al., 2012). However, this effect is unlikely in our current results as previous research has demonstrated that D1 receptor agonism enhances performance on attentional set-shifting or attentional control tasks (Nikiforuk, 2012; Pezze, Dalley, & Robbins, 2007), whereas we saw no main effect of raclopride, and an interaction with the MPH/raclopride rats actually reduced performance. Interestingly, the
interaction between MPH and raclopride was only evident in sign trackers, which is the only autoshaping phenotype mediated by D₂ receptors, whereas both sign trackers and goal tracking phenotypes are mediated by D₁ receptor function (Roughley & Killcross, 2019; Macpherson & Hikida, 2018). Although no main effect of raclopride was observed in attentional set-shifting, further research exploring the role of D₂ receptors following MPH exposure in sign trackers may provide a clearer understanding of MPH’s lasting changes in the brain.

Unexpectedly, raclopride hindered performance during the 20 cue discrimination reminder trials immediately prior to the set-shift. This finding may be indicative of impaired memory from the visual cue discrimination task. Raclopride has been shown to induce memory deficits in other learning paradigms. Infusions of raclopride into the infralimbic PFC immediately prior to extinction training impaired the recall of extinction the next day when rats were tested drug-free (Mueller, Bravo-Rivera, & Quirk, 2010). Similarly, raclopride impaired conditioned fear acquisition and retention in a fear-potentiated startle paradigm when injected into the amygdala (Greba, Gifkins, & Kokkinidis, 2001). Raclopride has also been shown to induce memory deficits during tasks when drug is on board. Von Huben and colleagues (2006) observed that rhesus macaques had impaired spatial working memory and visuo-spatial paired associative learning following administration of raclopride. The current findings suggest that raclopride did not impair rats’ abilities to form a new strategy (i.e., during the set-shift), but may impair memory of the previous strategy (i.e., visual cue discrimination). Significantly for the current findings, if memory of the previous strategy was impaired, it is difficult to predict how learning the new strategy or performing the set-shift may have been affected. However, in a reversal learning paradigm very similar to our attentional set-shifting paradigm, vervet monkeys that received 0.03 mg/kg raclopride displayed similar performance on retention/reminder trials
prior to their reversal test compared to controls (Lee, Groman, London, & Jentsch, 2007). As raclopride only hindered performance on the cue reminder trials and had no effect on performance during the set-shift, including no effect on regressive errors, we can speculate that raclopride only impairs memory from the cue discrimination test to the reminder trials, but not working memory performance within the set-shift.

The dose of raclopride was selected based on previous literature to yield behavioral effects, without impacting locomotor behavior. Previous research has shown that dopaminergic agonism and antagonism affect locomotion, with moderate to high doses (i.e., 0.5 – 8.0 mg/kg) of D2 antagonists impairing locomotion (Hillegaart & Ahlenius, 1987). However, the dose of 0.2 mg/kg raclopride was selected for the current experiment because 0.125 and 0.25 mg/kg doses were reported not to affect motor activity in an open field (Hillegaart & Ahlenius, 1987), but 0.2 mg/kg was effective at changing reaction time and performance on motivational tasks (Cheng & Liao, 2007; Marrow, Overton, & Clark, 1993; Smith & Smith, 2010). In Experiment 2, raclopride did not increase the number of omissions made during the set-shift, indicating that raclopride did not impair rats’ abilities to perform the motoric requirements in the operant chamber. Similarly, there were no differences between raclopride and saline injected rats for correct or incorrect responses during the set-shift, indicating normal motor performance. However, approximately one week following completion of attentional set-shift testing, rats were tested for locomotion in an open field test after a second injection of their same drug assignment of either saline or 0.2 mg/kg raclopride. Raclopride decreased distance traveled in the open field \((F(1,37)=26.09, p<.01)\), indicating raclopride-induced a locomotor impairment of forward locomotion. The open field data suggests that either the number of responses and omissions made in the operant chamber are not a good assessment of general locomotion or rats may have
had heightened sensitivity to a second injection of raclopride. However, one study was able to demonstrate a strong correlation between lever pressing on a variable-time fixed ratio schedule to locomotor activity on an actometer (McKerchar, Zarcone, & Fowler, 2005) and other studies successfully use within-subject designs for assessing D2 antagonism effects with no effect of repeated drug exposure on behavior (Amalric, Berhow, Polis, & Koob, 1993; Fowler & Liou, 1998).

Despite the vast majority of research implicating a crucial role of proper dopaminergic neurotransmission for optimal attentional set-shifting performance, the adrenergic system may also contribute to attentional set-shifting. For example, depleting norepinephrine in the PFC impairs set-shifting (McGaughy, Ross, & Eichenbaum, 2008; Tait et al., 2007). Lapiz and Morilak (2006) demonstrated that systemic injections of an α2 adrenergic autoreceptor antagonist, atipamezole (thus increasing norepinephrine), reduced the number of trials needed to reach criterion in rats on the digging bowl set-shifting paradigm. Further, microinjections of an α1 adrenergic receptor antagonist, benoxathian, into the mPFC in combination with atipamezole attenuated the enhanced performance observed following atipamezole alone, indicating that attentional set-shift performance is dependent on α1 adrenergic receptor mediation of the mPFC. Likely, both the dopaminergic and adrenergic systems work collectively to yield proper working memory task performance (Arnsten & Dudley, 2005). The current study focused on the dopaminergic influence on attentional set-shifting and did not assess the role of the adrenergic system, which may partially explain why raclopride alone did not affect performance on the set-shift.

The over-arching model for the current study hypothesized that PCA Index Score would mediate attentional set-shift, with raclopride disrupting performance in saline treated rats.
Although PCA Index Score was not a significant covariate for any of the dependent measures of the attentional set-shift, the distribution of PCA Index Scores allowed for an exploratory analysis of the effects of D2 antagonism in goal and sign trackers. Prior research has shown that systemic administration of the selective D2 antagonist eticlopride impaired conditioned performance of both sign and goal trackers, decreasing lever pressing and magazine entries respectively (Lopez, Karlsson, & O’Donnell, 2015). In contrast, only sign trackers responded to a D2 agonist, quinpirole hydrochloride, which decreased lever pressing in sign trackers but had no effect on magazine entries in goal trackers. These results indicate that D2 receptor modulation may differ between the two behavioral phenotypes (Lopez, Karlsson, & O’Donnell, 2015). Supporting this prior research, exploratory analyses on the current data revealed a main effect of raclopride as well as a MPH condition by raclopride condition interaction in sign trackers, but no drug effects in either goal trackers or intermediates. Lopez, Karlsson, and O’Donnell (2015) theorized that activation of presynaptic D2 autoreceptors, which are inhibitory, could reduce the ability to process salient cues. Paralleling their interpretation of their findings, our results indicate that sign trackers may be more sensitive to the effects of D2 antagonism, specifically in regard to processing salient cues, as they are the phenotype with the highest cue salience. Further, because the sign tracking phenotype is highly related to impulsive behavior (Flagel et al. 2010), sign trackers may be more sensitive to the reduced lever pressing effects of MPH, a drug clinically known to target impulsivity symptoms (Kratochvil et al., 2002; Malone & Swanson, 1993). In the current study, only sign trackers were sensitive to MPH and raclopride manipulations during the set-shift, which may explain why PCA Index Score as a continuous variable, which includes variance from all of the phenotypes, was not a significant covariate for set-shifting performance.
Finally, a potential reason why main effects of MPH condition or raclopride condition were not observed during the set-shift could be attributed to the procedures of autoshaping. All rats learn to associate either the left or the right lever with a reward during autoshaping, with the specific lever counterbalanced across rats. By testing the rats in five days of autoshaping and exposing them to either a left or a right lever only, rats may form an inherent bias or tendency to readily respond to one side or the other during the set-shift. Although side bias or side preference is assessed prior to attentional set-shift testing, the impact of being conditioned on one side during autoshaping may influence a rat’s ability to shift from a visual cue to only attending to one lever in the operant chamber. This is not the only instance in which researchers have purported a bias affecting set-shifting performance. Floresco, Block, and Tse (2008) suggest that rats have an innate bias towards adopting alternation strategies on spatial tasks. This bias could be further enhanced during the alternating lever training leading up to the set-shift testing. Thus, rats may learn the visual cue discrimination, which is essentially a randomized cued-alternation task, more readily in the attentional set-shifting paradigm than responding to one side alone because of this alternation bias. The current findings therefore suggest that our rats may have the opposite bias: a tendency to adopt a side response strategy, rather than an alternating strategy, due to conditioning to one side across five days of autoshaping. The successful performance seen during the set-shift in all groups but the MPH/raclopride group may provide evidence for this bias.

**Future Directions and Clinical Implications**

The current findings contribute to our understanding of the effects of MPH in a healthy adult sample on incentive salience and behavioral flexibility. As MPH did not increase incentive
salience or have long-lasting effects on D₂ circuitry, these findings may help alleviate public concern that recreational MPH use can enhance reward-related cues and thus have implications for addiction. However, MPH also did not enhance or hinder association learning in the current autoshaping paradigm, leading to questions about its effectiveness as a cognitive enhancer.

Despite high use of MPH as a cognitive enhancer (Smith & Farah, 2011), there is little research on the potential influence of recreational MPH use on cue salience, which may have implications for understanding the development of drug addiction and/or relapse behaviors as well as performance on executive functioning tasks, which are also DA-dependent. Further research investigating sign and goal tracking behavior is needed to understand the role of D₂ receptors in incentive salience versus association learning. In the current study, the tendency for MPH to reduce lever pressing as compared to saline controls and alter the clustering of PCA Index Scores indicates that MPH may have implications for learning processes in healthy individuals using the substance as a cognitive enhancer, albeit not contributing to lasting effects to influence attentional set-shifting. Further research should investigate the implications of incentive salience in reward and addiction-related behaviors and should target other reward-related brain regions, such as the nucleus accumbens.

The interaction between MPH administration and raclopride on increasing trials needed to reach criterion during the attentional set-shift warrants some clinical concern as MPH use in healthy individuals may impact the effects of later dopaminergic drug use. Little research has investigated the effects of MPH in healthy individuals on executive functioning tasks, with no research investigating the potential lasting effects after discontinued MPH use. The current experiment’s finding of an interaction between prior MPH administration and raclopride administration on set-shifting performance indicates that future research is needed to understand
how MPH may have lasting effects on D₂ receptor circuitry. One avenue for future research includes investigating the dopaminergic and adrenergic systems together to understand how they may modulate each other during the attentional set-shifting paradigm.

Although PCA Index Score was not a significant covariate for performance during the set-shift, sign trackers alone were sensitive to drug manipulations. The findings here provide the impetus to further investigate behavioral differences in goal and sign tracking phenotypes on cue-related executive functioning tasks. For our laboratory, the larger range of PCA Index Scores that were observed by omitting the light cue during autoshaping will allow us to investigate sign and goal trackers moving forward. This research may aid our understanding of the cue-related mechanisms driving motivated behaviors such as reward-seeking and addiction in goal and sign trackers. The exploration of sex differences in cue salience and performance during the attentional set-shift may also provide useful insight for the drug addiction body of literature. Sex differences are apparent in drug addiction, with females escalating more quickly to addiction and being at greater risk for relapse (Becker & Hu, 2008). Investigating differences in cue salience may provide a sex-dependent mechanism driving associative learning and cravings associated with cues. Further, males and females also differ in performance on the attentional set-shift, with females requiring fewer trials to meet criterion than males (McWaters, Errante, & Matuszewich, in preparation).

Overall, the current findings indicate that MPH does not affect incentive salience or association learning but may have long-lasting effects on dopaminergic circuitry that affect later dopaminergic drug use and executive functioning. Future research is needed to investigate the effects of MPH in healthy versus clinical populations on the development of incentive salience and later executive functioning performance and should also investigate the potential sex
differences in MPH use and incentive salience as males and females have vastly different addiction behavior profiles (for review, see Becker & Hu, 2008). Therefore, pharmacological cognitive enhancers, specifically MPH use, should be further researched as their use is on the rise in a large population of healthy individuals.
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