GLOBAL IMPACT OF ACTIVATION OF VENTRAL TEGMENTAL AREA DOPAMINE NEURONS

by

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Dopamine neurons in the ventral tegmental area (VTA) are dysregulated in numerous psychiatric disorders, including schizophrenia, addiction, and ADHD. VTA dopamine neurons send strong projections to forebrain areas such as the striatum and the prefrontal cortex (PFC) where they mediate various cognitive and affective processes; these processes range from working memory, attention, decision making, action selection to motivation. Despite the importance of VTA dopamine neurons, we have a limited understanding of how dopamine neuron activity modulates downstream neural activity. The current studies, thus, utilized optogenetics in Th::Cre rats to selectively activate VTA dopamine neurons and investigated how phasic VTA dopamine activity modulates global neural activity. Specifically, these studies examined global forebrain functional magnetic resonance imaging (fMRI) activity and local neural activity within PFC during optogenetic activation of VTA dopamine neurons.

The first study demonstrated that phasic VTA dopamine activity increased fMRI responses in several areas, including VTA-innervated limbic regions such as the ventral striatum. Surprisingly, the most prominent fMRI signal increase was observed in the dorsal striatum, which is not traditionally associated with VTA dopamine neurotransmission. These data suggest that there are more robust functional interactions between mesolimbic and non-limbic basal ganglia dopamine circuits than conventionally described. These findings also provide a potential
novel framework for understanding dopamine-dependent functions and interpreting data from human studies that primarily rely on fMRI as a non-invasive measure of neural activity.

The second study revealed that phasic activation of VTA dopamine neurons in freely moving animals elicited heterogeneous patterns of modulation in PFC neuron spiking activity on both transient and sustained timescales. In addition to the modulation of individual unit activity, dopamine stimulation also generated responses at the population level. Furthermore, dopamine neuron activation enhanced the power of PFC high gamma oscillations and phase amplitude coupling between theta and high gamma frequencies in the local field potentials (LFPs). The effects of VTA dopamine activity were also state-dependent as it generated a robust increase in LFP beta but not high gamma oscillations under isoflurane anesthesia. Thus, activation of dopamine neurons elicits diverse effects on PFC network activity that may be relevant for PFC-dependent cognitive processes.
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PREFACE

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1.0 INTRODUCTION

Ventral tegmental area (VTA) dopamine neurons are critically involved in diverse functions ranging from cognition, emotion, motivation to action, and dysfunctions of VTA dopaminergic neurotransmission have been implicated in psychiatric disorders such as schizophrenia, ADHD, depression, and addiction (Nestler & Carlezon Jr 2006, Robbins & Arnsten 2009, Salamone & Correa 2012, Seamans & Yang 2004, Viggiano et al 2003, Wise 2004). Decades of work on dopamine systems, including the VTA, have provided valuable insight into activation patterns of dopamine neurons, functions mediated by their activity, projection targets of these neurons, and downstream effects of dopamine release (Bjorklund & Dunnett 2007, Fields et al 2007, Grace et al 2007, Robbins & Arnsten 2009, Schultz et al 1997, Swanson 1982). However, a unified theory on how dopamine activity and subsequent dopamine release modulates downstream neural activity, globally in the brain and within specific regions such as prefrontal cortex (PFC) and striatum, is lacking. Furthermore, while human studies on dopamine function have been conducted in parallel to animal studies, a clear link between animal research that directly probes neural function via invasive methods and human research that relies heavily on hemodynamic measures such as blood-oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) needs to be established. This link will be crucial in applying the findings of basic
dopamine research to solving a plethora of psychiatric and neurological disorders including schizophrenia, addiction, Parkinson’s, Huntington’s, depression, and ADHD.

This dissertation addresses a broad overarching question: how does VTA dopamine activity modulate global neural activity in the forebrain? Specifically, the dissertation is concerned with two sub-questions: how does VTA dopamine activity a) modulate global neural activity, measured as hemodynamic signals with fMRI? and b) modulate spontaneous network activity in medial prefrontal cortex (mPFC)? Before delving more into the purpose and details of the dissertation, the present state of research on dopamine systems in the mammalian forebrain, with special emphasis on the PFC and striatum, is discussed in the following sections. Most of the dopamine neuroanatomy and function summarized in the introduction is focused on the rodent brain, but non-human primates and humans are also considered.

### 1.1 NEUROANATOMY OF MIDBRAIN DOPAMINE SYSTEM

#### 1.1.1 Dopamine in the mammalian brain

Dopamine is one of the mostly extensively studied monoamines in the brain. Over the past few decades, it has been studied in the context of almost all higher cognitive and emotional functions as well as psychiatric disorders and many neurological disorders. This saga started with the discovery of dopamine as a neurotransmitter in the 1960’s by Arvid Carlsson (Carlsson et al 1962). Subsequent efforts by many researchers to label dopamine producing neurons have resulted in the identification of at least 10 dopamine containing nuclei (A8-A17) in the
mammalian brain (Bjorklund & Dunnett 2007, Tritsch & Sabatini 2012). Of these nuclei, the two regions that have been most studied are VTA (A10) and substantia nigra compacta (SNc) (A9). VTA and SNc dopamine neurons have typically been studied in different behaviors/contexts: VTA in reward-related behaviors and consequently in disorders such as addiction, depression, and schizophrenia (Salamone et al 2016, Wise 2004), while SNc in motor functions and disorders such as Parkinson’s (DeLong 1990, Smith & Villalba 2008).

1.1.2 Projection patterns of midbrain dopamine neurons

Conventionally, projections of different midbrain dopamine nuclei to forebrain areas are considered to be distinct, with the notion that the A10 nucleus primarily projects to the PFC and the ventral striatum (VS) or nucleus accumbens (NAc) via the mesocorticolimbic pathway and the A9 nucleus preferentially innervates the dorsal striatum (caudate-putamen) (DS) via the nigrostriatal pathway. However, strictly speaking, there is considerable overlap of projections of midbrain nuclei as VTA dopamine neurons also project to the ventro-medial head of caudate, and SNc dopamine neurons send some cortical projections (Bjorklund & Dunnett 2007). While the relationship between VTA and SNc activity and the gradient of functional/anatomical connectivity between these regions is a topic that will be revisited multiple times, this dissertation will primarily focus on VTA dopamine neurons and terminal regions innervated by VTA, including the PFC and striatum.
1.1.3 Architecture of VTA: heterogeneity in VTA neurons

Although VTA is typically studied as one dopaminergic entity, it is not a homogenous structure. It is divided into sub-regions, which include the parabrachial pigmented nucleus (PBP) located laterally, paranigral nucleus (PN) located ventrally, and three other smaller midline nuclei (Morales & Margolis 2017); these VTA sub-regions seem to exhibit differences in cellular heterogeneity. VTA not only contains dopamine neurons (~60%) but also GABA (~35%) and glutamate neurons (2-3%) (Nair-Roberts et al 2008). VTA also contains combinatorial neurons that produce a combination of these different neurotransmitters (dopamine and glutamate, dopamine and GABA, and glutamate and GABA); the exact proportions of all these mixed cellular types are not entirely known (Morales & Margolis 2017). While neurons in the PBP and PN are mostly dopamine neurons and contain most of the dopamine release/regulation machinery such as dopamine transporter (DAT), vesicular monoamine transporter-2 (VMAT2) and D2 receptors, neurons in the midline nuclei seem to be more heterogeneous. Many neurons in the midline sub-regions express tyrosine-hydroxylase (TH) but not VMAT2, D2, and DAT. Further, most of the glutamate only neurons and combinatorial glutamate and dopamine neurons are concentrated along the midline and sparsely in the PBP, which forms most of the lateral VTA (Morales & Margolis 2017). While the current studies in this dissertation did not examine differences between VTA sub-regions, in view of the cell type diversity, they utilized a transgenic line of Th::Cre rats so as to exclusively target TH-containing VTA dopamine neurons.
1.2 TYPES AND DISTRIBUTION OF DOPAMINE RECEPTORS

1.2.1 Types of dopamine receptors and intracellular signaling mechanisms

Dopamine exerts its effects via two classes of receptors—D1 like and D2 like. D1 family contains D1 and D5 receptors while the D2 family consists of D2, D3, and D4 receptors. All dopamine receptors are metabotropic G-protein coupled receptors (GPCRs), which means that their effects are much slower compared to ionotropic receptors utilized by glutamate and GABA neurotransmitters (Tritsch & Sabatini 2012). D1 and D2 receptors have differential affinity for dopamine (D2 has a higher affinity than D1 receptors) and are thought to generally produce antagonistic effects (Seamans & Yang 2004). For example, stimulation of D1 and D2 receptors initiates opposite protein kinase-A (PKA)-coupled signaling cascades. D1 receptor stimulation activates $G_\alpha_s$ and $G_\alpha_{olf}$, which are positively coupled to cyclic AMP (cAMP) and PKA production, while D2 receptors activate $G_\alpha_i$ and $G_\alpha_o$, which negatively influence levels of PKA. By acting on PKA, these receptors can phosphorylate various ion channels, glutamate and GABA receptors, and transcription factors (Neve et al 2004, Tritsch & Sabatini 2012). Dopamine receptors can also modulate intracellular calcium levels and voltage and ligand gated channels via PKA independent pathways (Tritsch & Sabatini 2012).

1.2.2 Distribution of dopamine receptors

Of the dopamine receptors, D1 and D2 are expressed much more abundantly than D3, D4, and D5 receptors (Tritsch & Sabatini 2012), and the distribution of D1 and D2 receptors varies by
dopamine terminal region. A classic view about anatomical and functional architecture in the striatum is that D1 and D2 containing neurons are segregated into neuronal groups that form direct and indirect pathways. D1 is primarily expressed in striatal projection neurons (SPNs, also called medium spiny neurons or MSNs, which are principal neurons in the striatum) that terminate in substantia nigra reticulata (SNr, striatonigral or direct pathway), while D2 is primarily expressed in SPNs projecting to the globus pallidus externa (GPe, striatopallidal or indirect pathway) (Gerfen et al 1990, Mink 1996). However, recent studies suggest that D1 and D2 striatal circuits are not completely separated. There is emerging evidence of cross talk between these pathways (Calabresi et al 2014). Further, a distinct population of MSNs co-expresses D1 and D2 receptors, and these MSNs are potentially differently affected by dopamine activity compared to MSNs in direct and indirect pathways. About 2% of MSNs in the DS co-express D1 and D2 receptors, while a much higher frequency of these dual MSNs exists in the VS (7-14%), especially in the shell region of NAc (Gagnon et al 2017). The anatomical separation of D1 and D2 pathways in the VS has also been debated recently, as approximately 50% of cells in the dorsal ventral pallidum (VP, that is thought to be part of the indirect pathway in the ventral stream) receive inputs from D1 expressing MSNs while 89% receive inputs from D2 expressing MSNs in the NAc core (Kupchik et al 2015). Aside from MSNs (over 90% of which express D1 and D2 receptors), interneurons in the striatum also contain dopamine receptors. Cholinergic interneurons primarily contain (over 80%) D2 and D5 receptors whereas 50-70% of parvalbumin (PV)+, calretinin (CR)+, and neuropeptide (NPY)/somatostatin(SOM)/nitric oxide synthase (NOS) co-positive interneurons primarily express D5 receptors (Tritsch & Sabatini 2012). Another important feature of dopamine receptor distribution in the striatum is the
presence of presynaptic D2 receptors in dopamine terminals that might serve auto-receptor functions (Sesack et al 1994).

In the cortex, D1 and D2 receptors are intermixed among pyramidal neurons and interneurons are not as well segregated into different pathways. DA receptors are expressed in only a fraction of all pyramidal neurons (which are the principal projection neurons in the cortex), and D1 receptors are more abundant (~20% in layer 2/3, ~20-40% in layer 5 and ~30-60% in layer 6 pyramidal neurons) than D2 receptors in these neurons (Santana et al 2009). DA receptors are more widespread in interneurons; most of the interneurons that express DA receptors are PV+ fast-spiking (FS) interneurons, where up to 60% of PV+ neurons can express D1 receptors (Le Moine & Gaspar 1998, Tritsch & Sabatini 2012). About 25% of calbindin (CB)+ interneurons also express D1 receptors. D2 receptors are sparser and are mostly limited to PV+ FS interneurons (Le Moine & Gaspar 1998, Tritsch & Sabatini 2012). Although anatomical and functional segregation of D1 and D2 pathways has not been systematically tested in the PFC as in the striatum, a recent study suggested that D1/D2 receptors in PFC might be preferentially expressed on pyramidal neurons projecting to different output regions and might thus mediate different behavioral functions (Jenni et al 2017).

1.3 MODES OF DOPAMINE NEURON FIRING AND EXTRACELLULAR DOPAMINE LEVELS

Dopamine neurons exhibit two modes of spontaneous firing in anesthetized and awake animals: single spiking and burst firing. When dopamine neurons fire in single spike mode in vivo, they
emit spikes steadily but at an irregular interval with frequencies between 1 – 9 Hz. In the bursting mode, dopamine neurons fire a few spikes (typically 2-10 in anesthetized rats and up to 23 spikes in freely moving rats) with short inter-spike intervals that correspond to frequencies >10 Hz (Freeman et al 1985, Grace & Bunney 1984a, Grace & Bunney 1984b). While 20 Hz is considered the average intraburst frequency (Grace & Bunney 1984a), dopamine neurons can fire bursts with frequencies between 10 – 100 Hz and occasionally above 100 Hz (Hyland et al 2002, Kiyatkin & Rebec 1998). These two modes of firing in dopamine neurons are commonly referred to as tonic versus phasic firing (Grace 2016), although tonic and phasic modes of dopamine neurotransmission have been interpreted differently over the years. These terms were first used to describe two types of dopamine release in terminal regions, with phasic dopamine release referring to phasic or burst firing-driven transient surge of dopamine in the synaptic cleft and tonic dopamine release referring to spike-independent extrasynaptic release of dopamine driven by presynaptic NMDA activation by glutamatergic afferents. The latter mode of release would maintain sustained “background” extracellular levels of dopamine ([DA]₀) (Grace 1991, Nieoullon et al 1978). Subsequently, the definition of tonic and phasic dopamine transmission has been modified to relate post-synaptic dopamine levels to modes of dopamine firing (Grace 2016). The currently prevalent idea is that low “tonic” firing rates of dopamine neurons combined with various local factors, including cholinergic activation and NMDA activation via glutamatergic afferents (Cachope & Cheer 2014, Threlfell et al 2012), maintain background [DA]₀ in terminal regions in baseline conditions. Tonic [DA]₀ also depends on the number of dopamine neurons firing spontaneously, which is regulated by GABAergic inputs from areas such as the VP (Floresco et al 2003). These sustained background dopamine levels are typically detected by microdialysis measures (Grace 2016). In the presence of behaviorally salient stimuli
such as rewards and reward-predictive stimuli, dopamine neurons fire “phasically” by emitting transient bursts that last for several hundred milliseconds (Grace 2016, Schultz et al 1997) and that depend on glutamatergic and cholinergic inputs from areas such as the pedunculopontine tegmental nucleus (Floresco et al 2003). These bursts elicit phasic dopamine release transients in terminal regions (Garris et al 1994, Garris & Wightman 1994, Lavin et al 2005, Williams & Millar 1990) that can be detected by fast-scan cyclic voltammetry (FSCV) (Phillips et al 2003, Stuber et al 2008, Stuber et al 2005, Wanat et al 2009).

While the description of dopamine activity patterns as tonic and phasic has been very helpful in understanding dopamine function and in designing experiments to manipulate one type of activity versus another to affect specific functions (Bass et al 2013, Tsai et al 2009), one aspect of dopamine neuron firing that is not emphasized in the literature is that the spontaneous activity of dopamine neurons is not solely comprised of the slow irregular “tonic” firing both in anesthetized and awake states (Freeman et al 1985, Grace & Bunney 1984a). Especially in freely moving animals, dopamine neurons fire a lot more spontaneous spikes in bursts compared to the anesthetized condition (Freeman et al 1985), possibly because of the presence of many “uncontrolled” stimuli in the environment that can activate afferents to dopamine neurons; so spontaneous dopamine firing switches between tonic and burst modes of activity. Thus, spontaneous background $[DA]_0$ in terminal regions, especially in awake animals, may be maintained by both modes of firing. Phasic dopamine activity in response to behaviorally salient stimuli could simply elicit a big surge of dopamine over the background release because of the simultaneous bursting of many dopamine neurons.
1.4 FUNCTION OF ACTIVITY OF DOPAMINE NEURONS

1.4.1 Movement

Dopamine neurons in the SNc and their projections to the DS are critical in proper motor function as a) severe movement deficits in Parkinson’s disease is primarily associated with death of SNc dopamine neurons, and b) lesions of dopamine neurons in the nigrostriatal system can produce Parkinsonian symptoms (Carlsson 1964, Dauer & Przedborski 2003, Schultz 1982, Schultz 2007). Recently, sub-second phasic activity transients have also been detected in dopamine neuron axons in DS during locomotion, and transient optogenetic stimulation of these axons can rapidly initiate movement (Howe & Dombeck 2016).

How dopamine release in the striatum may impact movement is typically explained in relation to the expression of dopamine receptors in the direct and indirect pathways (discussed in section 1.2.2). The classic basal ganglia model postulated that activation of direct pathway MSNs facilitates movement while that of the indirect pathway MSNs inhibits movement (Alexander & Crutcher 1990, Kravitz & Kreitzer 2012). By increasing the excitability of direct pathway MSNs via D1 receptors and decreasing the excitability of indirect pathway MSNs via D2 receptors, dopamine is thought to facilitate movement (Kravitz & Kreitzer 2012). It is unclear what function MSNs that co-express D1 and D2 receptors mediate compared to selective D1 and D2 MSNs (Kravitz & Kreitzer 2012). Further, it is recently being appreciated that the basal ganglia circuitry might be more complicated than predicted by initial D1/D2 models, and that the coordinated activity between direct and indirect circuits might be necessary for proper movement initiation and execution (Calabresi et al 2014, Tecuapetla et al 2016).
Most studies investigating dopamine’s role in movement have focused on the DS, but there is some evidence for the involvement of mesoaccumbens projections in motor function as well, as local infusion of dopamine, D1 agonists, and amphetamine in the NAc induces an increase in locomotion (Cador et al 1995, Swanson et al 1997). Whether D1 and D2 pathways provide functionally segregated contributions to VS’s involvement in movement is not clear.

1.4.2 Reinforcement

One of the most popular functions ascribed to dopamine is its role in reward or pleasure; this notion originated from the classic experiment conducted by Olds and Milner in the 1950’s, which showed that electrical stimulation of certain regions of the brain such as the septum was highly reinforcing (Olds & Milner 1954). Subsequent studies suggested that catecholamines, including dopamine, might be important in driving intra-cranial self-stimulation (ICSS) for several reasons: a) the medial forebrain bundle (MFB), which contains midbrain dopamine projections ascending to striatal and cortical areas, is one of the regions that maximally supports ICSS, and b) dopamine directly enhances ICSS behavior (German & Bowden 1974, Jacques 1979, Kravitz & Kreitzer 2012, Redgrave 1978). Recently, optogenetic studies have directly confirmed dopamine’s role in reinforcement as dopamine neuron activation in VTA supports conditioned place preference and ICSS behavior (Adamantidis et al 2011, Tsai et al 2009, Witten et al 2011). While a much greater emphasis is placed on mesolimbic circuit’s involvement in reward and reinforcement, optogenetic stimulation of SNc dopamine neurons also robustly supports ICSS behavior (Ilango et al 2014). Recent studies have also shown that D1 and D2 pathways serve opposing roles in reinforcement in VS and dorso-medial striatum (Kravitz &
Kreitzer 2012, Kravitz et al 2012, Lobo et al 2010). Further, the reinforcing effects observed with ICSS could be explained in terms of incentive salience and learning rather than the rewarding effects of dopamine itself, per say. Thus, dopamine’s putative role in motivation and learning will be discussed in the following two sub-sections.

1.4.3 Motivation and salience

An emerging view of dopamine’s function is that dopamine doesn’t directly induce pleasure but rather modulates cue-triggered incentive salience (termed “wanting” behavior) that motivates animals to approach and work for rewards (Berridge 2007, Berridge & Kringelbach 2015). Along those lines, studies have shown that blockade of mesolimbic dopamine neurotransmission doesn’t impair food consumption but rather prevents animals from exerting effort to acquire food rewards (Salamone & Correa 2012, Salamone et al 2016).

The fact that mesolimbic dopamine neurons are not simply “reward neurons” is also evident from electrophysiological studies that have shown that dopamine neurons respond to aversive stimuli, such as restraint stress (Anstrom & Woodward 2005), footshock (Brischoux et al 2009), and tail pinch (Zweifel et al 2011). Further, there are distinct populations of dopamine neurons that encode motivational value and salience (Bromberg-Martin et al 2010, Matsumoto & Hikosaka 2009b). Value encoding neurons respond with opposite valences to rewarding and aversive stimuli and potentially drive seeking of positive outcomes and avoiding aversive outcomes. On the other hand, salience encoding neurons respond positively to both types of stimuli and potentially modulate orienting/alerting responses as well as increase general motivation/arousal (Bromberg-Martin et al 2010, Matsumoto & Hikosaka 2009b).
1.4.4 Learning

Dopamine neurons respond phasically via transient bursts to reward-predictive stimuli and unexpected reward delivery (Matsumoto & Hikosaka 2009b, Schultz 1998, Schultz et al 1997). Influential theories suggest these phasic responses serve as teaching signals, as these phasic signals code the discrepancy between predicted rewards and actual rewards, termed the reward prediction error, that is thought to guide learning (Montague et al 1996, Schultz 1998, Schultz 2013). The role of transient VTA dopamine activity in serving as a teaching signal has recently been directly supported by an optogenetics study (Steinberg et al 2013).

Dopamine neurons can guide aversive learning as well. They can partially encode aversion prediction errors, especially neurons that respond positively to cues that predict aversive outcomes (Matsumoto & Hikosaka 2009b). Activation of VTA dopamine neurons is also necessary for conditioning to a cue that predicts an aversive outcome (Zweifel et al 2011). Further, pairing of cues with phasic dopamine release in mPFC enhances subsequent learning of the relationship between those cues and rewarding/aversive outcomes, suggesting that dopamine activity can assist learning by increasing saliency of cues (Popescu et al 2016).

In addition to mediating transient learning of associations between cues and outcomes, dopamine can impact long-term consolidation. Dopamine antagonists administered either systemically or directly into PFC and striatum minutes after behavioral training can disrupt memory consolidation, demonstrating the necessity of post-training dopamine signaling in learning (Dalley et al 2005, Izquierdo et al 2007, Setlow & McGaugh 1998).
1.4.5 Frontal executive functions

Dopamine activity in PFC, that depends mostly on mesocortical projections from VTA dopamine neurons, is critical for many executive functions, including working memory, attention, behavioral flexibility, and probabilistic decision making; and these functions involve a complex interaction of different dopamine receptors (Chudasama & Robbins 2004, Floresco et al 2006, Granon et al 2000, Puig et al 2014, Ragozzino 2002, Sawaguchi & Goldman-Rakic 1991, St Onge et al 2011). Generally, it seems that an optimal level of dopamine and D1 receptor activation in PFC supports optimal behavioral performance, as there is an inverted U relationship between the amount of D1 receptor activation and cognitive functioning (Goldman-Rakic et al 2000, Seamans & Yang 2004). Consequently, when PFC dopamine levels are low such as in disorders including schizophrenia and Parkinson’s, D1 agonists can improve cognitive performance; when PFC dopamine levels are high due to stress, for example, D1 agonists impair while D1 antagonists improve behaviors such as working memory and attention.

1.5 DOPAMINE MODULATION OF NEURAL ACTIVITY IN STRIATUM

Dopamine has been shown to modulate a dozen different synaptic and cellular mechanisms in complex ways, and thus it has been difficult to decipher the exact nature of dopamine modulation of downstream neural activity. However, generally, they can act presynaptically to modulate the release of neurotransmitters. Postsynaptically, they can alter receptor properties to influence neurotransmitter detection or modulate synaptic integration and excitability by affecting various
ionic conductances (Tritsch & Sabatini 2012). For the sake of simplicity, the discussion in this and the following section will be limited to the modulation of excitability (measured in in-vitro studies or intracellular in-vivo studies) and spiking output of striatal neurons.

Striatum receives the densest innervation from midbrain dopamine neurons (Bjorklund & Dunnett 2007) and has the highest concentration of dopamine and dopamine receptors (Boyson et al 1986). While the initial view about dopamine modulation of striatal activity was that dopamine has an inhibitory effect on striatal neuronal excitability (Nicola et al 2000), a more recent and widely accepted perspective is that dopamine via D1 receptors increases and via D2 receptors decreases the excitability and spiking of striatal neurons (Gerfen & Surmeier 2011, Tritsch & Sabatini 2012). A closer look at the in vitro, in vivo (anesthetized), and in vivo (awake) studies does not necessarily paint such a coherent picture of dopamine activity modulation of striatal activity. However, a piece of the puzzle that emerges from the pool of contradictory studies is that dopamine may serve to enhance signal to noise ratio in the striatum.

1.5.1 In vitro studies

In vitro studies generally support the hypothesis that dopamine bi-directionally modulates the excitability of striatal MSNs. The membrane potential of striatal neurons fluctuates between a depolarized state termed the “up state”, that is driven mostly by excitatory inputs and during which neurons emit action potentials, and a hyperpolarized state termed the “down state”. Via modulation of K+ and Ca2+ conductances, D1 receptor activation increases the threshold for up-state transitions while increasing the duration of up-states and depolarization and firing during those states, so effectively increasing signal to noise ratio (Plotkin et al 2011, Tritsch & Sabatini
2012). On the other hand, D2 receptor activation facilitates up-state transitions but decreases the duration of and depolarization during those states (Plotkin et al 2011, Tritsch & Sabatini 2012). This fairly consistent view is only tempered by the fact that D1 activation also reduces voltage gated Na+ currents, which might somewhat decrease the excitability of striatal neurons (Tritsch & Sabatini 2012). Further, this simplified view of post-synaptic effects of dopamine neuron activation is complicated by evidence that dopamine axons in the striatum co-release glutamate and GABA, which can elicit excitatory and inhibitory effects respectively, on both direct and indirect pathway SPNs (Tecuapetla et al 2010, Tritsch et al 2012).

1.5.2 In vivo studies in anesthetized animals

Initial in vivo studies in anesthetized animals suggested that dopamine decreases the firing rate of striatal neurons in vivo (Nicola et al 2000, Siggins 1978, Yang & Mogenson 1984). Subsequent studies contradicted this finding by demonstrating that burst stimulation of the MFB elicits a slightly delayed excitatory response in striatal neurons that is D1 receptor dependent (Gonon 1997). Similarly, in support of the bidirectional modulation by D1/D2 receptors hypothesis, West and Grace (2002) showed in intracellular recordings in vivo that dopamine enhances excitability (assessed via measures such as resting membrane potential, membrane potential during up states, and the amount of current necessary to depolarize and induce spiking in neurons) of SPNs via D1 receptor activation and decreases their excitability via D2 receptor activation. Excitatory post-synaptic potentials (EPSPs) evoked by PFC stimulation were also enhanced by D2 blockade and reduced by D1 blockade in the striatum (West & Grace 2002), further bolstering the D1/D2 hypothesis. Tonic and phasic dopamine modulation might elicit
slightly different effects, as VTA burst stimulation (rather than tonic modulation of dopamine receptor activity as in the West and Grace (2002) study) increases depolarization of NAc neurons to an “up” like state but decreases firing during the up-state, and these up-states are maintained synergistically by both D1 and D2 receptors (Brady & O'Donnell 2004, Goto & O'Donnell 2001). Furthermore, VTA burst stimulation dampens PFC-stimulation induced EPSPs in NAc neurons by activating D2 but not D1 receptors (Brady & O'Donnell 2004). This dampening of afferent inputs to NAc while enhancing depolarization of NAc neurons might serve to filter out noise and increase the signal of relevant information, thus effectively increasing contrast.

1.5.3 In vivo studies in awake animals

Studies from awake animals do not necessarily lend support to the straightforward view about D1/D2 modulation of striatal neural activity. Initial studies recording single unit activity in the striatum of awake animals demonstrated that dopamine via D1 receptors decreases basal activity of striatal neurons as well as afferent-excitation induced firing of these neurons modeled as activity evoked by behaviorally relevant stimuli or by application of glutamate; however, the decrease of spontaneous activity is greater than that of evoked activity, implying a modulation of signal to noise ratio by dopamine (Kiyatkin & Rebec 1999, Nicola et al 2000, Rolls et al 1984). In contrast, recently it was shown that both D1 and D2 receptor blockade decreases firing rate of neurons in the DS along with decreased locomotion (Burkhardt et al 2009). While dopamine modulation of interneuron activity in the striatum has been relatively understudied, there is some evidence that dopamine increases the firing of FS interneurons via activation of D2 receptors in freely moving animals (Wiltschko et al 2010).
1.6 DOPAMINE MODULATION OF NEURAL ACTIVITY IN PFC

1.6.1 Architecture/anatomy of dopamine system in PFC

Mesocortical dopamine systems were discovered in the 1970’s by Ann Thierry and colleagues (Berger et al 1974, Thierry et al 1973). Dopamine projections to PFC primarily terminate in deep layers 5 and 6 (Berger et al 1991), and these projections can make direct synaptic connections with both pyramidal neurons and interneurons (Carr et al 1999, Carr & Sesack 2000, Sesack et al 1995a, Sesack et al 1998b, Sesack et al 1995b). Some of these synaptic contacts lack postsynaptic specializations (Carr et al 1999, Seguela et al 1988), which may contribute to dopamine’s actions in PFC via volume transmission. Another feature of dopamine architecture in PFC that may contribute to volumetric transmission is the predominant extra-synaptic localization of dopamine receptors (Caille et al 1996, Smiley et al 1994b, Yung et al 1995). Dopamine neurons largely make synaptic contacts with distal dendrites in both pyramidal neurons and interneurons in rats and primates (Carr et al 1999, Carr & Sesack 2000, Sesack et al 1995b), which is consistent with the supposed modulatory function of dopamine, as changes in ionic conductances at distal dendrites can only weakly affect somal activity. Even though there are fewer synaptic contacts onto GABA neurons compared to pyramidal neurons (Sesack et al 1995b), most of these direct GABA connections are specifically with PV+ FS interneurons (Sesack et al 1995a, Sesack et al 1998b). This suggests that dopamine might be able to influence pyramidal cell activity more strongly by modulation of PV+ interneurons, as these interneurons can directly inhibit the soma of pyramidal cells.
1.6.2 Dopaminergic modulation of neuronal PFC activity

After many decades of research on dopamine modulation of PFC activity, the nature of the relationship between dopamine activity and PFC neuronal activity has remained controversial. However, a general summary of findings from in vitro and in vivo studies is presented below.

1.6.2.1 In vitro studies

In vitro studies have shown that dopamine modulates intrinsic plasticity by affecting various ionic currents that control neuronal excitability. While there are some inconsistent findings (Gulledge & Jaffe 1998, Moore et al 2011), many studies have generally supported the idea that dopamine increases the intrinsic excitability of pyramidal neurons (increase the probability of spiking when depolarizing inputs are present) via D1 receptor activation (Buchta et al 2017, Gorelova & Yang 2000, Gulledge & Jaffe 2001, Lavin & Grace 2001, Penit-Soria et al 1987, Yang & Seamans 1996) and also amplifies NMDA mediated increases in cell excitability (Tseng & O'Donnell 2004, Wang & O'Donnell 2001). Similarly, dopamine has been shown to consistently increase the excitability of FS interneurons (Gorelova et al 2002, Kroner et al 2007, Seamans & Yang 2004, Zhou & Hablitz 1999).

A simplified view of dopamine modulation of PFC activity based on in vitro findings is summarized below. Dopamine seems to act via early activation of D2 receptors followed by late activation of D1 receptors (Seamans et al 2001). In the early D2 dominated state, D2 receptor activation decreases miniature inhibitory post-synaptic currents (mIPSCs) in FS interneurons and sensitivity of GABA-A receptors, thus decreasing evoked IPSCs (eIPSCs) in PFC pyramidal cells (Seamans et al 2001). The excitability of pyramidal neurons by D2 activation is either
decreased (Gulledge & Jaffe 1998, Tseng & O'Donnell 2004), increased (Moore et al 2011) or not affected at all (Gorelova & Yang 2000). As a result, in the D2 state, synaptic inputs have easy access to pyramidal neurons, but the peaks in activity induced by those inputs are small. Thus, the D2 dominated state might facilitate exploration as many inputs can be represented by PFC neurons, albeit weakly. On the other hand, in the late D1 dominated state, dopamine increases the intrinsic excitability of interneurons (Gorelova et al 2002, Zhou & Hablitz 1999) and slightly augments GABA-A receptor sensitivity, resulting in enhanced eIPSCs in pyramidal neurons (Seamans et al 2001). Furthermore, D1 receptor activation increases intrinsic excitability (Yang & Seamans 1996) and reduces intrinsic inhibition (Buchta et al 2017) in pyramidal neurons. D1 activation also reduces high threshold calcium spikes in apical dendrites of pyramidal neurons (Yang & Seamans 1996). Because of inhibition of calcium dendritic currents and enhancement of eIPSCs at the soma, long-range low threshold inputs from other cortical areas, for example, might have difficulty accessing PFC networks. However, robust inputs, either from local recurrent networks or from long range connections, that can cross the initial filter will be strongly represented because of the increased intrinsic excitability of pyramidal neurons. Computational biophysical models have supported these ideas and led to the generation of a dual state theory of dopamine modulation of PFC activity (Durstewitz & Seamans 2008, Seamans et al 2001, Seamans & Yang 2004). According to this theory, D1 dominated states support robust online maintenance of information, such as during working memory, while D2 states facilitate exploration and flexible switching between different representations, such as during initial learning. These evidence and ideas have also been interpreted to suggest that dopamine modulates the signal to noise ratio of cortical networks (Cohen et al 2002, Winterer & Weinberger 2004).
1.6.2.2 In vivo studies (non-human primates)

In monkey studies that employed extracellular recordings of neural activity in PFC, iontophoretic application of dopamine has been shown to primarily enhance both spontaneous activity and task-related activity, especially during movement related epochs and delay periods (Sawaguchi 2001, Sawaguchi & Matsumura 1985, Sawaguchi et al 1986, Sawaguchi et al 1988). Most of these effects were shown to be D1 dependent, and D2 usually did not elicit an effect, except general inhibition of all activity in one study (Williams & Goldman-Rakic 1995). Puig and colleagues, on the other hand, have demonstrated that D1 and D2 receptors work synergistically to enhance neural selectivity to preferred directions in a visuomotor learning task, mostly by suppressing firing to non-preferred directions (Puig & Miller 2012, Puig & Miller 2015). Several studies have also indicated a U-shaped response profile of neurons to preferred and non-preferred directions in the presence of D1 agonists/antagonists (Williams & Goldman-Rakic 1995) such that an optimal level of D1 activation is necessary for proper spatial tuning of PFC neurons.

1.6.2.3 In vivo studies (rodents)

Early in vivo recording studies in rodents demonstrated that activation of mesocortical projections has a powerful inhibitory influence on PFC activity. Initially, Bunney and colleagues demonstrated in awake animals that mPFC neurons respond to iontopheretic injections of dopamine by marked inhibition (Bunney & Aghajanian 1976). In ketamine-anesthetized animals, single pulses of VTA activity also transiently inhibit the firing of a majority of PFC neurons within milliseconds (Ferron et al 1984). Many subsequent studies replicated these findings and also indicated that the inhibitory effects depend more on activation of D2 receptors than D1
receptors (Godbout et al 1991, Sesack & Bunney 1989), with some involvement of GABAergic receptors (Pirot et al 1992a). While these initial studies led to the prevalent idea that dopamine inhibits PFC pyramidal neuron activity, dopamine’s effects appear to be much more complex. Some additional properties of the nature of dopamine modulation of PFC activity are discussed below.

Dopamine doesn’t always suppress firing in pyramidal neurons. Instead, it enhances signal to noise ratio in PFC neurons of anesthetized animals by suppressing their background firing but enhancing acetylcholine induced firing (Yang & Mogenson 1990). Intracellular recording studies have indicated that VTA burst stimulation elicits sustained depolarization or “up states” in bi-stable PFC neurons in a D1 dependent manner but reduces spiking during the up states (Lewis & O'Donnell 2000, Seamans et al 2003), which might serve as another method of filtering out irrelevant background activity. Other studies have shown timescale dependent modulation of PFC activity in that VTA stimulation initially induces EPSPs in pyramidal neurons, via glutamate receptor activation, and elicits either a delayed and long-lasting decrease in spontaneous firing of bi-stable PFC neurons or a prolonged enhancement of evoked firing in most PFC neurons (Lavin et al 2005).

The effect of dopamine on the activity of FS interneurons in vivo has been relatively understudied. Congruent with the initial hypothesis of dopamine’s actions in PFC and with evidence from many in vitro studies on dopamine’s enhancement of FS interneuron excitability, the findings of Tseng and colleagues indicated that NMDA stimulation of VTA increases the firing of PV+ FS interneurons, and this increase precedes the inhibition of firing of most pyramidal neurons (Tseng et al 2006). This result, however, was contradicted by a study that
demonstrated predominantly inhibitory effects of VTA stimulation on the firing of most interneurons (Tierney et al 2008).

Dopamine has also been shown to modulate “population level” properties of neurons. For example, amphetamine modulates neural state space organization in a working memory task such that low doses enhance separation of neural states between task epochs but facilitate convergence of neural trajectories within an epoch (as proposed by attractor models for working memory), while high doses destroy neural state separation between task epochs and reduce within-epoch neural trajectory convergence (Lapish et al 2015). Further, Iwashita (2014) demonstrated that VTA stimulation increases pattern similarity in population level responses elicited by visual cortex stimulations, implicating dopamine’s role in tuning sensory responses.

Thus, the effects of dopamine on PFC neuron activity are varied and complex, not simply excitatory or inhibitory. Some of the discrepancies among studies might also result from differences in methodologies to activate VTA dopamine neurons as well as differences in animal states (anesthetized versus awake) at the time of recording. Further, despite the apparent complexity, one nature of dopamine modulation that consistently emerges from various studies is the enhancement of signal to noise ratio or contrast in PFC neurons.

1.6.3 Dopaminergic modulation of PFC local field potentials (LFPs) and synchrony

LFPs, measured as low-pass filtered extracellular voltages, reflect a summation of various currents, including inhibitory and excitatory sub-threshold synaptic currents (reflecting inputs) as well as spiking of neurons (Buzsáki et al 2012). Many studies have indicated that the spectral power of different frequency components of the LFP signal reflect synchronization in local
networks (Buzsaki & Draguhn 2004, Fries 2005), and these synchronized oscillations correlate with diverse behaviors, including working memory, attention, and learning (Benchenane et al 2011, Fries et al 2001, Fujisawa & Buzsaki 2011, Puig et al 2014). LFPs also represent a very useful link between microscopic measures of spiking activity and macroscopic scalp electroencephalogram (EEG) recordings, which have an obvious clinical relevance (Buzsáki et al 2012).

Synchronized oscillations in LFPs can be an effective method for facilitating neural communication between regions. This can be done in one of two ways. First, neurons in one region can be synchronized to local oscillations so that they will fire simultaneously. The simultaneous firing of neurons during peaks of oscillations and inhibition during volleys would greatly enhance the post-synaptic impact of firing these neurons, as spikes from many neurons can coincide within a narrow time window (Benchenane et al 2011, Salinas & Sejnowski 2001).

Second, local oscillations in the sending region and the receiving region can be synchronized such that the receiving area is sensitive to the firing of neurons in the sending area in limited time windows, which might serve as a filtering mechanism that greatly enhances signal to noise (Benchenane et al 2011, Fries 2005, Fries 2015).

One of the most studied oscillations in the brain is gamma oscillations (30 – 100 Hz), which have been proposed to be driven by feedforward and feedback inhibition between interneurons (ING model) or by feedforward excitation of interneurons and feedback inhibition of pyramidal neurons (PING model) (Benchenane et al 2011). Optogenetic stimulation studies have demonstrated that the synchronized activity of PV+ FS interneurons is sufficient to generate gamma oscillations in the cortex (Cardin et al 2009, Sohal et al 2009). The mechanisms driving other types of oscillations, including theta and beta oscillations, are not as well understood,
especially in the cortex.

It has been suggested that dopamine may play an influential role in modulating oscillations, especially gamma rhythms, in the PFC and other forebrain areas (Uhlhaas & Singer 2010, Whittington et al 2011). This hypothesis is supported by many pieces of evidence: a) dopamine is involved in working memory and other cognitive functions that are correlated with theta and gamma oscillations (Benchenane et al 2010, Benchenane et al 2011), b) dopamine projections to PFC are well-situated to modulate PV+ FS interneurons and have been shown to modulate FS interneuron excitability in vivo and in vitro as discussed earlier in this section, and c) schizophrenia, which is characterized by reduced gamma oscillations in PFC, is also associated with hypodopaminergia in PFC (Uhlhaas & Singer 2010, Whittington et al 2011). Experimental studies manipulating dopaminergic transmission in PFC and hippocampus lend some support to this hypothesis, but the nature of dopamine modulation of LFPs appears to be much more complex. In vitro, dopamine via D1 receptors has been shown to reduce gamma oscillations in the hippocampus (Weiss et al 2003) but increase those oscillations in the same region via D4 receptors (Andersson et al 2012, Furth et al 2013). In vivo studies that have pharmacologically modulated dopamine transmission via systemic injection of amphetamine, for example, have reported either no effect on spontaneous oscillations in hippocampus (Ma & Leung 2000) or weak increases in spontaneous cortical gamma power that are associated with behavioral hyperactivity (Pinault 2008, Wood et al 2012); these increases are much weaker compared to those elicited by drugs that manipulate glutamatergic transmission such as MK-801. Similarly, cocaine via D1 receptors modulates gamma oscillations in PFC of anesthetized animals by reducing the bandwidth rather than increasing the power of these oscillations, which may result in tighter synchronization of neuronal networks (Dilgen et al 2013).
Aside from gamma oscillations, dopamine has been shown to influence rhythms in other frequency bands. For example, direct application of dopamine in PFC in anesthetized animals does not modulate the power of local oscillations but enhances the coherence of theta oscillations between PFC and hippocampus (Benchenane et al 2010). Furthermore, tonic dopamine modulation via D1 and D2 antagonists produces an aberrant increase in alpha and beta oscillations in the PFC during a visuomotor learning task in monkeys (Puig & Miller 2012, Puig & Miller 2015). During a working memory task in rats, a 4 Hz oscillation synchronizes PFC networks with VTA neurons, suggesting that VTA may modulate slower frequencies in PFC (although the directionality of oscillations was not established in this study) (Fujisawa & Buzsaki 2011). This study also showed that 4 Hz synchronization of PFC and VTA networks is associated with a corresponding synchronization of gamma rhythms that are phase-coupled to the 4 Hz rhythms. Along the lines of cross-frequency coupling, a recent study demonstrated dopaminergic enhancement of phase-amplitude coupling between delta and gamma rhythms but not of the power of any oscillations in PFC (Andino-Pavlovsky et al 2017). Hence, there is some evidence for dopaminergic modulation of gamma oscillations, but this might depend on various factors including the state of the animal, whether the animal is anesthetized or awake and moving versus performing a specific cognitive task; furthermore, synchronization in other frequency bands such as theta and beta might also be influenced by dopamine activity depending on those factors.

1.6.4  **Dopaminergic modulation of PFC information processing over multiple timescales**

One curious phenomenon involving the dopamine system that has puzzled researchers is that the
phasic activity of dopamine neurons is rapid and capable of driving fast behaviors such as learning, working memory, and error detection while theoretically the timescale of dopamine’s actions is limited by the metabotropic nature of dopamine receptors. To add to the sluggishness of dopamine’s actions due to receptor kinetics, most dopamine receptors are located extrasynaptically (Caille et al 1996, Smiley et al 1994b, Yung et al 1995), which means that dopamine’s postsynaptic effects may be primarily mediated by volume transmission (Lapish et al 2007). Additionally, dopamine is cleared slowly from the extracellular space in the PFC (Cass & Gerhardt 1995) due to a low number of DATs (Sesack et al 1998a). The slow clearance mechanism may further contribute to volumetric dopamine neurotransmission in PFC. Similarly, extracellular dopamine levels measured via microdialysis in terminal regions are sustained over many trials of a behavioral task and even after termination of the task, when the task involves cognitive behaviors such as working memory and set-shifting (Cheng et al 2003, Ostlund et al 2011, Phillips et al 2004, Stefani & Moghaddam 2006, van der Meulen et al 2007). Accordingly, studies have shown that VTA stimulation can elicit transient modulation of mPFC neural activity within milliseconds and seconds as well as prolonged modulation of evoked firing on the timescale of minutes to hours (Lapish et al 2007, Lavin et al 2005). One theory that attempts to reconcile this temporal anomaly in dopamine neurotransmission posits that the transient effects of dopamine neuron activity may be mediated by glutamate co-release from terminals while the delayed effects on the order of minutes to hours may depend on dopamine release and its slow diffusion into the extracellular space (Lapish et al 2007). This theory is particularly attractive because recent studies have identified glutamate co-release mechanisms in dopamine terminals (Morales & Margolis 2017, Tecuapetla et al 2010). Contradicting this theory, some studies have shown that transient effects of VTA activation can be blocked by the application of dopamine
antagonists (Godbout et al 1991, Pirot et al 1992a). However, in interpreting these studies, one must be aware that these drugs also modulate tonic dopamine levels (Lapish et al 2007), which could then influence the post-synaptic impact of glutamate. For example, glutamate co-release from dopamine terminals might transiently modulate PFC activity only when there is an optimal tonic dopamine drive in PFC. These are important considerations in studies examining postsynaptic impact of dopamine activity in PFC.

1.7 DOPAMINERGIC MODULATION OF FMRI SIGNALS

While animal researchers have devoted a lot of time and energy into understanding dopaminergic regulation of neural activity in rodents and non-human primates, the ultimate goal of these research efforts is to be able to understand brain function in humans and apply knowledge about normal dopamine functioning to treat disorders such as schizophrenia and addiction. As invasive research involving in vitro and in vivo electrophysiological recordings is rare and difficult to conduct in humans, fMRI measurement of hemodynamic signals as an indirect measure of neural activity remains a viable alternative. Many fMRI studies have shown hemodynamic activation, primarily BOLD signal increases, in mesolimbic and nigrostriatal systems during the performance of dopamine-dependent behaviors such as reward-prediction error signaling and various form of learning, in normal healthy subjects as well as impairment of these signal changes in schizophrenia (Kumari et al 2002, Morris et al 2012, Murray et al 2008, Reiss et al 2006, Simon et al 2015, Waltz et al 2009, Weickert et al 2009). In order to relate these fMRI signal changes observed in human studies to the valuable insights about dopamine
neurotransmission from animal studies, it is imperative that we first understand how dopamine neuron activity itself drives hemodynamic changes in downstream areas.

Several studies have previously examined the contribution of dopamine to hemodynamic signals. Systemic administration of drugs that release dopamine or bind to dopamine receptors/transporters, including cocaine, methylphenidate, amphetamine, D1 and D2 agonists/antagonists, produces changes in BOLD, CBVw (cerebral blood volume-weighted) and cerebral blood flow-weighted (CBFw) hemodynamic signals in dopaminergic systems such as the striatum, thalamus, and PFC among other regions of rats, monkeys, and humans (Breiter et al 1997, Chen et al 2004, Choi et al 2006, Howell et al 2002, Jenkins et al 2004, Mandeville et al 2013, Marota et al 2000). Generally, dopamine-elicited hemodynamic signal increases depend on the type of dopamine receptor activated, as D1 agonists increase while D2 agonists decrease fMRI signals (Choi et al 2006, Marota et al 2000). It should be noted, however, that these previously reported hemodynamic changes might not reflect local changes in dopamine transmission but could be explained by peripheral or global vascular effects of dopamine drugs and/or activation of complex long-range neural interactions mediated by glutamatergic and/or GABAergic systems. For example, a recent study showed that local activation of dopamine in V1 doesn’t produce hemodynamic changes otherwise elicited by systemic administration of dopamine drugs (Zaldivar et al 2014), suggesting that systemically administered dopamine can elicit indirect hemodynamic effects in a brain region.

How transient activation of VTA dopamine neurons modulates fMRI activity globally had been relatively unexplored until recently. Transient electrical stimulation of the MFB elicits positive BOLD signal changes in NAc and negative BOLD signal changes in striatum (Krautwald et al 2013). However, interpretation of these results is complicated by the presence of
dopaminergic, GABAergic, serotonergic, noradrenergic, and histaminergic projections in the MFB from VTA/SN, raphe nuclei, locus coeruleus, and other regions (Cheer et al 2005, Delfs et al 1998, Hashemi et al 2011, Krautwald et al 2013, Vertes 1991). In the past year, several studies, including ours, have utilized optogenetics in combination with fMRI (opto-fMRI) to circumvent most of these issues, and these will be discussed in Chapter 2.

1.8 PURPOSE OF DISSERTATION

What is evident from the summary of previous studies on dopamine modulation of downstream neural activity is that dopamine elicits complex effects because of the activation of different types of receptors, potential crosstalk between the receptor-specific pathways, production of responses at different timescales, and modulation of responses from the level of neurotransmitter release to receptor sensitivity to intrinsic excitability to spiking output. Because of the complexity of the dopamine system, perhaps it is necessary to examine neural activity at the level of networks and circuits to obtain a more integrated idea of what happens when all these different cellular and subcellular responses elicited by dopamine summate. Thus, we approached the study of dopamine modulation of downstream neural activity in a holistic manner by first examining global changes in neural activity, measured indirectly via fMRI, in the forebrain and then examining network-wide modulation of neural activity locally in mPFC, using measures of neuronal spiking and LFPs, in different behavioral states.
With the advent of optogenetics, researchers have been able to answer specific questions about circuits in cell-type and projection specific manner with great temporal precision (Deisseroth 2015). This technique has been particularly important in the study of VTA dopamine neurons because of the heterogeneity of VTA cell population as discussed in Section 1.1.3. While optogenetics has been widely used in *Th::Cre* and DAT::Cre transgenic rats and mice to selectively and phasically modulate VTA dopamine neurons and examine subsequent effects on various behaviors (Steinberg et al 2013, Tsai et al 2009, Witten et al 2011), there is a paucity of research on downstream neural effects of phasic direct activation of VTA dopamine neurons in vivo. All experiments in this thesis, thus, utilized the cellular and temporal precision of optogenetics to phasically modulate the activity of VTA dopamine neurons in *Th::Cre* rats in vivo. The use of optogenetics in *Th::Cre* rats overcomes many of the limitations of techniques employed by previous studies such as electrical stimulation of VTA or pharmacological manipulation of dopamine receptors and, thus, might help provide a more coherent and accurate picture of dopamine modulation of global neural activity.

By combining optogenetics with traditional tools that allow invasive and non-invasive measures of neural activity, such as fMRI and in vivo electrophysiology, this dissertation addresses how phasic activation of VTA dopamine neurons modulates neural activity in downstream areas of the rat forebrain. Chapter 2 examines global modulation of neural activity upon phasic optogenetic activation of VTA dopamine neurons, using BOLD and CBVw fMRI measures. BOLD fMRI signals are typically assessed by human fMRI studies, while CBVw signals are not traditionally utilized by human studies but might provide a better assessment of underlying neural activity. Chapter 3 investigates local modulation of network activity, that involves spiking of individual neurons and of a population of neurons as well as neural
synchronization via LFPs, in mPFC by VTA dopamine activity in freely moving and anesthetized animals. Chapter 4 summarizes the findings of Chapter 2 and 3 to provide a picture of state-dependent dopamine modulation of neural activity. Chapter 4 also attempts to determine the relationship between hemodynamic and electrophysiological signals. Further, it outlines potential future work that can additionally delineate the complex downstream effects of VTA dopamine activation.

1 Cell types in the striatum
The principal neurons in the striatum are SPNs or MSNs. There are at least six different types of interneurons in the striatum (Tepper et al 2010, Tritsch & Sabatini 2012), which are listed below.

a) Large aspiny cholinergic interneurons or tonically active neurons  
b) PV-expressing FS GABAergic interneurons  
c) NPY/SOM/ NOS-coexpressing low-threshold spiking GABAergic interneurons  
d) NPY only expressing GABAergic neurogliaforms,  
e) TH expressing GABAergic interneurons, and  
f) CR expressing GABAergic interneurons

2 Cell types in the PFC
Aside from pyramidal neurons, which are the principal neurons in the cortex, PFC contains different types of interneurons listed below (Kawaguchi & Kubota 1997).

a) FS interneurons, which contain PV. Two classes are basket cells and chandelier cells.  
b) Late spiking interneurons, including neurogliaforms  
c) Burst spiking and regular spiking non-pyramidal cells  
d) SOM containing Martinotii cells (some are CB positive) and vasoactive intestinal polypeptide (VIP) containing double bouquet cells (these are positive for cholecystokinin or CR)
2.0 Unexpected Global Impact of VTA Dopamine Neuron Activation As Measured by Opto-FMRI

2.1 Introduction

The mesolimbic dopamine circuitry figures prominently in many models of psychiatric disorders, such as addictive disorders (Everitt et al. 2008, Koob & Volkow 2010), schizophrenia (Grace 2000), and mood and anxiety disorders (Nestler & Carlezon Jr 2006, Polter & Kauer 2014). Central to this circuitry and the associated disorder models are dopamine neurons in the ventral tegmental area (VTA) that project to the traditional limbic regions, such as the nucleus accumbens (NAc), amygdala, and the prefrontal cortex (PFC) (Bjorklund & Dunnett 2007). The mesolimbic system is generally thought to be functionally distinct from dopamine-innervated basal ganglia regions, including the dorsal striatum (DS), which are implicated in action selection and movement disorders (Graybiel & Grafton 2015, Smith & Kieval 2000). Accordingly, with a few exceptions (Howes et al. 2013, Yoon et al. 2013), human investigations of addictive disorders (Volkow et al. 2012), schizophrenia (Juckel et al. 2006, Morris et al. 2012, Rolland et al. 2015), and mood disorders (Arrondo et al. 2015, Nauczyciel et al. 2013) have primarily focused on functional magnetic resonance imaging (fMRI) activity in VTA innervated limbic regions as a measure of dopamine-related dysfunction. Studies of “normal” VTA dopamine-related functions, such as reward prediction error signaling and encoding of
motivational salience, in healthy individuals have also focused on these limbic regions, especially the NAc region of the ventral striatum (VS) (Carter et al 2009, Morris et al 2012, O'Doherty et al 2003).

Despite the abundance of fMRI studies characterizing normal and abnormal blood oxygenation-level dependent (BOLD) signals in VS and other mesolimbic areas, whether VTA dopamine neuron activity and subsequent dopamine release directly contribute to these BOLD signals is unclear. Most theories on the origin of BOLD signals are centered on glutamate release (Attwell et al 2010, Lauritzen 2005), and the evidence for dopamine activity contributing to fMRI responses are from indirect observations. For example, systemic administration of psychostimulants, such as amphetamine and cocaine, and D1/D2 agonists/antagonists modulates fMRI signals (Chen et al 2004, Knutson & Gibbs 2007, Marota et al 2000). These effects, however, could be mediated by peripheral/global vascular changes, activation of other adrenergic or serotonergic systems, or alteration of glutamate signaling by psychostimulants (Underhill et al 2014). In addition, systemic drug studies cause artificially prolonged changes in dopamine release or receptor activation. This may not model the physiological mechanism of dopamine neuron signaling during behavior, in which dopamine neurons are transiently activated in response to cues and outcomes (Schultz 1998).

The current study sought to establish causation between phasic activation of VTA dopamine neurons and global fMRI responses in the forebrain. We combined optogenetics with fMRI (opto-fMRI) in Th::Cre rats, which allowed us to selectively and transiently stimulate VTA dopamine neurons while simultaneously measuring fMRI signals. We utilized endogenous BOLD contrast for fMRI because of its relevance to neural measures in human studies. The BOLD response, however, may be influenced by nonspecific contributions of large blood vessels
Mandeville & Marota 1999). Thus, we also examined cerebral blood volume-weighted (CBVw) fMRI changes using Feraheme as a contrast agent, which reduces sensitivity to large blood vessels and enhances spatial specificity to neural activity (Poplawsky et al 2015, Zhao et al 2006). We predicted that the strongest change in fMRI activity would be observed in regions such as the VS that are densely innervated by VTA dopamine neurons. We found, however, that basal ganglia regions such as the DS and globus pallidus (GP) that receive sparse or no VTA dopaminergic innervation were also activated, with activation being much stronger in DS than VS.

2.2 METHODS

2.2.1 Animals

Transgenic Th::Cre rats that express Cre recombinase under the control of the tyrosine hydroxylase (TH) promoter (Witten et al 2011) (gifted by Dr. Karl Deisseroth, Stanford University) and their wild-type litter mates were bred in house by pairing Th::Cre rats with wild-type Long Evans rats (Harlan, Frederick, MD). All rats were housed in a 12-hour reverse light/dark cycle with lights on at 7 pm. All experiments were performed in male adult rats (4-10 months old). Experiments were conducted according to the ethical guidelines of the Institutional Animal Care and Use Committee at the University of Pittsburgh.
2.2.2 Stereotaxic surgery

Under isoflurane anesthesia, Th::Cre and wild-type rats were unilaterally injected in the VTA with recombinant adeno-associated viral (AAV) vector constructs containing the gene encoding channelrhodopsin (ChR2) under the Cre promoter (AAV5-Ef1α-DIO-ChR2-eYFP, University of North Carolina Vector Core, Chapel Hill, NC, USA). Four 1 µL injections were made at four VTA sites (AP = −5.0 and 6.0 mm, ML = 0.7 mm, DV = −7.0 and 8.2 mm) (adapted from Witten et al. (2011)) at a rate of 0.1 µL min⁻¹ using a microsyringe (Hamilton Co., Reno, NV, USA) and a pump (World Precision Instruments, Sarasota, FL). For rats used in fMRI experiments, plastic (240-µm core diameter, 0.63 NA) or silica (200-µm core diameter, 0.22 NA) optical fibers (Doric Lenses, Quebec, Quebec, Canada) were then implanted dorsal to VTA (AP = −5.5 mm, ML = 0.6 - 0.7 mm, and DV = 7.0 - 7.3 mm). All AP/ML and DV measurements are in reference to Bregma and brain surface respectively (Paxinos & Watson 2007). Nylon screws (PlasticsOne, Roanoke, VA, USA) and dental cement were used to secure the implant. Optical fibers were implanted either during the viral infusion surgery or ~6 weeks later. Behavioral and fMRI experiments were conducted at least 6 weeks after the viral infusion surgeries.

Carbon fiber electrodes and optical fiber were acutely lowered into the striatum (DS and VS) and VTA respectively of Th::Cre rats immediately before the fast-scan cyclic voltammetry (FSCV) experiments, which were conducted at least 6 weeks from the viral infusion surgeries. The DS electrode was lowered at AP = 1 mm, ML = 3.6 mm, and DV = 4 mm at a 15º angle in order to accommodate the VS electrode next to it. The VS electrode was lowered straight into the NAc at AP = 1.4 mm, ML = 1.8 mm, and DV = 7.5 mm. Silica optical fibers (Doric Lenses) were also simultaneously lowered into VTA (AP = -5.4 mm, ML = 0.6 mm, and DV = 7 mm).
2.2.3 Characterizing virus integration

Post-imaging histology was used to establish colocalization of TH and ChR2-eYFP in dopamine cell bodies (see histology and immunohistochemistry below). All but two Th::Cre rats showed sufficient ChR2-eYFP integration in VTA.

2.2.4 Histology and immunohistochemistry

Immediately after the termination of fMRI experiments, animals were anesthetized with an intraperitoneal injection of pentobarbital (120 mg/kg equivalent, Euthasol, Virbac AH, Fort Worth, TX, USA) and transcardially perfused with 0.9% saline and 4% paraformaldehyde (PFA), diluted from 32% PFA solution (Electron Microscopy Sciences, Hatfield, PA, USA). Brains were fixed in 4% PFA overnight at 4°C and then transferred to a 20% sucrose solution. Frozen brains were sectioned at 35 µm, and optical fiber placements in VTA were verified by staining coronal brain slices with cresyl-violet. To examine the expression of ChR2-eYFP in dopamine neurons of VTA, VTA coronal slices were treated with 1% sodium borohydride in 0.1 M sodium phosphate buffer solution (PBS), rinsed with the buffer, and incubated in primary and secondary antibody solutions. The primary antibody solution contained mouse anti-TH antibody (1:2000; #MAB318, EMD Millipore, Billerica, MA, USA), chicken anti-EGFP antibody (1:1000; # ab13970, Abcam, Cambridge, MA, USA), 10% Triton-X and normal donkey serum dissolved in PBS. The secondary antibody solution consisted of Cy3-tagged donkey anti-mouse antibody (1:500; Jackson ImmunoResearch), Alexa Fluor 488-tagged donkey anti-chicken secondary antibody (1:500, Jackson ImmunoResearch, West Grove, PA, USA), 10% Triton-X
and normal donkey serum. After antibody reactions, sections were washed in PBS and mounted onto slides. A confocal laser scanning microscope (Olympus Fluoview FV1000, Olympus, Melville, NY, USA) was used to visualize expression of ChR2-eYFP in VTA dopamine neurons.

In order to quantify colocalization of TH and EYFP in dopamine cell bodies, Z-stack (step size: 1 μm) images were acquired at 40 X from random locations in the VTA of all Th::Cre rats that showed sufficient ChR2-eYFP integration. To better identify EYFP+ cell bodies in the VTA of these rats and accurately determine the specificity of ChR2 expression, slides from a subset of animals (n = 4 Th::Cre rats) were further processed with DAPI nuclear staining. These slides were coverslipped with a DAPI-containing mounting medium (Vectashield, Vector Labs, Burlingame, CA), and Z-stack (step size: 1 μm) confocal images were acquired either at 100 X or 40 X combined with 2.5 X True Zoom (Olympus Fluoview FV1000) from random locations in VTA to quantify triple colocalization of TH, ChR2-eYFP and DAPI. Image locations were randomly sampled within the right VTA by manually moving the microscope XY stage, such that the fields of view were non-overlapping and distributed throughout medial, middle, and lateral VTA. Image J was used to count the number of cell bodies.

After FSCV experiments, animals were immediately euthanized with an injection of chloral hydrate (400 mg/kg). The perfusion and histology procedures were identical to the description above, except 60 μm slices were obtained from the striatum in addition to 35 μm slices from the VTA. Striatal slices were examined under a fluorescence microscope to visualize tips of carbon fiber electrodes that were painted with a fluorescent DiI stain (ThermoFisher Scientific, Pittsburgh, PA).
2.2.5 Self-stimulation behavior

2.2.5.1 Behavior

A subset of rats was tested in an intra-cranial self-stimulation (ICSS) task 2-20 days before the fMRI experiment. While ICSS behavior is a crude measure of dopamine activation and was not used as an inclusion/exclusion criterion, we reasoned that it may provide a pre-histology assessment of virus integration. Behavioral experiments were conducted in an operant box (Coulbourn Instruments, Whitehall, PA, USA) equipped with a nose poke port. Optical fiber implants were connected to a patch cord (200-µm core diameter and 0.22 NA; Doric Lenses, Quebec, Quebec, Canada), attached to a 473-nm blue laser diode (OEM Laser Systems, Midvale, UT, USA), and controlled by a Master-8 stimulator (A.M.P.I., Jerusalem, Israel). Without prior training, rats were given two behavioral sessions over two consecutive days. Each session started as soon as the rats were placed in the operant box and lasted one hour. In the first session, nose poke ports were baited with sugar pellets to facilitate exploration, and nose pokes resulted in blue laser (~473 nm) VTA stimulation (20 Hz, 20 pulses, 5-ms pulse width, 5 - 8 mW output at the optical fiber tip at steady state). In the second session, nose pokes only resulted in VTA stimulation, and sugar pellets were not available.

2.2.5.2 Behavioral data analysis

The total number of nose pokes per session was quantified for each rat. Rats were categorized as behaviorally responsive to stimulation if they executed a total number of nose pokes greater than the upper 99.9% confidence limit of the total nose-pokes executed by wild-type controls in
Session 2. Based on this criterion, two \textit{Th::Cre} rats were categorized as behaviorally non-responsive (NB \textit{Th::Cre}), and these were the same rats with poor ChR-eYFP integration in VTA.

2.2.6 Imaging procedures during VTA stimulation

2.2.6.1 Animal preparation

Rats were induced with 5\% and maintained with 2\% isoflurane gas in a mixture of 30\% O\textsubscript{2} and 70\% N\textsubscript{2} gases during surgery. Rats were intubated and mechanically ventilated (TOPO dual mode ventilator, Kent Scientific, Torrington, CT, USA). The ventilation rate was maintained at 55 - 60 breaths/min, while the ventilation volume and ratio of the humidified medical air + O\textsubscript{2} gas mixture were adjusted to maintain oxygen levels at 27 - 28\% and end-tidal CO\textsubscript{2} at 3 - 4\% (Capnomac Ultima, Datex-Engstrom, Finland). The right femoral artery and vein were catheterized for physiological monitoring and administration of 5\% dextrose and contrast agent, respectively. Pancuronium bromide (1 - 2 mg/kg, i.v.) was administered as a muscle relaxant and paralytic.

For hemodynamic measurements, isoflurane was reduced and maintained at 0.7 - 1.0\%. The mean arterial blood pressure (MABP) was monitored through the arterial line and was maintained between 70 – 130 mmHg (MP150, BioPac Systems Inc., Goleta, CA, USA). In addition, the rat rectal temperature was maintained at 37 ± 1°C using a warm water circulator, and a 0.9\% saline, 5\% dextrose, and 0.33 mg/kg/h atropine supplemental fluid was administered intravenously at 1.0 mL/kg/h.
2.2.6.2 Optical stimulation of VTA

For VTA stimulation, a plastic patch cord (240-µm core diameter, 0.63 NA, 6-m long; Doric Lenses, Quebec, Quebec, Canada), attached to a 473-nm blue laser diode (CrystaLaser, Reno, NV, USA) and controlled by a Master-8 pulse generator (A.M.P.I., Jerusalem, USA), was connected to the implanted optical fiber with a zirconia sleeve (Doric Lenses, Quebec, Quebec, Canada). The light power output was calibrated at the tip of the optical fiber with a broadband power meter (Melles Griot, Carlsbad, CA). The right VTA was stimulated with a pulsed train of light (20 Hz, 5-ms pulse width, 20 s) at an optimal power level (2.5 - 7.5 mW) that elicited maximal activation in downstream areas but did not evoke a MABP change. The same laser power was used for BOLD and CBVw fMRI within the same rat. The onset and offset times for the pulse paradigms were controlled by the MRI acquisition computer.

2.2.6.3 MRI Experiments

All MRI experiments were performed on a 9.4-T/31-cm MR system interfaced by a DirectDrive console (Agilent Technologies, Santa Clara, CA) and an actively shielded gradient coil with a 40-G/cm peak gradient strength and 120-µs rise time (Magnex, Oxford, UK). The head of the rat was fixed in a non-magnetic head restraint with a bite bar and ear plugs. A custom-built surface coil was placed on the surface of the head and used for radio-frequency excitation and reception.

2.2.6.4 Anatomical MRI

Anatomical images (128 × 128 matrix size, 16 × 16 mm² field-of-view (FOV) (125 × 125 µm² in-plane resolution)) were acquired using a fast spin-echo sequence and the following parameters: 5.0-s repetition time (TR), train of 8 echoes per segment, 40.7-ms effective echo
time (TE), and 4 averages. Seven or 8 1-mm thick slices were acquired with no gap between slices, and the anterior commissure was always centered in the fourth slice.

2.2.6.5 fMRI data acquisition

We measured two types of fMRI contrasts: endogenous BOLD, followed by contrast-enhanced CBVw fMRI (single i.v. bolus of Feraheme (ferumoxytol, AMAG Pharmaceuticals, Waltham, MA, USA), 15 mg Fe/kg) using a gradient-echo echo-planar imaging sequence with the same FOV as the anatomical images: 16 x 16 mm², 64 x 64 matrix size (250 x 250 µm² in-plane resolution), 500-ms TR with 2 segments (1-s effective TR), 7 - 8 slices, and 2 dummy scans. The effective TE values were variable for BOLD (12 - 14 ms) and CBVw fMRI (2.5 - 14 ms). We varied the TE values to reduce susceptibility effects (i.e. to improve our baseline signal-to-noise ratio) while still maintaining a high detection sensitivity (i.e. contrast-to-noise ratio) in each animal. The optimal flip angle was determined by acquiring baseline fMRI images at increasing flip angles and selecting the one that had the maximal signal within the striatum. A single fMRI run consisted of 5 baseline (5 s) images followed by 4 concatenated VTA stimulation periods, each consisting of 20 stimulus-evoked (20 s) followed by 40 recovery images (40 s) for a total of 245 images per run.

2.2.7 fMRI data analysis

Data were pre-processed and individual functional maps were calculated using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). In addition, a quantitative region-of-interest (ROI) analysis on selected brain regions and a group analysis between rats were performed.
Group comparisons were made between wild-type rats and those $Th::Cre$ rats with sufficient ChR2 integration in VTA. Data from the $Th::Cre$ rats ($n = 2$) which showed poor ChR2 integration (which were the same rats that were behaviorally non-responsive) were analyzed as a separate group designated as NB $Th::Cre$.

### 2.2.7.1 Single animal functional maps

Reconstructed images were spatially realigned, linearly detrended and the normalized difference of the fMRI series (% signal change) was calculated ($[S_t - S_0] / S_0$, where $S_0$ is the mean of the baseline images and the second half of each of the four recovery periods) using home-written Matlab code (MathWorks, Natick, MA). Motion realignment was performed on a run-by-run basis by co-registering the average $S_0$ baseline fMRI image of each run to that of the first run using a rigid-body, three degree-of-freedom transformation (3-DOF: translations in x and y-axes, and rotation about the z-axis). Stimulus-evoked increases in CBV cause the raw CBVw fMRI signal to decrease. Therefore, the signs of the CBVw signal changes were reversed so that increased changes represent CBV increases. Repeated fMRI time series of the same stimulation type and parameters were concatenated for subsequent analysis in SPM8.

T-value maps were calculated using a general linear model in SPM8. The fMRI time series $\times$ 2 column design matrix consisted of a constant, baseline variable and a predicted hemodynamic response function (HRF) that was calculated by convolving the concatenated block-stimulation paradigm with the default SPM8 BOLD HRF and a previously reported CBVw impulse response function (Silva et al 2007). The effect estimates for each respective variable (β-maps) were then calculated from the pre-processed and concatenated fMRI time series. The resulting β-maps were then used to calculate the t-value functional maps. These functional maps
were co-registered to the anatomical images and linearly interpolated to a $128 \times 128$ matrix size (voxel size: $125 \, \mu m \times 125 \, \mu m \times 1 \, mm$) to match the anatomical space.

Baseline signal-to-noise ratio (SNR) and baseline blood volume (BV) index maps were also calculated to examine the influence of these factors on the fMRI activation maps. BOLD and CBVw SNR maps were calculated from the mean of the respective fMRI baseline images ($S_0$) divided by their standard deviation. BV index maps were calculated from the baseline pre- (pre-$S_0$) and post- (post-$S_0$) Feraheme injection images that were acquired with the same TE: $\ln (\text{pre-}S_0 / \text{post-}S_0) / \text{TE}$ (Kim et al 2013).

2.2.7.2 Group analysis

To perform voxel-wise statistical testing across rats, individual functional maps were normalized to a similar anatomical space using SPM8. These transformations were estimated using the anatomical image volumes from each rat, with one representative rat serving as the template. The resulting co-registered anatomical volumes were then averaged and, subsequently, used as the mean anatomical underlay for the group functional maps. Next, the estimated transformations were applied to the corresponding functional maps (previously registered to the anatomical images). The functional maps were then analyzed in SPM8 using one sample t-tests for each group ($Th::Cre$ and wild-type) in the 2nd-level analysis to generate group t-maps. A single covariate column for TEs was added in the analysis to exclude the effects of the variable TEs in the group functional maps, although we observed no significant effects of the varied TE for BOLD and only small effects caused by the varied TE for CBVw maps (data not shown). Voxel-wise and family-wise error correction thresholds for $Th::Cre$ group maps were set to $p < 0.025$. We chose a moderately conservative voxel-wise threshold of $p < 0.025$ to confidently display
meaningful fMRI activations in the Th::Cre group maps. We also applied a family-wise correction threshold of $p < 0.025$ (cluster size = 28 voxels) to correct for type-1 errors due to multiple comparisons after voxel-wise statistical tests. To verify that the selected thresholds were appropriate, group maps for Th::Cre rats were also generated at other thresholds (voxel-wise = $p < 0.025$, family-wise = none (cluster size = 0 voxels); voxel-wise and family-wise = $p < 0.05$, cluster size = 42 voxels; voxel-wise and family-wise = $p < 0.01$, cluster size = 19 voxels). The appropriate cluster sizes (19, 28 and 48 voxels for family-wise thresholds of $p < 0.01$, $p < 0.025$, and $p < 0.05$, respectively) were determined by the 3dClustSim function in AFNI (Analysis of Functional Neuroimages) software (Cox 1996) using information about the image sample size and voxel-wise threshold. Group maps for wild-type rats were shown at a low threshold without family-wise error correction (voxel-level $p < 0.025$ and cluster size > 0 voxels) to demonstrate no activation.

2.2.7.3 ROI analysis

ROIs (ipsilateral and contralateral to the stimulated side) were manually drawn on the high-resolution anatomical images of individual rats and corresponded to the brain regions defined by the Paxinos and Watson (2007) brain atlas. Even though group functional maps influenced our choice of ROIs, these ROIs included whole anatomical structures and were drawn blind to the spatial pattern of functional activation within those anatomical regions. DS was also blindly subdivided into four ROIs (dorsomedial, dorsolateral, ventromedial and ventrolateral). No voxel in the ROI mask was assigned to more than one ROI and some remaining partial volume effects are expected at the ROI boundaries due to the limited spatial resolution of fMRI.
For the ROI analyses, activation t-values (from single animal functional maps), time courses (% signal change across time), SNR, and BV of all voxels (un-thresholded) within each ROI were averaged for each rat. To compare activation t-values between groups ($Th::Cre$, NB $Th::Cre$ and wild-type) within each ROI, Brown-Forsythe ANOVAs that do not assume homogeneity of variances were performed. Post-hoc planned comparisons were conducted using two sample t-tests for unequal variances for significant ROIs between a) $Th::Cre$ rats and wild-type rats and b) wild-type rats and NB $Th::Cre$ rats.

To directly compare the relative t-value, BV, and SNR data across ROIs in the striatum only of $Th::Cre$ rats, ROI values were normalized by the sum of the values across the five ROIs for each animal. One-way repeated measures ANOVAs with ROI as the within-subjects factor were then utilized to compare these relative ROI measures across sub-divisions of the striatum, and Greenhouse-Geisser correction was applied whenever necessary. All statistical analyses for ROIs were conducted in SPSS (IBM, Armonk, NY, USA).

### 2.2.7.4 fMRI data exclusion criteria

To strictly eliminate controllable sources of hemodynamic contamination in our fMRI data, we removed fMRI runs with stimulation-evoked MABP fluctuations. For this, we correlated the measured MABP time course with the stimulation time course. If the Pearson’s correlation was significant at $p < 0.01$ and a MABP change during stimulation was greater than 1 SD from the baseline, then the corresponding fMRI run was removed. A run was excluded if a MABP change was observed during any of the four stimulations or if MABP data were not acquired. This resulted in the exclusion of all BOLD fMRI runs for 2 $Th::Cre$ rats. For all other rats, a total of 1
- 6 runs was analyzed per animal and fMRI contrast. One rat was also removed from CBVw group functional maps for Th::Cre rats because of poor anatomical image normalization.

2.2.8 Forepaw stimulation

2.2.8.1 Imaging

Following the completion of VTA stimulation and imaging experiments, additional CBVw fMRI images were acquired for wild-type and NB Th::Cre rats during forepaw stimulation to verify that their hemodynamic responses were intact. To stimulate the forepaw, non-magnetic needle electrodes were subcutaneously placed in both forepaws below digits two and four. Rats were maintained at 0.9 – 1.4% isoflurane, and electrical pulses (1.5 – 3.7 mA, 1 ms stimulus duration, 8 Hz) (Kim et al 2010a) were delivered to the left and/or right forepaws in a block design (10 – 20 s stimulus on, 50 s recovery, 8 – 12 repetitions). CBVw fMRI images were acquired using the same parameters as VTA stimulation experiments within each animal.

2.2.8.2 Forepaw data analysis

Single animal functional activation maps for forepaw stimulation were calculated similar to VTA stimulation activation maps, as explained in the previous section. For the ROI analysis, contralateral and ipsilateral primary somatosensory cortex (S1) ROIs (in reference to the stimulate forepaw side) were drawn blindly based on anatomical boundaries defined by the Paxinos and Watson (2007) atlas. fMRI activation t-values of all voxels (un-thresholded) within the S1 ROIs were averaged for each hemisphere and rat. Since both forepaws (left and right) were simultaneously stimulated for one wild-type rat, the average of both hemispheres served as
the contralateral data point, with no ipsilateral reference point. For all other rats, contralateral and ipsilateral activation t-values were separately calculated. Then, a two-sample t-test was conducted to compare activation in contralateral and ipsilateral S1.

2.2.9 FSCV methods

Before commencing the FSCV experiment, isoflurane was reduced from ~2% that was used for surgery to 1.25 – 1.75%. Isoflurane concentrations were adjusted for each animal to maintain light anesthesia without visible signs of waking.

2.2.9.1 FSCV

Carbon fiber electrodes were constructed as previously described (Shu et al 2013), except the exposed fiber tip was trimmed to 500 μm and electrodes were immersed in DiI stain immediately before experiments. Electrodes were calibrated pre- and post in standard dopamine solutions via a custom-built flow cell. All dopamine concentrations were determined from post-calibration data. FSCV was performed as described previously (Shu et al 2013), except for the use of two amplifiers (Keithley 428, Keithley Instruments, Inc., Cleveland, OH, USA) to simultaneously scan two electrodes. The potential 0 V vs. Ag/AgCl was applied between scans. Scans were performed by applying triangular potential waveforms (three linear sweeps to 1.0 V, −0.5 V, and 0 V at 400 V/s) at 10 Hz.
2.2.9.2 Optical stimulation parameters

Three different stimulation protocols were used to optogenetically activate VTA dopamine neurons. The first protocol was identical to the fMRI optical stimulation described in section 2.3.6.2 (20 Hz, 20 s, 5 ms pulse width, 5 mW power). The second protocol contained a train of 60 Hz pulses (pulse width = 2 ms, 5 mW power) delivered for 3 s. The third protocol contained a train of 60 Hz pulses delivered for 3 s at 1 mW. Three trials each of the first and second stimulation types were presented with an inter-trial-interval of at least 5 min. Only one trial of the third stimulation type was presented to each animal. Trials of different stimulation types were presented in a pseudo-randomized order.

2.3 RESULTS

The experimental design and post-imaging histology results are depicted in Figure 2.1. Th::Cre and wild-type rats received infusions of Cre-dependent AAV viral constructs encoding ChR2 in the VTA before optical fibers were implanted for unilateral optogenetic activation of VTA dopamine neurons (Figures 2.1A, 2.1B). After allowing > 6 weeks for viral integration, opto-fMRI experiments were conducted in lightly anesthetized rats (maintained at 0.7 -1 % isoflurane) in a 9.4-T MRI scanner (Figure 2.1C). Functional images were acquired while VTA was optically stimulated transiently for 20 s at 20 Hz, followed by 40 s of recovery (Figure 2.1C). We utilized unilateral, as opposed to bilateral, optogenetic control of VTA dopamine activity in combination with fMRI in order to separate ipsilateral, contralateral, and bilateral neuronal control.
Post-experiment histology confirmed that optical fibers targeted the right VTA (Figure 2.1D and Figure 2.2). Viral expression of ChR2 (61% of TH+ cell bodies co-expressed ChR2-eYFP) was highly specific to dopamine neurons in VTA (96% of ChR2-eYFP+ cell bodies also expressed TH) (Figures 2.1E, 2.1F and 2.1G). Activation of VTA dopamine neurons is reinforcing (Witten et al 2011); therefore, to gain a sense that we were sufficiently stimulating dopamine neurons in Th::Cre animals, some of the Th::Cre and wild-type rats were behaviorally characterized in an ICSS operant task before the opto-fMRI experiments. Out of the rats tested, most Th::Cre rats expressed robust ICSS behavior, but two Th::Cre rats and all wild-type rats executed very few nose-pokes in this task (Figure 2.1H). Histology confirmed that the two “behaviorally non-responsive” Th::Cre (NB Th::Cre) rats expressed low levels of ChR2 in VTA compared to the “behaviorally responsive” Th::Cre rats (examples shown in Figure 2.1H). None of the wild-type rats expressed ChR2 in VTA (example in Figure 2.1H).
Figure 2.1 Experimental design, histology, and behavior.
(A) Experimental design. A subset of rats (6 Th::Cre and 3 wild-type) were characterized behaviorally in an intra-cranial self-stimulation (ICSS) task prior to fMRI scanning. Two Th::Cre rats were excluded from the Th::Cre group fMRI data analysis due to low levels of ChR2 integration in VTA and weak expression of ICSS behavior (also see Figure 2.1H). (B) Schematic demonstrating infusion of AAV viral construct and implantation of an optical fiber in right VTA. (C) fMRI setup. Each fMRI run consisted of 5 s of baseline followed by 4 optical stimulation trials. In each trial, blue laser pulses were delivered to VTA at 20 Hz (5 ms width, power = 2.5 – 7.5 mW, 473 nm) for 20 s, followed by 40 s of rest. (D) Representative histological images from two Th::Cre rats showing ChR2 expression and implanted fiber tips (white arrowheads) in VTA. Scale bar: 130 µm. (E) Expression of ChR2-eYFP (green) in TH+ (red) neurons in VTA of Th::Cre rats. Scale bar: 40 µm. (F) Quantification of TH+ cell bodies (n = 2083 cells from 7 Th::Cre rats) that also express eYFP in VTA (measure of sensitivity of ChR2-eYFP expression). (G) Quantification of VTA cell bodies immunopositive for both eYFP and DAPI (n = 687 cells from 4 Th::Cre rats) that also express TH in VTA (measure of specificity of ChR2-eYFP expression). (H) (Top) ICSS behavior. Rats executed nose pokes to receive optical VTA stimulation (20 Hz, 5 ms pulse width, power = 5 – 8 mW, 473 nm). The average total number of nose pokes executed per session by Th::Cre, “behaviorally non-responsive” Th::Cre (NB Th::Cre) and wild-type rats is shown. (Bottom) Representative histological images show a differential amount of ChR2 (green fluorescence, top row) expression directly below the optical fiber tips in VTA of Th::Cre, NB Th::Cre, and wild-type rats. The bottom row shows TH expression in images acquired in the same field of view as the ChR2 images (top row). All images corresponding to a particular fluorescence stain (ChR2 or TH) were acquired using the same parameters, and white arrowheads point to optical fiber tips. Scale bars: 200 µm.
Figure 2.2 Histological verification of fiber placements for all rats used in fMRI experiments.

The middle row shows placements for two rats that were behaviorally non-responsive (NB Th::Cre). The first and last rows illustrate fiber placements for the remaining Th::Cre and wild-type rats, respectively.
2.3.1 Global fMRI responses to optogenetic stimulation of VTA dopamine neurons

Because of the obvious behavioral and histological separation, NB Th::Cre rats were excluded from Th::Cre group functional maps and analyzed as a separate “inadvertent control” group in the ROI analysis. Their individual functional maps also showed no activation during VTA optical stimulation for BOLD and CBVw fMRI contrasts (data not shown). Since all other Th::Cre rats (including those not characterized in ICSS) showed sufficient ChR2 integration in VTA (comparable to the Th::Cre example in Figure 2.1H) and similar activations in individual functional maps during VTA stimulation, they were included in the Th::Cre group analysis.

**Group-brain maps.** The group analysis revealed that optical stimulation of VTA dopamine neurons in Th::Cre rats increased BOLD signals in voxels mostly within the dorsal part of the striatum (Figures 2.3A, 2.3B, and 2.4). A few significantly activated voxels were also observed in the VS (includes the NAc), GP, and hippocampus. Since BOLD signals are influenced by baseline CBV (Mandeville & Marota 1999), we speculated that the observed robust activation in DS and relatively weak activation in the VS might result from a greater baseline CBV in the dorsal compared to the ventral striatal areas. To address this possibility, we examined fMRI signals using a CBV contrast method. CBVw fMRI has been shown to reduce large vessel artifacts and improve sensitivity around microvessels near the activated neurons (Poplawsky et al 2015, Zhao et al 2006). CBVw functional maps for Th::Cre rats showed significant activation in response to VTA dopamine stimulation in a larger number of voxels compared to BOLD maps, verifying the improved sensitivity of the CBVw method (Figures 2.3B and 2.4). Significant voxels were observed in both DS and VS, orbitofrontal cortex (OFC), medial prefrontal cortex (mPFC), ventral pallidum (VP), GP, hippocampus, amygdala complex,
and thalamus. However, activation was still concentrated in the dorsal part of the striatum. There was no significant change in BOLD or CBVw activity throughout the brain of wild-type rats upon optical VTA stimulation (Figure 2.3C). Activation maps in Figure 2.3C are shown at a low threshold (no family-wise correction) to demonstrate a lack of any activation. As a positive control, fMRI activation was observed in S1 during forepaw stimulation in wild-type and NB Th::Cre rats (Figure 2.5), indicating that their hemodynamic response was intact.
Figure 2.3 Group activation maps.

(A) Atlas figures overlaid on structural images to delineate regions-of-interest (ROIs) (mPFC = medial prefrontal cortex; OFC = orbitofrontal cortex; DS = dorsal striatum; VS = ventral striatum; S1 = primary somatosensory cortex; VP = ventral pallidum; GP = globus pallidus; Hipp = hippocampus; Amy = amygdala; Thal = thalamus; PS = posterior striatum). (B) Statistical t-value maps
overlaid on structural images illustrate increased BOLD and CBVw activity in the striatum and other ROIs after optical VTA stimulation in Th::Cre rats (n = 5 for BOLD and n = 6 for CBVw). Note that positive CBVw t-values represent blood volume increases during stimulation. Atlas overlays (same as A) mark the boundaries of ROIs. Voxel-wise and family-wise error correction (cluster size > 28 voxels) thresholds were set to p < 0.025. (See Figure 2.4 for group maps with different thresholds) (C) Wild-type (n = 4 for BOLD and CBVw) rats do not exhibit any increase in BOLD and CBVw activity in the whole brain even at a low threshold (voxel-level p < 0.025 and cluster size > 0 voxels) upon optical VTA stimulation. Color bar indicates t-values. Voxel size: 125 µm X 125 µm X 1 mm.
Figure 2.4 Group activation maps at different thresholds.

Group activation maps (same as Figure 2.3B) at different voxel-wise and cluster-wise thresholds. Statistical t-value maps overlaid on structural images for optical VTA stimulation in Th::Cre rats (n = 5 for BOLD and n = 6 for CBVw) at (A) voxel-wise threshold = p < 0.025; no family-wise threshold (cluster > 0 voxels); (B) voxel-wise threshold = p < 0.05; family-wise threshold = p < 0.05 (cluster > 42 voxels); and (C) voxel-wise threshold = p < 0.01; family-wise threshold = p < 0.01 (cluster > 19 voxels). Color bar indicates t-values.
Figure 2.5 Forepaw stimulation.

(A) Forepaw stimulation activation maps. Statistical t-value maps overlaid on structural images illustrate increased CBVw activity in right (top panel) and left (bottom panel) S1 of two representative wild-type rats after electrical stimulation of the contralateral forepaw (left forepaw for top panel and right forepaw for bottom panel). Voxel-wise and family-wise error correction (cluster size > 28 voxels) thresholds were set to $p < 0.025$, similar to VTA activation maps. White dashed lines delineate S1 in the structural images. (B) ROI analysis. Mean ± SEM t-values for correlation of CBVw signal changes within the entire S1 ROI with predicted hemodynamic response functions. S1 ROIs contralateral as well as ipsilateral to the stimulated forepaw were drawn on individual structural images for wild-type rats ($n = 4$) and NB $Th::Cre$ ($n = 2$) rats, and data from wild-type and NB $Th::Cre$ rats were combined. A two sample t-test resulted in a trend towards significant difference in t-values between S1 ROIs ($t(9) = 2.206$, $p = 0.055$) contralateral and ipsilateral to the stimulated side.
ROI analysis. To complement the group activation maps, we performed ROI analyses using un-thresholded data. The ROIs were chosen based on known output regions of VTA dopamine neurons (Berger et al 1991, Bjorklund & Dunnett 2007, Klitenick et al 1992, Swanson 1982, Yetnikoff et al 2014) and observed signal changes in group activation maps. A control ROI was also drawn in S1 to assess any non-specific changes from VTA stimulation, given that no activation is expected here. ROIs were drawn as depicted in Figure 2.3A. Note that the boundaries of VS ROIs were drawn around the NAc, and the olfactory tubercle was excluded.

We examined BOLD activation in ipsilateral (right) forebrain areas that receive substantial projections from VTA dopamine neurons and detected a significant difference between Th::Cre and wild-type rats in VS only (Figure 2.6A). In addition, we observed a significant difference in BOLD activation between Th::Cre and wild-type rats in basal ganglia regions—DS and GP—that receive sparse or no projections from VTA dopamine neurons (Bjorklund & Dunnett 2007). CBVw activation was significantly different between Th::Cre and wild-type rats in many ipsilateral limbic forebrain areas that receive substantial VTA dopaminergic innervation, including VS, VP, OFC, and amygdala, and in regions that receive sparse/no VTA dopamine innervation, such as DS, GP, thalamus, and hippocampus (Berger et al 1991, Bjorklund & Dunnett 2007, Klitenick et al 1992, Swanson 1982, Yetnikoff et al 2014) (Figure 2.6B). In the contralateral (left) hemisphere, only the thalamus CBVw activation was significantly different between Th::Cre and wild-type rats (Figure 2.7). Compared to the right DS activation (t-value > 1.5 for BOLD and CBVw), fMRI responses in other regions, including right VS, were much weaker (t-value < 1 for all other ROIs). Because activation was mostly specific to the stimulated hemisphere (except thalamus) and was absent in S1 (Figures 2.6A and 2.6B), it is unlikely that the observed BOLD and CBVw responses in Th::Cre rats were mediated
by residual blood pressure changes, global vascular effects or motion. There was no significant
difference in BOLD and CBVw activation between wild-type rats and NB Th::Cre rats for any
ROI, suggesting that the observed activation patterns were related to the levels of ChR2 in VTA
and expression of VTA dopamine stimulation-dependent ICSS behavior.
Mean ± SEM t-values for correlation of (A) BOLD and (B) CBVw signal changes within ROIs with predicted hemodynamic response functions. Right hemisphere ROIs were drawn on individual structural images for Th::Cre rats (n = 5 for BOLD and n = 7 for CBVw), NB Th::Cre rats (n = 2 for BOLD and CBVw), and wild-type rats (n = 4 for BOLD and CBVw). Brown-Forsythe ANOVAs yielded significant differences in activation t-values for BOLD in the following ROIs: VS (F(2,8) = 13.569, p = 0.008), DS (F(2,8) = 23.017, p = 0.006), and GP (F(2,8) = 6.843, p = 0.043) and for CBVw in the following structures: VS (F(2,10) = 34.546, p = 0.000), VP (F(2, 10) = 10.625, p = 0.006), OFC (F(2,10) = 7.4555, p = 0.015), amygdala (F(2,10) = 5.489, p = 0.025), DS (F(2, 10) = 29.262, p = 0.000), GP (F(2,10) = 14.061, p = 0.003), thalamus (F(2,10) = 8.131, p = 0.012), and hippocampus (F(2,10) = 8.624, p = 0.007). Post-hoc two sample t-tests resulted in significant differences in BOLD t-values between Th::Cre and wild-type rats in these ROIs: VS (t(7) = 4.376, p = 0.009), DS (t(7) = 4.879, p = 0.008), and GP (t(7) = 3.643, p = 0.012). CBVw t-values were significantly different between Th::Cre and wild-type rats in these ROIs: VS (t(9) = 5.639, p = 0.001), VP (t(9) = 3.013, p = 0.021), OFC (t(9) = 2.460, p = 0.038), amygdala (t(9) = 2.409, p = 0.040), DS (t(9) = 5.472, p = 0.001), GP (t(9) =3.397; p = 0.012), thalamus (t(9) = 2.817, p = 0.022), and hippocampus (t(9) = 2.523, p = 0.033). There was no significant difference between NB Th::Cre and wild-type
groups for any ROI. * = p < 0.05, ** = p < 0.01. (C) DS of Th::Cre rats was divided into four quadrants (DM = dorsomedial, DL = dorsolateral, VM = ventromedial, and VL = ventrolateral) as indicated in the anatomical images with overlaid atlas figures (top). BOLD and CBVw t-values, BOLD and CBVw baseline signal-to-noise ratio (SNR), and baseline blood volume (BV) were expressed as percentages of summed total values across the five subdivisions of striatum, including VS (bottom). One-way repeated measures ANOVAs yielded significant differences among sub-divisions of the striatum in relative activation t-values for BOLD (F(4, 16) = 53.067, p = 0.000, n = 5 rats) and CBVw (F(4, 24) = 26.913, p = 0.000, n = 7 rats, Greenhouse-Geisser corrected) contrasts. Repeated measures ANOVAs also resulted in significant differences among striatal subdivisions in relative BV (F(4, 24) = 16.623, p = 0.000, n = 7 rats) and relative SNR for BOLD (F(4, 16) = 76.71, p = 0.000, n = 5 rats) and CBVw (F(4, 24) = 37.34, p = 0.000, n = 7 rats) contrasts.
Mean ± SEM t-values for correlation of (A) BOLD and (B) CBVw signal changes within ROIs with predicted hemodynamic response functions. Left hemisphere ROIs were drawn on individual structural images for *Th::Cre* rats (n = 5 for BOLD and n = 7 for CBVw), NB *Th::Cre* rats (n = 2 for BOLD and CBVw), and wild-type rats (n = 4 for BOLD and CBVw). Brown-Forsythe ANOVAs for each ROI yielded significant differences in activation t-values in the **thalamus** (F(2,10) = 7.655, p = 0.013) and **amygdala** (F(2,10) = 6.386, p = 0.018). Post-hoc two sample t-tests resulted in a significant difference in CBVw response between *Th::Cre* rats and wild-type rats (t(9) = 2.455, p = 0.040) in the thalamus only. There was no significant difference between NB *Th::Cre* and wild-type groups for any ROI. * = p < 0.05.

Figure 2.7 Contralateral ROI analysis for VTA stimulation.
To further examine the spatial pattern of activation in the striatum of Th::Cre rats, we sub-divided the right DS into four ROIs. Medio-lateral divisions were drawn as described in the literature (Liljeholm & O’Doherty 2012, Van Waes et al 2012). Medial and lateral divisions of the DS are thought to be anatomically and functionally distinct because they receive different inputs and are respectively implicated in associative/limbic versus sensorimotor functions (Liljeholm & O’Doherty 2012). The medial and lateral divisions were further divided in half to get dorsal and ventral sub-divisions (Figure 2.6C). Relative activation t-values were significantly different among sub-divisions of the striatum, and the strongest BOLD and CBVw activations upon VTA stimulation were observed in the dorsomedial division, followed by the dorsolateral area of the DS (Figure 2.6C). The weakest activation was observed in the VS. To determine if baseline SNR and BV can explain the greater activation in DS compared to VS, we compared these factors across striatal subdivisions. Relative BV and SNR for BOLD and CBVw contrasts were significantly different among striatal subdivisions, and the smallest BV and SNR were observed in the VS (Figure 2.6C). However, a 3.33% average difference in BV and 4.57% (CBVw) to 5.43% (BOLD) average SNR difference between VS and the dorsomedial striatum are unlikely to account for the large difference (24.16 % for BOLD and 21.05 % for CBV, on average) in activation t-values between these regions. In general, in spite of statistically significant differences in BV and SNR, these measures were comparable across striatal subdivisions and may not explain the observed spatial distribution of fMRI activation. However, other possibilities cannot be ruled out, such as differences in neural-hemodynamic coupling mechanisms and/or the dynamic range of activating blood vessels between VS and DS subdivisions.
**Time courses.** The mean time courses across *Th::Cre* rats from significant ROIs are plotted in Figure 2.8 (no threshold applied). In the right DS, fMRI signals increased gradually and peaked at 1.5 ± 0.2% at 16.8 ± 2.6 s for BOLD and 2.0 ± 0.4% at 18.4 ± 1.6 s for CBVw, respectively. Compared to DS, the peak BOLD and CBVw signal changes in the right VS (BOLD: 1.2 ± 0.2% at 21.6 ± 3.3 s, CBVw: 1.7 ± 0.3% at 24.6 ± 2.4 s) were weaker. Percent signal changes in other activated ROIs were much weaker but exhibited similar slow temporal dynamics in which the signal peaked near the end of stimulation.
Figure 2.8 Time courses.

Mean ± SEM % BOLD (top) and CBVw (bottom) signal changes are plotted across time. Only ROIs that showed a significant difference in BOLD and CBVw activity between Th::Cre and wild-type rats in the ROI analysis (indicated in Figures 6A, 6B, and Figure 2.7) are plotted for Th::Cre (n = 5 for BOLD and n = 7 for CBVw) and wild-type rats (n = 4 for BOLD and CBVw). % signal changes for NB Th::Cre rats are not shown. % signal changes in the left hemisphere
ROIs corresponding to significant right hemisphere ROIs are also depicted. Optical stimulation started at $t = 0$ s and terminated at $t = 20$ s. All values are signal changes expressed as percentage of the baseline (-4 s to 0 s and 41 s to 55 s). Note that the signs of CBVw values are reversed so that increased signal changes represent increases in CBV.
2.3.2 Specificity of activation of VTA dopamine neurons

Because of the inevitable spread of virus and ChR2 expression to the adjacent structures, it is possible that some of the observed fMRI activations were mediated by stimulation of dopamine neurons in the SN and other midbrain nuclei. We are, however, confident that we primarily stimulated VTA dopamine neurons for several reasons. First, the optical fiber placements were medial in the VTA of most animals (Figures 2.9 and 2.10) and, thus, optical stimulation was unlikely to have strongly activated SN neurons. Second, regardless of the exact VTA optical fiber placement, we consistently observed a striking DS activation in the individual maps of all Th::Cre rats (excluding NB Th::Cre rats, example activation maps for two rats shown in Figure 2.11). This point is further demonstrated by directly comparing striatal activation between two rats with medial and lateral placements of optical fibers in VTA (Figure 2.11). Because of the very medial location of the fiber tip in rat #3460 and low laser output (power = 2.5 mW at fiber tip, irradiance = 1.83 mW/mm² at 500 µm from fiber tip), it is highly unlikely that SN dopamine neurons were activated during optical stimulation of VTA. The predicted blue light irradiance during stimulation even at the medial edge of SN was < 0.47 mW / mm², which is not sufficient to elicit action potentials (Boyden et al 2005). However, optical stimulation robustly increased CBVw activity in the DS of this rat (Figure 2.11A). In contrast, because of the lateral location of the fiber tip and higher laser output (power = 5 mW at fiber tip, irradiance = 3.66 mW/mm² at 500 µm from fiber tip) in rat #3446 (Figure 2.11B), there was a greater possibility of activating SN dopamine neurons with stimulation. Activation maps of this rat still revealed strong CBVw activation in the DS. The similarity of the fMRI activation maps between these two rats further
indicates that weaker VS versus stronger DS activation is not due to preferential stimulation of SN dopamine neurons.
Figure 2.9 Immunohistological images.

Immunohistological images from two representative Th::Cre rats showing ChR2 expression (green fluorescence) in the midbrain along the anterior-posterior axis of VTA. Boundaries of
VTA and SN are outlined with white and yellow dashed lines, respectively. Optical tracts terminating dorsal to VTA are highlighted with white dashed lines Scale bar: 300 µm.

Figure 2.10 Structural MRI images.

Structural MRI images (voxel size: 125 µm x 125 µm x 1 mm) show fiber tracts terminating in VTA region in all Th::Cre rats (excluding NB Th::Cre).
Figure 2.11 Individual histology and activation maps.

(Top) Schematics of approximate spread of blue light in the VTA and SN are overlaid on immunohistological images (red: TH, green: ChR2-eYFP) that show optical fiber tips at locations farthest from SN (A) and closest to SN (B). The predicted irradiance values (mW/mm²) calculated using the online irradiance calculator (http://www.stanford.edu/group/dlab/cgi-bin/graph/chart.php) are indicated for distances of 0.5 mm (first white line) and 1 mm (second white line) from the optical fiber tips. Scale bar: 0.5 mm. Dashed white lines mark the approximate boundary between VTA and SN. (Bottom) CBVw t-value statistical maps for two rats (A and B) illustrate individual responses in the striatum to VTA optical stimulation. Voxel-wise and family-wise error correction (cluster size > 19 voxels) thresholds were set to p < 0.01. Color bar: t-values.
2.3.3 Contribution of dopamine release to observed fMRI signals

Even though we were confident that optogenetic stimulation of VTA did not preferentially activate SN dopamine neurons, VTA dopamine neurons also send some sparse projections to the medial portion of the DS (Bjorklund & Dunnett 2007). To determine the contribution of dopamine release from these striatal projections to observed fMRI signals, we assessed the amount of dopamine release in DS and VS during phasic activation of VTA dopamine neurons. We acutely lowered carbon-fiber electrodes in the DS and VS simultaneously (Figures 2.12A, 2.12B and 2.12C) and measured phasic dopamine release in the two regions for 60 s from the start of 20 Hz, 20 s VTA stimulation (power = 5 mW) under light isoflurane anesthesia. Despite the sparsity of VTA dopamine projections to DS, we observed a strikingly larger extracellular dopamine concentration ([DA]₀) increase in DS compared to VS (Figure 2.12D). In fact, two rats with very medial placements of the optical fiber in VTA (Figure 2.12C) showed much stronger increases in [DA]₀ in DS compared to the rat with a slightly lateral fiber placement, suggesting that the DS [DA]₀ increase was not due to preferential activation of SN dopamine neurons. In the VS, most of these rats showed very low increases in [DA]₀. We also examined changes in [DA]₀ during transient VTA dopamine stimulation (3 s) at a faster rate (60 Hz, power = 5 mW). 60 Hz stimulation elicited a much larger increase in striatal [DA]₀ than 20 Hz stimulation (Figure 2.12E). The large difference in [DA]₀ increase between DS and VS was even more apparent after 60 Hz stimulation. To control for any potential spread of blue light to the SN, we also measured [DA]₀ after stimulation at 60 Hz for 3 s at a much lower power (1 mW) (Figure 2.12F). While the reduction of power also drastically reduced [DA]₀ increase after stimulation, we still observed substantial increases in DS [DA]₀ of all rats, while only one rat clearly showed an increase in VS
[DA]₀. This particular rat (Rat 1) exhibited comparable increases in [DA]₀ in DS and VS during stimulation with 60 Hz at 1 mW and 20 Hz at 5 mW; however, a 60 Hz stimulation at 5 mW resulted in a profoundly larger increase in DS compared to VS. These preliminary results (from n = 3 rats) clearly demonstrate a different pattern of dopamine release in the DS and VS following phasic VTA dopamine activation. Although this finding was surprising based on anatomical projections, it can potentially explain the differential fMRI activation in DS and VS observed in the present study.
Figure 2.12 Measurement of phasic dopamine release in DS and VS.

Optogenetic VTA dopamine neuron stimulation produced a greater dopamine release, measured with FSCV, in DS compared to VS of Th::Cre rats. (A) Carbon-fiber electrodes for FSCV were
acutely and simultaneously lowered into DS and VS. (B) Histological verification of electrode placements (marked by vertical or angled lines) in DS and VS. (C) Histological verification of optical fiber placements (marked by colored dots) in VTA. Atlas images are adapted from Paxinos and Watson (2007). (D) Optogenetic stimulation of VTA at 20 Hz, 20s (power = 5 mW) strongly increased dopamine release in DS but weakly in VS, under isoflurane anesthesia. Plot on the left shows group summary (n = 3 rats, average of 3 trials per rat) as mean +/- SEM dopamine concentration. Plots on the right show release patterns in individual rats. T = 0 s indicates stimulation onset, and blue rectangles mark stimulation period. (E) Dopamine release in DS and VS after optogenetic stimulation of VTA dopamine neurons at 60 Hz, 3 s (power = 5 mW). Plots show dopamine release (n = 3 rats, average of 3 trials per rat) for individual rats. (F) Dopamine release in DS and VS after optogenetic stimulation of VTA dopamine neurons at 60 Hz, 3 s at reduced laser power (power = 1 mW). Plots show dopamine release (n = 3 rats, one trial per rat) for individual rats.
2.4 DISCUSSION

The current study establishes causation between activation of VTA dopamine neurons and fMRI responses in VTA dopamine-innervated limbic regions that include the VS, VP, OFC, and amygdala. fMRI activation was also observed in regions that do not receive substantial VTA dopamine projections, such as thalamus, hippocampus, and basal ganglia regions (DS and GP) (Berger et al 1991, Bjorklund & Dunnett 2007, Klitenick et al 1992, Swanson 1982, Yetnikoff et al 2014). In particular, DS exhibited the most robust BOLD and CBVw signal increase, compared to other regions, upon activation of VTA dopamine neurons. These results, thus, suggest a potentially novel dynamic VTA–basal ganglia circuit associated with phasic activation of VTA dopamine neurons.

2.4.1 Contribution of dopamine neuron activation to hemodynamic signals.

Several mechanisms can explain the observed hemodynamic changes in downstream areas upon VTA dopamine neuron activation. The most straightforward explanation is that these changes depend on phasic dopamine release. Some of the areas that exhibited fMRI activation, such as VS, receive dense dopamine innervation from VTA, and thus direct dopamine release potentially drives activation in these regions. We also observed significant fMRI activation in regions, such as the DS and GP, that are not traditionally considered part of the VTA dopaminergic system (Smith & Kieval 2000). Decades of tracing studies have indicated that VTA dopamine neurons send sparse projections to DS, most of which terminate in the very medial edge of DS and none in GP (Bjorklund & Dunnett 2007). Therefore, we first thought that phasic dopamine release in
these regions may not primarily drive fMRI activation. However, we found that VTA dopamine activation elicits a profound dopamine release in DS that is much larger than in VS. This finding suggests several possibilities: a) VTA dopamine projections to DS are much denser than initially characterized; b) VTA dopamine terminals in DS, even though fewer in number, release more dopamine per synapse than terminals in VS; and c) phasic VTA dopamine activation may indirectly enhance dopamine release in DS.

In support of the first possibility, a recent study that employed newer viral-genetic strategies, which may be more sensitive than classical tracing methods and may allow better visualization of axonal arborization, demonstrated a dense and broad arborization in the DS of lateral NAc-projecting VTA dopamine axons (Beier et al 2015). While our own examination of striatal slices from some Th::Cre rats that expressed eYFP selectively in VTA showed a preferential innervation of VS compared to DS (data not shown), it is possible that our method is not sensitive to detect axonal arborizations. Furthermore, many studies have consistently shown that dopamine terminals in DS release a higher concentration of DA compared to those in VS for the same amount of phasic stimulation, suggesting a dorso-ventral gradient of dopamine release in the striatum (Calipari et al 2012). Thus, in addition to dense arborization of VTA dopamine collaterals in DS, VTA dopamine terminals in this region may release larger dopamine per synapse, which supports the findings of the FSCV experiment in the present study. Thus, the strong DS fMRI activation could be driven by direct dopamine release from VTA dopamine terminals in DS.

In spite of the large dopamine release in DS observed in the present study, whether dopamine itself can locally increase hemodynamic signals is not known. Many studies have suggested that dopamine inhibits activity in the striatum (Brady & O'Donnell 2004, Kiyatkin &
Rebec 1999, Nicola et al 2000, Rolls et al 1984, Tritsch et al 2012, Tritsch et al 2014). Further, fMRI activations are primarily attributed to changes in glutamate neurotransmission; blockade of local glutamate receptors profoundly reduces hemodynamic fMRI signals (Iordanova et al 2015), and > 80% of glucose metabolism in the rat cortex is estimated to be due to glutamate release and uptake (Sibson et al 1998). Given this body of evidence, it is possible that the robust fMRI response in DS is mediated, at least in part, by network-level interactions involving long-range basal ganglia-thalamic-cortical loops that may terminate in the striatum via convergent glutamate projections (Haber & Calzavara 2009, Voorn et al 2004) instead of/in addition to direct dopamine release. Convergence of glutamatergic afferents may also be a mechanism for enhancing dopamine release in the striatum, as electrical stimulation of cortical glutamate afferents or pharmacological activation of glutamate receptors greatly enhances striatal dopamine release (Nieoullon et al 1978, Youngren et al 1993). This way, fMRI activation may be mediated by a combination of glutamatergic and dopaminergic activation in DS.

There are several potential networks that may be involved in modulating DS glutamate activity. For example, VTA dopamine modulation of VS neurons can influence thalamic neurons (directly or indirectly via the pallidum) (Haber & Calzavara 2009, Nauta et al 1978). A change in thalamic activity may then influence DS fMRI signals via direct thalamic glutamate projections (Voorn et al 2004). Alternatively, VTA dopamine modulation of cortical areas may impact DS activity via cortico-striatal projections (Voorn et al 2004). This proposed divergence of signal from the VS to thalamic and cortical areas and eventual convergence of glutamatergic inputs to the DS may amplify fMRI responses in this region. Consistent with this potential mechanism, we observed significant fMRI responses to VTA dopamine stimulation in the thalamus, pallidum, and cortical areas such as the OFC. Further studies including pharmacology and recording from
DS and thalamic/cortical neuronal ensembles are necessary to delineate these potential mechanisms.

Of note, a recent study reported that activation of VTA dopamine neurons did not greatly increase forebrain BOLD fMRI signals (Helbing et al 2015), in contrast to our observations in the present study. We speculate that differences in the anesthesia choice and depth, lower BOLD sensitivity at the 4.7-T magnetic field, and length of VTA stimulation (8 s compared to 20 s) may contribute to this discrepancy. The latter possibility may play a significant role because the VTA stimulation-dependent activation time courses that we observed were very slow, and activation tapered off soon after the stimulus offset. In the current experiment, the percent BOLD signal change in DS was only 0.47 ± 0.24% at 8 s, which is expected to be less at 4.7-T and may be below the limits of detection. Other recent studies that also employed opto-fMRI in Th::Cre rats, on the other hand, have confirmed the patterns of BOLD and CBVw activation observed in the present study in anesthetized and awake animals (Decot et al 2017, Ferenczi et al 2016).

2.4.2 Functional relevance of VTA - DS activation.

Mesolimbic projections from VTA and nigrostriatal projections from SN are generally thought to be distinct dopaminergic systems that modulate processes mediated by VS and DS, respectively (Bjorklund & Dunnett 2007, Matsumoto & Hikosaka 2009a, Smith & Kieval 2000). While the lack of clear separation between VTA and SN and their projection targets has also been recognized (Bjorklund & Dunnett 2007), the present finding that activation of VTA dopamine neurons robustly increases DS fMRI activity suggests that there is considerable functional overlap between mesolimbic and nigrostriatal systems. Thus, activation of VTA dopamine
neurons may influence classic non-limbic basal ganglia-related motor/cognitive functions. It is interesting that within the DS, stronger activation was observed in dorsal areas, with the medial dorsal half exhibiting slightly greater activation than the lateral dorsal half. The medial (associative striatum) and lateral (sensorimotor striatum) divisions of DS receive different cortical and sub-cortical inputs and are involved in “cognitive control” functions and goal-directed motor-learning versus expression of habitual/skilled behaviors, respectively (Graybiel & Grafton 2015, Liljeholm & O’Doherty 2012). Further studies are necessary to determine the relation of the activation pattern within DS to the topography of inputs to DS and the functional consequence of VTA dopamine activation in associative/cognitive versus habitual behaviors.

2.4.3 Relevance to human neuroimaging and psychiatric disorders.

The existence of a functional VTA -> DS/GP circuit has implications for disorders that are traditionally associated with either the mesolimbic or non-limbic basal ganglia regions. For example, schizophrenia is considered primarily a disorder of the VTA dopaminergic system, and animal models of schizophrenia heavily focus on VTA innervated regions, in particular the VS/NAc (Csernansky & Bardgett 1998, Grace 2000). Human imaging studies, however, have shown abnormal hemodynamic activity in DS as well as VS in schizophrenia (Kumari et al 2002, Morris et al 2012, Murray et al 2008, Reiss et al 2006, Weickert et al 2009). Recent studies have further implicated dopamine neurotransmission in the associative regions of the DS, but not VS, in schizophrenia and the vulnerability to develop the disorder (Howes et al 2009, Kegeles et al 2010). While the human DS findings may have been interpreted as a departure from the involvement of limbic circuits in the pathophysiology of schizophrenia (Kegeles et al 2010), the
present data suggest that the DS dysfunctions are consistent with the “mesolimbic dopamine hypothesis” of schizophrenia.

Similarly, although most animal research on addiction focuses on the VTA to VS/NAc circuit, abnormal fMRI activity is observed in both DS and VS in addictive disorders (Claus et al 2011, Koob & Volkow 2010, Volkow et al 2012, Vollstädt-Klein et al 2010). Only recently has the crucial role of DS in addiction been recognized, and this has been explained in terms of alterations in mesostriatal projections from SN (Volkow et al 2012). Our data, however, suggest that DS abnormalities in addiction could result directly from cellular adaptations in and aberrant activation of VTA dopamine neurons. Evidence of heightened BOLD activity in both DS and VTA but moderate activation in NAc of heavy drinkers compared to social drinkers during exposure to alcohol-related cues (Claus et al 2011) supports the idea of an aberrant VTA to DS circuit in addiction.

2.4.4 Methodological considerations.

Several potential methodological concerns should be addressed. First, a recent study reported significant ectopic Cre expression in the VTA of Th::Cre mice (Lammel et al 2015), suggesting that optogenetic stimulation in Th::Cre transgenic lines may activate non-dopamine cells. The Th::Cre rat strain we used here, however, is a different genetic line, and we and others (Witten et al 2011) have observed very little ectopic expression of ChR2 in the VTA of these rats. Thus, it is unlikely that our results are due to significant activation of non-dopamine VTA neurons.

Second, optogenetic stimulation of VTA dopamine neurons is, obviously, an artificial method of activation and may result in synchronous firing of a large group of dopamine neurons.
This may not represent the spatial and temporal dynamics of VTA neuron firing that we typically observe during task performance (Kim et al 2010b, Totah et al 2013). Regardless, the results of the current study are important because they highlight the existence of VTA dopamine-dependent dynamic circuits that may come on-line during certain behaviors or pathological conditions.

Finally, the choice and level of anesthesia could have impacted our observed fMRI activation maps. For example, isoflurane anesthesia suppresses BOLD fMRI activation (Desai et al 2011), which could explain the paucity of significant BOLD activations outside the striatum in the present study. We accounted for some of this anesthesia-induced reduction in sensitivity by the use of a CBV contrast that dramatically increased fMRI activations in many areas.

2.4.5 Conclusions

Optogenetic stimulation of VTA dopamine neurons increased fMRI signals in regions, such as the VS, that are densely innervated by these neurons. Concomitant activation was observed in the dorsal striatal and pallidal regions of the basal ganglia that do not receive considerable dopamine innervation from the VTA. These results suggest a functional interaction between phasic activation of VTA dopamine neurons and basal ganglia systems, providing a novel circuit-level framework that may be critical for reassessing existing theoretical models about striatal-dependent normal functions and brain disorders such as schizophrenia and substance abuse disorder.
2.5 ACKNOWLEDGEMENTS

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3.0 MODULATION OF PFC NETWORK ACTIVITY BY VTA DOPAMINE NEURONS

3.1 INTRODUCTION

Dopamine neurotransmission in the prefrontal cortex (PFC) is critical for cognitive functions, including working memory, learning, attention, decision making as well as behavioral flexibility (Chudasama & Robbins 2004, Floresco et al 2006, Granon et al 2000, Puig et al 2014, Ragozzino 2002, Sawaguchi & Goldman-Rakic 1991, St Onge et al 2011) and has been implicated in cognitive deficits observed in schizophrenia, Parkinson’s, and ADHD (Nieoullon 2002, Robbins & Arnsten 2009). When animals perform cognitive behaviors, dopamine neurons fire phasically in response to salient events such as reward-predicting cues and unexpected rewards (Schultz et al 1997), and phasic dopamine firing elicits a large surge of dopamine release in PFC (Garris et al 1994, Garris & Wightman 1994, Lavin et al 2005, Williams & Millar 1990). There is considerable interest in understanding how phasic dopamine release modulates PFC neural activity; however, after decades of research, the nature of this modulation remains poorly understood (Puig et al 2014, Seamans & Yang 2004).

Many in vivo and in vitro studies have suggested that dopamine activity inhibits spontaneous PFC pyramidal neuron firing (Bunney & Aghajanian 1976, Godbout et al 1991, Pirot et al 1992a, Sesack & Bunney 1989) by increasing the excitability of fast-spiking (FS)
interneurons (Gorelova et al 2002, Kroner et al 2007, Seamans & Yang 2004, Tseng et al 2006, Zhou & Hablitz 1999). However, notable exceptions exist, with studies showing excitatory effects on pyramidal neurons (Sawaguchi 2001, Sawaguchi & Matsumura 1985, Sawaguchi et al 1986, Sawaguchi et al 1988) and inhibitory effects on interneurons (Tierney et al 2008). Some discrepancies among these studies could result from methodological differences, including in vitro versus in vivo (anesthetized versus awake) preparations, techniques utilized to modulate dopamine transmission in PFC (electrical stimulation, dopamine iontophoresis, or application of dopamine agonists/antagonists), and timescales of recording (Lapish et al 2007, Lavin et al 2005, Seamans & Yang 2004). Electrical stimulation activates GABA and glutamate neurons in midbrain dopamine nuclei (Morales & Margolis 2017), and dopamine pharmacology may not mimic timescales and concentrations of physiological dopamine transmission upon phasic activation of dopamine neurons. A few studies have directly activated midbrain dopamine neurons with recently available genetic techniques that allow for cell-type and/or temporal specificity and simultaneously measured PFC activity (Buchta et al 2017, Kabanova et al 2015, Popescu et al 2016), but none have systematically addressed the role of phasic dopamine activity in modulating neuronal spiking output of PFC neurons in vivo.

Cognitive processes such as working memory and attention are associated with local field potential (LFP) oscillatory phenomena in the PFC, especially in the gamma and theta range (Benchenane et al 2011, Fries et al 2001, Fujisawa & Buzsaki 2011, Puig et al 2014). Neural oscillations may play a fundamental role in information processing and could be a useful dynamic measure of network dysfunction in psychiatric disorders (Buzsáki & Draguhn 2004, Fries 2005, Fries 2009, Lisman & Buzsaki 2008, Uhlhaas & Singer 2012, von der Malsburg 2010). Influential theories have also linked PFC oscillations to dopamine neurotransmission
during cognition in normal conditions and in disorders such as schizophrenia (Benchenane et al 2011, Lisman 2012, Uhlhaas & Singer 2010, Whittington et al 2011). Accordingly, previous studies have suggested dopamine’s role in modulating PFC gamma oscillations (Pinault 2008, Wood et al 2012), alpha/beta oscillations (Puig & Miller 2012, Puig & Miller 2015), PFC-hippocampus theta coherence (Benchenane et al 2010), and delta-gamma coupling in PFC (Andino-Pavlovsky et al 2017) based on experiments involving pharmacological manipulations, including dopamine iontophoresis, systemic amphetamine injections, and D1/D2 receptor modulation; however, no study to the best of our knowledge has described a causal relationship between the phasic and selective activity of dopamine neurons and oscillations in the PFC.

Most dopamine projections to PFC in rodents originate in the ventral tegmental area (VTA) (Berger et al., 1991). Thus, in the present study, we phasically activated VTA dopamine neurons and examined PFC network activity, via measures of neuronal spiking and LFPs. To activate VTA dopamine neurons, we used optogenetic techniques in Th::Cre rat lines (Witten et al 2011) that expressed Cre-inducible channelrhodopsin-2 (ChR2) proteins in the VTA. This allowed us to selectively modulate neural activity of VTA dopamine neurons in a temporally precise manner using a blue laser (~473 nm) while simultaneously recording neural activity in the medial prefrontal cortex (mPFC) of freely moving and anesthetized rats. We examined both transient and prolonged effects of phasic activation of VTA dopamine neurons on PFC neural activity, as dopamine neurotransmission can modulate post-synaptic activity on multiple timescales (Lapish et al 2007).
3.2 METHODS

3.2.1 Subjects

Adult male transgenic Long Evans rats expressing Cre recombinase under the control of tyrosine hydroxylase promoter (Th::Cre) and wild-type littermates were used. Rats were housed in a 12-hour reverse light/dark cycle with lights on at 7 pm. Experiments were approved by and conducted according to the ethical guidelines of the Institutional Animal Care and Use Committee at the University of Pittsburgh.

3.2.2 Stereotaxic surgery, virus infusion, and cranial implantation

Cre-inducible recombinant adeno-associated viral (AAV) viral vector constructs containing the gene encoding ChR2 (AAV5-Ef1α-DIO-hChR2-eYFP) were obtained from the University of North Carolina Vector Core (Chapel Hill, NC, USA). Under isoflurane anesthesia, 1 µL injections were made at each of the four unilateral VTA sites (AP = -5.0 and -6.0 mm, ML = 0.6 – 0.7 mm, DV = -7.0 and -8.2 mm) at a rate of 0.1 µL min⁻¹ using a microsyringe (Hamilton Co., Reno, NV, USA) and a pump (World Precision Instruments, Sarasota, FL). Either in the same surgery or a few weeks after the viral infusion surgery, an optical fiber or an optrode (see below) was implanted in VTA unilaterally while an electrode or a microdialysis cannula was implanted in the ipsilateral mPFC. Optical fibers with metal cannulae (200 µm core diameter, 0.22 NA; Doric Lenses, Quebec, Quebec, Canada) were implanted in the VTA at the following coordinates: AP = -5.4 – (-5.8) mm, ML = 0.6 - 0.7 mm, DV = -7.0 mm. Optrodes were custom-
built in house by gluing an electrode array, consisting of 8 Teflon-insulated stainless steel wires, to an optical fiber; the wires extended below the optical fiber termination by ~ 500 um. The optrodes were implanted in the VTA at the following coordinates AP= -5.4 – (-5.8) mm, ML = 0.6 - 0.7 mm, DV = -7.7 mm. An electrode array consisting of 8 Teflon-insulated stainless-steel wires (NB Labs, Denison, TX) was implanted in the mPFC at the following coordinates: AP = 3.0 mm, ML = 0.7 mm, and DV = -3.3 mm. Microdialysis guide cannulae (CMA Microdialysis, Holliston, MA) were implanted at the following coordinates: AP = 3.0 mm, ML = 0.8 mm, and DV = -1.5 mm. All coordinates are given in mm relative to Bregma, and DV coordinates are relative to the brain surface; coordinates were adjusted for individual surgeries. Experiments were conducted at least 4 weeks from viral infusion surgeries.

3.2.3 Histology and immunohistochemistry

At the end of the experiment, animals were euthanized with an intraperitoneal injection of chlornal hydrate (400 mg/kg), transcardially perfused with 0.9% saline and 4% paraformaldehyde (PFA) (diluted from 32% PFA solution from Electron Microscopy Sciences, Hatfield, PA, USA) and decapitated. Brains were fixed in 4% PFA overnight at 4°C and then transferred to a cryoprotectant consisting of 20% sucrose. Brains were sectioned at 35 µm to get coronal slices of the VTA, and optical fiber/optrode placements in VTA were verified by staining slices with cresyl-violet. To examine the expression of ChR2-eYFP in dopamine neurons of VTA, VTA coronal slices were treated immunohistochemically as described in Section 2.3.4. To verify electrode and microdialysis cannula placements, 60 µm coronal slices of the mPFC were stained with cresyl-violet and observed under a light microscope.
3.2.4 Optical stimulation of VTA

For VTA stimulation, a patch cord (200-µm core diameter, 0.22 NA; Doric Lenses), attached to a 473-nm blue laser diode (OEM Laser Systems, Midvale, UT) and controlled by a Master-8 pulse generator (A.M.P.I., Jerusalem, Israel), was connected to the implanted optical fiber with a zirconia sleeve (Doric Lenses). Laser output was measured at the optical fiber tip before each experiment with a broadband power meter (Thor Labs, Newton, NJ). For most experiments in the study, VTA was stimulated with a repeated phasic stimulation sequence that utilized slow burst, fast burst, and sustained phasic protocols (Figure 3.1d). In the slow burst protocol, VTA was stimulated with 200 ms bursts (20 Hz, 5 ms pulse width, 4 pulses) repeated every 500 ms for 10 min. In the fast burst protocol, VTA was stimulated with 200 ms bursts (100 Hz, 5 ms pulse width, 20 pulses) repeated every 500 ms for 10 min. In the sustained phasic protocol, VTA was stimulated continuously for 5 s (20 Hz, 5 ms pulse width, 100 pulses), and the phasic trains were repeated every 10 s for 20 min. The sustained phasic stimulation was delivered for a longer period compared to other two protocols to acquire a sufficient number of transient trials (each 10-s period was considered a trial, ~120 total trials in 20 min). On the other hand, fast and slow burst stimulation protocols allowed for a greater number of transient trials (~1200 in 10 min).

Light output was measured to be 5 – 15 mW in Th::Cre and 9 – 15 mW in wild-type electrophysiology sessions. In microdialysis experiments, light output was measured to be ~10 mW in all Th::Cre and wild-type sessions.

For the final experiment, VTA was stimulated with a protocol that was identical to the paradigm used in the study described in Chapter 2. This experiment was conducted to facilitate comparison of electrophysiological mPFC responses observed in the current study to mPFC
fMRI responses described in Chapter 2. VTA was stimulated at 20 Hz for 20 s (pulse width = 5 ms, power = 5 mW) with a 120 s inter-stimulus interval.

### 3.2.5 Microdialysis Procedures

Rats (Th::Cre: n = 4 and wild-type: n = 3) were quickly and lightly anesthetized with isoflurane for insertion of the microdialysis probe (membrane length = 3 mm, CMA Microdialysis) into the guide cannulae without tearing the probe membrane. Probes were perfused with Ringer's solution (in mM: 37.0 NaCl, 0.7 KCl, 0.25MgCl₂, and 3.0 CaCl₂) at a flow rate of 2.0 μL/min during sample collection. Dialysate samples were collected every 20 min and immediately injected into an HPLC system for electrochemical detection of dopamine as described before (Adams & Moghaddam 1998). After establishing at least three stable baseline samples, VTA was stimulated with either the sustained phasic or the fast burst protocol for 20 min. Animals were allowed to recover from isoflurane inhalation for at least 90 min from probe insertion before collection of the first baseline sample used in data analysis. Each animal was run over multiple sessions (with at least 24 hours between sessions), but only one session per protocol per animal was used. Other sessions were removed because of leaking microdialysis probes and low sample volume, inability to separate dopamine peaks in the chromatogram, or patch cord breakage.

### 3.2.6 Electrophysiology Procedures

For awake recordings, the implanted electrodes were connected to a unity-gain junction field effect transistor headstage and lightweight cabling, which passed through a commutator and
enabled rats to move freely (Plexon, Dallas, TX). Signals were digitized at 40 kHz sampling rate, and then band pass filtered at 0.5 – 200 Hz (or 125 Hz in some cases) for LFP channels, at 300 Hz – 8kHz for mPFC spike channels and at 150 Hz – 8kHz for VTA spike channels. LFPs were amplified at 500X gain and downsampled to 1 kHz via an OmniPlex acquisition system (Plexon). The signal was referenced against a ground that was placed in the skull above the cerebellum.

To acquire neural spikes, continuous spike channel signals were thresholded using a threshold that exceeded baseline noise by at least 3 SD. Voltage waveforms that crossed the threshold were isolated into clusters using Plexon offline sorter (Plexon); clusters that were well isolated from other unit clusters and from noise were designated as single units (SUA) and clusters that did not separate well were classified as multi-units (MUA).

For LFP recordings under anesthesia, signals were digitized at 40 KHz sampling rate, band pass filtered at 0.5 –125 Hz, amplified at 2000 X gain and downsampled to 1kHz via a recorder software (Plexon). LFP signals were also notch-filtered (Q-factor = 1) at 60 Hz to prevent line noise contamination.

3.2.7 General recording session procedures

3.2.7.1 Awake mPFC recordings in repeated phasic stimulation sessions

These recordings were conducted in freely moving rats (Th::Cre: n = 17 and wild-type: n = 4). Rats were habituated to the recording chamber for 1-2 days prior to the first recording session. Each recording session consisted of 30 minutes of baseline recording, after which light pulses were delivered to the VTA using one of the repeated phasic stimulation protocols described
above. Recording continued for 60 minutes after stimulation onset. Only one stimulation protocol was delivered per session, and the order of the protocol across sessions was counterbalanced across animals (with at least 24 hours between sessions). Most animals were presented with a particular stimulation protocol only once. A few animals received multiple sessions of the same stimulation protocol because some sessions had to be removed due to suspected fiber breakage, instability of the laser power, and disruptive animal behavior during the experiment.

3.2.7.2 VTA optrode recording

A subset of Th::Cre rats (n = 4) used in the awake mPFC recordings were also implanted with optrodes in VTA to examine responses of VTA units to laser stimulation. Recordings in the VTA were conducted either in the same session as the mPFC recordings or in a separate session. In a few sessions for which mPFC recordings were not used for analysis, laser output was adjusted on-line (between 1-18 mW); power was increased to elicit activation in VTA units if no response was detected or reduced if multi-unit response or large field response to stimulation prevented isolation of single units.

3.2.7.3 Awake and anesthetized recordings in 20 Hz, 20 s stimulation sessions

These recordings sessions were conducted in a subset of Th::Cre rats (n = 5) after the repeated phasic stimulation sessions were completed. Awake recordings were conducted first, followed by anesthetized recordings with at least 24 hours between those two sessions. In anesthetized recording sessions, rats were induced at 4% isoflurane, after which isoflurane was gradually
reduced to 0.8 – 1.5% and maintained at that concentration for at least 10 min before commencing experiments.

### 3.2.7.4 Movement tracking

Movement activity was tracked throughout in most of the freely moving recording sessions via an infrared activity monitor (Coulbourn, Holliston, MA).

### 3.2.8 Data Analysis

Data analysis was conducted with custom scripts in Matlab (Mathworks) and SPSS statistical software (IBM). Hyunh-Feldt correction was applied for all repeated measures ANOVAs to correct for sphericity violation. Multiple comparison correction was applied for more than 3 post-hoc comparisons. Parametric or non-parametric statistical tests were used depending on the data.

### 3.2.8.1 Microdialysis data

Dopamine concentration (fmol/μL) for each sample was expressed as a percentage of average baseline dopamine concentration (baseline constituted three samples immediately before stimulation). For statistical analysis, % values were first log transformed to reduce the large variability in dopamine concentration increases upon stimulation. Repeated measures ANOVA was conducted on log transformed values, and one sample tests against baseline average were performed for post-hoc analysis; one tailed t-tests were used to test the hypothesis that stimulation elicited an increase in dopamine levels. A two way repeated measures ANOVA was
also run to compare mPFC extracellular dopamine responses to VTA stimulation in \textit{Th::Cre} and wild-type rats.

\subsection*{3.2.8.2 VTA optrode data}

To assess the responses of VTA single units to optogenetic VTA stimulation, peri-event histograms and rasters were examined. For each unit, the probability of spiking at different latencies from the time of a single blue laser pulse delivery was also calculated.

\subsection*{3.2.8.3 mPFC unit classification}

Single units recorded in mPFC were classified as regular spiking (RS) or FS based on their firing rate at baseline (before stimulation) and spike waveform characteristics (Homayoun & Moghaddam 2009). As FS units fire at higher frequencies and exhibit narrow spike waveform widths, units were identified as FS if firing rate $\geq 10$ Hz, valley width at half height $\leq 0.3$ ms, and peak to valley width $\leq 0.3$ ms. Based on this criterion, 7 units were classified as FS and the rest as RS units.

\subsection*{3.2.8.4 Movement analysis}

Based on digital movement scores and manual recordings of animal behavior, 3 min epochs in baseline, stimulation, and post-stimulation periods during which animals were continuously moving at comparable levels were manually identified. For \textit{Th::Cre} rats, two epochs (stim 1 and stim 2) were extracted during the stimulation period. As wild-type rats were less mobile, only one movement epoch was extracted during the stimulation period; to keep the number of epochs
for statistical comparison similar to *Th::Cre* rats, two movement epochs were isolated in the post-stimulation period. If at least two 3-min movement epochs in *Th::Cre* sessions and one 3-min epoch in wild-type sessions couldn’t be identified during the stimulation period, those sessions were removed from analyses that involved movement epochs only. As this method resulted in the exclusion of many wild-type sessions, data from all three stimulation protocols were combined for wild-type rats (n = 6 sessions from two rats) to increase the sample size. A similar method was used to identify quiescent epochs when animals were mostly still in baseline, stimulation, and post-stimulation periods. All extracted post-stimulation epochs occurred at > 15 min from offset of stimulation sequence.

### 3.2.8.5 Transient unit activity around each phasic stimulation train

**Individual unit response.** Transient responses of prefrontal units to phasic VTA stimulation were examined by binning spike counts (bin size = 1s for sustained phasic protocol and bin size = 0.05 s for fast and slow burst protocols) and calculating firing rates. Independent t-tests were conducted between each bin’s firing rate against mean baseline firing rate, and units were deemed to be significantly modulated if at least one post-stimulation bin was significantly different from baseline at p ≤ 0.001. P ≤ 0.001 criterion was more conservative than Bonferroni correction, but this criterion prevented false positives based on examination of rasters and histograms. Color maps were used to visualize responses of all units; values in the color map indicated changes in firing rate from baseline represented by t-values.

**Population response.** To examine changes in population activity patterns, a distance-based similarity measured was used. For this measure, each population state at a particular time was comprised of spike counts of N-units in the N-dimensional space, forming a spike count
vector. We then calculated Euclidean distance between population states during stimulation and baseline to assess whether the population states diverged over time from baseline as a result of phasic VTA dopamine activity.

Units with firing rates < 0.1 Hz were removed from this analysis. To get pseudo-baseline trials, the baseline period was binned into consecutive trials of the same duration as stimulation trials (10 s for sustained phasic stimulation and 0.5 s for fast and slow burst stimulation). Within each trial, spike counts were calculated in bins (bin size = 2 s for sustained phasic stimulation and 0.1 s for fast and slow burst stimulation). Spike counts for each bin were pooled across all recorded single and multi-units (not just simultaneously recorded) to get pseudo-population spike count vectors. Then, Euclidean distances were calculated between spike count vectors from pseudo-baseline trials and stimulation trials in each bin. One tailed two sample t-tests (Bonferroni corrected) between distances in the first bin and all other bins were conducted to assess if the population activity diverged over time because of VTA stimulation (which would correspond to increased distances, thus right t-tailed t-tests were used). We calculated population distances with raw spike counts and with normalized spike counts (Z-score normalized to the first bin). However, before normalization, units with firing rates < 1 Hz were removed. Inclusion of those low firing units would result in noisy population responses after normalization, as spike count = 1 in a bin would correspond to very high Z-scores for these units.

3.2.8.6 Sustained unit activity during repeated phasic VTA stimulation

Even though we recorded neural activity for 30 min prior to and 60 min after stimulation onset, we only conducted data analysis in the period -20 to 40 min for most analyses. In a few animals,
we examined the additional 10 min periods during baseline and post-stimulation to extract moving or quiescent epochs.

**Individual unit response.** Unit spike counts were binned into 120 s to get firing rates. Firing rates were Z-score normalized against baseline, and units were determined to be significantly activated or inhibited if firing rates post-stimulation were respectively greater or less than 99% confidence interval (corresponding to Z-scores > or < 1.96) of baseline in 3 consecutive bins (Wood et al 2012). Z-values rather than t-values were used to get confidence intervals and assess significance as there were no trials in this analysis, and each unit’s activity was assessed over the course of a session. Thus, firing rates across baseline bins were used to calculate baseline standard deviation for obtaining Z-values; t-values would, in this case, be highly influenced by the number of baseline bins. Proportions of units activated/inhibited were also calculated during the period of stimulation (20 min for sustained phasic and 10 min for fast and slow burst sessions) and during the entire post-stimulation period of 40 min from stimulation start. Fisher’s exact test was used to compare proportions between *Th::Cre* and wild-type rats.

**Population response.** To assess sustained population responses to prolonged VTA stimulation, Euclidean distances of spike count vectors were calculated between baseline and stimulation. Spike counts were pooled across all recorded single and multi-units to get pseudo population response vectors. Units with firing rates < 0.1 Hz were removed. The entire session was divided into epochs of 5 min duration, resulting in 4 baseline epochs (-20 to -15 min, -15 to -10 min, -10 to -5 min, and -5 to 0 min). Each epoch was further binned into 10 s consecutive trials (total 30 trials). Either raw spike counts or normalized spike counts (units with firing rates < 1 Hz were removed and spike counts were Z-score normalized to the first baseline epoch) were used for the distance analysis. All pairwise distances of spike count vectors were calculated
between each trial of an epoch and all trials of baseline epoch 2 and then averaged to yield one
distance value per trial. To determine whether the population response significantly diverged
from baseline after stimulation, distances for each epoch were compared against distances for
baseline epoch 3 using right-tailed two sample Bonferroni corrected t-tests.

When population activity was analyzed in movement epochs only, each epoch was
divided into 10 s consecutive trials (total: 18 trials). Spike count vector distances between each
trial of an epoch (stimulation or post-stimulation) and all trials of the baseline epoch were
averaged to yield a mean pairwise distance value per trial. To assess the divergence of population
activity upon stimulation, these mean pairwise distance values were compared between
stimulation and post-stimulation epochs using one-tailed Bonferroni-corrected two sample t-
tests.

3.2.8.7 Transient LFP response to each phasic stimulation train

Transient LFP data were analyzed using the continuous wavelet transformation or CWT (with
Morlet wavelets) function in Matlab. A CWT rather than fast-fourier transform (FFT) was used
for transient analysis of LFP power because the former method is especially suited to detect
transient changes in LFPs, and FFT frequency resolution depends on the bin size (which would
be around 0.1 s for fast and slow burst stimulation trials; this bin size would highly reduce the
frequency resolution and wouldn’t permit examination of power in low frequencies). LFPs were
detrended, artifacts were removed, and CWT was applied to LFP from each stimulation trial (that
was 10 s in duration for sustained phasic stimulation and 0.5 s for fast and slow burst
stimulation). LFP power at each frequency and time point was averaged across trials and Z-score
normalized to the total power within each frequency. Frequencies were binned into several
bands: delta (1–4 Hz), low theta (4.5–8 Hz), high theta (8–13 Hz), beta (14–30 Hz), low gamma (30–55 Hz), and high gamma (55–100 Hz). Averaged LFP power within each frequency band was divided into 1 s bins (for sustained phasic stimulation) or 0.05 s bins (for fast and slow burst stimulation), and repeated measures ANOVAs followed by Bonferroni corrected post-hoc paired t-tests against baseline were used to assess changes in LFP power within each frequency band over time.

3.2.8.8 Sustained LFP response to repeated phasic VTA stimulation

LFP was detrended, artifacts were removed, and data were analyzed using the Chronux toolbox (http://www.chronux.org). LFP spectral power was calculated using a multi-taper FFT with a sliding time window of 4 s in 2 s steps. A standard multi-taper approach was used that applied the 15 leading tapers to each window (time bandwidth product = 8). Each session’s spectrogram was Z-score normalized to baseline, and Z-scored LFP power for each frequency band was divided into 5 min bins. A repeated measures ANOVA (followed by post-hoc Bonferroni corrected paired t-tests against baseline) was conducted to detect any difference in spectral power across time.

For the analysis of LFPs in moving or quiescent epochs, a multi-taper FFT was conducted with the same parameters as in the whole session analysis. Each epoch’s spectrogram was Z-score normalized against the first 60 s of the baseline epoch (for visualization as a color map, the entire baseline period of 180 s was used for normalization). Z-scored LFP power values across all time points within an epoch were averaged for each frequency band, and the effect of stimulation was assessed by a repeated measures ANOVA followed by paired t-tests.
3.2.8.9 Assessment of phase locking of spikes to LFPs

To correct for phase lags in lower frequencies, LFPs were first aligned using FPAlign (Plexon). Signals were then band-pass filtered using eegfilt.m (using the least squares finite infinite response filter) function from the EEGLab toolbox (https://sccn.ucsd.edu/eeglab/index.php). Band-pass filtered LFPs were Hilbert transformed to extract instantaneous phases, and a circular distribution of phases for each unit was obtained by extracting phases corresponding to each spike. For the entire session analysis, LFPs and unit spike counts were divided into 5 min bins. Single units and multi-units were pooled, and only units that emitted at least 100 spikes within each bin were included. The circular statistic toolbox (Berens 2009) was used to perform Rayleigh’s Z-test for circular uniformity and determine if units were significantly phase locked to specific bands within each time bin. Proportions of significantly phase locked units were compared across time bins using Fisher’s exact tests. To determine the strength of phase-locking to each frequency band, phase locking values (PLVs) were computed for all units. PLVs correspond to the mean resultant length (MRL) of phase angles. In short, MRL is calculated as the modulus of the sum of unit vectors representing instantaneous LFP phases corresponding to each spike divided by the number of spikes (Siapas et al 2005). To assess a change in the strength of phase-locking over time, PLVs for all units (not just significantly phase locked units) were compared using Friedman’s ANOVA followed by Bonferroni-corrected signed rank tests.

For the movement epoch analysis, proportions of significantly phase locked units and PLVs for all units were calculated within 3 min epochs and compared across epochs using Fisher’s exact test for proportions and Friedman’s ANOVA respectively.
3.2.8.10 Assessment of phase amplitude coupling in LFPs

LFPs were band pass filtered using the EEG toolbox and Hilbert transformed within each band to extract instantaneous phases and amplitudes. Phase amplitude coupling between the phase of low frequencies (delta, low theta, and high theta) and the amplitude of higher frequencies (low gamma and high gamma) was assessed by calculating modulation indices (MI). MI was calculated using the get_mi.m function in the cross frequency toolbox (available from http://attention-circuits-control.org/doku.php?id=cross-frequency-coupling) that is based on Tort et al. (2010)’s method (Tort et al 2010). MI depends on the Kullback-Leibler distance between an empirical distribution containing amplitudes of one signal (high frequency in this study) over phase bins of another signal (low frequency in this study) and a uniform distribution.

For the entire session analysis, MI values for each frequency pair were calculated in 5 min bins and normalized for each animal by dividing by the average MI value in the session. Differences in MI values over time were assessed via a Friedman’s ANOVA followed by Bonferroni corrected signed rank tests. To rule out spurious effects, surrogate sets were built by randomly partitioning LFP time series into two slices and rearranging the order of the slices; 100 surrogate sets were built and the MIs for all surrogate time series were averaged to get a surrogate MI value per animal.

For the movement epoch analysis, MI values were normalized by the total MI value across epochs and were assessed for effects of stimulation using a Friedman’s ANOVA followed by signed rank tests.
3.2.8.11 LFP analysis in the 20 Hz, 20 s stimulation sessions

**Awake recordings.** LFPs were detrended, artifacts were removed, and a digital 60 Hz notch filter (Q-factor = 1) was applied. A multi-taper FFT (time bandwidth product = 3 and number of tapers = 5, window size = 1 s, step size = 0.1 s) was then conducted using the Chronux toolbox to generate a spectrogram for each trial. LFP power for each frequency and time point was averaged across trials and Z-score normalized against the pre-stimulation period -20 to -10 s. Z-scored LFP power in each frequency band was binned into 5 s bins, and a repeated measures ANOVA (followed by Tukey’s post-hoc tests) was utilized to determine significant changes in power over time.

Trials were classified based on animals’ behavior (assessed in terms of movement frequency) before and during stimulation. If the animal was fairly still/ quiescent during baseline and during stimulation (< 0.1 Hz movement), trials were designated as “still throughout”. If the animal was still (< 0.1 Hz movement) or exhibited low levels of movement (between 0.1 Hz to 1 Hz) during baseline but showed high levels of movement (> 1 Hz) during stimulation, the trials were identified as “movement increase” trials. If the animal was moving at high but comparable levels (> 1 Hz) before and after stimulation, trials were considered to be “movement throughout” trials. All other trial types were considered ambiguous and not included.

**Anesthetized recordings.** LFP processing procedures were similar to what is described for awake recordings, except that a 60 Hz notch filter was not applied during analysis as this was done during data acquisition. Trials were visually classified into two types based on whether the LFPs exhibited high frequency components or high-amplitude spindles along with reduction in low amplitude fast frequencies, and LFPs for the two trial types were separately analyzed.
3.3 RESULTS

We injected AAV5-DIO-ChR2-eYFP into the VTA to selectively target VTA dopamine neurons in \textit{Th::Cre} rats (Figure 3.1a) or to control for non-specific effects of stimulation in wild-type littermates. After a wait time of at least four weeks to allow for maximal virus expression, we recorded extracellular activity (units and LFPs) in mPFC while optically stimulating VTA with blue light in freely behaving rats (Figure 3.1b). All electrode implants were histologically verified to be in mPFC, and optical fiber placements were confirmed to be inside or just dorsal to VTA (Figure 3.2). We have previously verified highly specific and sensitive expression of ChR2 in VTA dopamine neurons of these \textit{Th::Cre} rats (Lohani et al 2017). In mPFC recording sessions, baseline activity was recorded for 30 minutes after which a particular stimulation protocol was delivered. Following stimulation offset, neural activity was recorded for at least 40 min (60 min from stimulation start) (Figure 3.1c).

During stimulation, one of three protocols was utilized to phasically activate VTA dopamine neurons. These protocols were chosen to mimic the phasic activity patterns observed in VTA dopamine neurons when animals are engaged in relevant behaviors. For example, when animals are performing reward-guided tasks, dopamine neurons fire in bursts with intra-burst frequencies between 15 – 100 Hz in response to various salient events of the tasks such as cues and rewards (Hyland et al 2002, Kiyatkin & Rebec 1998, Schultz et al 1997). In addition, these tasks typically last 10 – 20 min (Cheng et al 2003, Stefani & Moghaddam 2006), during which many trials are repeated; dopamine neurons, thus, burst fire repeatedly over the duration of the task. To mimic the repetitive bursting of dopamine neurons during behaviorally relevant states, we utilized two protocols: fast and slow burst stimulation protocols that contained a sequence of
bursts with burst width = 200 ms and intra-burst frequency = 20 Hz (slow) or 100 Hz (fast). Bursts were repeated with inter-burst frequency = 500 ms for 10 min (Figure 3.1d). We also employed a third protocol termed as sustained phasic stimulation to mimic enhanced phasic firing of dopamine neurons for many seconds. Sustained phasic increases in VTA dopamine neuron firing, although not typically reported in the literature, have been observed in a sustained attention task (Totah et al 2013). Similarly, a voltammetry study showed prolonged dopamine signaling in the ventral striatum between trial onset and goal reaching for a period of 5 – 10 s in a maze task (Howe et al 2013), which could be related to prolonged dopamine phasic firing (Niv 2013). In the sustained phasic protocol, a train of blue light pulses (20 Hz, train width = 5 s) was delivered every 10 s for a total of 20 min. These protocols allowed us to examine changes in neural activity transiently (on the order of milliseconds to seconds) around each burst or phasic train as well as on a prolonged timescale (on the order of minutes). The latter timescale is important because dopamine is a slow acting neuromodulator, partly due to metabotropic nature of dopamine receptors and extra-synaptic localization of dopamine receptors in terminal regions (Caille et al 1996, Smiley et al 1994a). Dopamine’s actions in PFC might be especially prolonged due to fewer dopamine transporters (DATs) (Sesack et al 1998a) that can cause dopamine released during phasic transients to accumulate over time in the extracellular space (Lapish et al 2007).
Figure 3.1 Histology, experimental schematic, and stimulation protocols.

a) Immunohistological images showing expression of ChR2-eYFP (left) and TH (right) in VTA of a representative Th::Cre rat. Scale bar indicates 550 µm. White triangles point to the termination of an optical fiber implant just dorsal to VTA. b) (Left) Schematic of simultaneous optogenetic stimulation of VTA and recording in mPFC. (Right) Schematic of recording sessions in freely moving rats. c) Recording timeline in a session. d) Illustrations of stimulation protocols.
Figure 3.2 Histological confirmation of electrode and optical fiber placements.

Histological confirmation of multi-wire electrodes in mPFC (left) and optical fibers in VTA (right) of Th::Cre and wild-type rats. Note that most optical fiber implants terminated dorsal to VTA so that blue light would illuminate the entire VTA.
3.3.1 Optogenetic stimulation entrained VTA dopamine neurons

In a subset of \(Th::Cre\) rats, VTA was implanted with an optrode instead of an optical fiber; the optrode allowed us to examine responses of VTA dopamine neurons to blue light stimulation in the region. Responses of three well-isolated VTA units to different stimulation protocols are shown in Figure 3.3. All units responded reliably to light pulses in the sustained phasic stimulation and slow burst stimulation protocols (probability of spiking with a latency of < 10 ms was > 90%) (Figure 3.3a, 3.3c, and Figure 3.4a, 3.4c), while units did not respond with high fidelity to the fast burst stimulation pulses (probability of spiking for unit 1 at a latency of < 10 ms was 28% and for unit 2 was 10%) (Figure 3.3b). In spite of the overall low probability of spiking during fast burst stimulation, Unit 1 showed more reliable responses to pulses presented earlier in the burst compared to later in the burst (Figure 3.3b). Furthermore, from observing the rasters and histograms (Figure 3.3b) as well as raw voltage traces (Figure 3.4b) for these units, it is apparent that fast burst stimulation elicited transient bursts with a few spikes emitted at a frequency of 10 – 100 Hz. We also did not observe an apparent change in light responsivity of dopamine neurons over time due to factors such as ChR2 desensitization or depolarization block, as their probabilities of spiking in response to light pulses were similar in early and late trials (Figure 3.4d).
Figure 3.3 Optogenetic stimulation entrained VTA dopamine neurons.

Three well-isolated VTA units (from n = 2 rats) responded to VTA optogenetic stimulation. Responses of units to different stimulation protocols are presented in a), b), and c). For each subfigure, leftmost panels show rasters (top) and corresponding peri-event histograms (bottom; bin size = 1 s (panel a), bin size = 0.1 s (panels b and c)). On rasters, trial numbers are shown on the left (note: total trial numbers are different for each raster). T = 0 indicates start of a burst or phasic train and blue rectangles mark the period of stimulation. Rightmost panels depict the probability of unit spiking at latencies 0-10 ms from the delivery of single blue light pulses.
Figure 3.4 Optogenetic stimulation entrained VTA dopamine neurons.

(a,b,c) Raw voltage traces for unit 2 whose responses to VTA stimulation with sustained phasic (a), fast burst (b), and slow burst (c) protocols are also displayed in raster and histogram format in Figure 3.3. Blue squares indicate when each blue light pulse was delivered. (d) Probability of unit spiking (within 10 ms) during early trials (first 1 min) and late trials (between 9-10 min from stimulation sequence start for fast and slow burst protocols; 19-20 min for sustained phasic protocol).
3.3.2 Optogenetic stimulation of VTA dopamine neurons increased mPFC dopamine levels

As dopamine is a neuromodulator and could elicit subtle effects on mPFC network activity, we first wanted to ensure that VTA optogenetic stimulation elicited sufficient dopamine release in mPFC. A different group of rats was implanted with microdialysis probes in mPFC and optical fibers in VTA (Figure 3.5a). We found that VTA optogenetic stimulation with the sustained phasic and fast burst protocols increased mPFC extracellular dopamine levels ([DA]₀) on average by >200% of baseline selectively in \textit{Th::Cre} but not wild-type rats. The increase in [DA]₀ was sustained during post-stimulation samples (Figure 3.5). These increases were similar to previously reported increases in [DA]₀ when animals are performing a reward-driven cognitive task (Stefani & Moghaddam 2006), suggesting that our stimulation protocols elicited physiologically relevant sustained dopamine release in mPFC. All probe and optical fiber placements were histologically verified to be in mPFC and inside/just dorsal to VTA respectively (Figure 3.6).
Figure 3.5 Phasic activation of VTA dopamine neurons increased [DA]₀ in mPFC.

a) Schematic of microdialysis setup. b) Sustained phasic stimulation of VTA dopamine neurons for 20 min significantly increased mPFC [DA]₀ (F(8,24) = 6.536, p = 0.001, repeated measures ANOVA) in stimulation sample 4 and post-stimulation sample 5 compared to baseline (sample 4: p = 0.002, sample 5: p = 0.043; t-tests) in Th::Cre rats (n = 4 rats, mean baseline [DA]₀ = 0.114 +/- 0.088 fmol). Sustained phasic stimulation in wild-type rats (n = 3 rats, mean baseline [DA]₀ = 0.136 +/- 0.026 fmol) did not increase [DA]₀ (F(8,16) = 1.016, p = 0.462). c) Fast burst stimulation of VTA dopamine neurons for 20 min significantly increased [DA]₀ (F (8,16) = 6.107, p = 0.005) in stimulation sample 4 and post-stimulation sample 5 (sample 4: p = 0.026, sample 5: p = 0.026; t-tests) in Th::Cre rats (n = 3 rats, mean baseline [DA]₀ = 0.109 +/- 0.052
Wild-type rats (n = 3 rats, mean baseline $[DA]_0 = 0.220 +/- 0.108 \text{ fmol}$) did not show an increase in $[DA]_0$ after fast burst VTA stimulation ($F(8,16) = 2.231$, $p = 0.228$). Furthermore, two way repeated measures ANOVAs indicated that the pattern of $[DA]_0$ change over time was significantly different between $Th::Cre$ and wild-type rats after stimulation (sustained phasic stimulation: $F(8,40) = 4.362$, $p = 0.001$, fast burst stimulation: $F(8,32) = 6.075$, $p = 0.000$; interaction effects). Error bars indicate mean +/- SEM. ** $p < 0.05$ and * $p < 0.01$. 
Figure 3.6 Histological verification of microdialysis probe and optical fiber placements.

Histological confirmation of microdialysis probes in mPFC (left) and optical fibers in VTA (right) of Th::Cre and wild-type rats.
3.3.3 Transient modulation of mPFC unit activity by phasic VTA dopamine activity

We recorded well-isolated single units ($Th::Cre$: $n = 131$ and wild-type: $n = 55$) and multi-units ($Th::Cre$: $n = 69$ and wild-type: $n = 24$) from freely moving rats. Most single units recorded in $Th::Cre$ rats were classified as RS or putative pyramidal units, and only seven units were classified as putative FS (Figure 3.7). We first examined the responses of individual units to each phasic VTA stimulation train (either a burst that lasted for 200 ms or a sustained train that lasted for 5 s). We found that only a handful of units responded transiently to VTA phasic stimulation. These units showed heterogeneous responses among them, as some were activated and others were inhibited (Figure 3.8a). Overall, only 7.5% of units (across all stimulation protocols) were transiently modulated by phasic VTA dopamine activity in $Th::Cre$ rats, and the proportion of activated and inhibited units was similar (Figure 3.8b and Table 3.1). 57% of FS units (compared to 6% of pyramidal units) were modulated by phasic dopamine activity; the small sample in the present data may, however, render this an unreliable indicator of the effects of dopamine activity on FS units. Nonetheless, it is possible that FS neurons are more likely to be modulated by transient VTA dopamine activity than pyramidal neurons. Interestingly, we observed transient activation and inhibition in both pyramidal and FS units (example units’ responses shown in Figure 3.9) in contrast to previous in vivo rodent studies that have primarily reported inhibition in pyramidal units and excitation in FS units (Pirot et al 1992b, Sesack & Bunney 1989, Tseng et al 2006). These findings suggest that phasic VTA dopamine activity can transiently modulate mPFC neuronal activity on the order of milliseconds to seconds in a few units, and these modulations can be heterogeneous in pyramidal and FS units. None of the units (SUA and MUA) recorded in wild-type animals showed significant response to stimulation (Table 3.1).
Phasic VTA dopamine activity did not transiently modulate population-level responses, which were assessed in terms of the similarity of population activity patterns (which will be described in detail in the next section) between baseline and stimulation (all $p > 0.05$ for comparison of Euclidean distances between baseline and stimulation trials, Bonferroni corrected).
Figure 3.7 Classification of mPFC units.

Classification of prefrontal units recorded in Th::Cre rats as regular spiking (RS) and fast-spiking (FS) units. Units were identified as FS if firing rate $\geq 10$ Hz, valley width at half height $\leq 0.3$ ms, and peak to valley width $\leq 0.3$ ms.
Figure 3.8 Transient responses of mPFC units to phasic VTA dopamine neuron activation.

a) Firing rate changes around each phasic train (that lasted for 5 s in the sustained phasic protocol and for 0.2 s in the fast burst and slow burst protocols) are depicted as t-value color maps for single units (SUA, top) and multi-units (MUA, bottom). Firing rate changes compared to pre-stimulation periods are represented by t-values. Each row indicates one unit, and bin size = 0.025 s for fast and slow burst stimulation and bin size = 0.25 s for sustained phasic stimulation. b) (Left) Percentage of units across all stimulation protocols that were significantly
activated/inhibited on a transient timescale by each phasic stimulation train. (Right) Summary of the number of regular-spiking (RS) or fast-spiking (FS) single units and multi-units significantly modulated by transient phasic stimulation trains (pooled across all protocols). A similar summary is depicted in Table 1.

**Table 3.1 Summary of transient modulation of mPFC unit activity.**

Summary of modulation of mPFC unit activity by transient stimulation trains (phasic bursts or sustained phasic events). Each cell indicates the total number of single units (SUA): regular spiking (RS) or fast-spiking (FS) units or multi-units (MUA) significantly activated or inhibited by each stimulation protocol. The rightmost column indicates the percentage of units across all three protocols that were significantly modulated by VTA optogenetic stimulation.

<table>
<thead>
<tr>
<th></th>
<th>Fast Burst</th>
<th>Slow Burst</th>
<th>Sustained Phasic</th>
<th>Total Units</th>
<th>% Modulated</th>
</tr>
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<tr>
<td></td>
<td>Activated</td>
<td>Inhibited</td>
<td>Activated</td>
<td>Inhibited</td>
<td></td>
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</table>
Figure 3.9 Example mPFC unit responses.

Transient unit responses for example regular-spiking (RS) (a) and fast-spiking (FS) (b) units in mPFC. Top plots are peri-stimulus histograms and bottom plots are rasters. For histograms, bin size = 1 s for units recorded in the sustained phasic stimulation session (identified here as histograms with x-axis that ranges between -2 to 8 s) and bin size = 0.05 s for units recorded in response to fast or slow burst protocols (identified here as histograms with the x-axis range between -0.1 to 0.4 s). Blue rectangles mark the time of stimulation.
3.3.4 Sustained modulation of mPFC unit activity by repeated phasic VTA dopamine activity

Unit activity was also examined on a longer timescale (bin size = 120 s). Phasic VTA dopamine activity significantly modulated about 7% of pyramidal units and 14% of multi-units during the period of stimulation that lasted for 10 – 20 min (Figure 3.10b and Table 3.2) across all stimulation protocols. Units were more likely to show delayed modulation after stimulation, as the percentage of units modulated during the entire period of 40 min from stimulation onset was higher than during the stimulation period alone (Figure 3.10c and Table 3.3). Most single units that responded to stimulation were pyramidal units. Across all stimulation protocols, only two FS units were modulated. Generally, the population of units that showed transient versus lasting responses did not completely overlap as well. Only four units across all protocols that showed transient responses also showed prolonged responses, suggesting that different ensembles of neurons might be modulated on different timescales. We also observed non-specific unit responses in wild-type rats, possibly reflecting gradual changes in the firing rates of some units over time (Figure 3.10b and Table 3.2). This was more apparent when examining changes over the entire session (Figure 3.10c and Table 3.3). Nonetheless, overall more units were modulated by VTA stimulation in Th::Cre rats (26%) compared to wild-type rats (13%) in the entire session, but the proportions of units activated/inhibited across groups were not significant (Fisher’s exact test, p > 0.05).
Figure 3.10 Sustained mPFC unit responses to phasic VTA dopamine neuron activation.

a) Firing rate changes during the entire period of stimulation and post-stimulation on a prolonged time scale (bin size = 120 s) are depicted as Z-value color maps for single units (SUA, top) and multi-units (MUA, bottom) recorded in Th::Cre rats. Firing rate changes compared to the pre-stimulation baseline period are represented by Z-values. Each row indicates one unit. b) Percentage of units (combined SUA and MUA) that were significantly activated/inhibited during the stimulation period only (that lasted for 20 min for the sustained phasic (SP) protocol and 10 min for the fast burst (FB) and slow burst (SB) protocols) in Th::Cre and wild-type rats. c) Percentage of units (combined SUA and MUA) that were significantly activated/inhibited during
and/or after stimulation (during a period of 40 min from stimulation start) in \(Th::Cre\) and wild-type rats.

Table 3.2 Summary of prolonged modulation of mPFC unit activity during stimulation period only.

Each cell indicates the total number of single units (SUA)—regular spiking (RS) or fast-spiking (FS) units—or multi-units (MUA) significantly activated or inhibited by each stimulation protocol. The rightmost column indicates the percentage of units across all three protocols that were significantly modulated by VTA optogenetic stimulation.

<table>
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<th></th>
<th>Fast Burst</th>
<th>Slow Burst</th>
<th>Sustained Phasic</th>
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Table 3.3 Summary of prolonged modulation of mPFC unit activity during the period of 40 min post stimulation start.

Each cell indicates the total number of single units (SUA)—regular spiking (RS) or fast-spiking (FS) units—or multi-units (MUA) significantly activated or inhibited by each stimulation protocol. The rightmost column indicates the percentage of units across all three protocols that were significantly modulated by VTA optogenetic stimulation.

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<th></th>
<th>Slow Burst</th>
<th></th>
<th>Sustained Phasic</th>
<th></th>
<th>Total Units</th>
<th>%Modulated</th>
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<td>21</td>
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Because of the observed bidirectional modulation (both activation and inhibition) of unit activity by VTA stimulation (which rendered averaging firing rates across all neurons to examine population activity less meaningful) and because proportions of activated/inhibited units in Th::Cre versus wild-type did not provide a clear picture of net modulation of mPFC neural activity, we examined activity patterns across the entire mPFC population to better understand neural ensemble response. A concept that has been very useful to examine population response is the firing rate or spike count vector (Bartho et al 2009, Stopfer et al 2003). Each vector consisting of spike counts of N-neurons, which can be denoted by a point in an N-dimensional space, represents the neural population state. The neural population state can change with time.
and stimulus presentation, and similarity measures, such as Euclidean distances between vectors, can be used to compare population states between different time points or stimulus conditions.

We utilized the Euclidean distance measure to assess VTA dopamine activity modulation of neural population state over time. For this analysis, we pooled all recorded units (not just simultaneously recorded) to obtain pseudo-spike count population vectors and calculated the spike count vector distance between the baseline period and all other stimulation/post-stimulation periods. We found that upon repeated phasic stimulation of VTA dopamine neurons, mPFC population response exhibited a significant divergence from baseline activity within 5 min from stimulation onset in Th::Cre rats but not wild-type rats. This response was sustained for up to 5 min after stimulation offset, after which it gradually returned to the pre-stimulation state (Figure 3.11). Fast burst stimulation of VTA dopamine neurons, on the other hand, produced a transient population response at 5–10 min from stimulation onset that was significantly different from baseline (Figure 3.11). Slow burst stimulation did not significantly modulate population activity during stimulation but elicited a delayed response at 15–25 min from stimulation onset (Figure 3.11). To rule out the possibility that these population level changes were determined mostly by units with high firing rates, we normalized the activity of units (units with firing rates <1 Hz were removed because after normalization, small changes in spike counts of these units elicited noisy population responses) before calculating distances. The findings were similar to before normalization, except that the delayed response upon slow burst stimulation was absent (Figure 3.12). These findings suggest that repeated phasic VTA stimulation can elicit significant population responses that are specific to activation of VTA dopamine neurons, are mostly time-locked to the duration of stimulation, and depend on the type of stimulation.
Differences in population response across stimulation protocols were comparable to the differences in the amount of behavioral modulation elicited by those protocols in Th::Cre rats. Sustained phasic stimulation significantly and robustly increased movement (movement index tracked all active events, including grooming, rearing, and locomotion) while fast burst stimulation produced weaker but significant movement increases (Figure 3.13). Slow burst stimulation did not change behavior compared to baseline. In wild-type rats, behavior was not significantly modulated by any stimulation protocol (repeated measures ANOVA, sustained phasic stimulation: F(11,33) = 1.296; fast burst stimulation: F(11,33) = 0.483; slow burst stimulation: F(11,11) = 0.591; all p > 0.05).

As an animal’s behavior in a freely moving preparation is very dynamic and because of the observed modulation of behavior by stimulation, we also examined population responses during baseline and after stimulation in similar behavioral states. For this, we identified movement only epochs in baseline, stimulation, and post-stimulation periods during which animals were moving at comparable rates (Figure 3.14 and 3.15). Even when movement behavior was similar to baseline, sustained phasic stimulation and fast burst stimulation significantly modulated population responses in the stimulation period (Figure 3.16 and 3.17), and results were comparable with or without unit spike count normalization. Interestingly, while significant population responses to slow burst stimulation were not detected when data from the whole session were analyzed irrespective of behavior (Figures 3.11 and 3.12), there was some significant population response during stimulation when movement only epochs were analyzed (Figure 3.16 and 3.17). This discrepancy may be explained by differences in average behavior during stimulation periods used in the whole session versus movement epoch only analysis. In the latter analysis, the stimulation period obviously consisted of only active behaviors. On the
other hand, many slow burst stimulation sessions used in the whole session analysis contained mostly quiescent behaviors during VTA activation, which resulted in low levels of average movement across sessions (Figure 3.13). These findings raise the possibility that VTA dopamine activity’s effects on mPFC population response depends on the behavioral state, whether an animal is engaged in active behaviors versus if the animal is mostly quiescent. This idea also fits with the observations that population responses (Figures 3.11 and 3.12) and animals’ behaviors (Figure 3.13) returned to baseline levels soon after the offset of sustained phasic and fast burst stimulation sequences, even though microdialysis experiments demonstrated that [DA]₀ was elevated for some time after stimulation offset (Figure 3.5). Hence, VTA dopamine activity and subsequent prolonged dopamine release may modulate mPFC population ensemble response in a behavioral state dependent manner. We did not examine population responses when animals were in quiescent states only, as most animals exhibited movement during sustained phasic and fast burst VTA stimulation periods. Slow burst stimulation sessions that contained quiescent epochs during stimulation did not yield a sufficient number of units to perform the population analysis.
Figure 3.11 Non-normalized mPFC population activity divergence during VTA dopamine activation.

Euclidean distances (mean +/- SEM) between population spike count vectors (SUA+MUA, non-normalized, units with firing rate < 0.1 Hz removed) at baseline (time -10 to -15 min before stimulation starts) and all other epochs (each epoch duration = 5 min) are shown for a) Th::Cre and b) wild-type rats. N indicates the total number of units in each condition, and blue rectangles mark the duration of stimulation (t = 0 corresponds to stim start). To assess the divergence of population activity over time upon stimulation, all distances were compared against the distance between two baseline epochs (-10 to -15 min to -5 to -10 min). Sustained phasic stimulation significantly increased Euclidean distances (compared to baseline) at 5 – 25 min from onset of stimulation sequence (5 – 10 min: p = 0.000; 10 – 15 min: p = 0.000; 15 – 20 min: p = 0.000; 20 – 25 min: p = 0.000; Bonferroni corrected right tailed t-tests). Fast burst stimulation transiently increased Euclidean distance at 5 – 10 min from stimulation onset (p = 0.023), and slow burst stimulation only produced delayed increases in distance at 15 – 25 min from stimulation onset (15 – 20 min: p = 0.022; 20 – 25 min: p = 0.000). Wild-type population activity for the most part did not show significant divergence from baseline, except for a non-specific increase in distance at 35 – 40 min from stimulation onset in the slow burst condition (p = 0.03). In the plots,
distances are displayed as mean +/- SEM normalized distances (distances for each bin were divided by mean distance between -10 to -15 min and -5 to -10 min epochs). *p < 0.05 and ** p < 0.01.

Figure 3.12 Normalized mPFC population activity divergence during VTA dopamine neuron activation.

Euclidean distances (mean +/- SEM) between population spike count vectors (SUA+MUA, Z-score normalized to a baseline epoch between -20 to -15 min, and units with firing rate < 1 Hz removed) at baseline (time -10 to -15 min before stimulation start) and all other epochs (each epoch duration = 5 min) are shown for Th::Cre and wild-type rats. N indicates the total number of units in each condition, and blue rectangles mark the duration of stimulation. To assess the divergence of population activity over time upon stimulation, all distances were compared against the distance between baseline epochs (-10 to -15 min to –5 to -10 min). Sustained phasic stimulation significantly increased population state divergence from baseline between 5 – 20 min from the onset of stimulation sequence (5 – 10 min: p = 0.000; 10 – 15 min: p = 0.000; 15 – 20
Fast burst stimulation increased Euclidean distances at 0–10 min from stimulation onset (0–5 min: \( p = 0.000 \); 5–10 min: \( p = 0.000 \)), and slow burst stimulation did not significantly increase distance at any time point. Wild-type population activity also did not show significant divergence from baseline (all \( p > 0.05 \)). Distances are displayed as mean +/- SEM normalized distances (divided by mean distance between -10 to -15 min and -5 to -10 min epochs). *\( p < 0.05 \) and **\( p < 0.01 \).

**Figure 3.13 Modulation of movement by VTA optogenetic stimulation in Th::Cre rats.**

Sustained phasic stimulation and fast burst stimulation significantly increased the amount of movement (repeated measures ANOVA: \( F(11,132) = 7.926, p = 0.000 \), sustained phasic stimulation; \( F(11,132) = 4.092, p = 0.000 \), fast burst stimulation). * and ** indicate the significance of each bin for comparison against baseline (post-hoc paired t-tests, Bonferroni corrected) at \( p < 0.05 \) and \( p < 0.01 \) respectively. Although a repeated measures ANOVA indicated a significant change in movement over time in the slow burst stimulation condition (\( F(11,99) = 2.198, p = 0.023 \)), post-hoc comparisons against baseline did not reveal significance (all \( p > 0.05 \), paired t-tests, Bonferroni corrected). Time = 0 indicates start of stimulation. Note that movement here does not refer to locomotion but shifts in body position elicited mostly by active behaviors such as locomotion, grooming, and rearing; movement events were detected with an infrared heat monitor.
Figure 3.14 Identification of movement epochs in Th::Cre rats.

a) Moving epochs (each 3 min long) were identified in baseline, stimulation, and post-stimulation periods, during which animals were moving at comparable rates. Stim 1 and 2 correspond to two different moving epochs during the stimulation period. b) Movement (mean +/- SEM) for each bin (bin size = 60 s) in each of the four epochs expressed as percentage of baseline bins. Movement was not significantly different from baseline at all time points (all $p > 0.05$; paired t-tests).
Figure 3.15 Identification of movement epochs in wild-type rats.

a) Moving epochs (each 3 min long) were identified in baseline, stimulation and post-stimulation periods, during which animals were moving at comparable rates. b) Movement (mean +/- SEM) for each bin (bin size = 60 s) in each of the four epochs expressed as percentage of baseline bins. All bins were not significantly different from baseline bin 1 (all p > 0.05; paired t-tests).
Figure 3.16 Non-normalized mPFC population activity divergence in moving periods only in \textit{Th::Cre} and wild-type rats.

Moving epochs (each 3 min long) were identified in baseline, stimulation and post-stimulation periods as shown in Figure 3.14 for \textit{Th::Cre} and Figure 3.15 for wild-type rats. Stim 1 and 2 correspond to two different moving epochs during the stimulation period in \textit{Th::Cre} rats, while post-stim 1 and post-stim 2 indicate two different moving epochs during the post-stimulation period of wild-type rats (see Methods). a) Euclidean distances of population spike count vectors (SUA+MUA, non-normalized, and units with firing rate < 0.1 Hz removed) between baseline and all other epochs (each epoch duration = 3 min) are shown for all stimulation protocols for \textit{Th::Cre} rats. b) For wild-type rats, data from all three stimulation protocols were pooled to increase sample size. As wild-type rats typically did not move during stimulation, many sessions had to be removed, resulting in a low sample size for each stimulation protocol. N indicates the total number of units in each condition, and blue rectangles mark the time of stimulation. To assess the divergence of population activity over time upon stimulation, all distances were compared against the distance between baseline and post-stim epoch (post-stim 2 in wild-type). Sustained phasic stimulation significantly increased population activity divergence from baseline.
during stimulation epochs compared to post-stimulation (stim 1: \( p = 0.000 \); stim 2: \( p = 0.000 \); Bonferroni corrected one tailed t-tests). Fast burst stimulation increased population divergence in all stimulation epochs compared to post-stimulation (stim1: \( p = 0.015 \), stim2: \( p = 0.000 \)), and slow burst stimulation only significantly increased the distance at Stim 2 (\( p = 0.046 \)). Wild-type population activity did not show significant divergence over time (all \( p > 0.05 \)). For clarity, distances are displayed as mean +/- SEM normalized distances (distances for each epoch were divided by mean distance between baseline and post-stimulation epochs). \( *p < 0.05 \) and \( **p < 0.01 \).

![Figure 3.17 Normalized mPFC population activity divergence in moving periods only in Th::Cre and wild-type rats.](image)

Moving epochs (each 3 min long) were identified in baseline, stimulation and post-stimulation periods as shown in Figure 3.14 for Th::Cre and Figure 3.15 for wild-type rats. Euclidean distances of population spike count vectors (SUA+MUA, Z-score normalized to baseline epoch,
and units with firing rate < 1 Hz removed) between baseline and all other epochs (each epoch duration = 3 min) are shown. N indicates the total number of units in each condition, and blue rectangles mark the time of stimulation. To assess the divergence of population activity over time upon stimulation, all distances were compared against the distance between baseline and post-stim epoch (post-stim 2 in wild-type). Sustained phasic stimulation increased Euclidean distances between stimulation epochs and baseline compared to baseline and post-stimulation (stim 1: p = 0.075 (trend); stim 2: p = 0.021; Bonferroni corrected one tailed t-tests). Fast burst and slow burst stimulation protocols increased population divergence in all stimulation epochs compared to post-stimulation (fast burst, stim1: p = 0.000, stim2: p = 0.028; slow burst, stim 1: p = 0.015, stim 2: p = 0.005). Wild-type population activity did not show significant increases in distance in stim 1 or post-stim1 epochs compared to post-stim 2 (all p > 0.05). For clarity, distances are displayed as mean +/- SEM normalized distances (distances for each epoch were divided by mean distance between baseline and post-stimulation epochs). *p < 0.05 and ** p < 0.01.
3.3.5 Modulation of mPFC LFPs by phasic VTA dopamine activity

We did not observe a strong modulation of mPFC LFPs on a transient timescale (Figure 3.18). Sustained phasic stimulation transiently increased high gamma power for the 5 s of light stimulation, but this increase was weak and the post-hoc tests did not yield significance (Figure 3.18a).
Figure 3.18 Transient modulation of mPFC LFPs by phasic VTA dopamine activity in Th::Cre rats.

a) LFP spectrogram (normalized to total LFP power within each frequency bin) depicts response to each phasic train in the sustained phasic stimulation protocol. Color bar indicates Z-scores. Sustained phasic stimulation significantly increased high gamma (HG) power ($F(9,126) = 2.112$, $p = 0.035$; repeated measures ANOVA), but the post-hoc comparisons against the pre-stimulation period were not significant ($p > 0.05$, Bonferroni corrected paired t-tests). It also significantly modulated low theta (LT) power ($F(9,126) = 2.607$, $p = 0.011$), but post-hoc comparisons did not show significant effects ($p > 0.05$). There was no significant modulation in other frequencies (low gamma (LG): $F(9,126) = 0.400$, $p = 0.933$; beta (Be): $F(9,126) = 0.836$, $p$...
= 0.568; high theta (HT): F(9,126) = 1.569, p = 0.141; delta (De): F(9,126) = 1.067, p = 0.391).
b) Fast burst stimulation did not significantly modulate LFP power in any frequency (high
gamma: F(9,126) = 1.248, p = 0.272; low gamma: F(9,126) = 1.390, p = 0.204; beta: F(9,126) =
0.613, p = 0.701; high theta: F(9,126) = 0.526, p = 0.704; low theta: F(9,126) = 0.115, p = 0.906;
delta: F(9,126) = 2.234, p = 0.117). c) Slow burst stimulation significantly modulated the power
of high gamma and beta (high gamma: F(9,99) = 2.137 , p = 0.037; beta: F(9,99) = 2.438, p =
0.033) but post-hoc comparisons did not yield significance (all p > 0.05, Bonferroni corrected).
Changes in the power of other frequency bands were non-significant (low gamma: F(9,99) =
0.896, p = 0.532; high theta: F(9,99) = 0.760, p = 0.556; low theta: F(9,99) = 0.479 , p = 0.655;
delta: F(9,99) = 2.077, p = 0.143). * indicates p < 0.05 for repeated measures ANOVA. Error
bars indicate mean +/- SEM.
On a prolonged timescale, VTA stimulation elicited various changes in mPFC LFPs. Upon VTA dopamine activation using the sustained phasic stimulation protocol, the most prominent increase in LFP power was detected in the broadband high gamma (55 – 100 Hz) frequency band (repeated measures ANOVA: $F(11,154) = 9.941$, $p = 0.000$) (Figure 3.19). Sustained phasic stimulation also increased power in the low gamma (30 – 55 Hz) frequency band (repeated measures ANOVA: $F(11,154) = 5.581$, $p = 0.000$) and decreased the power of lower frequencies, including beta (14 – 30 Hz) ($F(11,154) = 3.910$, $p = 0.000$), high theta (8 – 13 Hz) ($F(11,154) = 3.987$, $p = 0.000$), low theta (4.5 – 8 Hz) ($F(11,154) = 3.782$, $p=0.000$), and delta (1 – 4 Hz) ($F(11,154) = 7.370$, $p = 0.000$). Even though significant changes in power were observed in all frequencies, some even during baseline samples, these effects were much smaller compared to power increases in the high gamma band during stimulation (mean high gamma power at significant samples equaled Z-scored values > 0.5 while mean Z-values for all other frequencies were below 0.5) (Figure 3.19b). Fast burst stimulation elicited a weaker modulation of mPFC LFPs compared to the sustained phasic stimulation. Nonetheless, the most apparent increase in LFPs was in the high gamma frequency band (repeated measures ANOVA: $F(11,154) = 5.431$, $p = 0.000$). Fast burst stimulation also increased power in the low gamma ($F(11,154) = 3.119$, $p = 0.000$) band and decreased power in lower frequencies (beta: $F(11,154) = 2.357$, $p =0.014$; high theta: $F(11,154) = 3.243$, $p = 0.003$; low theta: $F(11,154) = 2.927$, $p =0.006$, and delta: $F(11,154) = 3.243$, $p =0.003$). But similar to the effects of the sustained phasic stimulation protocol, the strongest increase upon fast burst stimulation was observed in the high gamma band (mean Z-score values ranged between 0.3 – 0.4), while significant changes in other frequency bands were weaker (mean Z-score values < 0.3) (Figure 19b). Slow burst stimulation failed to elicit significant changes in LFPs (Figure 3.19a). While repeated measures ANOVAs indicated
significant differences in LFP power across time in all frequencies (high gamma: $F(11,121) = 2.131, p = 0.032$; low gamma: $F(11,121) = 2.557, p = 0.034$; beta: $F(11,121) = 2.094, p = 0.041$; high theta: $F(11,121) = 3.006, p = 0.016$; low theta: $F(11,121) = 3.002, p = 0.026$; $F(11,121) = 3.046, p = 0.019$), none of the post-hoc comparisons against baseline were significant (Figure 3.19b). As with the population unit response, these differences in LFP responses across protocols were related to the differences in behavioral modulation elicited by the varying stimulation patterns (Figure 3.13).

VTA stimulation did not modulate LFP power in wild-type rats (sustained phasic stimulation: $F(11,33) = 0.585$ (high gamma), $F(11,33) = 0.455$ (low gamma), $F(11,33) = 0.688$ (beta), $F(11,33) = 0.428$ (high theta), $F(11,33) = 0.466$ (low theta), $F(11,33) = 0.538$ (delta); fast burst stimulation: $F(11,33) = 0.566$ (high gamma), $F(11,33) = 0.919$ (low gamma), $F(11,33) = 0.283$ (beta), $F(11,33) = 0.548$ (high theta), $F(11,33) = 0.653$ (low theta), $F(11,33) = 0.642$ (delta); slow burst stimulation: $F(11,22) = 0.663$ (high gamma), $F (11,22) = 0.921$ (low gamma), $F(11,22) = 1.514$ (beta), $F(11,22) = 1.686$ (high theta), $F(11,22) = 2.435$ (low theta), $F(11,22) = 1.3734$ (delta); all $p > 0.05$).
Figure 3.19 Phasic VTA dopamine activation modulated LFPs on a prolonged timescale in Th::Cre rats.

a) Power spectrograms depict Z-score normalized LFP power for frequencies between 0 – 100 Hz. Color bar indicates Z-scores. b) Mean +/- SEM normalized power values for different frequency bands at different time bins (bin width = 5 min). T = 0 min corresponds to stimulation onset and blue rectangles mark the duration of stimulation. * and ** indicate p < 0.05 and p < 0.01 for comparison of each bin against baseline bin 1 (post-hoc paired t-tests; Bonferroni corrected). Error bars indicate mean +/- SEM.
Next, we examined modulation of LFPs by stimulation compared to baseline in epochs with similar levels of movement (Figure 3.20). We found that all stimulation protocols significantly increased the power of the high gamma band. Fast burst and slow burst stimulation also significantly increased power in the high theta and low gamma bands respectively (Figure 3.20). There was no modulation of LFP power in any frequency band across different movement only epochs in wild-type rats (Figure 3.21). These results demonstrate that VTA dopamine activation significantly increased mPFC high gamma power even when net movement was comparable between the stimulation and baseline epochs, suggesting that the high gamma power increase was not simply due to changes in arousal or gross differences in movement. As was observed in the population unit response, LFPs exhibited a stronger modulation by VTA slow burst stimulation when analyzing sessions and epochs that contained primarily active behaviors during stimulation (Figure 3.20) compared to the analysis of all sessions irrespective of behavior (Figure 3.19). Furthermore, the whole session analysis for the sustained phasic condition revealed that LFPs were modulated mostly during the period of stimulation, and this duration coincided with the total period of behavioral modulation (Figure 3.13); however, mPFC dopamine levels remained elevated for some time after stimulation offset (Figure 3.5). Hence, changes in mPFC LFP activity elicited by VTA dopamine activation might depend not only on the amount of local [DA]₀ release but also on the behavioral state of the animal.

To further test the idea that VTA dopamine stimulation-induced LFP power modulations depend on behavioral states, we examined LFPs when animals were in quiescent states before and after stimulation. As most animals were moving during VTA sustained phasic stimulation, we couldn’t analyze its effects on LFPs in the quiescent state. A few animals (n = 3) exhibited
mostly quiescent behavior during fast burst stimulation, and LFP analysis of these sessions demonstrated that VTA stimulation did not modulate LFP power in any frequency band (high gamma: $F(3,6) = 0.830, p = 0.484$; low gamma: $F(3,6) = 1.111, p = 0.408$; beta: $F(3,6) = 1.736, p = 0.260$; high theta: $F(3,6) = 1.717, p = 0.262$; low theta: $F(3,6) = 2.027, p = 0.212$; and delta: $F(3,6) = 1.507, p = 0.306$). A few animals ($n = 4$) also showed sustained quiescent behavior during slow burst stimulation; during quiescent epochs, VTA slow burst stimulation significantly increased high gamma power, but post-hoc tests were not significant (a trend towards increase at stim 1: $p = 0.095$) (Figure 3.22a). VTA stimulation also seemed to increase low gamma power in Stim 1 epoch, but this increase was not significant. Because of the low sample size for the quiescence analysis, we also combined sessions from fast burst and slow burst conditions. In the combined data, there was a slight increase in low and high gamma power during the first stimulation epoch, but this increase was non-significant. Instead, a more robust decrease in the power of low frequencies was detected (post-hoc comparisons: high theta: stim 1 ($p = 0.010$), low theta: stim 1 ($p = 0.011$), and delta: stim 1 ($p = 0.008$), stim 2 ($p = 0.044$), paired t-tests). Thus, when animals were in the quiescent state as opposed to moving, VTA dopamine stimulation more consistently decreased the power of lower frequencies than increased the power of higher frequencies. One caveat to the examination of quiescent epochs is that arousal levels were not measured, and thus animals may be in different arousal states during the identified quiescence periods, which may explain the observed LFP effects during stimulation.
Figure 3.20 Sustained changes in mPFC LFPs upon VTA dopamine activation during moving periods in Th::Cre rats.

Moving epochs (each 3 min long) were identified in baseline, stimulation and post-stimulation periods as shown in Figure 3.14. Stim 1 and 2 correspond to two different moving epochs during the sustained stimulation period. Power spectrograms (top panels) depict LFP power for frequencies within each epoch as Z-score normalized values. Color bar indicates Z-scores. Bar plots (bottom panels) show mean +/- SEM of normalized Z-score power values for high gamma, low gamma, high theta, and low theta frequency bands. a) Sustained phasic stimulation significantly increased the power of high gamma (F(3,30) = 3.235, p = 0.036; repeated measures ANOVA) in stim epoch 1 (p = 0.035; post-hoc paired t-test) and stim epoch 2 (p = 0.047). Beta power was also elevated (F(3,30) = 3.2079, p = 0.037) in stim epoch 1 (p = 0.041) and post-stim...
epoch 2 (0.0325) but not stim epoch 2 (p = 0.316). However, the meaning of this effect is unclear as it was not observed with other stimulation protocols and the power values fluctuated across epochs. b) Fast burst stimulation significantly increased high gamma power (F(3,24)=3.961, p = 0.020) and high theta power (F(3,24) = 5.875, p = 0.004). Post-hoc tests revealed a significant increase in high gamma in stim epoch 1 (p = 0.009), and a trend towards significant increase in stim epoch 2 (p = 0.079). High theta power was elevated in stim epoch 1 (p = 0.044) and stim epoch 2 (p = 0.046). c) Slow burst stimulation significantly increased high gamma (F(3,9) = 4.855, p = 0.0282) and low gamma power (F(3,9)= 6.8024, p =0.011). Post-hoc tests revealed a trend in high gamma increase in stim epoch 1 (p = 0.073), a significant increase in low gamma power in stim epoch 1 (p = 0.008) and a trend in stim epoch 2 (p = 0.065). * and ** indicate significance of repeated measures ANOVA at p < 0.05 and p < 0.01 respectively.
Figure 3.21 Sustained changes in mPFC LFPs upon VTA dopamine activation in moving periods in wild-type rats.

Moving epochs (each 3 min long) were identified in baseline, stimulation and post-stimulation periods as shown in Figure 3.15. PostStim 1 and PostStim 2 correspond to two different moving epochs during the post-stimulation period. All sessions (that used different stimulation protocols) were pooled (see Methods). Power spectrograms depict LFP power for each epoch as Z-score normalized values. Color bar indicates Z-scores. There was no difference in LFP power in any frequency band across epochs (High gamma: $F(3, 15) = 1.035, p = 0.41$; Low Gamma: $F(3,15) = 0.746, p = 0.541$; Beta: $F(3,15) = 2.031, p = 0.153$; High Theta: $F(3,15) = 0.053, p = 0.983$; Low Theta: $F(3,15) = 0.297, p = 0.827$; Delta: $F(3,15) = 2.208, p = 0.129$; repeated measures ANOVA).
Figure 3.22 Sustained changes in mPFC LFPs upon VTA dopamine activation in quiescent periods in Th::Cre rats.

Quiescent epochs (each 3 min long) were identified in baseline, stimulation and post-stimulation periods, during which animals showed sustained immobility. Stim 1 and 2 correspond to two different quiescent epochs during the sustained stimulation period. Power spectrograms (top panels) depict LFP power for each epoch as Z-score normalized values. Color bar indicates Z-scores. Bar plots (bottom panels) show mean +/- SEM of normalized Z-score power values for all frequency bands. a) Slow burst stimulation significantly increased the power of high gamma (F(3,9) = 4.613, p = 0.032, repeated measures ANOVA), but post-hoc comparisons only showed
a trend for Stim 1 (p = 0.095). b) When fast burst stimulation and slow burst stimulation sessions were combined for quiescent period analysis, stimulation significantly decreased power of high theta (F(3,18) = 4.883, p = 0.014), low theta (F(3,18) = 3.760, p = 0.030), and delta (F(3,18) = 4.126, p = 0.022) frequencies. * indicates significance of repeated measures ANOVA at p < 0.05.
In spite of the modulation of LFP power, VTA stimulation did not consistently increase phase-locking of mPFC units to local LFPs. In the sustained phasic stimulation sessions, 0 – 4.8% of units were phase locked to high gamma oscillations during the 10-min period preceding stimulation, while 15.9% were phase locked in 0 – 5 min, and 8 – 10% in 5 – 20 min following stimulation onset (Figure 3.23). However, these differences in proportions were not significant (p > 0.05; Fisher’s exact test). There was no difference in the proportion of units phase-locked to any frequency band (p > 0.05; all Fisher’s exact tests). There was also no difference in PLV, that measures the strength of phase-locking, for all units before and after stimulation for high gamma, low gamma, high theta, low theta, and delta frequency bands (Friedman’s ANOVA, p > 0.05). PLV values were elevated for beta frequency (p = 0.008; Friedman ANOVA), but the post-hoc comparisons against baseline did not yield significance. Similarly, stimulation with fast burst protocol did not elicit a significant change in the proportion of phase locked units for any frequency band (p > 0.05; Fisher’s exact tests) and PLVs for most frequencies (p > 0.905; Friedman ANOVA), except for delta band (p = 0.014; Friedman ANOVA; post-hoc comparisons were non-significant). Slow burst stimulation also did not produce significant differences in phase-locked unit proportions for most frequency bands except for high-theta band in the post-stim period of 25 – 30 min from stim-start (p = 0.015; Fisher’s exact test). There was no significant change in PLVs for all frequencies (p > 0.05; Friedman ANOVA). We also didn’t observe significant changes in the proportion of units phase-locked to most frequencies when analyzing moving epochs only, except for delta band during post-stimulation in the slow burst condition (p = 0.0211; Fisher’s exact test). Hence, we did not observe a significant modulation of
phase-coupling of spikes to high gamma oscillations by VTA dopamine neuron activation, even though high gamma band showed a clear increase in oscillation power. Some significant effects on spike-LFP coupling in other frequency bands were observed, but these effects were not consistent across different stimulation protocols.
Figure 3.23 Modulation of mPFC phase-locking.

Proportion of mPFC units phase-locked to local high gamma oscillations during sustained phasic VTA dopamine stimulation in $Th::Cre$ rats. Time = 0 indicates stimulation onset, and blue rectangles mark the duration of stimulation.
3.3.7 Modulation of phase amplitude coupling by phasic VTA dopamine activity

Previous studies have shown that theta and gamma oscillations can interact via cross-frequency coupling (Canolty et al 2006), which may be important for various cognitive functions, including working memory and learning (Canolty & Knight 2010, Lisman & Jensen 2013, Tort et al 2009). A recent study also indicated that dopamine can increase delta-gamma coupling in mPFC (Andino-Pavlovsky et al 2017). Thus, we examined if and how the phase of delta, low theta or high theta frequencies modulated the amplitude of low gamma or high gamma frequencies upon VTA dopamine activation.

Sustained phasic stimulation, in addition to increasing high gamma power, also increased coupling between high gamma and theta oscillations (Figure 3.24), in both high and low theta ranges (high theta to high gamma coupling: $\chi^2(7) = 15.066$, $p = 0.014$, Friedman’s ANOVA; post-hoc: n.s., signed-rank tests, Bonferroni corrected; low theta to high gamma: $\chi^2(7) = 26.353$, $p = 0.000$, Friedman’s ANOVA; post-hoc: $p (5 – 10 \text{ min}) = 0.003$; $p (10 – 15 \text{ min}) = 0.072$, signed rank with Bonferroni correction). These effects were not spurious changes due to random coupling by chance or simply because of increased power because surrogate LFPs ($n = 100$ surrogate time series obtained by splicing the time series into two random splits within each bin) did not show such coupling (low theta to high gamma: $\chi^2 (7) = 6.778$, $p = 0.450$; high theta to high gamma: $\chi^2 (7) = 4.470$, $p = 0.720$). Similarly, there was a trend towards significance in the increase of coupling between high theta and high gamma upon fast burst stimulation ($\chi^2 (7) = 13.178$, $p = 0.068$; surrogate analysis: $\chi^2 (7) = 5.578$, $p = 0.590$). Finally, we also observed a significant increase in low theta to low gamma coupling after slow burst stimulation ($\chi^2 (7) = 15.306$, $p = 0.032$; post-hoc: $p (0 – 5 \text{ min}) = 0. 024$, and $p (20 – 25 \text{ min}) = 0.034$, signed rank test.
with Bonferroni correction; surrogate analysis: $\chi^2(7) = 5.778$, $p = 0.057$). There was no change in the coupling of delta and high gamma or delta and low gamma frequencies after VTA dopaminergic stimulation with any protocol (all $p > 0.05$ for Friedman’s ANOVA), in contrast to the observation of a recent study (Andino-Pavlovsky et al 2017). Therefore, during periods when significant increases in high gamma power were observed upon VTA stimulation with sustained phasic and fast burst protocols, there was also a corresponding increase in the coupling between theta and high gamma frequencies. There was no significant phase-amplitude coupling between any frequencies tested for wild-type animals after sustained phasic stimulation ($n = 4$ rats), fast burst stimulation ($n = 4$ rats), or slow burst stimulation ($n = 3$ rats) (all $p > 0.05$, Friedman’s ANOVA).

When we examined cross-frequency coupling in movement epochs, enhanced coupling between high theta and high gamma was observed during fast burst stimulation ($\chi^2(3) = 17.667$, $p = 0.000$; post-hoc: $p$ (Stim 1) = 0.020, $p$ (Stim 2) = 0.004; surrogate analysis: $\chi^2(3) = 0.333$, $p = 0.953$) (Figure 3.25). There was also a trend toward significant increase in coupling between high theta and high gamma during slow burst stimulation ($\chi^2(3) = 6.600$, $p = 0.086$; surrogate analysis: $\chi^2(3) = 4.500$, $p = 0.212$). Fast burst stimulation also resulted in a decrease in delta to high gamma coupling ($\chi^2(3) = 8.333$, $p = 0.040$; post-hoc: $p$ (Stim 1) = 0.027, $p$ (Stim 2) = 0.004; surrogate analysis: $\chi^2(3) = 2.22$, $p = 0.532$), while sustained phasic stimulation resulted in a decrease in delta to low gamma coupling ($\chi^2(3) = 12.100$, $p = 0.007$; post-hoc: n.s.; surrogate analysis: $\chi^2(3) = 0.900$, $p = 0.826$). Delta to low gamma coupling increased in the post-stim period after slow burst stimulation ($\chi^2(3) = 8.100$, $p = 0.044$; post-hoc: n.s.; surrogate analysis: $\chi^2(3) = 0.300$, $p = 0.960$). Thus, while delta coupling to higher frequencies was either decreased during stimulation or increased during the post-stimulation period, theta to high gamma coupling
was elevated upon fast and slow burst stimulation in time bins (Figure 3.25) when enhanced high gamma power was also observed (Figure 3.20). There was no significant phase-amplitude coupling between any frequencies tested for wild-type animals (all \( p > 0.05 \), Friedman’s ANOVA; \( n = 6 \) sessions, sessions were pooled across all protocols from 2 wild-type animals, see Methods). Pooled together, our findings suggest that VTA dopamine activity may increase the coupling between theta and high gamma frequencies, especially during periods of elevated high gamma power, while it may not increase delta to gamma coupling as previously suggested (Andino-Pavlovsky et al 2017).
Figure 3.24 Modulation of mPFC LFP cross frequency coupling by VTA dopamine activation during the entire session.

Mean +/-SEM normalized modulation index (MI) values of phase-amplitude coupling in Th::Cre rats (sustained phasic: n = 15 rats, fast burst: n = 15 rats, slow burst: n = 12 rats). MI values are plotted across time to depict phase-amplitude coupling between delta and high gamma (DHG),
low theta and high gamma (LTHG), high theta and high gamma (HTHG), delta and low gamma (DLG), low theta and low gamma (LTLG), and high theta and low gamma (HTLG) for *Th::Cre* rats. T = 0 indicates stimulation onset, * and ** indicate significance of Friedman’s ANOVA at p < 0.05 and p < 0.01 respectively.
Figure 3.25 Modulation of mPFC LFP cross-frequency coupling by VTA dopamine activation during moving epochs.

Mean +/-SEM normalized cross-frequency coupling modulation index (MI) values for moving epochs in Th::Cre rats (sustained phasic: n = 12 rats, fast burst: n = 9 rats, slow burst: n = 4 rats). Moving epochs were identified as described in Figure 3.14. Cross frequency phase-amplitude
coupling was calculated between delta and high gamma (DHG), low theta and high gamma (LTHG), high theta and high gamma (HTHG), delta and low gamma (DLG), low theta and low gamma (LTLG), and high theta and low gamma (HTLG). * and ** indicate significance of Friedman’s ANOVA at p < 0.05 and p < 0.01 respectively.
3.3.8 Modulation of LFPs by a 20 Hz, 20 s continuous stimulation in awake and anesthetized states

Finally, in order to directly compare electrophysiological LFP responses to functional magnetic resonance imaging (fMRI) responses in mPFC during VTA dopamine activation (that were described in Chapter 2), a subset of Th::Cre animals (n = 5) used in the current study were also subjected to a different stimulation protocol that was utilized in the study in Chapter 2.

First, we stimulated VTA dopamine neurons at 20 Hz for 20 s (5 ms pulse width, power = 5 mW) (Figure 2.1) while rats were freely moving. Similar to the LFP response elicited by repeated phasic stimulation paradigms (Figure 3.19), the 20 Hz, 20 s protocol significantly increased the power of broadband gamma oscillations (Figure 3.26a). The increase in the power of high gamma oscillations (mean Z-scores at significant samples: 5 – 10 s = 2.457, 10 – 15 s = 2.203, 10 – 15 s = 2.345) was substantially higher than the increase in low gamma power (mean Z-scores at significant samples: 5 – 10 s = 0.841, 10 – 15 s = 1.166, 15 – 20s = 0.988). In order to determine the dependence of LFP changes on behavioral states, we classified trials based on whether animals were quiescent versus moving at baseline and during stimulation as well as post-stimulation periods. If VTA stimulation elicited a substantial change in animal’s behavioral state (or increase in movement), it produced a corresponding profound modulation of power in all mPFC LFP frequencies (Figure 3.26b). These LFP changes were characterized by a broadband increase in high gamma and low gamma power as well as a decrease in the power of lower frequencies, including beta, high theta, low theta, and delta. If VTA stimulation did not substantially change animals’ behavior, such as when animals were moving at comparable rates before and during stimulation, LFPs were weakly modulated (Figure 3.26c). While VTA
stimulation still significantly increased high gamma power, although weakly (mean Z-scores: 0 – 5 s = 0.409, 5 – 10 s = 0.689, 10 – 15 s = 0.254, 15 – 20 s = 0.364, 20 – 25 s = 0.074, 25 – 30 s = -0.046, 30 – 35 s = 0.290, 35 – 40 s = 0.334), low gamma power was not affected (Figure 3.26c). These results are consistent with the observation of behavioral state dependent modulation of LFPs by repeated phasic stimulation protocols (Figure 3.20 & 3.22). If animals stayed in a fairly quiescent state before and during stimulation, VTA stimulation did not increase high gamma power (Figure 3.26d). One caveat to the examination of quiescent trials is that these trials were very few in number, and the fifth rat was excluded because VTA stimulation always modulated the rat’s behavioral state. To summarize, VTA dopamine stimulation with the 20 Hz, 20 s protocol in freely moving animals substantially modulated high gamma power compared to other frequencies, but this modulation depended on the behavioral state of the animal.

Interestingly, under light isoflurane anesthesia, the same VTA stimulation pattern (20 Hz, 20 s, power = 5 mW) did not modulate high gamma power but instead robustly increased beta power (Figure 3.27). We found that when animals were under light anesthesia, their mPFC LFPs fluctuated between states with high frequency components (Figure 3.27a) and those with high-amplitude spindles (Figure 3.27d), possibly reflecting fluctuations in the internal anesthesia state of the animal. The increase in mPFC beta power also depended on the LFP state at the time of stimulation. If LFPs exhibited high frequency components, VTA stimulation increased the power of beta oscillations (Figure 3.27b and 3.27c). On the other hand, if VTA dopamine neurons were stimulated when LFPs contained high amplitude spindles, there was no modulation of LFP oscillations in any frequency (Figure 3.27e). Thus, phasic VTA dopamine activation elicited differential patterns of mPFC LFP modulation depending on the behavioral state (awake and moving, awake and still, versus anesthetized) and the current LFP state.
Figure 3.26 Modulation of mPFC LFPs by optogenetic VTA dopamine stimulation at 20 Hz for 20 s in freely moving Th::Cre rats.
LFPs were examined for all trials (n = 5 rats) (a), trials during which animals’ behavior changed from fairly quiescent or low levels of movement to high levels of movement upon stimulation (n = 5 rats) (b), trials during which animals were moving at high rates throughout (n = 5 rats) (c), trials during which animals where quiescent throughout (n = 4 rats) (d). (a) VTA dopamine stimulation significantly increased the power of high gamma (F(9,36) = 9.409, p = 0.018) and low gamma oscillations (F(9,36) = 10.423, p = 0.015). Power of beta, high theta, low theta, and delta frequencies were not significantly modulated (p > 0.05, repeated measures ANOVA). (b) When examining trials with substantial movement increases from the pre-stimulation baseline, VTA stimulation elicited a profound change in the power of LFP oscillations in all frequencies: high gamma (F(9, 36) = 11.579, p = 0.018), low gamma (F(9,36) = 13.192, p = 0.006), beta (F(9,36) = 7.171, p = 0.010), high theta (F(9,36) = 11.062, p = 0.016), low theta (F(9,36) = 7.450, p = 0.025), and delta (F(9,36) = 5.00, p = 0.015). (c) In trials during which animals were moving throughout the trial at comparable rates, a weaker but significant increase in the power of high gamma frequency was detected (F(9,36) = 5.291, p = 0.001). Low gamma power was not significantly modulated (F(9,36) = 2.813, p = 0.075). Beta (F(9,36) = 11.330, p = 0.000), high theta (F(9,36) = 7.075, p = 0.009), low theta (F(9,36) = 5.346, p = 0.015), and delta (F(9,36) = 10.678, p = 0.000) power were significantly modulated over time. Significance in beta power seemed to be driven by inconsistent fluctuations of power, even during pre-stimulation periods (as pre-stim period of -5 to 0 s showed a significant increase in beta power compared to -10 to -5 s). Accordingly, when post-hoc comparisons were conducted against -5 to 0 s instead of -10 to -5 s, none of the stimulation samples were significant (all p > 0.05). While high theta and low theta power were also modulated over time, the corresponding Z-score power values were small and fluctuated between increases and decreases across time. None of the post-hoc comparisons for delta power modulation yielded significance (p > 0.05, Tukey’s post-hoc) (d) During trials when animals were fairly quiescent, VTA stimulation produced either no change or a weak decrease in high gamma power (F(9,27) = 4.2930, p = 0.015). Beta power was inconsistently modulated over time (F(9,27) = 90.340, p = 0.021), as a significant decrease was detected even in the pre-stimulation period -5 to 0 s compared to -10 to -5 s (p < 0.05, Tukey’s post-hoc). *p <0.05 and ** p < 0.01 for post-hoc Tukey’s comparison against the pre-stimulation period -10 to -5 s. Error bars indicate mean +/- SEM. Color scale for the heat maps ranges from Z= -2 to 2.
VTA stimulation differentially impacted LFPs that appeared more “awake-type” with high frequency components (shown in panel a) compared to those that contained high amplitude “spindles” but reduced low amplitude high frequency components (panel d). b) LFP spectrogram (n = 5 rats) shows Z-score normalized power (normalized to pre-stimulation period of -20 s to -10 s) for frequencies between 0-100 Hz for “awake-type” LFPs. c) Z-scored power values in 5 s bins are plotted across time for beta frequency of LFPs displayed in panel b. VTA stimulation resulted in a significant and strong increase in beta LFP power (F(9,36) = 5.049, p = 0.000;
Tukey’s post-hoc: 5–10 s: $p = 0.018$; 10–15 s: $p = 0.008$; 15–20 s: $p = 0.005$). High gamma ($F(9,36) = 0.202, p = 0.930$), low gamma ($F(9,36) = 1.702, p = 0.125$), low theta ($F(9,36) = 1.113, p = 0.373$), high theta ($F(9,36) = 2.469, p = 0.116$), and delta ($F(9,36) = 0.826, p = 0.536$) frequencies were not significantly modulated. e) When LFPs contained spindles and reduced high frequency components, VTA stimulation did not significantly modulate power in any frequency: high gamma ($F(9,36) = 0.147, p = 0.976$), low gamma ($F(9,36) = 0.563, p = 0.778$), beta ($F(9,36) = 0.475, p = 0.767$), high theta ($F(9,36) = 1.142, p = 0.372$), low theta ($F(9,36) = 1.206, p = 0.338$), delta ($F(9,36) = 1.285, p = 0.304$). Error bars indicate mean +/- SEM.
3.4 DISCUSSION

Phasic activity of VTA dopamine neurons in freely moving animals elicited diverse modulation of network activity in the mPFC. Dopamine neuron activation modulated the spiking of some mPFC neurons on multiple timescales and elicited a sustained population or ensemble response. It enhanced high gamma oscillations as well as coupling between theta and high gamma oscillations. These neural changes depended partially on the behavioral state of the animal. Consistent with the state dependence idea, VTA dopamine activity enhanced beta oscillations instead of high gamma oscillations in anesthetized animals. These varying patterns of modulation at the level of single neurons, to a population of neurons, to network oscillations in mPFC might interact in complex ways to mediate the behavioral effects of phasic VTA dopamine neuron activation in cognitive and affective processes.

3.4.1 Differences between stimulation protocols

We observed that sustained phasic stimulation most robustly modulated neural activity in mPFC compared to fast and slow burst stimulation. mPFC activity showed the weakest response to slow burst stimulation. These differences in modulation of PFC activity corresponded to the amount of behavioral modulation elicited by the three stimulation patterns, potentially implying a differential amount of dopamine release in mPFC during each phasic train in each of these protocols. Although we conducted microdialysis to measure dopamine release in PFC over a timescale of several minutes, this method cannot detect phasic dopamine transients as it tracks a general increase in $[DA]_o$ averaged over minutes and may not be sensitive to small differences in
phasic dopamine release. Thus, differences between stimulation protocols could be explained in terms of a gradient of dopamine release across sustained phasic, fast burst, and slow burst protocols.

3.4.2 State dependent modulation of mPFC activity

The effects of VTA dopamine activity on mPFC activity may depend, at least partly, on the behavioral state of the animal. This idea is supported by many pieces of evidence from the current study. First, we found that the ability of a stimulation protocol to elicit strong population responses or gamma power LFP changes was correlated with its influence on behavioral state. For example, sustained phasic stimulation robustly modulated animal’s behavior in that animals switched from a mostly quiescent state to a state of active exploration; this protocol also produced the strongest modulation of mPFC neural activity. On the other hand, slow burst stimulation elicited weak behavioral and neural changes compared to baseline. Second, in the sustained phasic and fast burst conditions, modulation of neural activity, both population response and LFP gamma increase, was largely restricted to the period of stimulation (with some prolonged effects up to 5 min after stimulation offset); however, [DA]₀ in mPFC was elevated for >20 min after stimulation offset. This suggests that increased [DA]₀ is not always correlated with modulation of mPFC neural activity. Third, when we examined trials during which animals were fairly quiescent before and after stimulation with the 20 Hz, 20 s protocol, VTA dopamine activation did not elicit a significant change in gamma power of LFPs. In contrast, when animals drastically changed their behavioral state from quiescence to movement during stimulation, there was a corresponding profound modulation of broadband gamma in LFPs. Further, when we
examined periods of comparable movement between baseline and stimulation, we found that the relative LFP modulation during stimulation was weaker, but high gamma power increase was still observed. The weaker effect may be explained in terms of a differential modulation of dopamine activity itself by behavioral states, as midbrain dopamine neurons show higher activity (>20% increase in firing rate that is mostly due to increased bursting) during active waking versus quiet waking that is not related to phasic movement episodes (Steinfels et al 1983). Thus, when animals switch from quiescent to active movement states even during baseline, dopamine levels in mPFC are probably elevated and the increased [DA]₀ may mediate some increases in gamma oscillations. Therefore, comparison of mPFC activity between active waking/moving epochs during baseline and stimulation might show weaker additive effects of additional dopamine increases with phasic stimulation. The state dependence idea is even more robustly supported by evidence that under light isoflurane anesthesia, phasic VTA dopamine activity does not modulate high gamma oscillations but elicits an increase in beta oscillations.

Our findings suggest that dopamine release on a prolonged timescale due to phasic VTA dopamine activity may not strongly modulate mPFC activity, at least population activity and gamma oscillations, by itself but may instead impact local activity when many strong afferent inputs are impinging on PFC (Lavin et al 2005). In anesthetized and quiescent states, afferent inputs to PFC are reduced due to partial closing of thalamic gates (Angel 1991, Sanders et al 2012, Sterman & Bowersox 1981) compared to when animals are actively exploring an environment. This difference may explain the state dependence of modulation of mPFC activity.
3.4.3 VTA dopaminergic modulation of spiking activity

A prevalent idea in the field of PFC dopamine research is that VTA dopamine activity enhances the firing of FS interneurons and indirectly inhibits the firing of pyramidal neurons ((Seamans & Yang 2004, Sesack & Bunney 1989, Tseng et al 2006), but see (Lavin et al 2005, Tierney et al 2008)). However, the present study showed that while most PFC neurons do not respond transiently to phasic VTA dopamine neuron activation, FS and pyramidal neurons that respond show both activation and inhibition. This suggests that VTA dopamine activity can interact with downstream PFC neurons of the same cell type in many different ways, possibly by engaging different receptors and by eliciting direct or indirect effects via interneurons. Our results also suggest that phasic VTA dopamine neuron activity can transmit information on the order of milliseconds to seconds to PFC neurons. This finding is relevant in the context of a longstanding speculation in the field about whether phasic dopamine neuron firing, that is thought to guide fast cognitive behaviors such as learning of cue-outcome associations, error detection, and working memory, can itself transiently modulate PFC activity (Lapish et al 2007), as rapid dopamine neurotransmission in PFC is hindered by many factors. Dopamine receptors are metabotropic, and most dopamine receptors are located extrasynaptically in terminal regions (Caille et al 1996, Smiley et al 1994b, Yung et al 1995). Furthermore, fewer DATs in the PFC prevent rapid clearance of dopamine from the extracellular space (Cass & Gerhardt 1995, Sesack et al 1998a). Nonetheless, previous studies have shown rapid PFC responses to VTA electrical stimulation (Ferron et al 1984, Lavin et al 2005), but these responses could have been elicited by activation of glutamatergic or GABAergic projections (Nair-Roberts et al 2008). Which receptors may mediate the transient responses in PFC neurons observed in the present study is not known, but it
is possible that glutamate co-release from dopamine terminals (Morales & Margolis 2017) can mediate these fast responses in PFC neurons (Lapish et al 2007, Lavin et al 2005).

Repeated phasic VTA dopamine activity also modulated the responses of individual PFC units on the timescale of many minutes; however, individual unit responses were heterogeneous and noisy. Modulation of spiking activity of PFC neurons was best captured by the examination of population level responses. Sustained phasic and fast burst stimulation elicited population responses that were mostly specific to the duration of stimulation; slow burst stimulation, however, did not significantly modulate population states. These long-lasting effects of dopamine activity may be due to accumulation of dopamine in the extracellular space with repeated phasic stimulation coupled with activation of intracellular signaling cascades, which are inherently slow and protracted, via metabotropic dopamine receptors. Thus, our data suggest that repeated phasic activation of VTA dopamine neurons may result in a sustained PFC [DA]₀ increase which may modulate the organization of PFC firing patterns in consistent ways. This modulation of population level activity patterns on the timescale of minutes may be relevant for the processing of afferent inputs when animals are engaged in active exploratory behaviors. Thus, prolonged VTA dopamine activity may facilitate information processing in PFC over many minutes, which could translate into an enhancement of long-lasting cognitive processes, such as sustained attention (Lavin et al 2005, Sarter et al 2001).

3.4.4 VTA dopaminergic modulation of mPFC oscillations

Gamma oscillations can be divided into multiple bands, including low gamma (~30-55 Hz) and high gamma (~55-100 Hz) (Colgin et al 2009), which has also been called “mid-gamma” to
distinguish from very high frequencies > 100 Hz (Buzsaki & Wang 2012). We found that repeated phasic VTA dopamine activity increased most robustly the power of high gamma oscillations, although significant modulation of other oscillations, including low gamma, was also observed. Amphetamine, which increases dopamine levels, also enhances cortical high gamma power in freely moving rats (Wood et al 2012). Other pharmacological studies in vivo have shown either an increase in total cortical gamma power with amphetamine (Pinault 2008) or reduced bandwidth of optogenetically-evoked low gamma 40 Hz oscillations with cocaine administration (Dilgen et al 2013). It is unclear from these studies whether spontaneous high gamma power was also impacted by these drugs. In the present study, phasic VTA dopamine activity did not modulate mPFC oscillations on a transient timescale, except for a weak increase in high gamma power upon sustained phasic stimulation. It is possible that this weak effect on the order of seconds contributed partially to the sustained effects on the order of minutes.

Even though both high and low gamma have been to related to cognitive processes such as working memory and attention (Uhlhaas et al 2011), high gamma is emerging as the most impaired oscillatory range in patients of psychiatric disorders especially schizophrenia (Sun et al 2013) and relevant genetic models of the disorder (Carlson et al 2011). The precise mechanism for the generation of high gamma oscillations is not known (Uhlhaas et al 2011), but low and high gamma oscillations have different relationships with underlying theta rhythms, suggesting differential physiological correlates of these oscillations (Belluscio et al 2012). Several potential mechanisms for high gamma generation have been suggested, including network interactions between FS interneurons and pyramidal neurons in the presence of thalamic inputs (Suffczynski et al 2014) and electrical coupling via gap junctions (Buzsáki & Draguhn 2004, Uhlhaas et al 2011). Thus, phasic activation of dopamine neurons could regulate high gamma oscillations in...
the PFC via modulation of local synaptic or electrical coupling or by influencing afferent inputs to PFC, and these mechanisms could be highly relevant to the pathophysiology of dopamine-related cognitive deficits.

We found a dissociation between an increase in LFP power and an entrainment of local spikes to the underlying LFP. This type of dissociation has been previously observed during dopamine-dependent hyperkinesia states that are also associated with increases in theta and gamma power in cortex but decreased synchronicity to these LFP frequencies (Costa et al 2006). This dissociation could be explained by several possibilities: a) different processes may be involved in influencing LFP amplitude and entrainment of spikes to LFP, and dopamine may only affect the former process. b) Different ensembles of neurons than those recorded in the study, especially FS interneurons which were few in number in the current study, may be more synchronized to the gamma oscillations. c) The function of the observed LFP power modulation may be to tune the mPFC to receive afferent inputs in specific time windows (Benchenane et al 2011, Fries 2005, Fries 2015) rather than to modulate the temporal dynamics of outgoing spikes. This modulation of sensitivity to incoming information can serve as a filtering mechanism and can enhance signal to noise. Finally, we cannot rule out the possibility that our unit sample size may be too low to detect any weak effects of dopamine modulation on spike-LFP phase-locking.

Prolonged phasic VTA dopamine activity also enhanced mPFC theta to high gamma coupling during periods when high gamma power was observed to be elevated. This suggests two possibilities: a) that activation of VTA dopamine neurons could enhance the amplitude of high gamma oscillations by modulating the mechanism mediating coupling between theta and high gamma, and b) that dopamine activation could provide multiplexed code to mPFC by independently modulating high gamma power and organizing gamma cycles around theta; this
type of multiplexing is thought to be important in spatial working memory (Lisman & Jensen 2013). Finally, the co-modulation of high and low frequency oscillations also indicates that the increase in high gamma power may not be a simple artifact of spiking activity as has been previously suggested (Ray & Maunsell 2011). A previous study had indicated that dopamine release in mPFC increases delta-gamma coupling (Andino-Pavlovsky et al 2017), but we did not find evidence for that coupling. Modulation of gamma power by a 4 Hz oscillation in mPFC along with increased gamma/4 Hz coherence between VTA and mPFC has also been reported (Fujisawa & Buzsaki 2011), but the high gamma power in the current study was coupled to slightly higher frequencies in the theta range above 4 Hz.

3.4.5 Neuromodulatory effects of phasic VTA dopamine activity

Phasic VTA dopamine activation elicited diverse effects on mPFC network activity, but overall these effects were modest and heterogeneous. For example, sustained phasic stimulation consistently increased high gamma power, but the increases were smaller compared to gamma power increases elicited by the modulation of glutamatergic systems (Pinault 2008, Wood et al 2012). Further, only a handful of units were individually modulated on both transient and sustained timescales and their responses were heterogeneous; population analysis on sustained timescales captured these weak and heterogeneous but consistent modulation of spiking activity in mPFC unit population, but the population responses during stimulation diverged from baseline by only about 10 – 50 %. These effects support the classical idea of dopamine as a neuromodulator and are also consistent with dopamine projections to mPFC, which primarily terminate in the distal dendrites of pyramidal neurons and interneurons (Carr et al 1999, Carr &
Sesack 2000, Sesack et al 1995b). As a result, dopamine release or even glutamate co-release may not elicit robust effects on neuronal activity but may modulate various ion conductances at distal dendrites that can slowly and weakly impact somal activity.

### 3.4.6 Important considerations

We stimulated VTA dopamine neurons at the soma and not at the mPFC terminals; thus, optogenetic stimulation in the VTA potentially activated dopamine projections to other areas, especially the striatum. It is possible that the observed neural effects in mPFC are indirectly mediated by activation of these other projections instead of or in addition to activation of dopamine projections in mPFC. For example, dopamine release enhances gamma oscillations in the striatum (Costa et al 2006), and dopaminergic drugs such as amphetamine and apomorphine cause a switch in ventral striatal gamma from low gamma to high gamma oscillations (Berke 2009). These striatal effects may then modulate frontal gamma activity via basal ganglia-thalamic-cortical loops (Parent & Hazrati 1995). Another potential mechanism is the activation of the medial septum by VTA dopamine projections (Miura et al 1987), as the medial septum has been shown to strongly influence hippocampal theta and gamma oscillations (Ma & Leung 2000, Mitchell et al 1982). Thus, theta and gamma oscillations as well as coupling between them in mPFC could be influenced by the medial septum via direct projections (DeNardo et al 2015) or indirectly via the hippocampus (Thierry et al 2000). While future studies need to examine the specific role of local dopamine projections in modulating mPFC activity, we do not consider the simultaneous enhancement of dopamine release in multiple regions an issue. In fact, this may be behaviorally relevant as extracellular dopamine levels are elevated in multiple brain regions.
during the performance of the same behavior (Gambarana et al 2003, Stefani & Moghaddam 2006), and thus dopamine neurotransmission in many regions may interact to modulate brain-wide neural activity and subsequently behavior.

The present study did not examine the contribution of D1 or D2 receptor activation to the observed effects on spiking activity and LFP oscillations. While D1 receptors are more abundant in PFC (Santana et al 2009), several studies have also reported D2 mediated effects in this region (Godbout et al 1991, Sesack & Bunney 1989). Furthermore, D1 and D2 receptors can elicit complementary or antagonistic effects on neural activity (Burkhardt et al 2009); thus, examination of the type of receptor involved, D1 or D2 or even glutamate receptors, in mediating the observed effects will be an important future direction for the current research.

Dopamine neuron activity may elicit other effects in the PFC such as synaptic plasticity and modulation of excitability (without increasing firing rate) (Jay 2003, Seamans & Yang 2004) that cannot be measured with the technique used in the current study. Thus, while transient phasic VTA dopamine activity elicited weak or no effects on mPFC spiking and LFP activity, it may elicit robust changes in these other measures that can be functionally relevant for the learning of cue-outcome associations (Montague et al 1996, Schultz 1998, Schultz 2013, Steinberg et al 2013) and modulation of signal to noise in cortical networks (Cohen et al 2002, Winterer & Weinberger 2004). Finally, as the current study only examined the modulation of spontaneous awake neural activity, future research needs to explore whether phasic VTA dopamine activity during specific behavioral tasks elicits similar effects on mPFC network activity.
4.0 GENERAL DISCUSSION

4.1 SUMMARY OF FINDINGS

Our results demonstrate that phasic VTA dopamine neuron activation elicits diverse effects on hemodynamic activity in the forebrain and local neural activity in the mPFC. In the study outlined in Chapter 1, we combined optogenetic stimulation of VTA dopamine neurons in Th::Cre rats with fMRI measurement of BOLD and CBVw hemodynamic signals in the forebrain to assess the global effects of phasic VTA dopamine activity. We showed that VTA dopamine activity increases hemodynamic signals in both regions that receive dense and sparse/no innervation from VTA dopamine neurons. Surprisingly, the most prominent hemodynamic activation is observed in the DS that receives relatively sparse innervation from VTA compared to areas such as VS and amygdala, and these activation patterns correlate with patterns of dopamine release in DS and VS. Dopamine release patterns, on the other hand, do not match with known projection density of VTA dopamine neurons in striatal sub-divisions. These results highlight the complex functional, potentially a combination of direct and circuit-level, interactions of VTA dopamine neurons with forebrain areas. They also suggest that VTA dopamine neurons send distributed signals in the forebrain to potentially modulate various sensory, cognitive, and affective processes but specifically elicits robust effects in the striatum to critically impact behavioral outputs. Furthermore, these findings describe a causal relationship
between VTA dopamine activity and forebrain fMRI signals that are measured during dopamine-dependent tasks in healthy humans and patients of dopaminergic disorders.

In the study described in Chapter 2, we combined extracellular recordings of unit activity and LFPs in mPFC and optogenetic stimulation of VTA dopamine neurons in freely moving and anesthetized Th::Cre rats. VTA dopamine neurons were stimulated using different paradigms that resemble the phasic activity patterns of these neurons during cognitive and reward-driven behaviors. Microdialysis experiments indicated that these paradigms increase dopamine levels in mPFC of Th::Cre rats. We demonstrated that the phasic activation of VTA dopamine neurons modulates unit activity of some neurons on multiple timescales. It can elicit transient activation or inhibition in both pyramidal and FS units on the order of milliseconds and seconds as well as sustained responses on the order of minutes. Phasic VTA dopamine activity also modulates population states or ensemble responses on prolonged timescales. Modulation of mPFC activity on transient versus sustained timescales may depend on the co-release of glutamate versus dopamine respectively from dopamine terminals (Lapish et al 2007).

Phasic activation of VTA dopamine neurons also affects mPFC network activity on the timescale of minutes by influencing various oscillations, especially high gamma. High gamma power is increased, along with enhanced phase-amplitude coupling between theta and high gamma oscillations. The high gamma power increase is not associated with synchronized activity in mPFC pyramidal neurons, suggesting a different function of high gamma enhancement, such as increasing signal to noise by filtering incoming signals in specific time windows (Benchenane et al 2011, Fries 2005, Fries 2015). Most of these changes in network activity elicited by dopamine activity may depend partially on the behavioral state, whether the animal is awake and moving or awake and quiescent or anesthetized. Under light isoflurane anesthesia, for example,
VTA dopamine activation increases the power of beta rather than high gamma oscillations. Thus, our results show varied effects of phasic VTA dopamine activity on different components of the mPFC network, all of which may summate in complex ways to mediate dopamine-dependent prefrontal functions. Further, the observed slow, diverse and, in many cases, modest changes in cortical network activity during phasic VTA dopamine activity are consistent with the classical idea of dopamine as a neuromodulator.

Finally, our results from fMRI and in vivo electrophysiology studies in mPFC indicate that all neural activity changes may not directly elicit hemodynamic responses measured with fMRI. While phasic VTA dopamine activation generates a robust increase in mPFC LFP beta frequency power under light isoflurane anesthesia as demonstrated in Chapter 3, it elicits relatively weak fMRI responses in this region in an identical preparation, which was described in Chapter 2. This discrepancy may be explained by the fact that fMRI signals correlate more strongly with changes in gamma oscillations (Logothetis et al 2001, Niessing et al 2005). This observation could be important for understanding the neural origins of hemodynamic signals and for the interpretation of the results of human studies that primarily rely on fMRI as a non-invasive measure of neural activity.

4.2 MODULATION OF STRIATAL HEMODYNAMIC ACTIVITY

I propose that the observed unexpected and robust increase in DS BOLD and CBVw signals during phasic VTA dopamine activation is mediated by a combination of dopamine and glutamate release in DS. The proposed mechanism is depicted in Figure 4.1. First, VTA
dopamine activity may increase dopamine release in DS via direct projections. This is consistent with our observation of increased DS dopamine release measured with FSCV. However, we also observed a much larger increase in [DA]₀ in DS compared to VS, and this finding contradicts the projection patterns of VTA dopamine neurons in the striatum suggested by classical tracing studies (Bjorklund & Dunnett 2007). In section 2.5.1, I outlined several factors that could explain the large increase in [DA]₀ in DS. Briefly, there may be a dorso-ventral gradient of dopamine release (Calipari et al 2012) from VTA terminals in the striatum as depicted in Figure 4.2. Alternatively, a higher density of VTA dopamine terminals may innervate DS than previously suggested (Beier et al 2015). Dopamine release in DS could also be enhanced by glutamate release from cortical and subcortical afferents.
Figure 4.1 Schematic illustrating heightened glutamate and dopamine release in DS as a result of VTA dopamine activity.

1) Phasic VTA dopamine activity enhances dopamine release via direct projections to DS. 2) Phasic VTA dopamine activity modulates activity in various cortical and sub-cortical areas, including OFC and thalamus, which send dense glutamatergic efferents to the DS. This may result in an enhanced glutamate release in DS. 3) Enhanced glutamate release may further increase dopamine release in DS.
Our findings indicate that phasic dopamine activity increases activity in various cortical and sub-cortical areas, such as OFC and thalamus. Activation in OFC may be mediated by direct VTA dopamine projections (Berger et al 1991, Bjorklund & Dunnett 2007, Loughlin & Fallon 1984). As thalamic projections from VTA are weak (Yetnikoff et al 2014), with some innervation of medial thalamic areas (Swanson 1982), thalamic activation could be mediated by a combination of direct and indirect circuit-level effects. Many cortical and subcortical areas, including OFC and thalamus, send dense glutamatergic efferents to the DS (Burguière et al 2013, Hoover & Vertes 2011, Ongur & Price 2000, Voorn et al 2004). Thus, VTA dopamine activation may result in the divergence of signals to distributed cortical and subcortical areas and the eventual convergence onto DS via glutamatergic projections, which would elicit robust glutamate release in DS. As a result of this distributed processing, weak modulation of intermediate areas can still elicit a profound glutamate response in DS. The glutamate increase may then enhance dopamine release in DS via presynaptic modulation of NMDA receptors on dopamine terminals (Grace 1991, Nieoullon et al 1978, Youngren et al 1993). This mechanism may, thus, cause an amplification of both dopamine and glutamate release in DS (Figure 4.1).

The glutamate and dopamine responses in DS may strongly influence various neural processes in DS and elicit robust hemodynamic activation. As described in Section 2.5.1, glutamate activity is a strong predictor of hemodynamic responses (Iordanova et al 2015, Lauritzen 2005, Scott & Murphy 2012). Whether dopamine release can locally elicit hemodynamic signals is not known, but there is evidence for dopamine modulation of vasomotor responses in cortical vasculature (Krimer et al 1998). Thus, the robust fMRI activation in DS may result from a combination of dopamine and glutamate activation during optogenetic stimulation of VTA dopamine neurons.
Further studies are needed to determine the extent of glutamate and dopamine contribution to the observed hemodynamic signals in DS by pharmacologically manipulating local dopamine and glutamate transmission. Future studies should also combine optogenetic stimulation of VTA dopamine neurons with extracellular recordings in DS to determine the neural correlates of the observed fMRI signals and investigate differences in neural responses in DS and VS that may result in differences in fMRI activation in the two regions.
Figure 4.2 Schematic representation of differential dopamine release in DS and VS.

Even though VTA dopamine innervation is much stronger in VS and relatively sparse in DS, the pattern of dopamine release from VTA terminals may be different in these regions. A higher concentration of dopamine may be released per synapse in DS versus VS, resulting in a higher net \([DA]_0\) in DS during phasic activation of VTA dopamine neurons.
4.3 STATE DEPENDENT REGULATION OF MPFC NEURAL ACTIVITY BY PHASIC VTA DOPAMINE ACTIVATION AND RELATIONSHIP TO FMRI SIGNALS

Our findings suggest that phasic VTA dopamine activity’s effects on mPFC neural activity may be related to the behavioral state of the animal, which is described in detail in section 3.5.2. Based on our results from awake and anesthetized recordings, I have proposed a model to explain dopamine’s role in state dependent modulation of mPFC neural activity. This model is based on the idea that the cortical activation state is different across behavioral states due to differences in the amount of thalamic inputs to the cortex. When animals are engaged in active exploration and movement, the cortex receives a barrage of glutamatergic inputs from the thalamus. In anesthetized as well as awake but quiescent states, afferent inputs to PFC are reduced due to partial closing of thalamic gates (Angel 1991, Sanders et al 2012, Sterman & Bowersox 1981). I argue that dopamine’s effects on sustained timescales depend on the cortical activation state. Dopamine may modulate population-level neuronal responses and LFP high gamma oscillations specifically when mPFC is in the activated state. This hypothesis explains the robust effects of VTA dopamine activity on mPFC neural population states and high gamma oscillations when animals engage in active behaviors during stimulation and the rapid return of these effects to baseline levels post-stimulation, when animals also return to quiescent behavioral states, even though mPFC [DA]₀ is still elevated. This hypothesis is further supported by the evidence of a lack of high gamma increase upon VTA stimulation during low cortical activation states, such as under isoflurane anesthesia.

The proposed state-dependence model is illustrated in Figure 4.3. I will focus on dopamine’s effects on oscillations, but similar mechanisms may mediate population responses in
mPFC. The mechanism for the generation of high gamma oscillations is not well understood, but it has been proposed that they can arise from synaptic interactions between FS interneurons and pyramidal neurons or electrical coupling via gap junctions (Suffczynski et al 2014, Uhlhaas et al 2011). A recent computational modeling study demonstrated that the same cortical network consisting of FS interneurons and pyramidal neurons can generate high frequency oscillations in different frequency ranges, whether high gamma, low gamma, or beta, depending on the rate of external/thalamic input to the cortex (Suffczynski et al 2014). Specifically, activation of the cortex by high frequency external inputs generates high gamma oscillations. Reduction of input frequency reduces the frequency of oscillations in a systematic way.

I suggest two mechanisms via which dopamine could impact oscillations in the PFC: a) by modulating the strength of excitatory inputs to mPFC in a frequency dependent manner, and b) by enhancing GABAa receptor currents. The plausibility of the first mechanism is supported by evidence that dopamine can filter excitatory inputs to the hippocampus in a frequency dependent manner, i.e. it inhibits EPSPs elicited by low frequency excitatory inputs but enhances EPSPs from high frequency excitatory inputs (Ito & Schuman 2007). The proposed filtering mechanism is also consistent with the idea that dopamine enhances signal to noise ratio in cortical networks (Cohen et al 2002, Winterer & Weinberger 2004). In support of the second proposed mechanism, dopamine has been shown to enhance GABAa currents via D1 receptors (Seamans et al 2001).

Based on these experimental and computational evidence, I propose the following: when animals are engaged in active exploration and movement, the PFC receives a barrage of thalamic inputs. In the presence of these extrinsic high frequency excitatory inputs, the intrinsic excitation and inhibition between pyramidal neurons and FS interneurons will generate high gamma
oscillations in mPFC as suggested by Suffczynski (2014). Enhanced dopamine release will then increase the amplitude of existing high gamma oscillations by enhancing a) the strength of excitatory inputs to mPFC, and b) GABAa currents; both processes will increase the potency of excitation and inhibition between mPFC neurons (Figure 4.3A). When animals are in quiescent states during which the mPFC receives a reduced frequency of thalamic inputs, the mPFC oscillatory system is biased towards lower frequencies such as low gamma, and high gamma is absent. In this reduced cortical activation state, dopamine will reduce the strength of excitatory input to mPFC but will enhance GABAA currents. The net interaction of these two processes will result in no effect of dopamine on the dominant high frequency oscillation or low gamma (Figure 4.3B).
Figure 4.3 Hypothetical model for state-dependent modulation of mPFC oscillations by dopamine activity.

The model consists of pyramidal neurons (Py) and fast-spiking interneurons (FS) exerting rhythmic excitation and inhibition respectively. (A) In the awake and active exploration/movement state, a barrage of thalamic inputs impinges on mPFC (1). These high-frequency thalamic inputs interact with the Py-FS intrinsic cortical networks to generate high gamma oscillations. Dopamine enhances these oscillations by increasing the strength of thalamic excitatory inputs (2) and GABAa currents (3). (B) In the awake and quiescent state, thalamic inputs to mPFC are reduced in frequency (4). These low frequency thalamic inputs interact with the Py-FS intrinsic cortical network to generate low gamma instead of high gamma oscillations. Dopamine decreases the strength of low frequency thalamic excitatory inputs (5) and enhances GABAa currents (6), resulting in no net modulation of gamma oscillations. (C) In the anesthetized state, thalamic inputs to mPFC are highly reduced in frequency (7). Due to the high reduction of excitatory input frequency but enhancement of the time constant of GABAa receptors and GABAa currents by isoflurane (8), prominent beta oscillations are generated. As thalamic excitatory inputs are almost non-existent, dopamine produces a minimal impact on synaptic EPSPs elicited by any sparse inputs. Instead, dopamine enhances GABAa currents and subsequently beta oscillations.
Finally, under light isoflurane anesthesia, due to highly reduced frequency of excitatory inputs to mPFC, high gamma oscillations will be absent. Instead, the dominant high frequency oscillation of the cortical network will be shifted to beta oscillations. Many previous studies have observed prominent beta oscillations under light anesthesia (McCarthy et al 2008), and an inspection of LFPs recorded in our studies also supports this observation (Figure 4.4). Beta oscillations may be especially enhanced under anesthesia due to an increase in the time constant of GABAa receptors, which is \(~ 250\%\) by isoflurane (McCarthy et al 2008, Nishikawa & MacIver 2001), and enhanced GABAa currents (Bieda et al 2009). Thus, reduced excitatory thalamic inputs and an increased time constant of GABAa receptors under light isoflurane anesthesia may result in beta oscillations in the cortex. Dopamine will then elicit two effects as proposed: reduction of the strength of low frequency excitatory inputs and enhancement of GABAa currents. As the excitatory inputs to mPFC are already highly reduced under isoflurane, dopamine’s impact on synaptic EPSPs may be minimal. On the other hand, as beta oscillations in this state are primarily maintained by strong GABAa currents, beta oscillations may be especially sensitive to dopamine modulation of these currents, resulting in a pronounced enhancement of these oscillations by dopamine (Figure 4.3C). Thus, dopamine activity influences mPFC oscillations in a state dependent manner and it potentially does so by modulating the dominant high frequency oscillation in each state.
Figure 4.4 Raw mPFC LFPs recorded in an awake and moving state versus in an anesthetized state.

In the lightly anesthetized state (~1% isoflurane), the dominant high frequency oscillation is beta (for example, there are four oscillatory cycles between -0.6 s to -0.4 s, which corresponds to a frequency of 20 Hz). LFPs in an awake state, however, are characterized by gamma oscillations riding on slow theta oscillations.
This model on state-dependent modulation of oscillations also explains the apparent discrepancy between the observed neural activity and fMRI activity in mPFC. Our results indicate that even though phasic VTA dopamine activation strongly enhances beta oscillations under light isoflurane anesthesia, it elicits relatively weak fMRI activation in this region in an identical state. This discrepancy may be due to fMRI signals being more reflective of gamma oscillations in the cortex rather than beta oscillations. Previous studies have shown that gamma oscillations, particularly in the high gamma range > 50 Hz, are tightly correlated with BOLD fMRI signals (Logothetis et al. 2001, Niessing et al. 2005), and, interestingly, low frequency oscillations below 40 Hz can be negatively correlated with BOLD signals (Mukamel et al. 2005). This suggests that hemodynamic activity in the active exploration state may be much greater than in the anesthetized state. Future experiments should examine this idea by modulating VTA dopamine activity and measuring mPFC fMRI signals in awake animals that are head-fixed but performing an active behavior. Further studies are also needed to determine the relevance of the proposed GABAa mechanism and frequency-dependent modulation of excitatory inputs in mPFC by dopamine in generating oscillations.

4.4 NEURAL CORRELATES OF STRIATAL FMRI SIGNALS

The proposed model can be extended to dopamine modulation of striatal neural activity and fMRI signals. The origin of LFP gamma signals in striatum remains poorly understood, mostly because of the lack of excitatory neurons in the striatum (Berke 2009). However, it has been suggested that striatal gamma oscillations could result from interactions between FS interneurons...
in the presence of external inputs from afferent structures, such as cortex (Berke 2009). Synthesizing this idea with the computational cortical model of Suffczynski et al. (2011), I hypothesize that in the striatum, rhythmic inhibition and disinhibition between FS interneurons interacts with fast excitatory external inputs to generate high gamma oscillations (Figure 4.5). Dopamine may then enhance these high gamma oscillations via the mechanisms proposed in the previous section. This hypothesis fits well with the schematic described in Figure 4.1. In Figure 4.1, I described a mechanism for VTA dopamine-mediated activation of glutamatergic cortical and sub-cortical afferents, from areas such as OFC and thalamus, to DS. Even though thalamic-cortical gates are mostly closed under anesthesia (Schneider & Kochs 2007), thalamic-striatal connections may remain active. Thus, even under isoflurane anesthesia, it may be possible to elicit high gamma oscillations in the striatum via activation of cortical and thalamic afferents to DS, and these oscillations may be profoundly enhanced by the large increase in striatal \([DA]_0\). The increase in high gamma oscillations may correspond to robust BOLD and CBVw signals observed in this region. Future studies should measure LFP responses in DS to phasic VTA dopamine activation in anesthetized animals to directly test the proposed hypothesis.
Figure 4.5 LFP correlates of fMRI signals in DS.

Glutamate inputs from cortical and sub-cortical areas (1), such as OFC and thalamus, modulated by VTA dopamine activity may drive high gamma oscillations in a DS network consisting of fast-spiking (FS) interneurons. Dopamine increase in DS may enhance high gamma oscillations by increasing the strength of afferent signals and amplifying GABAa currents. The increased high gamma oscillations are correlated with strong fMRI responses in DS.
4.5 GENERAL SIGNIFICANCE OF FINDINGS

The studies described in this dissertation utilized optogenetics in \textit{Th::Cre} rats to selectively and phasically activate dopamine neurons in the VTA. By demonstrating diverse and some unexpected effects of phasic VTA dopamine activation on downstream neural activity, these studies have highlighted the complex nature of dopamine neuromodulation. This complexity has been previously recognized in the literature, but the potential contamination of results by activation of GABAergic/glutamatergic neurons or tonic rather than phasic modulation of extracellular dopamine levels by dopamine agonists/antagonists has been extensively debated. The selective and phasic activation of VTA dopamine neurons in our studies avoids these confounds. The heterogeneous and complex effects of VTA dopamine activity, in mPFC, striatum and other areas, identified in our studies may be how dopamine plays a role in almost all cognitive and affective processes and why dopamine is implicated in the pathophysiology of many psychiatric and neurological disorders.

The studies in this dissertation have also identified a potentially novel functional connection between VTA dopamine neurons and basal ganglia that may help us better understand dopamine-mediated striatal functions. By assessing the contribution of VTA dopamine activity to fMRI signals and exploring the relationship between neural activity and fMRI responses, these studies have also provided a valuable link between animal dopamine research, that uses a variety of reductionist and invasive techniques, and human research that cannot typically afford invasive neural investigations. Furthermore, the characterization of BOLD and/or other hemodynamic changes associated with VTA dopaminergic neurotransmission in this dissertation may be used for clinical purposes to assist the early
diagnosis of dopaminergic disorders such as schizophrenia, depression, and addiction by examining hemodynamic responses in tasks that depend on VTA dopamine activation. Finally, the dissertation has identified novel putative mechanisms through which dopamine neurons modulate cortical information processing. These mechanisms may be highly relevant for understanding dopamine’s impact on normal PFC-dependent cognition and for relating cognitive deficits in disorders such as schizophrenia and ADHD to dopaminergic dysregulation in PFC.

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