Frontostriatal Circuit, Receptor, and Neural Coding Mechanisms Underlying the Cognition Enhancing Actions of Psychostimulants

by

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Abstract

Psychostimulants exert dose-dependent alterations in frontostriatal cognitive function. Specifically, higher doses, associated with addiction, robustly impair frontostriatal-dependent cognition. In contrast, at low and clinically-relevant doses, psychostimulants enhance cognitive and behavioral function dependent on the prefrontal cortex (PFC) and extended frontostriatal circuitry. The procognitive actions of psychostimulants are observed in individuals with attention deficit hyperactivity disorder (ADHD) as well as in typical human and animal subjects. However, despite the widespread use of these drugs, the cognitive and neurophysiological underpinnings of their cognition-enhancing and therapeutic effects are poorly understood, limiting our ability to develop better treatments for frontostriatal cognitive dysfunction.

The goal of this thesis is to examine the neurocircuitry, receptor mechanisms and electrophysiological actions underlying the cognition-enhancing and cognition-impairing effects of low dose psychostimulants. Specifically, these studies addressed three major questions: 1) Does methylphenidate (MPH; Ritalin®) act directly within distinct frontostriatal subfields to improve or impair PFC-dependent cognitive function? 2) What are the receptor mechanisms underlying the procognitive actions of MPH? 3) What are the electrophysiological actions underlying the cognition improving vs cognition impairing actions of MPH within frontostriatal circuitry in animals tested in a spatial working memory task.

These studies demonstrate that: 1) Although MPH acts directly within the PFC to enhance performance in both working memory and sustained attention tasks, sustained attention displayed a right-shifted dose sensitivity; 2) This differential sensitivity to MPH between working memory and sustained attention involves the differential activation of PFC alpha-1 receptors; 3) MPH exerts complex, dose-dependent actions on frontostriatal neuronal signaling during a working memory task, with high doses suppressing task related neuronal representations and altering oscillations in local field potentials whereas low doses have minimal effects on frontostriatal signaling. Collectively, these studies provide new insight into the neurobiology of higher cognitive function and provides important information for the development of novel treatments for ADHD and other conditions associated with frontostriatal dysfunction.

Specific Aims

To better develop alternative ADHD treatments with comparable efficacy to psychostimulants it is important that we understand the neural mechanisms responsible for the cognition-enhancing/therapeutic actions of these drugs. Extensive evidence in human and animals demonstrates that low dose psychostimulants facilitate higher cognitive functions dependent on frontostriatal circuitry, whereas higher doses impair these same processes. The proposed studies examine the frontostriatal neurocircuitry, receptor mechanisms and electrophysiological actions involved in the dose dependent cognition-enhancing and cognition-impairing actions of MPH. This information will increase our understanding of the neurobiology underlying: 1) the cognition-enhancing actions of this widely used class of drugs; 2) higher cognitive function. Collectively, this knowledge is important in developing novel treatments for ADHD and other conditions associated with PFC dysfunction.

AIM 1. TO IDENTIFY THE DEGREE TO WHICH METHYLPHENIDATE (MPH; RITALIN®) ACTS WITHIN DISTINCT FRONTOSTRIATAL SUBFIELDS TO IMPROVE PFC-DEPENDENT COGNITION AS MEASURED IN A DELAYED-RESPONSE TEST OF SPATIAL WORKING MEMORY. (CHAPTER 2)

AIM 2. TO IDENTIFY THE POTENTIAL CIRCUIT AND RECEPTOR MECHANISMS UNDERLYING THE DIVERGENT DOSE-DEPENDENT COGNITIVE EFFECTS OF MPH ACROSS PFC-DEPENDENT COGNITIVE TASKS. (CHAPTER 3)

AIM 3. TO IDENTIFY THE ELECTROPHYSIOLOGICAL ACTIONS OF COGNITION IMPROVING VS COGNITION IMPAIRING DOSES OF MPH WITHIN FRONTOSTRIATAL CIRCUITRY DURING A SPATIAL WORKING MEMORY TASK. (CHAPTER 4)

Chapter 1

Introduction

Attention deficit hyperactivity disorder (ADHD), first described in 1902 (Still, 1902), is a cognitive/behavioral syndrome associated with attentional deficits, hyperactivity and impulsivity. Initially viewed as a childhood disorder, ADHD is now known to occur in adults, with a sizeable proportion of adult ADHD reflecting a continuance of the disorder from childhood (Barkley, 2002; Biederman et al., 1993; Mannuzza et al., 1991; Spencer et al., 1994; Weiss et al., 1985). Conservative estimates indicate ADHD affects 3-8% of the population (Visser et al., 2007). Importantly, this disorder is associated with increased risk in a number of domains including academic, social and health-related (Barkley et al., 2002; Hechtman et al., 2004; Mannuzza et al., 1997, 1993). As such, it is important that ADHD patients be identified and treated to mitigate these risks.

Although our understanding of the neurobiology of ADHD is still emerging, available evidence implicates a key role of the prefrontal cortex (PFC) in this disorder. The PFC plays a critical role in a variety of cognitive and behavioral processes affected in ADHD, including working memory, planning, attention, and response inhibition (Arnsten & Robbins, 2002; Miller & Cohen, 2001; Posner & Petersen, 1990). Collectively, these and other processes comprise a proposed 'executive function' of the PFC involved in the temporal organization of goal-directed behavior. Importantly, lesions of the PFC produce a variety of behavioral effects similar to those seen in ADHD, including hyperactivity, impulsivity and deficits in working memory and sustained attention (for review, Arnsten & Li, 2005), consistent with functional imaging studies demonstrating a dysfunction of the PFC and extended frontostriatal circuitry in ADHD (Bush et al., 2005; Vaidya et al., 1998).

Psychostimulants are a class of drugs most commonly associated with their potent arousing and behaviorally-activating actions, as well as the significant potential for abuse (Rebec & Bashore, 1984; Segal, 1975). Nonetheless, these drugs, particularly methylphenidate (**MPH**, Ritalin®) and amphetamine (Adderall®), are highly effective in treating a variety of cognitive and behavioral deficits in PFC-dependent function in children, adolescents and adults with attention deficit hyperactivity disorder (ADHD; Abikoff et al., 2004; Bradley, 1937; Greenhill, 2001; Hechtman et al., 2004; Scheffler et al., 2009; Wender et al., 1985), including working memory, attention, and impulsivity; with millions of prescriptions written annually.

Initially, these seemingly contradictory actions of psychostimulants were thought to reflect unique and 'paradoxical' effects in individuals with ADHD. However, subsequent work unambiguously demonstrated that the cognition-enhancing and behavioral-calming actions of psychostimulants are not limited to ADHD. Thus, when administered at low and clinically-relevant doses, psychostimulants improve a variety of behavioral and cognitive processes dependent on the prefrontal cortex (PFC) in human subjects with and without ADHD (Jl & G, 2001; Mehta et al., 2001; Rapoport et al., 1980). Consistent with this, the cognition-enhancing actions of low-dose psychostimulants have been recently recognized by the general population, with rising use of these drugs on and off college campuses to improve academic and work-related performance by individuals without ADHD (Maher, 2008; McCabe et al., 2005; Setlik et al., 2009). Moreover, these actions are not unique to humans, having been documented in typical animal subjects when administered at clinically-relevant doses; i.e. doses that produce clinically-relevant plasma concentrations in the 8-40 ng/mL range (Arnsten & Dudley, 2005; Berridge et al., 2006; Devilbiss & Berridge, 2008; Kuczenski & Segal, 2002; Swanson & Volkow, 2001; Zdrale et al., 2008). Of particular relevance, prior studies in our laboratory demonstrate that systemic MPH, at doses that produce clinically-relevant plasma concentrations, improves the

accuracy of rats performing a spatial delayed alternation test of working memory (**Figure 1**; Berridge et al., 2006), similar to that observed with all FDA approved treatments for ADHD (for review, Berridge & Devilbiss, 2011). Together, these observations indicate that an animal model of ADHD is not necessary to examine the neural mechanisms involved in the cognitive/therapeutic effects of low-dose stimulants. This is not a trivial advantage, given most animal models of psychopathology suffer from a high degree of uncertainty regarding the extent to which they model the neurobiology of a disorder, even while mimicking certain behavioral features of that disorder

Evidence suggests that the cognition-enhancing/therapeutic actions of psychostimulants may stem from direct action within the PFC (Bush et al., 2005; Dickstein et al., 2006). For example, in animals, clinically-relevant and cognition-enhancing doses of psychostimulants elevate extracellular catecholamine levels and increase responsiveness to afferent signals *preferentially* within the PFC (Berridge et al., 2006; Devilbiss & Berridge, 2008). Moreover, structural and functional imaging studies demonstrate psychostimulants reverse ADHD-associated hypofrontality (Bush et al., 2005; Rubia et al., 1999; Sheridan et al., 2007; Vaidya et al., 1998). Combined, these observations provide intriguing, though indirect, support that the cognition-enhancing/therapeutic effects of low-dose psychostimulants involve drug action directly within the prefrontal/frontal cortex.

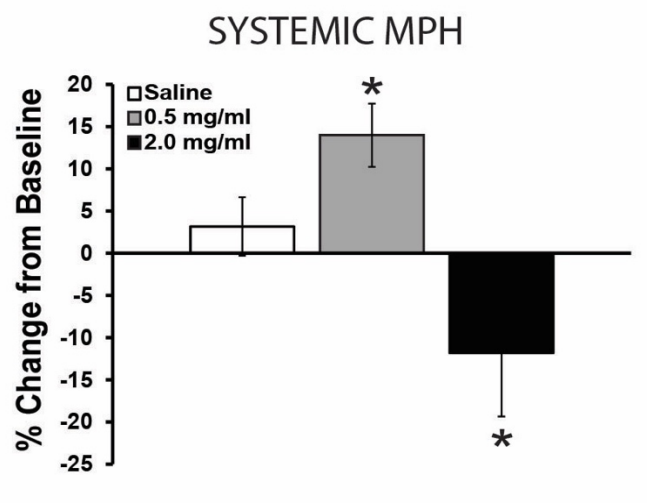

Figure 1. Effects of low-dose methylphenidate (MPH) on spatial working memory as measured in a delayed-alternation task. Shown are mean±SEM percent change in accuracy from baseline. At a dose of 0.5 mg/kg, IP MPH significantly improved performance, whereas 2.0 mg/kg MPH impaired performance in the same task. *$p<.05$ compared with vehicle-treated animals.

Frontostriatal Circuits in Cognition

The PFC represents one node in an extended network involved in response selection under ambiguous/distracting conditions. Response selection involves predicting potential outcomes associated with multiple response options, evaluating the quality of predicted outcomes, and outcome evaluation (for review, Johnson et al., 2007). This process likely involves the coordinated activity of a distributed network that includes the PFC, hippocampus, and the striatum (Dalley et al., 2004; Johnson et al., 2007; Pratt & Mizumori, 2001; Vertes, 2006).

The PFC displays a functional topographic organization with dorsal subfields associated with higher cognitive function and ventral regions primarily associated with affect- and motivation-related processes (Dalley et al., 2004). In the rat, the dorsal portion of the medial PFC, encompassing the dorsal anterior cingulate and dorsal prelimbic subfields, serves a critical role in higher cognitive function (Kesner, 2000), while the ventromedial PFC, comprised of the infralimbic and ventral prelimbic subfields, participates in affective/motivational processes (Dalley et al., 2004; Vertes, 2006, 2004). This functional heterogeneity within the PFC involves, in part, topographically-organized projections to downstream striatal subfields, forming distinct functional frontostriatal circuits (Chudasama & Robbins, 2006; Dalley et al., 2008; Dunnett et al., 2005). Of particular relevance is the circuitry connecting the PFC with the striatum, which involves both direct projections from the PFC to the striatum as well as multisynaptic projections to the PFC from the striatum (Dalley et al., 2004; Pratt & Mizumori, 2001; Vertes, 2006). A large body of evidence demonstrates that the dorsomedial striatum (**dmSTR**) receives direct projections from the dmPFC and is associated with more 'cognitive-like' processes, such as task-

switching (Johnson et al., 2007; Ragozzino et al., 2002). Additionally, there is also limited evidence that the ventromedial striatum (**vmSTR**) may be involved in the cognition enhancing actions of psychostimulants (Mair et al., 2002; Seamans & Phillips, 1994). For example, imaging studies have implicated structural and functional changes in fronto-striatal circuitry of ADHD patients, including hypoactivity of the striatum that is reversed with MPH (Lou et al., 1989; Rubia et al., 2009). Combined, these observations suggest that psychostimulants may additionally act in the striatum in order to enhance cognitive function.

Neurochemical Actions of Psychostimulants

As stated above, the two most commonly used psychostimulants in the treatment of ADHD are methylphenidate and amphetamine. At behaviorally activating doses, these drugs potently increase extracellular levels of norepinephrine (NE) and dopamine (DA) throughout the brain, largely by blocking NE and DA reuptake (Segal, 1975). Some psychostimulants, particularly amphetamine, actively stimulate DA efflux through the DA transporter (Kuczenski & Segal, 1994). Although amphetamine can also stimulate NE efflux and block serotonin reuptake, these actions only occur at high and clinically inappropriate doses (Florin et al., 1994). In contrast, MPH acts only to block NE and DA reuptake, neither inhibiting serotonin reuptake nor stimulating NE or DA efflux (Kuczenski & Segal, 1997).

Low and clinically-relevant doses of stimulants exert behavioral actions that are qualitatively different than higher and behaviorally-activating doses. As noted above, cognition-enhancing doses of psychostimulants appear to preferentially affect the PFC. This includes evidence from microdialysis studies in home-cage housed animals that demonstrate clinically-relevant doses of MPH produce a preferential elevation in extracellular NE and DA within the PFC. Specifically, in rats, doses of methylphenidate that elicit clinically-relevant plasma concentrations and improve PFC-dependent

behavioral function, produce prominent increases in extracellular levels of NE and DA within the PFC, while having substantially smaller effects on DA levels in the nucleus accumbens and NE levels in the medial septal area (Berridge et al., 2006). Moreover, in both the hippocampus and somatosensory cortex, these same doses of methylphenidate elevate NE levels similar to that seen in the medial septal area and well below that observed in the PFC (Drouin et al., 2006; Kuczenski & Segal, 2002; Kuczenski & Segal, 2001).

Overall, these observations indicate an enhanced sensitivity of PFC catecholamines to cognition-enhancing doses of psychostimulants. This preferential sensitivity of PFC NE and DA to low-dose stimulants may involve low DA transporter (DAT) levels within the PFC combined with an ability of the NE transporter (NET) to clear extracellular NE and DA (Carboni et al., 1990; Sesack et. al., 1998). Thus, due to competition between NE and DA for binding to the NET, increases in NE will result in less DA binding to the NET, resulting in an increase in extracellular DA levels in a feed-forward model. Conversely, increases in extracellular DA will result in greater competition for NE binding to the NET, resulting in an elevation in NE levels.

NE acts at three families of receptors: α1, α2, and β receptors, each comprised of multiple subtypes. All three families are found throughout the PFC (Nicholas et al., 1993a; Nicholas et al., 1993b; Pieribone et al., 1994). α2 receptors display a higher affinity for NE relative to α1 and β receptors and are thus preferentially engaged at lower rates of release (**Figure 2**; Arnsten, 2000b) . There are three subtypes of α_2 receptors, α2A, α2B, and α2C, with post-synaptic α2A receptors playing a critical role in the regulation of PFC-dependent cognition (MacDonald et al., 1997; Wang et al., 2007).

DA acts at two different families of receptors throughout the brain, including in the PFC: D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptors. Importantly, the 'dopamine (DA)'

D4 receptor binds both NE and DA and thus should be considered a generalized catecholamine receptor (Van Tol et al., 1991). While evidence strongly implicates D4 receptor signaling in cognition (Furth et al., 2013; Yuen et al., 2013; Zhong and Yan, 2014), our understanding of the cognitive actions of *NE signaling* at this receptor is limited.

Catecholamine modulation of PFC Function

Working Memory. As noted, the catecholamines, NE and DA are important neuromodulators affecting PFC-dependent behavior (Arnsten, 2007). These modulatory actions have been most intensively examined in delayed-response tests of working memory. Spatial working memory is a form of short-term memory used to guide goal directed behavior based on representational knowledge of spatial position. Catecholamine modulation of prefrontal function was first noted by Brozoski (1979), who demonstrated that selective inactivation of either neurotransmitter profoundly impaired performance on tasks requiring WM function. Subsequent work demonstrates that NE and DA exert inverted-U shaped dose-dependent facilitation of PFC-dependent cognition, with both low and high levels of DA/NE resulting in cognitive impairment (Arnsten & Li, 2005; Arnsten & Robbins, 2002). These modulatory actions of NE and DA involve multiple receptor subtypes. For DA, D1 receptor stimulation elicits an inverted-U shaped dose-dependent modulation of working memory performance (**Figure 2**; Arnsten, 2007). NE also acts within the PFC to exert an inverted-U shaped modulation of performance in tests of working memory such that *both* inadequate and excessive NE signaling is associated with an impairment in working memory performance while moderate levels of NE signaling is associated with optimal performance (**Figure 2**; Robbins and Arnsten, 2009). This inverted-U shaped function reflects the differential activation of NE receptor subtypes within the PFC. Thus, under low arousal conditions associated with low rates of NE release or other conditions associated with reduced NE signaling in the PFC (e.g. NE lesions of the PFC, aging) working memory is

impaired (Arnsten et al., 1996). These deficits in working memory are reversed by selective activation of high-affinity α2 receptors (Arnsten et al., 1996). Conversely, under conditions associated with moderate arousal levels and moderate rates of NE release, α2 receptor blockade degrades optimal working memory (Arnsten, 2000). In contrast to that seen with PFC α2 receptors, stimulation of α1 receptors in the PFC elicits a stress-like impairment in working memory (Arnsten and Castellanos, 2002). Moreover, while blockade of PFC α1 receptors has no effect on working memory under optimal conditions associated with moderate rates of NE release (Li and Mei, 1994), this reverses working memory impairment associated with high rates of NE release (e.g. stress; **Figure 2**; Arnsten and Castellanos, 2002; Birnbaum et al., 1999). For β-receptors, limited evidence indicates that β1 and β2 receptors in the PFC exert opposing actions on working memory; blockade of β1 or stimulation of β2 receptors in the PFC improves working memory (Ramos et al., 2008; Ramos et al., 2005)

Combined, this information suggests the hypothesis that psychostimulants exert working memory-enhancing effects via activation of α_2 and D1 receptors within the dmPFC. Consistent with this hypothesis, *systemic* administration of either a D1 antagonist (SCH23390) or an α_2-antagonist (idazoxan) prevents the beneficial effect of low-dose MPH on working memory when administered at doses that, on their own, do not affect working memory (Arnsten & Dudley, 2005). Of course, these latter studies do not determine the anatomical location of D1 and α_2-receptors involved in the cognitive effects of low-dose MPH.

Attentional Processes Psychostimulants have been typically viewed to *uniformly* affect PFC-dependent cognitive processes (Pievsky & McGrath, 2018; Solanto, 2001). However, increasing evidence in humans and animals demonstrates that subsets of PFC-dependent cognitive processes display differential dose sensitivity to these drugs. Specifically, working memory and response inhibition display relatively narrow inverted-U dose-dependent facilitation, while

behavioral calming and attention-enhancing effects are observed across a broader, right-shifted range of doses (Berridge et al., 2012; Sprague & Sleator, 1977; Tannock et al., 1989; Tannock, et al., 1995). In order to investigate the effects of MPH across distinct cognitive processes, an operant based signal detection test was employed to assess the effects of psychostimulants on sustained attention.

Different PFC dependent cognitive processes are modulated via different neural and receptor mechanisms (Arnsten & Li, 2005; Berridge et al., 2012; Dalley et al., 2004; Robbins & Arnsten, 2009). Indeed, NE exerts receptor-specific modulatory actions across PFC-dependent cognitive processes. As. noted above, PFC α1 receptors impair working memory, whereas, in contrast, PFC α1 receptors facilitate both flexible (attentional set shifting) and focused/sustained attention (Arnsten et al., 1999; Lapiz and Morilak, 2006; Berridge et al., 2012). Consistent with this, clinically relevant doses of methylphenidate maximally enhance working memory, while doses 4-fold higher maximally improve sustained attention via activation of α1 receptors (Berridge et al., 2012). Collectively, these observations suggest that MPH may additionally act via NE α1 receptors within the dmPFC to promote attentional processes.

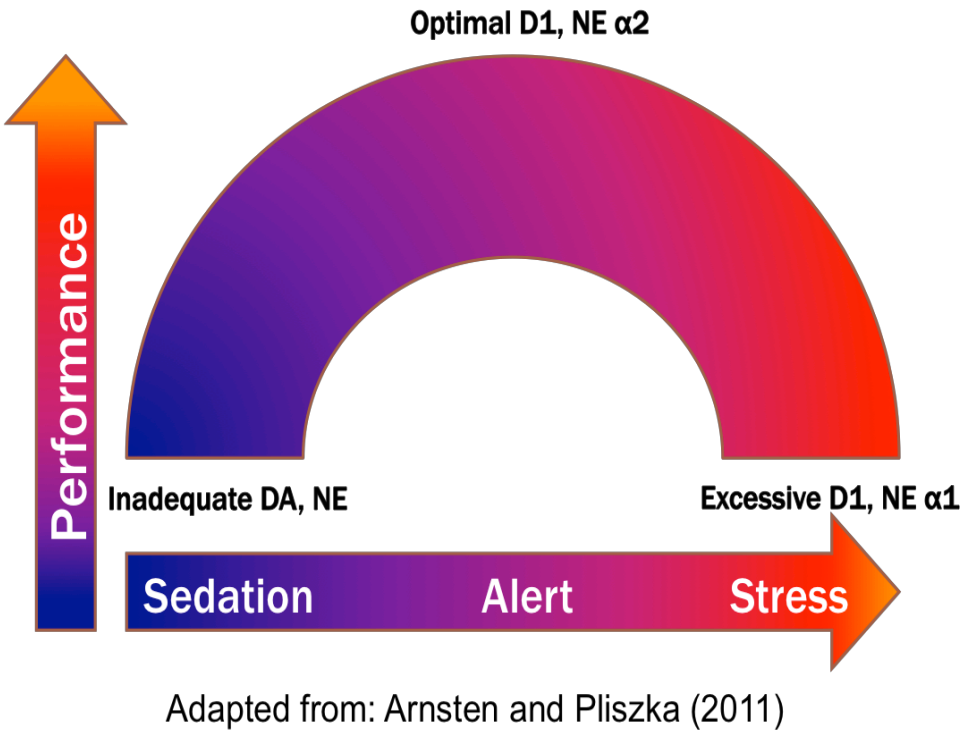

Figure 2. *Both* inadequate and excessive NE and DA signaling is associated with impairment in working memory performance whereas moderate levels of catecholamine signaling is associated with optimal performance.

Frontostriatal Electrophysiology

In vivo, PFC neurons display a diversity of task-related discharge patterns. One of the most intensely studied is the sustained discharge displayed by a subset of PFC neurons during the delay-portion of working memory tasks (*delay-related discharge*; Fuster and Alexander, 1971). Initially posited to represent information storage in working memory, currently there is much debate over the degree to which this activity reflects a storage function vs. a role in attention, the interpretation of rules/context, or other functions (Asaad et al., 2000; Lebedev et al., 2004; Miller & Cohen, 2001; Postle, 2006). Nonetheless, PFC delay-related discharge is positively correlated with optimal performance in these tasks, regardless of the specific cognitive/behavioral process it represents (Wang et al., 2007). In addition to delay-related

activity, PFC neurons are sensitive to other task-related events, including reward/outcome and decision-related processes (Batuev et al., 1990; Bouret & Sara, 2004; Devilbiss et al., 2016; Watanabe, 1996).

In the dorsolateral PFC of monkeys, neurons display delay-related discharge with spatial tuning during an oculomotor delayed response task (Arnsten, 2007). As reviewed above, optimal performance in PFC dependent tasks requires optimal levels of catecholamines; with dopamine acting at D1 receptors and NE acting at postsynaptic α_{2A} receptors. Supporting this, electrophysiological evidence indicates that α_{2A} receptor stimulation strengthens delay-related discharge for neurons tuned to behaviorally relevant spatial locations (increasing signal; Wang et al., 2007). In contrast, optimal D1 receptor stimulation weakens delay-related discharge to neurons tuned to behaviorally irrelevant spatial locations (increasing noise; Vijayraghavan et al., 2007). Combined, these actions improve the signal to noise ratio of tuning properties of PFC neurons. In contrast, high levels of arousal (i.e. stress) leads to NE acting at lower affinity α_1-receptors. and excessive DA acting at D1 receptors to reduce working memory through the overall suppression of PFC neuronal activity and collapse of spatial tuning (Arnsten, 2000a).

Previous work in our lab analyzed the effects of acute white noise-stress on task-related activity of PFC neurons in rats performing a delayed alternation test (Devilbiss et al., 2012). These studies demonstrate that *in vivo* PFC neurons display recurrent activation during and outside the delay interval (i.e. represent spiking history-related discharge; **Figure 3A**), and that this activity significantly contributes to ongoing, sustained discharge. Moreover, acute stress was observed to weaken the relative contributions of spiking history to PFC neuronal discharge during the delay period of a working memory test (**Figure 3B**). In a follow-up study, stress was further shown to suppress delay activity in PFC neurons strongly tuned to the delay phase (i.e. those that showed elevated activity during the delay period), while increasing delay activity in

PFC neurons not tuned to delay (Devilbiss et al., 2016). Combined, these actions decrease the signal to noise ratio, impairing signal processing within the PFC. Given stress impairs PFC-dependent cognition as measured in this test, high doses of MPH may mimic this effect, whereas an action opposite to that may be see with cognition-enhancing doses of MPH, strengthening of PFC delay sensitive neurons, while simultaneously reducing activation to neurons not tuned to the delay. Finally, cognition-impairing doses of MPH may affect task-related discharge outside the delay period in a similar manner as seen with stress, i.e. generally suppressing outcome related activity, whereas cognition-enhancing doses may a have an opposite effect.

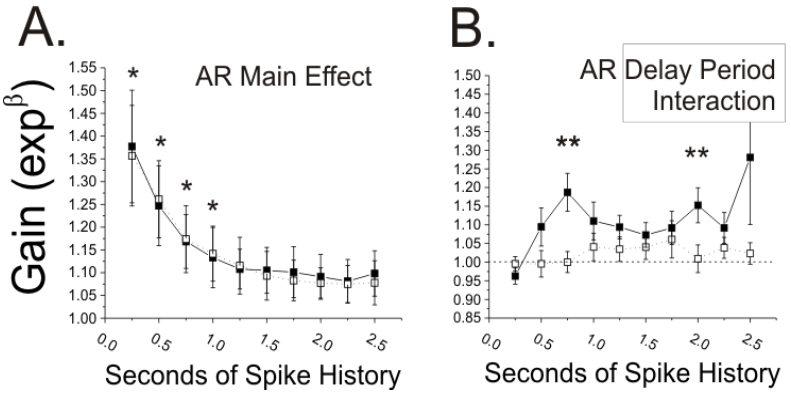

Figure 3. Stress-related changes in plPFC neuron spike-history effects. (A) Spiking gain measures of the contribution of spike history at different points back in time decay exponentially under baseline and stress conditions. (B) Delay-specific effects of spike history. Stress suppressed the effects of spike history specifically during delay period.

A variety of imaging, electrophysiological, and lesion studies demonstrate that the PFC is aided in supporting working memory through interactions with subcortical structures, including the striatum (Floresco et al., 1997; Frank et al., 2001; Levy et al., 1997a; Lewis et al., 2004; Spencer et al., 2012). This includes elevated striatal activity during the delay period of working memory tasks (Chiba et al., 2015; Histed et al., 2009; Postle & D'Esposito, 1999; Soltysik et al., 1975). In the rat, dorsomedial striatal neurons display tuning to both delay and reward related components of delayed response tasks (Akhlaghpour et al., 2016; Levy et al., 1997b).

Interestingly, this striatal activity may not be sustained throughout the delay as found in the PFC, but tiled across the delay period through the sequential activation of neighboring neurons (Akhlaghpour et al., 2016). Thus, in addition to alterations in PFC neuronal activity, this suggests that the cognition modulating actions may have additional actions on neuronal activity within the dmSTR. This includes the hypothesis that high doses may suppress whereas cognition-enhancing doses may elevate activity in neurons strongly tuned to the delay.

While spiking data looks at the activity of individual neurons, local field potentials (LFPs) reflect the slower, aggregate activity of many neurons within a small volume of brain tissue (Kajikawa & Schroeder, 2011). Oscillations in LFPs are thought to play an important role in allowing neural ensembles to talk to each other both within and between brain regions (Buzsáki & Draguhn, 2004). LFP oscillations are typically broken up into discrete frequency bands that are purported to correlate with distinct behavioral and cognitive functions, although the exact role of these oscillations is still debated (Hsieh & Ranganath, 2014; Klimesch, 1999; Lisman, 2010; Roberts; Roux & Uhlhaas, 2014). For example, theta activity in the frontal cortex is positively correlated with behavioral performance in working memory tasks (Gevins, 1997; Hsieh & Ranganath, 2014; Itthipuripat et al., 2013; Mitchell, et al., 2008), although see (Klimesch, 1999). This includes evidence that frontal cortical theta increases following successful completion of working memory tasks in humans and monkeys (Hsieh & Ranganath, 2014; Itthipuripat et al., 2013), and that rodent mPFC neurons are entrained to theta during successful but not unsuccessful working memory trials (Hyman et al., 2010) In the case of frontal alpha oscillations, evidence suggests that they may be most closely aligned with the ability to inhibit task irrelevant information (Roux & Uhlhaas, 2014). In particular, the power of alpha oscillations prior to distractors in a working memory task is positively correlated with performance (Bonnefond & Jensen, 2012). Lastly, frontal gamma oscillations are positively correlated with working memory load (Howard et al., 2003)

Prefrontal cortical dysfunction is associated with a variety of changes in frontal cortical oscillatory power. In particular, ADHD, which is associated with impaired working memory,

shows increases in frontal theta power (Barry et al., 2003; Clarke et al., 2007; Koehler et al., 2009), with psychostimulant treatment normalizing this increase (Clarke et al., 2007; Loo et al., 2004). This would appear in contrast to the evidence above where frontal theta is positively correlated with successful working memory performance. However, these seemingly contradictory observations may be related to the fact that increased theta power is also correlated with working memory load (Jensen & Tesche, 2002). Thus, increased theta in both ADHD and successful behavioral performance may reflect increases in effort, rather than a signature of working memory accuracy *per se*. ADHD, is also associated with aberrant changes in alpha oscillations (Koehler et al., 2009; Woltering et al., 2012) and psychostimulant treatment normalizing this activity (Loo et al., 2004). Lastly, there is limited evidence that frontal gamma oscillations are decreased in ADHD, with psychostimulant treatment reversing this hypoactivity (Wilson et al., 2013).

To date, there has been little work done on the role of neural oscillations in the dmSTR during working memory tasks. However there is evidence that theta activity is prominent in the striatum of rats performing spatial navigation tasks (DeCoteau et al., 2007), with increased theta power observed during decision making components of the task and increased gamma oscillations in response to reward (Tort et al., 2008). Thus, there exists a large gap in our understanding of how striatal oscillations contribute to working memory performance.

In order to better understand the neural mechanisms underlying the cognitive/therapeutic actions of these drugs, it is important to characterize their electrophysiological actions in animals engaged in a test of PFC-dependent function. The above reviewed information suggests the hypothesis that the divergent cognitive actions of low vs. higher doses of psychostimulants may be associated with opposing modulatory actions on task-related and oscillatory activity within dorsomedial frontostriatal circuitry. For these studies, we examined the effects of vehicle or MPH treatment on frontostriatal activity of three frequency bands: theta (4-7 Hz), alpha (7-12 Hz), and gamma (40-80 Hz) during the delay phase of a delayed spatial working memory test.

Chapter 2

Psychostimulants Act within the Prefrontal Cortex to Improve Cognitive Function

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Abstract

Background: At low and clinically-relevant doses, psychostimulants enhance cognitive and behavioral function dependent on the prefrontal cortex (PFC) and extended frontostriatal circuitry. These actions are observed in individuals with attention deficit hyperactivity disorder (ADHD) as well as in normal human and animal subjects. Despite the widespread use of these drugs, the sites of action involved in their cognition-enhancing and therapeutic effects are poorly understood. Indirect and/or correlative evidence suggests the cognition-enhancing/therapeutic effects of psychostimulants may involve actions directly within the PFC or extended frontostriatal circuitry. The current studies examined the degree to which methylphenidate (MPH; Ritalin®) acts within distinct frontostriatal subfields to improve PFC-dependent cognition as measured in a delayed-response test of spatial working memory.

Methods: Working memory performance was assessed following microinfusion of vehicle or varying doses of MPH (0.03-8.0 μg/500 nl) directly into the dorsomedial PFC (dorsal prelimbic and dorsal anterior cingulate cortex), the ventromedial PFC (infralimbic) and the dorsomedial striatum of rats (n=69).

Results: MPH infusion into the dorsomedial PFC, but not ventromedial PFC, elicited an inverted-U shaped facilitation of PFC-dependent cognition as measured in this task. The magnitude of this improvement was comparable to that seen with systemic administration. Additional studies demonstrated that although the dorsomedial striatum is necessary for accurate performance in this task, MPH infusion into this region did not affect working memory performance.

Conclusions: These observations provide the first definitive evidence that the PFC is a site of action in the cognition-enhancing and presumably therapeutic actions of low-dose psychostimulants.

Millions of prescriptions are written annually for psychostimulants, particularly methylphenidate (MPH; Ritalin) and amphetamine, to treat the cognitive and behavioral symptoms of attention deficit hyperactivity disorder (**ADHD**; Greenhill, 2001). Extensive research demonstrates that low doses of these drugs improve cognitive and behavioral processes dependent on the prefrontal cortex (PFC)/frontal cortex in ADHD patients. These observations are consistent with structural and functional imaging evidence implicating dysregulation of the PFC/frontal cortex and extended frontostriatal circuitry in this disorder (Bush et al., 2005; Castellanos & Tannock, 2002; Mehta et al., 2004; Shaw et al., 2009). Importantly, the cognition enhancing actions of psychostimulants are not unique to ADHD, as similar effects occur in normal humans and animals when administered at low and clinically-relevant doses (Berridge et al., 2006; Gamo et al., 2010; Kuczenski & Segal, 2002; Mehta et al., 2001). Indeed, this is evident through the increasing use of these drugs in the general population as cognitive enhancers (Wilens et al., 2008). Despite their widespread use, the neural circuitry responsible for the therapeutic and cognition-enhancing actions of low-dose psychostimulants is surprisingly poorly understood.

A number of observations suggest that the cognition-enhancing/therapeutic actions of these drugs may stem from direct action within the PFC (Bush et al., 2005; Dickstein et al., 2006). For example, in animals, clinically-relevant and cognition-enhancing doses of psychostimulants elevate extracellular catecholamine levels and increase responsiveness to afferent signals *preferentially* within the PFC (Berridge et al., 2006; Devilbiss & Berridge, 2008). Moreover, structural and functional imaging studies demonstrate psychostimulants reverse ADHD-associated hypofrontality (Bush et al., 2005; Rubia et al., 1999; Sheridan et al.,

2007; Vaidya et al., 1998). However, interpretation of these observations is confounded both by their correlational nature and the fact that systemic administration of psychostimulants, as used in all of these studies, can influence PFC neuronal activity indirectly via actions in regions that project to the PFC. Combined, these observations provide intriguing, though indirect, support that the cognition-enhancing/therapeutic effects of low-dose psychostimulants involve drug action directly within the prefrontal/frontal cortex.

An additional or alternative site of action in the cognition-enhancing/therapeutic actions of psychostimulants is the striatum, a region also implicated in the neuropathology of ADHD (Seidman et al., 2005). For example, a variety of observations indicate the striatum is anatomically and functionally connected with the PFC and plays a prominent role in cognitive/behavioral processes historically viewed as 'PFC-dependent' (Balleine et al., 2007; Balleine & O'Doherty, 2010). Additionally, studies in both humans and animals demonstrate that clinically-relevant doses of psychostimulants impact dopamine (DA) signaling within striatal regions (Clatworthy et al., 2009; Volkow et al., 2002), albeit to a lesser degree than seen in the PFC (Berridge et al., 2006).

To test whether the cognition-enhancing effects of psychostimulants involve direct action within the PFC and/or striatum, the current studies examined the effects of microinfusion of MPH into select PFC and striatal subfields on performance in a PFC-dependent delayed-alternation test of working memory (Kesner, 2000). Importantly, the pharmacology of performance in this test closely aligns with the pharmacology of ADHD (Berridge & Devilbiss, 2011; Berridge et al., 2006; Gamo et al., 2010), in contrast with other tests of PFC-dependent cognition (Berridge et al., 2012). This close alignment may reflect the fact that performance in these tasks is simultaneously dependent on a variety of cognitive and behavioral processes known to be affected in ADHD, including attention, working memory, and planning.

The medial PFC (mPFC) of rats is functionally and anatomically heterogeneous, with the dorsomedial PFC (dmPFC), encompassing the dorsal anterior cingulate (dAcg) and dorsal prelimbic subregions, implicated in higher cognitive function (Dalley et al., 2004; Kesner, 2000). In contrast, the ventromedial PFC (vmPFC) comprised of the infralimbic and ventral prelimbic subregions, is strongly associated with autonomic, visceromotor, and affective processes (Dalley et al., 2004; Mehta et al., 2004; Vertes, 2004). Consistent with this, the current studies demonstrate that MPH infusion into the dmPFC, but not vmPFC, improve working memory performance comparable to that seen with systemic administration.

The dorsomedial striatum (dmSTR) receives direct projections from the dmPFC. Moreover, pharmacological and lesion studies demonstrate that the dmSTR participates in higher cognitive functions typically associated with the dmPFC (Ragozzino, 2007; Voorn et al., 2004). Therefore, additional studies examined the degree to which MPH acts within the dmSTR to improve working memory performance. We first identified a region of the dmSTR that receives prominent and direct projections from the dmPFC. Subsequent studies demonstrated that reversible inactivation of the dmSTR impairs performance comparable to that seen with PFC inactivation, indicating the dmSTR is necessary for performance of this task. Nonetheless, MPH infusion into this region had no effect on PFC-dependent cognition as measured in this task.

Combined, these studies provide the first demonstration that the cognition-enhancing actions of psychostimulants believed to underlie the therapeutic effects in treatment of ADHD involve direct action within the PFC.

Methods and Materials

Animals: Male Sprague-Dawley rats (260-280 grams; Charles River, Wilmington, MA) were pair-housed in clear polycarbonate cages on a 13-11 hour light/dark cycle (lights on 06:00). Animals were fed *ad libitum* for the first 7 days and subsequently restricted to 15-17 grams of food per day following training/testing. Training/testing was conducted between 09:00 and 16:00 hours (typically 6 days/week). Rats were weighed twice weekly to confirm animals did not lose weight and were assigned a single experimenter who handled them extensively prior to behavioral testing. All facilities and procedures were in accordance with the guidelines regarding animal use and care put forth by the National Institutes of Health of the United States and were approved by the Institutional Animal Care and Use Committee of the University of Wisconsin.

Surgery: Following training in the T-maze (see below), rats were anesthetized with isoflurane and placed in a standard stereotaxic device with the skull flat. Indwelling stainless steel cannulae (25 ga.) were implanted bilaterally over either the dAcg/prelimbic PFC (A+3.0; L±0.8; V-0.2 mm measured from dura; Fig. 1A,C), infralimbic PFC (same coordinates as dAcg, with longer needle; Fig. 2A,B), or dmSTR (A+0.45; L±2.0; V-3.2 mm; Fig. 3B) and secured to the skull with stainless steel screws and dental acrylic (Plastics One, Roanoke, VA). Stainless steel stylets prevented occlusion of the cannulae and were replaced as needed to maintain patency.

Great care was taken to maintain the structural integrity of the mPFC in these experiments. Given the anatomical and behavioral evidence implicating the dorsal mPFC (dAcg, dorsal prelimbic PFC) in higher cognitive/behavioral processes (Dalley et al., 2004; Kesner,

2000), cannulae were only lowered 200 μm below dura to avoid cannula-related damage to this region.

Behavioral Training and Testing: Training and testing were similar to that described previously (Berridge et al., 2006). Black plastic sheeting surrounded the maze to obscure any external spatial cues. Animals were trained to enter the maze arm not chosen on the previous trial to gain food reward (1 chocolate chip/trial; 20 trials per session, 1 session per day). Between trials, rats were placed in the start box located at base of the T and prevented from exiting by a removable acrylic glass gate during the delay interval. Following surgery, rats resumed testing in the T-maze until performance reached pre-surgery levels. Delay intervals were used that resulted in 65%-80% accurate performance. Stable performance was defined as 2 consecutive days of 65-80% accuracy in which performance did not differ by more than 10%. Accuracy in performance improves gradually with testing at a given delay, thus it was necessary to extend the delay interval over several weeks of testing. For these studies, delay intervals ranged between 3 and 80 seconds (mean = 21 seconds). Performance at the same delay was further tested on the first two days following infusion to confirm stable performance. No consistent effects of any of the treatments were observed in the days following treatment. Thus, when a difference between the pre-infusion average and post-infusion average was greater than 10%, the animal was considered unstable and that treatment data point excluded.

All treatments were separated by at least two days. Before receiving a drug treatment, rats were given two mock infusions, consisting of an initial needle insertion followed by vehicle infusion 48 hours later. This permitted animals to acclimate to the mild restraint associated with the infusions, as well as minimize detrimental behavioral effects of tissue damage related to needle insertion.

Drug Infusion: Methylphenidate HCl (Sigma, St. Louis, MO; (0.035, 0.125, 0.5, 2.0, and 8.0 µg/500 nl)) and the $GABA_A$ agonist muscimol (Sigma, St. Louis, MO; 75 ng/500 nl) were dissolved in AECF (147 mmol/L NaCl, 1.3 mmol/L CaCl2, 0.9 mmol/L MgCl2, 2.5 mmol/L KCl; pH=7.4). 0.5 µl infusions of drug or AECF were made bilaterally through 33 gauge needles that projected below the guide cannulae by 1.6 mm for dAcg, 2.5 mm for prelimbic (See Fig. 1C), 4 mm for infralimbic (see Fig. 2B), and 3.5 mm for dmSTR infusions (See Fig. 3B). Infusions were performed using a microprocessor pump (Harvard Apparatus, South Natick, MA), set at a rate of 250 nl/minute for 2 minutes. Needles remained in tissue for 2 minutes following infusions after which the stylets were replaced. Rats were placed in their home cage for an additional 15 minutes before testing.

Histological Analyses of Drug Infusion Sites and Data Selection: At the end of testing, rats were deeply anesthetized with isoflurane and transcardially perfused with 10% w/v formaldehyde. Brains were stored in formaldehyde for a minimum of 24 hours prior to sectioning. Placement of injectors was verified in 40 µm-thick coronal sections stained with Neutral Red dye. Data from a given experiment were included only when histological analyses verified accurate placement of injectors and minimal tissue damage.

Striatal Retrograde Tracer Infusion: In a limited number of animals (n=4), the retrograde tracer Fluorogold (FG; Fluorochrome, Denver, CO) was infused into the dmSTR (see Surgery above) or dorsolateral STR (+0.45A, ±3.8L, -3.5V), using glass pipettes, as described previously (España et al., 2005). For these infusions, single-barrel glass micropipettes (15-25 µm diameter; Friedrich and Dimmock Inc. Millville, NJ) were filled with 2.0 % FG solution (dissolved in saline) as previously described. Once the infusion pipette was in position, FG was iontophoresed (5.0 µA, 15-minutes, 5-second pulses, 50% duty cycle) with the pipette remaining in place for an

additional 10-minutes. Animals were sacrificed 7 days following FG infusions. FG was visualized using immunohistochemical procedures as described previously (España et al., 2005). Briefly, animals were perfused with 4% paraformaldehyde, 40 μm sections were collected through the PFC and later incubated for 48 hours at 4° with rabbit anti-FG antibody (1:2,000; Chemicon International, Temecula, CA; cat# AB153) diluted in 0.01 M PBS-TX. After incubation, tissue was rinsed with 0.01 M PBS-TX, and incubated with donkey-anti-rabbit antibody (1:500; Jackson ImmunoResearch, West Grove, PA) for 90-minutes. Tissue was then rinsed with 0.01 M PBS-TX, exposed to rabbit PAP (1:500; Dako Corporation, Carpinteria, CA) for 90-minutes and rinsed with 0.01 M PBS-TX. Sections were reacted with diaminobenzidene (DAB; Vector Laboratories) to yield a brown precipitate.

Statistical Analyses: Given the number of infusions/animal was limited to four, it was not possible that every animal receive every dose of MPH. Thus, the effect of intra-PFC infusions on performance (change in % accuracy compared to baseline) was statistically analyzed using a between-subjects 1-way ANOVA. Post-hoc analyses were conducted comparing each dose to vehicle using Dunnett's t-tests. In addition, given systemically-administered psychostimulants improve working memory performance following a non-monotonic dose-response curve (inverted-U) a planned comparison tested the fit of data to a quadratic trend. For muscimol studies and 8.0 μg MPH vs. AECF, independent T-tests were used for analysis.

RESULTS

Cognitive Effects of Intra-PFC MPH Infusion

Given the likely involvement of rat dAcg in working memory performance (Kesner, 2000), we avoided placement of guide cannulae directly into this dorsal-most region of the rat mPFC and utilized small (33ga) infusion needles. As shown in Figure 1, this approach resulted in minimal damage to the mPFC. Vehicle infusions had minimal, non-significant effects on working memory performance (Figure 1; 0.75% ± 2.37% change from baseline, n=13). In contrast, direct infusion of MPH (500 nl) into the dmPFC resulted in an inverted-U-shaped dose-dependent improvement in working memory (Figure 1; $F(1,49)=8.36$, $p<0.01$ for quadratic fit of data). MPH maximally improved accuracy by 10.3% ± 2.7% at the 0.125 μg/hemisphere dose ($p=0.04$; n=14). Both a four-fold lower dose (0.031 μg/hemisphere, n=7) and a four-fold higher dose (0.5 μg/hemisphere, n=10) of MPH produced a non-significant trend for improvement (8.5% ± 3.9%, $p=0.17$; 5.8% ± 4.2%, $p=0.33$, respectively), while the highest dose (2.0 μg/hemisphere, n=10) had no distinguishable effects on performance relative to vehicle treatment (0.42% ± 3.0%, $p=0.84$).

When administered systemically, MPH impairs working memory performance at a dose 4-fold higher than one that elicits maximal cognition enhancement. Thus, it was surprising that when infused into the dmPFC at concentrations that were 4-fold higher (0.5 μg/hemisphere) and 16-fold higher (2.0 μg/hemisphere) MPH lacked cognition-impairing actions. Therefore, in a limited subset of subjects (n=6) we examined the effects of a 64-fold higher dose (8.0 μg/hemisphere) on delayed response performance. Performance of animals treated with this highest dose did not differ significantly from vehicle treatment (5.7% ± 4.8%, $t(17)=1.05$, $p=0.16$).

To assess whether the cognition-enhancing actions of MPH are limited to the dmPFC, additional studies examined the working memory effects of MPH when infused into the infralimbic subfield of the vmPFC. In contrast to that seen with more dorsally placed infusions, infusion of MPH into the vmPFC did not substantially alter performance at any dose examined (See Figure 2; vehicle (n=4) = -4.2 ± 5.3% change from baseline; 0.125 μg (n=7) = 4.4 ± 3.6%, p=0.46; 2.0 μg (n=4) = 6.0 ± 9.2%, p=0.43).

Cognitive Effects of Intra-Striatal MPH Infusion

Additional studies examined the involvement of the dmSTR in working memory performance. We initially identified a region of the dmSTR that receives prominent projections from the dmPFC using iontophoretic infusions of the retrograde tracer, Fluorogold (Fluorochrome, Denver, CO). As shown in Figure 3A, and consistent with prior observations (Mehta et al., 2004), placement of this tracer into the dmSTR resulted in robust labeling of neurons in the dmPFC but not the vmPFC. In contrast, retrograde tracer placement within the dorsolateral striatum resulted in minimal retrograde labeling within the dmPFC and more robust labeling within the vmPFC (data not shown). Subsequent intra-tissue infusions targeted the region of the dmSTR identified in these retrograde tracing studies that receives a prominent projection from the dmPFC.

Although the dmSTR is implicated in higher cognitive function, the degree to which this region contributes to performance in tests of working memory is unknown. Therefore, we next assessed the degree to which temporary inactivation of the dmSTR affects working memory performance using intra-dmSTR infusions of the GABA agonist, muscimol (75 ng/hemisphere). Temporary inactivation of the dmSTR profoundly impaired performance in this test (Figure 3C). Indeed, as shown in Figure 3C, the magnitude of this impairment was comparable to that seen

with mPFC inactivation (dmSTR, vehicle (n=13) = -1.9 ± 2.9% change from baseline; muscimol (n=8) = -48.7 ± 9.1%, t(19)=5.87, $p<0.001$: PFC, vehicle (n=17) = -1.66% ± 2.21%; muscimol (n=6) = -48.2% ± 10.1%, t(21)=6.78, $p< 0.001$).

Finally, additional studies examined whether MPH acts within the dmSTR to improve working memory performance (Figure 3D). For these studies, vehicle (n=13), 0.125 μg MPH (n=6) and 2.0 μg MPH (n=4) were infused into the dmSTR prior to testing. In contrast to that seen in the dmPFC, neither dose of MPH infused into the dmSTR infusion affected working memory performance (vehicle; -1.94% ± 2.92% change from baseline; 0.125 = 3.83% ± 3.46%, p=0.20; 2.0 = 2.47% ± 2.98%, p=0.35).

DISCUSSION

Despite the widespread use of psychostimulants as cognitive enhancers, surprisingly little is known about the neural circuitry involved in their cognitive/therapeutic actions. Although systemic administration of low-dose psychostimulants improves frontostriatal function, whether this reflects direct or indirect actions in the PFC or striatum is unclear. The current studies provide unambiguous evidence that psychostimulant action within the PFC is *sufficient* to promote higher cognitive function as assessed in a delayed-response test of working memory. Moreover, the magnitude of the cognition-enhancing effect of intra-PFC MPH was virtually identical to that seen with systemic administration of clinically-relevant doses of this drug (Arnsten & Dudley, 2005; Berridge et al., 2006; Devilbiss & Berridge, 2008). In contrast, while our results demonstrate the dmSTR is necessary for performance in this test of working memory, MPH infusion into the dmSTR failed to affect performance. These observations indicate a prominent role of select PFC/frontal cortex subfields in the cognition-enhancing and therapeutic actions of psychostimulants and other drugs used in the treatment of ADHD. Additionally, these results are consistent with previous research indicating a prominent role of the PFC/frontal cortex in the pathophysiology of ADHD.

Site of Action within the mPFC

The rat PFC is heterogeneous, with differing subfields associated with distinct cognitive, behavioral, affective, and physiological functions (Kesner, 2000). It has been posited that there exists a dorsal/ventral divide within the rat mPFC, with the dorsal aspects consisting of the dAcg and prelimbic subfields linked to 'cognitive' functions, while the ventrally-situated IL subregion is anatomically and functionally associated with processing of visceral, autonomic and affective

information (Mehta et al., 2004; Vertes, 2004, 2006). The current results are consistent with this proposed functional subdivision of the mPFC: MPH improved working memory performance when infused into the dorsal, but not ventral, mPFC. Additionally, the ability of MPH to improve working memory performance when infused into the dmPFC is consistent with prior lesion studies that implicate this region in egocentric-based motor memory and the temporal sequencing of behavior (Kesner, 2000). The current studies utilized an infusion volume that provides for consistent behavioral effects and is typical of studies that examine the cognitive/behavioral effects of catecholamine-related drugs in rats. However, this volume likely precludes making strong conclusions regarding the degree to which an infusion selectively influences the dAcg vs. dorsal prelimbic subfields of the mPFC. Future mapping studies using smaller infusion volume are needed to address this issue.

These observations are also consistent with functional imaging studies that indicate a dysregulation of the dorsolateral PFC and anterior cingulate in ADHD (Bush et al., 1999; Castellanos & Tannock, 2002; Seidman et al., 2006). Limited evidence suggests the rat dmPFC is functionally homologous to both of these regions in the human/primate (Seamans et al., 2008), Thus, the current results cannot necessarily be extrapolated to identify the degree to which the cognition-enhancing/therapeutic actions of psychostimulants in humans involve actions within the dorsolateral PFC vs. anterior cingulate. Further studies in non-human primates will need to determine the relative role of the anterior cingulate and PFC in the cognition-enhancing actions of psychostimulants.

A variety of cognitive processes affected in ADHD are dependent on the PFC, including working memory, sustained attention, impulsivity, and planning (Arnsten, 2006). The current studies utilized a well-characterized task of PFC-dependent function that requires a variety of cognitive and motivational processes, including working memory, attention, motivation and

response outcome evaluation (Arnsten & Dudley, 2005; Berridge et al., 2006; Devilbiss & Berridge, 2008). Given the pharmacology of performance in this task is closely aligned with the pharmacology of ADHD, our current results likely extend to the therapeutic actions of psychostimulants (Berridge et al., 2006; Kuczenski & Segal, 2002). This close alignment between the pharmacology of working memory performance and ADHD contrasts with that seen in other tests used to assess PFC-dependent function in animals, including sustained attention and attentional set-shifting (Berridge et al., 2012). Nonetheless, it will be of interest for future studies to examine the actions of intra-PFC infusions of MPH in additional tests of cognitive processes known to be affected in ADHD.

Both the magnitude and general inverted-U shaped dose-dependent actions of intra-PFC MPH-induced improvement in working memory performance are comparable to that seen with systemic administration of clinically-relevant doses of MPH (Arnsten & Dudley, 2005; Berridge et al., 2006; Devilbiss & Berridge, 2008). However, while systemically administered MPH *impairs* working memory performance when administered at a dose 4-fold higher than an optimally-improving dose (Devilbiss & Berridge, 2008), intra-PFC infusion of MPH at doses 16-fold (2 μg/hemisphere) and 64-fold (8 μg/hemisphere; data not shown) greater than the maximally-facilitating dose (0.125 μg/hemisphere) failed to hinder performance. These observations indicate that although psychostimulants act within the dmPFC to facilitate PFC-dependent cognition, the impairment of PFC-dependent function associated with higher doses of these drugs likely involves either actions outside the dmPFC or vmPFC or requires concurrent activity in these medial PFC subfields **and** regions outside the PFC. The hippocampus, medial-dorsal thalamus, and striatum are all implicated in higher cognitive processing (Vertes, 2006), including working memory (Floresco et al., 1999a; Wang & Cai, 2006). Thus, these regions

would be of particular interest in future studies exploring the neurocircuitry underlying the cognition-impairing actions of higher doses of psychostimulants.

Potential Receptor Mechanisms

Catecholamines act within the PFC to facilitate performance in tests of working memory in an inverted-U shaped manner (Arnsten & Li, 2005; Robbins & Arnsten, 2009). In the case of DA, this involves inverted-U shaped modulatory actions of DA D1 receptors (Vijayraghavan et al., 2007) For norepinephrine (NE), activation of postsynaptic α_2-receptors promotes, while activation of α_1-receptors impairs, working memory performance (Arnsten et al., 1999, 1996). α_2-receptors possess a higher affinity for NE than α_1-receptors (Arnsten, 2000b). Thus, it has been proposed that under conditions of moderate rates of NE release PFC α_2-receptor activation predominates, facilitating PFC-dependent function, whereas at higher rates of NE release (e.g. stress) α_1-receptors are engaged, impairing PFC-dependent function (Arnsten, 2000b)

Consistent with these behavioral observations, electrophysiological evidence suggests optimal catecholamine modulation is needed for proper PFC neuronal signal processing. For example, in monkeys, PFC α_2-and D1 receptors act in concert to produce optimal spatial tuning of delay-related neurons (Arnsten, 2007; Vijayraghavan et al., 2007). In contrast, stimulation of PFC α_1 receptors reduces neuronal responsiveness and decreases spatial tuning (Birnbaum et al., 2004). Combined, these observations suggest that there is an ideal level of both NE and DA signaling within the PFC that supports optimal signal processing abilities of PFC neurons and PFC-dependent behavior.

Microdialysis studies demonstrate that low and clinically relevant doses of psychostimulants preferentially elevate extracellular NE and DA within the PFC (Berridge et al., 2006). When combined with the current results, this information indicates that cognition-

enhancing actions of low-dose psychostimulants likely involve α_2 and/or D1 receptor activation within the PFC. Consistent with this, the cognition-enhancing effects of systemic MPH are prevented by pretreatment with either an α_2 or D1 receptor antagonist (Arnsten & Dudley, 2005).

Striatal Involvement in 'PFC-Dependent' Function

As reviewed above, evidence suggests that the striatum may be critically involved in both the pathophysiology of ADHD and the therapeutic/cognition-enhancing actions of psychostimulants. Of particular relevance to this discussion, frontostriatal projections are topographically organized, with the dmSTR of the rat receiving direct projections from the dmPFC (Voorn et al., 2004). Additionally, lesion and pharmacological studies suggest the dmSTR acts in concert with the dmPFC to support flexible, goal-directed behavior (Balleine & O'Doherty, 2010; Ragozzino, 2007). Thus, the current studies examined the degree to which the dmSTR is involved in working memory performance. We observed that inactivation of the dmSTR profoundly impaired performance of spatial delayed alternation, providing the first demonstration that the dmSTR is critically involved in performance of this task. Nonetheless, infusion of MPH into the dmSTR had no noticeable effect on performance in this task, indicating that this region does not play a prominent role in the cognition-enhancing or impairing actions of psychostimulants.

The ventromedial striatum (vmSTR), particularly the nucleus accumbens, has been implicated in ADHD as well as the regulation of impulsivity (Cardinal et al., 2001; Scheres et al., 2007; Volkow et al., 2011; Volkow et al., 2009). Functional imaging studies indicate that MPH-induced improvement in certain cognitive/behavioral tasks is associated with alterations in vmSTR activity (Dodds et al., 2008; Vaidya et al., 1998). Additionally, limited observations also indicate the vmSTR is involved in the performance of delayed response tasks (Mair et al., 2002;

Seamans & Phillips, 1994). Indeed, MPH-induced changes in DA receptor occupancy in the ventral striatum predict the magnitude of improvement in a spatial working memory task (Clatworthy et al., 2009). However, it should be noted that although it is not possible to image DA receptor/transporter occupancy in the PFC with current methodology, available evidence indicates there is likely a similar or stronger association between MPH-induced changes in DA/NE receptor occupancy within the PFC and changes in cognition (Berridge et al., 2006). Collectively, these observations indicate that vmSTR function may contribute to the cognitive/therapeutic effects low-dose psychostimulants. However, whether these effects involve direct or indirect actions of psychostimulants on the vmSTR remains to be determined. Future studies will address whether the cognition-enhancing effects of MPH involve actions within the vmSTR.

Conclusion

These results provide the first direct evidence that psychostimulants act within the PFC, but not the dmSTR, to improve PFC-dependent higher cognitive function, an action closely associated with the effective treatment of ADHD. The ability of intra-PFC psychostimulants to improve cognitive function was regionally selective, with infusions into the dorsal, but not the ventral, medial PFC improving PFC-dependent cognition. While the receptor mechanisms that support the cognition-enhancing actions of psychostimulants within the PFC remain to be definitively determined, previous research indicates a likely role of NE α_2 and/or DA D1 receptors. The fact that the PFC is a site of action in the cognition-enhancing actions of psychostimulants is consistent with the posited role of the PFC in the etiology of ADHD and suggests that selective targeting of the PFC may be of particular benefit in the treatment of ADHD and other conditions associated with PFC dysfunction.

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Figures

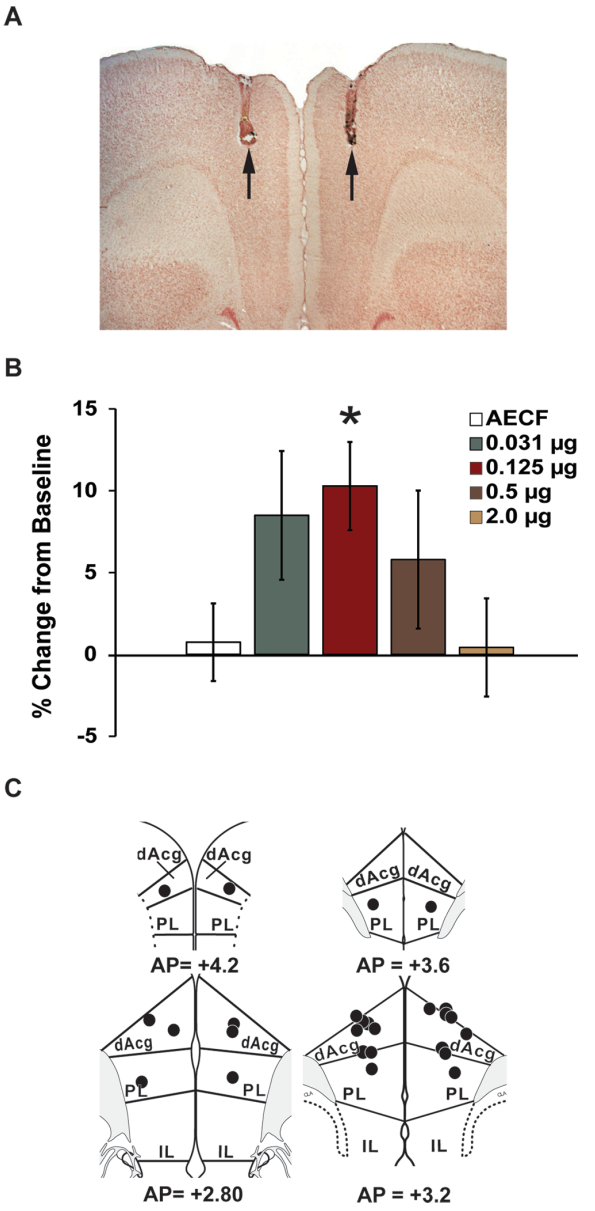

Figure 1. **MPH acts in the dmPFC to improve working memory performance in an inverted-U manner.** (**A**) Representative photomicrograph depicting an infusion site into dorsomedial prefrontal cortex (dmPFC). Note minimal damage to dorsal areas. (**B**) Infusion of MPH into the dmPFC improved working memory performance in an inverted-U dose-dependent manner, with 0.125 µg/hemisphere producing a maximal improvement. (**C**) Schematic diagram indicating all 0.125 µg infusion sites into the dmPFC. dAcg, dorsal anterior cingulate; PL, prelimbic; IL, infralimbic. Numbers represent AP level (Swanson, 1992). $^{*}P < 0.05$ relative to vehicle treatment.

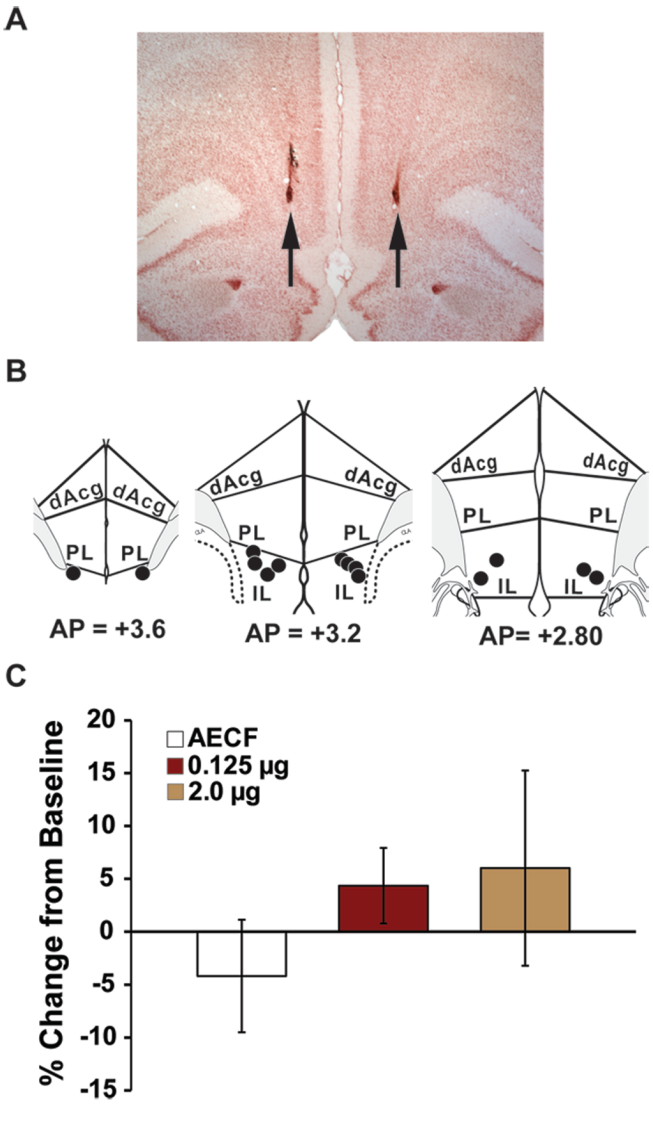

Figure 2. **MPH does not act in the vmPFC to improve working memory performance.** (**A**) Representative photomicrograph indicating site of MPH infusion into the infralimbic subregion of the mPFC. (**B**) Schematic of 0.125µg MPH infralimbic infusion sites. (**C**) Infusion of MPH into the IL PFC had no significant effect on working memory performance measured as the percent change from baseline (mean ± SEM). mPFC, medial prefrontal cortex; dAcg, dorsal anterior cingulate; PL, prelimbic; IL, infralimbic. Numbers represent AP level (Swanson, 1992).

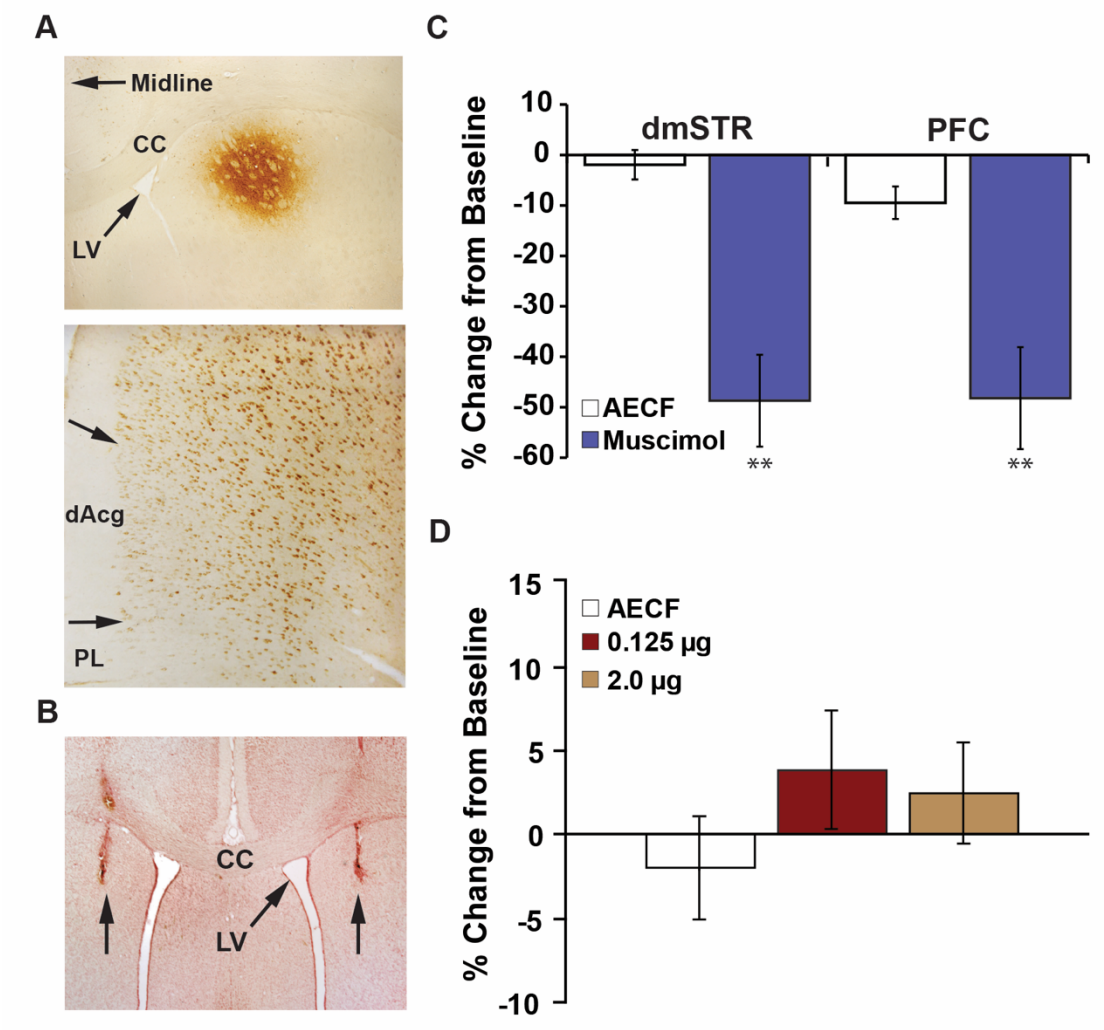

Figure 3. **The dmSTR receives projections from the dmPFC and is necessary for working memory.** (**A**) Fluorogold retrograde tracer infused into dmSTR (top panel) results in strong neuronal labeling of dorsomedial PFC (Top Panel, 40x; Bottom Panel, 200x; A+2.7). (**B**) Representative photomicrograph depicting muscimol/MPH dmSTR infusion sites. (**C**) Intra-dmSTR muscimol significant impaired working memory performance as measured by the percent change from baseline (mean ± SEM). The magnitude of this impairment is comparable to that seen with PFC inactivation, indicating a critical role of the dmSTR in working memory performance. (**D**) Intra-dmSTR infusion of MPH had no significant effect on working memory performance as measured by the percent change from baseline (mean ± SEM). dmSTR, dorsomedial striatum; dAcg, dorsal anterior cingulate; PL, prelimbic; IL, infralimbic. $^{**}P < 0.01$ relative to vehicle (AECF) treatment.

Chapter 3

Receptor and Circuit Mechanisms Underlying Differential Procognitive Actions of Psychostimulants

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Abstract

Psychostimulants, including methylphenidate (MPH), improve cognitive processes dependent on the prefrontal cortex (PFC) and extended frontostriatal circuitry. In both humans and animals, systemic MPH improves certain cognitive processes, such as working memory, in a narrow inverted-U shaped manner. In contrast, other processes, including attention-related, are improved over a broader/right-shifted dose range. The current studies sought to elucidate the potential circuit and receptor mechanisms underlying the divergent dose-dependent procognitive effects of psychostimulants. We first observed that, as with working memory, although sustained attention testing was highly dependent on multiple frontostriatal regions, only MPH infusion into the dorsomedial PFC improved task performance. Importantly, the dose-response curve for this action was right-shifted relative to working memory, as seen with systemic administration. Additional studies examined the receptor mechanisms within the PFC associated with the procognitive actions of MPH across working memory and sustained attention tasks. We observed that PFC α2 and D1 receptors contributed to the beneficial effects of MPH across both cognitive tasks. However, α1 receptors only contributed to MPH-induced improvement in sustained attention. Moreover, activation of PFC α1 receptors was sufficient to improve sustained attention. This latter action contrasts with the impairing actions of PFC α1 receptors reported previously for working memory. These results provide further evidence for a prominent role of the PFC in the pro-cognitive actions of MPH and demonstrate the divergent dose sensitivity across cognitive processes aligns with the differential involvement of PFC α1 receptors.

At low and clinically-relevant doses, psychostimulants, including methylphenidate (MPH, Ritalin), enhance cognition dependent on the prefrontal cortex (PFC) and extended frontostriatal circuitry (Arnsten & Dudley, 2005; Mehta et al., 2000; Spencer et al., 2015). These actions are observed in individuals with attention deficit hyperactivity disorder (ADHD) as well as healthy human and animal subjects (Berridge et al., 2006, 2012; Mehta et al., 2001, 2004). Converging observations indicate that drug action within the PFC contributes to the pro-cognitive effects of psychostimulants (for review, Spencer et al., 2015). This includes the fact that direct infusion of MPH and other ADHD-approved treatments into the PFC of rats and monkeys improves PFC-dependent cognition as measured in tests of working memory (Spencer et al., 2012; Tanila, Rama, & Carlson, 1996; Wang et al., 2007).

Psychostimulants have been typically viewed to *uniformly* affect a diversity of PFC-dependent cognitive processes (Pievsky & McGrath, 2018; Solanto, 2001). However, increasing evidence in humans and animals demonstrates that subsets of PFC-dependent cognitive processes vary in their sensitivity to these drugs. Specifically, working memory and response inhibition display relatively narrow inverted-U dose-dependent facilitation, while overt behavior and different forms of attention are improved across a broader and right-shifted range of doses (Berridge et al., 2012; Sprague & Sleator, 1977; Tannock et al., 1989). To date, the neural mechanisms responsible for the differential sensitivity of PFC-dependent cognitive processes to psychostimulants are unclear, representing a critical weakness in our understanding of this clinically important class of drugs.

The PFC extends topographically-organized projections to the striatum, forming functional frontostriatal circuits (Sesack et al., 1989; Voorn et al., 2004). In rodents, the dorsomedial PFC (dmPFC), encompassing the dorsal anterior cingulate and dorsal prelimbic PFC, and dorsomedial striatum (dmSTR) are strongly implicated in higher cognitive function (Ragozzino, 2007). Consistent with this, prior studies demonstrate that, in rats, working memory performance is highly dependent on the dmPFC and the dmSTR as well as the ventromedial striatum (vmSTR; Seamans & Phillips, 1994; Spencer et al. 2012, 2015). Relative to low and

clinically-relevant doses, higher doses of psychostimulants elicit larger and more uniform elevations in norepinephrine (NE) and dopamine (DA) throughout the brain (Berridge et al., 2006; Kodama et al., 2017; Kuczenski & Segal, 1997). Thus, differences in sensitivity to psychostimulants across cognitive processes may reflect broader actions of higher doses across frontostriatal circuitry. To test this, the current studies first examined the degree to which MPH acts within distinct PFC and striatal subfields to improve sustained attention, a form of focused attention previously demonstrated to display a right-shifted dose sensitivity to MPH (Berridge et al., 2012). Similar to that seen with working memory (Spencer et al., 2012), we observed that while multiple frontostriatal regions support sustained attention, only MPH infusion into the dmPFC improved this cognitive process. Importantly, as with systemic administration, the dose dependency of this action was right-shifted relative to that seen previously with working memory (Spencer et al., 2012). Combined, these observations indicate the differences in dose sensitivity across PFC-dependent cognitive processes to MPH arise from mechanisms intrinsic to the PFC.

One such mechanism could involve differential actions of PFC catecholamine receptors across tasks. Within the PFC, catecholamines exert non-monotonic modulatory actions on cognition that are both receptor subtype- and cognitive process-dependent. Thus, in the case of working memory, PFC DA D1 receptors elicit an inverted-U shaped modulatory action, with both low and high rates of activation associated with impairment (Vijayraghavan et al., 2007; Zahrt et al., 1997). PFC NE also exerts an inverted-U modulation of working memory. However, in this case the beneficial actions are mediated by high-affinity $\alpha 2$ receptors, while the impairing actions of high rates of NE release involve activation of lower affinity $\alpha 1$ receptors, (Arnsten & Pliszka, 2011). Consistent with this, the working memory enhancing actions of MPH are prevented by systemically administered $\alpha 2$ and D1 antagonists (Arnsten & Dudley, 2005). In contrast to that seen with working memory, activation of PFC $\alpha 1$ receptors improves *flexible attention* as measured in an attention set shifting task (Lapiz & Morilak, 2006). Based on these and other observations, it was initially proposed that high rates of NE release (e.g. stress) in the PFC promote flexible attention *at the expense* of focused attention via $\alpha 1$ receptor

activation (Aston-Jones, Rajkowski, & Cohen, 1999; Aston-Jones et al., 2000; Lapiz & Morilak, 2006). However, subsequent observations indicated that *both* flexible and focused attention display similar right-shifted dose sensitivities to MPH (Berridge et al., 2012). Moreover, MPH-induced improvement in focused attention is prevented by systemic administration of an α1 antagonist (Berridge et al., 2012).

Collectively, these observations suggest that the procognitive actions of higher doses of MPH involve PFC α1 receptors. To test this, and to better understand PFC catecholamine receptor mechanisms regulating focused attention, additional studies examined the degree to which intra-PFC MPH-induced improvement in working memory and sustained attention is dependent on local α1, as well as α2 and D1, receptors. We observed that PFC α1 receptors are necessary for MPH-induced improvement in sustained attention, but not working memory. Moreover, activation of PFC α1 receptors was sufficient to improve sustained (focused) attention, identical to that seen previously for flexible attention (Lapiz & Morilak, 2006). In contrast to the task-selective involvement of PFC α1 receptors, both α2 and D1 receptors in the PFC are necessary for the procognitive effects of MPH in both tasks.

These studies demonstrate a central role of the PFC in the procognitive actions of psychostimulants beyond those assessed in tests of working memory. Additionally, these studies further our understanding of the receptor mechanisms underlying the diverse procognitive actions of this widely used class of drugs as well as the neurobiology of PFC-dependent cognition.

Methods and Materials

Animals: Male Sprague-Dawley rats (260-280 grams; Charles River, Wilmington, MA) were pair-housed in polycarbonate cages on a 13-11-hour light/dark cycle (lights on 06:00). Animals were fed *ad libitum* for 7 days and subsequently restricted to 15-17 grams of food/day available at 16:00. Testing/training was conducted between 09:00-16:00 hours at the same time each day, ±1-hour. Animals were weighed twice weekly and handled extensively prior to testing. All facilities and procedures were in accordance with National Institutes of Health (USA) guidelines and approved by the Institutional Animal Care and Use Committee.

Surgery: Following training (see below), rats were anesthetized with isoflurane. Twenty-five ga. stainless steel cannulae were stereotaxically (flat skull) implanted bilaterally over the dmPFC (i.e. dorsal anterior cingulate/dorsal prelimbic PFC; A+3.0; L±0.8; V-0.2 mm below dura), vmPFC (ventral prelimbic/infralimbic PFC; same coordinates, longer needles), dmSTR (A+0.45; L±2.0; V-3.2 mm), or vmSTR (A+1.6; L±1.5; V-3.6 mm) and secured with stainless steel screws and acrylic cement (Plastics One, Roanoke, VA). Stainless steel stylets prevented occlusion of the cannulae.

Sustained Attention: Animals were trained and tested in an operant-based signal detection test of sustained attention, as previously described (Berridge et al., 2006, 2012). Briefly, on half of 100 discrete trials (selected at random, p=0.5) an LED was illuminated and two levers were projected into the chamber ("signal trials"). The signal length was variable, randomly selected from the following list: 0.125, 0.25, 0.375, 0.5, 0.625, 0.75, 0.875, 1.0-seconds, with replacement. On the other half of trials, no signal occurred, after which both levers were inserted ("no signal trials"). Levers remained in the chamber until a response was made, at which time they were retracted. On signal trials, a right lever press was scored as a "hit" and reinforced with sucrose (45 mg; Bio-Serv, Frenchtown, New Jersey) and a left lever press was scored as a "miss." On a no-signal

trial, a right lever press was scored as a "false alarm" and a left lever press was reinforced ("correct rejection"). For correct response trials, house lights were illuminated for 5-seconds. For incorrect trials, levers were retracted followed by a 5-second time-out (houselights off). Failure to respond within 5-seconds of lever insertion triggered lever retraction and a 5-second time-out. There was a variable inter-trial interval of 13-seconds on average (minimum 5-second). Trials with no response occurred infrequently and were excluded from analyses. Animals were trained until performance reached 70% to 85%. Dependent measures included the proportion of trials with a correct response (proportion of hits + proportion of correct rejection), probability of a hit (correct responses/number of signal trials), probability of a false alarm (correct responses/number of no-signal trials), and d', a relative measure of stimulus detection ability. d' = Z(N) - Z(S). Z(N) = Z score of the Noise Distribution = Z score of (1-probability of false alarms). Z(S) = Z score of the Signal = Z score of (1-probability of a hit) (Gescheider, 2013).

Sustained attention testing involved the use of several separate cohorts with treatments spread among the different cohorts. Due to variations in performance between cohorts, all treatment effects were measured against vehicle treatment. In most cohorts treatments were replicated within animals.

Working Memory: Prior to surgery, animals were trained in a T-maze (opaque black Plexiglas) with 10 trials/session, as described previously (Devilbiss et al., 2012, 2016). Animals were rewarded (~58 mg chocolate chip or 45 mg sucrose pellet) when they entered the maze arm not chosen on the previous trial. Between trials the animal was placed in a start box at the base of the maze with removable Plexiglas gate, with a zero-delay. Spatial cues were minimized with black plastic sheeting. Animal waste was removed by a dry tissue between trials and cleaned with 50% ethanol between animals. Treatments were counter-balanced within and across animals.

Following surgery, rats were retrained with 20 trials/session at zero-delay until pre-surgery levels. Delay intervals were then introduced that resulted in 65%-80% accuracy (5-120 seconds; mean = 42 ± 36-seconds). Stable pre-treatment performance (baseline) was defined as 2 consecutive days in which performance did not differ greater than 10%. In order to ensure stable performance, rats were run at the same delay for two days following treatment. Data were included in the analyses only when baseline and post-treatment performance did not differ by more than 10%.

Drug Infusion: MPH HCl (0.03µg , 0.125µg, 0.5µg, 2.0µg /500nl), atimpamezole (1.25 µg or 0.625 µg/500nl), SCH23390 (0.5µg/500nl), benoxathian (0.4µg/500 nl), or muscimol (75ng/500nl) were obtained from Sigma-Aldrich (St. Louis, MO), and dissolved in buffered artificial extracellular fluid (147 mMol NaCl, 1.3 mMol CaCl2, 0.9 mMol MgCl2, 2.5 mMol KCl; pH=7.4). Bilateral 500nl infusions of drug/vehicle were made at a rate of 250nl/minute using 33 ga. needles that projected below the guide cannulae (1.6–2.0 mm, dmPFC; 4 mm, vmPFC; 2.5 mm, dmSTR; 4 mm vmSTR). Needles remained in tissue for 2-minutes following infusions, after which stylets were replaced. Prior to the first treatment, rats received two mock infusions, consisting of an initial needle insertion and 48-hours later a vehicle infusion. Rats were placed in their home cage for 15-minutes following infusions. All treatments were separated by at least two days. To maintain tissue integrity and minimize infection, the number of treatments was limited to 10/animal.

Histological Analyses and Data Selection: Following testing, rats were deeply anesthetized and transcardially perfused with 3.7% w/v formaldehyde. Brains were stored in formaldehyde for at least 24-hours. Injector placement was verified in 40-µm thick, Neutral Red stained coronal sections. Data from a given experiment were included only when histological analyses verified accurate placement of injectors and a minimum of tissue damage.

Statistical Analyses: Given limits on the number of infusions, as well as limits on working memory delay length, it was not possible for all animals to receive all doses. Therefore, results were analyzed using mixed linear models in JMP Pro 12 with treatment as a fixed effect and subjects as a random effect. Sustained attention testing was run with several separate cohorts. Because there are modest variations in performance within and between cohorts, all sustained attention treatment effects were calculated relative to vehicle in a within-subjects design. In the vast majority of cases, treatments were replicated in the same animal. Muscimol effects were modeled with region (dmPFC, dmSTR, vmSTR), treatment (vehicle, muscimol) and treatment x region as fixed effects and planned comparisons were corrected for multiple comparisons with holm-bonferroni correction. To assess the regional and dose-dependent actions of MPH on sustained attention, we used separate models for each region. Two additional models were used for the sustained attention and working memory antagonist studies. For all models, subjects were treated as a random variable. Outside of muscimol testing, all planned comparisons to vehicle treatment were conducted using Dunnett's t-tests.

Results

Frontostriatal circuitry associated with MPH-induced improvement in sustained attention

Dependency of sustained attention on frontostriatal circuitry. In contrast to working memory, the frontostriatal neurocircuitry supporting sustained attention is poorly understood. Thus, we first examined the degree to which sustained attention is dependent on different frontostriatal regions using reversible inactivation via microinfusion of the GABA agonist, muscimol. Muscimol had a significant effect on performance ($F_{1,28.9}$=86.0, p<0.0001). Inactivation of the dmPFC (n=10; vehicle n=10) and dmSTR (n=7; vehicle n=7) reduced performance to chance levels, as assessed by a change in d' from vehicle, as well as other measures (Figure 1, Table 1; dmPFC, $t_{29=}$ 5.46; p<0.0001; d', dmSTR, t_{29}=-3.19; p=0.04). Inactivation of the vmSTR (n=8; vehicle n=8) produced a larger impairment in sustained attention (t_{29}=7.74; p<0.0001; Figure 1, Table I). However, in contrast to that seen with dmPFC or dmSTR inactivation, this reflected the fact that animals stopped responding in the presence of errors, potentially reflecting diminished motivation to engage in the task, rather than cognitive impairment *per se*.

Intra-PFC MPH infusions. MPH acts in the dmPFC, but not the vmPFC, to improve working memory in an inverted-U manner (Devilbiss et al., 2012, 2016). To test whether MPH acts directly in the dmPFC or vmPFC to modulate sustained attention, animals received infusions of vehicle or varying doses of MPH into the either region. As shown in Figure 2 and Table S2, infusion of MPH (500nl; 0.03µg n=6; 0.125µg n=8 ; 0.5µg, n=6; 2.0µg, n=13 ; vehicle, n=21, due to multiple cohorts) into the dmPFC elicited an inverted U-shaped improvement in sustained attention as measured by d' ($F_{4,66.8}$=4.15, p<0.01), with maximal improvement observed at the 0.5 µg/hemisphere dose (p=0.02) relative to vehicle. As observed with systemic administration (see Supplemental Figure S1), this inverted-U dose-response curve is right-shifted relative to that seen previously with intra-PFC MPH-induced improvements in working memory (Spencer et al., 2012). MPH infusion into the vmPFC had no significant effects on d' ($F_{4,24}$=1.81, p=0.16; see Figure 3, Supplemental Table S3; 0.03µg, n=7, p=1.0; 0.125µg, n=7, p=0.98; 0.5µg, n=7, p=1.0;

2.0μg, *n*=7, *p*=0.10; vehicle, *n*=7). The one exception to this was a trend for improvement with the highest dose of MPH, likely reflecting diffusion of the drug into the dmPFC at a lower concentration.

Intra-striatal MPH infusions. Additional studies examined the sustained attention effects of MPH within the dmSTR and vmSTR. As shown in Figure 3, similar to that observed previously for working memory (Spencer et al., 2012), neither MPH infusion into the dmSTR or vmSTR had a significant effect on sustained attention as measured by a change in d' from vehicle treatment (see also Supplemental Table S3; **dmSTR**: $F_{4,68.3}$=1.32, *p*=0.27; 0.03μg, *n*=7, p=1.0; 0.125 μg, *n*=12, *p*=0.71; 0.5 μg, *n*=11, *p*=0.87; 2.0 μg, *n*=12, *p*=0.35; vehicle, *n*=29, due to multiple cohorts; v**mSTR**: $F_{3,20.3}$=0.13, *p*=0.94; 0.125 μg, *n*=8, *p*=0.93; 0.5 μg, *n*=8, *p*=0.99; 2.0 μg, *n*=8, *p*=0.90; vehicle *n*=8).

Receptor Mechanisms underlying beneficial actions of MPH on Working Memory vs. Sustained attention

Additional studies examined if the difference in dose sensitivity across tasks reflects differences in PFC catecholamine receptor mechanisms. Animals received either intra-dmPFC infusions of vehicle, MPH, the α1 antagonist, benoxathian, α2 antagonist, atipamezole, or the D1 antagonist, SCH23390, either alone or with MPH. Animals were then tested in our working memory or sustained attention tasks. The dose of MPH used was the maximally-improving dose for each task (see Figure 2, Berridge et al., 2012). Doses of the antagonists were based on prior published work (Bondi et al., 2010; Granon et al., 2000; Tanila et al., 1996) as well as extensive pilot studies that identified doses subthreshold for altering performance (data not shown).

Working Memory. Animals received intra-PFC infusions of vehicle (*n*=13), 0.125μg MPH (*n*=11), MPH + α1 antagonist, (0.4 μg, *n*=11), MPH + α2 antagonist (1.25 μg, *n*=10), or MPH + D1 antagonist, (0.5 μg, *n*=9). As shown in Figure 4, there was an overall effect of treatment

(F_{85}=2.3, p=0.03) with MPH infusion into the dmPFC significantly improving performance relative to vehicle (t_{85}=2.99, p=0.02). When infused into the dmPFC alone, neither the α1 antagonist (n=9, t_{85}=0.97, p=0.89), α2 antagonist (n=10, t_{85}=0.75, p=0.97), nor the D1 antagonist (n=12, t_{85}=0.33, p=0.97), significantly affected performance. When co-infused with MPH, at a dose previously shown to block NE-dependent improvement in attention set shifting (Lapiz & Morilak, 2006), the α1 antagonist had no effect on MPH-induced improvement in working memory (t_{85}=2.75, p=0.04). In contrast, the α2 antagonist (t_{85} = 0.73, p=0.97) and the D1 antagonist (t_{85}=0.73, p=0.97) completely blocked the cognition-enhancing actions of intra-PFC MPH.

Sustained Attention. Animals received intra-PFC infusions of vehicle (n=45), 0.5μg MPH (n=21), MPH + α1 antagonist (0.5μg, n=16), MPH + α2 antagonist (0.625 μg, n=16), or MPH + D1 antagonist (0.5 μg, n=15). As shown in Figure 4 and Supplemental Table S3, there was an overall effect of treatment ($F_{282.3}$=3.87, p=0.0002), with infusion of 0.5μg MPH into the dmPFC significantly improving performance relative to vehicle ($t_{282.3}$=2.9, p=0.03). When infused into the dmPFC alone, no significant effects on performance were observed for any of the antagonists (α1, n=20, $t_{282.3}$=-0.22, p=1.0; α2, n=14, $t_{282.3}$=0.93, p=0.96; D1, n=20, $t_{282.3}$=-0.86, p=0.98). In contrast to that seen with working memory, α1 receptor blockade prevented the sustained attention improving action of MPH ($t_{282.3}$=1.42, p=0.72). Similar to that seen with working memory, MPH-induced improvement in sustained attention was prevented by dmPFC α2 ($t_{282.3}$=0.02, p=1.0) and D1 antagonists ($t_{282.3}$=0.35, p=1.0).

α-1 receptor stimulation in the dmPFC improves sustained attention. These latter observations suggest that α1 receptors in the PFC facilitate sustained attention. To directly test this, we examined the effects of intra-PFC infusion of the α1-agonist, phenylephrine, on sustained attention at a dose previously shown to impair working memory (Arnsten et al., 1999). As shown in Figure 4 and Supplemental Table S4, activation of dmPFC α1 receptors (n=13) improved sustained attention relative to vehicle treatment as measured by d' ($t_{282.3}$=3.68, p=0.002), indistinguishable from that of MPH ($t_{282.3}$=-0.91, p=0.36).

Discussion

Psychostimulants are the most effective and widely used treatment for ADHD, reversing core frontostriatal cognitive deficits associated with this disorder (Mehta et al., 2001; Rubia et al., 2011; Vaidya et al., 1998). Prior observations demonstrate a subset of PFC-dependent processes display a narrow inverted-U dose sensitivity to MPH (e.g. working memory, response inhibition), while others display a right-shifted dose sensitivity (flexible attention, focused attention, behavioral calming) (Berridge et al., 2012; Sprague & Sleator, 1977; Tannock et al., 1989; Tannock et al., 1995). Prior to the current studies, the mechanisms responsible for this differential sensitivity of PFC-dependent cognitive processes to psychostimulants had been unexplored, representing in a critical gap in our understanding of this widely used class of drugs. To initially address this, the current studies examined whether differences in dose sensitivity to MPH across tests of working memory vs. sustained attention arise from varying circuit and/or receptor mechanisms.

These studies provide the first demonstration that sustained attention is dependent on multiple frontostriatal regions, similar to that reported previously for working memory (Spencer et al., 2012). Nonetheless, as with working memory (Spencer et al., 2012), MPH only improved sustained attention when infused into the dmPFC, and not the vmPFC, dmSTR or vmSTR. Importantly, the dose response curve for this action was right-shifted relative to that previously seen with intra-dmPFC MPH in working memory (Spencer et al., 2012). This difference in dose sensitivity across tasks is identical to that seen with systemic administration (Berridge et al., 2012). Thus, regardless of route of administration, sustained attention is maximally improved at a dose 4-fold higher than the maximally working memory-improving dose. Additionally, regardless of route of administration, at this higher dose, working memory is no longer improved (Figure 2; Supplemental Figure 1; Berridge et al., 2012; Spencer et al., 2012). These observations demonstrate the differential dose-sensitivity of these cognitive processes to MPH involves, at least in part, mechanisms contained within the PFC.

Additional studies provided the first examination of the PFC receptor mechanisms underlying the procognitive actions of MPH across working memory and sustained attention. These studies demonstrate that α2 and D1 receptors within the PFC contribute to the procognitive actions of MPH observed in both tasks. In contrast, PFC α1 receptors only contribute to MPH-induced improvement in sustained attention. Consistent with this, activation of PFC α1 receptors was sufficient to improve sustained attention. This contrasts with the working memory impairing actions of PFC α1 receptors (Arnsten et al., 1999; Mao et al., 1999). Given α1 receptors display a lower affinity than α2 receptors, this could explain why sustained attention displays a right-shifted dose sensitivity relative to working memory. Collectively, these observations provide new insight into the neurobiology underlying both PFC-dependent cognition and the procognitive actions of psychostimulants used in the treatment of ADHD.

The PFC and procognitive actions of psychostimulants.

The current study demonstrates that both sustained attention and working memory depend on multiple nodes of frontostriatal circuitry (Spencer et al., 2012). This is consistent with the fact that ADHD is associated with frontostriatal dysfunction (Casey et al., 2007; Cubillo et al., 2012; Liston et al., 2011) and that clinically relevant doses of MPH increase catecholamine signaling broadly within this circuit (Berridge et al., 2006; Kodama et al., 2017). Nonetheless, only MPH action in the dmPFC, and not the vmPFC, dmSTR or vmSTR, is *sufficient* to improve both sustained attention and working memory (Spencer et al., 2012). The preferential sensitivity of the dmPFC vs. the vmPFC in the procognitive actions of MPH is consistent with a well-known functional topographical organization of the rodent medial PFC, with dorsal regions more closely associated with 'executive' cognitive processes (Vertes, 2004, 2006).

The two other ADHD-approved medications, α2 agonists and selective NE reuptake inhibitors, have been demonstrated to also act in the dorsal PFC of primates and rodents to improve working memory (Gamo et al., 2010; Mao et al., 1999; Tanila et al., 1996; Wang et al., 2007). Combined, these observations demonstrate a prominent role of the PFC in the

procognitive actions of all approved treatments for ADHD. Nonetheless, this does not rule out a role of the striatum in the therapeutic effects of psychostimulants. Given clinically-relevant doses of psychostimulants elevate striatal DA neurotransmission (Berridge et al., 2006; Kodama et al., 2017) it is possible that, when combined with drug action in the PFC, drug action in the striatum may contribute to greater efficacy of psychostimulants in ADHD relative to noradrenergic-selective treatments (Kolar et al., 2008).

Receptor mechanisms within the PFC differentially contribute to the procognitive actions of MPH

NE and DA act directly within the PFC to exert inverted-U shaped modulation of working memory (Arnsten & Li, 2005; Robbins & Arnsten, 2009). For NE, this reflects procognitive actions of high affinity postsynaptic α_2-receptors, engaged at lower rates of release, and working memory impairing actions of lower affinity α_1-receptors, engaged at higher rates of release (e.g. stress; Arnsten et al., 1999, 1996; Tanila et al., 1996). For DA, moderate D1 receptor activation promotes, while higher rates of activation impair working memory performance (Vijayraghavan et al., 2007). Consistent with these observations, the current studies demonstrate that *within the PFC*, α2 and D1, but not α1, are necessary for MPH-induced improvement in working memory. In contrast, MPH-induced improvement in sustained attention was dependent on all three receptor subtypes.

Prior studies demonstrate that activation of PFC α1 receptors promote a flexible form of attention as measured in an attention set shifting task (Lapiz & Morilak, 2006). Based on earlier observations, it was posited that PFC α1 receptors promote 'flexible' attention at the expense of focused attention (Aston-Jones et al., 2000). However, in our studies, activation of PFC α1 receptors improved sustained/focused attention, identical to flexible attention (Lapiz & Morilak, 2006). Collectively, these observations indicate that although α1 receptors differentially regulate distinct PFC-dependent cognitive processes, this cannot be ascribed to a *selective* enhancement of attentional/cognitive 'flexibility'. One important difference between tests of working memory

and tests of attention is the need in working memory tasks to actively maintain and protect from distractors information needed to make a subsequent action. Neurophysiologically, α2 and D1 receptors promote sustained activation of PFC neuronal activity during the delay interval of a working memory task, while α1 receptors degrade delay-related activity (Arnsten, 2007). Thus, stimulation of PFC α2/D1 receptors help maintain internally generated representations. Future studies will need to determine if the pro-attentional actions of α1 receptors results from an increased sensitivity to external stimuli, as seen with more posterior cortical and subcortical areas (Arnsten, 2000b) or more generally to increased attention to the environment.

Clinical Implications

The neurocircuitry underlying the therapeutic actions of psychostimulants remains poorly understood. The current observations add to a growing body of evidence that the procognitive actions of ADHD-approved drugs involve (to some extent) direct action in the PFC (Arnsten & Pliszka, 2011; Spencer et al., 2012). This provides important information for future drug discovery targeted at the development of novel treatments for ADHD (for example, Hupalo & Berridge, 2016).

Sprague and Sleator (Sprague & Sleator, 1977) first described a differential dose sensitivity across varying cognitive/behavioral processes to MPH in children with ADHD, an observation later confirmed by Tannock and colleagues (Tannock et al., 1989). Our observations suggest an involvement of PFC α1 receptors in the procognitive actions of moderately higher doses of psychostimulants. It remains to be determined whether the facilitation of these α1 sensitive processes contribute to the beneficial (behavioral calming and attention-improving) vs. detrimental (cognitive constriction) actions of psychostimulants and whether this differs across ADHD subtype (Diamond, 2005). Lastly, our results demonstrate a role of PFC α1 receptors in the regulation of focused attention, similar to that reported for flexible attention (Lapiz & Morilak, 2006). This may have relevance for the development of novel attention-enhancing compounds.

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Tables

Table 1. Muscimol (75ng/hemisphere) Inactivation Within Frontostriatal Nodes.

Region					
Vehicle	**d'**	**Prop Correct**	**Pr(hit)**	**Pr(FA)**	**No responses**
dmPFC	1.72 ± 0.17	0.77 ± 0.03	0.73 ± 0.05	0.16 ± 0.04	3.90 ± 2.58
dmSTR	1.42 ± 0.22	0.71 ± 0.04	0.55 ± 0.07	0.12 ± 0.05	0.71 ± 3.64
vmSTR	2.12 ± 0.21	0.83 ± 0.04	0.76 ± 0.06	0.10 ± 0.05	0.06 ± 3.40
Muscimol					
dmPFC	**0.65 ± 0.17****	**0.59 ± 0.03****	0.49 ± 0.05	0.28 ± 0.04	2.83 ± 2.58
dmSTR	**0.54 ± 0.22***	0.57 ± 0.04	0.37 ± 0.07	0.23 ± 0.06	1.86 ± 3.64
vmSTR	**0.13 ± 0.21****	**0.28 ± 0.04****	0.46 ± 0.06	0.41 ± 0.05	**44.25 ± 3.41****

The GABA-A agonist, Muscimol (75 ng/hemisphere), was used to inactivate three nodes in the frontostriatal network prior to testing in sustained attention task. Infusion into the dmPFC and dmSTR reduced sustained attention performance to near chance. In contrast vmSTR inactivation produced a more profound impairment, driven by animals no longer performing the task following a series of errors. *$p<0.05$, **$p<0.01$ compared to vehicle treatment.

Figures

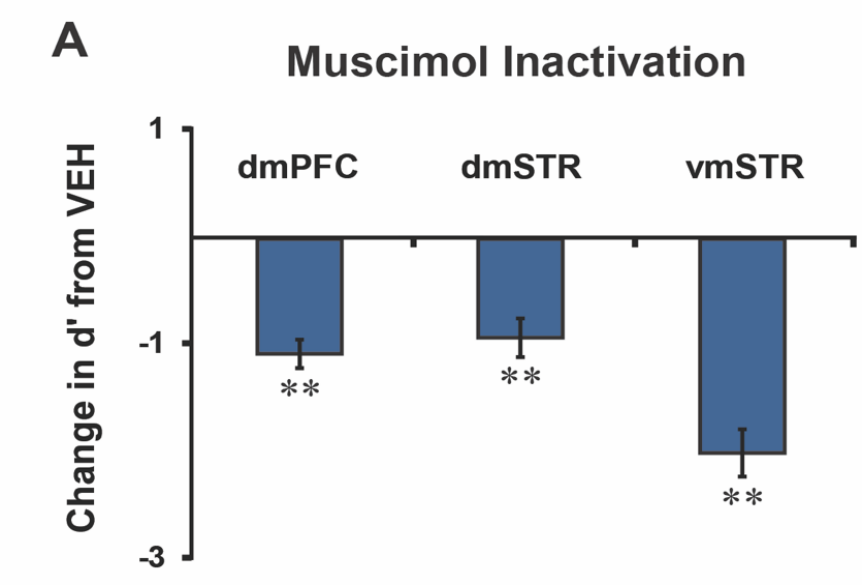


Figure 1. Frontostriatal circuit mechanisms supporting sustained attention. Shown are the effects of muscimol (75 ng/hemisphere) inactivation of the dmPFC, dmSTR or vmSTR on performance in a sustained attention task. Bars represent mean change in d' from vehicle treatment ± SEM . Performance was impaired, as measured by the absolute change in d' sensitivity compared to vehicle, with inactivation of all three regions. For the dmPFC and dmSTR, this resulted in nearly chance levels of performance (see Table I). In contrast vmSTR inactivation produced a more profound impairment, that largely reflects the fact that animals ceased performing the task following a series of errors. **$p<0.01$ compared to vehicle treatment.

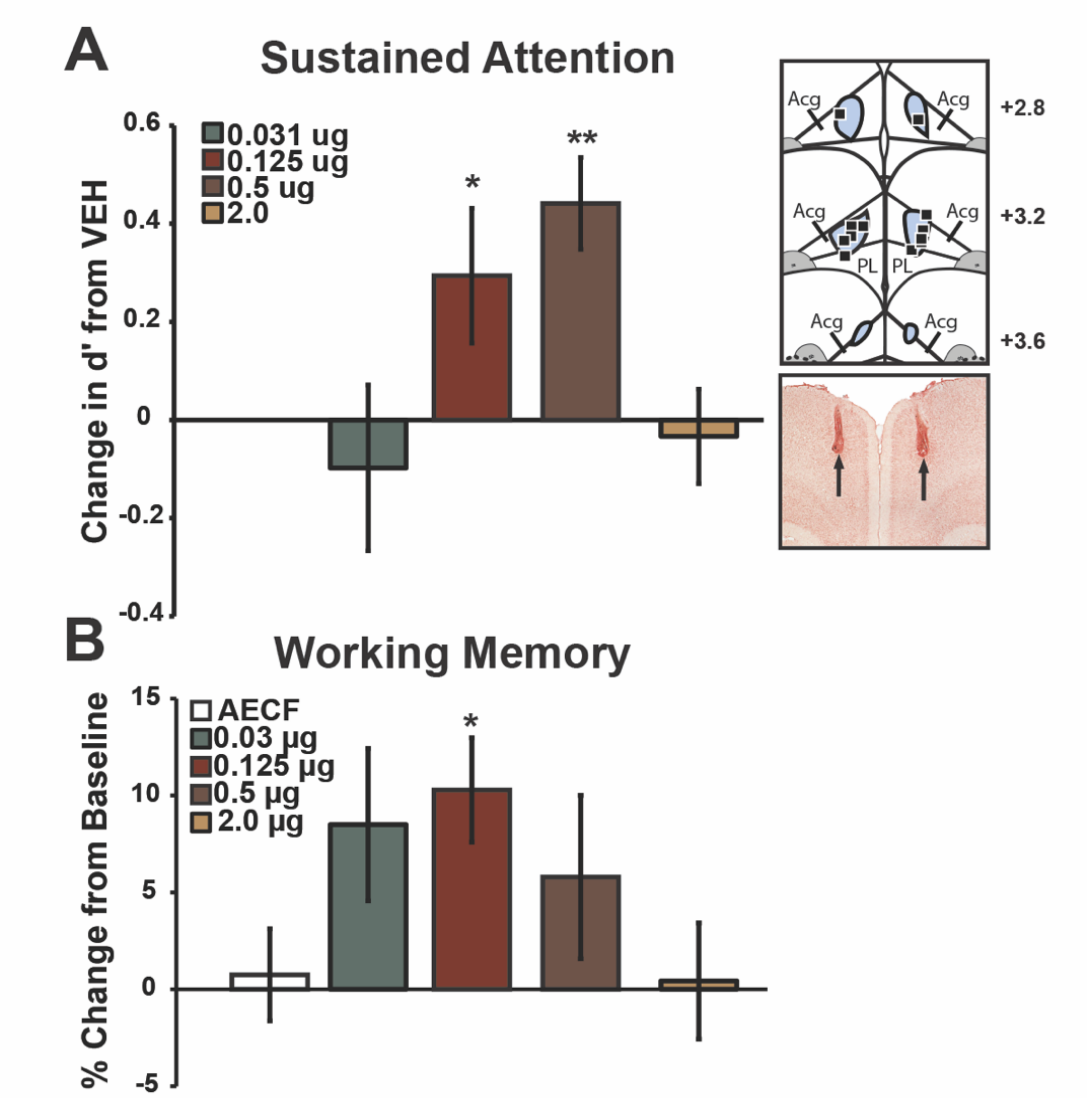


Figure 2. MPH acts in the dmPFC to improve both sustained attention and working memory. (A) *Left*: Infusion of MPH into the dmPFC (dorsal anterior cingulate/ dorsal prelimbic) improves sustained attention in an inverted-U shaped manner, with 0.5 µg producing maximal improvement in d' sensitivity compared to vehicle (mean ± SEM). *Top Right*: Schematic of all infusion sites for the 0.5 µg dose. Numbers represent A/P placement. *Bottom Right*: Representative photomicrograph of MPH infusion site in the dmPFC showing the main body of the needle track. Arrows indicate the ventral most location of the track within the dmPFC. Cannulae are only lowered into the dmPFC ~200 µm, minimizing damage. **(B)** Previously published data regarding the dose-response curve for MPH-induced improvement in working memory (Spencer et al., 2012). When compared with data presented in Panel A, it is clear that sustained attention displays right-shifted dose sensitivity relative to working memory. *P<0.05, **p<0.01 compared to vehicle treatment; Acg, dorsal anterior cingulate; PL, prelimbic.

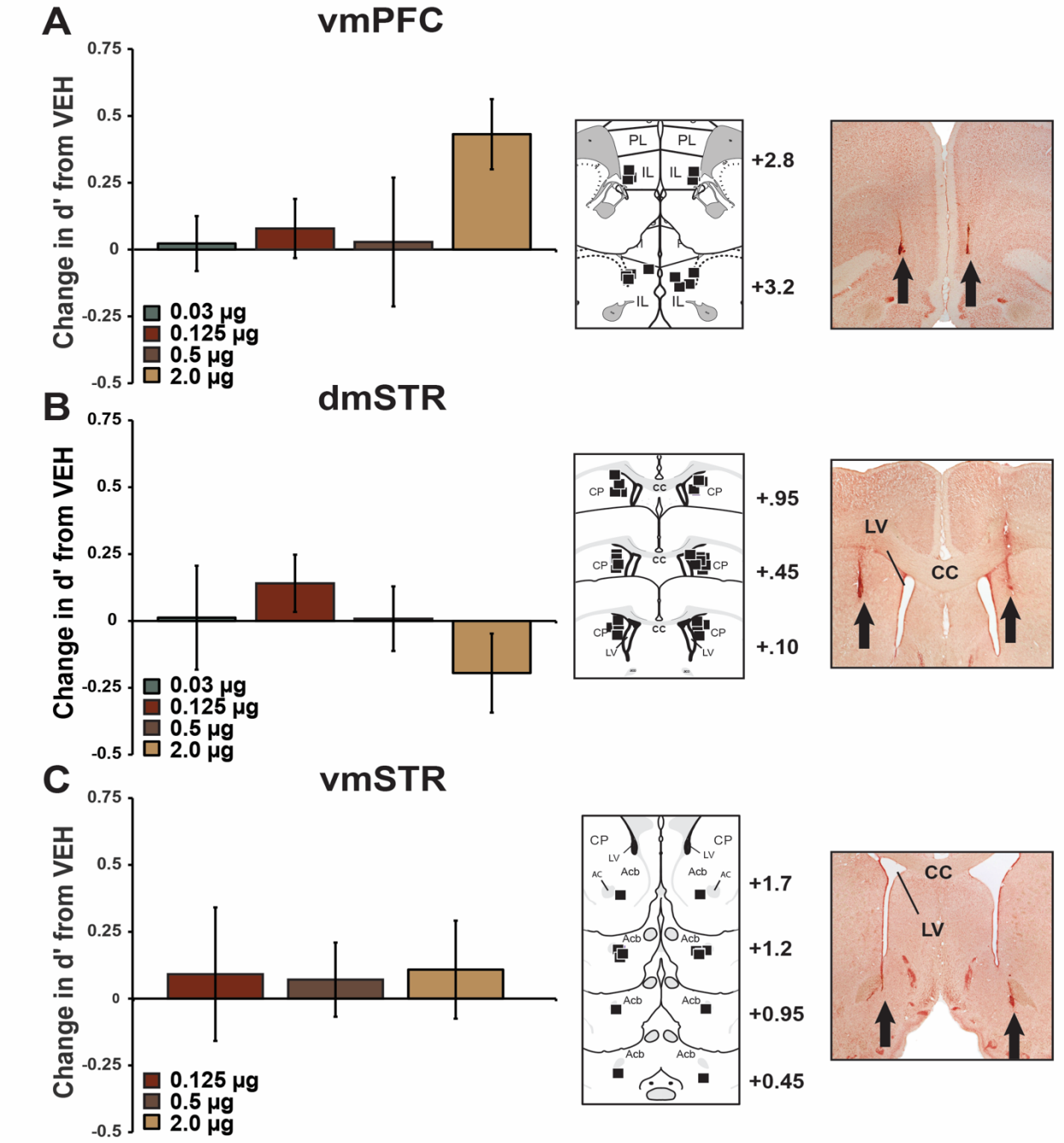


Figure 3. MPH actions outside the dmPFC. Infusion of MPH into the vmPFC **(A)** dmSTR **(B)** or vmSTR **(C)** had no effect on sustained attention as measured by change in d' compared with VEH treatment (mean±SEM). A trend for improvement was seen with the highest dose (2.0 µg) of MPH infused into the vmPFC, which likely reflects diffusion into the dmPFC (dorsal anterior cingulate/ dorsal prelimbic) at a lower concentration. Schematics depict all MPH infusion sites for each region. Numbers represent A/P placement. Photomicrographs depict representative infusion sites for each region. Arrows indicate ventral-most extent of infusion needles. *p<0.05; PL, prelimbic; IL, infralimbic; CP, caudate putamen; CC, corpus callosum; Acb, nucleus accumbens; LV, lateral ventricle

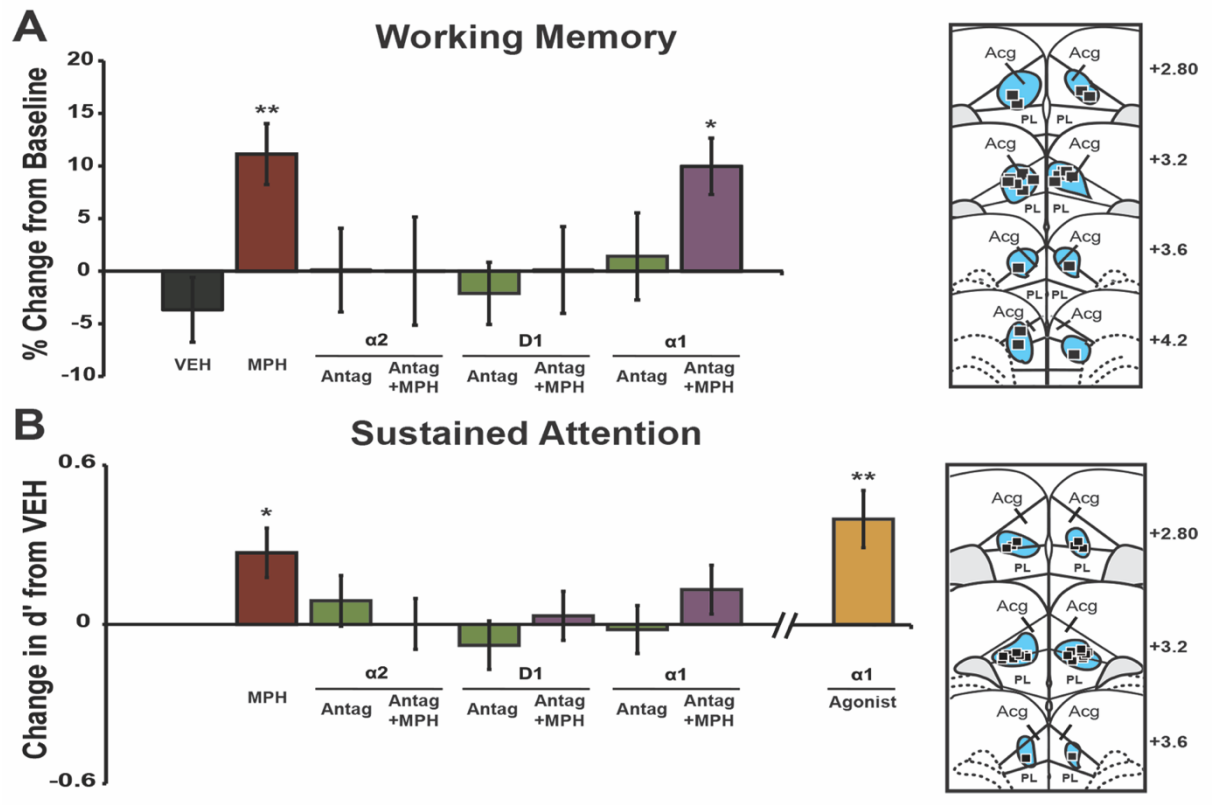
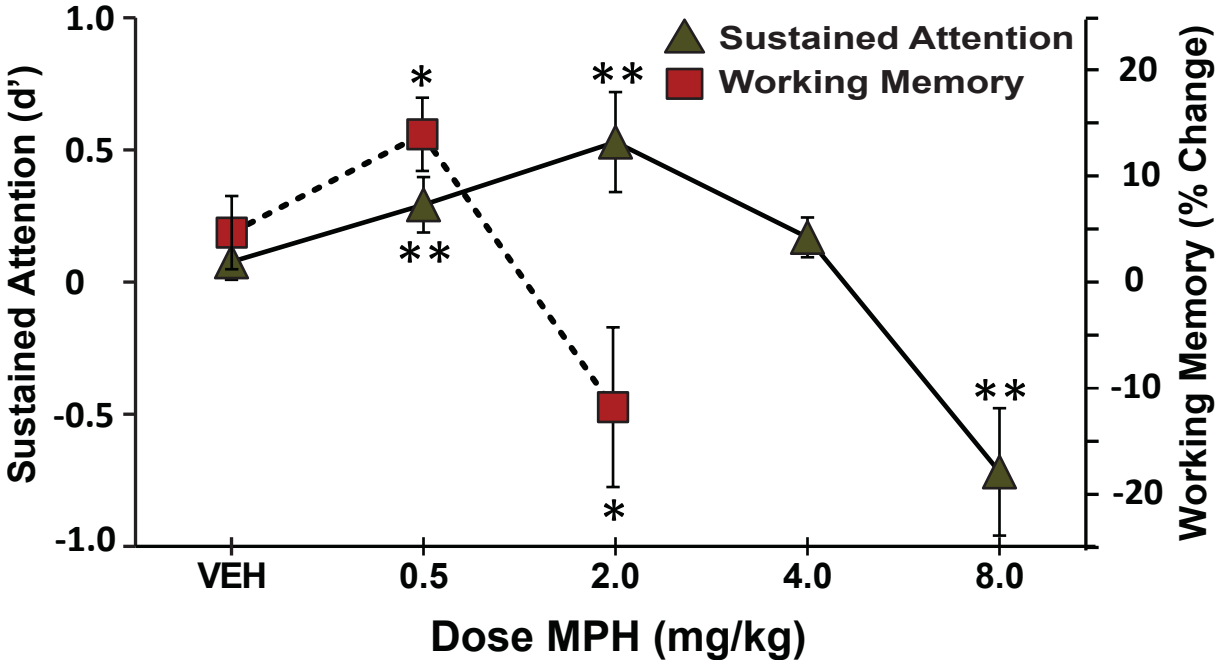


Figure 4. PFC receptor mechanisms underlying the divergent dose response curves of MPH across working memory and sustained attention. (A) The working memory-enhancing actions of intra-dmPFC MPH (0.125 μg) are blocked with co-infusion of the α2 antagonist atipamezole (1.25 μg) or the D1 antagonist SCH23390 (0.5 μg), but not the α1 antagonist benoxathian (0.4 μg) at doses that do not alter performance when given alone as measured by the percent change in performance from baseline. Schematic depicts all infusion sites with MPH (squares). (**B)** Similar to working memory, the sustained attention-enhancing actions of intra-dmPFC MPH (0.5 μg) were blocked with co-infusion of the α2 antagonist atipamezole (0.625 μg) or the D1 antagonist SCH23390 (0.5 μg). However, in contrast to working memory, the α1 antagonist, benoxathian (0.4 μg), also blocked intra-dmPFC MPH-induced improvements in sustained attention. Consistent with this latter observation, intra-dmPFC infusion of a dose of the α1 agonist, phenylephrine (0.1 μg), previously shown to impair working memory (Arnsten et al., 1999; Mao et al., 1999) significantly improved sustained attention, as measured by change in d' from VEH (mean ± SEM). The magnitude of improvement is comparable to that seen with intra dmPFC MPH (0.5 μg). Schematic depicts all infusion sites with MPH depicted (squares). Numbers represent A/P placement *$p<0.05$, **$p<0.01$ compared to vehicle or baseline. Acg, anterior cingulate; PL, prelimbic.

Supplemental Results

Supplemental Figure S1. Divergent dose response curves to *systemic* MPH in working memory and sustained attention.



Systemic MPH improves working memory in a narrow inverted-U shaped manner, whereas sustained attention is improved over a broader/right-shifted dose range showing continued improvement at doses that impair working memory. * P<0.05, ** p<.01; Data from (Berridge et al., 2012)

Supplemental Table S2. Dose Response of MPH infused into the dmPFC.

Drug	d'	Prop Correct	Pr(hit)	Pr(FA)	No responses
0.031µg MPH	0.138 ± 0.143	-0.022 ± 0.019	-0.029 ± 0.035	0.002 ± 0.027	-0.341 ± 0.357
0.125 µg MPH	**0.334 ± 0.127***	0.030 ± 0.016	0.043 ± 0.040	-0.029 ± 0.024	-0.344 ± 0.316
0.5 µg MPH	**0.406 ± 0.145***	0.026± 0.017	0.036 ± 0.035	-0.045 ± 0.027	-0.057 ± 0.358
2.0 µg MPH	0.034 ± 0.124	0.003 ± 0.016	0.013 ± 0.030	0.012 ± 0.023	-0.342 ± 0.324

The effect of various doses of MPH infused bilaterally into the dorsomedial PFC on change in performance from vehicle in a sustained attention task. Sustained attention performance was significantly elevated at a dose (0.125 µg MPH) that maximally enhanced working memory (R. C. Spencer et al., 2012). However, maximal improvement as measured by the sensitivity index d', was seen at a 4 fold higher dose (0.5 µg MPH), a dose that no longer improves working memory (R. C. Spencer et al., 2012). * $p<0.5$ compared to vehicle treatment.

Supplemental Table S3. Dose Response of MPH infused into the vmPFC, dmSTR, or vmSTR.

vmPFC	d'	Prop Correct	Pr(hit)	Pr(FA)	No responses
0.031 µg MPH	0.023 ± 0.186	-0.005 ± 0.029	0.001 ± 0.058	-0.004 ± 0.037	0.143 ± 0.458
0.125 µg MPH	0.079 ± 0.186	0.016 ± 0.029	0.054 ± 0.058	0.020 ± 0.037	0.429 ± 0.458
0.5 µg MPH	0.028 ± 0.186	-0.004 ± 0.029	-0.041 ± 0.058	-0.022 ± 0.037	0.429 ± 0.458
2.0 µg MPH	0.431 ± 0.186	0.048 ± 0.029	0.111 ± 0.058	-0.017 ± 0.037	0.429 ± 0.458
dmSTR					
0.031 µg MPH	-0.028 ± 0.180	-0.004 ± 0.027	0.021 ± 0.044	0.015 ± 0.032	0.000 ± 1.051
0.125 µg MPH	0.126 ± 0.128	0.012 ± 0.019	0.029 ± 0.031	0.002 ± 0.023	-0.785 ± 0.767
0.5 µg MPH	-0.099 ± 0.134	-0.021 ± 0.020	0.028 ± 0.033	0.050 ± 0.024	-0.238 ± 0.795
2.0 µg MPH	-0.206 ± 0.131	-0.023 ± 0.019	-0.013 ± 0.032	0.025 ± 0.024	-0.705 ± 0.705
vmSTR					
0.125 µg MPH	0.091 ± 0.221	-0.014 ± 0.048	-0.029 ± 0.054	-0.033 ± 0.046	0.188 ± 5.627
0.5 µg MPH	0.041 ± 0.230	-0.016 ± 0.049	-0.025 ± 0.056	-0.016 ± 0.047	-0.051 ± 5.848
2.0 µg MPH	0.108 ± 0.221	0.000 ± 0.048	-0.019 ± 0.054	-0.033 ± 0.046	0.188 ± 5.627

Various doses of MPH were infused into the vmPFC, dmSTR, or vmSTR prior to performing a sustained attention task. Infusion into any of these regions was not sufficient to alter sustained attention performance as assessed by a change from vehicle in a variety of signal detection measures.

Supplemental Table S4. Receptor mechanisms of intra-PFC MPH enhancement of Sustained Attention.

Drug	d'	Prop Correct	Pr(hit)	Pr(FA)	No responses
ATI	0.089 ± 0.096	0.006 ± 0.013	0.010 ± 0.022	0.007 ± 0.018	0.357 ± 0.592
ATI+MPH	0.002 ± 0.096	-0.006 ± 0.013	0.008 ± 0.022	0.013 ± 0.018	1.000 ± 0.592
BEN	-0.019 ± 0.090	-0.00 ± 0.013	-0.012 ± 0.021	-0.005 ± 0.017	0.260 ± 0.558
BEN+MPH	0.131 ± 0.092	0.006 ± 0.013	-0.019 ± 0.021	-0.030 ± 0.017	-0.396 ± 0.572
MPH	**0.270 ± 0.093 ***	0.028 ± 0.013	0.033 ± 0.021	-0.023 ± 0.017	-0.634 ± 0.577
PHEN	**0.397 ± 0.108 ****	0.036 ± 0.015	-0.012 ± 0.025	**-0.082 ± 0.020 ****	-0.174 ± 0.671
SCH	-0.078 ± 0.091	-0.007 ± 0.013	0.011 ± 0.021	0.018 ± 0.017	1.245 ± 0.564
SCH+MPH	0.033 ± 0.092	0.007 ± 0.013	-0.019 ± 0.021	-0.012 ± 0.017	-0.367 ± 0.572

The sustained attention-enhancing actions of intra-dmPFC MPH (0.5 μg) were blocked with co-infusion of the α2 antagonist atipamezole (ATI; 0.625 μg) or the D1 antagonist SCH23390 (SCH; 0.5 μg). However, in contrast to working memory, the α1 antagonist, benoxathian (BEN; 0.4 μg), also blocked intra-dmPFC MPH-induced improvements in sustained attention. Consistent with this latter observation, intra-dmPFC infusion of a dose of the α1 agonist, phenylephrine (PHEN; 0.1 μg), previously shown to impair working memory (A. F. Arnsten et al., 1999) significantly improved sustained attention, as measured by d'. The magnitude of improvement is comparable to that seen with intra dmPFC MPH (0.5 μg). All values are expressed as a change from vehicle. * P<.05, **p<0.01

Chapter 4

Differential Actions of Cognition-Enhancing vs. Cognition-Impairing Effects of Psychostimulants on Frontostriatal Signaling

Robert C. Spencer,* David M. Devilbiss, Andrea J. Martin, Rick L. Jenison, and Craig W. Berridge

Unpublished Manuscript

Abstract:

The dorsomedial prefrontal cortex (PFC) and extended frontostriatal circuitry play a critical role in higher cognitive function. Dysregulation of frontostriatal-dependent cognition is implicated in a variety of behavioral pathologies, including addiction and ADHD. Psychostimulants are well known to exert dose-dependent alterations in frontostriatal cognitive function. Specifically, higher doses, associated with addiction, robustly impair frontostriatal-dependent cognition. In contrast, low-doses used in the treatment of ADHD, improve PFC-dependent cognitive function. To date, the neurocircuitry and neural coding bases for these diverse cognitive actions of psychostimulants are unclear. The current studies examined the effects of cognition-enhancing and cognition-impairing effects of MPH on the neuronal spiking activity and local field potential (LFP) oscillatory activity within dorsomedial PFC and dorsomedial striatal (dmSTR) circuitry of rats engaged in a test of working memory. Cognition-impairing doses of MPH (8.0 mg/kg S.C.) robustly suppressed the neuronal representation of task-related events within frontostriatal circuitry in a region-specific manner. Within the dmPFC, high dose MPH robustly suppressed the spiking activity of neurons strongly tuned to delay (and to a lesser extend neurons tuned to reward), while activating neurons not tuned to positive response outcome feedback. In the dmSTR, cognition-impairing doses of MPH increased the activity of neurons strongly tuned to delay and decision point, while broadly activating neurons not tuned to task-related events. In contrast, cognition-enhancing doses (0.5 mg/kg S.C.) had minimal impact on task-related firing of either PFC or striatal neurons. In terms of delay-related LFP oscillatory activity, MPH elicited a dose-dependent decrease in theta (3-7 Hz) power in the PFC, but not the dmSTR. The highest, and cognition-impairing dose robustly increased alpha (8-12 Hz) and gamma (40-80 Hz) activity in both the dmPFC and dmSTR. These observations suggest the cognition-enhancing vs. cognition-impairing effects of psychostimulants do not simply reflect opposing alterations in frontostriatal neural coding.

Keywords: Prefrontal cortex, cognition, working memory, multichannel recording, Theta, Methylphenidate, Psychostimulants

Psychostimulants, including methylphenidate (MPH; Ritalin) are a class of drugs that elicit potent motor activating and arousal enhancing actions and possess significant potential for abuse (Rebec & Bashore, 1984; Segal, 1975). At doses associated with these actions, psychostimulants impair higher cognitive functions dependent on the prefrontal cortex (PFC) and the extended frontostriatal network, including, working memory, attention, and behavioral inhibition (Arnsten & Pliszka, 2011; Berridge et al., 2012; Devilbiss & Berridge, 2008; Spencer et al., 2015). Nonetheless, these drugs have been used since the 1930s to calm behavior and treat PFC-dependent cognitive deficits in Attention Deficit Hyperactivity Disorder (ADHD). Originally this was viewed as an action unique to ADHD. However, subsequent research unambiguously demonstrated that low and clinically-relevant doses of psychostimulants improve frontostriatal cognitive function broadly while being devoid of motor activating actions in healthy human and animal subjects, identical to that seen in ADHD (Berridge et al., 2006; Kuczenski & Segal, 2005; Mehta et al., 2001; Rapoport & Inoff-Germain, 2002; Tannock et al., 1989). Currently, the neural mechanisms underlying the divergent cognitive actions of psychostimulants are not well understood.

Psychostimulants elicit dose-dependent elevations in catecholamine (norepinephrine (NE) and dopamine, (DA)) signaling throughout the brain (Kuczenski & Segal, 1997). The cognitive functions of PFC catecholamines have been most intensively studied in tests of working memory (Arnsten & Li, 2005; Arnsten & Pliszka, 2011; Goldman-Rakic, 2000). Under these conditions, catecholamines exert potent and non-monotonic modulatory actions on PFC-dependent cognitive function, whereby too little or too much impairs performance in working memory tasks (Arnsten, 2000a). In contrast, at moderate levels of release, catecholamines act in the PFC to improve working memory via higher affinity noradrenergic $\alpha 2$ receptors as well as moderate activation of dopaminergic D1 receptors (Arnsten, 2000a). Electrophysiological studies demonstrate that PFC $\alpha 2$ receptors strengthen the activity of neurons tuned to the delay interval of working memory tasks, while moderate activation of dopaminergic D1 receptors decreases neuronal responses to task irrelevant stimuli (Arnsten, 2007). In contrast, at higher

rates of release, catecholamines impair working memory via activation of lower affinity noradrenergic α1 receptors as well as higher rates of D1 receptor activation (Arnsten, 2000a). These latter actions are associated with a robust suppression of delay related neuronal signaling in the PFC (Arnsten, 2007).

The PFC extends topographically organized projections to the striatum, forming functional frontostriatal circuits. In rats, the dorsomedial PFC (dmPFC) plays a prominent role in higher cognitive function and extends a prominent projection to the dorsomedial striatum (dmSTR; Gabbott et al., 2005; Spencer et al., 2012; Vertes, 2006, 2004). Evidence indicates that both regions are necessary for higher cognitive function (Spencer et al., 2012). Limited data also indicates that striatal neurons display task-related activity similar to that seen in the PFC (Akhlaghpour et al., 2016; Levy et al., 1997b; Stalnaker et al., 2010)

Currently, the frontostriatal neural coding mechanisms associated with the dose-dependent cognitive actions of psychostimulants are unknown. One simple hypothesis is that the opposing cognitive actions of low vs. higher doses of psychostimulants are associated with opposing modulatory actions on task-related spiking activity within dorsomedial frontostriatal circuitry. To test this, the current studies examined the effects of cognition-impairing vs. enhancing actions of MPH on task-related spiking activity of dmPFC (dorsal anterior cingulate and dorsal prelimbic PFC) and dmSTR neurons in rats tested in a working memory task. We observed that cognition-impairing doses of MPH robustly suppressed the activity of dmPFC neurons strongly tuned to delay (with a trend for suppressed reward), while activating neurons not tuned to a tone signaling successful outcome. In contrast, cognition impairing doses of MPH robustly increased dmSTR neurons strongly tuned to delay as well as those tuned to the decision point of the maze, while broadly increasing the activity of dmSTR neurons not tuned to task events. In contrast, cognition-improving doses had no significant impact on task-related firing of either PFC or striatal neurons.

Beyond spiking activity, oscillatory activity within discrete frequency bands contained in local field potentials (LFPs) is thought to help regulate the flow of information among neural

ensembles both within and between brain regions (Buzsáki & Chrobak, 1995; Salinas & Sejnowski, 2001). Theta (4-8 Hz), alpha (8-12 Hz), and gamma (30-100+ Hz) frequencies have been posited to play a role in PFC-dependent cognition and are dysregulated in ADHD (Clarke et al., 2007; Loo et al., 2004; Roux & Uhlhaas, 2014) Given these observations, as part of the current studies we also analyzed the dose-dependent actions of MPH on delay-related LFP oscillatory activity in the dmPFC and dmSTR. We observed that, MPH elicited a dose-dependent decrease in delay-related theta (3-7 Hz) power in the PFC, but not the dmSTR. Additionally, the cognition-impairing, but not improving, dose of MPH increased delay-related alpha (7-12 Hz) and gamma (40-80 Hz) oscillations in both the PFC and dmSTR.

Collectively, these observations indicate cognition impairing doses of MPH disrupt frontostriatal signaling of task related information in a region- and event-specific manner. In contrast, while MPH acts within the PFC to improve cognitive function (Spencer et al., 2012), this action is largely not associated with alterations in frontostriatal signaling. Therefore, the cognition-improving actions of psychostimulants do not merely reflect opposing electrophysiological actions within frontostriatal circuits seen with cognition-impairing doses.

Materials and Methods:

Subjects:

Male Sprague-Dawley rats (n=27; Charles River, Wilmington DE; 260-280g) were individually housed with environmental enrichment (Nylabone® chews) on a 13/11 hour light/dark cycle (lights on 0600h) with *ad libitum* water access. During training and testing, access to food was restricted to maintain motivation (15-20 g of standard chow). All facilities and procedures were in accordance with animal use and care guidelines of the National Institutes of Health of the United States and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison.

Working Memory Training:

Prior to surgery, animals were trained and tested in a T-maze constructed from opaque black Plexiglas, as described previously (Devilbiss et al., 2016). Animals were rewarded with a 45 mg sucrose pellet when they entered the arm of the maze not chosen on the previous trial (10 trials/session/day). After entering an arm, animals were given feedback in the form of a tone (Incorrect, 5 kHz; Correct, 10 kHz) by breaking an IR beam 2/3 of the way down the response arm (see Figure 1). Between trials, animals were placed in the start box of the maze and prevented from exiting by a Plexiglas gate for a brief, 0-second delay interval (i.e. the minimum time it takes to put down and remove the gate). Following training, animals were surgically implanted with recording electrodes and allowed to recover for 7–10 days with *ad lib* feeding. Following recovery, food-restriction was reinstated and training continued until 65-90% correct performance was obtained with delays ranging between 5-40 seconds. Sessions were separated by 2 hours. During training sessions, animals were tethered to a dummy wire harness similar to that used for electrophysiological recordings. Given performance improves over multiple testing sessions, delay duration was increased to maintain performance within the target accuracy range.

Surgery:

50 μM stainless steel, Teflon-coated electrodes (NB Labs, Dennison, TX) were implanted into the dmPFC and dmSTR of rats. For the dmPFC, an 8-wire linear array (250 uM separation) was used to target layer V along the rostro-caudal axis (A+3.0; L±0.8; V -2.5 to 2.7 mm). For the dmSTR, a 2x3x3 matrix (250 μm between wires and 250 μm between rows) was used (A+0.45; L±3.5; V -3.3 at 11° away from midline). Stainless steel screws (MX-0080-16B-C, Small Parts, Inc.) and dental acrylic (Plastics One, Roanoke, Virginia) were used to mount the electrode connectors to the skull. Animals were treated with buprenorphine (0.01 mg/kg subcutaneously; S.C.) and ampicillin (30 mg/kg S.C.) and allowed to recover for 7–10 days. 1 mL of saline was administered S.C. every hour during surgery.

Drugs:

MPH (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline (VEH; pH=7.4).

Testing procedures:

Animals were transported in their home cage to the recording room 2 hours prior to the first testing session and attached to a counterweighted tether/ 32-channel slip-ring commutator. Animals were able to freely move while single units were discriminated. The first, 30-trial baseline testing/recording session was conducted identically to the first session of training days Between recording sessions, animals were returned to their home cage and placed above the T-maze for 2 hours while remaining attached to the recording tether. Animals received VEH or 0.5 mg/kg or 8.0 mg/kg MPH S.C. 45 minutes prior to the second 31-trial testing session. In some cases, MPH treatment did not alter performance, thus sessions with less than ±14% change in performance were excluded.

Electrophysiology:

Single Unit Recordings. Animals were connected to an OmniPlex® Neural Data Acquisition System with counterbalanced cables attached to a freely rotating commutator. During the 2-hour habituation period, putative single 'units' of the dmPFC and dmSTR were discriminated in real time using online template matching algorithms to preliminarily discriminate action potentials exhibiting at least a 3:1 signal-to-noise ratio. Following unit sorting, animals remained tethered to the recording hardware and the quality of the discrimination was monitored throughout the remainder of the day. Precision timestamps of all task events and relevant animal behavior were captured with a combination of an infrared (IR) beam grid and high-resolution video capture and tracking (80 frames/sec) synchronized to the OmniPlex® electrophysiological hardware (Cineplex, Plexon, Dallas, Texas). Consumption of reward was time-stamped from the combined video capture and tracking of the experimenter hand delivering the reward, identifying the moment the animal bit down on the reward.

Neuron Identification: We identified wide spiking, putative excitatory output neurons by quantifying the peak-to-peak latency of the extracellular action-potential waveform (WS-type > 200μs), as previously described (Mitchell et al., 2007). Across multiple cortical regions most pyramidal neurons have broad action potentials, comprising 70 to 80% of all cortical neurons (e.g. Povysheva et al., 2006). Neurons with narrow action potentials and fast firing rates are typically interneurons (basket cells and chandelier cells). However, a small percentage of interneurons (10% to 15% of interneurons) also generate broad action potentials (Cauli et al., 1997). Thus, it is possible that a small percentage of wide spiking neurons were misclassified as pyramidal neurons in our study. However, given the proportion of WS-type relative to narrow spiking neurons in this dataset, this type of misclassification would likely comprise only a small number of neurons and therefore are unlikely to influence the overall results of this study.

Within the dmSTR, medium spiny neurons (MSNs) and fast-spiking (FS) interneurons were identified using the peak-to-valley duration (MSN > 250 μs; FS < 200 μs) and firing rate (MSN < 8 Hz) of extracellular action potential waveforms (Berke, 2009; Kim et al., 2007;

Stalnaker, et al., 2012). Again, although misclassification of neuron subtypes using these characteristics is possible, this would affect a small number of neurons and is unlikely to bias our overall results. Few FS neurons were identified and thus were not analyzed.

Distinct subpopulations of neurons selectively responded to each identified phase of the task (Delay, Branch, Correct Tone, and Reward receipt; see Figure 1). Due to the infrequency of error trials, this epoch was not analyzed. The selective response or "event-tuning" of a neuron was determined during baseline recording sessions by the Z-score of a neuron's spiking activity during a task interval versus the overall spiking activity of that neuron throughout the entire recording session. Z-scores thresholds were selected to identify groups of neurons with responses qualitatively similar to exemplar responses described by other laboratories (Fuster & Alexander, 1971; Hyman et al., 2010; Jung et al., 1998).

Consistent with prior studies in our laboratory, we observed no significant differences in baseline firing rates for left/right or correct/incorrect trials among all neurons or those strongly tuned to task events in the dmPFC or dmSTR (Devilbiss et al., 2016; Hyman et al., 2010).

Local Field Potential Analyses: Spectral Density. Local field potentials (LFPs) were recorded from all wires in each region, however, only one channel from each brain region per recording was analyzed. The channel was selected through visual inspection, ensuring a clear and stable signal with a minimum of noise. Signals were amplified, filtered (0.7-170 Hz), and digitized at a sample rate of 1 kHz. LFP signals were down-sampled to 125 Hz, pre-processed with a z-transform across all trials, and further transformed by taking the first derivative with respect to time (dV/dt). This normalization procedure suppresses motion artifact and tilts the spectrum up 6 dB/octave to enhance higher frequencies in the spectral decomposition. To reveal oscillatory activity, the LFP was analyzed using time-frequency continuous wavelet transform (cwt) using the Wavelet Toolbox in Matlab (MathWorks, Natick, MA). The signal was convolved with a series of Morlet wavelets with center frequencies ranging from 1-100 Hz and a length of two cycles. The result of these convolutions is the wavelet transform of the LFP, representing the

amplitude and phase of oscillations. Thus, LFP power was estimated by computing the square of the absolute value of the wavelet coefficient at each target frequency. These values were collapsed across time and averaged over trials.

Statistical Analyses

Single unit analyses. The mean spike rate during each task interval was determined on a trial-by-trial basis using Peri-Event Time Histogram (PETH) analysis. For each neuron, the % change in mean spike rate from baseline condition was calculated ((drug-baseline)/drug*100). For vehicle treatment, single sample T-Tests were used to determine if the percent change in firing from baseline was significantly different from 0. In order to assess the effect of drug on changes in firing rate across events, one-way ANOVAs were conducted in JMP Pro 12 for each treatment by tuning interaction. In order to further assess the effects of drug on changes in firing rate, additional one-way ANOVAs were conducted comparing treatment within each neuron type. Dunnett's tests were conducted to assess significant differences from VEH. Reported P values are adjusted for multiple comparisons.

LFP analyses. The effects of MPH on LFP power within distinct frequency bands were analyzed using planned T-tests comparing the percent change in spectral density from baseline seen in VEH to the same measure in MPH treatment groups. Reported *p* values are adjusted for multiple comparisons.

Histology:

Animals were deeply anesthetized with isoflurane while cathodal current (15μA) was passed through each electrode (referenced to the ground wire) for 10 seconds. Animals were then perfused with 3.7% formaldehyde after which brains were removed, and immersed in formaldehyde for at least 24 hr. Afterwards, brains were frozen and coronal sections (40 μm) were collected, mounted on slides and counterstained with neutral red. Animals were excluded if electrode placement was outside the targeted region.

Results:

Systemic MPH Elicits an Inverted-U Shaped Modulation of Working Memory Performance

Prior results from this lab demonstrated that MPH improves working memory performance in rats when administered in low and clinically relevant doses (0.5 mg/kg S.C.). In contrast, doses that are 4-16 fold higher than an enhancing dose (2.0 – 8.0 mg/kg MPH S.C.) impair working memory (Berridge et al., 2006; Devilbiss & Berridge, 2008). In the current studies we observed a similar pattern, with VEH treatment eliciting no significant change in working memory performance (3.4 ± 5.7%), 0.5 mg/kg MPH significantly improving performance by 26 ± 10% (t_{18}=-6.2, p<0.001) and 8.0 mg/kg MPH significantly impairing performance (-43.9 ± 22.6%, t_{18}=-6.6, p<0.001; Figure 1).

Neuron Response Properties Under Baseline Conditions

The majority of dmPFC neurons were classified as wide spiking (WS), output neurons (0.5 MPH, n = 111 of 114 neurons, 97%; 8.0 MPH, n= 140 of 142 neurons, 99%; VEH, 119 of 121 neurons, 98%). Due to the low number of NS dmPFC neurons isolated, this class of neurons was not further analyzed. Key task events in the T-maze were tracked during recording, including delay, branch (the decision point), reward, and correct or error tones that preceded reward or pickup (for error trials) and signaled correct or error response outcomes (Figure 1D). WS neurons were classified as displaying strong tuning to a given task event using a z-score comparing activity during the event to activity outside (see Methods). Neurons that were not classified as strongly tuned were considered not tuned to that event. As shown in Table S1, 14% of all dmPFC WS putative pyramidal neurons were strongly tuned to the delay interval, 13% to the branch point, 7% to the correct tone, and 9% to reward under baseline conditions (Table S1).

The majority of dmSTR neurons were classified as MSN-type i.e. Medium Spiny Neurons (0.5 MPH, n = 88 of 107 neurons, 82%; 8.0 MPH, n = 94 of 119 neurons, 79%; VEH, 94 of 102 neurons, 92%). Given the relatively small number of FS neurons, only MSNs were included in further analyses. As with the dmPFC, dmSTR neurons were strongly tuned to delay-

(20%) decision (20%), correct tone (10%), and reward (8%) events in the T-maze (Figure 1D, Table S1). Again, neurons not considered strongly tuned were classified as not tuned to the event. Interestingly, previous studies have not always shown sustained striatal activity during the delay period of working memory tasks (Tort et al., 2008). Here we saw many robust examples of striatal neurons displaying sustained activation during the delay period (Figure 1D).

MPH Effects on Neuronal Firing

PFC: As shown in Figure 2, relative to baseline, VEH treatment had minimal effects on the activity of PFC WS neurons strongly tuned to task events ($F_{3,56}$=0.57, p=0.64; Delay, -4.1±7.5%, t_{20}=-0.48, p=0.64; Branch, 0.84±12.5%, t_{11}=0.07, p=0.94; Reward, -6.6±10.8%, t_{11}=-0.6, p=0.77). The only exception to this was a modest *decrease* in the firing rate of neurons strongly tuned to correct tone (Tone, -16.7±10.2%, t_{14}=-2.24, p<0.05;). VEH treatment had no effect on neurons not tuned to task events ($F_{3,365}$=2.58, p=0.05; Delay, 5.0±4.0%, t_{93}=1.5, p=0.14; Branch, -3.94±4.5%, t_{98}=-0.99, p=0.32; Tone; -10.9±7.2%, t_{79}=-1.96, p=0.05; Reward, 3.9±5.1%, t_{95}=0.77, p=0.44).

A high and cognition-impairing dose of MPH (8.0 mg/kg) had no overall effect on the activity of neurons strongly tuned to task events ($F_{3,54}$=2.1, p=0.11; see Figure 2). Nonetheless, this dose elicited a robust suppression of task-related activity in PFC WS Delay tuned neurons (-35.75±8.3%; t_{49}=-2.82, p=.01 vs VEH). In contrast, high dose MPH did not significantly affect neurons strongly tuned to Branch (-14.9±9.2%; t_{44}=-1.01, p=0.49 vs. VEH), Tone (1.8±14.9%; t_{24}=1.03, p=0.52), or Reward (-33.3±10.8%; t_{30}=-1.75, p=0.16). High dose MPH had a significant overall effect on PFC WS neurons not tuned to task events ($F_{3,438}$=4.23, p=0.006). Planned comparisons revealed 8.0 mg/kg MPH increased the responsivity of strongly tuned neurons during correct tone presentation, but not to other events (Delay, -6.4±3.6%, t_{301}=-2.14, p=0.06; Branch, 7.7±4.3%, t_{298}=1.88, p=0.11; Tone, 20.8±6.3, t_{276}=3.31, p=0.002; Reward, 3.2±4.6%, t_{298}=-0.10, p=0.99; vs VEH).

As shown in Figure 2, at a dose that improved working memory (0.5 mg/kg), MPH had no significant effects on task-related activity for PFC WS neurons strongly tuned to T-maze task events ($F_{3,37}$=0.68, p=0.57; Delay, 3.9±9.2%, t_{49}=0.67, p=0.73; Branch, 4.6±12.0%, t_{44}=0.26, p=0.96; Tone, 7.3±17.6%, t_{49}=1.2, p=0.42; Reward, -16.8±12.5%, t_{30}=-0.62, p=0.77; vs VEH). For WS neurons not tuned to task events, there were no significant changes outside an increased response to presentation of correct tone ($F_{3,370}$=2.37, p=0.07; Delay, 1.1±3.9%, t_{301}=-0.70, p=0.70; Branch, -4.9±4.7%, t_{298}=-0.24, p=0.99; Tone, 9.7±6.6%, t_{276}=2.12, p=0.06; Reward, 11.1±5.2%, t_{298}=0.99, p=0.51; vs VEH).

dmSTR: VEH treatment had no significant overall effect on strongly tuned dmSTR MSNs ($F_{3,39}$=1.36, p=0.27; See Figure 2) Nonetheless, planned comparisons revealed a modest decrease in activity among dmSTR MSNs strongly tuned to delay (Delay, -14.8±12.1%, t_{17}=-2.67, p<0.05), while having no effect on dmSTR neurons tuned to other events in the task (Branch, -1.7±12.6%, t_{12}=-0.15, p=0.88; Tone, 19.7±33.3%, t_{5}=0.31, p=0.76; Reward, -2.4±13.6%, t_{5}=-0.2, p=0.85). Furthermore, VEH treatment had no effect on activity in dmSTR MSNs not tuned to task events ($F_{3,294}$=0.36, p=0.78; Delay, -3.2±6.6%, t_{68}=-1.3, p=0.21; Branch, 1.1±8.5%, t_{70}=0.24, p=0.81; Tone, 2.8±15.6%, t_{60}=0.31, p=0.76; Reward, 3.7±4.3%, t_{96}=0.9, p=0.39, see Figure 2).

Unlike the suppressive effects observed for delay-tuned neurons in the PFC, a cognition-impairing dose of MPH generally and broadly excited dmSTR neurons. For neurons tuned to task events, this dose had no significant overall effect on firing rate ($F_{3,43}$=1.59, p=0.21). Nonetheless, planned comparisons revealed significantly increased firing in dmSTR MSNs strongly tuned to delay (35.9±12.4%, t_{50}=2.92, p=0.01), with a trend for increased activity in Branch tuned neurons (36.2±11.3%, t_{52}=2.23, p=0.05 vs. VEH). In contrast, no effect was seen on MSNs tuned to the presentation of Correct Tone (91.5±33.3%, t_{23}=1.52, p=0.23), and neurons tuned to Reward (-5.3±11.8, t_{20}=-0.16, p=0.98 vs. VEH). For dmSTR MSNs not displaying task-related tuning, the high dose of MPH robustly and uniformly increased the activity of these

neurons compared to VEH ($F_{3,259}$=7.12, p<0.0001; Delay, 43.3±6.6%, t_{209}=4.97, p<0.0001; Branch, 52.1±8.6%, t_{192}=4.23, p<0.0001; Tone, 139.7±15.7%, t_{166}=6.2, p<0.0001; Reward, 94.2±11.1%, t_{219}=6.3, p<0.0001; see Figure 2).

A cognition-enhancing dose of MPH had no significant effects on the firing rate of dmSTR MSNs strongly tuned to task events relative to VEH ($F_{3,63}$=0.05, p=0.98; Delay, -4.7±=12.1%, t_{50}=0.59, p=0.78; Branch, -6.9±8.9%, t_{52}=-0.34, p=0.91; Tone, -7.8±14.3%, t_{23}=-0.69, p=0.70; Reward, -9.8±11.1%, t_{20}=-0.42, p=0.88; see Figure 2), nor among neurons not tuned to these events ($F_{3,233}$=0.94, p=0.42; Delay, 4.3±6.4%, t_{209}=0.82, p=0.63; Branch, -6.6±9.6%, t_{192}=-0.60, p=0.78; Tone, 10.0±17.5%, t_{166}=0.31, p=0.93; Reward, 1.9±11.4%, t_{219}=-0.15, p=0.98).

MPH Effects on Delay-Related LFP Spectral Density

Technical considerations: In the rat, neuronal oscillations between 4-12 Hz are generally considered in the theta band (Kramis et al., 1975; Li et al., 2014; O'Neill et al., 2013; Vertes, 2005). This differs from the human literature which generally classifies 4-8 Hz oscillations as theta and 8-12 Hz as alpha (Bonnefond & Jensen, 2012; Klimesch, 1999; Roux & Uhlhaas, 2014). Kramis et al (Kramis et al., 1975), 1975, first suggested two distinct frequency bands within rodent theta, each associated with unique behavioral and pharmacological properties. Type I, or atropine resistant theta (8-12 Hz) is associated with voluntary movement and REM sleep, while Type II, or atropine sensitive, theta (4-7 Hz) is seen during immobility. Nonetheless, in rodents, upper theta oscillations in the 8-15 Hz range have also been termed alpha (Anderson & Strowbridge, 2014; Tort et al., 2010), and correlate with the encoding of sensorimotor information (Nicolelis et al., 1995; Wiest & Nicolelis, 2003) similar to that seen in humans (Fransen et al., 2016). Importantly, there are both species and study-specific differences in the way frequency bands are defined (Buzsáki et al., 2013). Given this ambiguity in the literature in regard to naming frequency bands in the rodent, we have decided to identify 4-7 Hz as theta, 8-12 Hz as alpha, and 40-80 Hz as gamma for the purposes of this study.

PFC: As seen in Figure 3, within the dmPFC, MPH dose dependently decreased delay-related theta power compared to VEH (-4.3±0.93%). Specifically, the cognition-enhancing dose of MPH reduced theta power by -11±1.04% (t_{12}=-10.6; p<0.001) and the cognition-impairing dose by -28±3% (t_{12} = -8.4; p<0.001). Alpha and gamma power were increased following the cognition-impairing dose of MPH (16.8±3.6%, t_{22}=-8.4, p<0.001; 6.5±5.3%, t_{30}=-6.2, p<0.001 respectively compared to VEH). In contrast, the cognition-enhancing dose of MPH had no impact on alpha (-16.2±0.99%, t_{22}=1.6, p=0.12) or gamma (-2.3±0.27%, t_{30}=1.9, p=0.32) power relative to VEH (-14.1±0.86%, -1.8±0.42% respectively).

dmSTR: As seen in Figure 4, within the dmSTR, neither the cognition-impairing dose (-5.2±2.0%, T_{24}=-2.0, p=0.06), nor the cognition-enhancing dose (-12.7±1.2%, t_{24}=1.3, p=0.21) of MPH significantly affected theta activity relative to VEH (-10.8±0.82%). For alpha oscillations, the cognition-impairing dose significantly *increased* alpha power (15.9±1.2%; t_{22}=-20.9, p<0.001 compared to VEH) while the cognition-enhancing dose of MPH had no significant effect (-13.6±0.7% vs. -15.6±0.9% for VEH, t_{22}=-1.8, p=0.08). A similar pattern of effects was observed for gamma oscillations; the cognition-impairing dose of MPH *increased* gamma power (8.2±1.0%, t_{30}=-7.0, p<0.001), while the cognition-enhancing dose of MPH had no significant effect on power (-0.02±0.41% vs. 0.08±0.63% for VEH; t_{30}=0.14, p=0.89),

Discussion:

Psychostimulants exert dose dependent cognitive actions. In higher and abuse relevant doses, psychostimulants impair cognitive functions dependent on the PFC. Yet, at lower doses, they remain the most effective and widely used treatment for ADHD, reversing core frontostriatal cognitive deficits (Greenhill, 2001; Mehta et al., 2001; Rubia et al., 2011; Vaidya et al., 1998). The current studies sought to identify the neurophysiological mechanisms within dorsomedial frontostriatal circuitry that contribute to the dose-dependent cognitive actions of psychostimulants in animals engaged in a test of working memory.

MPH Effects of Neuronal Firing

Delayed-response tasks of working memory have been used extensively to study the neurobiological basis of PFC-dependent function. These tasks require information to be retained over short delay intervals and subsequently used to guide goal directed behavior. Sustained spiking activity within the PFC during the delay phase of working memory tasks has been posited to support the maintenance of working memory either directly or indirectly (Constantinidis et al., 2018; Curtis & D'Esposito, 2003; Fuster & Alexander, 1971; Goldman-Rakic, 1995). The current studies demonstrate that high, cognition-impairing doses of MPH, robustly suppress the firing of dmPFC neurons tuned to delay. This reduction in firing is consistent with prior observations indicating a positive correlation between delay related firing and working memory performance (Arnsten, 2009, 2015, Devilbiss et al., 2016). Additionally, by increasing the activity of dmSTR MSN neurons not tuned to delay, high dose MPH appears to degrade the representation of delay broadly within this frontostriatal circuit. Collectively, these actions likely contribute to the working memory-impairing actions of high dose MPH. High dose MPH also increased the activity of dmPFC neurons not tuned to the correct tone and reward. This latter action may contribute the ability of high dose psychostimulants to enhance attention to reward-related conditioned cues as seen in drug abuse and addiction (Field, 2006). Lastly, the large and widespread increase in firing among dmSTR neurons regardless of tuning

may result in part from the robust increases in dopamine seen in the striatum following higher, cognition-impairing doses of psychostimulants (Berridge et al., 2006; Kodama et al., 2017; Surmeier et al., 2007). This is consistent with previous observations that DA and high dose psychostimulants generally activate dmSTR neurons (Haracz et al., 1993; Rebec et al., 1997).

In contrast to the robust actions seen with a cognition-impairing dose of MPH, a clinically relevant and cognition-improving dose of MPH had no effect on task-related spiking activity in either the PFC or dmSTR within or outside the delay. This indicates that the neurophysiological mechanisms that support the ability of low dose psychostimulants to improve higher cognitive function are not simply the inverse of mechanisms that contribute to the cognition-impairing actions of higher doses. This is surprising given that prior studies from our lab demonstrate that in animals tested in a home-cage condition, cognition-enhancing doses of psychostimulants preferentially increased the responsivity of PFC neurons to punctate excitatory input driven by electrical stimulation of the ventral hippocampus, while having minimal effects on neurons outside the PFC (i.e. somatosensory cortex; Devilbiss & Berridge, 2008). This suggests that neurophysiological actions of low-dose psychostimulants are context dependent.

MPH Effects on Frontostriatal LFP Oscillatory Activity

As reviewed above**,** oscillatory activity within discrete frequency bands is believed to modulate neuronal signaling both within and between regions (Friston, 2011; Roux & Uhlhaas, 2014). For working memory, theta activity in the frontal cortex has been observed to be positively correlated with performance (Gevins, 1997; Hsieh & Ranganath, 2014; Itthipuripat et al., 2013; Mitchell et al., 2008). However, ADHD, which is associated with impaired working memory, is also associated with increased theta power (Barry et al., 2003; Clarke et al., 2007; Koehler et al., 2009), an effect normalized by psychostimulant treatment (Clarke et al., 2007; Loo et al., 2004). These seemingly contradictory observations may be related to the fact that increased theta power is also correlated with working memory load (Jensen & Tesche, 2002). Thus, increased theta may reflect increased effort, rather than a signature of working memory

accuracy *per se*. In the current study, a cognition-enhancing dose of MPH decreased theta power within the PFC during the delay phase of a working memory task similar to that seen following psychostimulant treatment in ADHD patients. However, an even greater suppression in delay-related theta was observed with a cognition-impairing dose. This latter action may be consistent with prior observations that acute stress (which impairs working memory) decreases frontal theta (Gärtner et al., 2014), potentially reflecting a collapse in the ability of frontal theta to support working memory.

In addition to theta, both alpha and gamma oscillations are also implicated in working memory. In particular, alpha power is positively correlated with increased working memory demands (Bonnefond & Jensen, 2012; Klimesch, 1999) and has been posited to protect working memory against distractors (Bonnefond & Jensen, 2012), while gamma oscillations correlate with working memory load (Howard et al., 2003). Nonetheless, ADHD is associated with increased alpha (Koehler et al., 2009; Snyder & Hall, 2006) and gamma (Herrmann & Demiralp, 2005) power. Again, this may reflect increased effort to maintain goal-directed processes. Here we show that cognition-impairing, but not improving, doses of MPH significantly elevated alpha and gamma frequencies in both the PFC and dmSTR. This is consistent with earlier studies in animals showing an increase in both oscillatory bands in the mPFC following high dose psychostimulant treatment . In contrast to that observed with the higher dose, the cognition-enhancing dose of MPH had no significant effects on either alpha or gamma activity.

Conclusions:

MPH exerts complex, dose dependent actions on frontostriatal signaling. At the high and cognition-impairing dose, MPH selectively suppressed key task-related responding in the dmPFC, and more generally activated neurons not tuned to task events in both the dmPFC and dmSTR. The simultaneous suppression of delay-tuned neurons within the PFC and the activation of neurons not tuned to task events within the dmPFC/dmSTR likely degrades goal-related signaling within this functional circuit. In contrast, low, cognition-enhancing doses of

MPH had little effect on rate coding within either PFC or dmSTR neurons. These actions were combined with decreased theta activity within the dmPFC and increased alpha and gamma oscillations in both the dmPFC and dmSTR. The degree to which these latter observations contribute to cognitive impairment or reflect compensatory responses to impaired network signaling is unclear. The robust effects of a cognition-impairing dose of MPH on frontostriatal signaling contrasted with the minimal effects observed with a cognition-enhancing dose. These studies demonstrate the neurophysiological mechanisms that support the cognition-enhancing actions of psychostimulants do not simply reflect the inverse of those mechanisms contributing the cognition-impairing effects of these drugs. Future work will need to apply different approaches, such as frontostriatal effective and functional connectivity or machine learning to determine how the procognitive doses of psychostimulants modulate frontostriatal activity.

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Figure 1

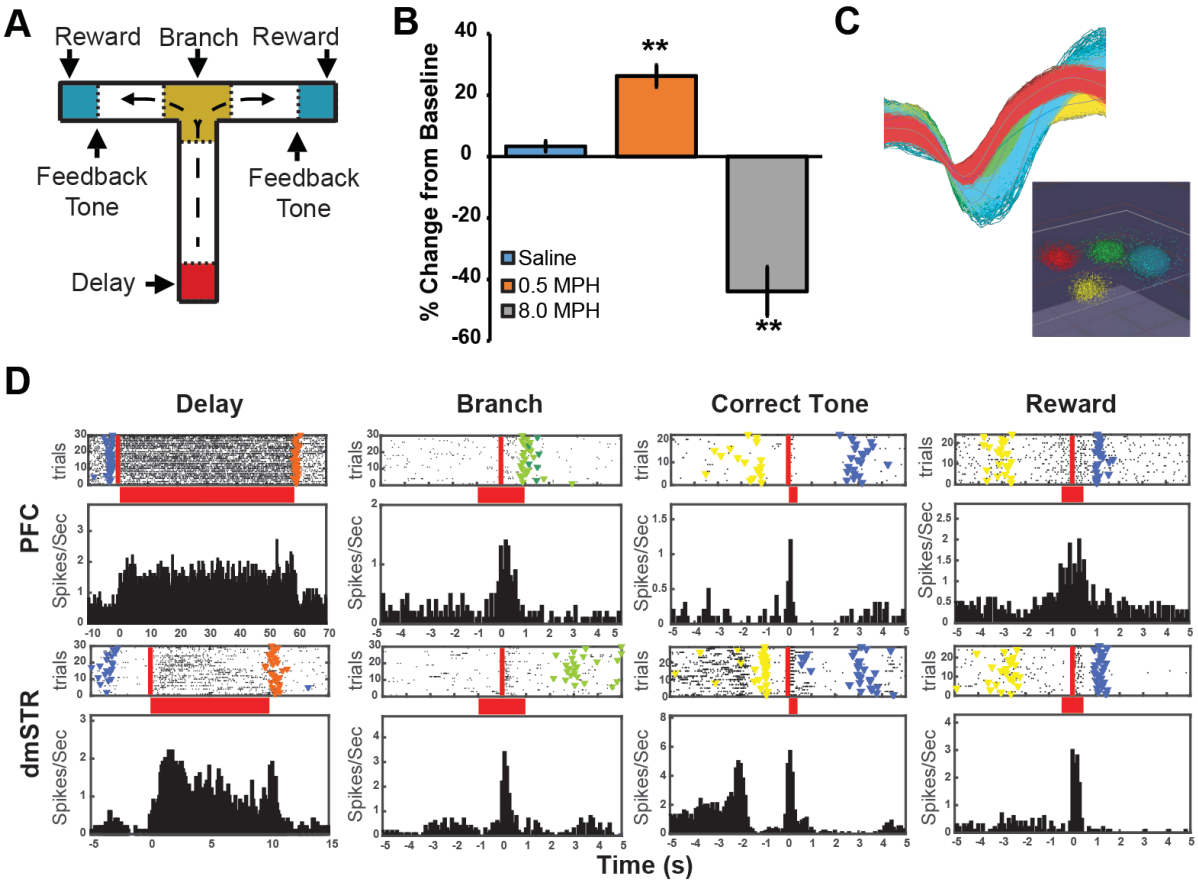

Figure 1. **(A)** Animals performed a spatial delayed alternation test of working memory while neuronal activity was recorded from the PFC and dmSTR along with timing of several behavioral components. **(B)** As shown previously (Berridge et al, 2006) performance in this task is modulated in an inverted-U fashion with low dose MPH (0.5 mg/kg S.C.) improving performance, and high dose MPH (8.0 mg/kg S.C.) impairing performance. **(C)** Individual neurons are identified by their unique waveform through online template matching (top) and separation in PCA space (bottom). **(D)** Exemplars of PFC (top) neurons and dmSTR (bottom) neurons firing to Task Related events in the T maze. Red indicates the interval analyzed for each event (delay length; Branch ±1s; Correct Tone +300ms; Reward ±0.5s). **p*<0.05, ***p*<0.01

Figure 2

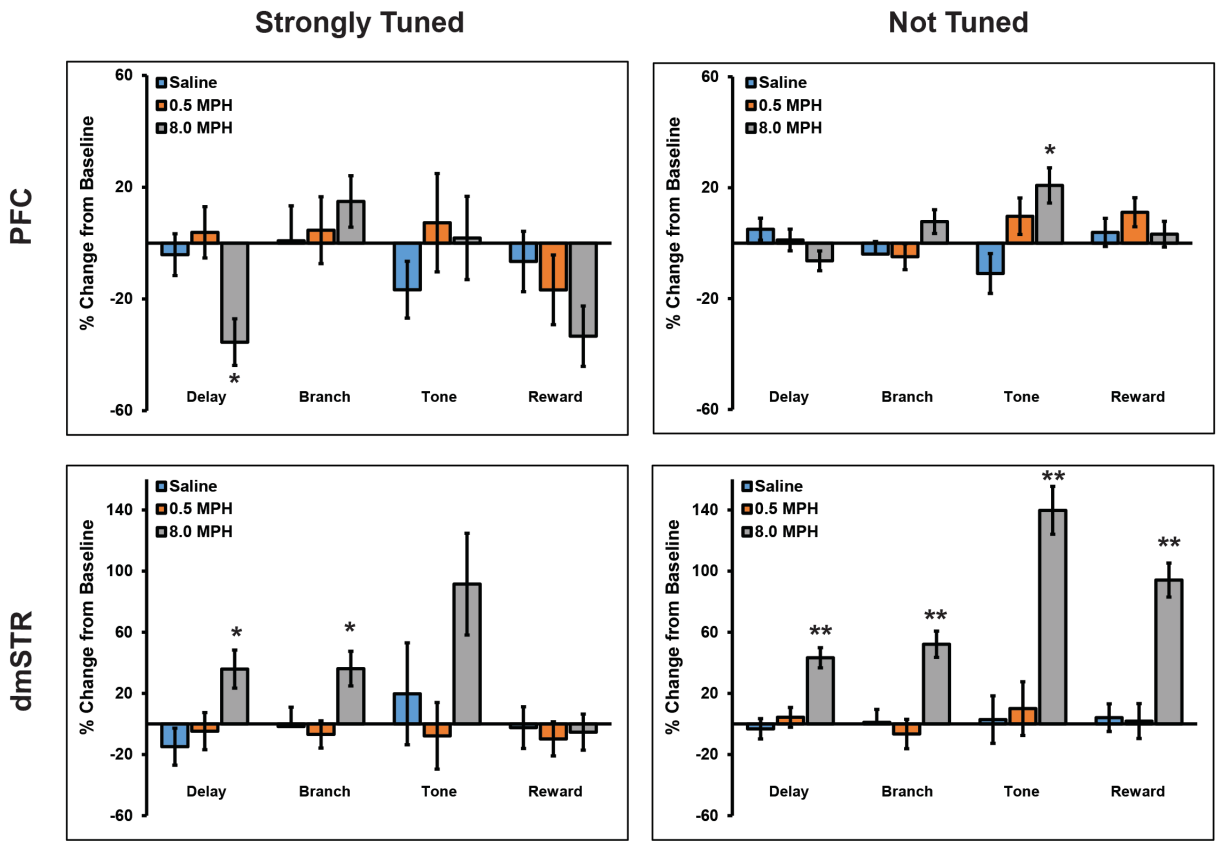

Figure 2. In the dmPFC **(top)**, *cognition-impairing* doses of MPH significantly suppress activity of neurons strongly tuned to delay (trend for reward) and non-selectively activated neurons not tuned to correct feedback signal (tone). In the dmSTR **(bottom)** *cognition-impairing* doses of MPH robustly increase firing of neurons strongly tuned to delay and branch as well as neurons not tuned to all key task events. In contrast *cognition-enhancing* doses of MPH have no significant effect on neuronal firing rates in either the PFC or striatum. *p<0.05, **p<0.01 compared to vehicle.

Figure 3

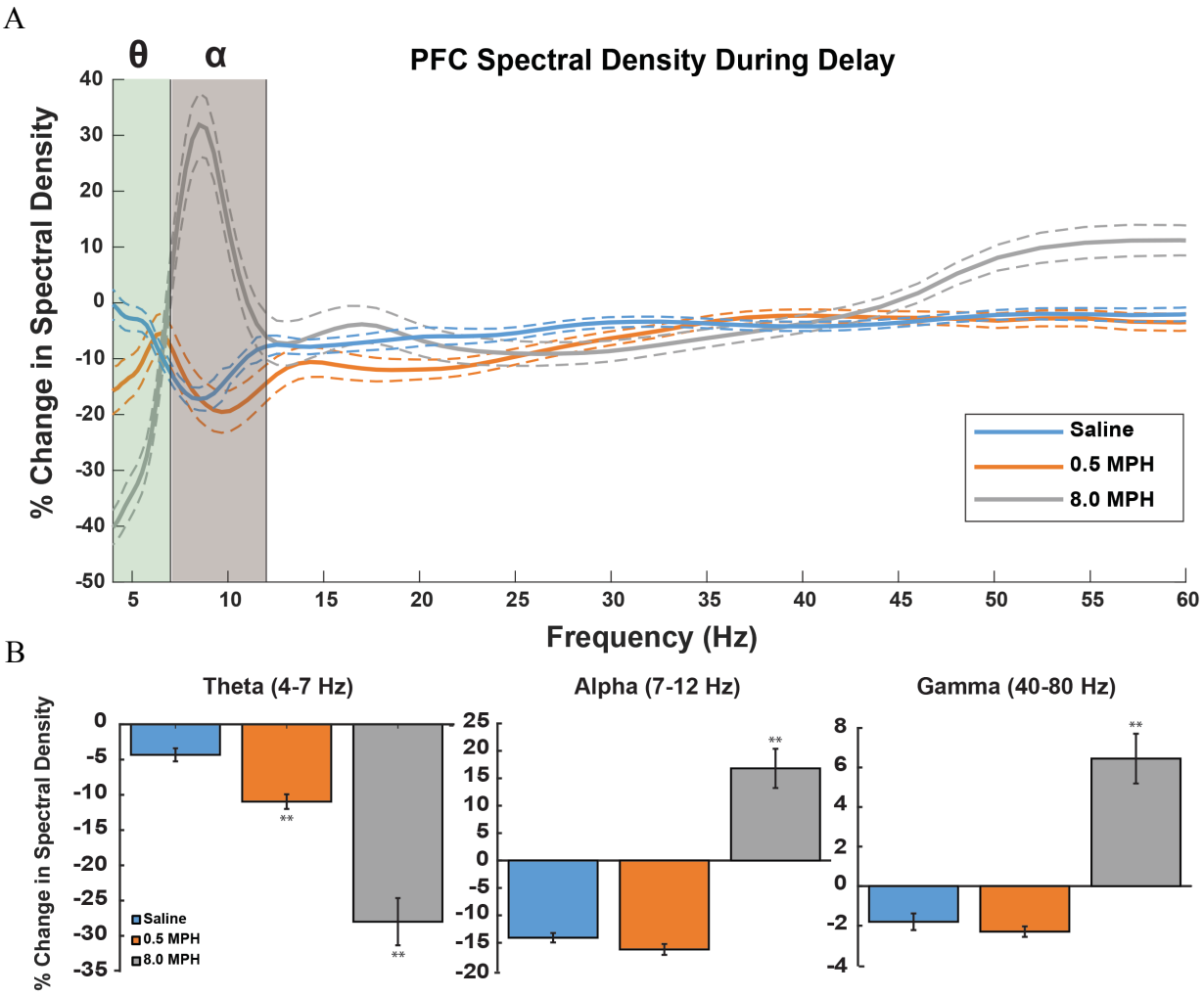

Figure 3. (A) Changes in PFC spectral density over the course of the delay phase in rats performing delayed alternation**. (B)** MPH exerts a dose dependent decrease in theta oscillations in the PFC. Cognition-impairing doses robustly increase alpha and gamma oscillations in the PFC, while Cognition-enhancing doses do not significantly differ from vehicle. $^{**}p<0.01$ compared to vehicle.

Figure 4

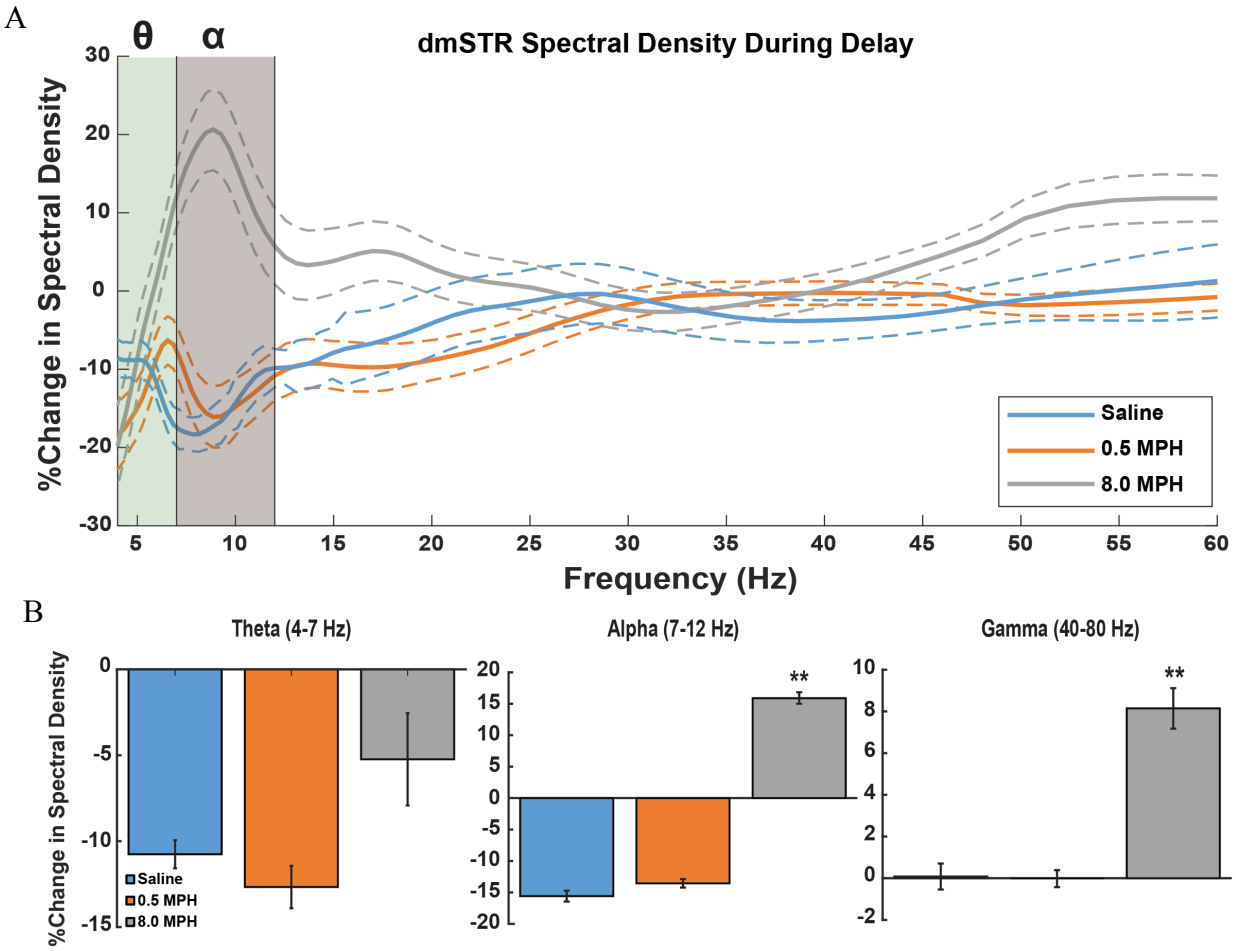

Figure 4. (A) Changes in dmSTR spectral density over the course of the delay phase in rats performing delayed alternation. **(B)** Cognition-enhancing and cognition-impairing doses of MPH have no effect on theta oscillations in the dmSTR. Cognition-impairing doses robustly increase alpha and gamma LFP oscillations in the dmSTR. $^{**}p<0.01$ compared to vehicle.

Supplemental Results

Supplemental Table 1. Population Sizes of Putative Pyramidal Neurons in the dmPFC and Medium Spiny Neurons in the dmSTR Displaying Strong Tuning to Task Events

Phase	**% Strongly Tuned WS PFC cells**	**% Strongly Tuned MS dmSTR**
Delay	14	20
Branch	13	20
Correct Tone	7	10
Reward	9	8

Chapter 5

Summary

The PFC plays a prominent role in the procognitive actions of psychostimulants

Psychostimulants are highly effective at treating ADHD, a disorder associated with frontostriatal dysfunction. Clinically relevant doses of MPH increase catecholamine signaling broadly within this circuit. The clinical literature has emphasized the potential involvement of the striatum in the therapeutic actions of these drugs in ADHD. In contrast, the current studies demonstrate that although sustained attention and working memory involve multiple nodes of frontostriatal circuitry, only MPH action within the dmPFC, and not the vmPFC, dmSTR or vmSTR, was *sufficient* to improve these cognitive processes. The preferential sensitivity of the dmPFC vs. the vmPFC in the procognitive actions of MPH is consistent with a well-known functional topographical organization of the rodent medial PFC, with dorsal regions more closely associated with 'executive' cognitive processes (Heidbreder & Groenewegen, 2003; Vertes, 2004, 2006).

In addition to MPH, α2 agonists and selective NE reuptake inhibitors, two other ADHD-approved medications, have also been shown to act directly in the dorsal PFC to improve working memory (Gamo et al., 2010; Mao et al., 1999; Ramos et al., 2006; Tanila et al., 1996; Wang et al., 2007). Combined, these observations demonstrate a prominent role of the PFC in the cognition-enhancing actions of all treatments approved for ADHD. However, this does not rule out a role for the striatum in the therapeutic effects of psychostimulants. The fact that clinically-relevant doses of psychostimulants elevate DA neurotransmission within the striatum (Berridge et al., 2006; Kodama et al., 2017) may explain why these drugs are more effective than the noradrenergic-selective treatments approved for ADHD, given the striatum is largely devoid of a noradrenergic innervation (Glowinski & Iversen, 1966). Thus, while MPH action in the striatum is not sufficient to improve higher cognitive function, it may nonetheless contribute to the therapeutic effects of psychostimulants in conjunction with actions in the PFC.

Accumulating evidence demonstrates that differing PFC-dependent cognitive processes display varying dose sensitivity to the procognitive actions of psychostimulants (Berridge et al.,

2012). The current studies demonstrate that these divergent dose response curves are recapitulated with intra-PFC infusion of MPH. Thus, whether administered systemically or directly into the PFC, sustained attention was maximally improved at a 4-fold higher dose relative to the maximally improving dose for working memory. At this higher dose, MPH no longer significantly improves working memory (Berridge et al., 2012; Spencer et al., 2012, Chapter 2). Moreover, the magnitude of the cognition-enhancing effect of intra-PFC MPH was virtually identical to that seen with systemic administration of clinically-relevant doses of this drug (Arnsten & Dudley, 2005; Berridge et al., 2006; Devilbiss & Berridge, 2008). Collectively these observations demonstrate the differential dose-sensitivity of these cognitive processes to MPH involves, at least in part, mechanisms contained within the PFC.

Taken together, the current studies provide unambiguous evidence that psychostimulant action within the dmPFC is *sufficient* to promote higher cognitive function as assessed in tests of working memory and sustained attention. In identifying the PFC as a key site of action in the procognitive effects of psychostimulants, this research provides important guiding information for future drug discovery research focused on ADHD. It is hoped further understanding of the neurobiology of the PFC will lead to the identification of novel, non-catecholamine targets for ADHD and other disorders of frontostriatal function (for example, Hupalo & Berridge, 2016).

Receptor mechanisms within the PFC differentially contribute to the procognitive actions of MPH

As detailed above, psychostimulants are typically thought to exert uniform actions across PFC-dependent cognitive functions. However, the work of Sprague and Sleator (Sprague & Sleator, 1977; Tannock et al., 1989) and others has demonstrated that MPH exerts dose dependent actions across cognitive/behavioral processes. For example, working memory and response inhibition display relatively narrow inverted-U dose-dependent facilitation, while behavioral calming and attention-enhancing effects are observed across a broader, right-shifted range of doses (Berridge et al., 2012; Sprague & Sleator, 1977; Tannock et al., 1989; Tannock et al., 1995). NE and DA act directly within the PFC to exert an inverted-U shaped modulation of

working memory performance (Arnsten & Li, 2005; Robbins & Arnsten, 2009), with optimal performance associated with optimal levels of DA acting at D1 receptors and NE acting at high affinity postsynaptic α_{2A} receptors. In contrast, stimulation of lower affinity α1 receptors within the PFC impairs working memory while promoting performance in an attention set shifting task that has a right shifted dose sensitivity to MPH vs. working memory. Consistent with this, the current studies demonstrate that *within the PFC*, α2 and D1, but not α1 receptors are necessary for MPH-induced improvement in working memory. In contrast, MPH-induced improvement of sustained attention (with a right shifted dose sensitivity to MPH) additionally requires activation of α1 receptors in addition to α2 and D1. The current results further demonstrate that PFC α1 receptor stimulation is sufficient to improve sustained attention.

As noted, activation of PFC α1 receptors promote a flexible form of attention, as measured in the attention set shifting task (Lapiz & Morilak, 2006). Based on these and other observations, it was posited that high levels of norepinephrine acting at PFC α1 receptors promote 'flexible' attention at the expense of focused attention (Aston-Jones et al., 2000). However, in our studies, activation of PFC α1 receptors improved sustained/focused attention, identical to that seen in attention set shifting (Lapiz & Morilak, 2006). Collectively, these observations indicate that although α1 receptors differentially regulate distinct PFC-dependent cognitive processes, these differences cannot be ascribed to a *selective* enhancement of attentional (or cognitive) 'flexibility'.

A significant finding of the current studies is that PFC α1 receptors are involved in the procognitive actions of moderately higher doses of psychostimulants. However, whether the facilitation of these α1 sensitive processes contribute to the beneficial (behavioral calming and attention-improving) vs. detrimental (cognitive constriction, over-focusing) actions of psychostimulants and whether this differs across ADHD subtype (Diamond, 2005) remains to be determined. These observations also raise clinical and preclinical questions regarding the degree to which higher doses that maximally control classroom behavior may exert detrimental actions in other functional domains via activation of α1 or other receptors within or outside the PFC.

Nonetheless, our studies demonstrate a role of PFC α1 receptors in the regulation of focused attention, similar to that reported for flexible attention (Lapiz & Morilak, 2006). Therefore, facilitation of α1 receptor signaling may prove useful in the development of attention-enhancing compounds.

Psychostimulants exert complex dose dependent actions on frontostriatal coding during a working memory task

Frontostriatal cognition/behavior is ultimately dependent upon neural coding within frontostriatal regions. The current studies demonstrated that MPH exerts complex, dose dependent actions on frontostriatal signaling. In particular, in the PFC we observed that a high and cognition-impairing dose of MPH potently suppressed activity of neurons strongly tuned to delay (with a trend for reduced reward signaling) while activating PFC neurons not tuned to feedback from a tone signaling reward. Within the dmSTR, high doses of MPH robustly increased activity of neurons not tuned to task events. The simultaneous suppression of delay tuned neurons within the PFC and strengthening of neurons not tuned to task events within the dmSTR impairs goal-related signaling within this dorsomedial frontostriatal circuit. In contrast, a low, cognition-enhancing dose of MPH had little effect on the task-related activity of neurons within either the PFC or dmSTR.

In terms of LFP activity, MPH dose dependently decreased theta (4-7 Hz) within the PFC, an action thought to reflect alterations in effort to maintain working memory (Khader et al., 2010). The fact that both cognition enhancing and cognition impairing doses of MPH decreased low theta was surprising. One possibility is that the modest reduction in low theta following a cognition-enhancing dose of MPH may reflect decreased effort needed to maintain memory (as seen following treatment in ADHD, Clarke et al., 2005), while the robust decrease following a cognition-impairing dose of MPH may indicate a collapse in effort and ability to maintain working memory processes (as seen in stress, Gärtner et al., 2014). Additionally, a cognition-impairing dose robustly increased alpha (7-12 Hz) and gamma (40-80 Hz) oscillations in both the PFC and dmSTR, mimicking elevations seen in ADHD patients. These actions are also

consistent with prior observations that high doses of psychostimulants increase power in both of these frequency bands within the mPFC of rats (Lapish et al, 2012, Berke, 2009). A possible interpretation of this data is that increases in alpha and gamma reflect an increased effort to maintain goal-directed processes Finally, the increase in alpha (7-12 Hz) oscillations following a high, and locomotor activating dose of MPH is consistent with evidence that more generally correlates power in this frequency with motoric movements (Kramis et al., 1975). In contrast, cognition-enhancing doses of MPH had little effect on LFP oscillatory activity. Collectively, these observations provide new insight into the modulation of neuronal activity within the frontostriatal network resulting from various doses of MPH.

Future Directions

Further studies will need to characterize the role of all catecholamine receptors on the cognition-modulating actions of MPH. As detailed in the introduction, in addition to α1, α2 and d1 receptors, catecholamines can bind to D2, β1 and β2 receptors. The current studies focused on α2 α1 and D1 receptors as their role in working memory function has been most extensively studied. However, there is limited evidence that the remaining receptors may also play important, and distinct roles in supporting working memory function. In particular, D2 receptor activation is associated with improvements in working memory tasks in monkeys (Arnsten et al., 1995) and humans (Mehta et al., 2001). There is also limited evidence that beta receptors are involved in higher cognitive functions, with β1 and β2 receptors in the PFC exert opposing actions on working memory; blockade of β1 or stimulation of β2 receptors in the PFC improves working memory (Ramos et al., 2005; Ramos et al., 2008). While this was outside the scope of the current set of experiments, additional studies should determine the role these catecholamine receptors play in the cognition-enhancing actions of psychostimulants *across* PFC-dependent tasks.

The current studies demonstrate that α-1 receptor stimulation in the PFC is sufficient to improve sustained attention to a similar degree as systemic administration of MPH. In order to compare with previous studies, the dose of phenylephrine used was identical to that previously

shown to impair working memory when infused into the PFC (Arnsten et al., 1999). However, in order to better characterize the effects of α-1 receptor stimulation in attentional tasks, a full dose response curve is warranted. It remains to be seen if further attentional enhancement is achievable with higher doses, or if there is an inverted U function, whereby lower and higher doses show diminishing returns, or even impairment.

Future studies should also address the neurocircuitry involved in the cognition impairing actions of high doses of psychostimulants. As detailed above, MPH action directly in the dmPFC of rats was sufficient to improve performance in both working memory and sustained attention tasks. However, unlike that seen with systemic administration, 4-fold and higher doses of psychostimulants did not impair cognitive function when infused into any of the frontostriatal regions investigated. This indicates that high doses of systemically administered psychostimulants impair PFC-dependent cognition 1) via actions outside the frontostriatal circuitry investigated, or 2) require the concurrent activation of multiple brain regions.

Finally, it is interesting that while cognition-impairing doses of MPH showed robust changes in frontostriatal coding, the effects of cognition-enhancing doses were relatively minor. Future work will need to apply different approaches, such as functional and effective connectivity measures and machine learning to identify how the procognitive actions of MPH modulate frontostriatal activity.

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