Role Of The Ventral Hippocampus In Exploration And Ventral Hippocampal Parvalbumin Neurons in Behaviors Relevant to Schizophrenia

by

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ABSTRACT

We conducted experiments to understand the role of Ventral Hippocampus (vHPC) projections to the Nucleus Accumbens (NAc) in exploratory locomotion, and to determine if the reduced vHPC parvalbumin neuron activity can result in behaviors associated with schizophrenia. Through the use of optogenetics, we activated vHPC neurons and vHPC terminals in the NAc. Both manipulations significantly increased locomotor activity in the open field. Selective inhibition of vHPC terminals in the NAc during a test for novel environment exploration significantly reduced preference for novel environments over familiar environments. DREADD-mediated inhibition of activation of vHPC parvalbumin neuron activity did not significantly alter amphetamine-induced locomotion. Overall, these experiments provide support for the role of the vHPC-NAc pathway in mediating exploratory behavior in novel environments, but it remains inconclusive whether dysregulated vHPC activity due to the loss of parvalbumin neurons leads to behaviors associated with schizophrenia.
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# TABLE OF CONTENTS

Acknowledgements................................................................. ii

Table of Contents................................................................................ iv

1. Introduction................................................................................. 1

1.1. Dorsal and Ventral Hippocampus in Spatial Processing.............. 1

1.2. vHPC and Emotion............................................................... 2

1.3. NAc and Goal-Directed Locomotion....................................... 3

1.4. vHPC and Goal-Directed Locomotion..................................... 5

1.5. vHPC Regulation of Dopamine Transmission.......................... 6

1.6. Connectivity Between the vHPC and NAc............................... 8

1.7. Role of the vHPC and NAc in Spatial Exploration..................... 9

1.8. Anterior Hippocampus and Schizophrenia.............................. 12

1.9. Parvalbumin Interneurons in the Hippocampus....................... 13

1.10. Parvalbumin Interneurons in Schizophrenia........................... 14

1.11. Tools to Control Neural Activity......................................... 16

2. Hypotheses and Experimental Aims.......................................... 18

2.1. Aim I................................................................................... 18

2.2. Aim II.................................................................................. 19

2.3. Aim III............................................................................... 20

3. General Materials and Methods............................................. 21

3.1. Animals............................................................................... 21

3.2. Surgery.............................................................................. 21

3.3. Behavioral Apparatuses....................................................... 22
3.4. Optogenetics Setup................................................................................................................... 22
3.5. AAV Vectors and Drugs............................................................................................................ 23
3.6. Perfusion and Histology............................................................................................................ 23
3.7. Immunohistochemistry............................................................................................................. 23

4. AIM I: To determine if the vHPC-NAc connection is sufficient for goal-directed
   locomotion.................................................................................................................................... 24
   4.1. Experiment 1- Anatomical Tracing....................................................................................... 24
   4.2. Experiment 2- Pharmacological Activation of the vHPC..................................................... 27
   4.3. Experiment 3- Optogenetic Activition of the vHPC.......................................................... 29
   4.4. Experiment 4 Optogenetic Activation of vHPC-NAc Terminals................................. 32

5. AIM II: To determine if the vHPC-Nac connection is necessary for novel
   environment exploration............................................................................................................... 35
   5.1. Experiment 5- Optogenetic Inhibition of vHPC-NAc Terminals.................................... 35

6. AIM III: To determine if vHPC parvalbumin neuron activity is associated with
   behavioral models of schizophrenia.............................................................................................. 39
   6.1. Experiment 6- DREADD Control of Neural Activity......................................................... 39
   6.2. Experiment 7- DREADD Inhibition of vHPC Parvalbumin Neurons................................. 44
   6.3. Experiment 8- PolyIC Model of Schizophrenia.................................................................... 54
   6.4. Experiment 9- DREADD Activation of vHPC Parvalbumin Neurons.............................. 56

7. Discussion..................................................................................................................................... 59

8. Conclusions.................................................................................................................................. 65

9. References..................................................................................................................................... 66
1. INTRODUCTION

1.1. Dorsal and Ventral Hippocampus in Spatial Processing

Understanding of the function of the hippocampal formation has been heavily influenced by its critical role in spatial learning and memory (Bird and Burgess, 2008). While regions of the hippocampal formation certainly subserve spatial processing, the hippocampal formation may not function as a unitary structure. Indeed, multiple lines of evidence support a functional segregation along its septotemporal axis. The hippocampal formation can be defined as consisting of the dentate gyrus, CA3, CA1, and subiculum (Amaral and Witter, 1989). Recent findings of a molecular distinction between the septal and temporal poles of the CA3 and CA1 support a functional dissociation between these regions (Thompson et al., 2008; Dong et al., 2009). A regional specificity in gene expression pattern was found above and below the rhinal fissure, making it a possible landmark demarcating the boundary between the septal pole – which includes the dorsal hippocampus (dHPC), and between the temporal pole or ventral hippocampus (vHPC) (Dong et al., 2009).

While the dHPC has been strongly implicated in complex spatial processing and navigation, support for the involvement of the vHPC in this function has been limited (Moser and Moser, 1998; O’Mara, 2005; Dong and Fanselow, 2010). Lesion studies in rodents have indicated that the integrity of the dHPC is necessary for spatial memory acquisition in tasks such as the Morris Water maze (Moser et al., 1993; Moser et al., 1995; Zhang et al., 2004). Lesions covering as little as 30-40% of the dHPC can significantly impair spatial learning (Moser et al., 1993; Moser et al., 1995; Richmond et al., 1999;
Bannerman et al., 1999; Pothuizen et al., 2004; Broadbent et al., 2004; Dillon et al., 2008).

In contrast, most vHPC lesion studies have not found a similar deficit (Moser et al., 1993; Richmond et al., 1999; Bannerman et al., 1999; Pothuizen et al., 2004), although contradictory findings have been reported (Gross et al., 1965; Riegert et al., 2004; Broadbent et al., 2004).

Further evidence for the dissociation between the dHPC and vHPC in spatial processing has come from neuronal recordings in behaving animals. Discharge correlates of neurons in freely-moving animals during the performance of spatial tasks have revealed differences in place fields in the dHPC and vHPC (Jung et al., 1994, Kjelstrup et al., 2008). Although place cells have been found along the entire longitudinal extent of the hippocampus, the vHPC contains fewer place cells with poorer spatial specificity compared to the dHPC (Jung et al., 1994). Moreover, Kjelstrup et al. (2008) reported a topographical gradient in the scale of spatial representation along the septotemporal axis of the hippocampus, with place fields smaller than 1 metre located in the septal pole and as large as 10 metres in the temporal pole. Place cells in the vHPC have been proposed to inherit their spatial specificity through converging inputs arriving from multiple place cells in the dHPC (Kjelstrup et al., 2008; O’Mara et al., 2009) via associational fibers (Amaral and Witter, 1989). Thus, compelling evidence exists for a preferential role of the dHPC over the vHPC in processing complex spatial representations for learning and memory.

1.2. vHPC and Emotion

Anatomical studies of the dHPC and vHPC have revealed non-overlapping efferent connections which may further underlie their functional separation. The dHPC has been found to preferentially target cortical areas subserving sensory and cognitive processing
consistent with its role in spatial navigation, such as the retrosplenial cortex and anterior thalamic nuclei (van Groen et al., 2004; Cenquizca and Swanson, 2007). On the other hand, the vHPC sends prominent projections to subcortical limbic structures pointing to its involvement in emotional processing (Swanson and Cowan, 1977). The vHPC directly innervates the basolateral amygdala for the modulation of contextual fear and anxiety (Richmond et al., 1999; Bannerman et al., 1999; Hale et al., 2008; Biedenkapp and Rudy, 2008), the periventricular zone of the hypothalamus through which it is believed to exert inhibitory control over autonomic and neuroendocrine responses driven by the hypothalamic-pituitary-adrenal axis (Henke, 1990; Lowry, 2002; Mueller et al., 2004; Herman and Mueller, 2006), and the nucleus accumbens (NAc) where it critically controls dopamine release possibly for the regulation of motivation and goal-directed locomotion (Groenewegen and Witter, 1987; Cooper et al., 2006; Grace et al., 2007). Thus, unlike the dHPC, the primary function of the vHPC may not be in the formation of detailed spatial representations for accurate spatial navigation. Rather, the vHPC may serve to encode broad representations of space for the processing of emotional context.

1.3. NAc and Goal-Directed Locomotion

The initiation of context-appropriate goal-directed actions may be particularly crucial for adaptability and survival (Mogenson et al., 1980; Zahm, 2000). The NAc has been proposed to be a critical neural substrate underlying adaptive motor responding since it relays self-preservation signals from limbic structures to motor systems (Mogenson et al., 1980; Zahm, 2000). Being a “limbic motor-interface”, the NAc is positioned to integrate information provided by limbic structures to promote appetitive motor responses (Mogenson et al., 1980; Phillips et al., 2008). Major limbic input arrives from dopaminergic
neurons in the ventral tegmental area (VTA), which facilitate responses to reward-associated cues (Fields et al., 2007), and from glutamatergic neurons of the amygdala, prefrontal cortex, and vHPC (Brog et al., 1993; Groenewegen et al., 1997; Papp et al., 2012). Through its primary output to the ventral pallidum, the NAc may influence voluntary motor movement. The ventral pallidum has been found to directly project to motor areas in the midbrain and brainstem such as the substantia nigra and pedunculopontine tegmentum (Mogenson et al., 1980; Groenewegen et al., 1993).

One of the most widely established responses mediated by the NAc is locomotor activity (Jones and Mogenson, 1980). Several studies have reported increased locomotor activity upon stimulation of the NAc with a variety of pharmacological agents including dopamine, dopamine agonists, and glutamate agonists (Pijnenburg et al., 1973; Jones and Mogenson, 1980; Wu et al., 1992; Wu et al., 1993). There is much evidence to suggest that locomotor activity is mediated by increased dopamine transmission in the NAc. First, local infusions of glutamate agonists into the NAc that increase locomotion can enhance levels of dopamine in the NAc (Giorguieff et al., 1977; Imperato et al., 1990; Cosford et al., 1994). Furthermore, locomotory activity levels are strongly correlated with the concentration of NAc dopamine (Hooks et al., 1991; Taeparvarapruk et al., 2000). Lastly, blockade of dopamine transmission in the NAc via dopamine antagonists or 6-hydroxydopamine (6-OHDA) denervation of dopaminergic terminals, abolishes locomotion induced by systemic and local amphetamine and glutamate agonist administration (Jones and Robbins, 1992; Wu et al., 1992; Meeker et al., 1998). It is important to note that locomotor activity facilitated by the NAc is thought not to reflect simple motor reflex or voluntary movement, but is instead adaptive, motivated, and goal-directed in nature (Zahm, 2000; Floresco, 2007).
1.4. vHPC and Goal-Directed Locomotion

Consistent with its connectivity to the NAc, the vHPC has also been robustly associated with goal-directed locomotion. Stimulation of the vHPC using a variety of methodologies reliably induces locomotor activity (Bast et al., 2003). This finding has been observed with microinfusions of pharmacological agents such as N-methyl-D-aspartate (NMDA) (Yang and Mogenson, 1987; Wu and Brudzynski, 1995; Brudzynski and Gibson, 1997; Legault and Wise, 1999; Bast et al., 2001a; Gimenez-Llort et al., 2002; Zhang et al., 2002a; Peleg-Raibstein and Feldon, 2006), carbachol (Brenner and Bardgett, 1998), and picrotoxin (Bast et al., 2001), as well as with electrical stimulation (Taeparvarapruk et al., 2000). In contrast, this behavioral effect has not been found upon NMDA stimulation of the dHPC (Zhang et al., 2002; Peleg-Raibstein and Feldon, 2006), further supporting a unique role for the vHPC in goal-directed locomotion.

Studies involving blockade of dopamine neurotransmission and inhibition of vHPC activity have provided evidence for a dopamine-dependent mechanism underlying the vHPC induction of locomotion. Systemic haloperidol administration and VTA lesions have been found to prevent hyperlocomotion following vHPC infusions of carbachol (Brenner and Bardgett, 1998) and NMDA (Wu and Brudzynski, 1995), respectively. Furthermore, vHPC lesions but not dHPC lesions have been shown to attenuate the hyperlocomotion accompanying acute systemic amphetamine (Caine et al., 2001; White et al., 2006; Lodge and Grace, 2007), behavioral sensitization to amphetamine (Lodge and Grace, 2008), and intra-NAc infusions of amphetamine (Burns, 1993). Experiments involving pharmacological inactivation of the vHPC have yielded similar results. Microinfusions of the NA+ channel blocker tetrodotoxin and the GABA\textsubscript{A} receptor agonist muscimol into the vHPC were also reported to decrease locomotor activity (Bast et al., 2001; Zhang et al.,
Overall, these studies suggest that vHPC activity is sufficient and necessary for spontaneous and psychostimulant-induced locomotion. However, the results of vHPC inhibition have been less consistent than vHPC activation, since findings of no effect or even enhanced amphetamine-induced locomotion have been reported with vHPC inhibition (Bardgett and Henry, 1999; Riegert et al., 2004; Degoulet et al., 2008).

1.5. vHPC Regulation of Dopamine Transmission

Microdialysis experiments have confirmed that the vHPC regulates dopamine transmission in the NAc. Both chemical and electrical stimulation of the vHPC can powerfully elicit dopamine release in the NAc (Brudzynski and Gibson, 1997; Blaha et al., 1997; Legault et al., 1999; Mitchell, 2000; Taepavarapruk et al., 2001; Moss et al., 2003; Taepavarapruk et al., 2008). Moreover, a direct link has been made between vHPC-induced elevation in dopamine concentration in the NAc and locomotor activity (Taepavarapruk et al., 2000). Brief high frequency electrical stimulation of the vHPC immediately elicits increased levels of dopamine efflux and locomotion that were directly correlated (Taepavarapruk et al., 2000). Interestingly, both increases in dopamine and locomotor activity were long lasting, remaining elevated well above baseline for more than 30 minutes after stimulation (Taepavarapruk et al., 2000).

vHPC-induced dopamine efflux may occur through several mechanisms in the NAc. First, glutamate released from the vHPC may act presynaptically on dopaminergic terminals arriving in the NAc (Seesak and Pickel, 1990; Blaha et al., 1997; Taepavarapruk et al., 2000). This view is supported by anatomical evidence that hippocampal and dopaminergic terminals converge onto single neurons in the NAc and are in close apposition to each other (Seesak and Pickel, 1990). However, a polysynaptic mechanism involving the VTA may
also contribute to vHPC-evoked dopamine release. Taepavarapruk et al. (2008) found that the increased dopamine concentration in the NAc following electrical stimulation of the vHPC was substantially reduced by VTA lidocaine infusions. Strong evidence has been provided by electrophysiological experiments for a multi-structural circuit involving the VTA and ventral pallidum (Legault et al., 2000; Floresco et al., 2001, 2003; Taepavarapruk et al., 2008), which exerts strong inhibitory influence over the VTA (Grace and Bunney, 1985). Thus, excitatory inputs from the vHPC are hypothesized to arrive onto GABAergic projection neurons (medium spiny neurons (MSNs)) in the NAc which in turn inhibit GABAergic projection neurons of the ventral pallidum, thereby disinhibiting dopaminergic neurons in the VTA (Floresco et al., 2001, 2003).

Dopamine neurons in the VTA are reported to have 3 distinct activity states: nonfiring, tonically firing, and burst firing (Grace, 2011). VTA dopamine neurons receive substantial GABAergic inhibition from the ventral pallidum, such that 50% of these neurons are tonically hyperpolarized (Grace and Bunney, 1985). Spontaneously active dopamine neurons display tonic single-spike firing at an average rate of 4.5 Hz, which is generated by endogenous pacemaker conductances (Grace and Bunney, 1984a; Grace, 1991; Grillner and Mercuri, 2002). Burst firing activity is observed as high frequency action potentials riding on a depolarizing wave and is associated with dopamine release (Grace and Bunney, 1984b; Grillner and Mercuri, 2002). Furthermore, burst firing is believed to be the relevant pattern of activity signaling reward (Hollerman et al., 1998), and requires NMDA receptor activation. Due to magnesium blockade of the NMDA receptor at hyperpolarized membrane potentials, only depolarized spontaneously active dopamine neurons can be made to burst fire (Lodge and Grace, 2006; Grace, 2011). It is through the control of the number of spontaneously active dopamine neurons in the VTA that the vHPC is believed to modulate
sensitivity of the dopamine system to salient events. Consistent with this view, NMDA stimulation of the vHPC was found to increase the proportion of spontaneously firing dopamine neurons in the VTA (Floresco et al., 2001, 2003). As hypothesized by Grace (2010), the polysynaptic activation of VTA dopamine neurons may be an important pathway through which the vHPC can modulate dopamine system responsiveness to salient environmental cues such as natural rewards.

1.6. Connectivity Between the vHPC and NAc Shell

Peleg-Raibstein and Feldon (2006) observed a selective increase in dopamine levels in the NAc shell but not in the core upon NMDA infusions into the vHPC. This finding strengthens the assumption raised by anatomical studies that the vHPC exerts its influence over goal-directed locomotion through a unidirectional connection with the NAc shell, an area termed the “hippocampal district” by Kelley and Domesick (1982) (Witter and Groenwegen, 1987; Brog et al., 1993). As with its other cortical and subcortical afferents, the hippocampus sends topographical projections to the NAc (Swanson and Cowan, 1977; Gaykema et al., 1991). Whereas the ventral region innervates the caudomedial NAc, projections from progressively dorsal regions extend rostrolaterally in the NAc (Swanson and Cowan, 1977; Kelley and Domesick, 1982; Witter and Groenwegen, 1987; Witter et al., 1990; Brog et al., 1993; Papp et al., 2012). In agreement with these anatomical findings, expression analyses of the immediate early gene marker of neural activity, cFos, following intra-vHPC NMDA infusion have revealed a preferential induction of cFos in the NAc shell compared to the core (Bardgett and Henry, 1999; Zornoza et al., 2005; Marie et al., 2010).

Electrophysiology recordings performed in anaesthetized rats have also provided evidence for a direct modulation of NAc activity by the vHPC. Yang and Mogenson (1984)
were one of the first to record from neurons in the NAc as the vHPC was electrically stimulated. This and other studies have found that single pulse stimulation of the vHPC can evoke spike firing in the medial NAc (Yang and Mogenson, 1984; Boeijinga 1990; Taeparvarapruk et al., 2008). Moreover, tetanic stimulation of the vHPC results in long-term potentiation of evoked potentials in the NAc (Boeijinga 1993; Belujon and Grace, 2008). Activity in the vHPC has also been strongly correlated with the depolarization of NAc MSNs (O’Donnell and Grace, 1995; Goto and O’Donnell, 2001; Goto and Grace, 2008). MSNs characteristically display membrane potential transitions from a highly hyperpolarized resting potential called the DOWN state (~ -78mV), to a slightly depolarized UP state (~ -65mV) (Goto and Grace, 2008). Interestingly, electrical stimulation of the vHPC can induce membrane potential shifts from the DOWN state to the UP state (O’Donnell and Grace, 1995). Furthermore, Goto and O’Donnell (2001) reported that local field potential changes in the vHPC are highly correlated with transitions to the UP state in NAc neurons, particularly those located in the medial NAc. These electrophysiological studies have revealed that activity in the NAc is sensitively controlled by excitatory inputs arriving from the vHPC and can occur in synchrony with vHPC activity.

1.7. Role for the vHPC and NAc in Spatial Exploration

A novel environment is a place never before encountered by the animal, and for which the animal has no spatial representation (O’Keefe and Nadel, 1978). Exploratory locomotion may be elicited by novel environments to facilitate the formation of an internal representation of the novel external space (O’Keefe and Nadel, 1978; Nadel, 2008). Thus, exploratory locomotion may be considered a type of goal-directed locomotion, where the goal is to acquire information about the surrounding space for the formation of a conceptual
map of the environment. Key evidence has separately emerged for the involvement of the vHPC and the NAc in mediating exploratory locomotion in novel environments.

The exploration of novel environments has been found to involve increased neurotransmission within the vHPC, suggesting that the vHPC may be involved in triggering exploratory locomotion. Following exposure to a novel context, immediate elevations in vHPC acetylcholine, glutamate, and aspartate are observed (Thiel et al., 1998; Bianchi et al., 2003; Takeda et al., 2006). The levels of these neurochemicals peak within the first 10 minutes following exposure and are significantly diminished 20 minutes after exposure. Novel environment-induced locomotor activity levels follow a similar pattern, also peaking within the first 10 minutes of exposure (Day et al., 1991; Bianchi et al., 2003). Furthermore, exposure to a novel environment is associated with increased cFos expression in the vHPC, particularly in the CA1 subfield (Hess et al., 1995; Hale et al., 2008). The rise in vHPC activity may be necessary for novel environment exploration since vHPC lesions but not amygdala or prefrontal lesions can significantly reduce locomotion in a novel open field (Burns et al., 1996).

Increased neurochemical transmission has also been found in the NAc shell “hippocampal district” during novel environment exploration. Several studies have observed dopamine release specifically in the NAc shell following exposure to a novel environment (Ladurelle et al. 1995; Rebec et al., 1997a; Rebec et al., 1997b; Feenstra et al., 2000; Legault and Wise, 2001), with those animals responding with higher levels of locomotion also showing greater dopamine release (Saigusa et al., 1999; Thiel et al., 1999). In conjunction with increased transmission in dopaminergic terminals in the NAc, increased impulse flow from glutamatergic afferents, possibly those arriving from the vHPC, have been implicated by elevated levels of glutamate (Bland et al., 1999; Ho et al., 2000). Similar
to the vHPC, cFos expression in the NAc is elevated following exploration of a novel environment (Badiani et al., 1998; Uslaner et al., 2001; Rinaldi et al., 2010), with higher expression in the NAc shell compared to the core (Badiani et al., 1998; Uslaner et al., 2001). In support of the view of enhanced activity in the NAc, Rothwell et al. (2010) saw long-term potentiation in MSNs located in the NAc shell but not in the core 24 hours after novel environment exposure. On the other hand, electrophysiological recordings have provided contrary results regarding novelty associated neuronal activity in the NAc. Wood and Rebec (2004) found that a higher number of neurons are inhibited in the NAc shell during the approach response to a novel context. Ample evidence has been provided by 6-OHDA lesion studies that the disruption of dopamine transmission in the NAc can impair exploratory behavior in novel environments (Fink and Smith, 1980; Evenden and Carli, 1985; Robbins and Koob, 1980). Likewise, a recent study in mice found that D2 receptor knockdown in NAc neurons decreased novel but not familiar environment locomotion (Fernandes et al., 2012).

To date, only one study has linked dopamine transmission in the NAc during novel environment exploration to vHPC activity. Free-choice novelty, a test of exploratory preference between a familiar context and a completely novel context, has been robustly associated with increased dopamine release in the NAc (Legault and Wise, 2001). The enhancement of dopamine was abolished by tetrodotoxin inactivation of the vHPC suggesting that neural activity in the vHPC is required to evoke NAc dopamine release during novel environment exploration (Rebec et al., 1997a; Rebec et al., 1997b, Legault and Wise, 2001).
1.8. Anterior Hippocampus in Schizophrenia

Dopamine transmission in the NAc has been proposed to mediate incentive-salience, a process whereby environmental or internal stimuli associated with reward can come to influence goal-directed behavior (Robinson and Berridge, 1993). Aberrant control of the dopamine signal, particularly by the vHPC, may result in salience attribution to irrelevant stimuli in the environment (Grace, 2010). Dysregulation of vHPC activity has been linked to schizophrenia since psychosis is characterized by excessive and inappropriate salience attribution (Kapur, 2003).

Over the last 50 years, dopamine system dysfunction has been the leading theory of the pathophysiology of schizophrenia. Support for this conceptualization stems primarily from observations that typical antipsychotics act by blocking dopamine receptors while psychostimulants that enhance dopamine transmission, such as amphetamine, can induce hallucinations and delusions (Carlsson and Lindqvist, 1963; Seeman, 2006). Despite evidence for altered dopamine transmission in schizophrenia, anatomical support for dopamine system pathology has not emerged, indicating that this may not be the primary site of pathogenesis in schizophrenia. Instead, diffuse neuroanatomical changes outside of the striatal dopamine system have been observed, including enlargement of the lateral ventricles, and reduced volume and disorganization of the hippocampus and frontal cortex (Gothe1ff et al., 2000). Thus, a hypothesis has been put forth that this disorder arises from dysfunctional limbic regulation of dopamine system activity, with particular emphasis on the anterior hippocampus, the human correlate of the vHPC in rodents (Grace, 2010). Indeed, similar to findings in rats with induced vHPC hyperactivity, schizophrenics exhibit elevated levels of striatal dopamine in response to amphetamine (Breier et al., 1997; Laruelle and Abi-Dargham, 1999; Peleg-Raibstein and Feldon, 2006).
The most notable abnormality in schizophrenia that has become the focus of recent theories of its pathophysiology is the loss of inhibitory GABA interneurons in the prefrontal cortex (PFC) and anterior hippocampus (Todtenkopf and Benes, 1998). The loss of GABAergic interneurons in the anterior hippocampus would diminish inhibitory control and therefore lead to hyperactivity in the structure (Behrens and Sejnowski, 2009; Lodge et al., 2009; Grace, 2011). Neuroimaging studies, revealing excessive excitatory neurotransmission in the anterior hippocampus of schizophrenia patients, have lent support for this theory (Schobel et al., 2009). In particular, a recent fMRI study found increased basal metabolism in the CA1 and subiculum subfields of the anterior hippocampus of schizophrenics experiencing delusions (Schobel et al., 2009). A positive correlation was also found between the severity of positive symptoms and cerebral blood volume in the CA1 and subiculum. The loss of inhibitory control over anterior hippocampal output may lead to the emergence of a hyperdopaminergic state (Grace, 2010).

1.9. Parvalbumin Interneurons in the Hippocampus

The GABAergic interneuron that has been heavily implicated in the pathophysiology of schizophrenia is the subtype containing the calcium-binding protein parvalbumin (Lewis et al., 2005). There are 3 classes of parvalbumin interneurons that represent 20% of all GABAergic cells in the hippocampus (Jinno and Kosaka, 2002; Baude et al., 2007). These classes are defined by their synaptic connectivity with principle neurons: axo-axonic cells terminate on axon initial segments, bistratified cells innervate dendrites, and basket cells innervate soma and proximal dendrites (Benes and Berretta, 2001). They also form extensive networks among themselves via dendrodendritic gap junctions (Baude et al., 2007). In particular, parvalbumin basket cells are thought to be the
most powerful type of GABAergic interneuron for entraining pyramidal cell activity, shaping neural network oscillations, and controlling hippocampal output (Freund, 2003; Baude et al., 2007). Evidence for these functions has mainly arisen from their unique morphology, firing pattern, and network connections. These basket cells have a dendritic tree spanning the strata oriens and radiatum, and dense axonal arborizations restricted to the stratum pyramidale (Klausberger et al., 2003). Basket cells comprise 60% of parvalbumin neurons in the CA1, and in addition to electrical synapses, they form chemical synapses with other parvalbumin interneurons. Furthermore, they exhibit fast-spiking activity at frequencies greater than 100Hz without accommodation (Freund, 2003; Klausberger, 2003). This firing pattern and the formation of extensive ensembles connected electrically and chemically especially equip parvalbumin basket cells to entrain pyramidal cells at gamma frequency (Freund, 2003). In particular, parvalbumin basket cells have been implicated in the generation of gamma oscillations within and between the hippocampus and prefrontal cortex during cognitive task performance (Freund and Katona, 2007). Sohal et al. (2009) showed that direct inhibition of parvalbumin interneurons using optogenetic tools suppresses gamma oscillations, while activation of these neurons can generate gamma oscillations in the PFC.

1.10. **Parvalbumin Interneurons in Schizophrenia**

Since parvalbumin interneurons appear to be crucial for regulating hippocampal output, their loss may result in excessive hippocampal activity and therefore increased drive of the mesoaccumbens dopamine system. Postmortem studies in schizophrenia patients have revealed a selective decrease in the number of parvalbumin interneurons in several brain structures including the medial prefrontal cortex, thalamus, entorhinal cortex, and
anterior hippocampus (Beasley and Reynolds, 1997; Lewis et al., 2001; Bitanihirwe et al., 2009; Pantazopoulous et al., 2007; Zhang and Reynolds, 2002). Zhang and Reynolds (2002) reported a decreased density of parvalbumin interneurons in all subfields of the hippocampus of schizophrenics compared to healthy controls, with no significant difference in the density of the calretinin class of inhibitory interneurons. Furthermore, depressed patients did not show a similar significant loss in parvalbumin interneurons.

In keeping with this theory, decreased parvalbumin interneuron immunoreactivity has also been observed in developmental animal models of schizophrenia. In the methylazoxymethanol (MAM) model of schizophrenia, prenatal delivery of the neurotoxin MAM at embryonic day 15 causes the selective loss of parvalbumin interneurons in the ventral hippocampus of adult rats (Lodge et al., 2009). The MAM-treated rats exhibit dopamine system dysfunction as indicated by increased locomotor activity in response to amphetamine (AMPH). Moreover, consistent with the circuit model proposed by Grace et al. (2007), MAM rats exhibit increased population activity in the VTA (Lodge et al., 2009; Penschuck et al., 2006).

Parvalbumin interneurons are also decreased in the polyribocytidilic acid (polyIC) maternal infection model of schizophrenia. Prenatal treatment with polyIC in mice on embryonic day 17 mimics maternal immune reaction to viral infection in the second trimester, which has been associated with increased risk of schizophrenia (Meyer et al., 2007, 2009; Boksa, 2010). PolyIC treated mice exhibit both a partial loss of vHPC and medial PFC parvalbumin neurons and increased amphetamine-induced locomotor activity (Meyer et al., 2008). polyIC induces pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, which affect fetal brain development, specifically neuronal survival, differentiation, and apoptosis (Boksa, 2010). Furthermore, activation of pro-inflammatory cytokines may
contribute to the oxidative stress observed in schizophrenia by inhibiting the antioxidant glutathione (Do et al., 2009). Interestingly, parvalbumin neurons have been found to be especially vulnerable to oxidative stress, suggesting a possible mechanism for their loss in schizophrenia (Behrens and Sejnowski, 2009).

In addition to the dopamine-mediated symptoms of schizophrenics, a loss of parvalbumin interneurons may account for their disrupted gamma oscillations during cognitive task performance (Uhlhaas et al., 2008). Diminished gamma oscillatory activity has also been observed in the vHPC and PFC of MAM and glutathione deficient animals exhibiting decreased vHPC parvalbumin immunoreactivity (Lodge, Behrens, and Grace, 2009; Steullet et al., 2010).

1.11. Tools For the Control of Neural Activity

Thus far, the methods used to manipulate vHPC activity have mainly been mechanical (electrical stimulation and lesioning) and pharmacological. In most cases, these methods have not permitted reversible and fine temporal control of neural activity, nor do they allow the targeting of specific cell types among a heterogeneous cell population. To address such methodological shortcomings, we used virus-mediated delivery of opsins or modified G-protein coupled receptors (DREADDs) in combination with the Cre/lox system.

Adeno-associated virus (AAV) is a non-replicating, non-pathogenic virus capable of transducing several cell types in many different species (Vasileva and Jessberger, 2005). Because of these properties, AAV has commonly been used as a gene delivery vector. AAV constructs containing a transgene of interest downstream of a specific promoter can be locally infused into specific brain regions (Rogan and Roth, 2011). This allows spatially restricted and potentially cell type specific expression of the transgene.
Cre recombinase is a DNA binding enzyme that binds loxP sites flanking a gene to mediate the excision, or the inversion of the gene when it is doubly floxed (‘FLEX’ switch). Transgenic mice expressing Cre recombinase under the control of a tissue-specific promoter can be used in conjunction with viral gene delivery of floxed transgenes. When Cre driver mice are infused with virus carrying a doubly floxed and inverted transgene, Cre will mediate the inversion and expression of that transgene in cell types determined by the tissue specific promoter. In most cases, expression of the transgene can be visualized by a fluorescent reporter protein signal.

Optogenetics describes a method for expressing ionotropic opsins to bidirectionally control cell activity with high temporal resolution. Channelrhodopsin-2 (ChR2) is a light-gated cation channel that when illuminated with blue light, allows for the influx of cations, primarily Na+ (Zhang et al., 2010; Bernstein and Boyden, 2011). Several studies have reported ChR2 to mediate action potential firing up to a frequency of around 20 Hz that is time-locked, on the order of milliseconds, to the delivery of pulses of light (Tsai et al, 2009; Tye et al., 2011). On the other hand, halorhodopsins inhibit cell activity by pumping Cl- into cells and therefore causing hyperpolarization when illuminated by green or yellow light (Bernstein and Boyden, 2011). Another advantage of the optogenetic system is the potential to control neural activity at specific synapses. Since opsins are trafficked to axon terminals, light may be delivered to the projection targets of expressing cells (Tye and Deisseroth, 2012).

The DREADDs system we used is comprised of the human M4 and M3 muscarinic G-protein coupled receptors modified to respond exclusively to the synthetic ligand clozapine-N-oxide. This system allows for bidirectional control of neural activity using systemic CNO. Whereas binding of CNO to the hM4D receptor activates the Gi signaling
pathway and subsequent hyperpolarization, binding of CNO to the hM3D receptor activates the G\textsubscript{q} signaling pathway for depolarization (Armbruster et al., 2007). Furthermore, this approach permits highly efficient temporal control of neural activity on the order of minutes and reversibility over hours (Pei et al., 2008; Ray et al., 2011).

2. HYPOTHESES AND EXPERIMENTAL AIMS

2.1. AIM I

While the dHPC has been implicated in spatial learning and memory, the vHPC has been more strongly linked to limbic system outputs as reviewed above. The vHPC is especially involved in goal-directed locomotion presumably through its direct projections to the NAc. Electrophysiology, microdialysis, and behavioral experiments have respectively indicated that activity in the vHPC heavily influences activity of neurons in the NAc, that this activity can elicit dopamine release in the NAc, and that this dopamine signal is critical for the induction of goal-directed locomotion.

However, thus far, studies examining the involvement of the vHPC in goal-directed locomotion have not been able to dissect the role of its various projections. It is plausible that the activation of other vHPC downstream targets with projections to the NAc such as the medial prefrontal cortex and amygdala could also mediate this behavior. Therefore, it remains unclear whether serial transmission from the vHPC exclusively to the NAc is sufficient to trigger goal-directed locomotion.

Hypothesis I: We propose that impulse flow from the vHPC exclusively to the NAc can trigger goal-directed locomotion.
AIM I: Our first aim is to determine if activity in vHPC projections to the NAc is sufficient to induce goal-directed locomotion. The experiments we conducted to address this aim involve the use of optogenetics to investigate the effect of activating either the vHPC or vHPC projections to the NAc on goal-directed locomotion.

2.2. AIM II

Separate literatures have emerged for the role of the vHPC and NAc in the exploration of novel environments. Increased activity has been found in both structures following exposure to novel environments, as indicated by enhanced cFos expression. Furthermore, novel environments also trigger neurochemical release in both structures. While increased ACh transmission is robustly observed in the vHPC, increased levels of glutamate and dopamine are observed in the NAc. Legault and Wise (2001) found that this novel-environment associated dopamine signal in the NAc is dependent on vHPC neural activity. Although these studies have independently linked the vHPC and the NAc to exploratory locomotion in novel environments, there is insufficient information to conclude that direct vHPC input to the NAc facilitates this behavior.

Hypothesis II: We propose that impulse flow from the vHPC to the NAc is need to drive novel environment exploration.

AIM II: Our second aim is to determine if activity in vHPC projections to the NAc is necessary for exploratory locomotion in novel environments. To address this aim, we used optogenetic methods to selectively silence vHPC projections to the NAc during a test of novel environment exploration and preference.
2.3. AIM III

An abundance of evidence points to the involvement of the anterior hippocampus in the pathophysiology of schizophrenia. Increased fMRI activity in the anterior hippocampus during delusions (Schobel et al., 2009, Heckers et al., 1998), may result from reduced inhibitory control due to the loss of parvalbumin interneurons (Behrens and Sejnowski, 2009; Lodge et al., 2009; Grace, 2011; Zhang and Reynolds, 2002). Since parvalbumin interneurons can powerfully control hippocampal network oscillations and output (Freund, 2003; Baude et al., 2007), parvalbumin neuron loss may result in increased hippocampal drive of the mesoaccumbens dopamine system. Animal models of schizophrenia that show decreased parvalbumin immunoreactivity in the vHPC, such as the MAM and polyIC models, also exhibit increased locomotor sensitivity to amphetamine. Although vHPC parvalbumin neuron loss has been correlated with schizophrenia, a direct causal relationship between decreased vHPC parvalbumin neuron activity and behavioral assays of schizophrenia has not been established. Additionally, the putative protective effect of activating remaining vHPC parvalbumin neurons in schizophrenia models has not been explored. Thus, it remains unclear whether recovering parvalbumin neuron functioning can reverse the deficits observed in schizophrenia.

Hypothesis III: We propose that decreased parvalbumin neuron activity in the vHPC contributes to the dopamine dysregulation observed in schizophrenia. Therefore, rescuing vHPC parvalbumin neuron activity should reverse dopamine-related symptoms in schizophrenia.

AIM III: Our third aim is to determine if inhibiting parvalbumin neuron activity in the vHPC can result in behaviors associated with schizophrenia, and whether increasing the activity of vHPC parvalbumin neurons can rescue these behaviors in an animal model of
schizophrenia. To this end, we used DREADDs to either inhibit vHPC parvalbumin neurons, or to activate vHPC parvalbumin neurons in the amphetamine sensitization model of schizophrenia, and examined the effect of these manipulations on amphetamine-induced locomotion.

3. GENERAL MATERIALS AND METHODS

3.1. Animals

Male C57BL/6 mice aged 3-4 months were used. They were individually housed at least 1 week prior to behavioral testing at a constant 12 hr light/dark cycle and were provided with food and water ad libitum.

3.2. Surgery

Mice were anaesthetized with isoflurane vaporized at a concentration of 5% during the induction phase and between 1 – 2% throughout surgery, with a constant oxygen flow of 0.2 L per minute. After being mounted into a stereotaxic device, a 28 gauge internal cannula was lowered into the vHPC (AP: -3.40 mm, DV: -4.75, ML: -2.75 mm, after Paxinos and Franklin, 2001). 0.1-0.2 µL of virus solution was infused through the internal cannula, which was connected by tygon tubing to a 10µL Hamilton needle syringe. This solution was infused at a rate of 0.1µL per minute using a pressure pump. Following the infusion, the internal cannula was left in place for 10 minutes to prevent solution backflow. For experiments requiring cannula implantation, a 24 gauge guide cannula was lowered into either the vHPC or the NAc shell (AP: 1.10 mm, DV: -3.75, ML: 0.65 mm),
and secured with surgical cement. The guide cannula was stoppered with a dummy cannula to prevent clogging.

3.3. Behavioral Apparatus

Open Field Locomotion. Two different sized open field arenas were used in these experiments, both of which were square shaped in plan and composed of particle board covered in black veneer. The larger open field arena measured 45 cm x 45 cm x 45 cm, and the smaller open field measured 30 cm x 30 cm x 30 cm. Open field testing was done under red light and locomotor activity was tracked and scored by AnyMaze.

Free-Choice Novelty. The apparatus was a rectangular clear acrylic box (45 cm x 20 cm x 30 cm) divided in half into 2 compartments by a separator that could be removed. The walls and floor of each compartment contained a different visual pattern, dots or stripes. This test was conducted under moderate white light illumination (0.5 mW). Locomotor activity and time spent in each compartment was tracked and scored by AnyMaze.

3.4. Optogenetics Setup

To deliver light to the brain, an optic fiber cable coupled to a laser source was inserted through the guide cannula. For neural activation experiments involving ChR2, a 473 nm blue laser was used along with a pulse generator to deliver 5ms pulse trains at a frequency of 20 Hz. For neural inhibition experiments involving halorhodopsin, continuous 532 nm of green light was delivered. A light intensity power ranging from 6mW to 40mW from the fiber tip was used depending on the experiment.
3.5. **AAV Vectors and Drugs**

AAV vectors were obtained from the University of North Carolina Vector Core. CNO was kindly given to us by the laboratory of Dr. Bryan Roth at UNC. d-Amphetamine was kindly donated by Dr. John Yeomans Laboratory in the Department of Psychology at the University of Toronto.

3.6. **Perfusion and Histology**

Immediately after completion of testing, mice were anaesthetized with Avertin and transcardially perfused with phosphate buffered saline followed by 4% paraformaldehyde. After perfusion, brains were removed and post-fixed overnight in 4% paraformaldehyde, then immersed in 30% PBS-sucrose solution until they sank to the bottom of the vial, fully absorbing the solution and thus sufficiently cryoprotected. Brains were coronally sectioned in the cryostat at a thickness of 40µm. Images of brain sections were then acquired using an Olympus FSX100 fluorescence microscope. Images were obtained with a 4.2x objective and are shown at up to 20x magnification.

3.7. **Immunohistochemistry**

Immunohistochemical staining was performed for parvalbumin and cFos protein. Briefly, sections containing the vHPC were blocked with 5% normal donkey serum in 0.1% PBS/Triton X for 1 hour, then incubated with rabbit polyclonal anti-parvalbumin primary antibody or with rabbit polyclonal anti-cFOS primary antibody for 72 hours (1:2000, Santa-cruz), followed by green fluorescent goat anti-rabbit secondary antibody (1:1000, AlexaFluor 488). For cFos staining, mice were injected with 2 mg/kg CNO 90 minutes before they were sacrificed.
Anatomical tracing experiments of the afferent and efferent connections of the vHPC were conducted to inform us of potential sites in the brain that could be examined in our experiments and to determine the reliability of opsin expression in vHPC downstream targets. We performed retrograde tracing from the vHPC to identify upstream structures that could regulate vHPC activity. Retrograde tracing from the NAc shell and anterograde tracing from the vHPC was conducted to confirm that the vHPC indeed projects to the NAc shell in mice and to determine the area of the vHPC that should be targeted in our mouse experiments (Kelly and Domesick, 1982; Groenewegen et al., 1987).

Methods

Retrograde Tracing. Mice were stereotaxically infused with the retrograde tracer cholera toxin subunit B conjugated to AlexaFluor (CTB) and after 7 days the brains were harvested and qualitatively examined for cell labeling (Conte et al., 2009). CTB-594 (red) was infused into the vHPC (0.7 μL) and the CA3, entorhinal cortex, amygdala, and septum were examined for labeling (n= 2). CTB-488 (green) was infused into the NAc shell (0.3 μL) and the pattern of labeling was examined in the vHPC.

Anterograde Tracing. AAV-mediated expression of ChR2 was used as the anterograde tracer. The vHPC was unilaterally infused with AAV carrying the gene for ChR2 fused to EYFP and under the control of the neuron-specific promoter human synapsin-1 (AAV-hsyn-ChR2-EYFP). vHPC downstream targets, specifically the NAc, amygdala, prefrontal cortex, and the hypothalamus were examined for projection labeling.
Results

Retrograde Tracing from the vHPC. CTB-594 was unilaterally infused into the ventral CA1 (N= 2) (Figure 1-1A). Labeling was observed in the ipsilateral and contralateral CA3 along the same septotemporal level as the area of the CA1 receiving the tracer (Figure 1-1B). This is consistent with the “lamellar” hypothesis of hippocampal organization (Amaral and Witter, 1989). Cell body labeling was also observed in the lateral entorhinal cortex (Figure 1-1A), basolateral amygdala (Figure 1C), medial septum, and the diagonal band (Figure 1-1D).

Figure 1-1. Images of CTB-594 retrograde tracer fluorescence (red) after infusion into the vHPC. (A) CTB-594 was infused into the ventral CA1. Labeling was observed in cells of the ipsilateral lateral entorhinal cortex (A), the ventral CA3 on the ipsilateral (A) and contralateral side (B), the basolateral amygdala (C), and the medial septum and diagonal band (D).

Retrograde Tracing from the NAc Shell. CTB-488 was unilaterally infused into the medial NAc (N= 5) (Figure 2-1A). Robust labeling was observed in the ventral half of the CA1 and subiculum, with densest labeling seen in the ventral tip (Figure 2-1B, C). Labelled cells in the VTA were also observed (Figure 2-1B)
Figure 2-1. Labeling in the vHPC following retrograde tracing from the NAc Shell. (A) Region of the medial NAc infused with CTB-488. (B) Retrogradely labeled cells in the VTA and ventral subiculum. (C) Magnified view of labeled cells in the ventral subiculum.

Anterograde Tracing from the vHPC. The ventral dentate gyrus, CA3, CA1, and subiculum were transduced by AAV as indicated by the EYFP signal (Figure 3-1A). Strongly labeled projections were observed in the basolateral amygdala, lateral and ventromedial hypothalamus, fimbria fornix, lateral septum, NAc shell, the prelimbic and infralimbic cortices of the medial prefrontal cortex, and the anterior olfactory nucleus (Figure 3-1B-E).

Figure 3-1. Representative images of ChR2-eYFP fluorescence (green) in the vHPC and its projection sites. Major fluorescence was observed in the (A) vHPC, (B) basolateral amygdala, fimbria fornix, ventromedial hypothalamus, (C) lateral septum, (D) nucleus accumbens, (E) prefrontal cortex, and anterior olfactory nucleus.
4.2. AIMI: EXPERIMENT 2- PHARMACOLOGICAL ACTIVATION OF vHPC

We performed local vHPC microinfusions of the cholinergic receptor agonist carbachol and the GABA_A receptor antagonist bicuculline in mice, to determine if we could replicate the locomotor effects observed in rats upon pharmacological activation of the vHPC.

Methods

Surgery. Mice were unilaterally implanted with a guide cannula in the vHPC (N= 16).

Open Field Testing and Infusion Procedure. Mice received 3-hour habituation sessions in the open field on 2 consecutive days prior to testing, and 30 minutes of habituation on test days immediately before receiving local infusions. Drug infusions were performed the same way as AAV infusions (as described in the Surgery methods) however, the internal cannula was only left in place for 2 minutes after infusion to prevent any additional stress caused by handling. Mice were either infused with 0.3uL of saline (N= 3), 0.3ug/0.3uL of carbachol (N= 11) or 0.25 ug/uL of bicuculline (N= 3). After the infusions, mice were immediately placed in the open field and distance travelled was measured for 100 minutes.

Results

Histology. For all animals, the tip of the internal cannula was located within the vHPC (Figure 1-2).

Open Field Locomotion. Infusion of carbachol and bicuculline increased locomotor activity compared to saline as indicated by a significant main effect of drug infusion (F(2, 14)= 9.14, P= 0.003) (Figure 2-2). Furthermore, locomotor activity significantly increased across
time (F(9, 126)= 6.87, P < 0.0001) and was significantly different between drug treatments (F(18, 126)= 6.48, P < 0.0001 time x drug interaction) (Figure 2-2). For carbachol infusion, locomotor activity was significantly higher than saline infusion at 30 minutes (p < 0.05), 40 minutes (p < 0.01), and 50 minutes (p < 0.05) (Figure 2-2). Locomotor activity was significantly higher at 40 minutes (p < 0.001), 50 minutes (p < 0.01), and 60 minutes (p < 0.01) after bicuculline infusion compared to saline infusion (Figure 2-2).

**Figure 1-2. Internal cannula placements in the vHPC.** Each spot represents an area where the internal cannula was placed.

**Figure 2-2. Effect of vHPC infusions of saline (N= 3), carbachol (CCh) (N= 11), or bicuculline (N= 3) on locomotor activity.** There was a significant effect of time (F9, 126 = 6.87, P < 0.0001) and drug infusion (F2, 14 = 9.14, P = 0.003), as well as a time x drug interaction (F18,126 = 6.48, P <0.0001).
4.3. AIM I: EXPERIMENT 3- OPTOGENETIC ACTIVATION OF vHPC

To determine if optogenetics can be reliably used to study the involvement of the vHPC in exploratory behavior, we examined whether optogenetic stimulation of the vHPC can induce hyperlocomotion similar to the effect that has been observed with pharmacological and electrical stimulation.

Methods

Surgery. A guide cannula was implanted unilaterally in the vHPC. Mice either received unilateral infusion of AAV-hsyn-ChR2-eYPF (0.2uL) (vHPC-ChR2 mice) through the guide cannula (N= 4) or no infusion (control) (N= 2).

Open Field Testing Procedure. One week after surgery, mice were habituated to the open field arena with the inserted optic fiber for 60 minutes immediately before receiving light stimulation. Blue light with a power of 6mW was delivered at 20 Hz for 5 seconds after 70 minutes and again after 90 minutes of being in the open field. Total distance travelled at 5-minute intervals was measured.

Results

Histology. In all mice infused with AAV-hsyn-ChR2-eYPF, ChR2-eYFP expression was observed in all subfields of the vHPC. The tip of the optic fiber was found to be located 1 mm above the vHPC (N= 4) (Figure 1-3).
Open Field Locomotion. Total distance travelled was significantly higher 5 minutes after light stimulation compared to baseline levels only for vHPC-ChR2 mice (light stimulation 1: t(3)= 7.49, p< 0.01); light stimulation 2: t(3)= 3.51, p< 0.05) (Figure 2-3). Repeated measures with one within and one between subjects factor revealed a significant time x group interaction (F(20, 80)= 4.99, p< 0.0001), and a significant difference in locomotor activity between groups (F(1, 4)= 130.79, p< 0.0001) (Figure 3-3). Change in locomotor activity was only significant over time for vHPC-ChR2 mice (F(21, 63)= 9.66, p<0.0001) (Figure 3-3). Moreover, locomotor activity of vHPC-ChR2 mice was significantly higher than that of control mice at 5 minutes (t(4)= 4.85, p< 0.01) and at 10 minutes (t(4)= 4.31, p< 0.05) after light stimulation 1, and at 5 minutes after light stimulation 2 (t(4)= 3.27, p< 0.05) (Figure 3-3).
Figure 2-3. Total distance travelled of vHPC-ChR2 (N= 4) and control mice (N= 2) at baseline and 5 minutes after light stimulation. vHPC-ChR2 mice ran significantly more after light stimulation 1 \([t(3)= 7.49, p< 0.01]\) and light stimulation 2 \([t(3)= 3.51, p< 0.05]\).

Figure 3-3. Distance travelled across time in 5-minute intervals after light stimulation of the vHPC (N= 6). Distance travelled was higher for vHPC-ChR2 mice (N= 4) compared to controls (N= 2) after light stimulation 1 (5 minutes: \(t(4)= 4.85, p< 0.01\); 10 minutes: \(t(4)= 4.31, p< 0.05\)) and light stimulation 2 (5 minutes: \(t(4)= 3.27, p< 0.05\)).
4.4. AIM I: EXPERIMENT 4- OPTOGENETIC ACTIVATION OF vHPC TERMINALS IN THE NAc

To determine whether vHPC projections specifically to the medial NAc are sufficient to induce exploratory locomotion, we exclusively activated ChR2-expressing vHPC projections in the medial NAc.

Methods

Surgery. 0.1uL of AAV-hsyn-ChR2-eYPF was infused unilaterally into the vHPC and a guide cannula was implanted unilaterally in the NAc (N= 6).

Open Field Testing Procedure. Two weeks after surgery, mice were habituated to the open field arena with the inserted optic fiber for 60 minutes immediately before receiving light stimulation. Blue light with a power of 30mW was delivered at 20 Hz for 15 seconds after 60 minutes and again after 90 minutes of being in the open field. Total distance travelled at 5-minute intervals was measured.

Results

Histology. ChR2-eYFP fluorescence was observed in cell soma located in all subfields of the vHPC, and was restricted to the area below the rhinal fissure (N= 6) (Figure 1-4A). vHPC terminals expressing ChR2-eYFP were observed in the medial NAc (Figure 1B). The tip of the implanted guide cannula was found directly above vHPC projections in the medial NAc (Figure 1-4B).
Figure 1-4. Images of ChR2-eYFP fluorescence in the vHPC and medial NAc, and NAc cannula implantation. (A) ChR2-eYFP expression was observed in cell soma in the vHPC. (B) The tip of the guide cannula was implanted above vHPC projections in the medial NAc.

Open Field Locomotion. Locomotor activity significantly differed across time (F(12, 60)= 6.97, p< 0.001) (Figure 2-4.). Total distance travelled in 5 minutes was higher than baseline only after the second light stimulation (t(5)= 4.99, p< 0.01) and approached significance after the first light stimulation (t(5)= 2.28, p< 0.07) (Figure 2-4, 3-4.)
Figure 2-4. Distance travelled across 5-minute time intervals after light stimulation of ChR2 expressing vHPC terminals in the NAc (N= 6). There was a significant main effect of time (F(12, 60)= 6.97, p< 0.001).

Figure 3-4. Total distance travelled 5 min before and 5 min after light stimulation of vHPC terminals in the NAc (N= 6). Locomotor activity was significantly greater than baseline after light stimulation 2 (t(5)= 4.99, p< 0.01).
5.1. AIM II: EXPERIMENT 5- OPTOGENETIC INHIBITION OF vHPC TERMINALS IN THE NAc

We examined whether activity in vHPC projections is necessary for novelty-induced locomotion and spatial novelty preference by specifically inhibiting halorhodopsin-expressing vHPC terminals in the medial NAc during the performance of these behaviors.

Methods

Surgery. Mice were bilaterally infused with 0.2uL AAV-hsyn-halorhodopsin-eYPF and bilaterally cannulated in the NAc (N= 7).

Free-Choice Novelty. Behavioral testing was performed 2 weeks after surgery. Mice were inserted with the optic fiber cable and habituated to one compartment of the 2-chamber apparatus for 30 minutes. After habituation, mice were placed in their home cages for 1 minute, at which point we started the delivery of continuous green light with a power of 40mW. They were then placed back into the apparatus with the partition separating the compartments removed, and allowed to freely explore both compartments for 5 minutes. Time spent in, distance travelled in, and number of entries into the familiar and novel compartments were measured. To determine baseline novelty preference and locomotion, 7 mice that did not have surgery were tested (no-surgery control mice).

Results

Histology. In all mice, halorhodopsin-eYFP fluorescence was observed bilaterally in cell soma located in all subfields of the vHPC (N= 7) (Figure 1-5A). Three mice displayed
halorhodopsin-eYFP fluorescence in vHPC terminals in the NAc (Halo-eYFP) (Figure 1-5B). Fluorescence was not observed in all other mice (surgery control mice, N= 4) (Figure 1-5C).

Figure 1-5. Representative halorhodopsin-eYFP fluorescence in the vHPC and medial NAc. Images are of (A) Bilateral halorhodopsin-eYFP expression in the vHPC (B) unilateral expression in the NAc (N= 1) (C) no expression in the NAc (N= 3).

Free-Choice Novelty. No-surgery control mice spent significantly more time in the novel compartment compared to the familiar compartment during the first 10 minutes of the test (after 5 minutes, t(6)= 10.29, p<0.0001; after 10 minutes, t(6)= 3.26, p< 0.05) (Figure 2-5). A significant time x compartment effect was found (F(5, 60)= 6.16, p< 0.001) with mice spending an average of 70% of their time in the novel compartment and 30% time in the familiar compartment within the first 5 minutes, and about 50% of their time in each compartment after 30 minutes (Figure 2-5). A significant main effect of time (F(5, 60)= 8.41, p< 0.00001) and time x compartment interaction (F(5, 60)= 0.0001) was also found for distance travelled (Figure 3-5). Locomotor activity was significantly higher in the novel compartment compared to the familiar compartment at 5 minutes (t(6)= 5.62, p< 0.001) and 10 minutes (t(6)= 3.09, p< 0.05) after the start of the test (Figure 3-5).
Figure 2-5. The percent time no-surgery control mice spend in the familiar and novel compartments during free-choice novelty preference (N= 7). Mice spent significantly more time in the novel compartment in the first 5 minutes (t(6)= 10.29, p<0.0001) and 10 minutes (t(6)= 3.26, p<0.05) of the test.

Figure 3-5. Distance travelled of no-surgery control mice in the familiar and novel compartments during free-choice novelty preference (N= 7). Locomotor activity in the novel compartment was significantly greater at 5 minutes (t(6)= 5.62, p< 0.001) and at 10 minutes (t(6)= 3.09, p< 0.05).
Similar to no-surgery control mice, surgery controls spent 70% time in the novel compartment and 30% time in the familiar compartment during the first 5 minutes of the test \((t(3)= 7.56, p< 0.01)\) (4-5). In contrast, mice expressing halorhodopsin in vHPC terminals in the NAc did not exhibit preference for the novel compartment as there was no significant difference in the percent time spent in either compartment \((t(2)= 0.739, p= 0.73)\). No significant difference in distance travelled in the novel and familiar compartments was observed for either groups, although there was a trend towards increased locomotor activity in the novel compartment for surgery controls \((t(3)= 2.59, p= 0.081)\) (5-5).

![Figure 4-5. The percent time surgery control (N= 4) and Halo-eYFP (N= 3) mice spend in the familiar and novel compartments during free-choice novelty preference with continuous 40mW green light delivery into the vHPC. Surgery control mice spent more time in the novel compartment \((t(3)= 7.56, p< 0.01)\) compared to the familiar compartment. Halo-eYFP mice did not spend significantly more time in either compartment \((t(2)= 0.739, p= 0.73)\).](image-url)
Figure 5-5. Distance travelled of surgery control (N= 4) and Halo-eYFP (N= 3) mice in the familiar and novel compartments during free-choice novelty preference with continuous 40mW green light delivery into the vHPC. No significant increase in locomotor activity was observed in the novel compartment for either group.

6.1. AIM III: EXPERIMENT 6- DREADD CONTROL OF vHPC ACTIVITY

To determine whether DREADDs are a reliable method for neuron activation and inhibition, we expressed hM3D and hM4D in neurons of all subtypes in the vHPC and performed electrophysiological recordings, cfos staining, and locomotor testing upon CNO administration. hM3D and hM4D expression in the vHPC was achieved by injecting AAV-hsyn-FLEX-hM3D-mCherry into the vHPC of mice expressing Cre recombinase under a broadly active promoter (BAct:cre mice).

Methods

Surgery. 0.2uL of AAV-hsyn-FLEX-hM3D-mCherry was infused unilaterally into the
vHPC of BAct:cre mice (N= 8).

In vitro response to CNO of hM4D and hM3D expressing neurons. As a proof of principle of the effect of hM3D and hM4D on neural activity, we prepared ventral hippocampal slices from BAct:cre mice expressing hM4D or hM3D and performed intracellular recordings from cells in the CA1 in the absence and presence of bath-applied CNO. These recordings were performed by Vivek Mahadevan in Dr. Melanie Woodin’s laboratory.

cFos Staining. BAct:cre mice expressing hM3D were injected with 1mg/kg i.p. of CNO (N= 2). After 90 minutes, they were sacrificed and the brains were harvested and stained for cFos.

Open Field Locomotion. After a 1-week recovery from surgery, mice were habituated to the open field for 3 hours one day prior to testing and for 30 minutes immediately before testing. Immediately following saline (10 mL/kg i.p.) injection on day 1, and CNO (1 mg/kg i.p.) injection on day 2, were placed in the open field for 3 hours.

Results

Histology. In all BAct:cre mice injected with AAV-FLEX-hM3D-mCherry, hM3D-mCherry fluorescence was observed in all subfields of the vHPC, and was restricted to the area below the rhinal fissure (N= 8) (Figure 1-6A).
*In vitro* response of hM3D and hM4D expressing cells to CNO. Whole-cell current-clamp membrane potential recordings were made from CA1 pyramidal neurons expressing hM3D in hippocampal slices. Upon CNO (10 uM) bath application, hM3D-mCherry expressing cells showed a depolarization in membrane potential from -66 mV to -54 mV, and an increase in firing rate (Figure 2-6). CNO application did not affect the membrane potential or the firing rate of neurons that did not express hM3D-mCherry (Figure 2-6). Whole-cell current-clamp membrane potential recordings were made from CA1 pyramidal neurons expressing hM4D-mCherry in hippocampal slices (Figure 3-6). Injection of a positive current (100 pA) for 2 s evoked robust action potential spikes in both hM4D-mCherry expressing and non-expressing neurons. Bath application of CNO (10 uM) induced a hyperpolarization of membrane potential from -65 mV to -72 mV only in hM4D-expressing neurons (Figure 3-6). Bath application of CNO also abolished current injection-evoked action potential firing in hM4D-mCherry expressing neurons (Figure 3-6). A similar abolition was not observed in neurons not expressing hM4D (Figure 3-6).

**Figure 2-6.** hM3D-mediated activation of CA1 pyramidal neurons recorded *in vitro*. (A) Bath application of CNO (10 uM) depolarized membrane potential and robustly increased the firing rate of hM3D-expressing neurons. (B) No effect on membrane potential or firing rate was observed in cell not expressing hM3D-mCherry.
Figure 3-6. hM4D-mediated inhibition of CA1 pyramidal neurons recorded in vitro

(A) Injection of a positive current (100 pA) for 2 s evoked robust action potential spikes in both hM4D-expressing and non-expressing neurons. (B) Bath application of CNO (10 uM) induced a hyperpolarization (from -65mV to -72mV) of the membrane potential only in hM4D-expressing neurons. Bath application of CNO abolished current injection-evoked action potential firing only in hM4D-expressing neurons.

cFos Staining. After 1mg/kg CNO injection, a higher number of cFos-positive cells were observed on the side of the vHPC expressing hM3D-mCherry compared to the side not expressing hM3D (Figure 4A-B). This enhanced cFos signal was observed in the region of the vHPC that overlapped with hM3D-mCherry expression (Figure 4C).
Figure 4-6. Images of cFos expression in the vHPC of mice unilaterally expressing hM3D. (A) Fewer cFos-positive cells were seen in the contralateral vHPC which did not express hM3D (B) compared to the ipsilateral vHPC. (C) The regions with increased cFos signal overlapped with the hM3D-mCherry signal (red).

**Open Field Locomotion.** There was no significant main effect of treatment (F < 1), but a significant main effect of time (F(17, 119)= 5.19, p< 0.000001) and time x treatment interaction (F(17, 119)= 3.95, p< 0.000001) on locomotor activity was found (Figure 5-6). One-way repeated measures ANOVA revealed a significant effect of time on locomotor activity following CNO injection (F(17, 119)= 7.24, p< 0.0000001) but not following saline injection (F(17, 119)= 1.133, p= 0.332) (Figure 5-6). Trend analysis indicated a significant linear trend following CNO injection (F(1, 7)= 106.44, p< 0.0001) suggesting that locomotor activity was highest early after CNO injection and declined over the 3 hour test session (Figure 5-6). Locomotor activity at 30 minutes (t(7)= 3.116, p< 0.05) and 70 minutes (t(7)= 2.50, p< 0.05) was higher for the CNO injection than for the saline injection (Figure 5-6).
Figure 5-6. Distance travelled across time in 10-minute intervals after saline injection and CNO injection (1mg/kg) of BAct:cre mice expressing hM3D in the vHPC (N= 8). There was a significant effect of time on locomotor activity after CNO injection (F(17, 119)= 7.24, p< 0.0000001), which was greater than locomotor activity following saline at 30 minutes (t(7)= 3.116, p< 0.05) and 70 minutes (t(7)= 2.50, p< 0.05).

6.2. EXPERIMENT 7: DREADD INHIBITION OF vHPC PARVALBUMIN NEURONS

We wanted to examine the effects of inhibition of parvalbumin neuron activity in the vHPC on spontaneous and amphetamine-induced locomotion. We expected that removing inhibitory transmission to vHPC pyramidal neurons would enhance vHPC activity and thus would increase both types of locomotion. In addition, we investigated the effects of polyIC treatment to determine if the parvalbumin loss and increased sensitivity to amphetamine reported by Meyer et al. (2008) can be reliably reproduced.
Methods

Animals. Experiments were conducted with parvalbumin:cre mice. The parvalbumin:cre driver line is a knockin line containing the Cre recombinase gene insert downstream of the parvalbumin translational STOP codon. The endogenous promoter and enhancer elements of the parvalbumin locus drive both parvalbumin and Cre recombinase expression. Two separate parvalbumin:cre lines were tested, differing in the mechanism by which Cre recombinase is expressed. The PV:2A-cre line contains the 2A peptide which mediates ribosomal skipping and co-translational cleavage of the initially fused parvalbumin and cre recombinase, leading to equimolar levels of these proteins. The PV:IRES-cre line contains an internal ribosomal entry site (IRES) upstream of Cre, enabling parvalbumin and Cre to be translated independently, rather than as a fusion product. This IRES mechanism leads to greater translation of the upstream endogenous sequences, (i.e. Parvalbumin gene) in comparison to the downstream IRES-mediated sequences, in this case Cre (Trichas et al., 2008).

Surgery. AAV-FLEX-hM4D-mCherry (0.2uL) was infused into the vHPC of PV:2A-cre (N= 8) and PV:IRES-cre mice (N= 7).

Immunohistochemistry. To verify that the hM4D receptor was indeed targeted to parvalbumin interneurons, and that our manipulations resulted in changes in neural activity, we performed fluorescent antibody staining for parvalbumin and cFos, respectively.

Open Field Locomotion Procedure. Mice were allowed 7 days to recover before behavioral testing. PV:2A-cre mice were tested in the larger open field, and PV:IRES-cre mice were tested in the smaller open field. Mice were habituated to the open-field for 3
hours one day prior to testing and for 30 minutes immediately before testing. Saline (10 mL/kg i.p.) was administered on day 1, CNO (2 mg/kg i.p.) on day 2, and CNO (2 mg/kg i.p.) or saline with a low dose of amphetamine (1 mg/kg i.p.) on the final test day. Mice were placed into the open field chamber immediately after the injections for 3 hours.

Results

Histology. hM4D-mCherry fluorescence was observed unilaterally in all subfields of the vHPC in PV:2A-cre mice (Figure 1-7A) and PV:IRES-cre mice (Figure 1-7B) infused with AAV-FLEX-hM3D-mCherry in the vHPC.

![Figure 1-7. Representative unilateral hM4D-mCherry expression in the vHPC of (A) PV:2A-cre and (B) PV:IRES-cre mice.](image)

Immunohistochemistry. After AAV-FLEX-hM4D-mCherry infusion into the vHPC, neurons expressing hM4D-mCherry were observed in both the PV:2A-cre and the PV:IRES-cre lines (Figure 2-7A-C). Staining of PV:2A-cre brain tissue for the parvalbumin protein revealed a high number of hM4D-mCherry cells that did not express parvalbumin (Figure 2-7A-C), as well as hM4D-mCherry expression in the alveus. This indicates that hM4D was not exclusively expressed in parvalbumin neurons and that it was
also likely expressed in pyramidal neurons. In contrast, a high specificity in hM4D targeting to parvalbumin neurons was observed in the PV:IRES-cre since most hM4D-mCherry expressing cells were double-labeled for parvalbumin (Figure 2-7D-F). Silencing neurons expressing hM4D-mCherry with 2mg/kg CNO resulted in increased vHPC cFos expression in the PV:IRES-cre line (Figure 3-7D-F) but not in the PV:2A-cre line (Figure 3-7A-C), indicating that in PV:IRES-cre mice, inhibition of hM4D-mCherry expressing neurons caused the disinhibition of surrounding neurons (Figure 3-7D-F).

Figure. 2-7. Parvalbumin immunoreactivity (green) in the vHPC of parvalbumin:cre mice expressing hM4D-mCherry (red). (A-C) Sections of the ventral CA1 of parvalbumin:2A-cre mice showing (A) hM4D-mCherry expression (B) fluorescently stained parvalbumin protein (C) and an overlay of these signals. (D-F) Sections of the ventral subiculum of parvalbumin:IRES-cre mice showing (A) hM4D-mCherry expression (B) fluorescently stained parvalbumin protein (C) and an overlay of these signals.
Figure 3-7. cFos expression (green) in the ventral subiculum of PV:2A-cre and PV:IRES-cre mice expressing hM4D-mCherry (red) after 5mg/kg CNO injection. PV:2A-cre cFos expression is shown for the (A) control side, (B) and hM4d expressing side of the vHPC (C) overlayed with hM4D expression. Similarly, PV:IRES-cre cFos expression is shown for the (A) control side, (B) and hM4d expressing side of the vHPC (C) overlayed with hM4D expression.

Open Field Locomotion. Total spontaneous locomotion in 3 hours was not significantly different between saline injection and CNO injection for either the PV:2A-cre mice (t(7)= 1.43, p = 0.196) (Figure 4-7A) or the PV:IRES-cre mice (t(6)= 1.35, p= 0.225) (Figure 4-7B). The total distance travelled of PV:2A-cre mice was significantly greater than of PV:IRES-cre mice after both saline (t(13)= 4.71, p< 0.001) and CNO injection (t(13)=...
4.91, p< 0.001) (Figure 4-7B), possibly because they were tested in larger open field arenas. For PV:2A-cre mice, two-way repeated measures ANOVA revealed no significant effect of time (F(17, 119)= 1.29, p= 0.212), treatment (F(1, 7)= 2.01, p= 0.196), and no time x treatment interaction (F(17, 119)= 1, p= 0.463) was found (Figure 5-7). For PV:IRES-cre mice, a significant main effect of time (F(17, 102)= 4.52, p< 0.000001) on distance travelled was observed but no main effect of treatment (F(1, 6)= 1.83, p= 0.225) or time x treatment interaction was found (F(17, 102)= 1.21, p= 0.271) (Figure 6-7). Next, we examined the effect of pre-injection with either saline or 2mg/kg CNO on amphetamine-induced locomotion. For PV:2A-cre mice, locomotor activity significantly changed over time following CNO pre-injection (F(2, 6)= 5.84, p< 0.05) but not following the amphetamine injection (F < 1) (Figure 7-7). Locomotor activity was not significantly higher 30 minutes after CNO injection compared to after amphetamine injection (F < 1) (Figure 8-7). For PV:IRES-cre mice, there was no significant difference between saline- and CNO-pre-injection across time (F < 1) and neither treatment significantly changed locomotor activity across time (time effect: F(2, 10)= 1.00, p= 0.401; time x treatment interaction: F(2, 10)= 2.06, p= 0.178) (Figure 9). Similarly, no significant differences in locomotor activity across time were observed between amphetamine with saline pre-injection and amphetamine with CNO pre-injection (main treatment effect: F < 1) and neither treatment significantly changed distance travelled across time (time effect: F < 1; time x treatment effect: F < 1) (Figure 9-7). Total distance travelled in 30 minutes was not significantly different between the pre-injection and amphetamine injection ((F(1, 5)= 4.27, p= 0.094) and mice receiving amphetamine after CNO pre-injection did not exhibit
significantly higher levels of locomotor activity compared to mice pre-injected with saline (F < 1).

Figure 4-7. Total distance travelled in 3 hours after 2mg/kg CNO injection and saline injection. No significant differences in distance travelled were observed between injections for either (A) PV:2A-cre (N= 8) or (B) PV:IRES-cre mice (N= 7).
Figure 5-7. Distance travelled across time in 10-minute intervals after saline injection and CNO injection (2mg/kg) into PV:2A-cre mice (N= 8). No significant differences in locomotor activity following saline and CNO injection across time were found (F(17, 119)= 1, p= 0.463).

Figure 6-7. Distance travelled across time in 10-minute intervals after saline injection and CNO injection (2mg/kg) into PV:IRES-cre (N= 7). No significant differences in locomotor activity following saline and CNO injection across time were found (F(17, 102)= 1.21, p= 0.271)
Figure 7-7. Distance travelled across time in 10-minute intervals after injection of 2 mg/kg CNO with 1 mg/kg amphetamine (N= 4) in PV:2A-cre. There was no significant effect of time following CNO pre-injection (F < 1) and amphetamine injection (F < 1).

Figure 8-7. Total distance travelled in 30 minutes following amphetamine (1mg/kg) in PV:2A-cre mice pre-injected with 2mg/kg CNO (N= 4). Locomotor activity was not significantly higher after CNO injection (F < 1).
Figure 9-7. Distance travelled across time in 10-minute intervals after injection of 2 mg/kg CNO with 1 mg/kg amphetamine (N= 4) or saline with amphetamine (N= 3) into PV:IRES-cre. No significant differences were found between treatment groups (F < 1) and amphetamine injection did not significantly change distance travelled across time for either group (F < 1).

Figure 10-7. Total distance travelled in 30 minutes following amphetamine (1mg/kg) in PV:IRES-cre mice pre-injected with saline (N= 3) or 2mg/kg CNO (N= 4). No significant differences were found between the pre-injection and amphetamine injection ((F(1, 5)= 4.27, p= 0.094) or between amphetamine treatments (F < 1).
6.3. AIM III: EXPERIMENT 8- POLYIC MODEL OF SCHIZOPHRENIA

Methods

PolyIC Administration. As previously described by Shi et al. (2003), 20 mg/kg polyIC was administered intraperitoneally to pregnant wildtype dams on embryonic day 17. Offspring of the polyIC treated dams were tested at 3 months of age for spontaneous and amphetamine-induced locomotion. Offspring of saline-treated dams served as controls.

Results

20 mg/kg polyIC administration to a pregnant dam on GD17 resulted in 4 offspring (polyIC mice) with variable parvalbumin protein expression in the vHPC (Figure 1-8A-C). While 3 polyIC-mice had similar levels of parvalbumin protein expression to saline-mice, one polyIC mouse showed a marked decrease in parvalbumin-immunoreactivity in the vHPC (Figure 1-8C). This reduction in parvalbumin was observed in all subfields of the vHPC (Figure 1-8C). The open field test revealed no significant differences in spontaneous locomotor activity between saline-mice and polyIC mice across time (time x group interaction: F < 1) (Figure 2-8). PolyIC-mice with parvalbumin expression in the vHPC (PolyIC-PV) did not significantly differ from saline-mice in locomotor activity across time after amphetamine injection (time x group interaction: F < 1) (Figure 3-8). Interestingly, the polyIC mouse without vHPC parvalbumin expression (PolyIC-no-PV) was the only mouse to exhibit an increased locomotor response to amphetamine (Figure 3-8).
Figure 1-8. Images of stained parvalbumin protein in the vHPC of (A) saline-treated mice and (B-C) polyIC treated mice. (C) No parvalbumin protein expression was detected in 1 polyIC-treated mouse.

Figure 2-8. Spontaneous locomotor activity of saline-treated (saline N= 4) and PolyIC-treated mice showing parvalbumin expression (PolyIC-PV, N=3) and no parvalbumin expression (PolyIC-no-PV, N= 1) in the vHPC across 2 hours. No significant differences in spontaneous locomotor activity were found between saline-mice and polyIC mice across time (F < 1).
Figure 3-8. Amphetamine-induced locomotor activity of saline-treated (saline N= 4) and of PolyIC-treated mice showing parvalbumin expression (PolyIC-PV, N=3) and no parvalbumin expression (PolyIC-no-PV, N= 1) in the vHPC across 1 hour. No significant differences in locomotor activity following 1mg/kg amphetamine was found between saline-mice and polyIC-PV mice across time (F < 1).

6.4. AIM III: EXPERIMENT 9- DREADD ACTIVATION OF vHPC PARVALBUMIN NEURONS

We expressed hM3D in vHPC parvalbumin neurons to determine whether enhancing parvalbumin neuron activity can suppress the hyperlocomotor response to amphetamine observed in the amphetamine sensitization model of schizophrenia.

Methods

Surgery. AAV-FLEX-hM3D-mCherry (0.2uL) was bilaterally infused into the vHPC of PV:2A-cre mice.

Amphetamine Sensitization Procedure. Sensitization to amphetamine began 1 week after surgery. Following a 3-hour habituation period to the open field and an initial injection of
saline, mice received 5 consecutive daily injections of amphetamine (1 mg/kg, i.p.) that were immediately followed by a 90-minute open field test. 30 minutes of habituation preceded each test session. After a 3-day injection-free period, mice received an injection of CNO (2 mg/kg) along with a challenge injection of amphetamine (1 mg/kg, i.p.), and again were placed into the open field for 90 minutes.

Additionally, a separate group of wild-type mice were sensitized to amphetamine following an alternate procedure. These mice received 8 consecutive daily amphetamine injections (1 mg/kg, i.p.) followed by challenge injections of amphetamine (1 mg/kg, i.p.) at 1 week and 3 weeks after the last consecutive injection. Data from the first 30 minutes after amphetamine injection were analyzed.

Results

Histology. Parvalbumin:2A-cre mice injected with AAV-FLEX-hM3d-mCherry expressed hM3D-mCherry bilaterally in all subfields of the vHPC (Figure 1-9).

![Figure 1-9. Bilateral vHPC hM3D-mCherry expression of parvalbumin:2A-cre mice.](image1)

Amphetamine Sensitization. The 5 consecutive daily injections of 1mg/kg amphetamine did not induce amphetamine sensitization in PV:2A-cre mice expressing hM3D, as indicated by no significant differences in distance travelled between treatments (F(4, 12)=
1.26, p= 0.340) (Figure 2-9). Furthermore, 2mg/kg CNO administered with the 3-day challenge injection of 1mg/kg amphetamine did not significantly attenuate locomotor activity compared to the last amphetamine injection (t(3)= 2.01, p= 0.137) (Figure 2-9).

The amphetamine sensitization procedure performed in WT mice resulted in a significant linear increase in locomotor activity across treatments (F(1, 3)= 38.87, p< 0.01) (Figure 3-9). The first amphetamine injection led to significantly more locomotor activity than the saline injection (t(3)= 5.68, p< 0.05), but mice did not show significantly greater distance travelled after the 8th consecutive amphetamine injection (t(3)= 2.08, p= 0.191) nor after the 1-week challenge injection (t(3)= 2.28, p= 0.108) compared to the first amphetamine injection (Figure 3-9). However, the 3-week amphetamine challenge resulted in greater locomotor activity compared to the first amphetamine injection (t(3)= 6.305, p< 0.01), indicating behavioral sensitization to amphetamine (Figure 3-9).

Figure 2-9. Total distance travelled 30 minutes after the first and fifth consecutive injection of amphetamine and after the 3-day challenge injection of amphetamine
with 2 mg/kg CNO. No significant differences were observed between treatments (F(4, 12)= 1.26, p= 0.340).

**Figure 3-9. Total distance travelled 30 minutes after saline, after the first and eighth consecutive amphetamine, and after the 1-week and 3-week amphetamine challenge injection.** There was a significant linear trend across treatments (F(1, 3)= 38.87, p< 0.01) and a significant difference between the first amphetamine injection and the 3-week amphetamine challenge (t(3)= 6.305, p< 0.01)

7. DISCUSSION

We conducted experiments to understand the role of vHPC projections to the NAc in goal-directed locomotion, specifically exploratory locomotion, and whether the dysregulated activity in this connection due to parvalbumin neuron silencing can result in behaviors associated with schizophrenia.

Our vHPC retrograde tracing experiment confirmed previous findings that the ventral CA1 receives afferents from structures internal and external to the hippocampus, such as the CA3 and medial septum respectively (O'Keefe and Nadel, 1978). The labeled
cells in the medial septum are most likely cholinergic in nature (Senut et al., 1989). If so, the medial septum may be the source of ACh that is released in the vHPC during novel environment exploration (Thiel et al., 1998; Bianchi et al., 2003; Takeda et al., 2006). Our pharmacological experiment involving intra-vHPC carbachol infusion supports the idea that increased cholinergic transmission in the vHPC is involved in triggering goal-directed locomotion.

Anterograde tracing from the vHPC and retrograde tracing from the NAc have provided converging evidence that the vHPC innervates the medial NAc. Activating this projection along with vHPC projections to other downstream targets by optogenetic stimulation of cell bodies in the vHPC, resulted in a robust increase in goal-directed locomotion. Locomotor activity was significantly increased compared to baseline and compared to control mice. Restricting the activation exclusively to vHPC terminals in the NAc resulted in a smaller induction of goal-directed locomotion. We have thus provided evidence that activity in the vHPC to NAc pathway alone is sufficient to trigger goal-directed locomotion. However, a possible limitation of our optogenetic activation experiments is that the stimulation frequency used may not be physiologically relevant for pyramidal cells of the hippocampus. While we provided light pulses at a constant frequency of 20Hz, pyramidal neurons are known to fire action potentials at theta frequency (4-10 Hz) during the performance of spatial and reward-related tasks (Martin and Ono, 2000; Tabuchi et al., 2000). Thus, future experiments involving optogenetic activation should strive to mimic the endogenous firing patterns of neurons in the vHPC.

Our next experiment investigated the effect of selectively inhibiting the activity of vHPC terminals in the NAc during novel environment exploration. Without manipulating
neural activity, mice significantly preferred novel environments over familiar environments, spending approximately 70% and 30% of their time in each respective context during the free-choice novelty test. Locomotor activity also reflected this preference for novelty since mice travelled greater distances in the novel environment compared to the familiar environment. Both measures of novelty exploration were reduced upon optogenetic inhibition of vHPC terminals in the NAc, suggesting that this connection is critical for driving novel environment exploration. However, this conclusion is based on data from only one mouse and one behavioral test. Further experiments must be conducted to replicate these findings with a larger sample size and with additional behavioral tests for novelty exploration. Although we provide evidence that the vHPC projections to the NAc are necessary for novel environment exploration, this connection may not be involved solely in this process. Findings from vHPC-NAc asymmetrical disconnection experiments suggest that serial transmission from the vHPC to the NAc may be crucial for intact performance on goal-directed spatial tasks (Floresco et al., 1997; Goto and Grace, 2005; Ito et al., 2008). Floresco et al. (1997) found that the disconnection of these structures impaired the accuracy of random foraging for food pellets in the radial arm maze. Rats with unilateral lidocaine infusions into the vHPC and the contralateral NAc made a higher number of entries into previously visited arms compared to saline infused rats. A similar deficit was reported in an associative learning task following vHPC-NAc disconnection (Goto and Grace, 2005). Unilateral infusions of lidocaine into the vHPC and a D1 antagonist into the NAc significantly slowed learning in the visual cue task (VCT) and in the response direction task (RDT). These rats required more trials to learn to make turns in a plus maze based on a visual cue (VCT) or a set
direction to obtain food reward. In both experiments however, a general reduction in locomotor activity or motivation to obtain food reward was not observed. Taken together, these studies suggest that in non-novel environments, the vHPC may be involved in providing spatial information to the NAc to guide goal-directed behavior. In novel environments however, the vHPC may specifically drive motivation to mediate the acquisition of a spatial representation of the surrounding context.

Our next set of experiments addressed whether the selective disruption of parvalbumin neuron activity could lead to behaviors associated with schizophrenia, and if a recovery of parvalbumin neuron activity could rescue these same behaviors in animal models of schizophrenia. To accomplish bidirectional control of neural activity, we used the DREADD system. *In vitro* recordings from neurons expressing either the hM4D or hM3D receptor established that CNO binding to these receptors effectively inhibits and activates action potential firing respectively. CNO administration increased cFos expression and locomotor activity in mice broadly expressing the hM3D receptor in all neuron subtypes of the vHPC. However, the locomotor effect was not as robustly observed across animals or as large in magnitude compared to ChR2-mediated activation of the vHPC.

AAV-FLEX-hM4D-mCherry infusion into parvalbumin:cre mice was performed to mediate the silencing of parvalbumin neuron activity. We expected a high concordance between hM4d-mCherry expression and parvalbumin-immunoreactivity, indicating specific targeting of the hM4D receptor to parvalbumin interneurons. However, this was only the case for the PV:IRES-cre line and not the PV:2A-cre line. Since the 2A-peptide sequence allows for equimolar expression of the endogenous and foreign gene, small
amounts of parvalbumin expression in non-GABAergic neurons would cause hM4D-mCherry expression in these neurons as well. Therefore, low specificity in the PV:2A-cre line may be explained by the higher sensitivity of the 2A-peptide sequence compared to the IRES sequence to endogenous gene expression (Madisen et al., 2010).

Open field locomotion tests revealed no effect of parvalbumin neuron inhibition on spontaneous or amphetamine-induced locomotion in either the PV:2A-cre or the PV:IRES-cre line. We predicted a behavioral effect in the PV:IRES-cre line since we observed increased cFos labeling in the region surrounding parvalbumin neurons expressing hM4D-mCherry. This result suggests that CNO binding to hM4D did indeed silence parvalbumin neurons and disinhibit postsynaptic neurons. However, since parvalbumin neurons only constitute approximately 20% of GABAergic neurons in the CA1 (Baude et al., 2006), it is possible that the proportion of vHPC pyramidal neurons disinhibited by hM4D-mediated parvalbumin neuron silencing may not have been sufficient to affect behavior. To clarify the validity of these findings, we must first rule out the possibility that the CNO dose used in this experiment was insufficient in completely silencing parvalbumin neuron firing. In vitro recordings from hM4D expressing parvalbumin neurons upon CNO application must be performed, and the behavioral experiments should be reproduced with higher doses of CNO. The polyIC experiment suggests that the near total loss of parvalbumin neurons enhances the response to amphetamine, since the one polyIC mouse exhibiting loss of parvalbumin-immunoreactivity in the vHPC also had increased amphetamine-induced locomotion.

Our final experiment examined whether increasing parvalbumin neuron activity can reduce vHPC output to the mesoaccumbens dopamine system, and therefore attenuate
the sensitivity of the dopamine system to amphetamine. This result would be consistent with previous reports that vHPC lesions can abolish amphetamine sensitization (Lodge and Grace, 2007). We found that hM3D-activation of parvalbumin neurons did not significantly attenuate amphetamine-induced locomotion in the amphetamine sensitization model of schizophrenia. However, there are several problems with this experiment. First, the amphetamine sensitization protocol used for PV:2A-cre mice expressing hM3D was not effective since mice did not exhibit an enhanced response to amphetamine after the consecutive injections. Because the response to amphetamine did not change from baseline, it is impossible to determine whether CNO had any effect on reducing amphetamine sensitivity. Contrarily, the alternate protocol used for wildtype mice led to a significant increase in amphetamine-induced locomotion 3 weeks after the last consecutive injection. Another limitation of this experiment is that only the PV:2A-cre line was used. As previously discussed, this line would not have allowed for the specific expression of hM3D in parvalbumin interneurons. Thus, to properly evaluate the effect of hM3D activation of parvalbumin neurons on amphetamine sensitization, we must reproduce this experiment in PV:IRES-cre mice using a more effective amphetamine sensitization protocol.
8. CONCLUSIONS

Converging lines of evidence have been presented for the role of the vHPC-NAc network in goal-directed locomotion. This connection is comprised of unidirectional projections from the vHPC to NAc. We have provided evidence for the involvement of serial transmission from the vHPC to the NAc in goal-directed locomotion, specifically exploratory locomotion in novel environments.

The exploratory locomotion triggered by the vHPC may aid other structures such as the dHPC in forming a cognitive map of the environment (O’Keefe and Nadel, 1978). Thus, the goal of vHPC-NAc mediated exploratory locomotion may be to facilitate the animal’s acquisition of features of its external environment. If however, activity in the vHPC-NAc network is dysregulated, the animal may be inappropriately driven to attribute salience to irrelevant stimuli, as is observed in schizophrenia. Altered anterior hippocampal processing in schizophrenia may result from the loss of parvalbumin inhibitory interneurons, and has been suggested to underlie increased sensitivity to amphetamine. Prior to our experiment, the behavioral correlates of selective vHPC parvalbumin neuron manipulation had not been investigated. Our preliminary results suggest that the silencing of vHPC parvalbumin neuron activity does not enhance amphetamine sensitivity. However, future research is necessary to confirm this finding.

Collectively, these experiments support the function of the vHPC to NAc pathway in exploration but it remains to be seen whether the inhibition of vHPC parvalbumin neurons can lead to behaviors associated with schizophrenia.
9. REFERENCES


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