Prefrontal cortical functional reorganization following early developmental ventral hippocampal perturbations

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Contents

Abstract

Early brain development is a critical window during which genetic and environmental factors can influence proper or improper establishment of brain pathways. Schizophrenia is a mental illness that arises from genetic and environmental perturbations to brain development, producing malfunctional pathways that mediate maladaptive behaviors. One such pathway implicated in schizophrenia pathology is the ventral hippocampal (vHPC) – prefrontal cortical (PFC) pathway which plays an important role in cognitive functioning. We sought to better elucidate the mechanisms by which developmental aberrations in this pathway emerge and cause cognitive deficits, building upon previous models. We established a framework through which we can dissect neurodevelopmental alterations using a combination of transgenic mice and viral constructs for cell-type specific and temporally specific manipulations of neuronal activity and electrophysiology to characterize functional changes. We applied these tools to identify that excitatory CaMKII expressing cells, but not inhibitory parvalbumin (PV) expressing cells, in the vHPC are critical to the functional maturation of the PFC. We found that the early developmental ablation of vHPC-CaMKII cells resulted in an increase in excitatory and decrease in inhibitory inputs onto PFC pyramidal cells. We also showed that early vHPC ablation results in deficits in PV cell density, decreased excitatory input onto PV cells, and a deficit in NMDA-receptor current in PV cells in the PFC. We therefore demonstrated how the excitatory: inhibitory network balance is impacted in the mature PFC following early vHPC perturbations by identifying functional cellular changes in both pyramidal and PV cells of the PFC. We further investigated the effects of these excitatory: inhibitory cellular changes by examining the *in vivo* local field potential of PFC neuronal network activity following early vHPC-CaMKII ablation. We found an excess in high gamma power, but reductions in theta, beta, and low gamma powers which may mediate the spontaneous working

memory deficits characterized in our mice. Early vHPC-CaMKII perturbations therefore produces synaptic inputs changes in the PFC leading to uncoordinated activity mediating oscillatory power changes that contribute to schizophrenia-relevant cognitive deficits. These findings may help in furthering our understanding of the neurodevelopmental underpinnings of schizophrenia.

Résumé

Le développement précoce du cerveau est une période critique au cours de laquelle des facteurs génétiques et environnementaux peuvent influencer l'établissement correct ou incorrect des voies cérébrales. La schizophrénie est une maladie mentale qui résulte de perturbations génétiques et environnementales du développement du cerveau, produisant des voies dysfonctionnelles qui médiatisent des comportements inadaptés. L'une de ces voies impliquées dans la pathologie de la schizophrénie est la voie hippocampique ventrale (vHPC) - corticale préfrontale (PFC), qui joue un rôle important dans le fonctionnement cognitif. Cependant, la manière dont les aberrations développementales de cette voie émergent et provoquent des déficits cognitifs reste à étudier. Nous avons donc établi un cadre permettant de disséquer les altérations neurodéveloppementales en utilisant une combinaison de souris transgéniques et de constructions virales pour des manipulations de l'activité neuronale et de l'électrophysiologie spécifiques à un type de cellule et à un moment donné, afin de caractériser les changements fonctionnels. Nous avons appliqué ces outils pour identifier que les cellules excitatrices exprimant le CaMKII, mais pas les cellules inhibitrices exprimant la parvalbumine (PV), dans le vHPC sont essentielles à la maturation fonctionnelle du PFC. Nous avons constaté que l'ablation des cellules vHPC-CaMKII au début du développement entraînait une augmentation des entrées excitatrices et une diminution des entrées inhibitrices dans les cellules pyramidales du PFC. Nous avons également montré que l'ablation précoce des cellules vHPC entraîne des déficits dans la densité des cellules PV, une diminution des entrées excitatrices dans les cellules PV et un déficit dans le courant des récepteurs NMDA dans les cellules PV de la PFC. Nous avons donc démontré comment l'équilibre entre le réseau excitateur et le réseau inhibiteur est affecté dans le PFC mature à la suite de perturbations précoces du vHPC en identifiant des changements cellulaires fonctionnels dans les cellules pyramidales et

les cellules PV du PFC. Nous avons également étudié les effets de ces changements cellulaires excitateurs/inhibiteurs en examinant le potentiel de champ local in vivo de l'activité du réseau neuronal du PFC à la suite d'une ablation précoce du vHPC-CaMKII. Nous avons constaté un excès de puissance gamma élevée, mais des réductions des puissances thêta, bêta et gamma basse, qui peuvent être à l'origine des déficits de mémoire de travail spontanée caractérisés chez nos souris. Les perturbations précoces de la vHPC-CaMKII produisent donc des changements au niveau des entrées synaptiques dans le PFC, ce qui conduit à une activité non coordonnée médiant les changements de puissance oscillatoire qui contribuent aux déficits cognitifs liés à la schizophrénie. Ces résultats pourraient nous aider à mieux comprendre les fondements neurodéveloppementaux de la schizophrénie.

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Contribution to original knowledge

The neonatal ventral hippocampal lesion rat model has been thoroughly established as an important heuristic model of schizophrenia. However, the mechanisms by which schizophrenia-like behaviors develop are ongoing investigations. By transitioning this model from rat to mice, we are able to expand our toolkits, including the use of transgenic lines and promoter dependent viral constructs, to dissect the neurodevelopmental hypothesis of schizophrenia. Using this framework presented in Chapter 2, we first identified that it is the ablation of CaMKII and not PV cells that are primarily responsible for the onset of PFC pathology. We then used whole-cell recordings to not only investigate pyramidal cell firing and how that compares to established literature within the rat model, but also visually identified PV expressing cells, which has not been conducted in the rat model. We showed that pyramidal cells show deficits in firing, and received increased excitatory but decreased mean inhibitory synaptic into inputs. In contrast, PV cells are reduced in density and show a reduced ability to sustain high-frequency firing, while receiving decreased excitatory inputs. We also contributed to the NMDA-receptor hypofunction hypothesis by demonstrating that NMDA-receptor current is reduced only in PFC PV cells, but in pyramidal cells, despite a decrease in NMDA-receptor subunit mRNA expression across the PFC. These results are published in Chapter 3. Finally, we performed local field potential analysis of frequency bands in the PFC in the CaMKII early viral ablation model and identified excess gamma frequency, and reduced occurrences of theta, beta, and low gamma frequencies depending on task-engagement, with a reduction in beta frequency specifically associated with incorrect decisions in a spontaneous Y-maze working memory assay. These results are presented in Chapter 4. This oscillation study is in contrast to the limited oscillation studies performed in the rat model, with one published electroencephalogram study examining cortical oscillations. Therefore, our studies contribute

needed insights into the cellular and oscillatory changes in the PFC that arises from early vHPC disruption, establishing a malleable model by which both the origins and consequences of altered neural development can be investigated in the context of schizophrenia.

Contribution of authors

In “Chapter 2: Neurodevelopmental insights into circuit dysconnectivity in schizophrenia”, I conducted the literature review and wrote the article, with the guidance and editing support of my supervisors Dr. Tak Pan Wong and Dr. Srivastava.

In “Chapter 3: Altered excitatory and decreased inhibitory transmission in the prefrontal cortex of male mice with early developmental disruption to the ventral hippocampus”, I conducted and analyzed the electrophysiology, cFos histology, and PFC PV histology experiments, while my colleague Dr. Sanjeev Bhardwaj performed the neonatal pup stereotaxic surgeries and performed the histological verification for ibotenic acid lesioning and viral ablation. I wrote the article, with the critical guidance and editing support of my supervisors Dr. Tak Pan Wong and Dr. Srivastava.

In “Chapter 4: Excess prefrontal cortical high gamma, and reduced theta, beta, and low gamma oscillations in mice with early developmental disruption to the ventral hippocampus”, I conducted the adult stereotaxic surgeries, acquired the electrophysiology data, performed the behavioral experiments and analyzed the data. Data analysis was conducted with the support of Dr. Eric Carmichael, who provided me with template codes. Dr. Sanjeev Bhardwaj performed the neonatal pup stereotaxic surgeries. I wrote the article, with the critical guidance and editing support of my supervisors Dr. Tak Pan Wong and Dr. Srivastava.

Figures and tables

Chapter 3: Altered excitatory and decreased inhibitory transmission in the prefrontal cortex of male mice with early developmental disruption to the ventral hippocampus

Table 1 Cell membrane properties.

Table 2 Summary of changes in spontaneous and miniature excitatory and inhibitory inputs onto PFC pyramidal and PV cells in P21 and adult mice following early developmental vHPC lesioning, or adult mice following population-specific vHPC ablation (CaMKII-vHPC ablated, PV-vHPC ablated).

Figure 1 Early vHPC lesioning results in firing deficits, altered excitatory and decreased inhibitory miniature inputs in PFC pyramidal cells of adult P60 mice.

Figure 2 Early vHPC lesioning results in decreased spontaneous and miniature inhibitory inputs, and increased miniature excitatory inputs onto PFC pyramidal cells of adolescent P21 mice.

Figure 3 Early CaMKII-vHPC viral ablation decreases vCA1 activity and PV-vHPC ablation increases dCA1 and vCA1 activity, as measured by cFos.

Figure 4 Early CaMKII-vHPC viral ablation reproduces some functional phenotypes of lesion mice, including altered excitatory inputs and decreased inhibitory inputs onto PFC pyramidal cells.

Figure 5 Early vHPC lesioning decreases PV cell density in the PFC.

Figure 6 Early vHPC lesioning alters PFC PV-firing output and decreases spontaneous excitatory inputs onto PV cells.

Figure 7 Early vHPC lesioning results in decreased NMDA-receptor subunit mRNA levels in PFC but PV-specific functional deficits in NMDA receptor–mediated current.

Supplementary Figure 1 Viral ablation of PV cells in PV-cre mice does not yield any significant changes in PFC pyramidal cell firing or synaptic inputs.

Abbreviations

vHPC	Ventral hippocampus
PFC	Prefrontal cortex
CaMKII	Ca^{2+} /calmodulin-dependent protein kinase II
PV	Parvalbumin
SST	Somatostatin
DT	Diphteria toxin
NVHL	Neonatal ventral hippocampal lesion
P#	Postnatal day
dHPC	Dorsal hippocampus

Chapter 1: Introduction and Comprehensive Literature Review

Introduction & Aims

Brain development is a process in which foundational pathways are established. These pathways, refined over time, mediate behaviors. Errors in development can underlie maladaptive behaviors. Schizophrenia is one such mental illness that is believed to arise from neurodevelopmental abnormalities. The neurodevelopmental hypothesis of schizophrenia suggests that genetic and environmental risk factors impinge on the structural and functional development of brain pathways to produce diverse symptoms of schizophrenia. The ventral hippocampal (vHPC)-prefrontal cortical (PFC) pathway has been implicated in schizophrenia pathology, particularly in mediating cognitive deficits. The underlying assumption is that abnormalities arise in the vHPC, which then impacts the maturation process of the PFC, which takes longer to develop. The objective of this thesis thus is to determine the nature of developmental abnormalities originating in the vHPC, and the consequences of these abnormalities on the functional maturation of the PFC. Specifically, we aimed to:

(1) Differentiate the contribution of hyper- versus hypo-activity of the vHPC on the development of schizophrenia-like pathology.
(2) Examine the functional changes in different neuronal cell types in the mature PFC subsequent to early developmental vHPC perturbations.
(3) Determine the summated impact and *in vivo* relevance of cellular changes in the mature PFC circuit function subsequent to early developmental vHPC perturbations.

These aims provide insights into the neurodevelopmental hypothesis of schizophrenia.

Comprehensive literature review

Preamble

Brain development is highly dynamic from in utero to adolescence. During this time, neurons are born, migrate, differentiate, and mature. This maturation process involves the building of efficacious connection points with other neurons, through synapse formation and myelination (Kolb and Gibb, 2011). Infant brains are already characterized with the establishment of major white matter connection pathways, peak synapse density, and peak myelination rate (Geng et al., 2012; Semple et al., 2013). This foundational structure of brain connectivity established in infants is then further refined through adolescence by synaptic pruning, before stabilizing by early adulthood (Semple et al., 2013). In utero to adolescence is therefore a critical stage during which abnormal developmental processes may later yield behavioral abnormalities.

Maturation rates vary by pathways, and by brain regions as well. Short-range pathways and primary cortical areas generally mature faster than long-range pathways and associative cortical areas (Geng et al., 2012; Collin and van den Heuvel, 2013). These topographic variations in maturation mirrors the brain's processing abilities and functional outputs. Short-range pathways and primary cortical areas support local information processing, while long-range pathways and associative areas, including the frontal lobe, support integrative information processing (Collin and van den Heuvel, 2013). The functional outputs of abnormal developmental processes will therefore depend on timing and location of the abnormality. Moreover, the effects of abnormalities can be compounded, given that associative circuits are built upon primary circuits. These features highlight the complexity of brain development, and the need for and importance of deciphering how abnormalities may develop within the brain and how they may impact behavioral outputs.

Schizophrenia is one such disorder that likely arises through abnormalities in brain development, and its symptoms can be understood as impairments in integrative information processing. Schizophrenia represents a broad spectrum including positive symptoms such as hallucinations, negative symptoms such as alogia, and cognitive symptoms such as deficits in working memory (American Psychiatric Institution 2013). These symptoms thus implicate dysfunction in long-range pathways and associative areas including the frontal lobe. The following sections will provide supporting evidence that schizophrenia emerges through abnormal brain development, and that its symptoms are an expression of dysfunctional communication between implicated brain regions, focusing on the ventral hippocampus and prefrontal cortex.

Neurodevelopmental Hypothesis of Schizophrenia

There are several lines of evidence that suggest mental illnesses, including schizophrenia, may originate through abnormal developmental processes. This evidence includes onset of illness, occurrence of risk factors, neuroimaging studies, and animal models of schizophrenia.

Onset of Illness

In Canada, half of all lifetime cases of mental illnesses begin before the age of 14, while three-quarters before the age of 24 (Spenrath et al., 2011). Similarly, schizophrenia is primarily diagnosed in late adolescence or in early twenties, with cases of childhood-onset and late-onset (beyond the age of 40 typically in females) also observable (Hafner et al., 1994). In addition to later onset, females and males tend to show different behavioral expressions. For example, female patients tend to show more affective symptoms, and perform better in cognitive tasks than male patients (Li et al., 2022). Independent of sex-specific initial onsets and presentation patterns,

schizophrenia onset is progressive, with a common prodromal phase in which subthreshold positive symptoms, such as odd beliefs or perceptual disturbances, impaired role functioning, abnormal moods, and social withdrawal, develop well in advance of psychosis (George et al., 2017; Hafner et al., 1994). This prodromal phase is experienced by 80-90% of schizophrenia patients (Yung and McGorry, 1996), and has a mean duration of 4.8 years (median 2.33) (Hafner and Maurer, 2006).

The comparison of developmental milestones achieved in children who later develop schizophrenia versus healthy children further emphasizes that schizophrenia is a neurodevelopmental disorder. Follow-up studies show that children who develop adult schizophrenia can be slower to achieve motor (standing, walking) and speech milestones (Sorensen et al., 2010; Fish et al., 1965; Isohanni et al., 2001). These children are also slower to develop indexing processing speed, attention, visual-spatial problem-solving skills, and working memory; and show deficits in verbal and visual reasoning that persist into adulthood (Reichenberg et al., 2010).

Early childhood motor and speech milestone delays, in addition to cognitive delays and deficits that persist into adulthood, followed by a progressive prodromal to psychosis transition highlights the developmental and progressive onset of schizophrenia.

Risk Factors

Risk factors for schizophrenia occur during critical periods of brain development: prenatal and early childhood, and adolescence. These risk factors can be further subdivided into environmental

and genetic risk factors. Environmental risk factors include maternal malnutrition, maternal stress, prenatal or postnatal infections that occur during in utero and perinatal stages of brain development, child abuse that occurs during early stages of brain development, and cannabis use that occurs during pubertal stages of brain development (Dean and Murray, 2005).

Genetic and epigenetic risk factors are additionally implicated in neurodevelopment (Birnbaum and Weinberger, 2017). Schizophrenia-associated risk genes, including AHI1, MTHFR, RELN, TRKA, DRD2, DISC1, GRIN2A, RGS4, GRM3, and CACNA1C, play important functions in neurodevelopment (Badner and Gershon, 2002; Harrison & Weinberger, 2005; Schizophrenia Working Group of the Psychiatric Genomics, 2014; Jones et al., 2011). Genome-wide association studies reveal copy number variants associated with schizophrenia that also overlap with developmental disorders such as intellectual disability, attention-deficit hyperactivity disorder, and autism-spectrum disorder (Grayton et al., 2012). This overlap positions schizophrenia along a continuum of neurodevelopmental disorders (Owen et al., 2011). Post-mortem studies further highlight that both genetic and epigenetic markers of schizophrenia are developmentally expressed, such as in fetal tissues, rather than in late adolescence or adult tissue at the time of diagnosis (Birnbaum and Weinberger, 2017; Tao et al., 2014). The fact that some epigenetic markers are more relevant in fetal tissues rather than in adult (Jaffe et al., 2015; Jaffe et al., 2016) suggest that these epigenetic risk factors are mediating an upstream cause of schizophrenia, rather than the expression of psychosis or other schizophrenia-related symptoms. Genome-wide association studies further implicate genes involved in placental health that can predict complicated pregnancies, an important environmental risk factor for schizophrenia (Ursini et al., 2018).

Environmental, genetic, and epigenetic risk factors of schizophrenia thus impinge on neurodevelopmental processes. These abnormalities in neurodevelopment then become evident as altered structural and functional brain connectivity, as demonstrated by neuroimaging studies.

Neuroimaging Abnormalities

Neuroimaging studies demonstrate altered brain developmental processes in schizophrenia. Isolated mild ventriculomegaly, or the enlargement of the lateral cerebral ventricles, detected in utero of the fetus brain is associated with the later development of mild neurodevelopmental abnormalities (Gilmore et al., 1998). These neurodevelopmental abnormalities are similar to those observed in children at high-risk of schizophrenia (Gilmore et al., 1998). While the in-utero studies are suggestive, neuroimaging studies in neonates at high-risk of schizophrenia, born to mothers diagnosed with schizophrenia, show altered morphological and white matter networks. These alterations include reduced global efficiency, longer connection distance, reduced hub nodes and edges, and reduced subcortical-cortical fiber numbers (Shi et al., 2012). Genetic schizophrenia common variants in neonates were additionally negatively correlated with grey and white matter volumes of the superior temporal gyrus, white matter volume of the frontal lobe, and total white matter volume (Le et al., 2023). Mancini et al., (2019) further shows that hippocampal volume is reduced in genetically high-risk subjects of schizophrenia with a 22q11 deletion, and that the hippocampal volume further decreases with the onset of psychosis that begins and spreads from the CA1 layer. These studies highlight how risk factors of schizophrenia impact global brain network connectivity well before the expression of behavioral abnormalities and symptoms of schizophrenia.

Excess synapses are established in early development, which then undergo a long process of pruning throughout adolescence, before stabilizing into adulthood. This pruning process is enhanced in schizophrenia patients and is thought to underly the reduced cortical thickness observed in patients (Glausier and Lewis, 2013; Cannon 2015). In fact, the lower the cortical thickness, the more likely a high-risk subject is to develop psychosis (ENIGMA 2021; Chung et al., 2015; Cannon et al., 2015). Environmental factors, such as stressful events, in childhood and adolescence may therefore trigger excess synaptic pruning (Paolicelli et al., 2017), thereby facilitating the development of psychosis.

Both genetic and environmental factors impinge on neurodevelopmental processes to then mediate the structural and functional dysconnectivity patterns observed in schizophrenia (Nath et al., 2021). These patterns implicate a wide-range range of brain regions and pathways. What then is the causal contribution of developmental alterations in specific brain regions and pathways to schizophrenia pathology?

The Neonatal Ventral Hippocampal Animal Lesion Model of Schizophrenia

Neurodevelopmental animal models of schizophrenia are largely epidemiological based, replicating environmental risk factors in the development of schizophrenia. These models include maternal infection models, and social isolation (Powell 2013). While these models provide the ability to mechanistically investigate the way in which an environmental risk factor may contribute to schizophrenia pathophysiology, they do not allow the direct assessment of a brain region and pathway's activity and how that influences schizophrenia pathogenesis.

The neonatal ventral hippocampal lesion model (nVHL), on the other hand, involves the bilateral excitotoxic lesioning of the vHPC in postnatal day 7 rat pups, which is analogous to the third trimester in humans (Dobbing and Sands, 1979), and this produces a range of schizophrenia-like behavioral deficits (Lipska et al., 1993). Importantly, the onset of these deficits mirrors that in schizophrenia patients. Negative-like symptoms, including social deficits, and cognitive-like symptoms, including working memory deficits, both emerge at pre-pubertal stages, prior to the development of positive-like symptoms, which include deficits in prepulse inhibition and dopamine agonist-induced locomotor hyperactivity, which emerge post-adolescence (Tseng et al., 2008). The onset and progression of schizophrenia-like behaviors is produced only when the vHPC is lesioned neonatally, not when lesioned in adults (Lipska et al., 1993). The neonatal lesioning of other brain regions implicated in schizophrenia pathology, such as the mediodorsal thalamus (Lipska et al., 2003), has failed to reproduce a similar schizophrenia-like behavioral syndrome. These results suggest that the vHPC is a core developmental hub of schizophrenia pathology.

nVHL-Induced Behaviors: A Focus on the PFC

Our lab, amongst others, has previously shown that these behavioral symptoms are likely mediated through abnormal functionality in other brain regions, such as the PFC. For example, the adult lesioning of the PFC normalizes hyperlocomotion to novelty and amphetamine (Lipska et al., 1998), and normalizes the nucleus accumbens response to mesolimbic stimulation in nVHL rats (Goto and O'Donnell, 2004). In addition, neonatal PFC lesioning restores both hyperlocomotion activity and grooming behavior in nVHL rats, but fails to rescue deficits in social interactions (Flores et al., 2005). These results suggest that neonatal vHPC lesioning mediates at least some

schizophrenia-like behaviors through the abnormal development and function of other brains regions, including the PFC.

While other brain regions may be involved in schizophrenia-related pathology in NVHL animals, including the thalamus, nucleus accumbens, ventral tegmental area, and amygdala (Tseng et al., 2009), we chose to examine the PFC because of its significant role in cognitive processing for which treatments of schizophrenia are of limited effectiveness (Cannon 2015), its structural and functional importance in executive control through which it connects to and feedbacks onto multiple different brain regions (Tseng et al., 2009), and its consistent implication in schizophrenia pathology based on neuroimaging and histological meta-analyses (Dong et al., 2018, Ellison-Wright et al., 2008, Vitolo et al., 2017, Berdenis van Berlekom et al., 2020). Furthermore, there is a direct excitatory connection between the vHPC and the PFC, and vHPC inputs are important in modulating PFC development (Ahlbeck et al., 2018), which has a protracted maturation period (Arain et al., 2013). The PFC is therefore highly vulnerable to developmental insults into adolescence through this protracted maturation period. Finally, the malfunctional vHPC-PFC pathway is recognized as a significant schizophrenia endophenotype (Bahner and Meyer-Lindenberg, 2017).

nVHL-induced Molecular and Synaptic Effects in the PFC

nVHL induced behavioral changes that are dependent on PFC activity may be mediated through altered synaptic transmission functions in the PFC. Histological, mRNA, and protein expression analysis reveals diverse molecular changes associated with glutamatergic, GABAergic, dopaminergic, cholinergic, and serotonergic systems in nVHL rats. Glutamatergic changes include

increased cortical NMDA receptor subunit 1 mRNA expression (Rawas et al., 2009), and a shift towards flip GluR1 and GluR3 mRNA expression, associated with a slower desensitization of AMPA receptors (Stine et al., 2001). GABAergic changes include a reduction in parvalbumin-positive interneuron number (François et al., 2009), developmentally altered GABA(A) receptor alpha1 subunit receptor mRNA expression, with decreased expression prepubertally and increased expression postpubertally (Endo et al., 2007), and decreased glutamate decarboxylase-67 mRNA expression (Lipska et al., 2003). Dopaminergic changes include increased netrin-1 receptor DCC protein expression, involved in dopaminergic brain wiring, in both prepubertal and postpubertal nHVL rats (Flores et al., 2009). Serotonergic changes include increased serotonergic receptor 5-HT2A-like binding and serotonin transporter-like binding (Mitazaki et al., 2020). Importantly, many of these changes can interact. For example, altered glutamatergic and dopaminergic signaling converge onto deep layer PFC pyramidal neurons to influence excitability. nVHL rats show an increased ability of NMDA and of a dopamine 1 receptor agonist to induce deep layer pyramidal cell excitability, and a decreased ability of a dopamine 2 receptor agonist to reduce pyramidal cell excitability (Tseng et al., 2007). In addition, a dopamine 2 receptor agonist fails to increase fast-spiking interneuron activity in the PFC of nvHL rats (Tseng et al., 2008). Increased cholinergic release in response to a dopamine-1 like receptor agonist has also been observed in nVHL rats (Laplante et al., 2004).

In addition to neurotransmitter system changes, nVHL rats show immune alterations, including altered cytokine expressions (Joseph et al., 2018) and microglial activity in the PFC (Hui et al., 2019). These immune changes may impact neuronal activity through increased microglial synaptic pruning (Sellgren et al., 2019), resulting in the reduction of pyramidal cell dendritic spine densities

and complexity observed in the PFC of nVHL rats (Ryan et al., 2013). These reductions in spine densities are in turn associated with an increase in miniature excitatory input amplitude and a decrease in miniature inhibitory input frequency onto PFC layer V pyramidal cells (Ryan et al., 2013).

Thus, neonatal vHPC lesioning produces a variety of molecular changes which in turn converge to impact cellular activity. These results highlight the need to better understand the impact on cellular function, that is the electrical activity of the cells, which is the main mechanism by which neurons communicate, in order to better make sense of the complexity of PFC molecular alterations. Delineating these functional changes may in turn provide better insight into how the PFC contributes to schizophrenia-relevant behaviors.

We have thus shown that the vHPC is a core hub of schizophrenia pathology, and that some of these schizophrenia-like behaviours in nVHL animals are mediated in part by the PFC. The next section will therefore thoroughly investigate and compare the structure and functions of both the vHPC and PFC in order to better understand how their interaction may mediate certain schizophrenia-like behaviors.

The Ventral Hippocampus and the Prefrontal Cortex

Ventral Hippocampal Structure, Development, and Function

The hippocampus, or hippocampal formation, comprises of several subdomains including the dentate gyrus, the *Cornu Ammonis* areas CA1, CA2, and CA3, and the subiculum (Witter and Amaral 2004). This circuitry runs along the entirety of the hippocampus, with information flowing

generally from the dentate gyrus, processed by the pyramidal neurons of the CA3, CA1 areas, prior to exiting via the subiculum (Vakilna et al., 2021). These regions develop differentially. Regions projecting to subcortical structures, including the CA2 and subiculum, appear to mature the earliest, followed by the CA1, with the dentate gyrus and its downstream CA3 target maturing the last (Lavenex and Banta Lavenex, 2013). The CA1 reached adult-like hippocampal volumes by 1-year of age in rhesus macaques (Lavenex and Banta Lavenex, 2013). This is in contrast to the continued maturation of the prefrontal cortex throughout adolescence, which reaches peak grey matter volume by 40 months, within the adolescent age of 2-4 years in rhesus macaques, prior to normal reductions in volume (Knickmeyer et al., 2010). Thus, the main projecting cells of the hippocampal CA1 pyramidal cells are established well before the full maturation of the PFC (Ahlbeck et al., 2018).

While the basic circuitry is consistent throughout the hippocampus, the hippocampus can be further divided into dorsal and ventral portions, with the ventral portion located medial to the amygdala within the temporal lobe (Witter and Amaral 2004). This ventral portion in rodents is equivalent to the anterior hippocampus in primates based on functional and structural similarities (Witter and Amaral 2004). Moreover, the vHPC can be distinguished from the dHPC through the expression of molecular markers, its connectivity patterns with other brain regions, electrophysiological properties, and function (Fanselow and Dong 2010; Milior et al., 2016; Jung et al., 1994; Tao et al., 2021). Briefly, the vHPC is enriched in markers including *Grp*, *Grin3a*, and *Cpne2* (Fanselow and Dong 2010; Cembrowski et al., 2016). vHPC pyramidal neurons show lower spatial resolution (Jung et al., 1994), are more excitable, have an increased probability of

glutamate release and of GABAergic inputs, and are less plastic compared to dorsal HPC pyramidal neurons (Milior et al., 2016).

In addition to altered local information processing, extrahippocampal connections further differentiate the vHPC. vHPC CA1 pyramidal neurons bidirectionally connect with the amygdala and ventral tegmental area, in addition to directly projecting to the shell of the nucleus accumbens, olfactory bulb, lateral septum, bed nuclei of the stria terminalis, PFC, and hypothalamus (Fanselow and Dong, 2010; Tao et al., 2021; Tseng et al., 2008). Thus, the low spatial resolution of vCA1 neurons (i.e. fewer place cells) in addition to the connectivity patterns help explain the limited role of the vHPC in spatial learning, in contrast to its extensive role in emotional and affective state processing (Fanselow and Dong, 2010).

Prefrontal Cortical Structure, Development, and Function

The PFC plays a pivotal role in executive functions and emotion regulation. These functions are reflected by its anatomical connectivity patterns. The PFC establishes reciprocal connections with multiple different cortical areas. These anatomical connections enable integrative information processing, thereby facilitating higher-order executive functions (Hathaway and Newton 2023). In addition, the PFC functions in emotion regulation through its connections with areas that release mood-altering neurotransmitters, including dopamine, norepinephrine, and serotonin (Hathaway and Newton 2023). In primates, the PFC can be further subdivided into the dorsolateral and orbitofrontal PFC. Whereas the dorsolateral area has been associated with functions related to planning, strategy, and executive functions, the orbitofrontal area has been associated with the

inhibition of primal survival responses (Hathaway and Newton 2023). In contrast to primate neuroanatomy, the use of PFC is more varied in rodent literature (Laubach et al., 2018). It is therefore important to define what we mean by the PFC before proceeding. In our studies, the rodent PFC refers to the medial PFC area. The medial PFC comprises of the prelimbic, infralimbic, and anterior cingulate areas (Giustino and Maren, 2015; Franklin and Paxinos, 2013). However, because ventral hippocampal inputs primarily project to the prelimbic and infralimbic areas (Hoover and Vertes 2007), we will be focusing on these two regions when referring to the PFC.

The PFC has a typical cortical laminar structure. Pyramidal cells are the primary cells involved in local and long-range communication, distributed throughout layers II to VI (Giustino and Maren, 2015). Within the PFC, the prelimbic and infralimbic regions vary in their laminar organization. For example, layer II neurons innervate layer I more frequently, making the infralimbic layer II layer appear broader than the prelimbic. Whereas layers II, III and V are more distinct in the prelimbic, the layers are more homogenous in the infralimbic, with similar cell sizes and densities, but smaller cell somas compared to the prelimbic (Giustino and Maren, 2015).

In regard to connectivity, the prelimbic and infralimbic areas share similar inputs and outputs, but distributions of projections vary. For example, the prelimbic cortex receives a mixture of cortical and limbic inputs, including indirect inputs from the dorsal hippocampus, direct inputs from the ventral hippocampus, and inputs from the ventral tegmental area. In contrast, the infralimbic cortex receives inputs primarily from subcortical limbic structures, including preferential inputs from the ventral hippocampus (Liu and Carter, 2018), in addition to prelimbic cortical inputs (Hoover and Vertes 2007). In regards to outputs, both regions project to the amygdala and thalamus but vary in

target sites. For example, the infralimbic primarily projects to the medial, basomedial, central, and cortical nuclei of the amygdala, whereas the prelimbic projects to the central and basolateral nuclei of the amygdala (Vertes et al., 2004). The prelimbic projects to the anteromedial nucleus of the thalamus but the infralimbic does not, whereas other thalamic nuclei are shared (Vertes et al., 2004). In addition, the prelimbic area primarily projects to the insular cortex, nucleus accumbens, and thalamus, and the infralimbic area primarily projects to the lateral septum, bed nucleus of stria terminalis, and hypothalamus, in addition to prefrontal, orbital, insular, and entorhinal cortices (Vertes et al., 2004). These differential anatomical connectivity patterns suggest the prelimbic may be more analogous to the primate dorsolateral PFC and function in cognitive processes, while the infralimbic may be more analogous to the primate ventromedial orbital PFC and function in visceral/autonomic processes (Vertes et al., 2004; Uylings et al., 2003).

Further investigations into the behavioral contributions of the prelimbic and infralimbic PFC areas, however, have challenged a dichotomic behavioral separation of the two regions. The prelimbic cortex, for example, has been associated with the expression of conditioned fear (Corcoran et al., 2007). In contrast, infralimbic cortical activity has been associated with fear extinction (Giustino and Maren, 2015; Soler-Cedeno et al., 2016). Yet, recent studies highlight a role for the prelimbic cortex in extinction learning through projections to the infralimbic area (Marek et al., 2018; Watanabe et al., 2021). Similarly, prelimbic activity has been associated with reward and drug seeking behavior, while infralimbic activity has been associated with inhibiting reward and drug seeking behavior (Willlcocks et al., 2013; Di Pietro et al., 2006). However, neural recordings suggest the PFC in prelimbic and infralimbic areas might encode outcome-based contexts, rather than a simple dichotomy of activation versus inhibition of behavior (Moorman and Aston-Jones,

2015; Moorman et al., 2015). These results highlight a more complicated and nuanced manner in which the PFC mediates behavior. Nonetheless, these studies demonstrate that both the prelimbic and infralimbic PFC areas function in integrating contextual information in the execution of complex behaviors.

vHPC – PFC Connection and Function

vHPC projections to the PFC provide an extensive amount of contextual information for PFC processing. The vHPC CA1/subiculum has direct excitatory projections to the PFC (Fanselow and Dong, 2010; Tao et al., 2021). These projections input primarily onto layer V pyramidal cells in the prelimbic and infralimbic regions, in addition to some pyramidal cells of layers II/III in both cortices (Liu and Carter 2018, Song et al., 2022). Independent of layer, vHPC inputs primarily onto pyramidal cells that project to other cortical areas (Liu and Carter 2018).

Importantly, these projections are present early in development. Longitudinal imaging studies in humans demonstrate the continued maturation of the dorsolateral PFC (Luna et al. 2015; Cohen et al. 2016; Crone and Steinbeis 2017) and of the hippocampal-ventromedial PFC functional connectivity across adolescence and into early adulthood (Calabro et al., 2020). More precise dissections using mouse models show that vHPC CA1 cells project as early as postnatal day 8 into the PFC, and help entrain PFC activity. This entrainment was not observed with dorsal CA1 cells (Ahlbeck et al., 2018). A recent study further shows that this entrainment is deficient in a dual-hit genetic-environmental schizophrenia mouse model involving heterozygous DISC1 allele mice with embryonic injection of a viral mimetic polyinosinic-polycytidylic acid (Song et al., 2022). Interestingly, PFC projecting vHPC have a unique transcription profile, enriched in oxidative and

metabolic pathways, when compared to subcortically projecting vCA1 cells (Gergues et al., 2020). These results highlight the intimate relationship between the vHPC and PFC, with vHPC inputs playing a key role in shaping PFC maturation.

This vHPC-PFC relationship mediates a variety of functions (Hong and Kaang, 2022). Optogenetic modulation of vHPC activity onto layer V PFC pyramidal cells shows its causal regulation of social memory (Phillips et al., 2019). vHPC inputs additionally carry important contextual information that influences conditioned fear expression. vHPC inputs activate the prelimbic region and inhibit the infralimbic region through feed-forward inhibition to induce fear renewal (Wang et al., 2016), and inhibition of vHPC-prelimbic connections during training impairs fear memory recall (Twinning et al., 2020). Finally, vHPC inputs to the PFC mediate anxiety-related behaviors. Inhibition of this pathway disrupts anxiety and the PFC representation of aversion, and this disruption is associated with reduced theta synchrony (Padilla-Coreano et al., 2016).

vHPC projections therefore play a significant role in the development of the PFC and its continued integrative functioning in cognitive processes. The next section will examine how each of these regions have been implicated in schizophrenia pathology and abnormal integrative processing.

vHPC-PFC Dysfunction in Schizophrenia

Dysfunction in the vHPC-PFC pathway is expressed in different forms. These forms include neuroimaging abnormalities in schizophrenia patients, electrophysiological characterizations of functional connectivity in patients and animal models of schizophrenia, cellular substrates of

dysfunction, including interneuron populations, and molecular substrates of dysfunction, including NMDA receptors.

Neuroimaging Implications of vHPC-PFC Dysfunction in Schizophrenia

Several meta-analyses implicate dysfunction in the anterior hippocampus (vHPC) and PFC in schizophrenia patients, with hippocampal atrophy being one of the hallmarks of schizophrenia pathology (Roeske et al., 2021; Minzenberg et al., 2009; Jaaro-Peled et al., 2010; Nath et al., 2021). Key insights from longitudinal studies include the fact that high-risk patients of schizophrenia show accelerated brain volume reductions in the prefrontal cortex and hippocampal CA1 area and the extent of this reduction can predict psychosis onset and severity (McIntosh et al., 2011; Cannon et al., 2015; Ho et al., 2003). The meta-analyses highlight the robustness of the PFC and hippocampal contributions to schizophrenia pathology, and longitudinal imaging studies emphasize the developmental and progressive onset of abnormalities, in addition to their association with symptom onset. Meta-analyses further show that schizophrenia patients have reduced activation of the executive functioning network, including the dorsolateral PFC, which in turn may contribute to cognitive deficits of schizophrenia (Minzenberg et al., 2009). Moreover, hippocampal volume is consistently reduced, although fewer studies make distinctions between the anterior and posterior hippocampus (Roeske et al., 2021).

Interestingly, while brain volumes across different regions appear consistently reduced, functional magnetic resonance imaging suggest activity in these regions can vary. For example, the PFC is associated with increased activity at baseline in the performance of tasks, but reduced activity with increasing load of a working memory task (Whitfield-Gabrieli et al., 2009). Similarly, the anterior

hippocampus shows increased activity at baseline, but reduced activation in a scene processing task (McHugo et al., 2018). Whereas the structural changes may be partly explained, for example, with excessive synaptic pruning documented in schizophrenia patients resulting in decreased neuronal complexity (Roeske et al., 2021; Cannon 2015; Mallya and Deutch, 2018), understanding the underpinnings of more nuanced changes in functional activity in these regions requires further investigation.

Electrophysiological Characterizations of Functional vHPC-PFC Changes

Electroencephalogram (EEG) recordings of schizophrenia patients, along with preclinical research into the cellular mechanisms mediating brain oscillations, provide some insights into the cellular substrates of altered functional connectivity in schizophrenia. Electrical brain activity patterns can be categorized into five prominent oscillatory frequency bands: delta (0.5-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-30Hz), and gamma (30-100Hz) (Perrottelli et al., 2021; Uhlhaas and Singer, 2013).

Delta

Delta oscillations are a slow wave activity that may contribute to cognitive processing by inhibiting interferences during performance of a mental task (Harmony 2013). Resting-state EEG recordings in schizophrenia patients show increased delta band power. This excess delta activity can be observed in the prefrontal cortex of first-episode schizophrenia patients (Pascual-Marqui et al., 1999) and subjects at high-risk of schizophrenia (van Tricht et al., 2014). In contrast, task evoked delta activity is reduced in schizophrenia patients (Bates et al., 2009; Ergen et al., 2008; Doege et

al., 2010). Altered delta oscillations may be mediated through changes in dopamine signaling in the fronto-striato-thalamic loop (Dandash et al., 2017).

Theta

Theta oscillations play an important role in memory, synaptic plasticity, and top-down control exerted via long-range synchronization (Uhlhaas and Singer, 2013). Resting EEG recordings show increased theta amplitudes in chronic schizophrenia patients, but not in first-episode or high-risk subjects (Ranlund et al., 2014; Perrottelli et al., 2021).

Animal studies show that medial PFC theta oscillations synchronize with vHPC generated theta oscillations, and that this synchrony reflects spatial working memory performance (O'Neill et al., 2013). In line with this, encephalogram studies have shown impaired theta increases in the medial temporal lobe, and impaired theta phase coupling between the medial PFC and medial temporal lobe during a spatial memory task in schizophrenia patients (Adams et Al., 2020). Dorsolateral PFC recordings additionally show impaired theta-gamma coupling associated with working memory deficits in schizophrenia (Barr et al., 2017). Together, these results indicate that theta communication within and between prefrontal and hippocampal areas is disrupted in schizophrenia patients, contributing to cognitive deficits.

Alpha

Alpha oscillations are associated with attention, inhibition, and long-range synchronization (Uhlhaas and Singer, 2013). In general, schizophrenia patients appear to show reduced alpha power that appears in early psychosis (Perrottelli et al., 2021). Slower alpha oscillations are associated with perceptual discrimination deficits in a visual attention task in schizophrenia

patients (Ramsay et al., 2021). In contrast, one study applied diffusor tensor imaging along with EEGs to show that reduced white matter hippocampal connectivity is associated with increased anterior alpha activity (Begre et al., 2003). While this study consisted of a small sample size, it suggests that abnormal hippocampal connectivity may contribute to aberrant alpha oscillations in frontal regions. Alpha oscillations are thought to be generated in superficial cortical layers, which propagate from higher order to lower cortical areas, and into the thalamus (Halgren et al., 2019).

Beta

Beta oscillations are important in long-range synchronizations (Koppell et al., 2000), and may be involved in the maintenance of current sensorimotor and cognitive states (Engel and Fries 2010). At rest, increased beta activity, including in frontocentral areas, are observed in schizophrenia patients (Venables et al., 2009; Narayanan et al., 2014). These aberrant beta signaling patterns are more prominent later in schizophrenia rather than earlier (Perrottelli et al., 2021).

During task performances, schizophrenia patients show reduced beta band phase synchrony in a speech task (Ford et al., 2007), and in a Gestalt perception task (Uhlhaas et al., 2006). Additionally, schizophrenia patients show increased beta synchronization to irrelevant stimuli compared to relevant stimuli (Liddle et al., 2016). These results show that beta activity is important in sensory perceptions, which is altered in schizophrenia patients. These beta perturbations are likely mediated by altered cellular changes in deeper cortical layers, in which beta oscillations are generated (Roopun et al., 2008; Uhlhaas and Singer, 2013).

Gamma

Gamma oscillations have received extensive attention in schizophrenia pathology. These are fast oscillations, generated by parvalbumin interneurons which synchronize pyramidal cell activity in superficial cortical layers II/III (Lewis et al., 2012; Roopun et al., 2008; Uhlhaas and Singer, 2013). They mediate a variety of processes, including perception, attention, memory, consciousness, motor control, and synaptic plasticity (Uhlhaas and Singer, 2013). They tend to mediate short-range synchronizations although long-range synchronizations are observable (Hirano and Uhlhaas, 2021).

Whereas both increases in gamma power, mainly from frontal regions, and no change in gamma power have been observed at rest in schizophrenia patients, deficits in gamma power during performances of various cognitive tasks have been more consistently documented (Perrottelli et al., 2021; Hunt et al., 2017; Hirano and Uhlhaas 2021). For example, schizophrenia patients show deficits in gamma power in the frontal cortex in a working memory task. This contrasts with healthy controls who show increasing gamma power with increasing memory loads and better performance (Cho et al., 2006; Basar-Eroglu et al., 2007; Shin et al., 2011). Importantly, recent experiments trying to normalize gamma power in frontal regions using EEG-based neurofeedback have found improved task performance in the working memory n-back task, which was associated with normalization of gamma activity (Singh et al., 2020). Gamma oscillations may therefore be a critical mechanism by which cognitive deficits can be normalized in schizophrenia patients.

Moreover, gamma oscillations may vary depending on illness stage. Clinically high-risk patients show increased gamma power, which was correlated with psychotic symptoms. In contrast, first-

episode and schizophrenia patients showed altered gamma powers, wherein reduced gamma power correlated with clinical symptoms (Grent-'t-Jong et al., 2018).

Mechanisms Underlying Schizophrenia Oscillatory Changes

Schizophrenia patients therefore exhibit aberrant oscillatory activity across a wide-range of frequencies. The directionally of changes can vary depending on illness stage, task-engagement versus at rest, and brain area. While these studies reveal there are functional changes in communication patterns, at short-range and long-range distances, experimental animal work help us to better decipher the molecular basis underlying altered connectivity. Two prominent hypotheses have emerged from this work: dysfunction in parvalbumin interneurons and NMDA-receptor hypofunction.

Parvalbumin-Interneuron Dysfunction

Interneurons are largely GABAergic cells that function in modulating circuit activity. There is a large diversity in interneuron types, that can be distinguished by morphology, electrical properties, synaptic connectivity, and molecular expression. Despite the diversity, there are three groups that account for almost all GABAergic interneuron populations: parvalbumin (PV)-expressing cells (~40%), followed by somatostatin (~30%), and 5HT3aR containing cells (~30%) (Tremblay et al., 2016; Rudy et al., 2011; Lee et al., 2010). Unsurprisingly, the complex pathophysiology of schizophrenia implicates all three groups (Dienel and Lewis, 2019). Yet, PV-cells have been more thoroughly investigated in its contributions to schizophrenia pathology.

There are several reasons why PV-cells are of interest in our neurodevelopmental vHPC-PFC context of schizophrenia pathogenesis. First, PV-interneurons have a protracted maturation period relative to glutamatergic cells (Arain et al., 2013) and other interneurons. Whereas PV-cell maturation occurs during the second through fourth postnatal weeks (Che et al., 2018), other interneuron types mature earlier. For example, somatostatin cells in deep cortical layers mature within the first postnatal week in mice. Disruption of early somatostatin synaptic inputs, but not late, onto PV-cells prevents the synaptic maturation of thalamocortical inputs onto PV-cells (Tuncdemir et al., 2016). Similarly, 5HT3aR cells are an important source of early inhibition in a transient thalamocortical circuit involved in the formation of sensory maps, that is present within the first postnatal week in mice (Che et al., 2018). PV cells are therefore more vulnerable to developmental insults during their protracted maturation period.

Second, the maturation of PV cells coincides with the maturation of brain areas. The maturation of PV-cell electrophysiological cellular properties, including reduced action potential duration and propagation time, and inhibitory input decay time constant, is associated with increased coherence in hippocampal gamma waves, reflecting a mature neuronal network that can then mediate cognitive processes (Doischer et al., 2008). This maturation was characterized between postnatal day 6 to 25 in the hippocampus of mice. Another marker of brain maturation is the development of perineuronal nets, which are extracellular matrix structures that frequently surround cortical PV cells and in turn stabilize synaptic connections. This stabilization limits plasticity, and is thought to demarcate the end of the heightened plasticity defining critical periods of development (Hensch 2005). Perineuronal net development surrounding PV cells increases from juvenile, through adolescence, and into adult age (between P24 to P70) in the PFC (Baker et al., 2017). Moreover,

activity of PV cells can regulate the extent of perineuronal net density (Devienne et al., 2021), highlighting the importance of the maturation of PV cells in the maturation of brain networks, both functional (gamma) and structural (perineuronal net) features of an adult brain.

Third, extensive data suggests deficits in PV-cells in schizophrenia patients, which may mediate cognitive deficits. A meta-analysis of post-mortem tissue identifies reduced densities of PV-cells in the frontal cortex of schizophrenia patients, and may be more affected in cortical layers III and IV (Kaar et al., 2019; Lewis et al., 2012). Reductions in PV-cell density has also been documented across hippocampal subfields in schizophrenia patients (Zhang and Reynolds, 2002; Konradi et al., 2011). Moreover, PV-expressing basket cells, as opposed to PV-expressing chandelier cells, may be more impacted. Basket cells innervate the perisomatic region of pyramidal cells, whereas chandelier cells innervate the axon initial segment (Dienel and Lewis, 2019). Protein expression analyses show reductions in GAD67 in putative PV-basket cells, but not in chandelier cells in the PFC of schizophrenia patients (Rocco et al., 2016). Causal investigations into the role of PV in schizophrenia have shown that knockdown of PV in the vHPC increases VTA dopamine population activity, while knockdown of PV in the medial PFC results in deficits in social interaction and reversal learning (Perez et al., 2019).

Finally, deficits in gamma oscillations in schizophrenia patients have been linked with PV dysfunction. PV-cells, particularly basket cells, are important in the generation of gamma activity, whereas the role of PV-cell chandelier cells in gamma generation is less clear (Dienel and Lewis, 2019). Interestingly, chronic, but not acute, suppression of PV-cell activity produces a degraded neuronal ensemble mediating aberrant gamma activity in schizophrenia mouse models (Hamm et

al., 2017). This finding suggests a developmental or chronic component of PV dysfunction is necessary to produce schizophrenia-like pathologies.

Thus, observations of PV deficits in the PFC and hippocampus of schizophrenia patients, in addition to PV-linked deficits of gamma oscillations suggest PV-expressing interneurons are critical to schizophrenia pathology. However, while other interneuron types are additionally implicated, the longer maturation period of PV cells posits them as a key substrate for neurodevelopmental based alterations in the vHPC-PFC circuitry. In line with this, vHPC cells project directly onto PV cells in the PFC (Liu et al., 2020). Thus, early perturbations to vHPC inputs may influence PFC development through PV cell altered maturation.

NMDA-Receptor Hypofunction

Interest in the NMDA-receptor hypofunction hypothesis of schizophrenia emerged with the discovery that NMDA-receptor antagonists, such as ketamine, can induce psychosis in otherwise healthy individuals (Nakazawa and Sapkota, 2020). Reduced NMDA-receptor subunit NR1 expression has been documented both in the PFC (Beneyto and Meador-Woodruff, 2008; Weickert et al., 2013; Cohen et al., 2015) and significantly in the dentate gyrus of the hippocampus (Law and Deakin, 2001; Gao et al., 2000). Altered NMDA-receptor signaling in the PFC and hippocampus may contribute to cognitive deficits and psychosis, respectively (Cohen et al., 2015).

NMDA-receptor hypofunction does not impact all cell types equally. Administration of an NMDA-receptor antagonist in awake rats decreases the firing rate of GABAergic interneurons, while increases that of pyramidal neurons through disinhibition in the medial PFC (Homayoun and

Moghaddam, 2007). Similarly, ketamine greatly reduces excitatory postsynaptic inputs onto GABAergic cells compared to pyramidal cells in cultured hippocampal neurons (Fan et al., 2018). These results suggest that the NMDA-receptor hypofunction likely impacts synaptic NMDA-receptor activity onto inhibitory interneurons more so than pyramidal cells (Nakazawa and Sapkota, 2020). In line with this, reduced NR2A subunit expression has been observed specifically in PV-interneurons of the dorsolateral PFC in schizophrenia patients (Cohen et al., 2015). Moreover, blocking the NR2A subunit in cultured cortical interneurons results in decreased GAD67 and PV expression, similar to expression patterns observed in schizophrenia patients (Cohen et al., 2015). Similarly, knocking out the NR1 subunit of NMDA receptors in about half the cortical and hippocampal interneuron population during early postnatal mouse development results in the post-adolescent onset of schizophrenia-like behaviours, and decreased GAD67 and PV expression (Belforte et al., 2010). These effects were not observed with adult NR1 knockout. These same mice also show altered vHPC-PFC connectivity. The medial PFC exhibits increased activity, decreased entrainment to hippocampal theta oscillations, and reduced PFC potentials evoked by vHPC stimulation (Alvarez et al., 2020).

NMDA-receptor mediated vulnerabilities in PV-cells have also been linked to altered gamma oscillations (Hasam-Handerson et al., 2018). Mice with reduced NR1 expression in PV-cells show increased spontaneous gamma activity in the somatosensory cortex, but reduced gamma power during sensory stimulation. These gamma alterations were associated with deficits in working memory and associative learning (Carlen et al., 2012). In addition to gamma oscillations, NR1 deletion in PV cells also affects theta oscillations in the hippocampal CA1 area (Korotkova et al., 2010).

These results suggest that NMDA-receptor hypofunction may contribute to schizophrenia pathology through perturbations in PV development and function. However, the effects of NMDA-receptor hypofunction are not necessarily restricted to PV-cells, and in fact may likely act through multiple cell types in early development (Pelkey et al., 2017). For example, NMDA-receptor hypofunction may also contribute to exacerbating synaptic pruning of glutamatergic cells (Cannon 2015).

Nonetheless, both the PV dysfunction and NMDA-receptor hypofunction hypotheses of schizophrenia highlight that perturbations in development, including in PFC and vHPC regions, can impact both short-range and long-range communication between cells, thereby influencing network oscillatory activities and information sharing capacities. Therefore, deciphering how these perturbations come about is important to better understanding the neurodevelopmental mechanistic basis of schizophrenia.

Dissecting the neurodevelopmental vHPC-PFC pathway of schizophrenia

This comprehensive literature review has demonstrated that the pathogenesis of schizophrenia is neurodevelopmental based. Genetic and environmental risk factor studies, neuroimaging studies, and experimental studies examining mechanisms underlying schizophrenia, including PV dysfunction and NMDA-receptor hypofunction, show that these factors and mechanisms act within early developmental to adolescent periods. Ultimately, these factors impact the functional connectivity of brain regions. This review has highlighted the critical role that the vHPC-PFC

pathway plays in mediating cognitive deficits of schizophrenia. Altered connectivity, within the local circuits and between the two regions, mediates altered behavioral expressions.

How can we causally investigate the neurodevelopmental origins of schizophrenia? Chapter 2 of this thesis examines the potential applications of transgenic animal models and viral tools that allow for cell-type and temporally specific reading and modulation of electrical activity in neuronal circuits in a neurodevelopmental context. Chapters 3 and 4 apply these tools in the investigation of vHPC-PFC neurodevelopmental contributions to schizophrenia pathology. Chapter 3 identifies which vHPC cell type population contributes to PFC altered functional maturation and explores the nature of this altered maturation using electrophysiology to examine both local pyramidal and interneuron activities, and explores both the PV-receptor dysfunction and NMDA-receptor hypofunction hypotheses within the PFC. Chapter 4 attempts to address how these local PFC alterations contributes to population level activity, and how this activity may relate to behavioral outputs. This thesis therefore explores the neurodevelopmental circuit mechanisms underlying vHPC-PFC functional dysconnectivity relevant to schizophrenia.

Chapter 2: Neurodevelopmental insights into circuit dysconnectivity in schizophrenia

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Highlights

- Schizophrenia is a disorder of brain dysconnectivity of developmental origins.
- Important brain connectivity alterations in schizophrenia are highlighted.
- We review limitations of current animal models of dysconnectivity.
- New methods to interrogate the roles of specific developing circuits are discussed.

Abstract

Schizophrenia is increasingly being recognized as a disorder of brain circuits of developmental origin. Animal models, however, have been technically limited in exploring the effects of early developmental circuit abnormalities on the maturation of the brain and associated behavioural outputs. This review discusses evidence of the developmental emergence of circuit abnormalities in schizophrenia, followed by a critical assessment on how animal models need to be adapted through optimized tools in order to spatially and temporally manipulate early developmental events, thereby providing insight into the causal contribution of developmental perturbations to schizophrenia.

Keywords

Schizophrenia; Circuit; Dysconnectivity; Development

1. Introduction

Schizophrenia patients exhibit a range of symptoms. These include positive symptoms (hallucinations, paranoid delusions, distorted perceptions and beliefs), negative symptoms (diminished emotional expression, avolition), disorganized thought and speech and impaired cognition (executive function, memory and social cognition problems) (Substance Abuse and Mental Health Services Administration, 2016; American Psychiatric Association, 2013). At one level, these symptoms can be conceptualized as errors in integrative information processing. Hallucinations, for example, are a product of improper integration of inner speech and external sources, wherein the inner speech is misattributed to an external source (Stein and Richardson, 1999; Friston, 1998). Working memory deficit, on the other hand, may depend on uncoordinated information maintenance and information updating (Moser et al., 2018).

These errors in integrative information processing are hypothesized to be mediated by misconnectivity or dysconnectivity of multiple brain circuits. In contrast to the more straightforward concept of misconnectivity, i.e., wrong targeting of afferents, dysconnectivity may encompass complex neural rearrangements and may be defined as abnormal functional integration among brain circuits (Stephan et al., 2009). This hypothesis, first postulated in the early-to-mid 90s, has since garnered ample evidence indicative of brain dysconnectivity in schizophrenia (Friston and Frith, 1995; Weinberger, 1993; Friston et al., 2016). In 2009, the National Institute of Mental Health proposed the Research Domain Criteria that recognizes mental health disorders as disorders of brain circuits (Insel and Cuthbert, 2009; Insel et al., 2010). Our conceptual understanding of schizophrenia as a disorder of brain circuits has therefore evolved slowly since the term was first coined. Whereas neuroimaging studies have illuminated on the nature of dysconnectivity in schizophrenia, we still understand very little on the direct relationships between risk factors to dysconnectivity to behaviour. Although the disorder is clearly neurodevelopmental in nature (Weinberger, 1986), we have yet to uncover how dysconnectivity may emerge (risk to dysconnectivity), and whether dysconnectivity sufficiently explains schizophrenia (dysconnectivity to behaviour).

This review will briefly summarize the patterns of dysconnectivity observed in schizophrenia. It will then argue the pivotal role that neurodevelopmental animal models play in further elucidating the relevance of dysconnectivity to schizophrenia. Finally, it will discuss future technical evolvements of neurodevelopmental animal models that are necessary for advancing schizophrenia research into the next decade.

2. The nature of dysconnectivity in schizophrenia

Schizophrenia patients show both structural and functional circuit deficits. Meta-analyses of structural magnetic resonance imaging (MRI) in first-episode schizophrenia patients reveal decreases in grey matter volume in most brain sites, including cortical, subcortical, limbic, and cerebellar areas; but an increase in grey matter volume in the putamen (Ellison-Wright et al., 2008; Glahn et al., 2008; Williams, 2008; Birur et al., 2017). These grey matter deficits become more pronounced and progress into surrounding regions in chronic schizophrenia patients (Ellison-Wright et al., 2008; Glahn et al., 2008; Williams, 2008; Vita et al., 2012). These deficits have been attributed to decreases in cell complexity, such as dendritic branching and spine density, rather than cell loss (Coyle et al., 2016). Similarly, voxel-based morphometry and diffusor tensor imaging (DTI) reveal widespread decreases in white matter tracts, particularly of long-range pathways including interhemispheric, frontal-temporal-limbic, and cortico-cerebellar pathways (Birur et al., 2017; Vitolo et al., 2017; Kelly et al., 2018).

Despite widespread deficits in the structural integrity of the brain, functional connectivity, that is the correlated activity between brain regions, shows greater variability in schizophrenia patients. For example, concurrent decreases in white matter and functional connectivity are observed in some frontal and temporal regions, whereas a discrepant increase in connectivity is observed in some frontal regions, in addition to parietal and occipital regions (Skudlarski et al., 2010; Sun et al., 2017; Cocchi et al., 2014; van den Heuvel et al., 2013). These functional connectivity patterns can also differ depending on whether the brain is at rest or engaged in a task, and the type of task it is engaged in (Birur et al., 2017; Dong et al., 2018; Giraldo-Chica and Woodward, 2017; Zhuo

et al., 2018; Godwin et al., 2017; Nielsen et al., 2017; Mier et al., 2017; Abram et al., 2017; Bahner and Meyer-Lindenberg, 2017). Schizophrenia is therefore characterized largely by structural brain deficits with irregular functional hypo−/hyper- connectivity patterns. The fact that these functional abnormalities are not necessarily coupled with structural abnormalities further emphasizes that schizophrenia is an expression of dysconnectivity, rather than misconnectivity.

3. The emergence of dysconnectivity

Longitudinal imaging studies demonstrate that dysconnectivity emerges prior to the onset of schizophrenia and has a progressive element. Individuals at-risk for developing schizophrenia exhibit accelerated reductions in whole brain volume, including prefrontal, temporal, and hippocampal areas (McIntosh et al., 2011; Ho et al., 2017). First-episode schizophrenia patients show progressive deficits in grey matter (Mane et al., 2009), white matter (Whitford et al., 2007), and global efficiency and integration of pathways (Sun et al., 2016). These features suggest that dysconnectivity arises through developmental aberrations.

In line with this, schizophrenia risk factors primarily exert their effects during developmental periods (Rapoport et al., 2012). Risk factors such as maternal stress, postnatal infection, and cannabis use affect early and adolescent brain development (Dean and Murray, 2005). Genetic and epigenetic risk factors are additionally implicated during early brain development (Birnbaum and Weinberger, 2017; Weinberger, 2017). A study of human brain transcriptome around GWAS-identified risk loci found enrichment for developmentally regulated genes and shifting isoform usage across pre- and postnatal life (Jaffe et al., 2018).

These risk factors, then, have cumulative and widespread effects on the processes of brain development. These factors have a particularly detrimental effect on the development of long-range pathways due to their longer maturation process compared to short-range pathways (Geng et al., 2012). Given that long-range pathways support integrative information processing (Collin and van den Heuvel, 2013), deficits in their development may produce symptoms of schizophrenia that reflect deficits in integrative information processing. Similarly, risk factors may influence the maturation of interneurons through their protracted developmental period. Interneurons are important in sculpting neural activity; disruption to their function may alter both local and long-range connectivity (Le Magueresse and Monyer, 2013; Swanson and Maffei, 2019; Zecevic et al., 2011). Therefore, dysconnectivity may be a product of abnormal brain development.

4. Dysconnectivity may mediate behavioural deficits of schizophrenia

There is a large amount of evidence supporting the presence of dysconnectivity in schizophrenia, and its developmental origins. However, does dysconnectivity explain the behavioural deficits of schizophrenia? Correlative neuroimaging studies suggest that dysconnectivity patterns are indeed associated with behavioural deficits. The rapidity and extent by which reductions in brain volume occur in the prefrontal cortex and hippocampal CA1 area in at-risk individuals can predict the onset and severity of psychosis (McIntosh et al., 2011; Cannon et al., 2015; Ho et al., 2003). Cases in which volumetric changes are arrested or reverse course, such as in the temporal grey matter in first-episode schizophrenia, are associated with better outcomes (Schaufelberger et al., 2011). In contrast, lateral ventricular enlargement predicts poorer outcomes, and progressive volumetric decreases in frontal lobe grey and white matter correlate with negative symptoms and deficits in executive functioning (Ho et al., 2003). Longitudinal functional connectivity studies further show

that the normalization of hyper- or hypo- connectivity with or without treatment (i.e. naturally) is associated with symptom improvement (Kraguljac et al., 2016; Keshavan et al., 2017; Anticevic et al., 2015; Duan et al., 2015).

Despite this evidence, our conclusions are limited given that this data remains correlative. In that regard, animal models can function in dissecting the causal contribution of brain dysconnectivity to behavioural output, thereby confirming that schizophrenia is a disorder of brain dysconnectivity.

5. Neurodevelopmental animal models of schizophrenia

Neurodevelopmental animal models of schizophrenia often mimic the early environmental risk factors for schizophrenia, such as prenatal maternal infection and early life stressors (Kim et al., 2015; Stilo and Murray, 2019; Boksa, 2010). These models have been valuable in establishing a causal relationship between these risk factors and dysconnectivity patterns of the brain (Sigurdsson, 2016). For example, DTI in the methylazoxymethanol acetate (MAM) and maternal immune activation (MIA) models shows white matter deficits in long-range pathways such as the corpus callosum and cingulum (Chin et al., 2011; Li et al., 2010). Longitudinal MRI in the MIA model further shows decreases in prefrontal cortical, hippocampal, and striatal volumes (Crum et al., 2017; Piontkewitz et al., 2012; Guma et al., 2019). Moreover, female offspring show a delayed onset of these structural deficits, the extent of hippocampal loss predicts the severity of psychosis-like symptoms, and adolescent administration of antipsychotics partially reverses these structural deficits (Piontkewitz et al., 2012). Neuroimaging has been a valuable translational tool demonstrating the similar dysconnectivity patterns produced in neurodevelopmental animal models as in schizophrenia patients. While several studies have pursued the mechanisms by which

this dysconnectivity may occur, such as altered microglial-synaptic interactions (Hui et al., 2018) and altered functional maturation of parvalbumin interneurons (Canetta et al., 2016), these studies do not show whether dysconnectivity alone explains schizophrenia-like behaviours. In addition to central changes in the brain, peripheral changes in bone marrow and gut microbiome composition can contribute to schizophrenia-like behavioural outputs in the MIA model, presumably through interactions with the brain (Estes and McAllister, 2016). Epidemiological-based neurodevelopmental models of schizophrenia (Powell, 2010) therefore provide evidence for the mechanisms by which various risk factors may impinge on brain connectivity, but heuristic neurodevelopmental models are necessary to show that direct manipulation of connectivity patterns can alone produce schizophrenia-like behaviours.

6. The neonatal ventral hippocampal lesion model of schizophrenia

If we accept that schizophrenia is a disorder of brain circuits of neurodevelopmental origins, then animal models of schizophrenia must involve a direct circuit manipulation during early development. Most models either directly manipulate circuit function, but in adults, thereby ignoring the developmental component of schizophrenia; or mimic a genetic or environmental risk factor, such as the maternal immune activation model, which produces dysconnectivity but does not directly assess the causal contribution of dysconnectivity alone to behavioural deficits (Crum et al., 2017; Piontkewitz et al., 2012; Guma et al., 2019).

The neonatal ventral hippocampal lesion (nVHL) model of schizophrenia, on the other hand, involves the lesioning in neonatal rats of the ventral hippocampus (vHPC), equivalent to the anterior hippocampus in humans which constitutes a central hub in schizophrenia dysconnectivity

patterns (Bahner and Meyer-Lindenberg, 2017; Lipska et al., 1993). Importantly, neonatal lesioning but not adult lesioning produces schizophrenia-like behavioural deficits that emerge post-adolescence, thereby paralleling the progression of schizophrenia onset in humans (Lipska et al., 1993).

This model therefore has been valuable in demonstrating that circuit abnormalities originating during development but not adulthood produce schizophrenia-like deficits. Yet, there are limitations to this model. Lesioning of the vHPC is a spatially and temporally limited method of dissecting brain circuitry and function and can lead to additional effects such as changes in immune functions that may contribute to the development of schizophrenia-relevant features (Joseph et al., 2018). The following section will discuss the limitations of this model and how newer evolving techniques can help address these limitations.

7. Cell-type resolution: targeting specific areas and projection pathways

The current nVHL model involves the infusion of an excitotoxin into the vHPC in postnatal day 7 rats (Lipska et al., 1993). The excitotoxin, usually ibotenic acid, is more limited to the injected area, with minimal spread and damage to adjacent areas and fibers, compared to other toxins (kainic acid) and other techniques (electrolysis, aspiration) (Jarrard, 1989). Even if these effects are minimal, they are not absent. Moreover, within the vHPC, there are multiple cell types that can be classified based on their expression of specific gene markers and their projection site.

Gene markers can be used to delineate different areas within the vHPC. For example, Htr2c and Coch expression can be used to delineate CA1 from CA3, respectively, and are specific to the

ventral areas and not dorsal (Fanselow and Dong, 2010; Thompson et al., 2008). These vHPC subfields play differential roles in information processing and behavioural output. For example, adult rats trained in an approach-avoidance conflict paradigm, in which interaction with a conflict cue (the simultaneous presentation of an appetitive (sucrose) and aversive (foot shock) cue) is examined, shows that ventral CA1 inactivation with muscimol increases avoidance to the conflict cue whereas ventral CA3 inactivation increases approach to the conflict clue (Schumacher et al., 2018). In contrast, lesioning the full vHPC with the NMDA neurotoxin increases approach to the conflict cue (Schumacher et al., 2016). These distinctions in behavioural output are important given that schizophrenia patients differ in their expression of behavioural approach and avoidance, and show differences in hippocampal subfield volumes that can be correlated with different cognitive measures (Felice Reddy et al., 2014; Baglivo et al., 2018; Mathew et al., 2014). These circuit dissections in animal models will therefore enable us to causally assess population specific contributions to behavioural output.

Cells expressing the same gene marker may still differ in their projection sites. Differential vHPC information processing by these projection sites may contribute to differential behavioural outputs relevant to schizophrenia (Grace, 2016; Vanes et al., 2019). For example, the vHPC can regulate ventral tegmental dopaminergic release through projections to the nucleus accumbens, and increased dopaminergic outputs may mediate positive symptoms of schizophrenia (Floresco et al., 2001; Lodge and Grace, 2007; Mikell et al., 2009). On the other hand, vHPC projections to the prefrontal cortex may mediate cognitive deficits of schizophrenia, such as working memory impairments (Bahner and Meyer-Lindenberg, 2017; Sigurdsson and Duvarci, 2016). However, the vHPC also has projections to the nucleus reuniens of the thalamus, which may also function in

working memory, and also has projections to the prefrontal cortex (Viena et al., 2018; Dolleman-van der Weel et al., 2019). Are vHPC projections to the prefrontal cortex versus the nucleus reuniens processing information for cognition similarly? Is the nucleus reuniens simply acting as a relay to the prefrontal cortex? The above studies suggest not, given that inactivating the nucleus reuniens disrupts working memory, despite having intact direct projections from the vHPC to the prefrontal cortex. If these two pathways are differentially processing cognitive information, then what are those differences and how do these differences interact? To better understand these questions, it is important to be able to manipulate pathway-specific projections, singly and in combination.

Efferent projections from the vHPC may not be solely responsible for the onset of schizophrenia-like deficits. Afferent projections from the nucleus reuniens, which receives infralimbic prefrontal cortical input, to the vHPC can regulate ventral tegmental dopaminergic release as well (Zimmerman and Grace, 2016). In fact, aberrant activity, rather than the absence of vHPC projection output may mediate dysconnectivity in other circuits, such as the dopaminergic system (Lodge and Grace, 2007). In support of this idea, vHPC lesions that spare the entorhinal cortex and dorsal hippocampus produce hyperlocomotion in response to amphetamine, suggesting that altered activity in these regions may at least partly contribute to schizophrenia-like behaviours (Swerdlow et al., 2001). These results suggest that it is important to examine the effects of altered activity in dysconnectivity.

8. Manipulating the balance between excitation and inhibition

Ventral hippocampal cells can be distinguished based on whether they are excitatory or inhibitory. Generally, projection cells in the brain tend to be excitatory. However, it may be presumptuous to assume that excitatory projection cells are the primary source of dysconnectivity. In schizophrenia patients, cumulative evidence shows impairment in inhibitory function in the brain. There is decreased GAD67 expression across multiple brain areas, including the hippocampus (Benes et al., 2007; Thompson et al., 2009), decreased GABA uptake sites in the hippocampus (Reynolds et al., 1990), and decreased parvalbumin-immunoreactivity across all subfields of the hippocampus in the anterior (equivalent to the rodent vHPC) but not posterior regions (Benes et al., 1998; Zhang and Reynolds, 2002; Falkai et al., 2016). Although it is hypothesized that decreases in interneuron function may "compensate for an upstream deficit in pyramidal cell excitation" (Lewis et al., 2012), it is also possible that interneuron deficits arise independently through other means. In fact, interneurons can have a protracted maturation process, making them vulnerable to diverse insults that may occur during development (Do et al., 2015). For example, enhancing oxidative stress can decrease hippocampal parvalbumin-interneuron immunoreactivity without any significant changes to pyramidal cell activity (Steullet et al., 2010). Environmental stress exposures during the critical period of early adolescence, but not adulthood, decreases the number of parvalbumin-positive interneurons and perineuronal nets (an extracellular matrix structure surrounding mature parvalbumin-interneurons and providing protection against oxidative stress), and results in vHPC hyperactivity (Gomes et al., 2019). A combination of biological and environmental stressors can further increase vulnerability to stressors (Gomes and Grace, 2017). Animal models that inhibit interneuron function in the vHPC are able to replicate some schizophrenia-related deficits (Nguyen et al., 2014; Perez et al., 2019), while restoring interneuron function can normalize vHPC activity

(Donegan et al., 2019; Gill et al., 2011). Do the same dysconnectivity patterns therefore emerge when excitatory versus inhibitory cell populations are targeted in the vHPC? An answer to this question will help illuminate on the origins of dysconnectivity.

9. Future evolvement: developmental applications of transgenic, viral, and electroporation techniques

In order to achieve cell-type resolution targeting populations marked by specific gene markers, projection site, or neurotransmission type, in the vHPC, it is necessary to utilize increasingly available and diverse transgenic mouse lines, and viral vectors. Given the difficulty in generating transgenic rats (Pradhan and Majumdar, 2016), improving spatial resolution in the nVHL model may necessitate transitioning into a mouse model equivalent, although this use may be circumvented with the production of viral vectors that can carry larger and therefore more diverse constructs (Holehonnur et al., 2015). For example, Htr2c-cre and PV-cre mouse lines have been used in conjunction with viral vectors expressing opsins or DREADDs in order to manipulate cell-type specific population activity (Nguyen et al., 2014; Li et al., 2018a). Projection-specific pathways can also be examined through the use of retrogradely transported viruses, such as CAV-2. For example, CAV-2-cre expression in the nucleus accumbens combined with AAV-DREADD expression in the vHPC allows manipulation of nucleus accumbens specific projecting cells (Phillips et al., 2019). In order to circumvent challenges of CAV-2 tropism (i.e. the preferential expression in certain cell types due to the presence of receptors for that virus), additional viruses can be used to express the receptors for the virus in the target projection neurons or other retrograde viruses can be used (Li et al., 2018b).

Although these tools for cell-type specific targeting have been available for many years, they have most frequently been applied in adult animals. Yet, schizophrenia is a neurodevelopmental disorder. And we know that neurodevelopmental manipulations of circuits do not necessarily result in the same behavioural phenotypes as adult manipulations, as seen with the nVHL model (Lipska et al., 1993). Challenges of neonatal manipulations include the fact that the expression of cell-type markers may evolve throughout development (i.e. may not be present in early development), and the technical difficulties in neonatal manipulations (i.e. high mortality, and imprecise targeting of regions in a small brain). Nonetheless, several labs have recently optimized protocols for developmental manipulations in a cell-type specific manner. Procedures for intracranial viral injections in neonatal mice as early as six hours after birth and for in utero electroporation have been established (Kim et al., 2013; He et al., 2018). These techniques can be sophisticatedly utilized in altering the function of specific cell type populations in a temporally specific manner. For example, Ahlbeck et al., 2018 demonstrate the presence of vHPC CA1 projections to the prelimbic area of the prefrontal cortex through intracranial retrograde and anterograde tracer injections in postnatal day 1 mice; and the ability of these projections to induce oscillatory activity within the prelimbic area through optogenetic manipulation of CA1 activity achieved through in utero electroporation (Ahlbeck et al., 2018). Although in utero electroporation still yielded reduced litter sizes (Ahlbeck et al., 2018), valuable insight was gained on vHPC-prefrontal developmental connectivity. Future investigations into the role of vHPC dysconnectivity in schizophrenia will therefore involve these tools optimized for early developmental manipulations.

10. The temporal modulation of signaling

The lesioning of the vHPC abolishes all inputs from this region to other areas. Yet imaging studies reveal the presence of both hypo- and hyper- connectivity and activity in schizophrenia patients. In a task in which images of scenes were presented, first-episode schizophrenia patients show reduced anterior hippocampal activity during scene-image processing (task), but elevated anterior hippocampal activity during scrambled-image processing (baseline) (McHugo et al., 2019). In fact, the greater the baseline activation, the lower the task activation; and this effect is specific to the anterior but not posterior hippocampus. Hippocampal activity changes may also be subfield specific. Schizophrenia patients show trends towards increased CA1 cerebral blood volume, but decreased CA2/3 cerebral blood volume (Talati et al., 2014). In the prefrontal cortex, schizophrenia patients show hyperactivity at baseline, but a reduced suppression of activity during a working memory task. Reduced suppression of medial prefrontal cortical activity is associated with impaired working memory (Whitfield-Gabrieli et al., 2009). In regard to connectivity, functional neuroimaging studies demonstrate reduced resting-state connectivity between the anterior hippocampus and medial prefrontal cortex (Zhou et al., 2008), and altered connectivity expressed by persistent activation during a working memory task between the dorsolateral prefrontal cortex and hippocampal formation (Meyer-Lindenberg et al., 2005).

These results demonstrate the dynamic connectivity and activity patterns in the prefrontal cortex and hippocampus. Moreover, high risk patients for psychosis show elevated resting perfusion, indicative of elevated activity (Allen et al., 2018; Allen et al., 2016). The neonatal vHPC lesion model therefore fails to recapitulate this dynamic activity pattern, and may not necessarily reflect hippocampal activity during stages preceding the onset of schizophrenia. Does activating the

vHPC in neonates produce the same schizophrenia-like behavioural deficits observed in the lesion model? This question can be addressed by using the transgenic and viral vector techniques described previously in order to express opsins or DREADDs to bidirectionally manipulate vHPC activity.

In addition to conferring the ability to bidirectionally manipulate activity, opsins in particular can be used to temporally mimic oscillatory activity deficits observed in schizophrenia patients. Electroencephalography (EEG) recordings reveal deficits in gamma power and theta-gamma coupling, which are associated with impairments in working memory (Woo et al., 2010; Barr et al., 2017). In contrast, schizophrenia patients selected for equal performance in working memory assays as healthy controls show compensatory changes in gamma oscillations (So et al., 2018). Whereas EEG techniques have poor spatial resolution, simultaneous electrophysiological recordings in the vHPC and medial prefrontal cortex of rodents confirm that long-range theta and gamma synchrony is important for spatial working memory (Sigurdsson et al., 2010; O'Neill et al., 2013; Spellman et al., 2015). Inactivating the vHPC with muscimol or prefrontal cortical somatostatin interneurons impairs hippocampal-prefrontal synchrony (O'Neill et al., 2013; Abbas et al., 2018). These synchronous events develop during early postnatal development, primarily driven by vHPC inputs to the prefrontal cortex (Ahlbeck et al., 2018; Brockmann et al., 2011). Optogenetic activation of vHPC CA1 pyramidal cells at theta frequencies (8 Hz) in postnatal day 8–10 mice increases oscillatory activity in the prelimbic cortex at all frequency bands (Ahlbeck et al., 2018). This effect was not observed when dorsal hippocampal cells were optogenetically activated, or with other optogenetic stimulation frequencies (4 Hz and 16 Hz). What, if any, are the effects of disrupting the temporal structure of vHPC-prefrontal cortical signaling during early

development on post-adolescent hippocampal-prefrontal cortical signaling and associated behaviours? EEGs in high risk clinical subjects and in siblings of schizophrenia patients suggest gamma deficits may exist in the absence of expression of psychosis (Reilly et al., 2018; Leicht et al., 2016). Therefore, manipulating the temporal structure of early developmental signaling using optogenetics is relevant to understanding how schizophrenia develops. These studies may provide further direction for treatments of schizophrenia that involve activity manipulations, such as deep brain stimulation or transcranial magnetic stimulation.

11. Conclusion

There is ample evidence supporting that circuit dysconnectivity of developmental origins may mediate behavioural deficits in schizophrenia. Animal models of research in causally demonstrating the relationship between neonatal circuit dysfunction and post-adolescent schizophrenia-like behavioural deficits must involve modern tools that provide high spatial and temporal resolution. These tools would allow for better circuit dissection, which can be used to form a library of information on how specific circuit abnormalities lead to specific behavioural effects; and may identify pre-symptomatic biomarkers. This information archive can then be used in the eventual transformation of medicine into personalized medicine – where patient-specific neuroimaging identification of circuit abnormalities can be used to target treatments in a circuit-specific manner. The emergence of these studies will therefore play a fundamental role in schizophrenia research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abbas AI, Sundiang MJM, Henoch B, et al. Somatostatin Interneurons Facilitate Hippocampal-Prefrontal Synchrony and Prefrontal Spatial Encoding. Neuron. 2018;100(4):926–939.e3. doi:10.1016/j.neuron.2018.09.029

Abram SV, Wisner KM, Fox JM, et al. Fronto-temporal connectivity predicts cognitive empathy deficits and experiential negative symptoms in schizophrenia. Hum Brain Mapp. 2017;38(3): 1111-1124.

Ahlbeck J, Song L, Chini M, et al. Glutamatergic drive along the septo-temporal axis of hippocampus boosts prelimbic oscillations in the neonatal mouse. Elife. 2018;7:e33158. Published 2018 Apr 10. doi:10.7554/eLife.33158

Allen P, Azis M, Modinos G, et al. Increased Resting Hippocampal and Basal Ganglia Perfusion in People at Ultra High Risk for Psychosis: Replication in a Second Cohort. Schizophr Bull. 2018;44(6):1323–1331. doi:10.1093/schbul/sbx169

Allen P, Chaddock CA, Egerton A, et al. Resting Hyperperfusion of the Hippocampus, Midbrain, and Basal Ganglia in People at High Risk for Psychosis. Am J Psychiatry. 2016 Apr 1;173(4):392-9. doi: 10.1176/appi.ajp.2015.15040485.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Washington, DC: APA. 2013

Anticevic A, Hu X, Xiao Y, et al. Early-course unmedicated schizophrenia patients exhibit elevated

prefrontal connectivity associated with longitudinal change. J Neurosci. 2015;35(1):267–286. doi:10.1523/JNEUROSCI.2310-14.2015

Baglivo V, Cao B, Mwangi B, et al. Hippocampal Subfield Volumes in Patients With First-Episode Psychosis. Schizophr Bull. 2018;44(3):552–559. doi:10.1093/schbul/sbx108

Bahner F and A Meyer-Lindenberg. Hippocampal-prefrontal connectivity as a translational phenotype for schizophrenia. Eur Neuropsychopharmacol. 2017 Feb;27(2):93-106. doi: 10.1016/j.euroneuro.2016.12.007

Barr MS, Rajji TK, Zomorrodi R, et al. Impaired theta-gamma coupling during working memory performance in schizophrenia. Schizophr Res. 2017 Nov;189:104-110. doi: 10.1016/j.schres.2017.01.044.

Benes FM, Kwok EW, Vincent SL, et al. A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressives. Biol Psychiatry. 1998 Jul 15;44(2):88-97.

Benes FM, Lim B, Matzilevich D, Walsh JP, et al. Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. Proc Natl Acad Sci USA. 2007;104(24):10164–10169. doi:10.1073/pnas.0703806104

Birnbaum R, Weinberger D. Genetic insights into the neurodevelopmental origins of schizophrenia. Nat Rev Neurosci. 2017;18, 727–740. https://doi.org/10.1038/nrn.2017.125

Birur B, Kraguljac NV, Shelton RC, et al. Brain structure, function, and neurochemistry in schizophrenia and bipolar disorder-a systematic review of the magnetic resonance neuroimaging literature. NPJ Schizophr. 2017;3:15. Published 2017 Apr 3. doi:10.1038/s41537-017-0013-9

Boksa, P. Effects of prenatal infection on brain development and behavior: a review of findings

from animal models Brain Behav. Immun., 24 (6) (2010), pp. 881-897, 10.1016/j.bbi.2010.03.005

Brockmann MD, Pöschel B, Cichon N, et al. Coupled oscillations mediate directed interactions between prefrontal cortex and hippocampus of the neonatal rat. Neuron. 2011 Jul 28;71(2):332-47. doi: 10.1016/j.neuron.2011.05.041.

Canetta S, Bolkan S, Padilla-Coreano N. Maternal immune activation leads to selective functional deficits in offspring parvalbumin interneurons Mol. Psychiatry, 21 (7) (2016), pp. 956-968, 10.1038/mp.2015.222

Cannon TD, Chung Y, He G, et al. Progressive reduction in cortical thickness as psychosis develops: a multisite longitudinal neuroimaging study of youth at elevated clinical risk. Biol Psychiatry. 2015;77(2):147–157. doi:10.1016/j.biopsych.2014.05.023

Chin CL, Curzon P, Schwartz AJ et al. Structural abnormalities revealed by magnetic resonance imaging in rats prenatally exposed to methylazoxymethanol acetate parallel cerebral pathology in schizophrenia Synapse., 65 (5) (2011), pp. 393-403, 10.1002/syn.20857

Cocchi L, Harding IH, Lord A, et al. Disruption of structure-function coupling in the schizophrenia connectome. Neuroimage Clin. 2014;4:779–787. Published 2014 May 9. doi:10.1016/j.nicl.2014.05.004

Collin G, van den Heuvel MP. The ontogeny of the human connectome: development and dynamic changes of brain connectivity across the life span. Neuroscientist. 2013;19(6): 616-628.

Coyle JT, Balu DT, Puhl MD, Konopaske GT. History of the Concept of Disconnectivity in Schizophrenia. Harv Rev Psychiatry. 2016;24(2):80–86. doi:10.1097/HRP.0000000000000102

Crum WR, Sawiak SJ, Chege W, et al. Evolution of structural abnormalities in the rat brain

following in utero exposure to maternal immune activation: A longitudinal *in vivo* MRI study. Brain Behav Immun. 2017;63:50–59. doi:10.1016/j.bbi.2016.12.008

Dean K, Murray RM. Environmental risk factors for psychosis. Dialogues Clin Neurosci. 2005;7(1):69–80.

Do KQ, Cuenod M, Hensch TK. Targeting Oxidative Stress and Aberrant Critical Period Plasticity in the Developmental Trajectory to Schizophrenia. Schizophr Bull. 2015;41(4):835–846. doi:10.1093/schbul/sbv065

Dolleman-van der Weel MJ, Griffin AL, Ito HT, et al. The nucleus reuniens of the thalamus sits at the nexus of a hippocampus and medial prefrontal cortex circuit enabling memory and behavior. Learn Mem. 2019;26(7):191–205. Published 2019 Jun 17. doi:10.1101/lm.048389.118

Donegan JJ, Boley AM, Yamaguchi J, et al. Modulation of extrasynaptic GABAA alpha 5 receptors in the ventral hippocampus normalizes physiological and behavioral deficits in a circuit specific manner. Nat Commun. 2019;10, 2819.

Dong D, Wang Y, Chang X, et al. Dysfunction of Large-Scale Brain Networks in Schizophrenia: A Meta-analysis of Resting-State Functional Connectivity. Schizophr Bull. 2018;44(1):168–181. doi:10.1093/schbul/sbx034

Duan HF, Gan JL, Yang JM, et al. A longitudinal study on intrinsic connectivity of hippocampus associated with positive symptom in first-episode schizophrenia. Behav Brain Res. 2015;283: 78-86.

Ellison-Wright I, Glahn DC, Laird AR, et al. The anatomy of first-episode and chronic schizophrenia: an anatomical likelihood estimation meta-analysis. Am J Psychiatry. 2008;165(8):1015–1023. doi:10.1176/appi.ajp.2008.07101562

Estes ML, McAllister AK. Maternal immune activation: implications for neuropsychiatric disorders Science., 353 (6301) (2016), pp. 772-777, 10.1126/science.aag3194

Falkai P, Steiner J, Malchow B, et al. Oligodendrocyte and Interneuron Density in Hippocampal Subfields in Schizophrenia and Association of Oligodendrocyte Number with Cognitive Deficits. Front Cell Neurosci. 2016;10:78. Published 2016 Mar 30. doi:10.3389/fncel.2016.00078

Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures?. Neuron. 2010;65(1):7–19. doi:10.1016/j.neuron.2009.11.031

Felice Reddy L, Green MF, Rizzo S, et al. Behavioral approach and avoidance in schizophrenia: an evaluation of motivational profiles. Schizophr Res. 2014;159(1):164–170. doi:10.1016/j.schres.2014.07.047

Floresco SB, Blaha CD, Yang CR, et al. Modulation of hippocampal and amygdalar-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection. J Neurosci. 2001;21(8): 2851-2860.

Friston KJ. The disconnection hypothesis. Schizophr Res. 1998;30(2): 115-125.

Friston KJ, Frith CD. Schizophrenia: a disconnection syndrome? Clin Neurosci. 1995;3(2): 89-97.

Friston K, Brown HR, Siemerkus J, Stephan KE. The dysconnection hypothesis (2016). Schizophr Res. 2016;176(2-3):83–94. doi:10.1016/j.schres.2016.07.014.

Geng X, Gouttard S, Sharma A, et al. Quantitative tract-based white matter development from birth to age 2years. Neuroimage. 2012;61(3):542–557. doi:10.1016/j.neuroimage.2012.03.057

Gill KM, Lodge DJ, Cook JM, Aras S, Grace AA. A novel α5GABA(a)R-positive allosteric

modulator reverses hyperactivation of the dopamine system in the MAM model of schizophrenia Neuropsychopharmacology., 36 (9) (2011), pp. 1903-1911, 10.1038/npp.2011.76

Giraldo-Chica M, Woodward ND. Review of thalamocortical resting-state fMRI studies in schizophrenia. Schizophr Res. 2017;180:58–63. doi:10.1016/j.schres.2016.08.005

Glahn DC, Laird AR, Ellison-Wright I, et al. Meta-analysis of gray matter anomalies in schizophrenia: application of anatomic likelihood estimation and network analysis. Biol Psychiatry. 2008;64(9):774–781. doi:10.1016/j.biopsych.2008.03.031

Godwin D, Ji A, Kandala S, Mamah D. Functional Connectivity of Cognitive Brain Networks in Schizophrenia during a Working Memory Task. Front Psychiatry. 2017;8:294. Published 2017 Dec 22. doi:10.3389/fpsyt.2017.00294

Gomes FV, Grace AA. Prefrontal cortex dysfunction increases susceptibility to schizophrenia-like changes induced by adolescent stress exposure Schizophr. Bull., 43 (3) (2017), pp. 592-600, 10.1093/schbul/sbw156

Gomes FV, Zhu X, Grace AA. The pathophysiological impact of stress on the dopamine system is dependent on the state of the critical period of vulnerability [published online ahead of print, 2019 Sep 5] [published correction appears in Mol Psychiatry. 2019 Sep 27;:] Mol. Psychiatry (2019), 10.1038/s41380-019-0514-1

Grace AA. Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression Nat. Rev. Neurosci., 17 (8) (2016), pp. 524-532, 10.1038/nrn.2016.57

Guma E, Plitman E, Chakravarty MM. The role of maternal immune activation in altering the

neurodevelopmental trajectories of offspring: A translational review of neuroimaging studies with implications for autism spectrum disorder and schizophrenia. Neurosci Biobehav Rev. 2019;104: 141-157.

He CX, Arroyo ED, Cantu DA, Goel A, Portera-Cailliau C. A Versatile Method for Viral Transfection of Calcium Indicators in the Neonatal Mouse Brain. Front Neural Circuits. 2018;12:56. Published 2018 Jul 23. doi:10.3389/fncir.2018.00056

Ho BC, Andreasen NC, Nopoulos P, et al. Progressive structural brain abnormalities and their relationship to clinical outcome: a longitudinal magnetic resonance imaging study early in schizophrenia. Arch Gen Psychiatry. 2003 Jun;60(6):585-94.

Ho NF, Holt DJ, Cheung M, et al. Progressive Decline in Hippocampal CA1 Volume in Individuals at Ultra-High-Risk for Psychosis Who Do Not Remit: Findings from the Longitudinal Youth at Risk Study [published correction appears in Neuropsychopharmacology. 2019 Nov;44(12):2144]. Neuropsychopharmacology. 2017;42(6):1361–1370. doi:10.1038/npp.2017.5

Holehonnur R, Lella SK, Ho A, Luong JA, Ploski JE. The production of viral vectors designed to express large and difficult to express transgenes within neurons. Mol Brain. 2015;8:12. Published 2015 Feb 24. doi:10.1186/s13041-015-0100-7

Hui CW, St-Pierre A, El Hajj H, et al. Prenatal immune challenge in mice leads to partly sex-dependent behavioral, microglial, and molecular abnormalities associated with Schizophrenia Front. Mol. Neurosci., 11 (13) (2018), 10.3389/fnmol.2018.00013 Published 2018 Feb 8

Insel TR, and Cuthbert BN. Endophenotypes: bridging genomic complexity and disorder heterogeneity. Biol Psychiatry. 2009;66(11): 988-989.

Insel T, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. Am J Psychiatry. 2010;167(7): 748-751.

Jaffe AE, Straub RE, Shin JH, et al. Developmental and genetic regulation of the human cortex transcriptome illuminate schizophrenia pathogenesis. Nat Neurosci. 2018;21(8): 1117-1125.

Jarrard LE. On the use of ibotenic acid to lesion selectively different components of the hippocampal formation. J Neurosci Methods. 1989 Sep;29(3):251-9.

Joseph AT, Bhardwaj SK, Srivastava LK. Role of Prefrontal Cortex Anti- and Pro-inflammatory Cytokines in the Development of Abnormal Behaviors Induced by Disconnection of the Ventral Hippocampus in Neonate Rats. Front Behav Neurosci. 2018;12:244. Published 2018 Oct 22. doi:10.3389/fnbeh.2018.00244

Kelly S, Jahanshad N, Zalesky A, et al. Widespread white matter microstructural differences in schizophrenia across 4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. Mol Psychiatry. 2018;23(5):1261–1269. doi:10.1038/mp.2017.170

Keshavan MS, Eack SM, Prasad KM, et al. Longitudinal functional brain imaging study in early course schizophrenia before and after cognitive enhancement therapy. Neuroimage. 2017 May 1;151:55-64. doi: 10.1016/j.neuroimage.2016.11.060.

Kim JY, Ash RT, Ceballos-Diaz C, et al. Viral transduction of the neonatal brain delivers controllable genetic mosaicism for visualising and manipulating neuronal circuits *in vivo*. Eur J Neurosci. 2013;37(8):1203–1220. doi:10.1111/ejn.12126

Kim DR, Bale TL, Epperson CN. Prenatal programming of mental illness: current understanding of relationship and mechanisms Curr. Psychiatry Rep., 17 (2) (2015), Article 5, 10.1007/s11920-014-0546-9

Kraguljac NV, White DM, Hadley N, et al. Aberrant Hippocampal Connectivity in Unmedicated Patients With Schizophrenia and Effects of Antipsychotic Medication: A Longitudinal Resting State Functional MRI Study. Schizophr Bull. 2016;42(4):1046–1055. doi:10.1093/schbul/sbv228

Le Magueresse C, Monyer H. GABAergic interneurons shape the functional maturation of the cortex Neuron., 77 (3) (2013), pp. 388-405, 10.1016/j.neuron.2013.01.011

Leicht G, Vauth S, Polomac N, et al. EEG-Informed fMRI Reveals a Disturbed Gamma-Band-Specific Network in Subjects at High Risk for Psychosis. Schizophr Bull. 2016;42(1):239–249. doi:10.1093/schbul/sbv092

Lewis DA, Curley AA, Glausier JR, Volk DW. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci. 2012;35(1):57–67. doi:10.1016/j.tins.2011.10.004

Li Q, Cheung C, Wei R, et al. Voxel-based analysis of postnatal white matter microstructure in mice exposed to immune challenge in early or late pregnancy Neuroimage., 52 (1) (2010), pp. 1-8, 10.1016/j.neuroimage.2010.04.015

Li SJ, Vaughan A, Sturgill JF, et al. A Viral Receptor Complementation Strategy to Overcome CAV-2 Tropism for Efficient Retrograde Targeting of Neurons. Neuron. 2018 Jun 6;98(5):905-917.e5. doi: 10.1016/j.neuron.2018.05.028.

Li X, Chen W, Pan K, et al. Serotonin receptor 2c-expressing cells in the ventral CA1 control attention via innervation of the Edinger-Westphal nucleus. Nat Neurosci. 2018 Sep;21(9):1239-1250. doi: 10.1038/s41593-018-0207-0.

Lipska BK, Jaskiw GE, Weinberger DR. Postpubertal emergence of hyperresponsiveness to stress

and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia. Neuropsychopharmacology. 1993;9(1): 67-75.

Lodge DJ, Grace AA. Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia. J Neurosci. 2007;27(42):11424–11430. doi:10.1523/JNEUROSCI.2847-07.2007

Mane A, Falcon C, Mateos JJ, et al. Progressive gray matter changes in first episode schizophrenia: a 4-year longitudinal magnetic resonance study using VBM. Schizophr Res. 2009 Oct;114(1-3):136-43. doi: 10.1016/j.schres.2009.07.014

Mathew I, Gardin TM, Tandon N, et al. Medial temporal lobe structures and hippocampal subfields in psychotic disorders: findings from the Bipolar-Schizophrenia Network on Intermediate Phenotypes (B-SNIP) study. JAMA Psychiatry. 2014;71(7): 769-777.

McHugo M, Talati P, Armstrong K, et al. Hyperactivity and Reduced Activation of Anterior Hippocampus in Early Psychosis. Am J Psychiatry. 2019 Dec 1;176(12):1030-1038. doi: 10.1176/appi.ajp.2019.19020151.

McIntosh AM, Owens DC, Moorhead WJ, et al. Longitudinal volume reductions in people at high genetic risk of schizophrenia as they develop psychosis. Biol Psychiatry. 2011 May 15;69(10):953-8. doi: 10.1016/j.biopsych.2010.11.003

Meyer-Lindenberg AS, Olsen RK, Kohn PD, et al. Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. Arch Gen Psychiatry. 2005 Apr;62(4):379-86.

Mier D, Eisenacher S, Rausch F, et al. Aberrant activity and connectivity of the posterior superior temporal sulcus during social cognition in schizophrenia. Eur Arch Psychiatry Clin Neurosci. 2017;267(7): 597-610.

Mikell CB, McKhann GM, Segal S, et al. The hippocampus and nucleus accumbens as potential therapeutic targets for neurosurgical intervention in schizophrenia. Stereotact Funct Neurosurg. 2009;87(4):256–265. doi:10.1159/000225979

Moser DA, Doucet GE, Ing A, et al. An integrated brain-behavior model for working memory. Mol Psychiatry. 2018;23(10):1974–1980. doi:10.1038/mp.2017.247

Nguyen R, Morrissey MD, Mahadevan V, et al. Parvalbumin and GAD65 interneuron inhibition in the ventral hippocampus induces distinct behavioral deficits relevant to schizophrenia. J Neurosci. 2014;34(45):14948–14960. doi:10.1523/JNEUROSCI.2204-14.2014

Nielsen JD, Madsen KH, Wang Z, et al. Working Memory Modulation of Frontoparietal Network Connectivity in First-Episode Schizophrenia. Cereb Cortex. 2017;27(7): 3832-3841.

O'Neill PK, Gordon JA, Sigurdsson T. Theta oscillations in the medial prefrontal cortex are modulated by spatial working memory and synchronize with the hippocampus through its ventral subregion. J Neurosci. 2013;33(35):14211–14224. doi:10.1523/JNEUROSCI.2378-13.2013

Perez SM, Boley A, Lodge DJ Region specific knockdown of Parvalbumin or Somatostatin produces neuronal and behavioral deficits consistent with those observed in schizophrenia. Transl Psychiatry. 2019;9,264. https://doi.org/10.1038/s41398-019-0603-6

Phillips ML, Robinson HA, Pozzo-Miller L. Ventral hippocampal projections to the medial prefrontal cortex regulate social memory. Elife. 2019;8:e44182. Published 2019 May 21. doi:10.7554/eLife.44182

Piontkewitz Y, Arad M, Weiner I. Tracing the development of psychosis and its prevention: what can be learned from animal models. Neuropharmacology. 2012;62(3): 1273-1289.

Powell SB. Models of neurodevelopmental abnormalities in schizophrenia Curr. Top. Behav.

Neurosci., 4 (2010), pp. 435-481, 10.1007/7854_2010_57

Pradhan BS, Majumdar SS. An Efficient Method for Generation of Transgenic Rats Avoiding Embryo Manipulation. Mol Ther Nucleic Acids. 2016;5(3):e293. Published 2016 Mar 8. doi:10.1038/mtna.2016.9

Rapoport JL, Giedd JN, Gogtay N. Neurodevelopmental model of schizophrenia: update 2012 Mol. Psychiatry, 17 (12) (2012), pp. 1228-1238, 10.1038/mp.2012.23

Reilly TJ, Nottage JF, Studerus E, et al. Gamma band oscillations in the early phase of psychosis: A systematic review. Neurosci Biobehav Rev. 2018 Jul;90:381-399. doi: 10.1016/j.neubiorev.2018.04.006.

Reynolds GP, Czudek C, Andrews HB. Deficit and hemispheric asymmetry of GABA uptake sites in the hippocampus in schizophrenia. Biol Psychiatry. 1990;27:1038–1044.

Schaufelberger MS, Lappin JM, Duran FL, et al. Lack of progression of brain abnormalities in first-episode psychosis: a longitudinal magnetic resonance imaging study. Psychol Med. 2011;41(8): 1677-1689.

Schumacher A, Villaruel FR, Ussling A, et al. Ventral Hippocampal CA1 and CA3 Differentially Mediate Learned Approach-Avoidance Conflict Processing. Curr Biol. 2018;28(8): 1318-1324 e1314.

Schumacher A, Vlassov E, Ito R. The ventral hippocampus, but not the dorsal hippocampus is critical for learned approach-avoidance decision making. Hippocampus. 2016 Apr;26(4):530-42. doi: 10.1002/hipo.22542.

Sigurdsson T. Neural circuit dysfunction in schizophrenia: insights from animal models Neuroscience., 321 (2016), pp. 42-65, 10.1016/j.neuroscience.2015.06.059

Sigurdsson T, Duvarci S. Hippocampal-Prefrontal Interactions in Cognition, Behavior and

Psychiatric Disease. Front Syst Neurosci. 2016;9:190. Published 2016 Jan 26. doi:10.3389/fnsys.2015.00190

Sigurdsson T, Stark KL, Karayiorgou M, et al. Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. Nature. 2010;464(7289):763–767. doi:10.1038/nature08855

Skudlarski P, Jagannathan K, Anderson K, et al. Brain connectivity is not only lower but different in schizophrenia: a combined anatomical and functional approach. Biol Psychiatry. 2010;68(1):61–69. doi:10.1016/j.biopsych.2010.03.035

So RP, Kegeles LS, Mao X, et al. Long-range gamma phase synchronization as a compensatory strategy during working memory in high-performing patients with schizophrenia. J Clin Exp Neuropsychol. 2018 Sep;40(7):663-681. doi: 10.1080/13803395.2017.1420142.

Spellman T, Rigotti M, Ahmari SE, et al. Hippocampal-prefrontal input supports spatial encoding in working memory. Nature. 2015;522(7556):309–314. doi:10.1038/nature14445

Stein J, and Richardson A. Cognitive disorders: A question of misattribution. Curr Biol. 1999;9(10): R374-376.

Stephan KE, Friston KJ, Frith CD. Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. Schizophr Bull. 2009;35(3):509–527. doi:10.1093/schbul/sbn176.

Steullet P, Cabungcal JH, Kulak A, et al. Redox dysregulation affects the ventral but not dorsal hippocampus: impairment of parvalbumin neurons, gamma oscillations, and related behaviors. J Neurosci. 2010;30(7):2547–2558. doi:10.1523/JNEUROSCI.3857-09.2010

Stilo SA, Murray RM. Non-genetic factors in schizophrenia Curr. Psychiatry Rep., 21 (10) (2019), p. 100 Published 2019 Sep 14 https://doi.org/10.1007/s11920-019-1091-3

Substance Abuse and Mental Health Services Administration Impact of the DSM-IV to DSM-5 Changes on the National Survey on Drug Use and Health [Internet]. Rockville (MD): Substance Abuse and Mental Health Services Administration (US) Jun. Table 3.22, DSM-IV to DSM-5 Schizophrenia Comparison. Available from https://www.ncbi.nlm.nih.gov/books/NBK519697/

Sun Y, Chen Y, Lee R, et al. Disruption of brain anatomical networks in schizophrenia: A longitudinal, diffusion tensor imaging based study. Schizophr Res. 2016;171(1-3): 149-157.

Sun Y, Dai Z, Li J, et al. Modular-level alterations of structure-function coupling in schizophrenia connectome. Hum Brain Mapp. 2017;38(4): 2008-2025.

Swanson OK, Maffei A. From hiring to firing: activation of inhibitory neurons and their recruitment in behavior Front. Mol. Neurosci. (2019), 10.3389/fnmol.2019.00168;12:168. Published 2019 Jul 3

Swerdlow NR, Halim N, Hanlon FM, Platten A, Auerbach PP. Lesion size and amphetamine hyperlocomotion after neonatal ventral hippocampal lesions: more is less Brain Res. Bull., 55 (1) (2001), pp. 71-77, 10.1016/s0361-9230(01)00492-0

Talati P, Rane S, Kose S, et al. Increased hippocampal CA1 cerebral blood volume in schizophrenia. Neuroimage Clin. 2014;5:359–364. Published 2014 Jul 22. doi:10.1016/j.nicl.2014.07.004

Thompson CL, Pathak SD, Jeromin A, et al. Genomic anatomy of the hippocampus. Neuron. 2008;60(6): 1010-1021.

Thompson M, Weickert CS, Wyatt E, Webster MJ. Decreased glutamic acid decarboxylase(67) mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders. Journal of psychiatric research. 2009;43:970–977.

van den Heuvel MP, Sporns O, Collin G, et al. Abnormal rich club organization and functional

brain dynamics in schizophrenia. JAMA Psychiatry. 2013;70(8): 783-792.

Vanes LD, Mouchlianitis E, Patel K, et al. Neural correlates of positive and negative symptoms through the illness course: an fMRI study in early psychosis and chronic schizophrenia Sci. Rep., 9 (1) (2019), Article 14444 Published 2019 Oct 8 https://doi.org/10.1038/s41598-019-51023-0

Viena TD, Linley SB, Vertes RP. Inactivation of nucleus reuniens impairs spatial working memory and behavioral flexibility in the rat. Hippocampus. 2018;28(4):297–311. doi:10.1002/hipo.22831

Vita A, De Peri L, Deste G, et al. Progressive loss of cortical gray matter in schizophrenia: a meta-analysis and meta-regression of longitudinal MRI studies [published correction appears in Transl Psychiatry. 2013;3:e275]. Transl Psychiatry. 2012;2(11):e190. Published 2012 Nov 20. doi:10.1038/tp.2012.116

Vitolo E, Tatu MK, Pignolo C, et al. White matter and schizophrenia: A meta-analysis of voxel-based morphometry and diffusion tensor imaging studies. Psychiatry Res Neuroimaging. 2017;270: 8-21.

Weinberger, DR. A connectionist approach to the prefrontal cortex. J Neuropsychiatry Clin Neurosci. 1993;5(3): 241-253.

Weinberger DR. The neurodevelopmental origins of schizophrenia in the penumbra of genomic medicine. World Psychiatry. 2017;16(3): 225-226.

Weinberger DR. The pathogenesis of schizophrenia: a neurodevelopmental theory. The Neurology of Schizophrenia (eds RA Nasrallah & DR Weinberger): 387– 405. Elsevier, 1986.

Whitfield-Gabrieli S, Thermenos HW, Milanovic S, et al. Hyperactivity and hyperconnectivity of

the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. Proc Natl Acad Sci USA. 2009;106(4): 1279-1284.

Whitford TJ, Grieve SM, Farrow TF, et al. Volumetric white matter abnormalities in first-episode schizophrenia: a longitudinal, tensor-based morphometry study. Am J Psychiatry. 2007;164(7): 1082-1089.

Williams LM. Voxel-based morphometry in schizophrenia: implications for neurodevelopmental connectivity models, cognition and affect Expert. Rev. Neurother., 8 (7) (2008), pp. 1049-1065, 10.1586/14737175.8.7.1049

Woo TU, Spencer K, McCarley RW. Gamma oscillation deficits and the onset and early progression of schizophrenia. Harv Rev Psychiatry. 2010;18(3):173–189. doi:10.3109/10673221003747609

Zecevic N, Hu F, Jakovcevski I. Interneurons in the developing human neocortex Dev. Neurobiol., 71 (1) (2011), pp. 18-33, 10.1002/dneu.20812

Zhang ZJ, Reynolds GP. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophr Res. 2002 May 1;55(1-2):1-10.

Zhou Y, Shu N, Liu Y, et al. Altered resting-state functional connectivity and anatomical connectivity of hippocampus in schizophrenia. Schizophr Res. 2008 Mar;100(1-3):120-32. doi: 10.1016/j.schres.2007.11.039.

Zhuo C, Wang C, Wang L, et al. Altered resting-state functional connectivity of the cerebellum in schizophrenia. Brain Imaging Behav. 2018;12(2):383–389. doi:10.1007/s11682-017-9704-0

Zimmerman EC, Grace AA. The nucleus Reuniens of the midline thalamus gates prefrontal

hippocampal modulation of ventral tegmental area dopamine neuron activity J. Neurosci., 36 (34) (2016), pp. 8977-8984, 10.1523/JNEUROSCI.1402-16.2016

Bridge: Neurodevelopment insights from vHPC-PFC dissection of dysconnectivity

Chapter 2 establishes that schizophrenia is a disorder of dysconnectivity that emerges during development. It provides a methodological framework for dissecting early developmental changes contributing to schizophrenia pathogenesis. This framework included use of transgenic mouse lines and viral tools for the cell-type specific and temporally specific bidirectional manipulation of schizophrenia-implicated brain region activities.

The next chapter applies the tools mentioned above to explore which vHPC cell population is involved in the generation of PFC functional deficits and characterizes the nature of these PFC deficits in different cell populations. We utilized transgenic mouse lines along with a viral tool in order to ablate either CaMKII expressing, or PV expressing cells in the vHPC of young pre-pubescent mouse pups. We then utilized whole-cell recordings to characterize changes in pyramidal cell and PV interneuron activity in the PFC once mice reached adulthood. Briefly, our results highlight that CaMKII-vHPC cells are responsible for PFC deficits, and that both pyramidal cells and interneurons show functional alterations in the mature PFC. The detailed insights gained from the application of the methodological framework established in the previous chapter are laid out in the following chapter: “Altered excitatory and decreased inhibitory transmission in the prefrontal cortex of male mice with early developmental disruption to the ventral hippocampus”.

Chapter 3: Altered excitatory and decreased inhibitory transmission in the prefrontal cortex of male mice with early developmental disruption to the ventral hippocampus

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Abstract

Ventral hippocampal (vHPC)-prefrontal cortical (PFC) pathway dysfunction is a core neuroimaging feature of schizophrenia. However, mechanisms underlying impaired connectivity within this pathway remain poorly understood. The vHPC has direct projections to the PFC that help shape its maturation. Here, we wanted to investigate the effects of early developmental vHPC perturbations on long-term functional PFC organization. Using whole-cell recordings to assess PFC cellular activity in transgenic male mouse lines, we show early developmental disconnection of vHPC inputs, by excitotoxic lesion or cell-specific ablations, impairs pyramidal cell firing output and produces a persistent increase in excitatory and decrease in inhibitory synaptic inputs onto pyramidal cells. We show this effect is specific to excitatory vHPC projection cell ablation. We further identify PV-interneurons as a source of deficit in inhibitory transmission. We find PV-interneurons are reduced in density, show a reduced ability to sustain high-frequency firing, and show deficits in excitatory inputs that emerge over time. We additionally show differences in vulnerabilities to early developmental vHPC disconnection, wherein PFC PV-interneurons but not pyramidal cells show deficits in NMDA receptor–mediated current. Our results highlight mechanisms by which the PFC adapts to early developmental vHPC perturbations, providing insights into schizophrenia circuit pathology.

Keywords

animal model, electrophysiology, dysconnectivity, parvalbumin interneuron, schizophrenia

Introduction

The ventral hippocampal (vHPC)-prefrontal cortical (PFC) circuitry plays important roles in cognition and social behaviors (Sigurdsson and Duvarci 2016; Phillips et al. 2019; Sun et al. 2020). These behaviors are notably impaired in schizophrenia with limited treatment options available (Goff et al. 2011). Altered functional connectivity at baseline and during task processing within this pathway has been observed in at-risk subjects and patients of schizophrenia (Meyer-Lindenberg et al. 2005; McHugo et al. 2019), and in genetic and environmental animal models of schizophrenia, suggesting that vHPC-PFC dysfunction may be a core pathophysiological characteristic of this disorder (Bähner and Meyer-Lindenberg 2017). Schizophrenia is widely believed to be a neurodevelopmental disorder; however, the mechanisms by which vHPC-PFC dysfunction emerge in adolescence/early adulthood are poorly understood.

The vHPC has direct excitatory projections to the PFC, which sculpt PFC activity patterns at an early age (Brockmann et al. 2011; Ahlbeck et al. 2018). Early developmental risk factors are thought to impinge on vHPC function (Froudist-Walsh et al. 2017; Murray et al. 2017; Cachia et al. 2020), which in turn would affect its influence on the development of other brain regions. Accordingly, neonatal, but not adult, lesioning of the vHPC in postnatal day 7 (P7) rats produces schizophrenia-relevant behaviors that emerge postpubertally, including working memory and social behavioral deficits (Lipska et al. 1993; Lipska and Weinberger 2000; Tseng et al. 2009). This putative neurodevelopmental model of schizophrenia shows cellular and molecular changes in the PFC, including altered microglia (Hui et al. 2019), loss of pyramidal cell dendritic spines (Flores et al. 2009), and altered glutamatergic (Stine et al. 2001), GABAergic (Lipska et al. 2003; Endo et al. 2007; François et al. 2009), adrenergic (Bhardwaj et al. 2014), and dopaminergic

transmission (Flores et al. 2009)–associated mRNA and proteins. While previous studies have found differences in pyramidal and fast-spiking interneuron functional responses to dopaminergic receptor agonists or dopaminergic pathway stimulation in the lesion model (O'Donnell et al. 2002; Tseng et al. 2007, 2008), how differences in excitatory and inhibitory markers translate functionally requires further investigation.

To address this question, we previously recorded pyramidal cell activity in adult rats following neonatal vHPC lesioning and found deficits in inhibitory input frequency onto pyramidal cells (Ryan et al. 2013). However, the potential source of deficit in inhibitory transmission was not investigated. There is still a limited understanding of possible differences in spiking properties and basal synaptic transmission in different PFC cell types, including pyramidal and interneuron populations that shape PFC output and therefore behavior. This limitation is imposed by the genetic inflexibility of rat models, preventing the identification and manipulation of specific cell type populations (Pradhan and Majumdar 2016). We therefore wanted to investigate the effects of early developmental vHPC manipulations on PFC functional organization using transgenic mice with viral delivery of diphtheria toxin (DT). This method enabled us to dissect the differential contributions of vHPC populations on PFC maturation, by cell-type specific ablations using CaMKII-cre or PV-cre transgenic mouse lines and an adeno-associated virus (AAV) with cre-dependent expression of DT subunit in the vHPC. This method also allowed us to determine differences in PFC pyramidal and PV-interneuron functional maturation patterns, by using a transgenic PV-tdTomato mouse line enabling visual identification of PV-expressing cells in the PFC.

We found early developmental excitotoxic vHPC lesioning in mice impairs PFC pyramidal cell spiking output and increases excitatory and decreases inhibitory synaptic inputs from early adolescence to adulthood. These synaptic effects were reproduced through virally targeted partial ablation of CaMKII-expressing vHPC cells, but not PV-expressing vHPC cells. PFC PV-expressing interneurons in turn are lower in cell density, have a reduced ability to sustain high-frequency firing, and show deficits in excitatory synaptic inputs that emerge in adulthood. Moreover, PV but not pyramidal cells show functional deficits in NMDA receptor–mediated currents. Our results therefore provide mechanistic insights into PFC adaptations to early vHPC disconnection.

Materials and methods

Animals

Male transgenic mice of C57BL6 background were used, including CaMKII-cre (Jackson Laboratory; stock no. 005359) and PV-cre (Jackson Laboratory; stock no. 017320) mice. Male mice data are presented here primarily due to a more pronounced and more similar behavioral progression of schizophrenia-like behaviors compared to females (Tseng et al. 2009), and a lack of significant electrophysiological findings in female mice. PV-cre mice were crossed with Ai9 mice (Jackson Laboratory; stock no. 007905) to label PV expressing cells with the fluorescent protein tdTomato (PV-tdTomato). Mice were housed under controlled conditions of light (12 h light:12 h dark), temperature, and humidity with ad libitum access to food and water. All experiments were approved by the Facility Animal Care Committee at the Douglas Research Centre and were in accordance with the Canadian Council on Animal Care guidelines.

Stereotaxic surgery

Mouse litters were randomly divided into control or experimental groups. To lesion the vHPC, 0.25 µL of ibotenic acid (Tocris, 10 µg/µL, lesion mice) was stereotaxically injected bilaterally into the vHPC of PV-tdTomato mice. About 0.25 µL of phosphate-buffered saline (PBS, 0.1 M, pH 7.4) was injected in sham controls. To ablate specific cell-type populations in the vHPC, 0.25 µL of AAV8/EF1.flex.dtA.mCherry (University of North Carolina Vector Core Centre; titer = 3.7 × 1012 genome copies per mL, DT mice) was stereotaxically injected. dtA encodes diphtheria toxin subunit A, which blocks protein synthesis (abbreviated as DT in mice in which toxin was injected). The injection was performed bilaterally into the vHPC in CaMKII-cre mice to ablate excitatory vHPC CaMKII neurons (Liu and Murray 2012) or PV-cre mice to ablate vHPC PV neurons. Control mice for viral ablations were injected with 0.25 µL of AAV8.DIO.mCherry (Canadian Neurophotonics Platform, Laval University; titer = 1.7 × 1013 genome copies per mL). All injections were performed in postnatal day 14 (P14) mice using the following coordinates from bregma for the vHPC: anterior/posterior: −2.8; lateral: ±2.8; dorsal/ventral: −3.2.

Histological verification of lesion and cell ablations

For PV-tdTomato mice, the ventral half of the brain during brain extraction for electrophysiology was snap frozen and stored in −80 °C. About 50-µm-thick vHPC coronal slices were obtained using a cryostat microtome (Leica). Sections were mounted on gelatin-precoated glass slides and stained with cresyl violet (0.5%).

For CaMKII-cre or PV-cre DT ablated mice, P60 mice were anesthetized with a ketamine/xylazine mixture prior to transcardial perfusion with PBS solution followed by 4% paraformaldehyde

fixative. Fixed brains were sectioned into 40-µm-thick coronal slices using a vibratome (VT1200s, Leica). Sections were washed in PBS, prior to blocking in 0.3% Triton-X and normal goat serum. Sections stained with PV antibody (Sigma, P-3088) were incubated for 2 h at room temperature before overnight 4 C incubation with the primary antibody at 1:1000. Sections stained with CaMKII antibody (Invitrogen, 13-7300) underwent a 30-min antigen retrieval step in 3% H2O2, followed by incubation with the primary for 2 h at room temperature before overnight incubation at 4 C at 1:500. Secondary Alexa Fluor 488 conjugated goat antimouse antibody (Life Technologies, A11034) was used at 1:2,000, incubated for 2 h. Sections were finally mounted on slides in DAPI containing mounting medium. At least three sections were examined and averaged per animal.

PFC PV-cell density measures

PV-tdTomato P60 mice were perfused with PBS solution followed by 4% paraformaldehyde fixative and fixed brains were collected and sectioned into 30-µm-thick coronal sections by vibratome. Sections were washed with PBS and mounted on slides using DAPI containing VectaShield mounting medium (Vector Laboratories). Endogenous PV-tdTomato signaling was imaged using the Olympus BX-63 microscope. Cell counts were performed using Image J by the experimenter blind to experimental conditions. Cell density was calculated as the number of PV cells over manually delineated PFC prelimbic and infralimbic layers using the Allen Brain Atlas as reference and using ImageJ. At least three sections were examined and averaged per animal.

cFos-positive cell density measures

CaMKII-cre and PV-cre P14 mice were unilaterally injected in the vHPC with AAV.DIO.mCherry in one hemisphere and AAV/EF1.flex.dtA.mCherry in the contralateral hemisphere. At P60, 90 min following a 10-min open-field exploration to stimulate cFos expression (Hale et al. 2008), the mice were perfused with PBS solution followed by 4% paraformaldehyde fixative. Unilateral viral ablations had no effect on open-field exploration between groups, likely because the viral ablations were unilateral as opposed to bilateral. Fixed brains were nicked in the cortical tissue to mark the DT viral infusion side and then sectioned using a vibratome to yield 30-µm-thick vHPC slices. Sections were washed in PBS, incubated for 30 min in 0.3% $NaBH_4$, washed in 0.1% Triton-X, and incubated for 1 h in 3% normal goat serum, followed by overnight incubation in 1:500 rabbit polyclonal cFos antibody (Sigma, F137). Secondary Alexa Fluor 488 conjugated goat antirabbit antibody (Life Technologies, A11034) was used at 1:1,000, incubated for 3 h. Sections were finally mounted on slides in DAPI containing mounting medium. At least three sections were examined, with the number of cFos positive cells counted over manually delineated hippocampal areas using the Allen Brain Atlas as reference and using ImageJ, and densities were averaged per animal.

RNA extraction and qRT-PCR

Brains were quickly extracted from decapitated mice and stored at −80 C until further use. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) were performed as described previously (Joseph et al. 2018). Briefly, 0.5-mm-thick PFC tissue was dissected. RNA extraction was performed using Trizol (Catalog No. 15596026; Thermo Fisher Scientific) and the Purelink RNA minikit (Catalog No.12183018A; Thermo Fisher Scientific). RNA quality was

assessed using Nanodrop. Two micrograms of RNA were used for cDNA synthesis using the high-capacity cDNA reverse transcription kit (Catalog No. 4368814; Applied Biosystem).

qPCR was performed using SYBR green PCR master mix (Catalog No. A6001; Promega). The following primers were used: GAPDH (AGCCCAGAACATCATCCCTG), PV (CTGGACAAAGACAAAAGTGGC), GluA1 (ACCCTCCATGTGATCGAAATG), GluN1 (AAATGTGTCCCTGTCCATACTC), GluN2A (TGGTGATCGTGCTGAATAAGG), and GluN2B (AAGGAGAGGAAGTGGGAGAG). qPCR was performed using Applied Biosystem real-time PCR 7500 with the following cycling conditions: initial denaturation at 95 C for 10 min, followed by 40 cycles of denaturation at 95 C for 15 s, annealing at 60 C for 1 min and elongation at 72 C for 1 min. Changes in RNA expression was quantified using the $2-\Delta\Delta CT$ method.

Slice preparation for electrophysiology

Two-month-old mice were anesthetized with isoflurane before being decapitated. The brain was rapidly removed and immersed in ice-cold carbogenated (95% O2 and 5% CO2) artificial cerebrospinal fluid (ACSF) containing in (mM): 125 NaCl, 2.5 KCl, 2 CaCl2, 2 MgCl2, 1.25 NaH2PO4, 26 NaHCO3, and 25 glucose (310–320 mOsm). All reagents were obtained from Sigma-Aldrich (St Louis, MO, USA), unless stated otherwise. About 300-µm-thick PFC coronal slices were obtained using a vibratome (VT1200s, Leica). Slices were subsequently incubated in carbogenated ACSF for 1 h at 32 C and then for 1 h at room temperature prior to recording. Sham and lesion mice or control and virally ablated mice were interleaved to ensure similar recording conditions across groups.

Electrophysiological recordings

Whole-cell recordings were performed as previously described in layer V of the PFC (Tse et al. 2011; Ryan et al. 2013). Slices were transferred to a recording chamber perfused continuously with carbogenated ACSF. Recordings were performed at room temperature. Patch pipettes were pulled from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL, USA). For voltage-clamp recordings of miniature postsynaptic activity, patch pipettes were filled with intracellular solution composed of (in mM): 110 Cs-Gluconate, 17.5 CsCl, 10 HEPES, 2 MgCl2, 0.5 ethylene glycol tetraacetic acid (EGTA), 4 ATP, and 5 QX-314 (pH 7.25, 280–290 mOsm). For current-clamp recordings of spiking and voltage-clamp recordings of spontaneous postsynaptic activity, the intracellular solution component Cs-gluconate was replaced with 120 mM of K-gluconate, CsCl was replaced with KCl, and QX-314 was not present.

For miniature excitatory and inhibitory postsynaptic activity, 0.5 μM of TTX (Alomone Labs, Jerusalem, Israel) was added to the perfusing ACSF to block voltage-gated sodium channels. Whole-cell patch-clamping was visualized using an upright microscope (Olympus) equipped with IR-DIC and fluorescent optics (for PV-tdTomato cell visualization). Once patched, cells were injected with 200 ms hyperpolarizing pulses to measure the input resistance. Cells were held at −60 mV to measure excitatory postsynaptic activity or +10 mV to measure inhibitory postsynaptic activity.

For NMDA receptor–mediated excitatory postsynaptic current (EPSC) recordings, slices were sequentially perfused with ACSF (with 2 mM magnesium) to measure AMPA receptor–mediated current and low magnesium (0.1 mM) ACSF solution to measure AMPA and NMDA receptor–

mediated composite currents, or vice versa with a minimum 5-min washout period between solutions. Cells were clamped at −60 mV throughout the recording. The change in NMDA receptor–dependent current was calculated as the difference in area of mEPSCs under low and high magnesium divided by the area under high magnesium (Gingrich et al. 2004). For evoked NMDA and AMPA receptor–mediated current recordings, slices were perfused with regular ACSF (with 2 mM magnesium) containing 20 μM picrotoxin. Evoked responses were recorded in cells clamped at −60 mV and +40 mV using the same stimulus strength.

All recordings were performed using a MultiClamp 700B amplifier (Molecular Devices), filtered at 2 kHz, sampled at 10 kHz, and acquired with the pCLAMP 10 program (Molecular Devices). Cells with an access resistance greater than 25 MΩ were omitted; mean values per group of recorded cells are in Table 1. No electronic compensation for series resistance was applied. Data were analyzed offline using the Mini Analysis Program 6.0.3 (Synaptosoft, Decatur, GA, USA) for synaptic events, and Clampfit (Molecular Devices) for input resistance and firing properties.

Experimental design and statistical analyses

Statistical analyses were performed using GraphPad Prism 6. The Shapiro–Wilk test and Levene's test were used to assess assumptions of normality and equality of variances, respectively. A two-way or three-way analysis of variance (ANOVA) with simple main effect and interaction analyses, or the nonparametric Kruskal–Wallis H test with post-hoc application of the Mann–Whitney U test using a Bonferroni corrected α-value was used for the statistical analysis of multiple groups. An unpaired t-test or the nonparametric Mann–Whitney U test was used for the statistical analysis between two groups. The Kolmogorov–Smirnov (K-S) test was used to examine differences in

sample distributions for cumulative probability plots, in which the interevent interval of the first 50 consecutive events from each recording was cumulated and compared between groups. A critical value of 0.05 was used for all tests except the K-S test in which a critical value of 0.01 was used. All data are presented as mean ± SEM. Number of recorded cells and animals are provided within each figure.

Results

Synaptic reorganization in the PFC after ibotenic vHPC lesion

To confirm whether early developmental vHPC lesioning in mice produces similar changes in PFC pyramidal cell functional activity as previously characterized in rats (Ryan et al. 2013), we bilaterally injected ibotenic acid into the vHPC in P14 mice and performed whole-cell voltage-clamp slice recordings of PFC pyramidal cells from adult (P60) mice. While mouse injections were slightly delayed compared to the rat model due to technical limitations, this age still corresponds to peak brain growth prior to a plateauing in brain volume by P20 in both rats and mice (Semple et al. 2013). Although sham and lesion mice show no significant differences in cell membrane properties, such as resting membrane potential or action potential threshold similar to previous findings (Table 1), a two-way ANOVA analysis reveals a significant main effect of condition (sham vs. lesion), in which pyramidal cells of lesion mice show deficits in spiking output in response to current injections ($F(1,380) = 58.47$, $P < 0.001$; Fig. 1A). No significant interaction effect was observed between mouse condition (sham vs. lesion) and current injected, although there may be a trend ($F(18,380) = 1.59$, $P = 0.06$). This deficit in spiking output may be partially attributable to changes in action potential properties. Lesion mice show a reduced action potential amplitude ($t(20) = 2.44$, $P = 0.02$; Fig. 1A) with no significant changes in fast afterhyperpolarization

amplitude (sham: 6.3 mV ± 0.7, lesion: 5.9 mV ± 0.5) or in rheobase (sham: 106pA ± 11, lesion: 97pA ± 12).

To assess whether a deficit in pyramidal cell output in lesion mice may additionally be due to changes in synaptic excitatory and inhibitory inputs, we examined the frequency and amplitude of action potential–dependent (spontaneous) and action potential–independent (miniature) excitatory and inhibitory postsynaptic currents (sEPSC, sIPSC, mEPSC, mIPSC). No significant differences were observed in sEPSC or sIPSC frequency or amplitude between sham and lesion mice (Fig. 1B and C). While no significant changes in mean mEPSC frequency was observed, a significant difference in the cumulative distribution of mEPSC inputs was found (K-S analysis, $D = 0.113$, $P = 0.006$, Fig. 1B). This discrepancy between mean versus cumulative distribution likely reflects an altered distribution in inputs, such that while the overall total number of events over the recorded time (i.e. frequency) was similar between conditions, the time interval between events varied. Examining the mEPSC cumulative distribution plot, we see lesion mice show a greater cumulation of lower interevent interval mEPSC events relative to control ($D = 0.12$, $P = 0.003$), suggestive of increased mEPSC excitation. We additionally observe in the mEPSC amplitude cumulative distribution with more events of reduced amplitude in lesion mice ($D = 0.191$, $P < 0.001$). In contrast, a significant decrease in mean mIPSC frequency ($t(16) = 2.24$, $P = 0.04$) and an according change in mIPSC interevent interval distribution ($D = 0.184$, $P < 0.001$) in lesion mice were observed (Fig. 1C). No significant changes in the amplitude of mIPSC events were observed (Fig. 1C). These results suggest vHPC lesioning in P14 mice produces changes in pyramidal cell functional properties. Some of these changes, such as altered action potential properties and altered distribution in mEPSC interevent intervals, contrast with findings from the

rat model (Ryan et al. 2013), suggesting that slight differences in lesioning age and/or different mechanisms may be at play. In the lesion mouse model, deficits in spiking output may be attributable to changes in action potential properties and altered miniature event frequency, including a deficit in inhibitory inputs.

Schizophrenia is characterized by progressive changes in brain structure and functional connectivity, with changes observable prior to the onset of schizophrenia-like symptoms (McIntosh et al. 2011; Ho et al. 2017). To find out if the synaptic reorganization we observed in P60 mice occurs in younger brains after lesion, we recorded changes in spontaneous and miniature synaptic inputs onto pyramidal PFC cells in P21 mice, a week following lesioning. While no significant changes in mean sEPSC and sIPSC frequency or amplitude were observed (Fig. 2A and B), we found a significant difference in the cumulative distribution in sIPSC interevent intervals ($D = 0.184$, $P < 0.001$; Fig. 2B) between sham and lesion mice. sIPSC inputs in lesion mice tend to be of larger interevent intervals. In addition, we found a trend toward increased mean mEPSC frequency ($t(13) = -1.8$, $P = 0.09$; Fig. 2A) and a significant decrease in mean mIPSC frequency ($t(17) = 2.635$, $P = 0.02$; Fig. 2B) in lesion mice. These results were accordingly accompanied by significant changes in the cumulative distribution in mEPSC interevent intervals ($D = 0.34$, $P < 0.001$; Fig. 2A) and mIPSC ($D = 0.166$, $P < 0.001$; Fig. 2B) interevent intervals, and a significant decrease in mIPSC amplitude ($t(17) = 3.444$, $P = 0.003$; $D = 0.265$, $P < 0.001$ Fig. 2B) but not mEPSC amplitude. These results demonstrate progressive changes in PFC synaptic inputs onto pyramidal cells, some of which normalize over time (such as sIPSC events) while others persist into adulthood (such as decreased mIPSC frequency and greater proportion of decreased mEPSC interevent intervals) (Table 2).

Synaptic reorganization in the PFC after selective ablation of excitatory or inhibitory (PV) vHPC neurons

The vHPC has direct projections to the PFC (Liu and Carter 2018), but altered activity within spared areas of the hippocampus may instead mediate schizophrenia-like behavioral deficits in the rat lesion model (Swerdlow et al. 2001). To differentiate the effects of projection neurons as opposed to altered activity, we ablated CaMKII-expressing vHPC cells (presumed projection cells) or PV-expressing vHPC cells (altered vHPC activity by ablating local interneurons). To do this, we used CaMKII-cre or PV-cre transgenic mice and injected a viral vector expressing diphtheria toxin (DT) that is dependent on the Cre-recombinase enzyme for expression. This toxin induces cell death by blocking cell protein synthesis (Brockschnieder et al. 2004) and was injected bilaterally in the vHPC in P14 mice, leading to the reduction in CaMKII or PV-expressing cell density (Fig. 4A; Supplementary Fig. 1A). To confirm that ablation of CaMKII-expressing and PV-expressing vHPC cells led to a predicted respective decrease and increase in vHPC activity, we stained for the immediate early gene marker cFos in adult (P60) mice and counted cFos-positive cells by area (not colabeled with CaMKII or PV markers). Two-way ANOVA analysis reveals a significant interaction effect of cell-type ablation and virus use on vHPC CA1 (vCA1) cFos-positive cell density ($F(1,8) = 29.34$, $P = 0.00063$). Viral-ablation of CaMKII-expressing cells results in decreased cFos-positive vCA1 cell density (post-hoc Tukey's HSD test, $P = 0.03$), whereas viral-ablation of PV-expressing cells results in increased cFos-positive vCA1 cell density compared to control ($P = 0.001$) (Fig. 3). Whereas the decreased cFos cell density was localized to the vCA1 in the CaMKII-ablation group, the increased cFos cell density was also present in the dorsal CA1 in the PV-ablation group ($F(1,8) = 10.26$, $P = 0.01$; post-hoc Tukey's HSD test, $P = 0.007$). This increase in the dCA1 may reflect the ablation of PV cells that may be innervating

CA1 cells in dorsal and ventral areas, or other differential innervation patterns (Lee et al. 2014). No significant differences in cFos-positive cell density were observed in the caudal dentate gyrus or CA3 areas in either groups (Fig. 3). The viral ablation of CaMKII- versus PV-expressing vHPC cells therefore contributed to hypo- and hyperexcitability in the vCA1, respectively.

We next performed whole-cell recordings in PFC pyramidal cells in adult (P60) mice to examine the differential effects of these ablated vHPC populations. In contrast to the lesion model, viral-ablation of vHPC CaMKII or PV-expressing cells has no effect on pyramidal cell firing output (Fig. 4B; Supplementary Fig. 1B). No significant effects were observed in mean sEPSC and sIPSC frequency or amplitude, although more events with reduced sEPSC interevent intervals were observed following CaMKII-vHPC ablation ($D = 0.209$, $P < 0.001$, Fig. 4C). In regard to miniature activities, while no significant differences in mean mEPSC frequency or amplitude was observed in either model, a similar distribution to sEPSCs with reduced interevent intervals was observed in the CaMKII-cre vHPC ablated model ($D = 0.186$, $P < 0.001$; Fig. 4C). In addition, a significant decrease in mean mIPSC frequency in the CaMKII-cre vHPC ablated model was observed ($H(3,39) = 10.918$, $P = 0.01$; post-hoc Mann Whitney U, $P = 0.006$; Fig. 4D), accompanied by a significantly changed mIPSC interevent interval distribution ($D = 0.256$, $P < 0.001$, Fig. 4D). More mIPSC events in CaMKII-cre vHPC ablated group were also of lower amplitude than controls ($D = 0.218$, $P < 0.001$; Fig. 4D). None of these changes were observed in the PV-cre vHPC ablated model (Supplementary Fig. 1). These results demonstrate that the CaMKII-cre vHPC ablation model recapitulates the ibotenic acid lesion model with respect to changes in mEPSC and mIPSC interevent intervals and decreased mean mIPSC frequency (Table 2). Changes in PFC functional

inputs therefore may primarily arise through the ablation of projection cells from the vHPC to the PFC.

Anatomical and functional changes in PFC PV neurons after vHPC lesion

Given that changes in inhibitory inputs onto PFC pyramidal cells appear to be a consistent finding in different variants of the vHPC lesion model, we wanted to identify the source of altered inhibitory activity in the PFC. We characterized PFC PV-interneurons, given that this interneuron population preferentially receives inputs from the vHPC compared to other interneuron subgroups (Liu and Carter 2018; Marek et al. 2018; Phillips et al. 2019), has a protracted maturation period (Doischer et al. 2008), and has been implicated in schizophrenia pathophysiology (Lewis et al. 2012). To examine changes in PFC-PV cells, we performed excitotoxic vHPC lesions in P14 transgenic PV-tdTomato mice, in which fluorescent PV-expressing cells can be visually identified. We first examined whether a deficit in pyramidal inhibitory inputs was due to a decrease in PV-cell density. A three-way ANOVA between region, layers, and condition reveals a significant main effect of condition (sham vs. lesion), with lesion mice showing a deficit in PV-cell density ($F(1,36) = 21.26$, $P < 0.001$; Fig. 5). Post-hoc Tukey's HSD test reveals that these deficits were specific to layers 2/3 and 5, but not layer 6. Cell density counts in layer 1 were omitted since there were little to no cells expressing PV. Finally, the differences in cell density were the same across the prelimbic and infralimbic areas. No significant interaction effect was observed between the mouse condition (sham vs. lesion), the PFC area (prelimbic vs. infralimbic), and the PFC layer ($F(2,36) = 0.12$, $P = 0.88$; Fig. 5). A deficit in pyramidal inhibitory inputs may therefore be attributable to a decreased density in PV-expressing cells.

In addition to a decreased density of PV-positive cells, we wanted to characterize the functional properties of remaining PV cells. While previous studies have shown altered dopaminergic inputs in presumed PV cells in the rat model (Tseng et al. 2008), these cells were selected for recordings based on their fast-spiking properties, which may not be exclusive to PV cells (Ma et al. 2006; Golomb et al. 2007). We thus performed whole-cell recordings in visually identified PV-interneurons of PFC slices in PV-tdTomato mice, to better assess changes in functional properties specific to this population of cells (Fig. 6A). We first assessed changes in spiking output in response to varying current injections of 1 s duration. While no significant differences in resting membrane potential, action potential threshold (Table 1), or frequency of firing (number of spikes/duration of firing) were observed (Fig. 6A), we found variable changes in the firing patterns. Specifically, at lower current injections, PV cells from lesion mice appear to be more excitable based on an increased number of spikes (Mann Whitney U: 60pA, $P = 0.008$; 80pA, $P = 0.02$) and increased duration of firing (Mann Whitney U: 60pA, $P = 0.008$; 80pA, $P = 0.01$; Fig. 6A). In contrast, at higher current injections, while PV cells from sham mice are able to sustain high-frequency firing throughout the 1-s pulse current injection, PV cells from lesion mice are only able to sustain this firing frequency for a significantly reduced duration of time (Mann Whitney U: for currents including and above 260pA, $P < 0.05$; Fig. 6A). No significant differences in interspike interval (sham: 23.2 ms ± 4.6, lesion 21.9 ms ± 2.8), action potential amplitude (sham: 53.4 mV ± 3.0, lesion 51.8 mV ± 4.4) and fast afterhyperpolarization (sham: 15.4 mV ± 0.5, lesion 15.1 mV ± 0.7) were observed.

We next assessed whether PFC PV cells show changes in synaptic inputs. While no significant changes in mean sEPSC or mEPSC frequency were observed, we found sEPSC events tend to be

of greater interevent intervals in lesion mice ($D = 0.246$, $P < 0.001$; Fig. 6B). No changes in sEPSC or mEPSC amplitudes were observed (Fig. 6B). We also attempted to measure inhibitory inputs (sIPSC and mIPSC) onto PV cells but failed to record an ample number of responses in both sham and lesion animals during our recording window. These results suggest that early developmental vHPC lesioning alters action potential–dependent excitatory inputs onto PV cells.

Similar to PFC pyramidal cells, we wanted to examine the progressive nature of changes in synaptic inputs onto PV cells. We therefore recorded PFC PV cells in P21 mice, one-week postlesioning. We found no significant differences in mean sEPSC and mEPSC frequency; however, a greater proportion of both sEPSC and mEPSC inputs were of lower amplitude (Fig. 6C). Thus, inputs to the PV evolve over time (Table 2).

NMDA receptors function as important sensors of local circuit glutamatergic levels, and deficits in function are implicated in schizophrenia pathology (Bygrave et al. 2019). To determine whether NMDAR hypofunction may be expressed in the PFC of our lesion model, we performed qRT-PCR to examine the mRNA levels of different glutamate receptors in PFC tissue. We found a significant decrease in mRNA levels of NMDA receptor subunits GluN1 ($U(6,6) = 32$, $P = 0.02$), GluN2A ($U(6,6) = 34$, $P = 0.009$), and GluN2B ($U(6,6) = 34$, $P = 0.009$), but not AMPA receptor subunit GluA1 (Fig. 7A). We next recorded pyramidal and PV PFC cell activity to see if a decrease in NMDA receptor mRNA levels translates to a functional deficit in NMDA receptor current. To do this, we recorded EPSCs under low (unblocked NMDA receptors) and high (blocked NMDA receptors) magnesium conditions in order to differentiate the NMDA component of currents (Fig. 7B). While pyramidal cells do not show a deficit in NMDA receptor–mediated current, PV cells

show a significant decrease in NMDA receptor–mediated current in lesion mice ($t(11) = 2.29$, $P = 0.04$; Fig. 7B). To further confirm a deficit in NMDA receptor–mediated currents, we measured the ratio of NMDA to AMPA currents evoked by stimulation in PV cells and found a significant deficit in the NMDA:AMPA current ratio ($t(10) = 2.615$, $P = 0.03$; Fig. 7C). Despite tissue level changes in NMDA-receptor subunit mRNA expression, our electrophysiological recordings suggest that PV but not pyramidal PFC cells show functional deficits in NMDA receptor–mediated currents in vHPC lesion mice.

Discussion

We have characterized progressive changes in the functional properties of pyramidal and PV PFC cells following early developmental vHPC perturbations. We found that early developmental lesioning of the vHPC in mice produces deficits in pyramidal cell spiking output and reduces the ability of PV cells to sustain high-frequency firing patterns. We also found altered distributions in action potential–dependent (spontaneous) and action potential–independent (miniature) excitatory and inhibitory synaptic inputs emerging at least one-week postlesioning, including more mIPSC events with increased interevent intervals and mEPSC events with decreased interevent intervals in PFC pyramidal cells, which persisted into adulthood. We further highlight the differential susceptibility to NMDA receptor current deficiencies of PV but not pyramidal PFC cells. Moreover, we showed the differential contribution of vHPC projection cells versus altered vHPC activity on PFC maturation, wherein ablating CaMKII-vHPC presumed projection cells recapitulated changes observed in the mouse lesion model compared to ablating PV-vHPC cells. Our study therefore provides important functional insights into the differential maturation patterns of different cell types in the PFC, and the differential effects of different vHPC populations on PFC maturation.

Our data show that early developmental vHPC lesioning produces deficits in pyramidal spiking output. While this deficit may be a result of ablated direct projections from the vHPC to the PFC, it may also be mediated by altered activity in other regions. In addition to the PFC, the vHPC has direct projections to the nucleus accumbens, which results in dopaminergic release (Grace 2012). Altered dopaminergic signaling following vHPC lesioning may contribute to deficits in pyramidal cell spiking output and changes in action potential properties. Studies in the neonatal rat vHPC lesion model show differences in pyramidal responses to dopamine receptor 1 and 2 (D1 and D2) agonists, wherein the D1 agonist has an enhanced excitatory effect on pyramidal cells while the D2 agonist fails to inhibit pyramidal cell spike activity in lesion rats compared to controls (Tseng et al. 2007). This altered dopaminergic signaling may mediate a decreased firing capacity (Anderson et al. 2019) and modulate the action potential waveform in our model (Cantrell et al. 1999; Seamans and Yang 2004; Few et al. 2007; Yang et al. 2013). Interestingly, we did not observe a significant deficit in pyramidal cell firing following virally targeted partial ablation of vHPC-CaMKII-expressing cells. This absence of effect may be due to other mechanisms, such as an extensive immune response to the lesion as opposed to the viral ablation, or spared connections. It would be of interest to manipulate vHPC-PFC versus vHPC-nucleus accumbens pathways in order to gain a better understanding of the different mechanisms mediating altered pyramidal firing.

PFC pyramidal cells were also found to exhibit decreased mIPSC frequency. This decrease was observed as early as P21 and was found in both the ibotenic acid lesion model and CaMKII-vHPC ablated model. We further identified PFC PV cells as a possible source of this deficit in mIPSC onto pyramidal cells. We found a decrease in PV cell density, consistent with previous findings in

the rat vHPC lesion model and in schizophrenia patients (François et al. 2009). We further showed that these cells fail to sustain high-frequency firing and have reduced NMDA receptor–mediated currents in contrast to pyramidal cells despite a tissue-wide decrease in mRNA of NMDA-receptor subunits. While knocking-out NMDA receptors in either pyramidal or PV interneurons can produce schizophrenia-like behavioral deficits (Bygrave et al. 2019), our results suggest that PV cells are particularly vulnerable to developing a functional deficit in NMDA receptor–mediated current. This deficit may be more evident in PV cells due to the smaller contribution of NMDA receptors to EPSCs in PV cells compared to pyramidal cells (Gonzalez-Burgos and Lewis 2012). Alternatively, PV cells may be more vulnerable to oxidative stress, which has been linked with both NMDA-receptor hypofunction (Hasam-Henderson et al. 2018; Hardingham and Do 2016) and a decreased ability to sustain high-frequency firing (Inan et al. 2016). In fact, vHPC lesioning in the rat model was shown to increase oxidative stress levels in the brain (Negrete-Díaz et al. 2010). Therefore, PFC PV cell deficits in firing capacity and NMDA-receptor hypofunction may be through mechanisms related to increased susceptibilities to oxidative stress.

Both PFC pyramidal and PV cells showed changes in excitatory synaptic inputs. While pyramidal cells show more mEPSC events with lower interevent intervals from early adolescence into adulthood, PV cells show more sEPSC events with increased interevent intervals that emerges in adulthood only. The greater proportion of lower mEPSC interevent intervals in pyramidal cells may be compensatory to the developmental ablation of excitatory vHPC inputs. The absence of change in mEPSC frequency in PV cells in early adolescence may reflect the prolonged maturation process of excitatory synapses onto PV cells (Chung et al. 2017). Although PV cells show more mEPSC and sEPSC events with lower amplitudes at P21, these changes were normalized by

adulthood. In adulthood, while more mEPSC events with decreased interevent intervals in pyramidal cells may reflect a synapsing population with an increased vesicular release probability, the more sEPSC events with increased interevent intervals in PV cells may reflect a synapsing population with diminished action potential activity. PFC pyramidal cells may therefore be receiving a greater proportion of inputs from external regions. In the case of PV cells, the synapsing population with decreased action potential activity may include local pyramidal cells, given that we have shown they have a reduced firing ability. In line with this, vHPC projections primarily target pyramidal cells (89%) compared to 4% of PV cells, demonstrating differences in patterns of innervation (Phillips et al. 2019). Additional experimentation using retrograde labeling techniques may be used to target pyramidal versus PV cell innervating vHPC populations in order to isolate differences in functional phenotype.

In addition to changes in the distribution of interevent intervals, we observed accompanying changes in amplitudes. Particularly, recordings from P21 mice largely show altered amplitude distributions. Pyramidal cells tend to show reduced mIPSC amplitude, whereas PV cells tend to show reduced sEPSC and mEPSC amplitudes, which normalize in adult mice for both cell types. These results highlight the altered expression of synaptic abnormalities in adolescent versus adult mice in the PFC.

Using transgenic mice, we were able to examine differences in the functional maturation of molecularly identified cell types in the PFC. In addition, we were able to examine the effects of manipulating different populations of the vHPC on PFC maturation. Ablating CaMKII-vHPC cells reproduced a PFC functional phenotype similar to the mouse lesion model (similar changes in

mEPSC and mIPSC interevent interval distributions, and decreased mean mIPSC frequency), but these changes were not observed in the PV-vHPC ablation model. These results suggest that the ablation of vHPC projection inputs to the PFC plays a more important role in sculpting PFC functional activity. In line with this, activity deprivation decreases PV interneuron maximal firing rate, with a more pronounced effect when this deprivation occurs at an earlier age (P16–18) (Miller et al. 2011; Miyamae et al. 2017). Thus, ablating CaMKII-vHPC cells may influence PFC PV interneuron maturation during their protracted development period (Doischer et al. 2008). Although ablating PV-vHPC cells produced no observable functional changes in the PFC, schizophrenia patients often exhibit vHPC hyperactivity that can be observed in at-risk patients (Heckers and Konradi 2015). It is possible that both hypo- and hyperactivity in PFC-vHPC projection cells may influence PFC maturation. However, despite increased cFos levels in PV-vHPC ablated mice, this hyperactivity may not have been of sufficient strength to influence PFC activity patterns. Perhaps other interneuron populations may contribute to vHPC hyperactivity, particularly during early developmental stages given PV cells are slow to mature. Additional research that examines the differential effects of hypo- and hyperactivity on sculpting PFC excitation:inhibition balances will be necessary for better understanding schizophrenia pathology, and achievable by using modern genetically flexible tools.

The rat neonatal vHPC lesion model has proven to be an important neurodevelopmental model of schizophrenia-related behavioral disturbances (Lipska and Weinberger 2000). From this, we have utilized a combination of transgenic mice and viral tools to better decipher how early developmental changes in the vHPC can influence PFC functional organization. However, similar to past animal model studies, our study was limited to male mice. Future studies would further

expand by using female mice. With our male model, we were able to reproduce certain electrophysiological abnormalities with only partial ablation of vHPC cells, thereby making our model more physiologically relevant than complete excitotoxin ablation of the vHPC. Schizophrenia, however, is characterized by abnormal circuit dysfunction in multiple brain regions (Ellison-Wright et al. 2008; Glahn et al. 2008; Williams 2008; Vita et al. 2012). Our model therefore is limited in its investigation in circuit dynamics from a single locus, the vHPC. Moreover, the vHPC has connections with multiple regions and differential connections within a region, as aforementioned. Circuit dissections will require an assortment of tools, such as retrograde labeling, tools that bidirectionally manipulate activity, and transgenic mouse lines used in the current study to elucidate effects between cell types (ex. PFC pyramidal vs. PV cells) and activity (ex. vHPC hypo- vs. hyperactivity) (Nath et al. 2021). Finally, while our study identified individual cell changes in spiking properties and synaptic inputs, how these functional changes summate at a population level will be needed in order to better understand how these changes influence circuit dynamics.

We have identified differences in PFC pyramidal and PV cell functional properties that emerge following early developmental vHPC lesioning and have dissected the differential contribution of vHPC populations on PFC maturation. These findings may provide mechanistic insights into how developmental aberration in vHPC circuits may shape the maturation of PFC neurons and behavioral symptoms relevant to schizophrenia.

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Tables

Table 1

Cell membrane properties.

Cell properties	**Pyramidal cells**		**PV cells**	
	Sham 20 cells, 6 mice	**Lesion 20 cells, 6 mice**	**Sham 16 cells, 7 mice**	**Lesion 17 cells, 9 mice**
Access resistance (MΩ)	18.0 ± 1.3	16.6 ± 1.3	14.7 ± 1.1	16.3 ± 1.4
Input resistance (MΩ)	273.1 ± 20.7	277.5 ± 26.1	191.4 ± 18.9	259.6 ± 42.5
Resting membrane potential (mV)	−67.4 ± 1.9	−66.2 ± 2.4	−63.1 ± 2.1	−62.3 ± 2.8
Action potential threshold (mV)	−43.0 ± 0.5	−40.7 ± 1.2	−39.0 ± 2.2	−41.6 ± 0.9

No significant differences were observed in access resistance of recorded cells, input resistance, resting membrane potential, or action potential threshold between sham and lesion mice for both pyramidal and PV interneurons.

Table 2

Summary of changes in spontaneous and miniature excitatory and inhibitory inputs onto PFC pyramidal and PV cells in P21 and adult mice following early developmental vHPC lesioning, or adult mice following population-specific vHPC ablation (CaMKII-vHPC ablated, PV-vHPC ablated).

PFC cell type	**Synaptic input recording summary**	**~P21**	**Adult (P60)**		
		Sham vs. Lesion	**Sham vs. Lesion**	**CaMKII-cre control vs. DT**	**PV-cre control vs. DT**
Pyramidal cell	sEPSC	No change	No change	More events with decreased interevent interval	No change
	sIPSC	More events with increased interevent interval	No change	No change	No change
	mEPSC	More events with decreased interevent interval	More events with decreased interevent interval and reduced amplitude	More events with decreased interevent interval	No change
	mIPSC	Significantly decreased mean and more events with increased interevent interval and	Significantly decreased mean and more events with increased interevent interval	Significantly decreased mean and more events with increased interevent interval and	No change

PFC cell type	Synaptic input recording summary	~P21	Adult (P60)		
		Sham vs. Lesion	Sham vs. Lesion	CaMKII-cre control vs. DT	PV-cre control vs. DT
		reduced amplitude		reduced amplitude	
PV interneuron	sEPSC	More events with reduced amplitude	More events with increased interevent interval	N/A	N/A
	mEPSC	More events with reduced amplitude	No change	N/A	N/A

Figures

Fig. 1

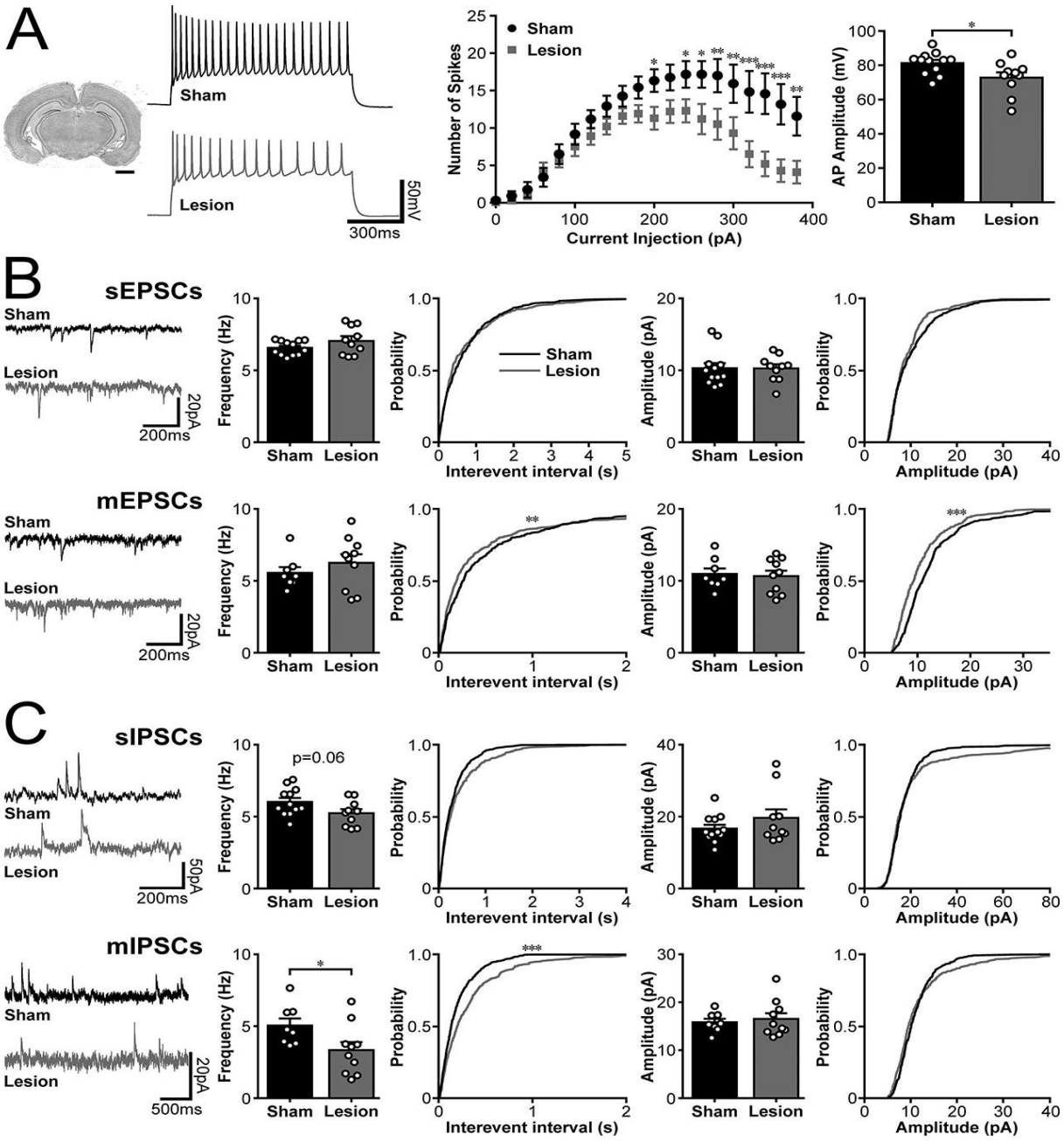


Fig. 1 Early vHPC lesioning results in firing deficits, altered excitatory and decreased inhibitory miniature inputs in PFC pyramidal cells of adult P60 mice. A) Representative image of a lesion brain (scale bar: 1 mm) and spiking output traces from sham vs. lesion mice. A two-way ANOVA

reveals a significant main effect of condition (sham vs. lesion) ($F(1,380) = 58.47$, ***$P < 0.001$), with post-hoc analysis revealing decreased spikes in response to increasing current injections in lesion mice (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). These spikes additionally display a reduced amplitude in lesion mice ($t(20) = 2.44$, *$P = 0.02$). N = 12 sham cells, 10 lesion cells; 3 mice per group. B, C) Left to right: representative traces, mean frequency, cumulative distribution of interevent intervals, mean amplitude, and cumulative distribution of amplitudes. B) Lesion mice show no significant changes in sEPSCs but do show altered distributions in mEPSC interevent intervals ($D = 0.12$, **$P = 0.003$) and amplitude ($D = 0.191$, ***$P < 0.001$), suggestive of more mEPSC events with short interevent intervals and small amplitudes in the lesion group relative to control. C) Lesion mice show no significant changes in sIPSCs but show a significant decrease in mIPSC frequency ($t(16) = 2.24$, *$P = 0.04$) and significantly changed mIPSC interevent interval distribution ($D = 0.184$, ***$P < 0.001$). N = 12 sham cells, 10 lesion cells; 3 mice per group for sEPSC and sIPSC data recorded in same cells as for spiking. N = 8 sham cells, 10 lesion cells; 3 mice per group for mEPSC and mIPSC data.

Fig. 2

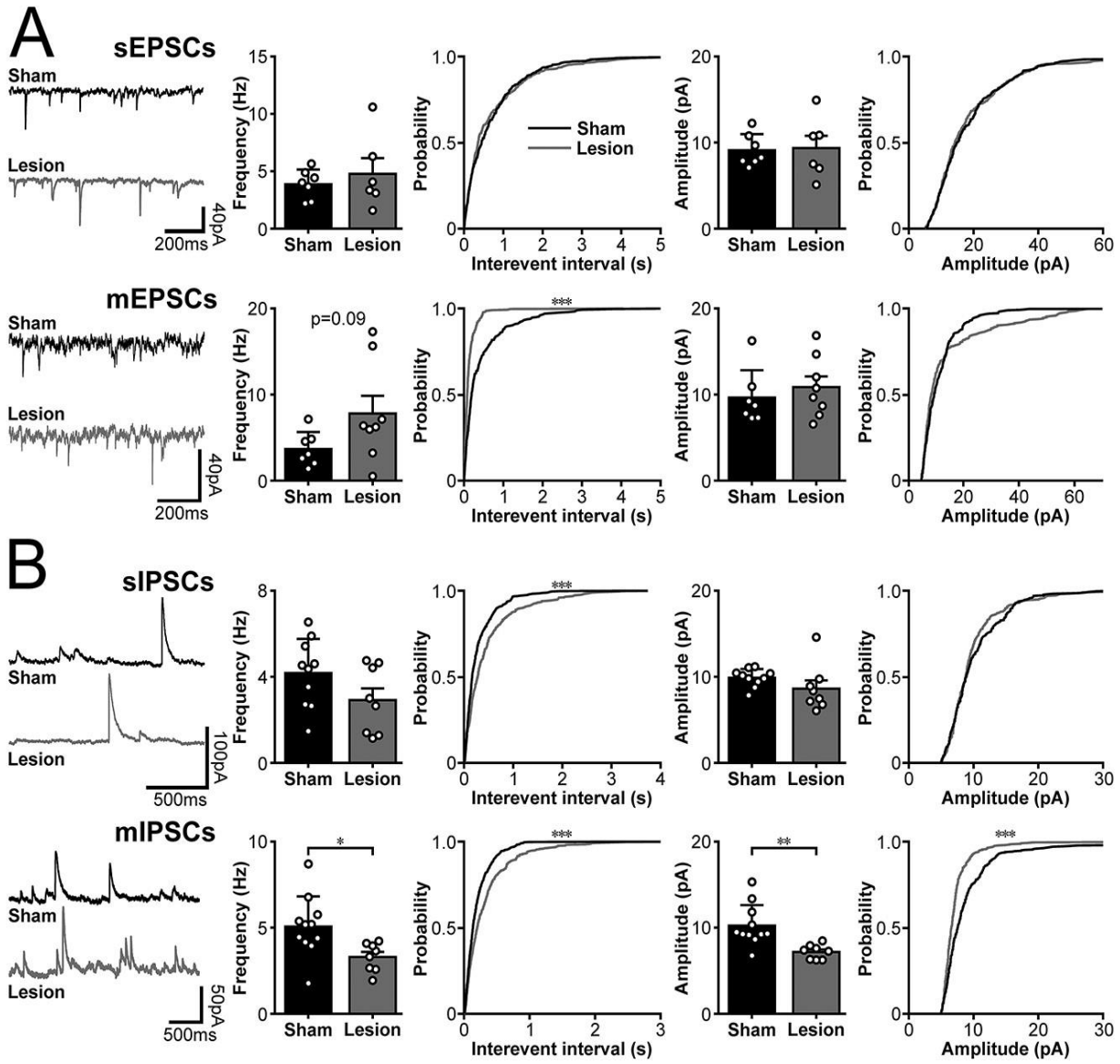


Fig. 2 Early vHPC lesioning results in decreased spontaneous and miniature inhibitory inputs, and increased miniature excitatory inputs onto PFC pyramidal cells of adolescent P21 mice. Representative traces, mean frequency, cumulative interevent interval distribution, mean amplitude, and cumulative amplitude distribution. A) P21 lesion mice show no significant changes in sEPSCs but do show a trend toward increased mEPSC frequency ($P = 0.09$) and a significantly changed mEPSC interevent interval distribution ($D = 0.34$, $^{***}P < 0.001$). B) P21 lesion mice

show a significantly altered sIPSC ($D = 0.184$, $^{***}P < 0.001$) interevent interval distribution, suggestive of a greater number of sIPSC events with reduced interevent intervals; and a decreased mean mIPSC frequency ($t(17) = 2.635$, $^{*}P = 0.02$), with an accordingly altered distribution in interevent intervals ($D = 0.166$, $^{***}P < 0.001$). mIPSCs are also of reduced amplitude by mean ($t(17) = 3.444$, $^{**}P = 0.003$) and distribution ($D = 0.265$, $^{***}P < 0.001$). N = 7 sham cells, 6 lesion cells; 3 mice per group for spontaneous input recordings. N = 11 sham cells, 8 lesion cells; 3 mice per group for miniature input recordings.

Fig. 3

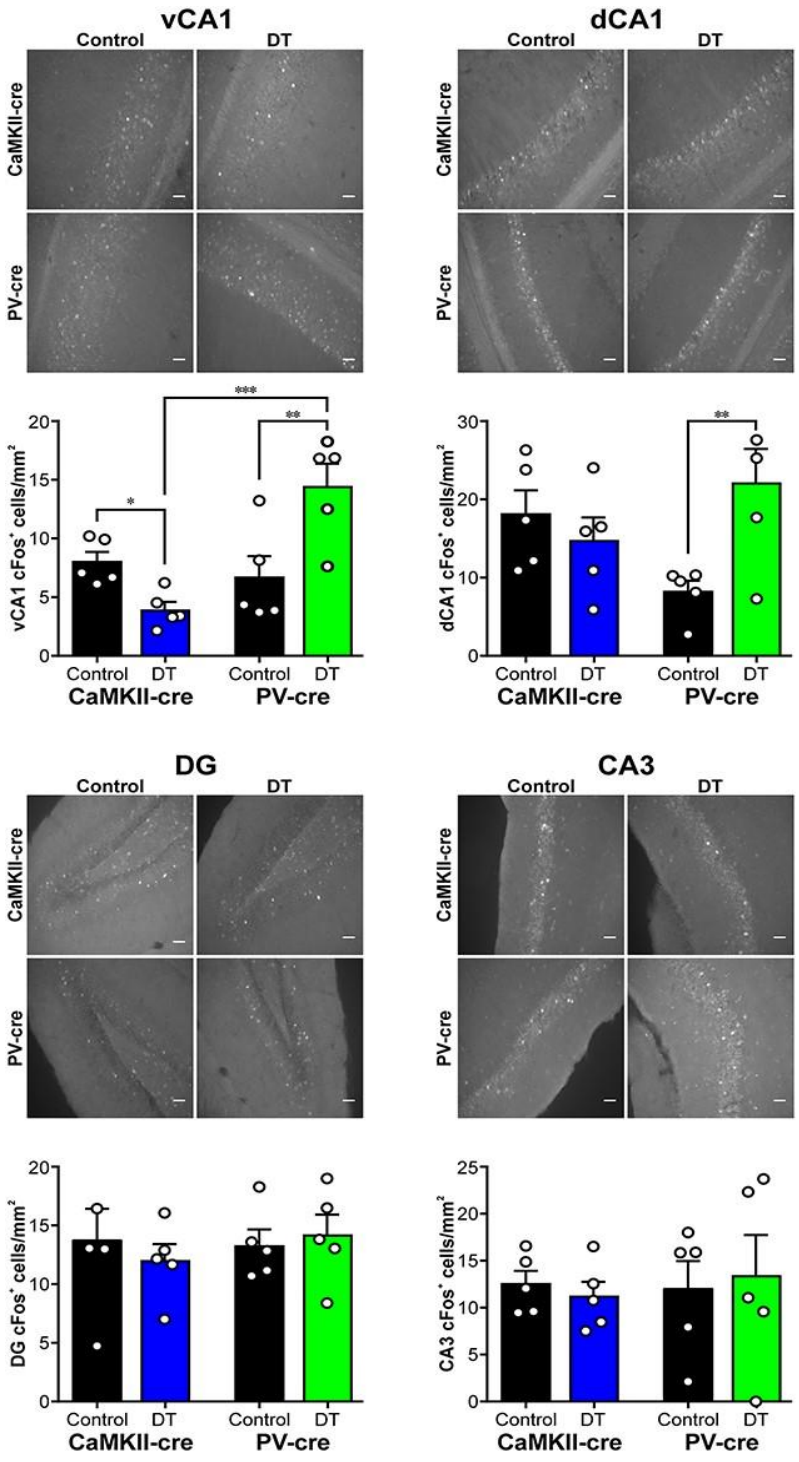


Fig. 3 Early CaMKII-vHPC viral ablation decreases vCA1 activity and PV-vHPC ablation increases dCA1 and vCA1 activity, as measured by cFos. Representative cFos stainings above, and

cFos cell density counts below in the vCA1, dCA1, DG, and CA3 of posterior brain sections. vCA1 DT infusion in CaMKII-cre mice decreases cFos density ($F(1,8) = 29.34$, $P = 0.00063$; Tukey's HSD test, *$P = 0.03$) and in PV-cre mice increases cFos cell density in vCA1 (Tukey's HSD test, **$P = 0.001$). cFos density measures between DT infused CaMKII-cre and PV-cre mice also significantly differ (Tukey's HSD test, ***$P = 0.0006$). vCA1 DT infusion in PV-cre mice increases cFos-positive cell density in the dCA1 ($F(1,8) = 10.26$, $P = 0.01$; Tukey's HSD test, **$P = 0.007$). No significant differences were observed in the DG and CA3. $N = 5$ mice per group. Scale bar: 50 μm.

Fig. 4

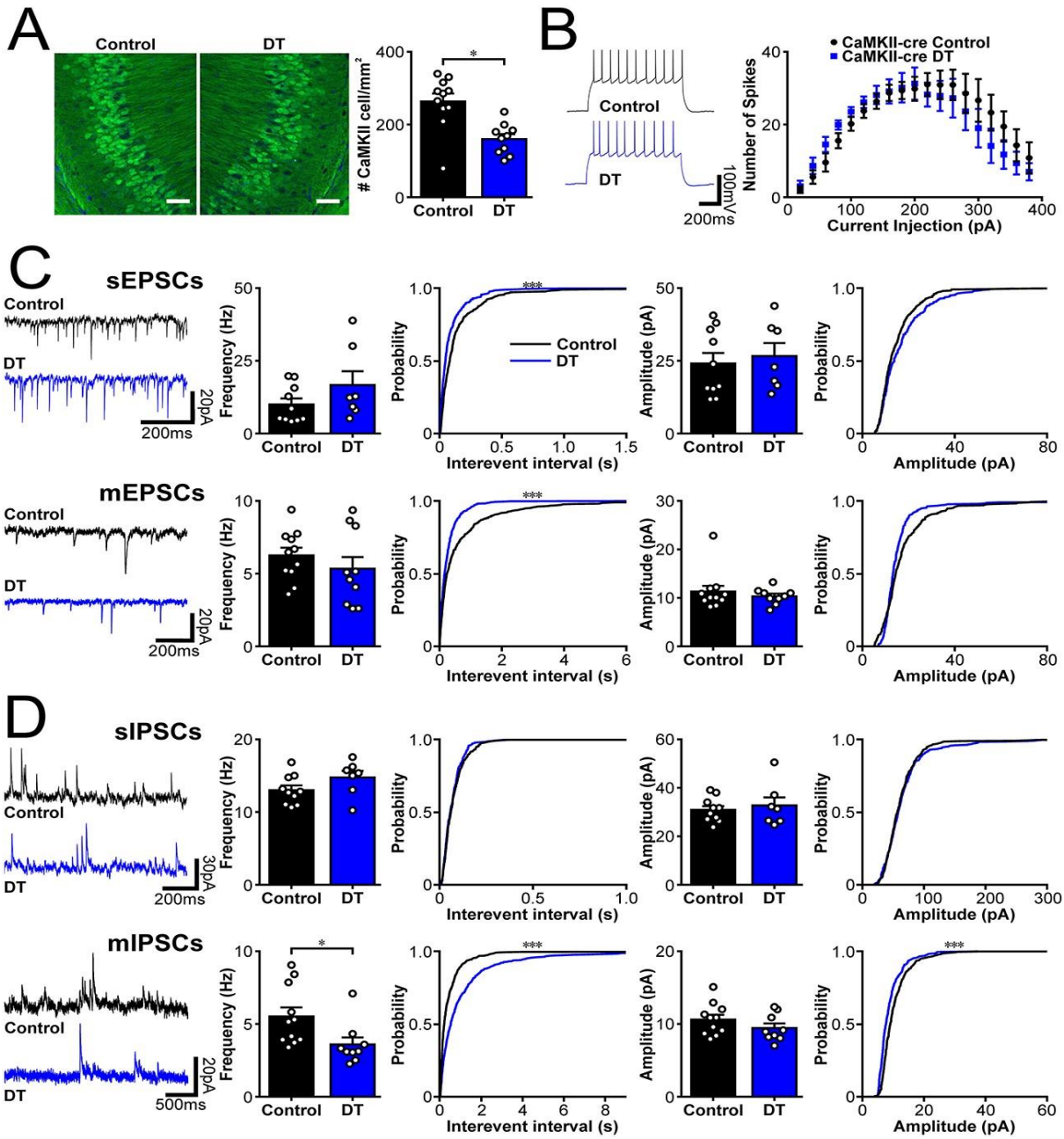


Fig. 4 Early CaMKII-vHPC viral ablation reproduces some functional phenotypes of lesion mice, including altered excitatory inputs and decreased inhibitory inputs onto PFC pyramidal cells. A) Representative CaMKII staining in CaMKII-cre control or virally ablated mice, confirming a decrease in cell-type marker according to targeted ablation ($t(19) = 3.938$, $P = 0.0009$). Scale bar:

50 μm. B) Representative spiking traces, and mean spiking frequency in CaMKII-vHPC control and virally ablated mice. No significant differences in spiking output in response to different current injections were observed. CaMKII-vHPC ablated group: N = 11 control cells, 8 DT cells; 4 mice per group. C, D) Representative traces, mean frequency, cumulative interevent interval distribution, mean amplitude, and cumulative amplitude distribution. C) Virally ablated mice show altered distributions in sEPSC ($D = 0.209$, $^{***}P < 0.001$) and mEPSC ($D = 0.186$, $^{***}P < 0.001$) interevent intervals, suggestive of more events with reduced interevent intervals. D) Virally ablated CaMKII mice show no changes in sIPSCs, but decreased mean mIPSC frequency ($H(3,39) = 10.918$, $^{*}P = 0.01$), and more mIPSC events with increased interevent interval ($D = 0.256$, $^{***}P < 0.001$), and reduced amplitude ($D = 0.218$, $^{***}P < 0.001$). N = 10 control cells, 7 DT cells; 4 mice per group for sEPSC and sIPSC data. N = 11 control cells, 9 DT cells; 3 mice per group for mEPSC and mIPSC data.

Fig. 5

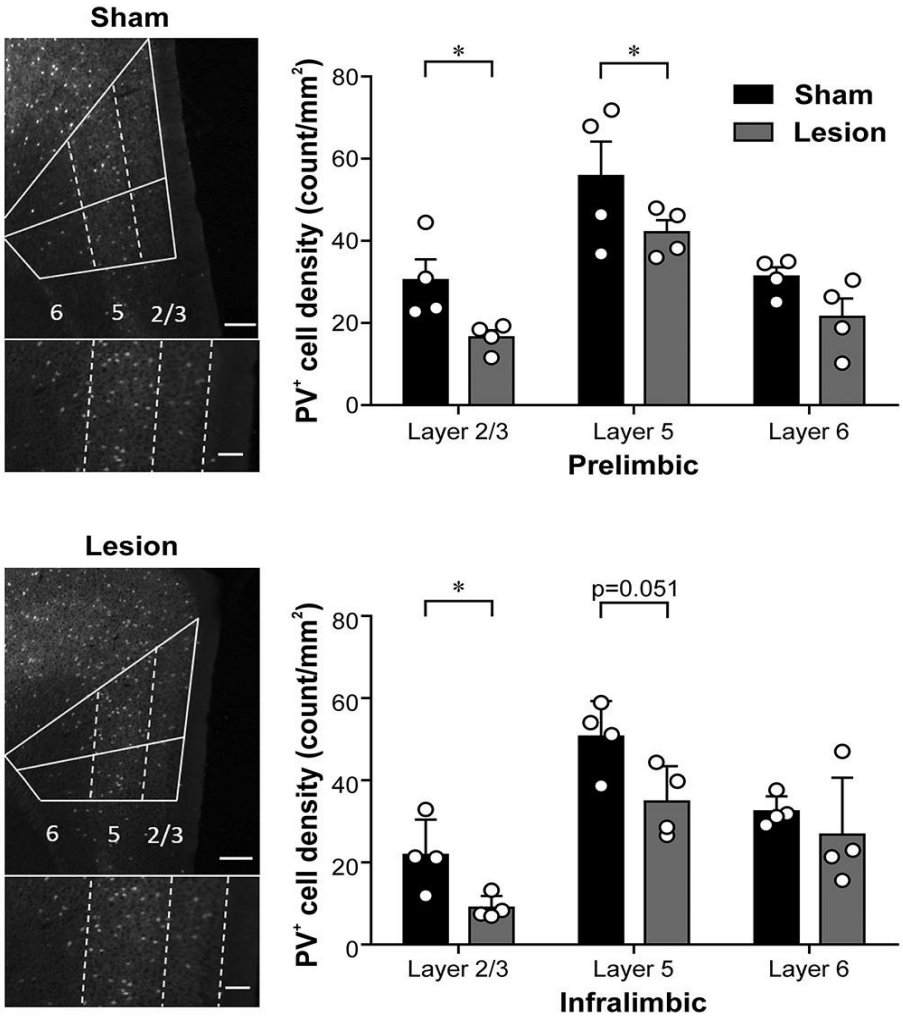


Fig. 5 Early vHPC lesioning decreases PV cell density in the PFC. Three-way ANOVA reveals decreased PV cell density in the prelimbic (top panel) and infralimbic areas (bottom panel) of the prefrontal cortex in lesion mice in layers 2/3 (*$P < 0.05$) and 5 (*$P < 0.05$), but not layer 6 ($F(1,36) = 21.26$, $P < 0.001$, $n = 4$ mice per group). Scale bar (100 μm, inset: 50 μm).

Fig. 6

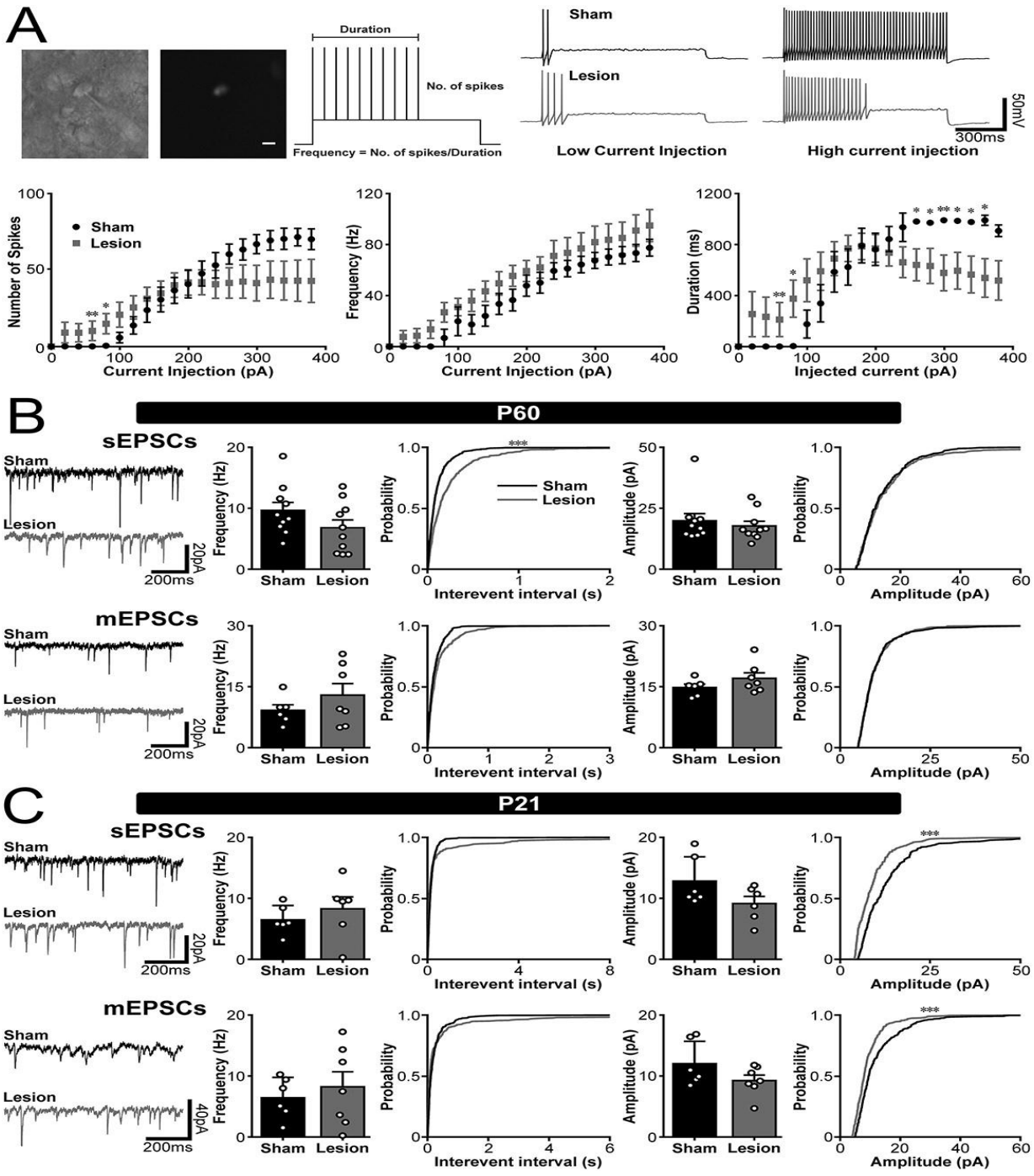


Fig. 6 Early vHPC lesioning alters PFC PV-firing output and decreases spontaneous excitatory inputs onto PV cells. A) Infrared and fluorescent image (scale bar: 10 μm), schematic, and representative spiking traces from recorded PFC PV-tdTomato expressing cell. While no

significant differences in frequency of firing is observed between sham and lesion mice, at lower current injections (60pA and 80pA), PV interneurons from lesion mice appear to show increased excitability as indicated by increased number of spikes and spiking duration, and at higher current injections (≥260 pA), PV interneurons show a failure to sustain high-frequency firing (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). $N = 10$ cells from 4 sham mice, 9 cells from 5 lesion mice. B, C) Representative traces, mean frequency, cumulative interevent interval distribution, mean amplitude, and cumulative amplitude distribution. B) Adult lesion mice show more sEPSC events with increased interevent interval ($D = 0.246$, ***$P < 0.001$), but no changes in mEPSCs. sEPSC recordings: $N = 10$ cells from 4 sham mice, 10 cells from 5 lesion mice; mEPSC recordings: $N = 8$ cells from 3 sham mice, 10 cells from 4 lesion mice. C) P21 lesion mice show sEPSCs ($D = 0.263$, ***$P < 0.001$) and mEPSCs ($D = 0.2$, ***$P < 0.001$) of lower amplitude by distributions. sEPSC recordings: $N = 6$ cells per group; 3 mice per group. mEPSC recordings: $N = 6$ sham cells, 7 lesion cells; 3 mice per group.

Fig. 7

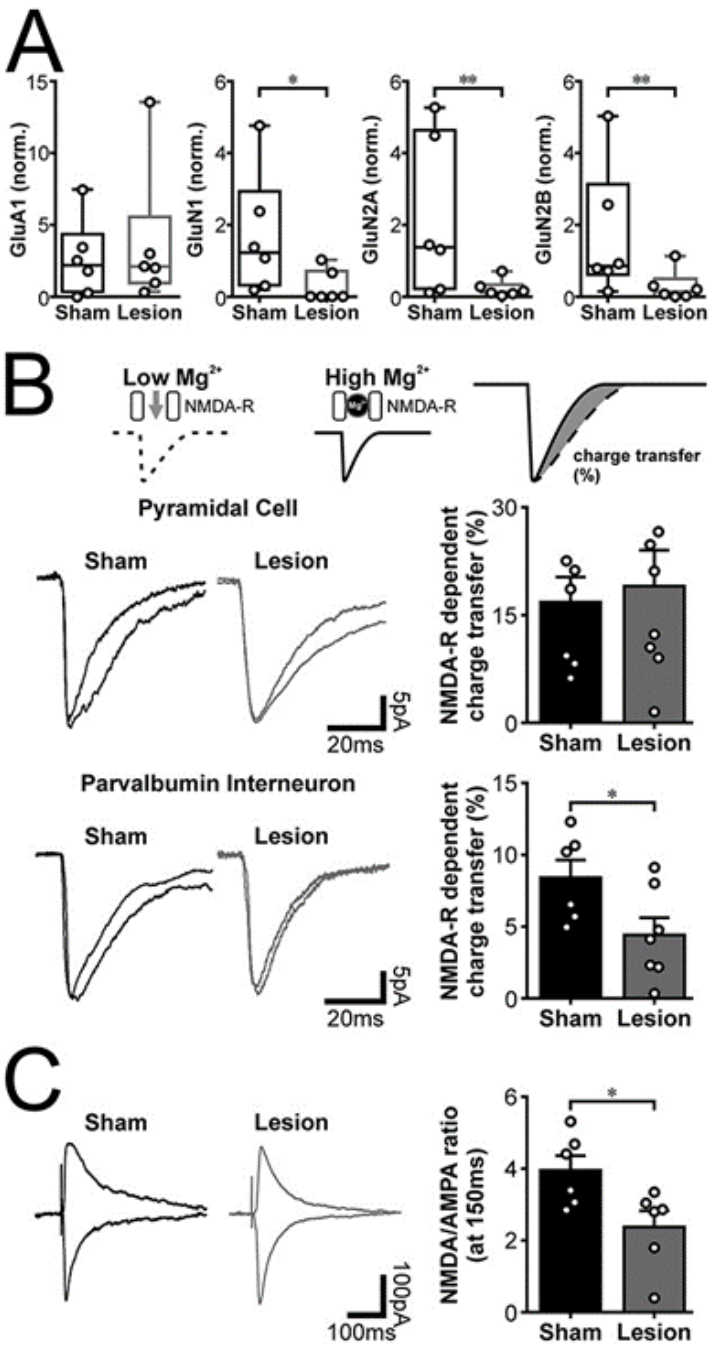
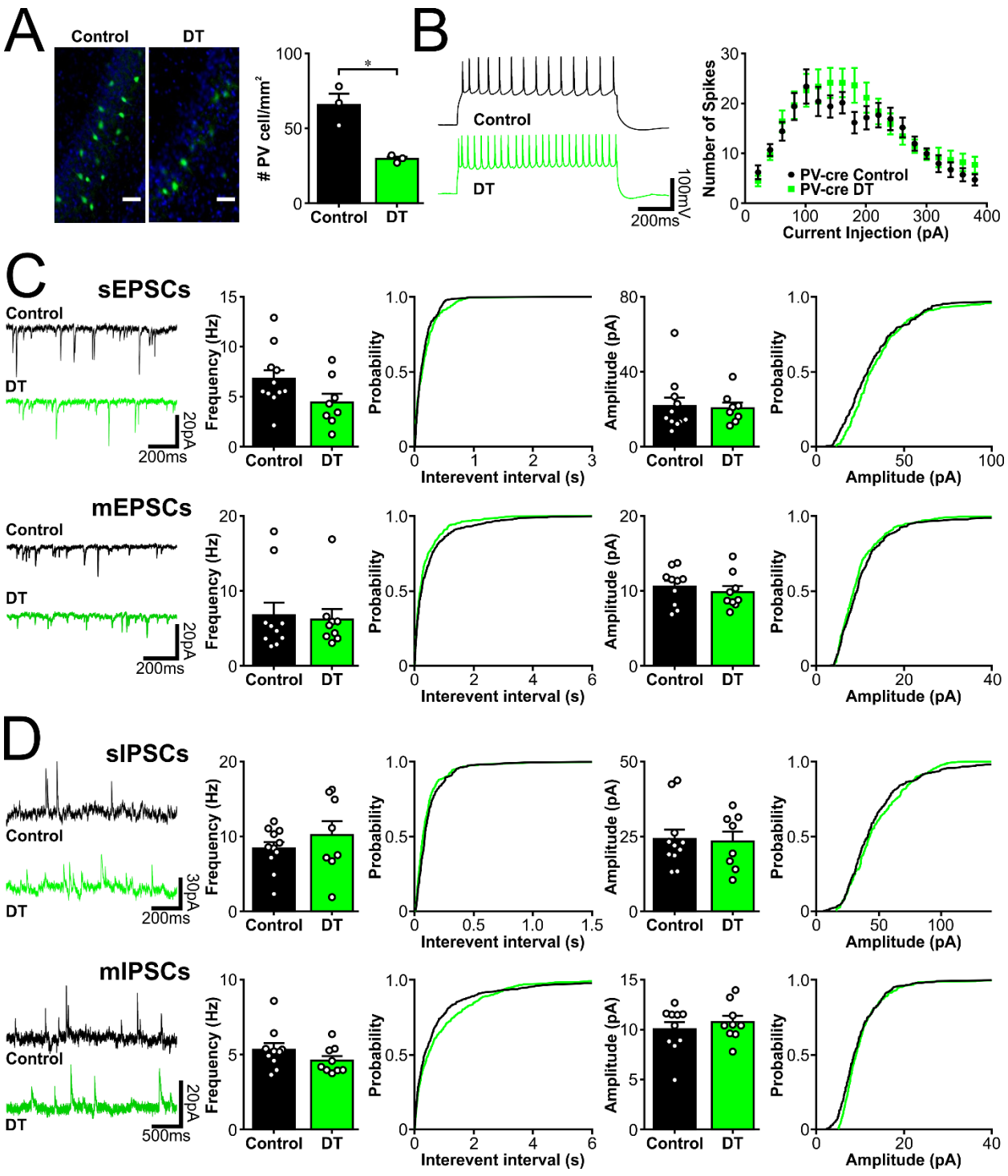


Fig. 7 Early vHPC lesioning results in decreased NMDA-receptor subunit mRNA levels in PFC but PV-specific functional deficits in NMDA receptor–mediated current. A) qRT-PCR analysis of AMPA-receptor GluA1 subunit expression, and NMDA-receptor GluN1, GluN2A, and GluN2B subunit expression (normalized to GAPDH expression). 2−ΔΔCT method of quantification reveals

no changes in GluA1, and a significant decrease in GluN1 ($U(6,6) = 32$, $^{*}P = 0.02$), GluN2A ($U(6,6) = 34$, $^{**}P = 0.009$), and GluN2B expression ($U(6,6) = 34$, $^{**}P = 0.009$) in lesion mice compared to sham mice ($n = 6$ mice per group). Box plot whiskers represent minimum and maximum. B) Schematic of recordings obtained under and low Mg+2 (composite AMPA and NMDA receptor–mediated current, dotted line) and high Mg+2 conditions (AMPA receptor–mediated current, solid line). Difference in area normalized to area at high Mg+2 conditions was used to calculate charge transfer. Sample traces and bar graph showing no significant differences in NMDA receptor–mediated current in PFC pyramidal cells ($n = 7$ sham cells, 8 lesion cells; 3 mice per group). Sample traces and bar graph showing a significant deficit in NMDA receptor–mediated current in PFC PV interneurons ($t(11) = 2.29$, $^{*}P = 0.04$; $n = 6$ cells from 3 sham mice, 7 cells from 4 lesion mice). C) Sample traces of evoked NMDA and AMPA responses. Ratio of NMDA (150 ms poststimulus at +40 mV): AMPA current (amplitude at −60 mV) shows a significant decrease in ratio in lesion mice compared to sham ($t(10) = 2.615$, $^{*}P = 0.03$; $n = 6$ cells per 3 mice per group).

Supplementary Figure 1



Supplemental Figure 1: Viral ablation of PV cells in PV-cre mice does not yield any significant changes in PFC pyramidal cell firing or synaptic inputs. (A) Representative PV vHPC staining in

PV-cre control and virally ablated mice, confirming a decrease in cell-type marker according to targeted ablation ($t(4) = 4.691$, $p=0.009$). Scale bar: 30 μm. (B) Representative spiking traces, and mean spiking frequency in PV-vHPC control and virally ablated mice. No significant differences in spiking output in response to different current injections were observed. PV-vHPC ablated group: n=8 control cells from 4 mice, 8 DT cells from 3 mice for spiking data. (C-D) Representative traces, mean frequency, cumulative interevent interval distribution, mean amplitude, and cumulative amplitude distribution. (C) No significant changes were observed in excitatory spontaneous or miniature inputs. N=11 control cells from 4 mice, 8 DT cells from 3 mice for sEPSC and sIPSC data. (D) No significant changes were observed in excitatory spontaneous or miniature inputs. N = 10 control cells, 9 DT cells; 3 mice per group for mEPSC and mIPSC data.

References

Ahlbeck J, Song L, Chini M, Bitzenhofer SH, Hanganu-Opatz IL. Glutamatergic drive along the septo-temporal axis of hippocampus boosts prelimbic oscillations in the neonatal mouse. Elife. 2018:7:e33158.

Anderson EM, Gomez D, Caccamise A, McPhail D, Hearing M. Chronic unpredictable stress promotes cell-specific plasticity in prefrontal cortex D1 and D2 pyramidal neurons. Neurobiol Stress. 2019:10:100152.

Bähner F, Meyer-Lindenberg A. Hippocampal-prefrontal connectivity as a translational phenotype for schizophrenia. Eur Neuropsychopharmacol. 2017:27:93–106.

Bhardwaj S, Tse Y, Ryan R, Wong TP, Srivastava LK. Impaired adrenergic-mediated plasticity of prefrontal cortical glutamate synapses in rats with developmental disruption of the ventral hippocampus. Neuropsychopharmacology. 2014:39:2963–2973.

Brockmann MD, Pöschel B, Cichon N, Hanganu-Opatz IL. Coupled oscillations mediate directed interactions between prefrontal cortex and hippocampus of the neonatal rat. Neuron. 2011:71:332–347.

Brockschnieder D, Lappe-Siefke C, Goebbels S, Boesl MR, Nave KA, Riethmacher D. Cell depletion due to diphtheria toxin fragment a after Cre-mediated recombination. Mol Cell Biol. 2004:24:7636–7642.

Bygrave AM, Kilonzo K, Kullmann DM, Bannerman DM, Kätzel D. Can N-methyl-D-aspartate receptor Hypofunction in schizophrenia be localized to an individual cell type? Front Psych. 2019:10:835.

Cachia A, Cury C, Brunelin J, Plaze M, Delmaire C, Oppenheim C, Medjkane F,

Thomas P, Jardri R. Deviations in early hippocampus development contribute to visual hallucinations in schizophrenia. Transl Psychiatry. 2020:10:102.

Cantrell AR, Scheuer T, Catterall WA. Voltage-dependent neuromodulation of Na+ channels by D1-like dopamine receptors in rat hippocampal neurons. J Neurosci. 1999:19:5301–5310.

Chung DW, Wills ZP, Fish KN, Lewis DA. Developmental pruning of excitatory synaptic inputs to parvalbumin interneurons in monkey prefrontal cortex. Proc Natl Acad Sci U S A. 2017:114:E629–E637.

Doischer D, Hosp JA, Yanagawa Y, Obata K, Jonas P, Vida I, Bartos M. Postnatal differentiation of basket cells from slow to fast signaling devices. J Neurosci. 2008:28:12956–12968.

Ellison-Wright I, Glahn DC, Laird AR, Thelen SM, Bullmore E. The anatomy of first-episode and chronic schizophrenia: an anatomical likelihood estimation meta-analysis. Am J Psychiatry. 2008:165:1015–1023.

Endo K, Hori T, Abe S, Asada T. Alterations in GABA(a) receptor expression in neonatal ventral hippocampal lesioned rats: comparison of prepubertal and postpubertal periods. Synapse. 2007:61:357–366.

Few WP, Scheuer T, Catterall WA. Dopamine modulation of neuronal Na(+) channels requires binding of a kinase-anchoring protein 15 and PKA by a modified leucine zipper motif. Proc Natl Acad Sci U S A. 2007:104:5187–5192.

Flores C, Bhardwaj SK, Labelle-Dumais C, Srivastava LK. Altered netrin-1 receptor expression in dopamine terminal regions following neonatal ventral hippocampal lesions in the rat. Synapse. 2009:63:54–60.

François J, Ferrandon A, Koning E, Angst MJ, Sandner G, Nehlig A. Selective reorganization of GABAergic transmission in neonatal ventral hippocampal-lesioned rats. Int J Neuropsychopharmacol. 2009:12:1097–1110.

Froudist-Walsh S, Bloomfield MA, Veronese M, Kroll J, Karolis VR, Jauhar S, Bonoldi I, McGuire PK, Kapur S, Murray RM, et al. The effect of perinatal brain injury on dopaminergic function and hippocampal volume in adult life. Elife. 2017:6:e29088.

Gingrich JR, Pelkey KA, Fam SR, Huang Y, Petralia RS, Wenthold RJ, Salter MW. Unique domain anchoring of Src to synaptic NMDA receptors via the mitochondrial protein NADH dehydrogenase subunit 2. Proc Natl Acad Sci U S A. 2004:101:6237–6242.

Glahn DC, Laird AR, Ellison-Wright I, Thelen SM, Robinson JL, Lancaster JL, Bullmore E, Fox PT. Meta-analysis of gray matter anomalies in schizophrenia: application of anatomic likelihood estimation and network analysis. Biol Psychiatry. 2008:64:774–781.

Goff DC, Hill M, Barch D. The treatment of cognitive impairment in schizophrenia. Pharmacol Biochem Behav. 2011:99(2):245–253.

Golomb D, Donner K, Shacham L, Shlosberg D, Amitai Y, Hansel D. Mechanisms of firing patterns in fast-spiking cortical interneurons. PLoS Comput Biol. 2007:3:e156.

Gonzalez-Burgos G, Lewis DA. NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull. 2012:38:950–957.

Grace AA. Dopamine system dysregulation by the hippocampus: implications for the pathophysiology and treatment of schizophrenia. Neuropharmacology. 2012:62:1342–1348.

Hale MW, Hay-Schmidt A, Mikkelsen JD, Poulsen B, Shekhar A, Lowry CA. Exposure

to an open-field arena increases c-Fos expression in a distributed anxiety-related system projecting to the basolateral amygdaloid complex. Neuroscience. 2008:155:659–672.

Hardingham GE, Do KQ. Linking early-life NMDAR hypofunction and oxidative stress in schizophrenia pathogenesis. Nat Rev Neurosci. 2016:17:125–134.

Hasam-Henderson LA, Gotti GC, Mishto M, Klisch C, Gerevich Z, Geiger J, Kovács R. NMDA-receptor inhibition and oxidative stress during hippocampal maturation differentially alter parvalbumin expression and gamma-band activity. Sci Rep. 2018:8:9545.

Heckers S, Konradi C. GABAergic mechanisms of hippocampal hyperactivity in schizophrenia. Schizophr Res. 2015:167:4–11.

Ho NF, Holt DJ, Cheung M, Iglesias JE, Goh A, Wang M, Lim JK, de Souza J, Poh JS, See YM, et al. Progressive decline in hippocampal CA1 volume in individuals at ultra-high-risk for psychosis who do not remit: findings from the longitudinal youth at risk study. Neuropsychopharmacology. 2017:42:1361–1370.

Hui CW, Bhardwaj SK, Sharma K, Joseph AT, Bisht K, Picard K, Tremblay M-È, Srivastava LK. Microglia in the developing prefrontal cortex of rats show dynamic changes following neonatal disconnection of the ventral hippocampus. Neuropharmacology. 2019:146:264–275.

Inan M, Zhao M, Manuszak M, Karakaya C, Rajadhyaksha AM, Pickel VM, Schwartz TH, Goldstein PA, Manfredi G. Energy deficit in parvalbumin neurons leads to circuit dysfunction, impaired sensory gating and social disability. Neurobiol Dis. 2016:93:35–46.

Joseph AT, Bhardwaj SK, Srivastava LK. Role of prefrontal cortex anti- and pro-

inflammatory cytokines in the development of abnormal behaviors induced by disconnection of the ventral hippocampus in neonate rats. Front Behav Neurosci. 2018:12:244.

Lee SH, Marchionni I, Bezaire M, Varga C, Danielson N, Lovett-Barron M, Losonczy A, Soltesz I. Parvalbumin-positive basket cells differentiate among hippocampal pyramidal cells. Neuron. 2014:82:1129–1144.

Lewis DA, Curley AA, Glausier JR, Volk DW. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci. 2012:35:57–67.

Lipska BK, Weinberger DR. To model a psychiatric disorder in animals: schizophrenia as a reality test. Neuropsychopharmacology. 2000:23:223–239.

Lipska BK, Jaskiw GE, Weinberger DR. Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia. Neuropsychopharmacology. 1993:9:67–75.

Lipska BK, Lerman DN, Khaing ZZ, Weickert CS, Weinberger DR. Gene expression in dopamine and GABA systems in an animal model of schizophrenia: effects of antipsychotic drugs. Eur J Neurosci. 2003:18:391–402.

Liu X, Carter AG. Ventral hippocampal inputs preferentially drive corticocortical neurons in the Infralimbic prefrontal cortex. J Neurosci. 2018:38:7351–7363.

Liu X, Murray KD. Neuronal excitability and calcium/calmodulin-dependent protein kinase type II: location, location, location. Epilepsia. 2012:53(Suppl 1):45–52.

Ma Y, Hu H, Berrebi AS, Mathers PH, Agmon A. Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. J Neurosci. 2006:26:5069–5082.

Marek R, Jin J, Goode TD, Giustino TF, Wang Q, Acca GM, Holehonnur R, Ploski JE, Fitzgerald PJ, Lynagh T, et al. Hippocampus-driven feed-forward inhibition of the prefrontal cortex mediates relapse of extinguished fear. Nat Neurosci. 2018:21:384–392.

McHugo M, Talati P, Armstrong K, Vandekar SN, Blackford JU, Woodward ND, Heckers S. Hyperactivity and reduced activation of anterior hippocampus in early psychosis. Am J Psychiatry. 2019:176:1030–1038.

McIntosh AM, Owens DC, Moorhead WJ, Whalley HC, Stanfield AC, Hall J, Johnstone EC, Lawrie SM. Longitudinal volume reductions in people at high genetic risk of schizophrenia as they develop psychosis. Biol Psychiatry. 2011:69:953–958.

Meyer-Lindenberg AS, Olsen RK, Kohn PD, Brown T, Egan MF, Weinberger DR, Berman KF. Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. Arch Gen Psychiatry. 2005:62:379–386.

Miller MN, Okaty BW, Kato S, Nelson SB. Activity-dependent changes in the firing properties of neocortical fast-spiking interneurons in the absence of large changes in gene expression. Dev Neurobiol. 2011:71:62–70.

Miyamae T, Chen K, Lewis DA, Gonzalez-Burgos G. Distinct physiological maturation of Parvalbumin-positive neuron subtypes in mouse prefrontal cortex. J Neurosci. 2017:37:4883–4902.

Murray RM, Bhavsar V, Tripoli G, Howes O. 30 years on: how the neurodevelopmental hypothesis of schizophrenia morphed into the developmental risk factor model of psychosis. Schizophr Bull. 2017:43:1190–1196.

Nath M, Wong TP, Srivastava LK. Neurodevelopmental insights into circuit dysconnectivity in schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry. 2021:104:110047.

Negrete-Díaz JV, Baltazar-Gaytán E, Bringas ME, Vazquez-Roque RA, Newton S, Aguilar-Alonso P, León-Chávez BA, Flores G. Neonatal ventral hippocampus lesion induces increase in nitric oxide [NO] levels which is attenuated by subchronic haloperidol treatment. Synapse. 2010:64:941–947.

O'Donnell P, Lewis BL, Weinberger DR, Lipska BK. Neonatal hippocampal damage alters electrophysiological properties of prefrontal cortical neurons in adult rats. Cereb Cortex. 2002:12:975–982.

Phillips ML, Robinson HA, Pozzo-Miller L. Ventral hippocampal projections to the medial prefrontal cortex regulate social memory. Elife. 2019:8:e44182.

Pradhan BS, Majumdar SS. An efficient method for generation of transgenic rats avoiding embryo manipulation. Mol Ther Nucleic Acids. 2016:5:e293.

Ryan RT, Bhardwaj SK, Tse YC, Srivastava LK, Wong TP. Opposing alterations in excitation and inhibition of layer 5 medial prefrontal cortex pyramidal neurons following neonatal ventral hippocampal lesion. Cereb Cortex. 2013:23:1198–1207.

Seamans JK, Yang CR. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol. 2004:74:1–58.

Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol. 2013:106-107:1–16.

Sigurdsson T, Duvarci S. Hippocampal-prefrontal interactions in cognition, behavior and psychiatric disease. Front Syst Neurosci. 2016:9:190.

Stine CD, Lu W, Wolf ME. Expression of AMPA receptor flip and flop mRNAs in the nucleus accumbens and prefrontal cortex after neonatal ventral hippocampal lesions.

Neuropsychopharmacology. 2001:24:253–266.

Sun Q, Li X, Li A, Zhang J, Ding Z, Gong H, Luo. Ventral hippocampal-prefrontal interaction affects social behavior via Parvalbumin positive neurons in the medial prefrontal cortex. iScience. 2020:23:100894.

Swerdlow NR, Halim N, Hanlon FM, Platten A, Auerbach PP. 2001. Lesion size and amphetamine hyperlocomotion after neonatal ventral hippocampal lesions: more is less. Brain Res Bull 55:71–77.

Tse YC, Bagot RC, Hutter JA, Wong AS, Wong TP. Modulation of synaptic plasticity by stress hormone associates with plastic alteration of synaptic NMDA receptor in the adult hippocampus. PLoS One. 2011:6:e27215.

Tseng KY, Lewis BL, Lipska BK, O'Donnell P. Post-pubertal disruption of medial prefrontal cortical dopamine-glutamate interactions in a developmental animal model of schizophrenia. Biol Psychiatry. 2007:62:730–738.

Tseng KY, Lewis BL, Hashimoto T, Sesack SR, Kloc M, Lewis DA, O'Donnell P. A neonatal ventral hippocampal lesion causes functional deficits in adult prefrontal cortical interneurons. J Neurosci. 2008:28:12691–12699.

Tseng KY, Chambers RA, Lipska BK. The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Behav Brain Res. 2009:204:295–305.

Vita A, De Peri L, Deste G, Sacchetti E. Progressive loss of cortical gray matter in schizophrenia: a meta-analysis and meta-regression of longitudinal MRI studies. Transl Psychiatry. 2012:2:e190.

Williams LM. Voxel-based morphometry in schizophrenia: implications for

neurodevelopmental connectivity models, cognition and affect. Expert Rev Neurother. 2008:8:1049–1065.

Yang J, Ye M, Tian C, Yang M, Wang Y, Shu Y. Dopaminergic modulation of axonal potassium channels and action potential waveform in pyramidal neurons of prefrontal cortex. J Physiol. 2013:591:3233–3251.

Bridge: The *in vivo* relevance of altered PFC cellular functional maturations to behavior

Chapter 3 successfully applies the methodological framework established in Chapter 2. This application yielded insights into the vHPC cell type population contributing to PFC dysfunction and into the nature of PFC dysfunction. CaMKII-vHPC cell ablation in early development results in increased probability of excitatory events and reduced inhibitory synaptic inputs onto PFC pyramidal cells. However, how do these synaptic changes impact communication of information within the PFC, and in turn impact PFC dependent behaviors?

The next chapter therefore examines changes in PFC connectivity by measuring local field potential activity (LFP) in awake, behaving mice. These LFP recordings yield information on how different frequency bands are impacted, each of which have been associated with specific functions and mechanisms of generations, as established in the comprehensive literature review. These LFP recordings were obtained at baseline, and when mice were engaged in tasks that involve vHPC-PFC activity including an anxiety-based task and a working memory task. LFP changes in frequency bands could then be correlated with changes in behavioral performance. Our results are laid out in the next chapter. This chapter thus makes sense of the alterations in synaptic inputs observed in the previous chapter by placing the results in a behavioral context relevant to schizophrenia pathology.

Chapter 4: Excess prefrontal cortical high gamma, and reduced theta, beta, and low gamma oscillations in mice with early developmental disruption to the ventral hippocampus[#]

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[#]to be submitted (Cerebral Cortex)

Abstract

Functional connectivity between the ventral hippocampus (vHPC) and prefrontal cortex (PFC) mediates cognitive behaviors; dysfunction in this pathway is an important endophenotype in schizophrenia. This connectivity exists early in development, with vHPC excitatory inputs playing an important role in sculpting PFC maturation. What are the impacts of perturbing these vHPC inputs early in development on PFC functional connectivity? We explore this question using a combination of a viral vector, transgenic mouse line, and *in vivo* electrophysiology to assay PFC oscillatory activity that correlative with cognitive functions. We addressed this question by specifically ablated CaMKII cells in the vHPC of postnatal day 14 male mice, and measured local field potential activity in the PFC when mice were of adult age. We found excess high gamma power in vHPC-CaMKII ablated mice when animals were in the home cage, or performed open-field, and spontaneous alternation Y-maze tasks. In contrast, we found deficits in theta and low gamma power in the open-field and Y-maze tasks. Finally, we found a significant deficit in beta power in vHPC-CaMKII ablated mice present when these mice made incorrect spontaneous alternation decisions in the Y-maze. Our results highlight that perturbation to early developmental vHPC inputs significantly impacts PFC functional maturation in a long-lasting manner, with alterations in local functional connectivity patterns across various frequency ranges. These alterations, in turn, may contribute to deficits in cognitive functions, including working memory.

Keywords

animal model, electrophysiology, local field potentials, prefrontal cortex, schizophrenia

Introduction

Communication between the ventral hippocampus (vHPC) and prefrontal cortex (PFC) plays important roles in various cognitive and social functions (Sigurdsson and Duvarci 2016; Phillips et al. 2019; Sun et al. 2020). Moreover, dysfunction in this pathway is a commonly observed endophenotype in schizophrenia patients, and is associated with cognitive deficits such as working memory impairments (Meyer-Lindenberg et al. 2005; McHugo et al. 2019; Bähner and Meyer-Lindenberg 2017). Elucidating the mechanisms underlying vHPC and PFC communication is therefore relevant to both normal cognitive functioning and abnormal functioning in disease states.

The vHPC connects directly to the PFC through excitatory projections present in early development, and helps in the functional development of the PFC (Brockmann et al. 2011; Ahlbeck et al. 2018). Whereas previous studies have characterized the impacts of vHPC activity manipulation on PFC activity and behaviors in adults (Phillips et al. 2019; O'Neill et al., 2013; Cernotova et al., 2021), limited research exists on the impact of early vHPC perturbations to PFC functional development (Ahlbeck et al., 2018; Oberlander et al., 2019). Moreover, the majority of these limited studies characterized *in vitro* activity changes in the PFC (Ryan et al., 2013; O'Donnell et al. 2002; Tseng et al. 2007, 2008) and were conducted in a neonatal vHPC rat lesion model of schizophrenia, thereby limiting more finetuned manipulations of the vHPC using genetic tools (Nath et al., 2021). We therefore wanted to investigate how early vHPC perturbation of specific neurons impacts *in vivo* changes in PFC functional connectivity using genetic tools.

We have previously demonstrated that the early and specific ablation of CaMKII-expressing cells in the vHPC, achieved using a viral vector and a transgenic mouse line, results in increased

excitatory and decreased inhibitory inputs onto PFC pyramidal cells, based on whole-cell recordings performed in slice (Nath et al., 2023). However, how these synaptic input changes translate to the population activity of the PFC and whether these changes are still relevant *in vivo* remains to be determined. Moreover, *in vivo* recordings of local field potential activity enable us to correlate changes in the frequency bands to behavioral functions, such as anxiety and working memory, both of which are associated with vHPC-PFC pathway connectivity (Hong and Kaang 2022).

This study therefore examines the effect of early vHPC-CaMKII ablation on population level activity in PFC by measuring local field potentials (LFPs) *in vivo*. LFPs were examined at baseline in home cage, in an open-field arena to assay for anxiety, and in a spontaneous alternation Y-maze task to assay for working memory. Altered frequency bands were then correlated to behavior. We found excess high gamma power in the PFC following early vHPC-CaMKII ablation. Reduced theta and low gamma power were also characterized in the open-field arena and spontaneous alternation Y-maze task. Additionally, reduced beta power was observed when vHPC-CaMKII mice made incorrect spontaneous alternation decisions. We therefore provide evidence of local field connectivity changes in the PFC, that may be mediated through altered cellular changes previously characterized.

Materials and methods

Animals

Male CaMKII-cre transgenic mice of C57BL6 background were used (Jackson Laboratory; stock no. 005359). Male mice data are presented here due to a more pronounced and behaviorally similar

progression of schizophrenia-like behaviors compared to females (Tseng et al. 2009), and a lack of significant slice electrophysiological findings in females in this model (Nath et al., 2023). Mice were housed under controlled conditions of light (12 h light:12 h dark), temperature, and humidity with ad libitum access to food and water. All experiments were approved by the Facility Animal Care Committee at the Douglas Research Centre and were in accordance with the Canadian Council on Animal Care guidelines. 9 experimental and 9 control mice were used for all experiments.

CaMKII-vHPC cell ablation stereotaxic surgery

Stereotaxic surgeries for the ablation of CaMKII-vHPC cells were performed as described previously (Nath et al., 2023). Briefly, 0.25 µL of AAV8/EF1.flex.dtA.mCherry (University of North Carolina Vector Core Centre; titer = 3.7 × 10*12 genome copies per ml, DT mice) was bilaterally stereotaxically injected into the vHPC of P14 mouse pups. This vector expresses diphteria toxin A (dtA), which blocks protein synthesis, thereby ablating CaMKII expressing cells in the transgenic line (Liu and Murray 2012). Control mice were bilaterally stereotaxically injected with 0.25 µL of AAV8.DIO.mCherry (Canadian Neurophotonics Platform, Laval University; titer = 1.7 × 10*13 genome copies per mL). P14 mouse vHPC injection coordinates from bregma were: 2.8 mm posterior, 2.8 mm lateral, and 3.2 mm ventral.

Electrode implant stereotaxic surgery for LFP recordings

Once control and CaMKII-ablated mice reached about 3 months of age, mice were implanted with a headstage (EIB-18, Neuralynx) with 4 tungsten filaments (0.1 mm diameter each) inserted into the medial PFC (from bregma: 1.7 mm anterior, 0.4 mm lateral, 1.5 mm ventral). Ground and

reference screws were placed above the frontal cortex, opposite to the implant side, and the cerebellum, respectively. The electrodes and headstage array were secured to the skull with metabond and dental cement (Patterson Dental). Neural recordings were amplified using a headstage preamplifier (HS-18, Neuralynx) before being digitized at 2 kHz using a Digital Lynx SX recording system (Neuralynx) and stored with Cheetah Software (Neuralynx). Signals were bandpass filtered between 0.2-1000 Hz.

Behavioral assays: home cage, open-field, spontaneous alternation y-maze

Mice were handled and habituated to the behavioral assay room for at least three days prior to experiments. All assays were performed under dimly lit conditions. 10-min LFP recordings were obtained in each assay: home cage, an open-field arena, and in the spontaneous alternation Y-maze. For the open-field arena, a large grey opaque box of 60 cm dimensions was used. Movement was tracked using ANY-Maze software. For the Y-maze, a black polypropylene set-up with three identical arms of 10 cm width, 40 cm length, and 16 cm height, separated by 120°, was used. Mouse were placed in one arm and allowed to freely explore all three arms. The total number of arm entries and the number of spontaneous alternations were noted. Percent alternation was calculated as the number of spontaneous alternations/(the total number of arm entries – 2) x 100%.

LFP Spectral Analysis

LFPs were analyzed as described previously (Carmichael et al., 2019; Carmichael et al. 2017). Power-spectral densities were computed using Welch's method on the first derivate of the data (MATLAB pwelch(diff(data)) and normalized to the maximum value. Relative band power was calculated for each frequency of interest, including delta (1-4 Hz), theta (4-12 Hz), beta (12-30

Hz), low gamma (30-55Hz), and high gamma frequencies (65-120Hz), and normalized to the total band power in the range of 0-120Hz (MATLAB bandpower(data)). Analyses of bandpower excluded the range 55-65Hz to avoid the 60Hz noise signal. All four channels, wherein one channel equates to one implanted tungsten filament, per mouse were used in the analysis, and the full 10-min LFP recording for home cage and open-field arena were used. For the spontaneous alternation Y-maze task, time stamps at which a mouse would enter the decision zone and exit the decision zone were noted (the decision zone being the center of the Y-maze). These decision times were divided into correct versus incorrect decisions. LFPs were accordingly separated into correct versus incorrect segments, and analyzed as done with home cage and open-field.

Histological verification of electrode insertion in PFC

Brains were extracted from sacrificed mice and coronally sectioned (35 μm, Leica) prior to staining with 0.5% cresyl violet solution for the verification of the electrode implant site in the PFC.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 6. The Shapiro–Wilk test and Levene's test were used to assess assumptions of normality and equality of variances, respectively. An unpaired t-test was used for the statistical analysis between two groups. A critical value of 0.05 was used for all tests, and marked with * for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$. All data are presented as mean ± SEM.

Results

Increased high gamma PFC power following early vHPC CaMKII-ablation in home cage

To determine the effect of early vHPC-CaMKII DT cell ablation on PFC local functional connectivity, we ablated CaMKII cells in P14 mice and measured PFC LFP activities once mice reached adulthood (P90-120). (Fig. 1). Power-spectral density analysis suggests altered powers at a wide range of oscillatory frequencies, including delta (1-4 Hz), theta (4-12 Hz), beta (12-30 Hz), low gamma (30-55Hz), and high gamma frequencies (65-120Hz), when mice are in a home cage setting (Fig. 1). Quantifying relative band power, we see that early developmental vHPC-CaMKII DT ablation significantly increased high gamma ($t(16) = -2.3$, $p = 0.04$) band powers compared to controls (Fig. 1).

Reduced theta and altered gamma PFC power following early vHPC CaMKII-ablation in an open-field

To determine how PFC LFP power dynamics may vary in a different environment, we examined LFP activity in mice placed in an open-field arena. Early vHPC-CaMKII DT ablation had no significant impact on mean speed (control: 0.10 ± 0.01 m/s DT: 0.09 ± 0.02 m/s) and time spent in the inner zone, or center, of the open-field arena (Fig. 2), suggesting no significant difference in anxiety-like behavior between the ablated and control groups. When examining the power-spectral densities and the quantified relative band powers, however, we see a significant reduction in relative band power at theta ($t(16) = 2.2$, $p = 0.04$) and low gamma ($t(16) = 2.2$, $p = 0.04$) frequencies, and a significant increase at high gamma frequency ($t(16) = -3.0$, $p = 0.009$) (Fig. 2). These results demonstrate that early vHPC-CaMKII DT ablation results in a general deficit in theta power and in an abnormally excess high gamma power across different environments, whereas the additional reduction in low gamma power is specific to the open-field arena. vHPC-CaMKII DT ablated mice thus show abnormal PFC local field connectivity in baseline states.

Deficits in spontaneous alternation Y-maze task associated with altered theta, beta, gamma oscillations in vHPC-CaMKII DT ablated mice

The PFC contributes to working memory processing (Lara and Wallis 2015; Yang et al., 2014). Given that lesioning the PFC produces deficits in the spontaneous alternation working memory assay (Lalonde 2002), we wanted to investigate how altered PFC local connectivity patterns in vHPC-CaMKII DT mice might influence behavior in the spontaneous alternation working memory assay. Mice were allowed to freely explore a Y-maze while recording PFC LFPs, and the percentage of alternations was measured. We found a significant reduction in spontaneous alternations following early developmental vHPC-CaMKII DT ablations ($t(16) = 4.9$, $p < 0.001$), but no significant changes in total number of arm entries (Fig. 3). These results suggest that PFC-relevant behavioral deficits, such as working memory, are induced by early vHPC-CaMKII DT cell ablation.

To better assess how altered LFP in PFC might mediate abnormal working memory processing, we compared PFC LFPs in control versus vHPC-CaMKII DT ablated mice. No significant differences in frequency band powers were observed when examining oscillations during performance of the full Y-maze task (Supplementary Figure 1). However, to better relate oscillatory activities to correct versus incorrect performance in the task, we differentiated LFPs between correct versus incorrect spontaneous alternation decisions. Similar to baseline home cage recordings, vHPC-CaMKII DT mice show significant deficits in theta power in both correct ($t(16) = 2.4$, $p = 0.03$) and incorrect alternation decisions ($t(16) = 3.2$, $p = 0.005$), and significant increases in high gamma power in both correct ($t(16) = -3.6$, $p = 0.003$) and incorrect ($t(16) = -3.2$, $p =$

0.006) alternation decisions compared to controls. In addition, similar to the novel open-field environment recordings, a significant reduction in low gamma power was observed in both correct ($t(16) = 3.1$, $p = 0.007$) and incorrect ($t(16) = 3.4$, $p = 0.004$) alternation decisions. Cumulative to these oscillatory changes, we additionally observed a significant deficit in beta power in the spontaneous alternation Y-maze task, but only when comparing control to vHPC-CaMKII DT activity at incorrect decision points ($t(16) = 3.0$, $p = 0.008$) (Fig. 4). These findings highlight a cumulation of abnormal PFC processing at various frequency ranges, with abnormal processing at theta, beta, and gamma processing potentially mediating deficits in spontaneous alternation based working memory in vHPC-CaMKII DT mice.

Discussion

The early disruption of vHPC signaling negatively impacts PFC functional maturation. We have shown this through the early ablation of vHPC-CaMKII cells using DT, which results in a cumulation of oscillatory deficits at various frequencies. At baseline in home cage, vHPC-CaMKII DT mice show abnormally increased high gamma power. In an open-field arena, these mice show deficits in theta and low gamma, in addition to excess high gamma power. Finally, in a spontaneous alternation Y-maze task, vHPC-CaMKII DT mice show decreases in theta, beta, and low gamma power, in addition to excess high gamma power. These abnormal PFC local field activities may mediate errors in spontaneous alternations. This data thus provides some insight into the circuit mediated alternations in functional connectivity contributing to schizophrenia-related behavioral deficits.

These oscillatory changes reflect the abnormal communication patterns between PFC cell populations. We have previously shown that early vHPC disruption through lesioning in mice results in a loss of PV cells, a reduced ability of PV cells to sustain high frequency firing, deficits in NMDA-receptor mediated current in PV cells, reduced pyramidal cell spiking activity, and increased probability of excitatory inputs and decreased inhibitory inputs onto pyramidal cells in the PFC (Nath et al., 2023). Additional studies have characterized a wide range of PFC abnormalities following neonatal vHPC lesioning in rats including altered dopaminergic signaling influencing both glutamatergic and GABAergic transmission (Tseng et al., 2007; Tseng et al., 2008), increased serotonergic transmission (Mitazaki et al., 2020), and increased acetylcholine release in stressed lesioned rats (Laplante et al., 2004). Moreover, one study found reduced power at 1-8Hz, 9-14Hz, and 15-30Hz in rats based on electroencephalogram recordings (Valdés-Cruz et al., 2012). Thus a wide range of altered signaling following early vHPC disruption may potentially contribute to the changes in theta, beta, and gamma power processing observed in the PFC.

Specifically, following early vHPC-CaMKII DT ablation, we characterized an increased probability of excitatory inputs and decreased inhibitory inputs onto layer V pyramidal cells (Nath et al., 2023). The net effect of these synaptic input changes is an enhanced excitability in the PFC. This increased excitability may reflect the excess high gamma power observed in our home cage baseline recordings, and throughout all the behavioral assays. In line with this, increased excitability is associated with increased occipital gamma band power in clinically high-risk patients of schizophrenia (Grent-'t-Jong et al., 2018). Schizophrenia patients also show hyperactivity in the PFC at baseline, and a reduced suppression of activity during a working memory task, which correlates with impaired working memory (Nath et al., 2021; Whitfield-

Gabrieli et al., 2009). Thus, excess activity in the PFC may contribute to working memory deficits associated with spontaneous alternation. Whether and how different synaptic transmissions (e.g., dopamine, GABA, serotonin) may influence the oscillatory changes observed following early vHPC-CaMKII ablation requires further investigation.

We observed deficits in PFC theta power when vHPC-CaMKII DT mice were in an open-field arena and in the spontaneous alternation Y-maze. Importantly, the hippocampus functions in the generation of theta oscillations, and these hippocampal theta oscillations in turn entrain PFC activity (Siapas et al., 2005; Kupferschmidt and Gordon, 2018). We have previously shown that early vHPC-CaMKII DT ablation results in decreased CA1 activity as measured by cFos-positive cell density (Nath et al., 2023). Thus, the ablation of vHPC-CaMKII cells likely disrupts hippocampal theta generation, which may impact long-range theta synchrony with the PFC. This synchronous theta oscillation between the vHPC mediates both anxiety-like (open-field arena related) and working memory expressions (Y-maze related) (Adhikari et al., 2011; O'Neill et al., 2013; Sigurdsson et al., 2010). While adult vHPC inactivation does not influence theta power in the PFC (O'Neill et al., 2013), the early inactivation of the vHPC may produce deficits in PFC theta power. For example, lidocaine treatment in the hippocampus of P6-8 male rats results in deficits in PFC slow theta-alpha bursts tightly coupled with superimposed gamma oscillations at about P14 (Brockmann et al., 2011). Schizophrenia patients accordingly show deficits in theta power in the medial temporal lobe, and in theta coupling between the PFC and temporal lobe, in a spatial memory task (Adams et al., 2020). Thus, early vHPC perturbation produces PFC deficits in theta power, and the disruption to long-range vHPC-PFC theta synchrony, mediated by vHPC-CaMKII ablation, may mediate the observed spatial spontaneous working memory deficits in the

Y-maze. Validation of vHPC theta deficits following vHPC-CaMKII DT ablation, and the effects on synchrony between the PFC and vHPC will require further experiments.

Whereas an excess in high gamma power was observed throughout recordings, we characterized a deficit in low gamma power in the open-field and Y-maze task. Gamma power is thought to be locally generated primarily through the activity of fast-spiking interneurons (Cardin et al., 2009). The difference in powers may reflect distinct neuronal ensembles (Catanese et al., 2016), or fast-spiking populations (van der Meer and Reddish, 2009), mediating the distinct effects. Single-unit recordings of these fast-spiking interneurons correlated to PFC gamma oscillations would be of interest to better elucidate this relationship.

We observed a deficit in beta power in vHPC-CaMKII DT mice but only when mice made incorrect spontaneous alternation decisions. Beta oscillations are thought to be generated through deep layer cortical interactions between pyramidal and interneurons, and may play a role in working memory by clearing out past information (Schmidt et al., 2019). Thus, deficits in beta in incorrect decisions in vHPC-CaMKII DT mice may indicate interference from past information of a visited arm, thereby impairing spontaneous alternation. While this beta deficit was not present in control animals when comparing incorrect to correct decisions, other mechanisms may be at play in yielding the incorrect decision. Nonetheless, a deficit in beta power may still contribute to increased probability of spontaneous alternation errors in vHPC-CaMKII DT ablated mice. This deficit in beta may be mediated through the observed changes in excitatory and inhibitory inputs onto pyramidal cells in layer V pyramidal cells (Nath et al., 2023).

This study was limited to examining LFP powers in the PFC. Future experiments must involve unit recordings simultaneous to field potential recordings to more directly relate PFC cell population changes, as characterized previously (Nath et al., 2023), to the LFP changes, thereby providing more detailed mechanistic insights into PFC cellular activity and local activity. In addition, coherent recordings between the PFC and vHPC would be valuable given the important role of the communication between these two regions in various cognitive processes. Ideally, long-range synchronous vHPC-PFC activity would be examined from development into adulthood, to better ascertain the impacts of early vHPC disruption to PFC maturation. Moreover, whereas we ablated vHPC-CaMKII cells, future experiments that specifically ablated vHPC-CaMKII-PFC projecting cells, such as through retrograde tools, would better specify whether the alterations in the PFC are a result of direct connections with from the vHPC to the PFC, or indirectly through other regions.

In conclusion, our study demonstrates that early vHPC-CaMKII DT ablation results in abnormally high gamma power, and deficits in theta, beta, and low gamma which may contribute to spontaneous alternation working memory deficits. These results highlight the significant role of early vHPC inputs to PFC functional maturation.

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Figures

Fig. 1

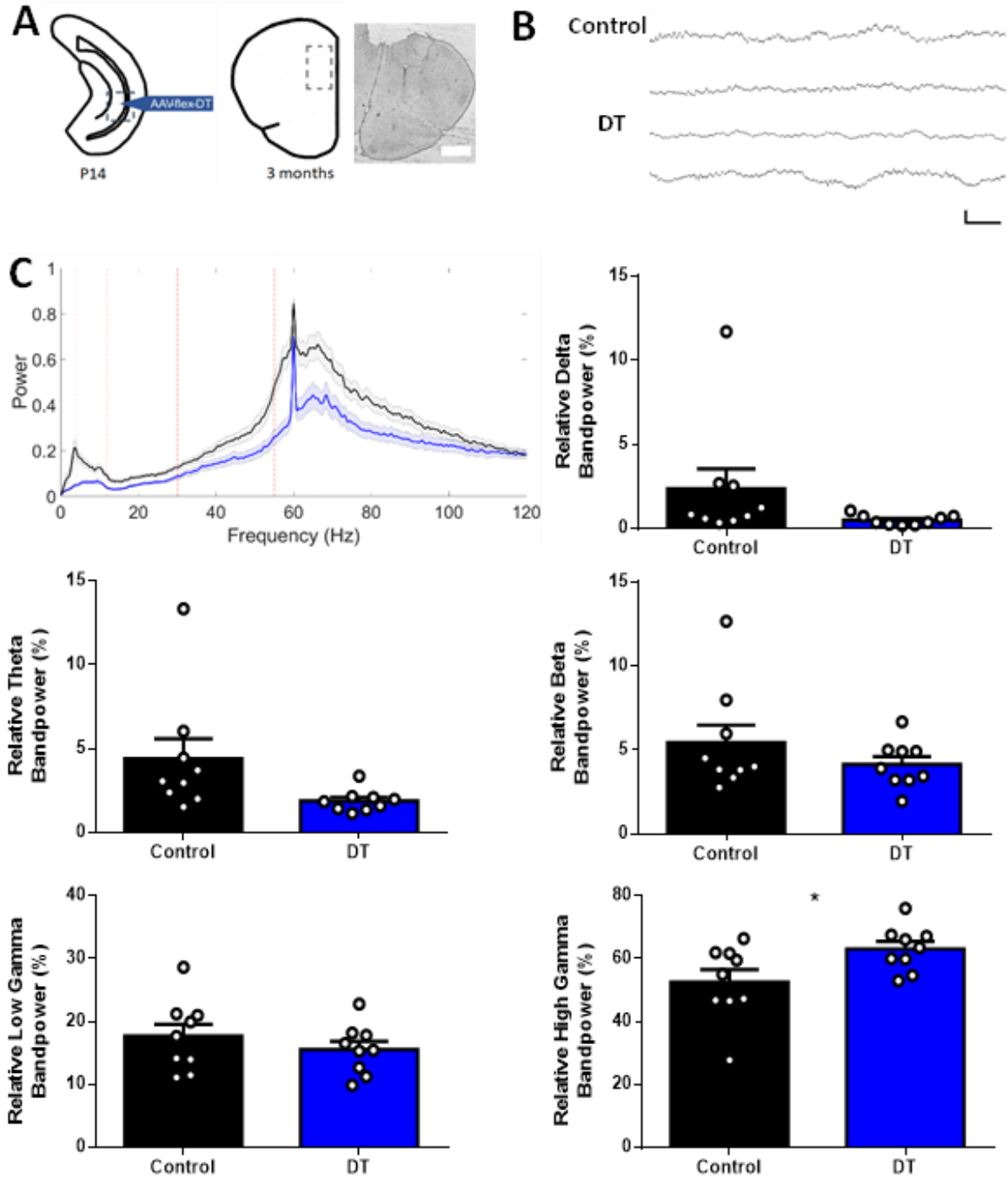
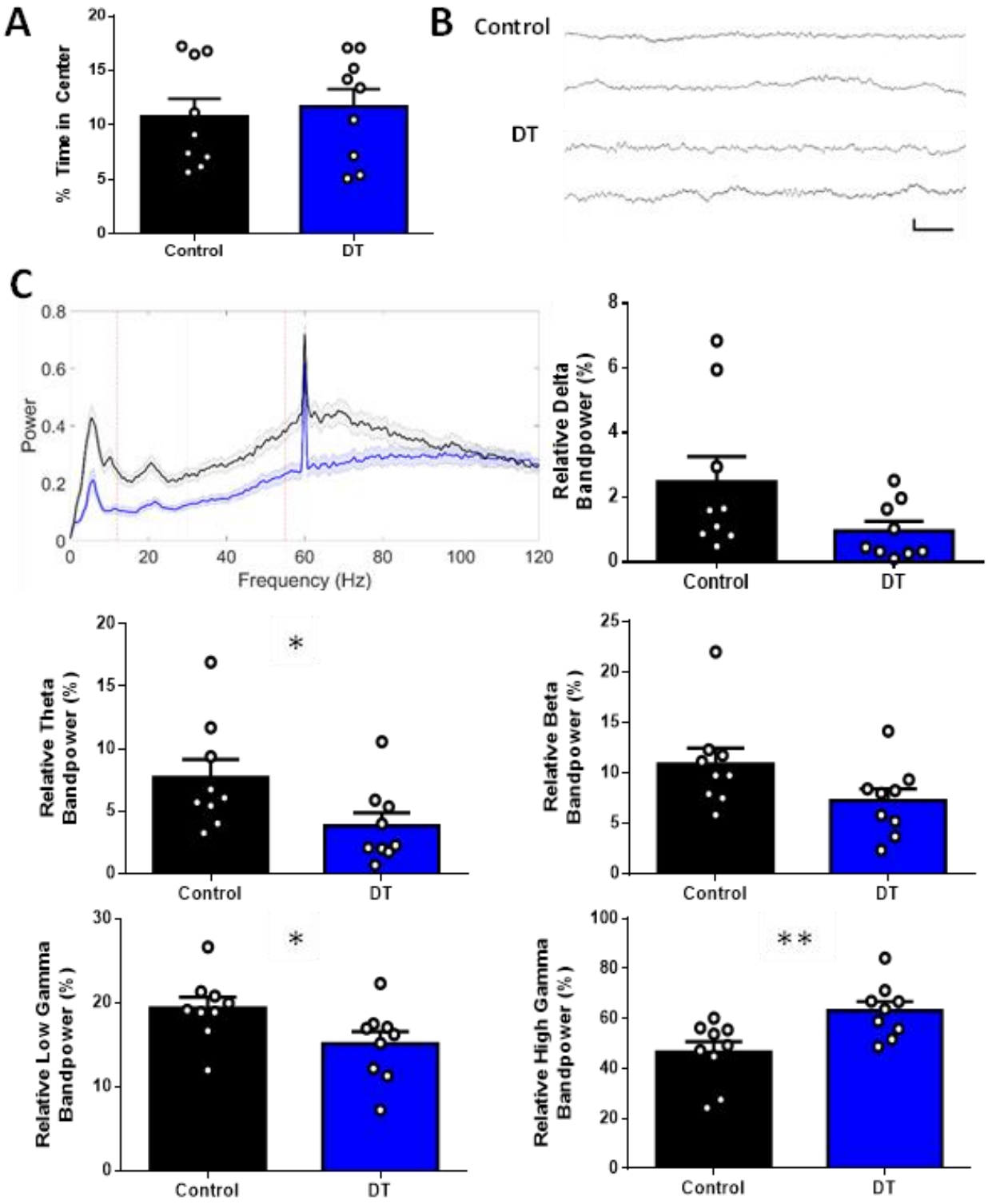


Fig. 1 Early vHPC-CaMKII DT ablation significantly increased high-gamma power in baseline home cage recordings of adult mice. A) Schematic showing viral vector expression targeted to the vHPC in CaMKII-cre P14 mice, and LFP recordings in PFC at 3 months of age. Representative image of electrode placement in PFC (scale bar: 1 mm). B) Raw LFP traces from two different mice in control and DT mice (scale bars: 0.1 s by 250 μV). C) Post-spectral density and relative bandpower quantifications with control represented in black and DT mice in blue. We found significantly increased high-gamma power in vHPC-CaMKII DT mice compared to controls ($t(16) = -2.3$, $*p = 0.04$). N = 9 mice per group.

Fig. 2


A
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Power
Frequency (Hz)
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Relative Beta Bandpower (%)
Relative Low Gamma Bandpower (%)
Relative High Gamma Bandpower (%)
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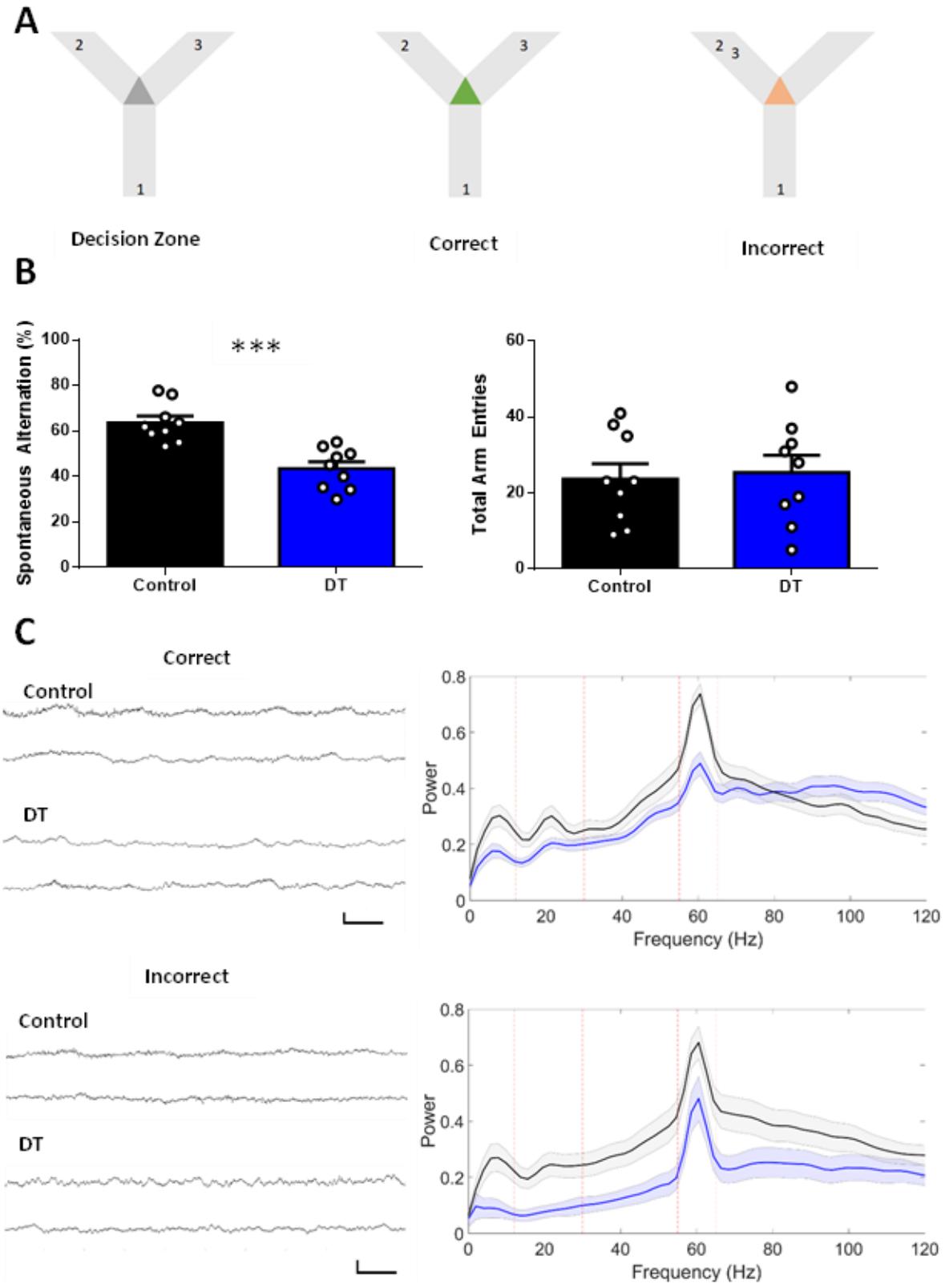
Fig. 2 Early vHPC-CaMKII DT ablation significantly decreased theta and low gamma power, and increased high-gamma power in an open-field arena. A) No significant differences were observed in the time spent in the center of the open-field between groups. B) Raw LFP traces from two different mice in control and DT mice (scale bars: 0.1 s by 250 μV). C) Post-spectral density and relative bandpower quantifications. We found significantly decreased theta ($t(16) = 2.2$, $*p = 0.04$) and low gamma ($t(16) = 2.2$, $*p = 0.04$) power, and a significant increase at high gamma power ($t(16) = -3.0$, $**p = 0.009$). N = 9 mice per group.

Fig. 3


A
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Decision Zone
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Spontaneous Alternation (%)
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Fig. 3 Early vHPC CaMKII DT ablation produces deficits in spontaneous alternation behavior, associated with changes in PFC LFPs. A) Schematic of spontaneous alternation Y-maze task. The center of the maze was considered the decision zone. If a mouse made a correct turn, then the LFP recording during the time spent in the decision zone prior to turning was considered correct. Likewise for an incorrect turn. B) Early vHPC-CaMKII DT ablation produces significant deficits in percent spontaneous alternations ($t(16) = 4.9$, $^{***}p < 0.001$), without altering total number of arm entries. C) Representative raw LFP traces (scale bars: 0.1 s by 250 μV) and post-spectral densities for correct versus incorrect decisions, demonstrating changes in frequency powers. N = 9 mice per group.

Fig. 4

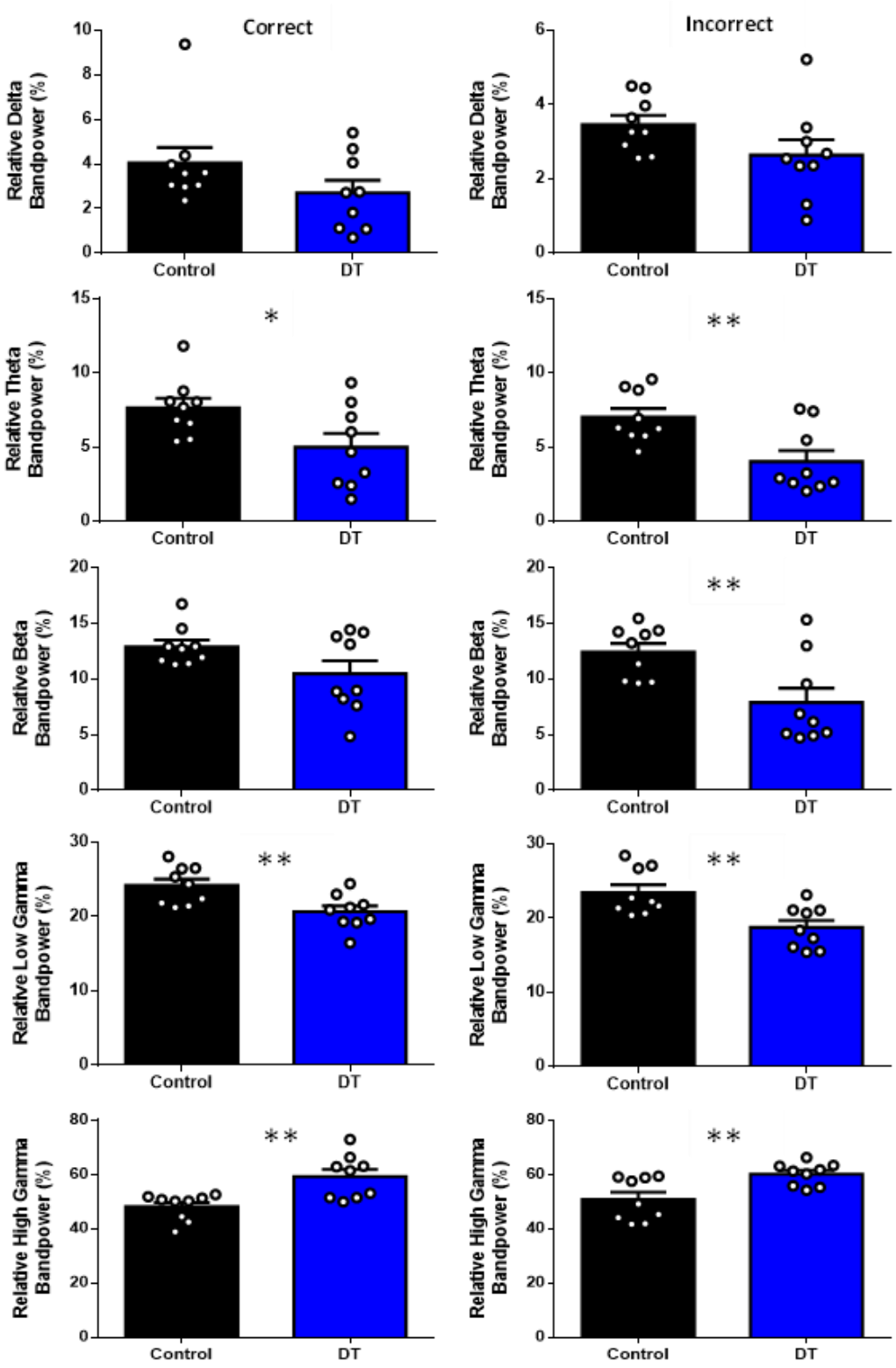
Correct
Incorrect
Relative Delta Bandpower (%)
Relative Theta Bandpower (%)
Relative Beta Bandpower (%)
Relative Low Gamma Bandpower (%)
Relative High Gamma Bandpower (%)
Control
DT
*
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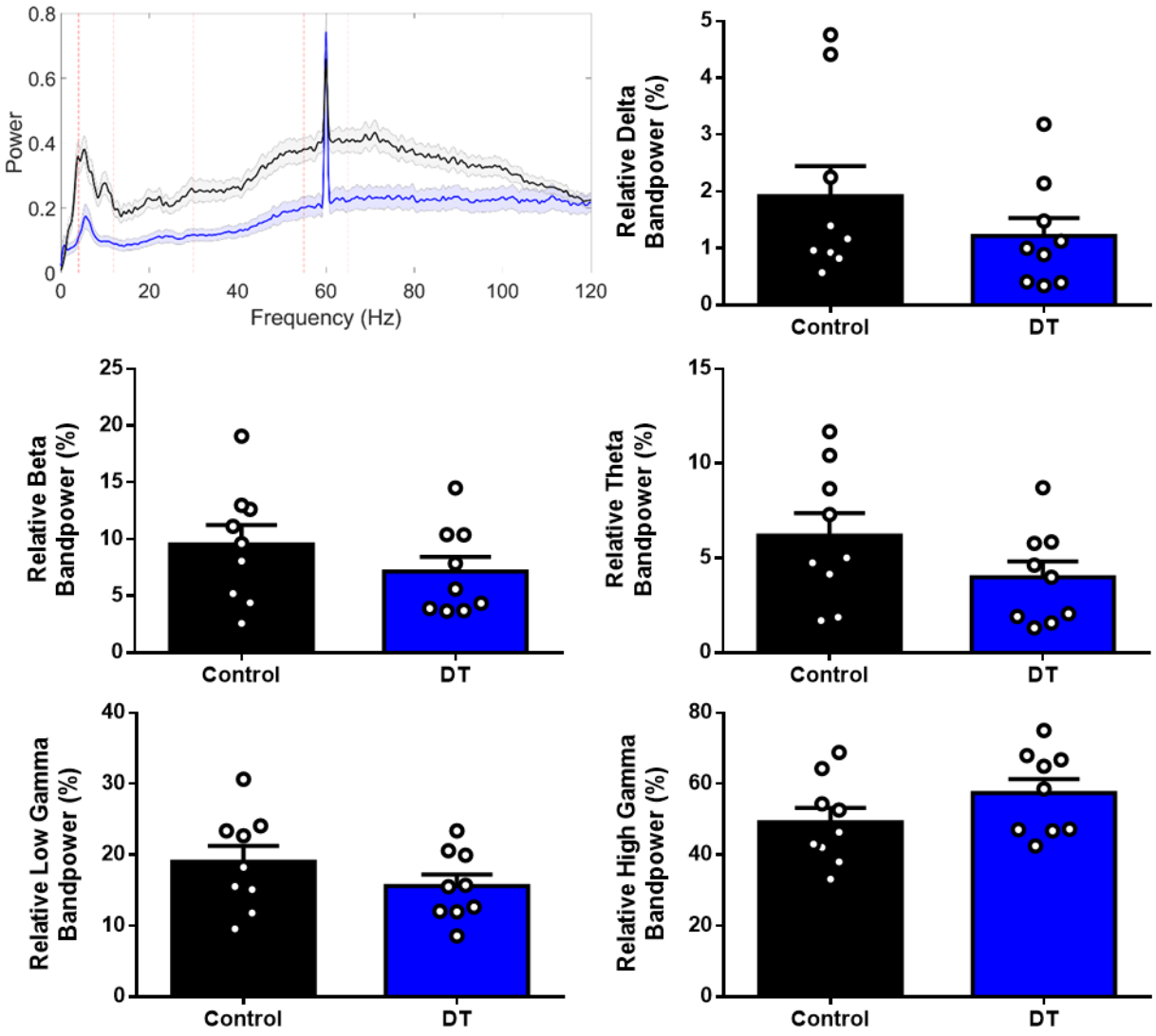
Fig. 4 Spontaneous alternation deficits in vHPC-CaMKII DT mice is associated with reduced theta, beta, low gamma, and excess high gamma power. vHPC-CaMKII DT mice show significant deficits in theta power in correct ($t(16) = 2.4$, $*p = 0.03$) and incorrect ($t(16) = 3.2$, $**p = 0.005$) alternation decisions, beta power in incorrect alternation decisions only ($t(16) = 3.0$, $**p = 0.008$), low gamma power in correct ($t(16) = 3.1$, $**p = 0.007$) and incorrect ($t(16) = 3.4$, $**p = 0.004$) alternation decisions, and increased high gamma power in correct ($t(16) = -3.6$, $**p = 0.003$) and incorrect ($t(16) = -3.2$, $**p = 0.006$) alternation decisions compared to controls. *N = 9 mice per group.*

Supplementary Figure 1



Supplementary Figure 1 No significant differences in frequency band powers when examining PFC oscillations throughout the full spontaneous Y-maze task. Power spectral density for the full spontaneous Y-maze task, without differentiating correct and incorrect decisions. Control and vHPC-CaMKII DT mice show a similar power spectral density profile, as in open-field and homecage baseline recordings. However, band power analysis reveals no significant differences at each of the frequency bands. N = 9 mice per group.

References

Adams RA, Bush D, Zheng F, et al. Impaired theta phase coupling underlies frontotemporal dysconnectivity in schizophrenia. Brain. 2020;143(4):1261-1277. doi:10.1093/brain/awaa035

Adhikari A, Topiwala MA, Gordon JA. Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. Neuron. 2010;65(2):257-269. doi:10.1016/j.neuron.2009.12.002

Ahlbeck J, Song L, Chini M, Bitzenhofer SH, Hanganu-Opatz IL. Glutamatergic drive along the septo-temporal axis of hippocampus boosts prelimbic oscillations in the neonatal mouse. Elife. 2018:7:e33158

Bahner F and A Meyer-Lindenberg. Hippocampal-prefrontal connectivity as a translational phenotype for schizophrenia. Eur Neuropsychopharmacol. 2017 Feb;27(2):93-106. doi: 10.1016/j.euroneuro.2016.12.007

Bitzenhofer SH, Pöpplau JA, Chini M, Marquardt A, Hanganu-Opatz IL. A transient developmental increase in prefrontal activity alters network maturation and causes cognitive dysfunction in adult mice. Neuron. 2021;109(8):1350-1364.e6. doi:10.1016/j.neuron.2021.02.011

Brockmann MD, Pöschel B, Cichon N, et al. Coupled oscillations mediate directed interactions between prefrontal cortex and hippocampus of the neonatal rat. Neuron. 2011 Jul 28;71(2):332-47. doi: 10.1016/j.neuron.2011.05.041

Cardin JA, Carlén M, Meletis K, et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature. 2009;459(7247):663-667. doi:10.1038/nature08002

Catanese J, Carmichael JE, van der Meer MA. Low- and high-gamma oscillations deviate

in opposite directions from zero-phase synchrony in the limbic corticostriatal loop. J Neurophysiol. 2016;116(1):5-17. doi:10.1152/jn.00914.2015

Carmichael JE, Gmaz JM, van der Meer MAA. Gamma Oscillations in the Rat Ventral Striatum Originate in the Piriform Cortex. J Neurosci. 2017;37(33):7962-7974. doi:10.1523/JNEUROSCI.2944-15.2017

Carmichael JE, Yuen MM, van der Meer MAA. Piriform cortex provides a dominant gamma LFP oscillation in the anterior limbic system. bioRxiv. 2019; doi.org/10.1101/861021

Cernotova D, Stuchlik A, Svoboda J. Transient Inactivation of the Medial Prefrontal Cortex and Ventral Hippocampus Impairs Active Place Avoidance Retrieval on a Rotating Arena. Front Neural Circuits. 2021;15:634533. Published 2021 Apr 28. doi:10.3389/fncir.2021.634533

Grent-'t-Jong T, Gross J, Goense J, et al. Resting-state gamma-band power alterations in schizophrenia reveal E/I-balance abnormalities across illness-stages. Elife. 2018;7:e37799. Published 2018 Sep 27. doi:10.7554/eLife.37799

Hong I, Kaang BK. The complexity of ventral CA1 and its multiple functionalities. Genes Brain Behav. 2022;21(7):e12826. doi:10.1111/gbb.12826

Kupferschmidt DA, Gordon JA. The dynamics of disordered dialogue: Prefrontal, hippocampal and thalamic miscommunication underlying working memory deficits in schizophrenia. Brain Neurosci Adv. 2018;2:2398212818771821. doi:10.1177/2398212818771821

Lalonde R. The neurobiological basis of spontaneous alternation. Neurosci Biobehav Rev. 2002;26(1):91-104. doi:10.1016/s0149-7634(01)00041-0

Laplante F, Stevenson CW, Gratton A, Srivastava LK, Quirion R. Effects of neonatal ventral hippocampal lesion in rats on stress-induced acetylcholine release in the prefrontal cortex. J Neurochem. 2004;91(6):1473-1482. doi:10.1111/j.1471-4159.2004.02831.x

Lara AH, Wallis JD. The Role of Prefrontal Cortex in Working Memory: A Mini Review. Front Syst Neurosci. 2015;9:173. Published 2015 Dec 18. doi:10.3389/fnsys.2015.00173

Liu X, Murray KD. Neuronal excitability and calcium/calmodulin-dependent protein kinase type II: location, location, location. Epilepsia. 2012:53(Suppl 1):45–52

McHugo M, Talati P, Armstrong K, et al. Hyperactivity and Reduced Activation of Anterior Hippocampus in Early Psychosis. Am J Psychiatry. 2019 Dec 1;176(12):1030-1038. doi: 10.1176/appi.ajp.2019.19020151

Meyer-Lindenberg AS, Olsen RK, Kohn PD, et al. Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. Arch Gen Psychiatry. 2005 Apr;62(4):379-86

Mitazaki S, Nakagawasai O, Onogi H, et al. Role of prefrontal cortical 5-HT2A receptors and serotonin transporter in the behavioral deficits in post-pubertal rats following neonatal lesion of the ventral hippocampus. Behav Brain Res. 2020;377:112226. doi:10.1016/j.bbr.2019.112226

Nath M, Bhardwaj SK, Srivastava LK, Wong TP. Altered excitatory and decreased inhibitory transmission in the prefrontal cortex of male mice with early developmental disruption to the ventral hippocampus. Cereb Cortex. 2023;33(3):865-880. doi:10.1093/cercor/bhac107

Nath M, Wong TP, Srivastava LK. Neurodevelopmental insights into circuit dysconnectivity in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2021;104:110047. doi:10.1016/j.pnpbp.2020.110047

Oberlander VC, Xu X, Chini M, Hanganu-Opatz IL. Developmental dysfunction of prefrontal-hippocampal networks in mouse models of mental illness. Eur J Neurosci. 2019;50(6):3072-3084. doi:10.1111/ejn.14436

O'Donnell P, Lewis BL, Weinberger DR, Lipska BK. Neonatal hippocampal damage alters electrophysiological properties of prefrontal cortical neurons in adult rats. Cereb Cortex. 2002:12:975–982.

O'Neill PK, Gordon JA, Sigurdsson T. Theta oscillations in the medial prefrontal cortex are modulated by spatial working memory and synchronize with the hippocampus through its ventral subregion. J Neurosci. 2013;33(35):14211-14224. doi:10.1523/JNEUROSCI.2378-13.2013

Phillips ML, Robinson HA, Pozzo-Miller L. Ventral hippocampal projections to the medial prefrontal cortex regulate social memory. Elife. 2019;8:e44182. Published 2019 May 21. doi:10.7554/eLife.44182

Ryan RT, Bhardwaj SK, Tse YC, Srivastava LK, Wong TP. Opposing alterations in excitation and inhibition of layer 5 medial prefrontal cortex pyramidal neurons following neonatal ventral hippocampal lesion. Cereb Cortex. 2013;23(5):1198-1207. doi:10.1093/cercor/bhs111

Schmidt R, Herrojo Ruiz M, Kilavik BE, Lundqvist M, Starr PA, Aron AR. Beta Oscillations in Working Memory, Executive Control of Movement and Thought, and Sensorimotor Function. J Neurosci. 2019;39(42):8231-8238. doi:10.1523/JNEUROSCI.1163-19.2019

Siapas AG, Lubenov EV, Wilson MA. Prefrontal phase locking to hippocampal theta oscillations. Neuron. 2005;46(1):141-151. doi:10.1016/j.neuron.2005.02.028

Sigurdsson T, Duvarci S. Hippocampal-Prefrontal Interactions in Cognition, Behavior and Psychiatric Disease. Front Syst Neurosci. 2016;9:190. Published 2016 Jan 26. doi:10.3389/fnsys.2015.00190

Sigurdsson T, Stark KL, Karayiorgou M, Gogos JA, Gordon JA. Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. Nature. 2010;464(7289):763-767. doi:10.1038/nature08855

Sun Q, Li X, Li A, Zhang J, Ding Z, Gong H, Luo. Ventral hippocampal-prefrontal interaction affects social behavior via Parvalbumin positive neurons in the medial prefrontal cortex. iScience. 2020:23:100894.

Tseng KY, Chambers RA, Lipska BK. The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Behav Brain Res. 2009:204:295–305

Tseng KY, Lewis BL, Hashimoto T, et al. A neonatal ventral hippocampal lesion causes functional deficits in adult prefrontal cortical interneurons. J Neurosci. 2008;28(48):12691-12699. doi:10.1523/JNEUROSCI.4166-08.2008

Tseng KY, Lewis BL, Lipska BK, O'Donnell P. Post-pubertal disruption of medial prefrontal cortical dopamine-glutamate interactions in a developmental animal model of schizophrenia. Biol Psychiatry. 2007;62(7):730-738. doi:10.1016/j.biopsych.2006.10.012

Valdés-Cruz A, Negrete-Díaz JV, Magdaleno-Madrigal VM, et al. Electroencephalographic activity in neonatal ventral hippocampus lesion in adult rats. Synapse. 2012;66(8):738-746. doi:10.1002/syn.21563

van der Meer MA, Redish AD. Low and High Gamma Oscillations in Rat Ventral Striatum have Distinct Relationships to Behavior, Reward, and Spiking Activity on a Learned Spatial

Decision Task. Front Integr Neurosci. 2009;3:9. Published 2009 Jun 11. doi:10.3389/neuro.07.009.2009

Whitfield-Gabrieli S, Thermenos HW, Milanovic S, et al. Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia [published correction appears in Proc Natl Acad Sci U S A. 2009 Mar 17;106(11):4572]. Proc Natl Acad Sci U S A. 2009;106(4):1279-1284. doi:10.1073/pnas.0809141106

Yang ST, Shi Y, Wang Q, Peng JY, Li BM. Neuronal representation of working memory in the medial prefrontal cortex of rats. Mol Brain. 2014;7:61. Published 2014 Aug 28. doi:10.1186/s13041-014-0061-2

Chapter 5: General Discussion and Future Directions

Summary

The overarching goal of this thesis was to examine the neurodevelopmental mechanisms underlying schizophrenia pathogenesis. The comprehensive literature review provided supporting evidence that schizophrenia is a brain disorder of dysconnectivity and that these dysconnectivity patterns emerge during early development. While several brain regions are implicated, we focused on two core regions: the vHPC and PFC. Dysfunction in this pathway constitutes an important schizophrenia endophenotype that impacts diverse cognitive processes. Chapter 2 outlined the tools necessary in order to dissect neurodevelopmental mechanisms underlying schizophrenia. These tools included the use of transgenic animal models, viral tools, and directed expression of molecules which would allow the bidirectional manipulation of activity in temporally specific windows and in specific cell type populations. These tools were then applied in both Chapters 3 and 4. Whereas the former chapter identified vHPC-CaMKII cell populations as significant contributors to altered PFC functional maturation, including increased probability of excitatory inputs, and decreased inhibitory inputs onto pyramidal cells, the latter chapter examined the impact of the integration of these synaptic changes onto PFC oscillatory activity and associated behavioral changes. We identified an excess in high gamma, and reductions in other oscillatory powers including theta, beta, and low gamma, which may contribute to impaired working memory in mice with early vHPC-CaMKII ablations. This thesis therefore successfully achieved a neurodevelopmental investigation into schizophrenia pathogenesis, identifying vHPC-CaMKII cells as significant contributors to altered cellular and LFP activities in the PFC and characterizing the nature of these changes in the PFC.

Targeting the vHPC-PFC circuitry

Neuroimaging studies in schizophrenia patients identify multiple brain regions with altered functional capacities. Based on these neuroimaging studies, we can investigate how each different brain region and their connectivity contributes to a schizophrenia phenotype in animal models. We have previously established that the vHPC is a core hub of schizophrenia pathology; early developmental lesioning of this region reproduces a range of schizophrenia-like behavioral abnormalities with a temporal emergence parallel to schizophrenia patients, and produces brain alterations similar to what is observed in schizophrenia patients (see Chapter 1). This region, however, projects to multiple different other regions. In addition to the PFC, the vHPC has direct projections to the thalamus, amygdala, nucleus accumbens, and ventral tegmental area (Fanselow and Dong 2010). Moreover, these regions additionally project to the PFC (Fanselow and Dong 2010). Therefore, the ablation of vHPC-CaMKII cells may impact PFC activity through both direct projections but also through indirect projections via other vHPC targeted regions.

One way to specifically examine vHPC-PFC connectivity would be to utilize a retrograde virus injected into the PFC combined with a drug that targets the retrogradely infected cells in the vHPC. Our lab has been interested in optimizing this technique in order to achieve vHPC-PFC pathway specificity for early developmental perturbations. Similarly, it would be advantageous to utilize an anterograde trans-synaptic tracer in order to record electrical activity specifically from PFC cells with vHPC synaptic inputs compared to PFC cells without. This trans-synaptic tracer would need to be injected prior to vHPC manipulation, such as ablation, since subsequent to ablation the synaptic inputs would be eliminated. Moreover, the temporal expression of the use of multiple constructs would need to be coordinated in order to still achieve early developmental perturbations

in the vHPC. These experiments, albeit more technically challenging, would help delineate vHPC-PFC specific contributions to schizophrenia pathogenesis, delineating the effects of direct vHPC inputs compared to potential indirect inputs mediated through other brain regions.

The use of such trans-synaptic graded transport tools would also be advantageous in determining the mechanism by which PFC dysfunction emerges. We found deficits in both pyramidal and PV cells following early vHPC lesioning in mouse pups. These deficits included reduced pyramidal cell firing, increased excitatory and decreased inhibitory inputs onto pyramidal cells, in contrast to decreased PV cell density, decreased excitatory inputs onto PV cells, and a deficit in NMDA-receptor mediated current in PV but not pyramidal cells in the PFC (Nath et al., 2023). It would be of interest to ablate pyramidal PFC projecting vHPC cells and examine the effects on PFC functional activity, and to contrast these effects to when PV PFC projecting vHPC cells are ablated. The results of these experiments may provide insight into whether PFC dysfunction originates from PFC pyramidal cells, PFC PV cells, or both. It might also help address, for example, why there is an increase in excitatory inputs onto pyramidal cells but decrease onto PV cells. Is it a differential effect of vHPC synaptic connectivity? Or, is it a result of a compensatory mechanism occurring in local PFC synaptic connectivity in response to ablated vHPC inputs? This trans-synaptic labeling approach could also be applied to other interneuron population targets in the PFC, including somatostatin (SST) and 5HT3aR cells, to examine how early vHPC perturbations impacts interneuron activities with different maturation windows (Arain et al., 2013; Tuncdemir et al., 2016; Che et al., 2018). These results could help determine factors in interneuron maturation that make them vulnerable to developmental insults.

Another method of examining the impacts of early vHPC perturbations on schizophrenia pathogenesis would be to compare and contrast the effects on different brain regions, and at different time points (early versus adulthood). For example, an underlying assumption of our studies is that the protracted maturation of the PFC (Arain et al., 2013) makes it susceptible to early developmental vHPC insults. Is this true in regions with vHPC projections, that are already relatively more mature in early development, such as the thalamus (Fair et al., 2010)? Is the nature of functional cellular changes in regions targeted by the vHPC the same? For example, would the thalamus, amygdala, and nucleus accumbens also show an increase in excitatory and decrease in inhibitory inputs, or excess high gamma frequency power similar to the PFC? Finally, are the observed changes the same when vHPC inputs are ablated in early development versus adulthood? A separate set of control experiments would also be needed to show that these effects are specific to vHPC projections, and not to another set of projections. Addressing these questions through an extensive battery of experiments would provide insight into how one core region may produce a range of schizophrenia-like behaviors through similar or different mechanisms between projection pathways.

In line with this, whereas our findings showed that vHPC-CaMKII ablation results in functional cellular deficits in the PFC but not early vHPC-PV cell ablation, the latter may still be relevant to schizophrenia pathogenesis but via other brain regions. This difference may explain the different behavioral expressions observed with early vHPC-CaMKII versus early vHPC-PV cell ablations, where the former is associated with more cognitive deficits and the latter with more dopamine-dependent behavioral deficits (unpublished data from our lab). Thus, both the examination of the impacts of early vHPC perturbations on diverse regions, in addition to the effects of pathway

specific manipulations using more precise tools are important in better dissecting the neurodevelopmental schizophrenia circuitry.

Independent of pathway specificity, it would still be necessary to examine how other pathway contributions to the PFC may have been impacted following early vHPC disruption. Compensatory changes may occur. For example, the increase in excitatory synaptic input probability observed in the PFC, despite ablation of vHPC-CaMKII excitatory projection cells, may be mediated through local or distant compensatory changes (discussed in Chapter 3). Similarly, how the vHPC may reorganize following an early developmental perturbation would be worth examining, as was done with cFos labeling in Chapter 3.

Manipulating vHPC activity in early development

Whereas the vHPC is a core hub region in schizophrenia functional dysconnectivity, one important question to be addressed is whether the early developmental ablation of CaMKII or PV cells in the vHPC is relevant to schizophrenia pathogenesis. The neonatal vHPC lesioning rat model produces a range of schizophrenia-like behaviors with a progression parallel that in schizophrenia patients (Lipska et al., 1993), yet there is no strong evidence that there are substantial reductions in total hippocampal cell numbers in schizophrenia patients (Allen et al., 2016). Neuroimaging studies further consistently show a reduction in anterior hippocampal volume yet baseline hyperactivity, which contrasts to the lesion model where the absence of the vHPC necessarily results in the absence of vHPC activity (McHugo et al., 2020; McHugo et al., 2018; Talati et al., 2014; Schobel et al., 2009). This model therefore suffers a little from construct validity, but has a strong face validity (Tseng et al., 2009).

Developing better construct validity while maintaining strong face validity would indicate improved modeling of schizophrenia pathogenesis and therefore understanding. The ablation of vHPC CaMKII or PV cell model may be short on construct validity, but it improves on this dimension by examining cellular markers implicated as risk factors for schizophrenia. Mutations in CaMKII alpha have been identified in schizophrenia patients, with some mutations resulting in impaired CaMKII autonomous kinase activity, which is important for long-term potentiation (Brown et al., 2021). Moreover, CaMKII alpha heterozygous knockout mice produce an immature dentate gyrus, and schizophrenia-like behaviors such as impaired working memory and aggressive behavior (Yamasaki et al., 2008). Similarly, decreased PV cell density has also been found in the hippocampus (Zhang and Reynolds, 2002; Konradi et al., 2011), which may reflect a reduction in PV expression, rather than PV cell death (Lewis et al., 2012). Knockout of PV in the hippocampus also produces schizophrenia-like behaviors (Perez et al., 2019).

Even if extensively reduced cellular numbers may not be associated with vHPC dysfunction in schizophrenia, our studies indicate that vHPC CaMKII cell populations are relevant at least to the onset of PFC deficits; therefore, future studies may need to manipulate the activity of this cellular population as opposed to ablating it.. Nath et al., 2021 already established the importance of using bidirectional and temporally specific manipulators of activity. Subsequent experiments can therefore examine the following: (1) How is vHPC CA1 pyramidal cell activity impacted in transgenic models involving schizophrenia-risk genes? The use of a transgenic mouse would enable us to examine vHPC CA1 pyramidal cell activity across development, as opposed to only examinations at adulthood. This experimental paradigm can also be performed with environmental

stressors. The ultimate goal here is to characterize thoroughly potential vHPC CA1 pyramidal cell activity dysfunction in early development. (2) Does manipulating vHPC activity in early development in the same pattern characterized in (1) contribute to the development of reduced vHPC volume and increased hyperactivity as observed in schizophrenia patients? This experiment would help us understand the progression of vHPC changes in schizophrenia, and would provide evidence that early vHPC activity aberrations result in schizophrenia related anterior hippocampal phenotypes, thereby improving construct validity. (3) What are the impacts on PFC functional maturation and vHPC-PFC dependent behaviors when vHPC cell activity is manipulated during early development based on activity patterns characterized in (1). Future investigations would therefore require tools to temporally manipulate activity in early development, specifically in vHPC-CaMKII cells given our findings, to better ascertain what aspect of vHPC input signals is relevant to vHPC dysfunction and to altered PFC functional maturation.

vHPC functional aberrations may exist prior to the onset of psychosis, and the adolescent exposure to risk factors, such as cannabis, may in turn trigger psychosis. Thus, another way of assessing the relevance of the vHPC in schizophrenia pathology, that can build upon the aforementioned experiments, would be to perturb vHPC activity as characterized in (1), and additionally examine the effects of an environmental exposure on vHPC activity. Would such exposure trigger psychosis? While human imaging studies reveal reductions in hippocampal volume can be associated with the onset of psychosis (see Chapter 1), this would provide a causal link and provide a potential path for prevention for psychosis.

Targeting an early developmental time window

The aforementioned approaches on how to best manipulate vHPC in early development in a way that would be more relevant to schizophrenia pathology also highlights the importance of identifying a proper temporal window over which a construct would act. The lesioning method with ibotenic acid, for example, applied in our P14 mouse pups and in the traditional nVHL rat model at P7 had a more immediate effect compared to the viral expression of diphtheria toxin, which leads to cell death within two to three days following expression (Brockschnieder et al., 2004). The viral construct also influences expression, with AAV, including AAV8 used in our studies, peaking in expression within two weeks (Penrod et al., 2015; Klein et al., 2006). While our perturbation still acts within pre-pubescent and pubescent time windows, it would be of interest to explore either transgenic animals or faster expressing constructs that can modulate activity at different time points, including potentially in utero. This would allow us to differentiate the impacts of the same manipulation in the vHPC at different time windows, in utero, post-natal, and pubescent, and relate such vHPC activity changes with various risk factors occurring at different stages of development (Birnbaum and Weinberger, 2017).

Examining causality: vHPC-PFC manipulations to normalize behavioral deficits

By applying tools to specifically target vHPC-PFC projections with bidirectional temporally directed manipulators of activity, we would also be able to investigate whether normalizing such activity patterns in adult mice can rescue schizophrenia-like behavioral deficits. We found that early ablation of vHPC-CaMKII cells was associated with excess high gamma, but reduced theta, and low gamma frequencies during the spontaneous Y-maze alternation task, in addition to reduced beta frequency power specifically at incorrect decisions. These altered PFC oscillations may be associated with deficits in the spontaneous Y-maze task. To confirm this, however, we could

investigate whether optogenetically modulating firing activities in the PFC, such as at beta frequency, helps improve performance in this task.

This rescue approach can also be applied to cellular activities within the PFC to determine how that impacts PFC oscillations. For example, would increasing the reduced inhibitory synaptic inputs onto pyramidal cells characterized *in vitro* using chemogenetic or optogenetic approaches normalize the excess gamma frequencies observed *in vivo*? In addition to performing single-unit *in vivo* recordings in the PFC to complement our LFP analyses, these rescue experiments would better establish causal relationships between cellular and circuit PFC changes in activity.

Similarly, we found a deficit in NMDA-receptors in PV cells but not pyramidal cells. While several studies have already investigated the effects of NMDA-receptor inhibition or knockout in pyramidal versus PV cells on schizophrenia-like behaviors and pathology (Homayoun and Moghaddam, 2007; Bygrave et al., 2016; Rompala et al., 2013; Jami et al., 2021; Lee and Zhou, 2019), it would be of interest to determine the developmental relevance of such NMDA-receptor inhibition patterns in PV cells and the rescue of PV NMDA-receptor deficits on PFC function.

Thus, whereas our studies identified vHPC-CaMKII cells as important contributors to altered PFC functional maturation, and examined the nature of these PFC changes both at cellular and field levels, future experimentation must be conducted in order to refine our understanding of this circuitry, including improved pathway specificity, relevant early developmental manipulations of activity, and assessments for causality.

Sex-specific differences in schizophrenia

An important limitation to our studies was that the experiment was conducted in male mice. Whereas initial experiments were conducted in both male and female mice, the lack of significant findings related to PFC activity changes following early vHPC excitotoxic lesioning led us to focus in on male mice for subsequent experiments (mentioned in Chapter 3). Schizophrenia pathogenesis in females varies; they are more likely than males to develop late-onset schizophrenia (around 40 years of age, see Chapter 1). This late-onset has been speculated to be related to hormones, where estrogen is thought to exert a protective effect against schizophrenia (Gogos et al., 2015). Future experimentation therefore requires further investigation into how vHPC-PFC altered function may contribute to schizophrenia pathogenesis in females. For example, do PFC deficits simply occur at later ages in female mice, compared to at P60 as characterized in our experiments (Chapter 3)? Our findings from male mice can still help us determine the pathogenesis of schizophrenia in female mice. For example, we can examine the effect of estrogen inhibition or estrogen activation on vHPC-PFC activity in male and female mice. The inhibition of estrogen in females may trigger PFC changes similar to that observed in males. In contrast, enhancing estrogen signaling subsequent to early vHPC perturbations may rescue PFC functional deficits, such as hypofunction in NMDA receptors observed in PV cells (El-Bakri et al., 2004; Chapter 3).

Integrating our findings into schizophrenia

The neurodevelopmental hypothesis of schizophrenia suggests schizophrenia originates in early development based on the occurrence of genetic, epigenetic, and environmental risk factors, and neuroimaging studies highlighting abnormal structural and functional connectivity patterns present in clinically high-risk subjects well before the onset of psychosis including in neonates (Chapter

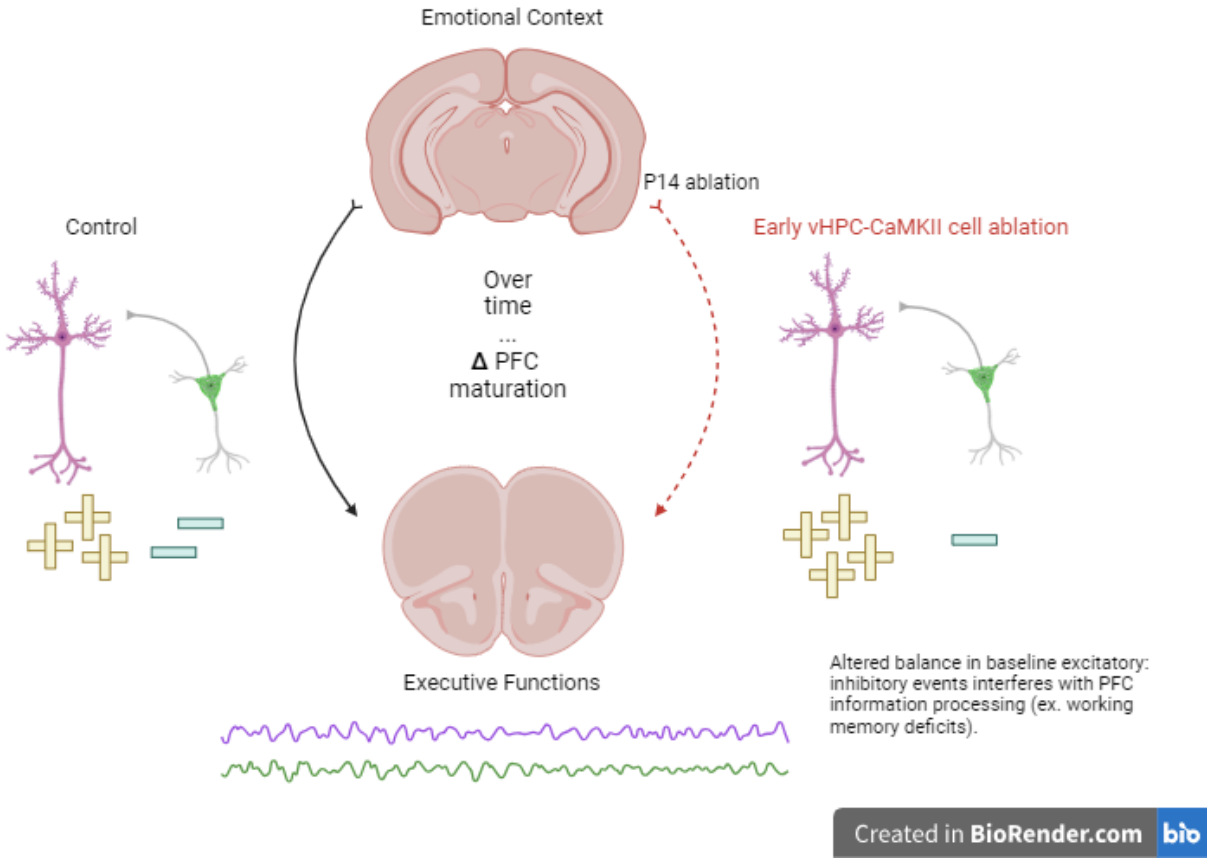
1). While there are multiple and diverse methods by which risk factors can impinge on neural circuitry, which brain regions and how their activity patterns contribute to the developmental progression of schizophrenia remain not well understood.

Our results show that the vHPC is a core region in the developmental onset of schizophrenia, by demonstrating its translatability from the nVHL rat model to our mouse model, and the relevance of different time points (P7 in rats, versus P14 in mice) albeit still during critical periods of brain development.

With respect to schizophrenia patients, our studies suggest that early developmental insults, genetic and environmental, likely impinge on anterior hippocampal/vHPC function. Given the important role of the vHPC in emotional and global memory functions (Geng et al., 2019; Decker et al., 2020), aberrations in vHPC activity produces further deficits in the still developing PFC, mediated by CaMKII excitatory projections. The PFC, therefore, adapts its circuitry to the aberrant information received from the vHPC. These adaptations occur in the form of altered excitatory synaptic input changes and decreased inhibitory synaptic inputs. This decrease in inhibitory inputs may be related to PV cell deficits, which we found to show reduced NMDA-receptor dependent current, which in turn may decrease PV expression through enhanced oxidative stress (Hasam-Henderson et al., 2018) resulting in reduced PV cell density measures characterized in our model following early vHPC lesioning. The net effect of these altered synaptic inputs *in vitro* therefore appears to be that of general increased excitation, which would be in line with the frequent observations of increased baseline activity in schizophrenia patients (Chapter 1).

We then investigated how these cellular changes may be related to *in vivo* PFC activity. Whereas a deficit in PV cell may be expected to lead to deficits in gamma oscillations (Hasam-Henderson et al., 2018), we found excess gamma activity at baseline and during task performance. Interestingly, the induction of oxidative stress, either via NMDA-receptor hypofunction or a reduction in the antioxidant glutathione (also a risk factor in schizophrenia) in PV cells, can lead to both decreases and increases in gamma band power (Hasam-Henderson et al., 2018). Moreover, schizophrenia patients also show deficits in PV yet occurrences of excess gamma activity (Chapter 1), therefore the relationship between PV and gamma remains to be further investigated. However, our findings of altered probability of excitatory inputs onto both pyramidal and PV cells in the vHPC-CaMKII ablation and vHPC lesion model suggests that the changes in synaptic connectivity is more complicated (as opposed to a simple excess), and this complicated probability distribution of excitatory synaptic inputs might underlie uncoordinated activity between PFC cells, and therefore reduced power across various oscillations, including theta, beta, and low gamma oscillations, wherein power is a reflection of the synchronous activity of cells at specific frequencies. Given that beta and theta oscillations are thought to be generated by pyramidal cell activity, the altered excitatory transmission may underlie the power deficits. These alterations in turn may underlie the observed working memory deficits in our studies, and in turn may explain cognitive deficits observed in schizophrenia patients mediated by altered PFC functional maturation due to early developmental insults to vHPC activity.

Summary Figure



Summary Figure: Early vHPC perturbation through ablation of CaMKII expressing cells results in PFC functional changes as the rodent develops. These changes include a general pattern of increased excitatory events and reduced sources of inhibition and decreased inhibitory inputs. These synaptic changes alters the oscillatory activities of the PFC, resulting in a general excess in high gamma activity at baseline, but reductions in other oscillatory frequencies of theta, beta, or low gamma depending on task performance. This enhanced 'noisiness' of the PFC interferes with its executive function abilities, a core feature of schizophrenia. This figure was created with BioRender.

Conclusion and summary

Early insults to brain development significantly impacts the processing of contextual information by the anterior hippocampus in humans, which is conveyed to the developing PFC, resulting in aberrations in its functional connectivity and therefore cognitive behavioral expressions. We have shown this through the early vHPC manipulation in mice, wherein the ablation of vHPC-CaMKII cells is responsible for the onset of PFC deficits, including increased excitatory and decreased inhibitory synaptic inputs onto pyramidal cells, and excess high gamma frequency, yet reduced theta, beta, and low gamma frequencies. We have briefly explored the molecular mechanistic underlying of these functional deficits, identifying deficits in NMDA-receptor current in PV cells and reductions in PV cell density following early vHPC lesioning. These changes in the PFC is in turn associated with cognitive deficits, including deficits in spontaneous working memory. Our results therefore highlight the need for treatments that target early development, and in studying these developmental aberrations, we will be able to potentially identify targets that can re-open a window for the re-establishment of proper functional connectivity and therefore alleviation of schizophrenia related behavioral deficits.

Reference list

Adams RA, Bush D, Zheng F, Meyer SS, Kaplan R, Orfanos S, Marques TR, Howes OD, Burgess N. Impaired theta phase coupling underlies frontotemporal dysconnectivity in schizophrenia. Brain. 2020 Apr 1;143(4):1261-1277. Doi: 10.1093/brain/awaa035. PMID: 32236540; PMCID: PMC7174039

Allen KM, Fung SJ, Weickert CS. Cell proliferation is reduced in the hippocampus in schizophrenia. Aust N Z J Psychiatry. 2016;50(5):473-480. doi:10.1177/0004867415589793

Amaral DG, Witter MP. Hippocampal formation. In: Paxinos G, editor. The rat nervous system. 3rd ed. Academic Press; San Diego, CA: 2004.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 2013. 5th ed. Arlington, VA: Author.

Arain M, Haque M, Johal L, et al. Maturation of the adolescent brain. Neuropsychiatr Dis Treat. 2013;9:449-461. doi:10.2147/NDT.S39776

Badner JA, Gershon ES. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol Psychiatry. 2002;7(4):405-411. doi:10.1038/sj.mp.4001012

Bähner F, Meyer-Lindenberg A. Hippocampal-prefrontal connectivity as a translational phenotype for schizophrenia. Eur Neuropsychopharmacol. 2017;27(2):93-106. doi:10.1016/j.euroneuro.2016.12.007

Baker KD, Gray AR, Richardson R. The development of perineuronal nets around parvalbumin gabaergic neurons in the medial prefrontal cortex and basolateral amygdala of rats. Behav Neurosci. 2017;131(4):289-303. doi:10.1037/bne0000203

Barr MS, Rajji TK, Zomorrodi R, Radhu N, George TP, Blumberger DM, Daskalakis ZJ. Impaired theta-gamma coupling during working memory performance in schizophrenia. Schizophr Res. 2017 Nov;189:104-110. Doi: 10.1016/j.schres.2017.01.044. Epub 2017 Jan 29. PMID: 28148460.

Basar-Eroglu C, Brand A, Hildebrandt H, Karolina Kedzior K, Mathes B, Schmiedt C. Working memory related gamma oscillations in schizophrenia patients. Int J Psychophysiol. 2007;64:39–45.

Bates AT, Kiehl KA, Laurens KR, Liddle PF. Low-frequency EEG oscillations associated with information processing in schizophrenia. Schizophr Res. 2009;115(2-3):222-230. doi:10.1016/j.schres.2009.09.036

Begré S, Federspiel A, Kiefer C, Schroth G, Dierks T, Strik WK. Reduced hippocampal anisotropy related to anteriorization of alpha EEG in schizophrenia. Neuroreport. 2003 Apr 15;14(5):739-42. Doi: 10.1097/00001756-200304150-00016. PMID: 12692474.

Belforte JE, Zsiros V, Sklar ER, et al. Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. Nat Neurosci. 2010;13(1):76-83. doi:10.1038/nn.2447

Beneyto M, Meador-Woodruff JH. Lamina-specific abnormalities of NMDA receptor-associated postsynaptic protein transcripts in the prefrontal cortex in schizophrenia and bipolar disorder. Neuropsychopharmacology. 2008;33(9):2175–86.

Berdenis van Berlekom A, Muflihah CH, Snijders GJLJ, et al. Synapse Pathology in Schizophrenia: A Meta-analysis of Postsynaptic Elements in Postmortem Brain Studies. Schizophr Bull. 2020;46(2):374-386. doi:10.1093/schbul/sbz060

Birnbaum R, Weinberger DR. Genetic insights into the neurodevelopmental origins of schizophrenia. Nat Rev Neurosci. 2017;18(12):727-740. doi:10.1038/nrn.2017.125

Boos HB, Aleman A, Cahn W, Hulshoff Pol H, Kahn RS. Brain volumes in relatives of patients with schizophrenia: a meta-analysis. Arch Gen Psychiatry. 2007;64(3):297-304. doi:10.1001/archpsyc.64.3.297

Brockschnieder D, Lappe-Siefke C, Goebbels S, Boesl MR, Nave KA, Riethmacher D. Cell depletion due to diphtheria toxin fragment A after Cre-mediated recombination. Mol Cell Biol. 2004;24(17):7636-7642. doi:10.1128/MCB.24.17.7636-7642.2004

Brown CN, Cook SG, Allen HF, et al. Characterization of six CaMKIIα variants found in patients with schizophrenia. iScience. 2021;24(10):103184. Published 2021 Sep 27. doi:10.1016/j.isci.2021.103184

Buzsáki G. Theta oscillations in the hippocampus. Neuron. 2002 Jan 31;33(3):325-40. Doi: 10.1016/s0896-6273(02)00586-x. PMID: 11832222.

Bygrave AM, Masiulis S, Nicholson E, et al. Knockout of NMDA-receptors from parvalbumin interneurons sensitizes to schizophrenia-related deficits induced by MK-801. Transl Psychiatry. 2016;6(4):e778. Published 2016 Apr 12. doi:10.1038/tp.2016.44

Calabro FJ, Murty VP, Jalbrzikowski M, Tervo-Clemmens B, Luna B. Development of Hippocampal-Prefrontal Cortex Interactions through Adolescence. Cereb Cortex. 2020;30(3):1548-1558. doi:10.1093/cercor/bhz186

Cannon TD, et al. Progressive reduction in cortical thickness as psychosis develops: a multisite longitudinal neuroimaging study of youth at elevated clinical risk. Biol Psychiatry. 2015;77:147–157

Cannon TD. How Schizophrenia Develops: Cognitive and Brain Mechanisms Underlying Onset of Psychosis. Trends Cogn Sci. 2015;19(12):744-756. doi:10.1016/j.tics.2015.09.009

Carlén M, Meletis K, Siegle JH, et al. A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. Mol Psychiatry. 2012;17(5):537-548. doi:10.1038/mp.2011.31

Cembrowski MS, Bachman JL, Wang L, Sugino K, Shields BC, Spruston N. Spatial Gene-Expression Gradients Underlie Prominent Heterogeneity of CA1 Pyramidal Neurons. Neuron. 2016;89(2):351-368. doi:10.1016/j.neuron.2015.12.013

Che A, Babij R, Iannone AF, et al. Layer I Interneurons Sharpen Sensory Maps during Neonatal Development. Neuron. 2018;99(1):98-116.e7. doi:10.1016/j.neuron.2018.06.002

Cho RY, Konecky RO, Carter CS. Impairments in frontal cortical gamma synchrony and cognitive control in schizophrenia. Proc Natl Acad Sci U S A. 2006;103:19878–19883.

Chung Y, et al. Prodromal Symptom Severity Predicts Accelerated Gray Matter Reduction and Third Ventricle Expansion Among Clinically High Risk Youth Developing Psychotic Disorders. Mol Neurospsychiatry. 2015;1:13–22

Cohen AO, Breiner K, Steinberg L, et al. When Is an Adolescent an Adult? Assessing Cognitive Control in Emotional and Nonemotional Contexts [published correction appears in Psychol Sci. 2017 Mar;28(3):399-402]. Psychol Sci. 2016;27(4):549-562. doi:10.1177/0956797615627625

Cohen SM, Tsien RW, Goff DC, Halassa MM. The impact of NMDA receptor hypofunction on GABAergic neurons in the pathophysiology of schizophrenia. Schizophr Res. 2015;167(1-3):98-107. doi:10.1016/j.schres.2014.12.026

Collin G, van den Heuvel MP. The ontogeny of the human connectome: development and dynamic changes of brain connectivity across the life span. Neuroscientist. 2013;19(6):616-628. doi:10.1177/1073858413503712

Corcoran KA, Quirk GJ. Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. J Neurosci. 2007;27(4):840-844. doi:10.1523/JNEUROSCI.5327-06.2007

Crone EA, Steinbeis N. Neural Perspectives on Cognitive Control Development during Childhood and Adolescence. Trends Cogn Sci. 2017;21(3):205-215. doi:10.1016/j.tics.2017.01.003

Dandash O, Pantelis C, Fornito A. Dopamine, fronto-striato-thalamic circuits and risk for psychosis. Schizophr Res. 2017;180:48-57. doi:10.1016/j.schres.2016.08.020

de Zwarte SMC, Brouwer RM, Agartz I, et al. The Association Between Familial Risk and Brain Abnormalities Is Disease Specific: An ENIGMA-Relatives Study of Schizophrenia and Bipolar Disorder. Biol Psychiatry. 2019;86(7):545-556. doi:10.1016/j.biopsych.2019.03.985

Dean K, Murray RM. Environmental risk factors for psychosis. Dialogues Clin Neurosci. 2005;7(1):69-80. doi:10.31887/DCNS.2005.7.1/kdean

Decker AL, Duncan K, Finn AS, Mabbott DJ. Children's family income is associated with cognitive function and volume of anterior not posterior hippocampus. Nat Commun. 2020;11(1):4040. Published 2020 Aug 12. doi:10.1038/s41467-020-17854-6

Devienne G, Picaud S, Cohen I, et al. Regulation of perineuronal nets in the adult cortex by the activity of the cortical network [published online ahead of print, 2021 May 27]. J Neurosci. 2021;41(27):5779-5790. doi:10.1523/JNEUROSCI.0434-21.2021

Di Pietro NC, Black YD, Kantak KM. Context-dependent prefrontal cortex regulation of cocaine self-administration and reinstatement behaviors in rats. Eur J Neurosci. 2006;24(11):3285-3298. doi:10.1111/j.1460-9568.2006.05193.x

Dienel SJ, Lewis DA. Alterations in cortical interneurons and cognitive function in schizophrenia. Neurobiol Dis. 2019;131:104208. doi:10.1016/j.nbd.2018.06.020

Dobbing J, Sands J. Comparative aspects of the brain growth spurt. Early Hum Dev. 1979;3(1):79-83. doi:10.1016/0378-3782(79)90022-7

Doege K, Jansen M, Mallikarjun P, Liddle EB, Liddle PF. How much does phase resetting contribute to event-related EEG abnormalities in schizophrenia?. Neurosci Lett. 2010;481(1):1-5. doi:10.1016/j.neulet.2010.06.008

Doischer D, Hosp JA, Yanagawa Y, et al. Postnatal differentiation of basket cells from slow to fast signaling devices. J Neurosci. 2008;28(48):12956-12968. doi:10.1523/JNEUROSCI.2890-08.2008

Dong D, Wang Y, Chang X, Luo C, Yao D. Dysfunction of Large-Scale Brain Networks in Schizophrenia: A Meta-analysis of Resting-State Functional Connectivity. Schizophr Bull. 2018;44(1):168-181. doi:10.1093/schbul/sbx034

Ellison-Wright I, Glahn DC, Laird AR, Thelen SM, Bullmore E. The anatomy of first-episode and chronic schizophrenia: an anatomical likelihood estimation meta-analysis. Am J Psychiatry. 2008;165(8):1015-1023. doi:10.1176/appi.ajp.2008.07101562

El-Bakri NK, Islam A, Zhu S, et al. Effects of estrogen and progesterone treatment on rat hippocampal NMDA receptors: relationship to Morris water maze performance. J Cell Mol Med. 2004;8(4):537-544. doi:10.1111/j.1582-4934.2004.tb00478.x

El-Rawas R, Saadé NE, Thiriet N, Atweh S, Jaber M, Al-Amin HA. Developmental changes in the mRNA expression of neuropeptides and dopamine and glutamate receptors in neonates and adult rats after ventral hippocampal lesion. Schizophr Res. 2009;113(2-3):298-307. doi:10.1016/j.schres.2009.05.009

Endo K, Hori T, Abe S, Asada T. Alterations in GABA(A) receptor expression in neonatal ventral hippocampal lesioned rats: comparison of prepubertal and postpubertal periods. Synapse. 2007;61(6):357-366. doi:10.1002/syn.20393

Engel AK, Fries P. Beta-band oscillations--signalling the status quo? Curr Opin Neurobiol. 2010 Apr;20(2):156-65. doi: 10.1016/j.conb.2010.02.015. Epub 2010 Mar 30. PMID: 20359884.

ENIGMA Clinical High Risk for Psychosis Working Group, Jalbrzikowski M, Hayes RA, et al. Association of Structural Magnetic Resonance Imaging Measures With Psychosis Onset in Individuals at Clinical High Risk for Developing Psychosis: An ENIGMA Working Group Mega-analysis. JAMA Psychiatry. 2021;78(7):753-766. doi:10.1001/jamapsychiatry.2021.0638

Ergen M, Marbach S, Brand A, Başar-Eroğlu C, Demiralp T. P3 and delta band responses in visual oddball paradigm in schizophrenia. Neurosci Lett. 2008;440(3):304-308. doi:10.1016/j.neulet.2008.05.054

Fair DA, Bathula D, Mills KL, et al. Maturing thalamocortical functional connectivity across development. Front Syst Neurosci. 2010;4:10. Published 2010 May 18. doi:10.3389/fnsys.2010.00010

Fan LZ, Nehme R, Adam Y, et al. All-optical synaptic electrophysiology probes mechanism of ketamine-induced disinhibition. Nat Methods. 2018;15(10):823-831. doi:10.1038/s41592-018-0142-8

Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures?. Neuron. 2010;65(1):7-19. doi:10.1016/j.neuron.2009.11.031

Fish B, Shapiro T, Halpern F, Wile R. The prediction of schizophrenia in infancy: 3. A ten-year follow-up report of neurological and psychological development. Am J Psychiatry. 1965;121:768-775. Doi:10.1176/ajp.121.8.768

Flores C, Bhardwaj SK, Labelle-Dumais C, Srivastava LK. Altered netrin-1 receptor expression in dopamine terminal regions following neonatal ventral hippocampal lesions in the rat. Synapse. 2009;63(1):54-60. doi:10.1002/syn.20584

Flores G, Silva-Gómez AB, Ibáñez O, Quirion R, Srivastava LK. Comparative behavioral changes in postpubertal rats after neonatal excitotoxic lesions of the ventral hippocampus and the prefrontal cortex. Synapse. 2005;56(3):147-153. doi:10.1002/syn.20140

Folsom DP, Lebowitz BD, Lindamer LA, Palmer BW, Patterson TL, Jeste DV. Schizophrenia in late life: emerging issues. Dialogues Clin Neurosci. 2006;8(1):45-52. doi:10.31887/DCNS.2006.8.1/dfolsom

Ford JM, Roach BJ, Faustman WO, Mathalon DH. Synch before you speak: auditory hallucinations in schizophrenia. Am J Psychiatry. 2007 Mar;164(3):458-66. doi: 10.1176/ajp.2007.164.3.458. PMID: 17329471.

François J, Ferrandon A, Koning E, Angst MJ, Sandner G, Nehlig A. Selective reorganization of GABAergic transmission in neonatal ventral hippocampal-lesioned rats. Int J Neuropsychopharmacol. 2009;12(8):1097-1110. doi:10.1017/S1461145709009985

Franklin KBJ, Paxinos G. The mouse Brain in Stereotaxic Coordinates. 2013. Fourth edition. ed. Amsterdam: Academic Press.

Fusar-Poli P, Borgwardt S, Crescini A, et al. Neuroanatomy of vulnerability to psychosis: a voxel-based meta-analysis. Neurosci Biobehav Rev. 2011;35(5):1175-1185. doi:10.1016/j.neubiorev.2010.12.005

Gao XM, Sakai K, Roberts RC, Conley RR, Dean B, Tamminga CA. Ionotropic glutamate receptors and expression of N-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia. Am J Psychiatry. 2000;157(7):1141-1149. doi:10.1176/appi.ajp.157.7.1141

Geng F, Redcay E, Riggins T. The influence of age and performance on hippocampal function and the encoding of contextual information in early childhood. Neuroimage. 2019;195:433-443. doi:10.1016/j.neuroimage.2019.03.035

Geng X, Gouttard S, Sharma A, et al. Quantitative tract-based white matter development from birth to age 2years. Neuroimage. 2012;61(3):542-557. doi:10.1016/j.neuroimage.2012.03.057

George M, Maheshwari S, Chandran S, Manohar JS, Sathyanarayana Rao TS. Understanding the schizophrenia prodrome. Indian J Psychiatry. 2017;59(4):505-509. doi:10.4103/psychiatry.IndianJPsychiatry_464_17

Gergues MM, Han KJ, Choi HS, et al. Circuit and molecular architecture of a ventral hippocampal network [published correction appears in Nat Neurosci. 2021 Nov;24(11):1637]. Nat Neurosci. 2020;23(11):1444-1452. doi:10.1038/s41593-020-0705-8

Gilmore JH, van Tol J, Kliewer MA, et al. Mild ventriculomegaly detected in utero with ultrasound: clinical associations and implications for schizophrenia. Schizophr Res. 1998;33(3):133-140. doi:10.1016/s0920-9964(98)00073-5

Giustino TF, Maren S. The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear. Front Behav Neurosci. 2015;9:298. Published 2015 Nov 9. doi:10.3389/fnbeh.2015.00298

Glausier JR, Lewis DA. Dendritic spine pathology in schizophrenia. Neuroscience. 2013;251:90-107. doi:10.1016/j.neuroscience.2012.04.044

Gogos A, Sbisa AM, Sun J, Gibbons A, Udawela M, Dean B. A Role for Estrogen in Schizophrenia: Clinical and Preclinical Findings. Int J Endocrinol. 2015;2015:615356. doi:10.1155/2015/615356

Goto Y, O'Donnell P. Prefrontal lesion reverses abnormal mesoaccumbens response in an animal model of schizophrenia. Biol Psychiatry. 2004;55(2):172-176. doi:10.1016/s0006-3223(03)00783-2

Grayton HM, Fernandes C, Rujescu D, Collier DA. Copy number variations in neurodevelopmental disorders. Prog Neurobiol. 2012;99(1):81-91. doi:10.1016/j.pneurobio.2012.07.005

Grent-'t-Jong T, Gross J, Goense J, et al. Resting-state gamma-band power alterations in schizophrenia reveal E/I-balance abnormalities across illness-stages. Elife. 2018;7:e37799. Published 2018 Sep 27. doi:10.7554/eLife.37799

Hafner H, Maurer K, Loffler W, et al. The epidemiology of early schizophrenia. Influence of age and gender on onset and early course. Br J Psychiatry. 1994;23(Supp.):29–38.

Häfner H, Maurer K. Early detection of schizophrenia: current evidence and future perspectives. World Psychiatry. 2006;5(3):130-138.

Halgren M, Ulbert I, Bastuji H, Fabó D, Erőss L, Rey M, Devinsky O, Doyle WK, Mak-McCully R, Halgren E, Wittner L, Chauvel P, Heit G, Eskandar E, Mandell A, Cash SS. The

generation and propagation of the human alpha rhythm. Proc Natl Acad Sci U S A. 2019 Nov 19;116(47):23772-23782. doi: 10.1073/pnas.1913092116. Epub 2019 Nov 4. PMID: 31685634; PMCID: PMC6876194.

Hamm JP, Peterka DS, Gogos JA, Yuste R. Altered Cortical Ensembles in Mouse Models of Schizophrenia. Neuron. 2017;94(1):153-167.e8. doi:10.1016/j.neuron.2017.03.019

Harmony T. The functional significance of delta oscillations in cognitive processing. Front Integr Neurosci. 2013;7:83. Published 2013 Dec 5. doi:10.3389/fnint.2013.00083

Harrison PJ, Weinberger DR. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence [published correction appears in Mol Psychiatry. 2005 Apr;10(4):420] [published correction appears in Mol Psychiatry. 2005 Aug;10(8):804]. Mol Psychiatry. 2005;10(1):40-5. doi:10.1038/sj.mp.4001558

Hasam-Henderson LA, Gotti GC, Mishto M, et al. Author Correction: NMDA-receptor inhibition and oxidative stress during hippocampal maturation differentially alter parvalbumin expression and gamma-band activity. Sci Rep. 2018;8(1):13170. Published 2018 Aug 29. doi:10.1038/s41598-018-31011-6

Hathaway WR, Newton BW. Neuroanatomy, Prefrontal Cortex. [Updated 2023 May 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499919/

Hensch TK. Critical period plasticity in local cortical circuits. Nat Rev Neurosci. 2005;6(11):877-888. doi:10.1038/nrn1787

Herbener ES. Emotional memory in schizophrenia. Schizophr Bull. 2008;34(5):875-887. doi:10.1093/schbul/sbn081

Hirano Y, Uhlhaas PJ. Current findings and perspectives on aberrant neural oscillations in schizophrenia. Psychiatry Clin Neurosci. 2021;75(12):358-368. doi:10.1111/pcn.13300

Ho BC, Andreasen NC, Nopoulos P, Arndt S, Magnotta V, Flaum M. Progressive structural brain abnormalities and their relationship to clinical outcome: a longitudinal magnetic resonance imaging study early in schizophrenia. Arch Gen Psychiatry. 2003;60(6):585-594. doi:10.1001/archpsyc.60.6.585

Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. J Neurosci. 2007;27(43):11496-11500. doi:10.1523/JNEUROSCI.2213-07.2007

Hong I, Kaang BK. The complexity of ventral CA1 and its multiple functionalities. Genes Brain Behav. 2022;21(7):e12826. doi:10.1111/gbb.12826

Hoover WB, Vertes RP. Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct. 2007;212(2):149-179. doi:10.1007/s00429-007-0150-4

Hui CW, Bhardwaj SK, Sharma K, et al. Microglia in the developing prefrontal cortex of rats show dynamic changes following neonatal disconnection of the ventral hippocampus. Neuropharmacology. 2019;146:264-275. doi:10.1016/j.neuropharm.2018.12.007

Hunt MJ, Kopell NJ, Traub RD, Whittington MA. Aberrant Network Activity in Schizophrenia. Trends Neurosci. 2017;40(6):371-382. doi:10.1016/j.tins.2017.04.003

Isohanni M, Jones PB, Moilanen K, et al. Early developmental milestones in adult schizophrenia and other psychoses. A 31-year follow-up of the Northern Finland 1966 Birth Cohort. Schizophr Res. 2001;52(1-2):1-19. doi:10.1016/s0920-9964(00)00179-1

Jaaro-Peled H, Ayhan Y, Pletnikov MV, Sawa A. Review of pathological hallmarks of schizophrenia: comparison of genetic models with patients and nongenetic models. Schizophr Bull. 2010;36(2):301-313. doi:10.1093/schbul/sbp133

Jami SA, Cameron S, Wong JM, Daly ER, McAllister AK, Gray JA. Increased excitation-inhibition balance and loss of GABAergic synapses in the serine racemase knockout model of NMDA receptor hypofunction. J Neurophysiol. 2021;126(1):11-27. doi:10.1152/jn.00661.2020

Jones CA, Watson DJ, Fone KC. Animal models of schizophrenia. Br J Pharmacol. 2011;164(4):1162-1194. doi:10.1111/j.1476-5381.2011.01386.x

Joseph AT, Bhardwaj SK, Srivastava LK. Role of Prefrontal Cortex Anti- and Pro-inflammatory Cytokines in the Development of Abnormal Behaviors Induced by Disconnection of the Ventral Hippocampus in Neonate Rats. Front Behav Neurosci. 2018;12:244. Published 2018 Oct 22. doi:10.3389/fnbeh.2018.00244

Jung MW, Wiener SI, McNaughton BL. Comparison of spatial firing characteristics of units in dorsal and ventral hippocampus of the rat. J Neurosci. 1994;14(12):7347-7356. doi:10.1523/JNEUROSCI.14-12-07347.1994

Kaar SJ, Angelescu I, Marques TR, Howes OD. Pre-frontal parvalbumin interneurons in schizophrenia: a meta-analysis of post-mortem studies. J Neural Transm (Vienna). 2019;126(12):1637-1651. doi:10.1007/s00702-019-02080-2

Klein RL, Dayton RD, Leidenheimer NJ, Jansen K, Golde TE, Zweig RM. Efficient neuronal gene transfer with AAV8 leads to neurotoxic levels of tau or green fluorescent proteins. Mol Ther. 2006;13(3):517-527. doi:10.1016/j.ymthe.2005.10.008

Knickmeyer RC, Styner M, Short SJ, et al. Maturational trajectories of cortical brain development through the pubertal transition: unique species and sex differences in the monkey revealed through structural magnetic resonance imaging. Cereb Cortex. 2010;20(5):1053-1063. doi:10.1093/cercor/bhp166

Kolb B, Gibb R. Brain plasticity and behaviour in the developing brain. J Can Acad Child Adolesc Psychiatry. 2011;20(4):265-276.

Konradi C, Yang CK, Zimmerman EI, et al. Hippocampal interneurons are abnormal in schizophrenia. Schizophr Res. 2011;131(1-3):165-173. doi:10.1016/j.schres.2011.06.007

Kopell N, Ermentrout GB, Whittington MA, Traub RD. Gamma rhythms and beta rhythms have different synchronization properties. Proc Natl Acad Sci U S A. 2000 Feb 15;97(4):1867-72. doi: 10.1073/pnas.97.4.1867. PMID: 10677548; PMCID: PMC26528.

Korotkova T, Fuchs EC, Ponomarenko A, von Engelhardt J, Monyer H. NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. Neuron. 2010;68(3):557-569. doi:10.1016/j.neuron.2010.09.017

Laplante F, Srivastava LK, Quirion R. Alterations in dopaminergic modulation of prefrontal cortical acetylcholine release in post-pubertal rats with neonatal ventral hippocampal lesions. J Neurochem. 2004;89(2):314-323. doi:10.1111/j.1471-4159.2004.02351.x

Laubach M, Amarante LM, Swanson K, White SR. What, If Anything, Is Rodent Prefrontal Cortex?. eNeuro. 2018;5(5):ENEURO.0315-18.2018. Published 2018 Oct 25. doi:10.1523/ENEURO.0315-18.2018

Lavenex P., Banta Lavenex P. Building hippocampal circuits to learn and remember: insights into the development of human memory. Behav. Brain Res. 2013;254:8–21.

Law AJ, Deakin JF. Asymmetrical reductions of hippocampal NMDAR1 glutamate receptor mRNA in the psychoses. Neuroreport. 2001;12(13):2971-2974. doi:10.1097/00001756-200109170-00043

Le H, Dimitrakopoulou K, Patel H, et al. Effect of schizophrenia common variants on infant brain volumes: cross-sectional study in 207 term neonates in developing Human Connectome Project. Transl Psychiatry. 2023;13(1):121. Published 2023 Apr 10. doi:10.1038/s41398-023-02413-6

Lee G, Zhou Y. NMDAR Hypofunction Animal Models of Schizophrenia. Front Mol Neurosci. 2019;12:185. Published 2019 Jul 31. doi:10.3389/fnmol.2019.00185

Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. The Journal of neuroscience: the official journal of the Society for Neuroscience. 2010;30:16796–16808.

Lewis DA, Curley AA, Glausier JR, Volk DW. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci. 2012;35(1):57-67. doi:10.1016/j.tins.2011.10.004

Li X, Zhou W, Yi Z. A glimpse of gender differences in schizophrenia. Gen Psychiatr. 2022;35(4):e100823. Published 2022 Aug 30. doi:10.1136/gpsych-2022-100823

Liddle EB, Price D, Palaniyappan L, Brookes MJ, Robson SE, Hall EL, Morris PG, Liddle PF. Abnormal salience signaling in schizophrenia: The role of integrative beta oscillations. Hum Brain Mapp. 2016 Apr;37(4):1361-74. doi: 10.1002/hbm.23107. Epub 2016 Feb 8. PMID: 26853904; PMCID: PMC4790909.

Lillrank SM, Lipska BK, Bachus SE, Wood GK, Weinberger DR. Amphetamine-induced c-fos mRNA expression is altered in rats with neonatal ventral hippocampal damage. Synapse.

1996;23(4):292-301. doi:10.1002/(SICI)1098-2396(199608)23:4<292::AID-SYN7>3.0.CO;2-2

Lipska BK, al-Amin HA, Weinberger DR. Excitotoxic lesions of the rat medial prefrontal cortex. Effects on abnormal behaviors associated with neonatal hippocampal damage. Neuropsychopharmacology. 1998;19(6):451-464. doi:10.1016/S0893-133X(98)00045-1

Lipska BK, Jaskiw GE, Weinberger DR. Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia. Neuropsychopharmacology. 1993;9(1):67-75. doi:10.1038/npp.1993.44

Lipska BK, Lerman DN, Khaing ZZ, Weickert CS, Weinberger DR. Gene expression in dopamine and GABA systems in an animal model of schizophrenia: effects of antipsychotic drugs. Eur J Neurosci. 2003;18(2):391-402. doi:10.1046/j.1460-9568.2003.02738.x

Lipska BK, Luu S, Halim ND, Weinberger DR. Behavioral effects of neonatal and adult excitotoxic lesions of the mediodorsal thalamus in the adult rat. Behav Brain Res. 2003;141(2):105-111. doi:10.1016/s0166-4328(03)00027-5

Liu X, Carter AG. Ventral Hippocampal Inputs Preferentially Drive Corticocortical Neurons in the Infralimbic Prefrontal Cortex. J Neurosci. 2018;38(33):7351-7363. doi:10.1523/JNEUROSCI.0378-18.2018

Liu X, Dimidschstein J, Fishell G, Carter AG. Hippocampal inputs engage CCK+ interneurons to mediate endocannabinoid-modulated feed-forward inhibition in the prefrontal cortex. Elife. 2020;9:e55267. Published 2020 Oct 9. doi:10.7554/eLife.55267

Luna B, Marek S, Larsen B, Tervo-Clemmens B, Chahal R. An integrative model of the maturation of cognitive control. Annu Rev Neurosci. 2015;38:151-170. doi:10.1146/annurev-neuro-071714-034054

Mallya AP, Deutch AY. (Micro)Glia as Effectors of Cortical Volume Loss in Schizophrenia. Schizophr Bull. 2018;44(5):948-957. doi:10.1093/schbul/sby088

Mancini V, Sandini C, Padula MC, et al. Positive psychotic symptoms are associated with divergent developmental trajectories of hippocampal volume during late adolescence in patients with 22q11DS. Mol Psychiatry. 2020;25(11):2844-2859. doi:10.1038/s41380-019-0443-z

Marek R, Xu L, Sullivan RKP, Sah P. Excitatory connections between the prelimbic and infralimbic medial prefrontal cortex show a role for the prelimbic cortex in fear extinction. Nat Neurosci. 2018;21(5):654-658. doi:10.1038/s41593-018-0137-x

McHugo M, Armstrong K, Roeske MJ, Woodward ND, Blackford JU, Heckers S. Hippocampal volume in early psychosis: a 2-year longitudinal study. Transl Psychiatry. 2020;10(1):306. Published 2020 Sep 1. doi:10.1038/s41398-020-00985-1

McHugo M, Talati P, Armstrong K, et al. Hyperactivity and Reduced Activation of Anterior Hippocampus in Early Psychosis [published correction appears in Am J Psychiatry. 2019 Dec 1;176(12):1051] [published correction appears in Am J Psychiatry. 2019 Dec 1;176(12):1056]. Am J Psychiatry. 2019;176(12):1030-1038. doi:10.1176/appi.ajp.2019.19020151

McHugo M, Talati P, Woodward ND, Armstrong K, Blackford JU, Heckers S. Regionally specific volume deficits along the hippocampal long axis in early and chronic psychosis. Neuroimage Clin. 2018;20:1106-1114. doi:10.1016/j.nicl.2018.10.021

McIntosh AM, Owens DC, Moorhead WJ, et al. Longitudinal volume reductions in people at high genetic risk of schizophrenia as they develop psychosis. Biol Psychiatry. 2011;69(10):953-958. doi:10.1016/j.biopsych.2010.11.003

Milior G, Di Castro MA, Sciarria LP, et al. Electrophysiological Properties of CA1 Pyramidal Neurons along the Longitudinal Axis of the Mouse Hippocampus. Sci Rep. 2016;6:38242. Published 2016 Dec 6. doi:10.1038/srep38242

Minzenberg MJ, Laird AR, Thelen S, Carter CS, Glahn DC. Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia. Arch Gen Psychiatry. 2009;66(8):811-822. doi:10.1001/archgenpsychiatry.2009.91

Mitazaki S, Nakagawasai O, Onogi H, et al. Role of prefrontal cortical 5-HT2A receptors and serotonin transporter in the behavioral deficits in post-pubertal rats following neonatal lesion of the ventral hippocampus. Behav Brain Res. 2020;377:112226. doi:10.1016/j.bbr.2019.112226

Moorman DE, Aston-Jones G. Prefrontal neurons encode context-based response execution and inhibition in reward seeking and extinction. Proc Natl Acad Sci U S A. 2015;112(30):9472-9477. doi:10.1073/pnas.1507611112

Moorman DE, James MH, McGlinchey EM, Aston-Jones G. Differential roles of medial prefrontal subregions in the regulation of drug seeking. Brain Res. 2015;1628(Pt A):130-146. doi:10.1016/j.brainres.2014.12.024

Nakazawa K, Sapkota K. The origin of NMDA receptor hypofunction in schizophrenia. Pharmacol Ther. 2020;205:107426. doi:10.1016/j.pharmthera.2019.107426

Narayanan B, O'Neil K, Berwise C, Stevens MC, Calhoun VD, Clementz BA, Tamminga CA, Sweeney JA, Keshavan MS, Pearlson GD. Resting state electroencephalogram oscillatory

abnormalities in schizophrenia and psychotic bipolar patients and their relatives from the bipolar and schizophrenia network on intermediate phenotypes study. Biol Psychiatry. 2014 Sep 15;76(6):456-65. doi: 10.1016/j.biopsych.2013.12.008. Epub 2013 Dec 24. PMID: 24439302; PMCID: PMC5045030.

Nath M, Bhardwaj SK, Srivastava LK, Wong TP. Altered excitatory and decreased inhibitory transmission in the prefrontal cortex of male mice with early developmental disruption to the ventral hippocampus. Cereb Cortex. 2023;33(3):865-880. doi:10.1093/cercor/bhac107

Nath M, Wong TP, Srivastava LK. Neurodevelopmental insights into circuit dysconnectivity in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2021;104:110047. doi:10.1016/j.pnpbp.2020.110047

Nicole O, Pacary E. CaMKIIβ in Neuronal Development and Plasticity: An Emerging Candidate in Brain Diseases. Int J Mol Sci. 2020;21(19):7272. Published 2020 Oct 1. doi:10.3390/ijms21197272

O'Neill PK, Gordon JA, Sigurdsson T. Theta oscillations in the medial prefrontal cortex are modulated by spatial working memory and synchronize with the hippocampus through its ventral subregion. J Neurosci. 2013 Aug 28;33(35):14211-24. Doi: 10.1523/JNEUROSCI.2378-13.2013. PMID: 23986255; PMCID: PMC3756763.

Owen MJ, O'Donovan MC, Thapar A, Craddock N. Neurodevelopmental hypothesis of schizophrenia. Br J Psychiatry. 2011;198(3):173-175. doi:10.1192/bjp.bp.110.084384

Padilla-Coreano N, Bolkan SS, Pierce GM, et al. Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. Neuron. 2016;89(4):857-866. doi:10.1016/j.neuron.2016.01.011

Paolicelli RC, Ferretti MT. Function and Dysfunction of Microglia during Brain Development: Consequences for Synapses and Neural Circuits. Front Synaptic Neurosci. 2017;9:9. Published 2017 May 10. doi:10.3389/fnsyn.2017.00009

Pascual-Marqui RD, Lehmann D, Koenig T, et al. Low resolution brain electromagnetic tomography (LORETA) functional imaging in acute, neuroleptic-naive, first-episode, productive schizophrenia. Psychiatry Res. 1999;90(3):169-179. doi:10.1016/s0925-4927(99)00013-x

Pelkey KA, Chittajallu R, Craig MT, Tricoire L, Wester JC, McBain CJ. Hippocampal GABAergic Inhibitory Interneurons. Physiol Rev. 2017;97(4):1619-1747. doi:10.1152/physrev.00007.2017

Penrod RD, Wells AM, Carlezon WA Jr, Cowan CW. Use of Adeno-Associated and Herpes Simplex Viral Vectors for *In vivo* Neuronal Expression in Mice. Curr Protoc Neurosci. 2015;73:4.37.1-4.37.31. Published 2015 Oct 1. doi:10.1002/0471142301.ns0437s73

Perez SM, Boley A, Lodge DJ. Region specific knockdown of Parvalbumin or Somatostatin produces neuronal and behavioral deficits consistent with those observed in schizophrenia. Transl Psychiatry. 2019;9(1):264. Published 2019 Oct 21. doi:10.1038/s41398-019-0603-6

Powell SB. Models of neurodevelopmental abnormalities in schizophrenia. Curr Top Behav Neurosci. 2010;4:435-481. doi:10.1007/7854_2010_57

Ramsay IS, Lynn PA, Schermitzler B, Sponheim SR. Individual alpha peak frequency is slower in schizophrenia and related to deficits in visual perception and cognition [published correction appears in Sci Rep. 2021 Oct 11;11(1):20497]. Sci Rep. 2021;11(1):17852. Published 2021 Sep 8. doi:10.1038/s41598-021-97303-6

Rocco BR, Lewis DA, Fish KN. Markedly Lower Glutamic Acid Decarboxylase 67 Protein Levels in a Subset of Boutons in Schizophrenia. Biol Psychiatry. 2016;79(12):1006-1015. doi:10.1016/j.biopsych.2015.07.022

Roeske MJ, Konradi C, Heckers S, Lewis AS. Hippocampal volume and hippocampal neuron density, number and size in schizophrenia: a systematic review and meta-analysis of postmortem studies. Mol Psychiatry. 2021;26(7):3524-3535. doi:10.1038/s41380-020-0853-y

Rompala GR, Zsiros V, Zhang S, Kolata SM, Nakazawa K. Contribution of NMDA receptor hypofunction in prefrontal and cortical excitatory neurons to schizophrenia-like phenotypes. PLoS One. 2013;8(4):e61278. Published 2013 Apr 16. doi:10.1371/journal.pone.0061278

Roopun AK, Kramer MA, Carracedo LM, Kaiser M, Davies CH, Traub RD, Kopell NJ, Whittington MA. Period concatenation underlies interactions between gamma and beta rhythms in neocortex. Front Cell Neurosci. 2008 Apr 8;2:1. doi: 10.3389/neuro.03.001.2008. PMID: 18946516; PMCID: PMC2525927.

Rudy B, Fishell G, Lee S, Hjerling-Leffler J. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev Neurobiol. 2011;71(1):45-61. doi:10.1002/dneu.20853

Ryan RT, Bhardwaj SK, Tse YC, Srivastava LK, Wong TP. Opposing alterations in excitation and inhibition of layer 5 medial prefrontal cortex pyramidal neurons following neonatal ventral hippocampal lesion. Cereb Cortex. 2013;23(5):1198-1207. doi:10.1093/cercor/bhs111

Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511(7510):421-427. doi:10.1038/nature13595

Schobel SA, Lewandowski NM, Corcoran CM, et al. Differential targeting of the CA1 subfield of the hippocampal formation by schizophrenia and related psychotic disorders. Arch Gen Psychiatry. 2009;66(9):938-946. doi:10.1001/archgenpsychiatry.2009.115

Sellgren CM, Gracias J, Watmuff B, et al. Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. Nat Neurosci. 2019;22(3):374-385. doi:10.1038/s41593-018-0334-7

Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Prog Neurobiol. 2013;106-107:1-16. doi:10.1016/j.pneurobio.2013.04.001

Shi F, Yap PT, Gao W, Lin W, Gilmore JH, Shen D. Altered structural connectivity in neonates at genetic risk for schizophrenia: a combined study using morphological and white matter networks. Neuroimage. 2012;62(3):1622-1633. doi:10.1016/j.neuroimage.2012.05.026

Shin YW, O'Donnell BF, Youn S, Kwon JS. Gamma oscillation in schizophrenia. Psychiatry Investig. 2011;8(4):288-296. doi:10.4306/pi.2011.8.4.288

Singh F, Shu IW, Hsu SH, Link P, Pineda JA, Granholm E. Modulation of frontal gamma oscillations improves working memory in schizophrenia. Neuroimage Clin. 2020;27:102339. doi:10.1016/j.nicl.2020.102339

Soler-Cedeño O, Cruz E, Criado-Marrero M, Porter JT. Contextual fear conditioning depresses infralimbic excitability. Neurobiol Learn Mem. 2016;130:77-82. doi:10.1016/j.nlm.2016.01.015

Song L, Xu X, Putthoff P, Fleck D, Spehr M, Hanganu-Opatz IL. Sparser and Less Efficient Hippocampal-Prefrontal Projections account for Developmental Network Dysfunction in a Model of Psychiatric Risk Mediated by Gene-Environment Interaction. J Neurosci. 2022;42(4):601-618. doi:10.1523/JNEUROSCI.1203-21.2021

Sørensen HJ, Mortensen EL, Schiffman J, Reinisch JM, Maeda J, Mednick SA. Early developmental milestones and risk of schizophrenia: a 45-year follow-up of the Copenhagen Perinatal Cohort. Schizophr Res. 2010;118(1-3):41-47. doi:10.1016/j.schres.2010.01.029

Spenrath MA, Clarke ME, Kutcher S. The science of brain and biological development: implications for mental health research, practice and policy. J Can Acad Child Adolesc Psychiatry. 2011;20(4):298-304.

Stine CD, Lu W, Wolf ME. Expression of AMPA receptor flip and flop mRNAs in the nucleus accumbens and prefrontal cortex after neonatal ventral hippocampal lesions. Neuropsychopharmacology. 2001;24(3):253-266. doi:10.1016/S0893-133X(00)00212-8

Talati P, Rane S, Kose S, et al. Increased hippocampal CA1 cerebral blood volume in schizophrenia. Neuroimage Clin. 2014;5:359-364. Published 2014 Jul 22. doi:10.1016/j.nicl.2014.07.004

Tao R, Cousijn H, Jaffe AE, et al. Expression of ZNF804A in human brain and alterations in schizophrenia, bipolar disorder, and major depressive disorder: a novel transcript fetally regulated by the psychosis risk variant rs1344706. JAMA Psychiatry. 2014;71(10):1112-1120. doi:10.1001/jamapsychiatry.2014.1079

Tao S, Wang Y, Peng J, et al. Whole-Brain Mapping the Direct Inputs of Dorsal and Ventral CA1 Projection Neurons. Front Neural Circuits. 2021;15:643230. Published 2021 Apr 14. doi:10.3389/fncir.2021.643230

Tremblay R, Lee S, Rudy B. GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. Neuron. 2016;91(2):260-292. doi:10.1016/j.neuron.2016.06.033

Tseng KY, Chambers RA, Lipska BK. The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Behav Brain Res. 2009;204(2):295-305. doi:10.1016/j.bbr.2008.11.039

Tseng KY, Lewis BL, Hashimoto T, et al. A neonatal ventral hippocampal lesion causes functional deficits in adult prefrontal cortical interneurons. J Neurosci. 2008;28(48):12691-12699. doi:10.1523/JNEUROSCI.4166-08.2008

Tseng KY, Lewis BL, Lipska BK, O'Donnell P. Post-pubertal disruption of medial prefrontal cortical dopamine-glutamate interactions in a developmental animal model of schizophrenia. Biol Psychiatry. 2007;62(7):730-738. doi:10.1016/j.biopsych.2006.10.012

Tuncdemir SN, Wamsley B, Stam FJ, et al. Early Somatostatin Interneuron Connectivity Mediates the Maturation of Deep Layer Cortical Circuits. Neuron. 2016;89(3):521-535. doi:10.1016/j.neuron.2015.11.020

Twining RC, Lepak K, Kirry AJ, Gilmartin MR. Ventral Hippocampal Input to the Prelimbic Cortex Dissociates the Context from the Cue Association in Trace Fear Memory. J Neurosci. 2020;40(16):3217-3230. doi:10.1523/JNEUROSCI.1453-19.2020

Uhlhaas PJ, Linden DE, Singer W, Haenschel C, Lindner M, Maurer K, Rodriguez E. Dysfunctional long-range coordination of neural activity during Gestalt perception in schizophrenia. J Neurosci. 2006 Aug 2;26(31):8168-75. doi: 10.1523/JNEUROSCI.2002-06.2006. PMID: 16885230; PMCID: PMC6673788.

Ursini G, Punzi G, Chen Q, et al. Convergence of placenta biology and genetic risk for schizophrenia. Nat Med. 2018;24(6):792-801. doi:10.1038/s41591-018-0021-y

Uylings HB, Groenewegen HJ, Kolb B. Do rats have a prefrontal cortex?. Behav Brain Res. 2003;146(1-2):3-17. doi:10.1016/j.bbr.2003.09.028

Vakilna YS, Tang WC, Wheeler BC, Brewer GJ. The Flow of Axonal Information Among Hippocampal Subregions: 1. Feed-Forward and Feedback Network Spatial Dynamics Underpinning Emergent Information Processing. Front Neural Circuits. 2021;15:660837. Published 2021 Aug 27. doi:10.3389/fncir.2021.660837

van Tricht MJ, Ruhrmann S, Arns M, et al. Can quantitative EEG measures predict clinical outcome in subjects at Clinical High Risk for psychosis? A prospective multicenter study. Schizophr Res. 2014;153(1-3):42-47. doi:10.1016/j.schres.2014.01.019

Venables NC, Bernat EM, Sponheim SR. Genetic and disorder-specific aspects of resting state EEG abnormalities in schizophrenia. Schizophr Bull. 2009 Jul;35(4):826-39. doi: 10.1093/schbul/sbn021. Epub 2008 Apr 1. PMID: 18381357; PMCID: PMC2696373.

Vertes RP. Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse. 2004;51(1):32-58. doi:10.1002/syn.10279

Vitolo E, Tatu MK, Pignolo C, et al. White matter and schizophrenia: A meta-analysis of voxel-based morphometry and diffusion tensor imaging studies. Psychiatry Res Neuroimaging. 2017;270:8-21. doi:10.1016/j.pscychresns.2017.09.014

Walter A, Suenderhauf C, Harrisberger F, et al. Hippocampal volume in subjects at clinical high-risk for psychosis: A systematic review and meta-analysis. Neurosci Biobehav Rev. 2016;71:680-690. doi:10.1016/j.neubiorev.2016.10.007

Wang Q, Jin J, Maren S. Renewal of extinguished fear activates ventral hippocampal neurons projecting to the prelimbic and infralimbic cortices in rats. Neurobiol Learn Mem. 2016;134 Pt A(Pt A):38-43. doi:10.1016/j.nlm.2016.04.002

Watanabe M, Uematsu A, Johansen JP. Enhanced synchronization between prelimbic and infralimbic cortices during fear extinction learning. Mol Brain. 2021;14(1):175. Published 2021 Dec 11. doi:10.1186/s13041-021-00884-6

Weickert CS, Fung SJ, Catts VS, Schofield PR, Allen KM, Moore LT, Newell KA, Pellen D, Huang XF, Catts SV, Weickert TW. Molecular evidence of N-methyl-D-aspartate receptor hypofunction in schizophrenia. Mol. Psychiatry. 2013;18(11):1185–92.

Willcocks AL, McNally GP. The role of medial prefrontal cortex in extinction and reinstatement of alcohol-seeking in rats. Eur J Neurosci. 2013;37(2):259-268. doi:10.1111/ejn.12031

Yamasaki N, Maekawa M, Kobayashi K, et al. Alpha-CaMKII deficiency causes immature dentate gyrus, a novel candidate endophenotype of psychiatric disorders. Mol Brain. 2008;1:6. Published 2008 Sep 10. doi:10.1186/1756-6606-1-6

Yung AR, McGorry PD. The prodromal phase of first-episode psychosis: past and current conceptualizations. Schizophr Bull. 1996;22(2):353-370. doi:10.1093/schbul/22.2.353

Zhang ZJ, Reynolds GP. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophr Res. 2002;55(1-2):1-10. doi:10.1016/s0920-9964(01)00188-8

Zhou Y, Shu N, Liu Y, et al. Altered resting-state functional connectivity and anatomical connectivity of hippocampus in schizophrenia. Schizophr Res. 2008;100(1-3):120-132. doi:10.1016/j.schres.2007.11.039

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