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Characterizing neuroanatomical changes in parvalbumin and perineuronal nets in a rat DISC-1 knock out model

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CHARACTERIZING NEUROANATOMICAL CHANGES IN PARVALBUMIN AND PERINEURONAL NETS IN A RAT DISC-1 KNOCK OUT MODEL

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ABSTRACT

BACKGROUND: Schizophrenia is a debilitating disorder that has a profound impact on quality of life due to the presence of both cognitive deficits and psychotic symptoms. Despite having significant global economic and social costs and a worldwide prevalence of 1%, schizophrenia is still not well understood. Research has been making strides in uncovering the pathophysiology and the etiology that drive this disease, ranging from genetic abnormalities, disrupted circuitry, changes in microarchitecture, to impaired synaptic connectivity. Evidence suggests that disrupted-in-schizophrenia-1 (DISC1) driven genetic disturbances in fast-spiking parvalbumin (PV) neurons and their surrounding perineuronal nets (PNNs) likely contribute to schizophrenia etiology as they are part of the microcircuits required for working memory, a cognitive function that has been consistently impaired in schizophrenic patients.

OBJECTIVE: To identify the neuroanatomical changes in PV neurons and surrounding PNNs in the superficial and deep layers of the prelimbic and infralimbic prefrontal cortex of a rat DISC-1 knockout model.

METHODS: 19 DISC1-KO male rats and 15 wildtype rats were treated with saline or MK-801. They were sacrificed between P268-269 and brains were

extracted and separated at the corpus callosum. After fixing and preserving, the brains were sliced then stained to visualize parvalbumin and perineuronal nets with immunohistochemistry. Slices were imaged and analyzed for PV, PNN, and PV+PNN counts in the superficial and deep regions of the prelimbic and infralimbic cortices. Averages counts within each group were taken and analyzed via 2-way ANOVAs for each brain region and dependent variable.

RESULTS: DISC1-KO rats displayed the following trending changes: decreased PV cells in deep layers of infralimbic and decreased PNNs throughout the prelimbic cortex. MK-801 appears to increase the number of unsheathed PV cells in the superficial layers of prelimbic and infralimbic cortex. It decreased the number of PNNs in the prelimbic of wildtype animals but not in the DISC1-KO cohort. MK-801 moderately increased PV counts in DISC1-KO.

CONCLUSIONS: This DISC1-KO model is a promising model of schizophrenia as we see the same directionality of decreases in PV and PNN as post mortem human studies. Furthermore, MK-801 is seen to have an increasing trend effect on PV cells, which should be considered when interpreting findings in future studies that look at these markers.

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LIST OF ABBREVIATIONS

BSA.....Bovine Serum Albumin

CSP .....Cavum Septum Pellucidum

CNS ..... Central Nervous System

DISC1-KO.....Disrupted in Schizophrenia1 Knock Out

DISC1 ..... Disrupted in Schizophrenia 1

DLPFC.....Dorsolateral Prefrontal Cortex

DTNBP1..... Human Dystrobrevin Binding Protein 1

ECM.....Extracellular Matrix

GAD67 ..... Glutamic Acid Decarboxylase 67

HOM .....Homozygous

IL.....Infralimbic

IPSC ..... Inhibitory Postsynaptic Current

Kal-7 .....Kalirin-7

MRI .....Magnetic Resonance Imaging

mPFC..... Medial Prefrontal Cortex

NDE1 ..... Nuclear Distribution Element Like

NDS .....Normal Donkey Serum

NOP .....Novel Object Preference

NPP ..... Novel Place Preference

NRG1..... Neuroregulin1

PB ..... Phosphate Buffer

PBS..... Phosphate Buffer Saline

PBST ..... Phosphate Buffer Saline with Triton-X

PET..... Positron Emission Tomography

PFC.....Prefrontal Cortex

PL .....Prelimbic

PNN .....Perineuronal Net

PPI ..... Pre-pulse Inhibition

PV .....Parvalbumin

SPECT.....Single Photon Emission Computed Tomography

TNIK.....Traf- and Nck- interacting Kinase

WT .....Wild Type

INTRODUCTION

Schizophrenia

Schizophrenia is a complex psychiatric disorder that has an estimated prevalence rate of 0.5-1% worldwide (Bitanihirwe & Woo, 2014; Dienel & Lewis, 2018; Najjar & Pearlman, 2015; Picchioni & Murray, 2007). It is characterized by the presence of symptoms broadly categorized into three categories: cognitive, positive and negative (Bitanihirwe & Woo, 2014; Meyer & Feldon, 2010; Patel, Cherian, Gohil, & Atkinson, 2014; Woo, 2014). Positive symptoms are defined as features that appear due to the disease process but are not normally present; this includes paranoia, delusions, and hallucinations (Meyer & Feldon, 2010; Woloszynowska-Fraser et al, 2017; Woo, 2014; Gejman et al., 2010). Negative symptoms are those that are should be present but are otherwise reduced or absent, which includes apathy, withdrawal, and a flattened affect (Gejman, Sanders, & Duan, 2010; Meyer & Feldon, 2010; Woo, 2014). Cognitive impairment spans broadly into executive functions, notably working memory and sustained attention. As these deficits are seen in 98% of schizophrenia patients (Keefe, Eesley, & Poe, 2005) and present long before psychosis onset (Dienel & Lewis, 2018; Heinrichs, 2005; Keefe et al., 2005; Tripathi, Kar, & Shukla, 2018; Woloszynowska-Fraser, Wulff, & Riedel, 2017), cognitive impairment is arguably the most pervasive and significant symptom type for this disease (Albus et al., 2006; Bilder et al., 2000; Heinrichs, 2005). It persists even throughout medication and stabilization of psychotic symptoms (Bilder et al., 2000; Bowie & Harvey,

2006; Erlenmeyer-Kimling et al., 2000; Heinrichs, 2005; Hill, Schuepbach, Herbener, Keshavan, & Sweeney, 2004; Hoff et al., 1999). Furthermore, the extent of impairment is the most effective predictor of long-term functional outcomes and prognosis (Dienel & Lewis, 2018; M. F. Green, Kern, Braff, & Mintz, 2000; Michael F. Green, 2016; Kahn & Keefe, 2013). Currently, there are no effective treatments options for these cognitive impairments (Bowie & Harvey, 2006; Goff, Hill, & Barch, 2011), which highlights the importance of understanding the neurobiological underpinnings of these deficits for future targeted treatments.

Schizophrenic symptoms typically manifest in the late teens to mid 30s (American Psychiatric Association, 2013; Mueser & Jeste, 2008; Picchioni & Murray, 2007; Woo, 2014) with prodromal cognitive disturbances usually present long before the first psychotic episode (Dienel & Lewis, 2018; KANE & LENCZ, 2008). The first signs of cognitive impairment can appear during early childhood and progress insidiously through the prodromal phase in adolescence (Kane & Lencz, 2008). These deficits are thought to be the result of aberrant processes during active neurodevelopment which culminates into a fully blown disease when the dysfunctional regions or circuits become mature or active (KANE & LENCZ, 2008; Marenco & Weinberger, 2000). This assumption is supported by the delayed maturation of the prefrontal cortex, an area strongly implicated in cognitive deficits associated with schizophrenia (Giedd, 2004; Gogtay et al., 2004; Huttenlocher & Dabholkar, 1997; Marenco & Weinberger, 2000).

The delayed onset of the full manifestation of the disease is suggestive of neurodevelopmental origins. However, the precise etiology and pathophysiology has yet to be ascertained. It is largely understood that it has a multifactorial cause, which includes genetic (Buxbaum et al., 2008; Niendam et al., 2018; Saetre et al., 2008; Schumacher et al., 2009), social (Boydell et al., 2001; Veling et al., 2007) and environmental contributions (Dalman, Allebeck, Cullberg, Grunewald, & Köster, 1999; P. B. Jones, Rantakallio, Hartikainen, Isohanni, & Sipila, 1998; KANE & LENCZ, 2008; Marenco & Weinberger, 2000). It is thought that the interaction of multiple susceptibility genes and environmental factors leads to the manifestation of the disease (Maynard, Sikich, Lieberman, & LaMantia, 2001; Picchioni & Murray, 2007). The presence of a single vulnerability gene, such as disrupted in schizophrenia-1 (DISC1) (Jaaro-Peled, 2009; Saetre et al., 2008; Schumacher et al., 2009), does not necessarily equate to developing the clinical syndrome; however, it may confer a small susceptibility effect (Lewis & Levitt, 2002; Sanders et al., 2008) that can be additive with other vulnerability genes, like neuroregulin1 (NRG1), human dystrobrevin binding protein 1 (DTNBP1), and nuclear distribution element like (NDE1) (Burdick et al., 2008; Kamiya et al., 2006; Lipska et al., 2006). Due to the multiple implicated factors and heterogeneity of the disease, it is plausible that the disease requires a two-hit model (Bayer, Falkai, & Maier, 1999; Feigenson, Kusnecov, & Silverstein, 2014). The initial hit, genetic or environmental, would disrupt an aspect of neurodevelopment, priming the underlying neurocircuitry to be vulnerable to a

second hit which will exacerbate the abnormal processes to yield the clinical disease (Maynard et al., 2001).

Epidemiological and cohort studies provide further support for the neurodevelopmental origin theory – via disruption of typical neural development in utero – as associations between the prenatal exposure to infection and increased schizophrenia prevalence have been described (A. S. Brown et al., 2000; Alan S. Brown, 2011; Alan S. Brown et al., 2004; Alan S. Brown & Derkits, 2010). Interestingly, the prenatal period has been noted to be the most vulnerable to any epigenetic disruptions that could have long-lasting effects (Kundakovic & Jaric, 2017; Nestler, Peña, Kundakovic, Mitchell, & Akbarian, 2016). However, not all cases of prenatal infections lead to developing schizophrenia, which suggests a second component or “hit” may be necessary.

Monozygotic studies show that there is not a 100% concordance rate (Kendler & Robinette, 1983; Onstad, Skre, Torgersen, & Kringlen, 1991; Pepper, Pathmanathan, McIlrae, Rehman, & Cardno, 2018), indicating there is an interaction between genetics and environment. In a study that compared the rates of schizophrenia between adoptees of typical biological parents raised by schizophrenic parents and adoptees of schizophrenic biological parents raised by normal parents, the former group had an incidence rate of 18.8% and the latter had 10.7% (Wender, Rosenthal, Kety, Schulsinger, & Welner, 1974). This indicates that both genetics and environment are risk factors, but that genetics may confer greater risk (Roth, Lubin, Sodhi, & Kleinman, 2009).

Epigenetic changes in genes have been noted in schizophrenia (for a review, see (Roth et al., 2009; Teroganova, Girshkin, Suter, & Green, 2016). These observed changes are seen in DNA methylation (Abdolmaleky et al., 2006; Liao et al., 2015) and histone modifications (Huang et al., 2007; Sharma, Grayson, & Gavin, 2008), both of which are influenced by environmental factors and are mechanisms that regulate gene expression (Liao et al., 2015). Some of the genes that exhibit differential methylation in schizophrenia, such as DISC1, are involved in neurodevelopment, further implicating aberrant processes in early development as a likely contributor to schizophrenia (Liao et al., 2015). In a study that profiled genome wide DNA methylation in the blood derived DNA of individuals carrying the chromosome 1 and 11 translocation and their family members that are non-carriers, it found 13 significant differentially methylated regions that surrounds the breakpoints of the translocation. Indeed, four of the regions were found to be mapped to the DISC1 gene (McCartney et al., 2018). The consequences of these epigenetic changes are seen in altered expression in genes that are implicated in prefrontal dysfunction related to GABAergic neurons, such as the decreased expression of glutamic acid decarboxylase 67 (GAD67), an enzyme that synthesizes GABA (Huang et al., 2007).

Beyond the abnormalities in gene expression, there are overall gross neuroanatomical changes observed in the neuroanatomy of schizophrenic patients via imaging and post mortem studies. Previous evidence has indicated that patients that go on to develop schizophrenia have a greater prevalence of

physical anomalies that are indicative of an early life disruption in development (Alan S. Brown, 2011). Specifically, morphological analyses show an increased prevalence of cavum septum pellucidum (CSP), a neuroembryonic marker of incomplete closure of the septal leaflets (Alan S. Brown, 2011). Septal leaflets are an important indicator of normal fetal neurodevelopment and abnormalities can result in psychiatric disturbances such as psychosis (Li et al., 2011; Supprian, Sian, et al., 1999), which can be visualized through magnetic resonance scans. Additionally, magnetic resonance imaging (MRI) studies have shown a significant difference in the CSP between schizophrenia patients and normal controls (Beraldi et al., 2018; Degreef et al., 1992). Although its specific role in the disorder is unclear, a large CSP is considered to be a marker of abnormal development of the limbic system (Beraldi et al., 2018), a system that is thought to play a significant role in affective and cognitive symptoms seen in patient populations (Nishijo, Rafal, & Tamietto, 2018; Rolls, 2015; Supprian, Heils, et al., 1999)

Imaging studies on post-mortem brains of schizophrenic patients have revealed insight into abnormalities seen in various brain regions. These progressive structural changes in the brain were also identified via imaging in high-risk patients that are close relatives of a schizophrenic patients. A multi-site mega-analysis conducted by Rozycki et al. (2018) found robust group differences between healthy and schizophrenic patients in both positive and negative directions, in which the amygdala, hippocampus and prefrontal cortex was

smaller in schizophrenia and lateral third ventricle and pallidum were larger compared to healthy controls. Notably, there was an evident gray matter volume loss compared to healthy controls in the prefrontal cortex (PFC), temporal cortex, parietal cortex, insula, and amygdala, with the greatest deficit seen in the PFC (Goghari, MacDonald, & Sponheim, 2014; Karlsgodt, Sun, & Cannon, 2010; Rozycki et al., 2018). These regional abnormalities are consistent with the observed symptoms in schizophrenia as they, the prefrontal cortex in particular, critically contribute to cognition (Goghari et al., 2014) and emotional processing (Glotzbach et al., 2011). Furthermore, the schizophrenic group was found to have significantly greater pallidum and ventricular volumes with considerable effect sizes (Rozycki et al., 2018). These changes are hypothesized to be the cumulative result of changes in neuroplasticity, neuronal maturation, and an altered neurodevelopmental trajectory (Buckley, 2005; Karlsgodt et al., 2010; Meyer-Lindenberg & Tost, 2014; Tripathi et al., 2018). The agents of these neurodevelopmental and neuroplasticity changes are still being investigated; however, it is suspected that mutations in the DISC1 gene causes widespread changes that have downstream effects on the GABAergic neuron population, extracellular matrix integrity, and mitochondrial function, which in turn throws the normal neurodevelopmental course array (Norkett, Modi, & Kittler, 2017; Ye et al., 2017).

A bevy of evidence implicates abnormalities in the PFC as part of the pathophysiology of schizophrenia. Imaging studies utilizing positron emission

tomography (PET) and single photon emission computed tomography (SPECT) saw decreased blood flow and metabolism in the PFC of schizophrenic patients performing a cognitive task, indicating prefrontal dysfunction (Andreasen et al., 1992; Goldman-Rakic & Selemon, 1997; Kawasaki et al., 1993; Liddle et al., 1992). The PFC has been noted to be an integral component of working memory and dysfunctions in this area align with the canonical deficits seen in working memory tasks of schizophrenics. Furthermore, studies show increases in the neuronal density of the PFC, specifically in the dorsolateral PFC (DLPFC), alongside a reduction in cortical thickness (Francine M. Benes, McSparren, Bird, SanGiovanni, & Vincent, 1991; Goldman-Rakic & Selemon, 1997; Selemon, 2004; Selemon, Rajkowska, & Goldman-Rakic, 1998). Increased densities were especially noted in cortical layers 2, 3, 4 and 6, with no observable changes in the neuronal count (Goldman-Rakic & Selemon, 1997; Selemon, 2004; Selemon et al., 1998). This suggests there is actually a decrease in neuropil, a network of cell processes and synapses. A loss of neuropil may confer a loss of terminals and changes in the synaptic stability and maintenance, which may lead to impaired connectivity (E. G. Jones, 1997).

Additional studies also have shown consistent changes to the GABAergic neurotransmission in the PFC with associated decreases in expression of GAD67 mRNA and protein (Dienel & Lewis, 2018; Woo, 2014). The GABAergic system plays an important role in the inhibition of the excitatory pyramidal cells throughout the brain. Its dysfunction can lead to an imbalance in excitation and

inhibition (Voineskos, Rogasch, Rajji, Fitzgerald, & Daskalakis, 2013), and this balance is considered necessary for normal executive processes. This suggests that GABA plays a pivotal role in producing the cognitive deficits seen in schizophrenia. Furthermore, there has been evidence of hypofunction at the NMDA receptor (Voineskos et al., 2013), which has influence over GABA interneuron firing (Woo, 2014). Studies have also shown evidence of lower gamma oscillatory power in the PFC of schizophrenic patients when compared to healthy controls. The gamma frequency of oscillations has been associated with working memory function, in which the power of oscillations increases with more demanding cognitive tasks. Mounting evidence shows that parvalbumin (PV), a protein expressed on a subset of GABAergic interneurons, contributes to the generation of these network oscillations, which are also coupled with the inhibitory activity. Post mortem studies show a well characterized, marked decrease in PV interneurons in the hippocampus and medial PFC (mPFC; Lewis, Hashimoto, & Volk, 2005). It has been hypothesized that the observed dysfunction in these inhibitory mechanisms may be downstream effects of a genetic mutation.

While there have been strides in our understanding of the development of schizophrenia, there is a lack of consistency in the findings as not all patients have the same presumed etiological cause. Despite these inconsistencies, there are certain genes that have been identified that seem to increase the risk of developing schizophrenia significantly. Thus, elucidating the impact of these

genes on neurodevelopment will only serve to uncover the factors that drive the disease.

Disrupted in Schizophrenia 1 (DISC1)

DISC1 is considered a strong candidate susceptibility gene for schizophrenia and other psychiatric disorders, like bipolar affective disorder, and recurrent major depression (Brandon et al., 2009; Pletnikov et al., 2008). The DISC1 gene was first identified through a study in a large Scottish family with a high penetrance of the translocation between chromosomes 1 and 11, which was found to co-segregate with the psychiatric disorders listed previously (Brandon et al., 2009; Norkett et al., 2017; Pletnikov et al., 2008). Inheritance of this translocation could confer a 50-fold increased risk compared to the general population (Brandon et al., 2009; Norkett et al., 2017). The DISC1 gene, which encodes for the DISC1 protein that has a large globular N-terminus, a self-association domain and a coiled coil rich C-terminus, was discovered on chromosome 1 when analyzing the interrupted regions from the translocation (Brandon et al., 2009; Norkett et al., 2017). There are three hypothesized effects of the translocation: abnormal transcripts encoding DISC1 1-597 and 60 to 69 new amino acids from chromosome 11, a truncated DISC1 transcript ending at a breakpoint, or haploinsufficiency due to no expression of the abnormal transcript (Norkett et al., 2017; Pletnikov et al., 2008).

DISC1 has been identified as a key player in the regulation of neuronal processes during development, such as growth (Kamiya et al., 2005), expansion

and migration (Kim et al., 2009; Tripathi et al., 2018; Wu et al., 2017). Research also shows its role is much more widespread as it is a key regulator of cortical neuronal precursor cell (NPC) proliferation, neuronal differentiation, mitochondrial homeostasis, intracellular trafficking, and intracellular signaling (Burdick et al., 2008; Deng et al., 2017; Mao et al., 2009; Norkett et al., 2017; Pletnikov et al., 2008). Its broad range of functions is likely due to the fact DISC1 can have over 100 potential interaction partners, such as kinesin motor proteins, Lis1, Nde1, phosphodiesterases, and mitochondrial proteins (Norkett et al., 2017). Primarily, the DISC1 protein is known to act as a scaffolding protein that assists with protein complex formations through its multiple motifs (Pletnikov et al., 2008). The truncated form of the protein could result in the loss of interactions with these binding partners, which may lead to aberrant systems that ultimately results in schizophrenia like symptoms (Morris, Kandpal, Ma, & Austin, 2003).

Beyond its role in ATP production, DISC1 is thought to contribute to mitochondrial calcium buffering since knockdown models have shown increased time in returning to baseline intramitochondrial calcium levels (Norkett et al., 2017). The symptoms of schizophrenia could be secondary to impaired ATP production, calcium buffering, and decreased dendritic outgrowth from altered regulation. These molecular abnormalities may impair network connectivity, which could contribute to the onset of schizophrenia (Norkett et al., 2017). Due to its interaction with proteins involved in neurodevelopment and neurosignaling, DISC1 is hypothesized to be a critical hub protein that exists as the junction for

major proteins involved in normal brain development (Ye et al., 2017). Beyond the knowledge of Ndel1/Nde1 being a modulatory component of the dynein complex and having a role in regulating brain development through cell cycle control, not much is known about the exact functions behind its interaction with DISC1. However, it has been noted that Ndel1 has been associated with DISC1 in psychiatric disorders and the complete removal of Ndel1 in mice has been found to be embryonically lethal. Furthermore, it has been found that the c-terminal of DISC1 is necessary for Ndel1/Nde1 binding (Brandon et al., 2009). When the 1, 11 balanced translocation mutation truncates this terminal by deleting residues 598 to 854 and disrupting its binding to Ndel1/NDe1, microcephaly was observed in both mice and humans (Alkuraya et al., 2011). This indicates the relevance of the DISC1/Ndel1/Nde1 interaction for normal development. However, all are scaffolding proteins capable of interacting with several other components, rendering it difficult to make clear interpretations of their roles.

In the experiments conducted by Ye et al., DISC1 was found to regulate neurogenesis through its connections with Ndel1/Nde1. Interruptions of the DISC1/Ndel1 interaction resulted in mitotic delays due to difficulty of the chromatids to separate after metaphase. Presumably, this is due to Ndel1's role in recruiting the dynein complex, which includes centrosomes and kinetochores, to the necessary machinery in the cell cycle. DISC1 is assumed to regulate Ndel1's kinetochore localization, a necessary event for anaphase, and thus

influence cell cycle progression. Disruptions in this interaction were found to cause delayed cell cycle progression and reduced proliferation of these neural stem cells in the ventricular zone of the cortex. Due to its role in cognition and emotional processing, impaired neurogenesis in the cortex seems to be a promising contributor to the symptoms of schizophrenia (Burdick et al., 2008; Ye et al., 2017). This suggests DISC1 may have a role in schizophrenia pathophysiology through the disruption in interaction with NDEl1/NDe1.

Neuroplasticity is an important process for development and maturation as it is the ability of a circuit to respond to experience and learning. Altered neuroplasticity is thought to be a potential driver behind cognitive deficits. Studies have suggested a possible role of DISC1 in neuroplasticity due to its localization at the synapse. In postmortem imaging studies, a reduction in dendritic spine density on cortical pyramidal neurons has been consistently observed (Garey et al., 1998; Glantz & Lewis, 2000; Rosoklija et al., 2000). The excitatory postsynaptic compartments are found in the dendritic spines and the spines' morphology has been associated with synaptic plasticity and neuronal activation (Yuste & Bonhoeffer, 2001). In support of this theory, truncated DISC1 gene mouse models found a significant decrease in synaptic spines in the dentate gyrus with corresponding impairment in working memory (Brandon et al., 2009; Glausier & Lewis, 2013; Mao et al., 2009). The exact mechanisms of how DISC1 regulates the actin cytoskeleton, a component critical for spine formation and neuroplasticity, is largely unknown. It is suspected it may be through its

interactions with kalirin-7 (Kal-7), a rac1-GTP exchange factor and a unit of synaptic signaling pathways, and Traf-and nck-interacting kinase (TNIK), a kinase linked to actin cytoskeleton regulation (Tropea, Hardingham, Millar, & Fox, 2018). Kal-7 has been found to be decreased in schizophrenia and TNIK has been linked to the impaired cortical function observed in the disease (Coba et al., 2012; MacLaren, Charlesworth, Coba, & Grant, 2011).

Parvalbumin

Parvalbumin (PV) is a calcium binding protein that is selectively expressed on GABAergic neurons within the brain (Caroni, 2015; Dienel & Lewis, 2018). There are two main subtypes of PV-expressing cells and are named based on their projection targets: PV basket cells and PV chandelier cells. The chandelier cells target the axon initial segment of the pyramidal cells and has a slower inhibitory postsynaptic current (IPSC) decay than the basket cells (Dienel & Lewis, 2018). PV-containing basket cells target the perisomatic region of pyramidal cells, which consists of the cell body, proximal dendrites and initial axon segment, and are characterized by their fast-spiking properties with rapid IPSC decay (Dienel & Lewis, 2018; Galarreta & Hestrin, 2002). This fast spiking phenotype serves to drive the gamma oscillatory activity, which is thought to coordinate large ensembles of neuronal activity that is necessary for memory and cognitive processes (Dienel & Lewis, 2018; Kim et al., 2016; Veres, Nagy, & Hájos, 2017.). Mounting evidence suggests that these PV+ interneurons play a critical role in working memory function through regulating the synchronization of

large ensembles of principal neurons firing for specific brain states and generating network oscillations necessary for higher order cognition (Kim et al., 2016, 2016; Veres et al., 2017.). This synchronization allows for efficiency in neural processing for local networks of neurons. Its presence is required for normal brain activity since its dysfunction has been seen to result in impaired higher order processing (Haenschel et al., 2009; Kucewicz et al., 2014; Williams & Boksa, 2010). For this reason and others, aberrations in PV interneurons have long been considered putative targets of dysfunction in schizophrenia research.

Gamma frequency of oscillations in the PFC has been associated with working memory function, in which the power of the oscillations corresponds to the cognitive load (Dienel & Lewis, 2018). In schizophrenia, there has been evidence of lower gamma oscillatory power in relation to the demands of the cognitive task (Dienel & Lewis, 2018). Interestingly, changes in PV interneurons function and distribution in the PFC has been seen in postmortem pathology, which further supports the role of PV cell dysfunction in the maintenance of working memory processes through its influence over gamma frequency (Lewis et al., 2012; Murray et al., 2015).

Consistently, changes to GABAergic neurotransmission in the PFC has been seen in postmortem tissue of schizophrenia with associated lower levels of GAD67 mRNA and protein (Dienel & Lewis, 2018; Woo, 2014). GAD67 is significant since it synthesizes most of the cytosolic GABA (Egerton, Modinos, Ferrera, & McGuire, 2017). Furthermore, GAD67 and PV are activity dependent

gene products in which greater activity reflects greater expression (Hensch, 2005). The decrease in GAD67 is presumed to confer a weakened inhibition on the PV basket cells (Dienel & Lewis, 2018).

Evidence shows that decreased inhibition from the PV basket cells is driven by the hypofunction of NMDA receptors on these cells, as less GABA synthesis is detected (Voineskos et al., 2013; Woo, 2014). NMDA hypofunction has long been implicated in the cognitive symptoms of schizophrenia, as the balance between excitatory and inhibitory neurotransmission appears to be mediated through its activity (Voineskos et al., 2013). Antagonizing the receptor was found to prevent the GABA interneurons from firing so the pyramidal neurons are no longer inhibited, resulting in an imbalance in excitation and inhibition (Voineskos et al., 2013). This balance was found to be necessary for executive processes, suggesting NMDA hypofunction is part of the pathophysiology of schizophrenia (Voineskos et al., 2013).

Although evidence suggesting alterations of NMDA receptors in schizophrenic subjects have been mixed, rodent studies have found reduced frequency and amplitudes of the miniature IPSC after administration of ketamine, a NMDA receptor antagonist (Mathew & Hablitz, 2011). Furthermore, the ablation of the NR1 subunit of the receptor in preadolescent mice were found to result in spatial working memory deficits and lower expression of GAD67 and PV in adulthood (Woo, 2014). These findings imply that impaired signaling through NDMA receptors on PV cells, either through deficit of its subunits or its

antagonism, has downstream effects of decreasing GAD67 and PV expression and thus, may lead to dysfunction in gamma oscillations and working memory (Dienel & Lewis, 2018; Woo, 2014). And lending support to the developmental theory of schizophrenia, NMDA receptor antagonism was found to produce deficits in PV and GAD67 only during prepuberty but not post-puberty (Woo, 2014).

Low doses of non-competitive NMDAR antagonists were found to induce positive, negative and cognitive symptoms of schizophrenia in healthy subjects (Krystal et al., 1994) and reintroduce these symptoms in stabilized schizophrenics (Lahti, Koffel, LaPorte, & Tamminga, 1995). Rodent studies that ablate or decrease expression of NMDAR subunits such as NR1 subunit were found to produce similar deficits in behavior (Belforte et al., 2010; Duncan et al., 2004). It was found that NMDAR antagonists may preferentially target cortical GABAergic cells since findings show acute administration results in hyperactivity of pyramidal cells (Jackson, Homayoun, & Moghaddam, 2004; Suzuki, Jodo, Takeuchi, Niwa, & Kayama, 2002) with an overall net hyperactivity in the brain (Miyamoto, Leipzig, Lieberman, & Duncan, 2000). Likewise, chronic administration was found to decrease expression of both PV and GAD67 in the cortex (Cochran et al., 2003; Keilhoff, Becker, Grecksch, Wolf, & Bernstein, 2004; Morrow, Elsworth, & Roth, 2007), which substantiates claims that NMDAR hypofunction is linked to PV cell dysfunction. The observed decrease in cortical inhibition in schizophrenic patients is suggestive of the vulnerable nature of PV

cells (Daskalakis et al., 2002; Wobrock et al., 2008). This decrease is presumed likely to be due to the dysfunction in the neurons itself (Nakazawa et al., 2012). It is reasonable to believe PV cells are susceptible to NMDAR hypofunction as they have develop over an extensive period of time, which allows any disruptions to have profound impacts on their development (Nakazawa et al., 2012). This makes it a compelling source to study in schizophrenia since cortical PV cell disruption has been associated with other mental disorders such as major depressive disorder (F. M. Benes & Berretta, 2001). Furthermore, the hypofunction of NMDAR on PV neurons was found to disrupt the gamma frequency synchrony, the basis for higher order cognitive function, which was found to be disrupted in human studies.

Perineuronal Nets

Perineuronal nets (PNNs) are reticular structures that are comprised of condensed components of the extracellular matrix (ECM) including proteoglycans, hyaluronan, and chondroitin sulfate that interact to form a lattice-like structure around the soma, dendrites, and axons of mature neurons (F. M. Benes & Berretta, 2001). Due to its appearance at the end of the critical period, it is assumed that the PNNs stabilize the synapses and the mature neural network by restricting neuroplasticity (Lensjø, Lepperød, Dick, Hafting, & Fyhn, 2017; Pantazopoulos, Woo, Lim, Lange, & Berretta, 2010; Paylor et al., 2018; Tsien, 2013). Furthermore, the density of PNNs has been observed to increase with age, suggesting the formation of these structures are experience or activity

dependent (Hensch, 2005). They are found widely distributed throughout the brain—in the entorhinal cortex, prefrontal cortex, hippocampus, and amygdala (Bitanihirwe & Woo, 2014) and preferentially surrounds fast spiking PV neurons (N. P. Morris & Henderson, 2000). It is important to note that PNNs are dynamic in their form, and can reorganize and remodel in response to synaptic plasticity (Berretta, Pantazopoulos, Markota, Brown, & Batzianouli, 2015).

PNNs are thought to be critical structures in regulating synaptic plasticity (Thompson et al., 2018) and the development and neuroprotection of the central nervous system (CNS); processes all found to be dysfunctional in schizophrenia (Bitanihirwe & Woo, 2014; Paylor et al., 2018). PNN expression increases as the brain is approaching the period of network stability and the end of heightened plasticity, which coincides with the end of adolescence (Paylor et al., 2018). Interestingly, PNNs have been found to be decreased in the PFC, amygdala, superior temporal cortex and entorhinal cortex in post mortem tissues of schizophrenic patients (Berretta et al., 2015; Bitanihirwe & Woo, 2014; Enwright et al., 2016; Mauney et al., 2013; Pantazopoulos et al., 2010; Paylor et al., 2018). Furthermore, these findings corresponded with cognitive impairments when replicated in animal models (Paylor et al., 2016, 2018; P. Steullet et al., 2017).

Due to its polyanionic nature, PNNs are assumed to be involved in maintaining ion homeostasis in the local environments around metabolically active neurons like fast spiking PV interneurons (Bitanihirwe & Woo, 2014). Since the ECM establishes the local Cl- concentration, a disruption in PNNs could alter

the amount of inhibition a pyramidal cell receives from the PV interneurons; this PV-pyramidal cell circuit is thought to key for gamma oscillatory activities generated for cognitive tasks (Enwright et al., 2016). There has been evidence of dysregulation of the antioxidant systems and increased amount of oxidative stress in schizophrenia (Do et al., 2000; Flatow, Buckley, & Miller, 2013; Yao, Leonard, & Reddy, 2006). PV cells have been shown to be especially vulnerable to damage from this type of stress (Hasenstaub, Otte, Callaway, & Sejnowski, 2010; Inan et al., 2016; Kann, Papageorgiou, & Draguhn, 2014), but are usually protected by the PNNs from this damage (Bitanihirwe & Woo, 2014; Cabungcal et al., 2013; Enwright et al., 2016; Morishita, Cabungcal, Chen, Do, & Hensch, 2015).

The relationship between PNN and PV and its importance in producing the cognitive symptoms of schizophrenia is compelling. PNN formation has been seen to be concurrent with PV neuron and inhibitory neural network maturation, which further implicates its relevance in memory and learning (Bitanihirwe & Woo, 2014). And since the firing properties of PV neurons largely establish normal gamma oscillations, deficits in PNNs are suggested to contribute to abnormal oscillatory activity (Pascal Steullet, Cabungcal, Cuénod, & Do, 2014) and thus altered cognition (Bitanihirwe & Woo, 2014). Furthermore, there is evidence that a loss of PNNs in the hippocampus corresponded with increased dopaminergic activity and increased firing rate of pyramidal cells, producing an overall hyperexcitability in the brain. These conditions make the hippocampus

susceptibility to dopamine hyperfunction, which is thought to be the source behind the positive symptoms of schizophrenia (Bitanihirwe & Woo, 2014).

Beyond PV protection, PNNs were also found to regulate PV function (Bernard & Prochiantz, 2016; Cabungcal et al., 2013a, 2013b), specifically the PV cells' excitability (Balmer, 2016; Lensjø et al., 2017) and dopamine signaling. Influence over these properties allows the PNNs to exert influence over network oscillatory activity (Pascal Steullet et al., 2014). Therefore, PNN dysfunction may be driving the network dysfunction seen in schizophrenia (Berretta, 2012; Berretta et al., 2015). This would corroborate the past findings suggesting that disturbed GABAergic signaling from decreased PV and GABA markers contribute to the cognitive impairment-related schizophrenic symptoms (Hashimoto et al., 2008; Takanori Hashimoto et al., 2003; Huang et al., 2007; Volk, Austin, Pierri, Sampson, & Lewis, 2000). Altered neurodevelopment after PNN reorganization further implicates its role in the pathophysiology of the disease (Dityatev et al., 2007; Lensjø et al., 2017; Liu et al., 2013; Pizzorusso et al., 2002). Studies found that the proteolysis of PNNs can create an environment that is optimal for synaptic plasticity, which may lead to instability in the extracellular matrix (Murase, Lantz, & Quinlan, 2017; Wen, Afroz, et al., 2018; Wen, Binder, Ethell, & Razak, 2018) and the rewiring of the networks (Pollock, Everest, Brown, & Poulter, 2014), all of which can lead to abnormal development from network dysfunction. Likewise, disruption in these structures were found to reopen these critical periods of plasticity which can cause destabilized synapses and thus

compromised neural circuits (Lensjø et al., 2017; Paylor et al., 2018). The dysfunction of neural circuitry seen after PNN dysfunction may be mediated by the reduced inhibition from PV cells and thus altered balance between the inhibitory and excitatory activities (Lensjø et al., 2017).

These findings can be synthesized together to highlight the importance of both PNNs and PVs in the pathophysiology of schizophrenia. Due to the body of evidence supporting the notion that PNNs are developmentally regulated and activity dependent (Hensch, 2005), they appear towards the end of the critical period, and the closure of synaptic plasticity is necessary for proper network function, it provides substantial support that PNNs are necessary for typical neurodevelopment. Additionally, with the wealth of research provided on DISC1 and its role in neurodevelopment, there seems to be support that DISC1 may be driving both the dysfunction seen in inhibitory circuits and abnormalities in PNNs. Therefore, the present work utilizes a novel DISC1-KO model to see if DISC1 dysfunction precedes the loss of PV and PNNs seen in schizophrenia postmortem tissue and animal models.

SPECIFIC AIMS

Due to observed prefrontal disruptions in a subset schizophrenic population with a DISC1 gene disruption, we are attempting to characterize the neuroanatomical changes in a novel rat DISC1-KO model to see the if this model can replicate the neuroanatomical findings seen in schizophrenia, and whether it can serve as a more translational model for future schizophrenia research. Since DISC1 has been implicated in being critical for typical neurodevelopment, and schizophrenia is purported to have neurodevelopmental origins, we hypothesize that a complete knock out of this gene will produce marked abnormalities within the PFC evidenced by decreased expression of PV and PNNs, both of which are critical for higher-order cognitive functioning. We anticipate the addition of MK-801 to the knock out will produce an effective later-life challenge akin to the diathesis-stress model, and elicit even more drastic deficits, as it will further antagonize the NMDA receptors on the PV cells and produce greater dysfunction in these cells.

METHODS

All animal procedures were reviewed and approved by Colby College's Institutional Animal Care and Use Committee and conducted in accordance with federally regulated standards.

Animals

Typical and transgenic Sprague-Dawley rats (Horizon Discovery) were acquired to serve as breeders and included three wild-type (WT) males, three WT females, three males with biallelic deletion within the DISC1 gene (DISC1-KO), and three DISC1-KO females. All breeders were postnatal day (P) 48 upon arrival. Females and males of the same genotype were paired together for breeding on P122. Males were removed after 7 days, after which pregnant dams were left undisturbed in their cages until the birth of their litters. The following day (P1), the pups were toe-clipped for genotype identification and cross-fostered to evenly distribute sex and genotypes amongst the WT and HOM moms, such that all litters were mixed genotypes. Pups were weaned on P24 and pair-housed in same-sex pairs in individually ventilated, clear polycarbonate caging in a temperature and humidity-controlled vivarium with access to food and water ad libitum. A total thirty-four genotyped male pups were utilized for the present study and group breakdown was as follows: DISC1-KO (n=19) and WT (n=15).

Dizocilpine Injections

Rats in each gene group were randomly assigned to treatment groups of either injections of 0.3mg/kg of dizocilpine (MK-801; Sigma-Aldrich) or saline. All

rats in the MK-801 groups were administered intraperitoneal injections on testing days (P45, P97-P101, P102, P121-126, P152, P165-166, P186-189, P192-196, P207-208, P234, and P240-242). No crossover treatments occurred between groups. See figure 1A for details on dosing schedule.

Behavioral Assays

A battery of tests was conducted on the rats across behavioral testing paradigms and prior to euthanasia to assess the role of genotype and NMDA antagonist administration on subsequent behavior. These behavioral assays included: social interaction, two pre-pulse inhibition (PPI) tests, Morris Water Maze to assess both special and working memory, novel place preference (NPP) test, and three novel object preference (NOP) tests. Social interaction/play habituation occurred on P35 and tested on P39. PPI habituation occurred on P42, assessed on P44, and tested the first time on P42 and again on P102 after 5 days of sub-chronic MK-801 injections. Spatial memory test #1 was conducted from P121 to P126. The 3 days of the first NOP habituation were from P149 to P151 and testing occurred on P152. A second NOP test and a NPP test was conducted on P165 and P166, respectively. Working memory tests began from P186 to P208 with a 26-day break and resumed from P234 to P242. NOP #3 and spatial memory test #2 were conducted only on the saline group. NOP #3 habituation occurred from P248 to 249, then tested on P250. Spatial memory test #2 took place from P252 to P255, P263 and P268. Refer to Figure 1A for comprehensive methodological timeline.

Brain Tissue Collection

Rat brains were obtained through rapid decapitation and immediate extraction for all groups from P268 to P269. The brains were sectioned into separate hemispheres at the corpus callosum and separately fixed and stored in 4% paraformaldehyde solution (in phosphate buffer (PB); pH= 7.3-7.4) for three weeks of post-fixation before being transferred to 0.1% sodium-azide solution for long term storage until ready for sectioning. All brains were transferred into 30% sucrose solution in 0.1M phosphate buffer saline (PBS) for cryoprotection for 4 days or until they sank, whichever came first, and then sliced with a freezing microtome (Leica, Buffalo Grove, IL) into 40μm coronal sections. Six series of sections of the PFC were stored free-floating in freezing solution at -20°C until processed for immunohistochemistry analyses.

Immunohistochemistry

One series of tissue slices per brain were randomly selected to stain for PV and PNNs, and all sections were spaced approximately 240μm from one another. Free-floating sections were first washed (2x5min) in PBS then blocked with 5% normal donkey serum (NDS; Jackson ImmunoResearch, West Grove, PA) with 0.3% triton-X in PBS solution (PBST) and 1% bovine serum albumin (BSA; ThermoFisher Scientific) for 1 hour on an agitator at room temperature. The slices were then incubated in primary antibody solution: biotin conjugate Lectin from wisteria floribunda agglutinin (WFA; 1:000, Sigma; for visualization of PNNs), and anti-parvalbumin (raised in rabbit, 1:10,000, ThermoFisher; for

visualization of PV) in 0.3% PBST for two nights at 4°C on an agitator. After the primary incubation period, slices were rinsed 3 times in 5-minute increments in 0.3% PBST and transferred into secondary antibody solution consisting of 0.3% PBST, streptavidin conjugate 488 (1:3000, ThermoFisher) which tags WFA, and donkey anti-rabbit secondary antibody Alexa 594 (1:500, abcam), to visualize PV. After incubating for 3 hours on an agitator at room temperature, sections were washed 3 times for 5 minutes in PBS. Hemisphere sections were mounted onto glass slides and dried before being cover-slipped with ProLong Gold antifade mounting medium (Invitrogen).

Imaging

A Keyence All-in-One Fluorescence Microscope BZ-X710 was used to image the stained sections of the PFC. The slices of each brain were visualized at 4x magnification to select 4 to 5 slices for inclusion (approximately between 3.72mm to 2.28mm relative to Bregma) and all photomicrographs used for analyses were taken at 20x magnification. Images of the superficial (cortical layers 1-3) and deep layers (cortical layers 4-6) of both prelimbic (PL) and infralimbic (IL) regions of the PFC, guided by the rat brain atlas (Swanson, 2004), were taken at 20x under both red (PV) and green (PNN) fluorescence (see photomicrograph locations in Figure 2B). 3 pictures were taken per slice per fluorescent channel: 1 superficial PL (20x), 1 deep PL (20x), 1 IL (20x), for a total of 6 pictures per slice. With each brain having 4 to 5 selected slices, 24-30 images were taken in total for each brain and stored for later analysis via ImageJ.

Superficial and deep layers of the PL were determined on the microscope and side by side images were taken. Caution was taken to ensure no cells were double counted in these images by leaving small gap between the two collected images. Because all layers of the IL can be visualized in one image under 20x, only one photo was taken. The superficial and deep layers of IL were later determined by editing in a line down the middle of the photo, separating it into right (superficial) and left (deep) regions for the brains with the midline on the left and the opposite for when the midline is on the right. See Figure 2B.

Each photomicrograph was analyzed using ImageJ software (NIH) for PV and PNN quantification. PV cells and PNNs in the superficial and deep regions of the IL and PL were counted using the multi-point tool. The number of PV cells surrounded by PNNs was measured by overlaying both PV and PNN images and counting the PV cells that are surrounded by PNNs. PV cells and PNNs that had distinct edges and fluoresced enough to distinguish from background were counted. In order to ensure accurate and reproducible counting, all images were taken at the same fluorescent intensity/exposure during microscopy.

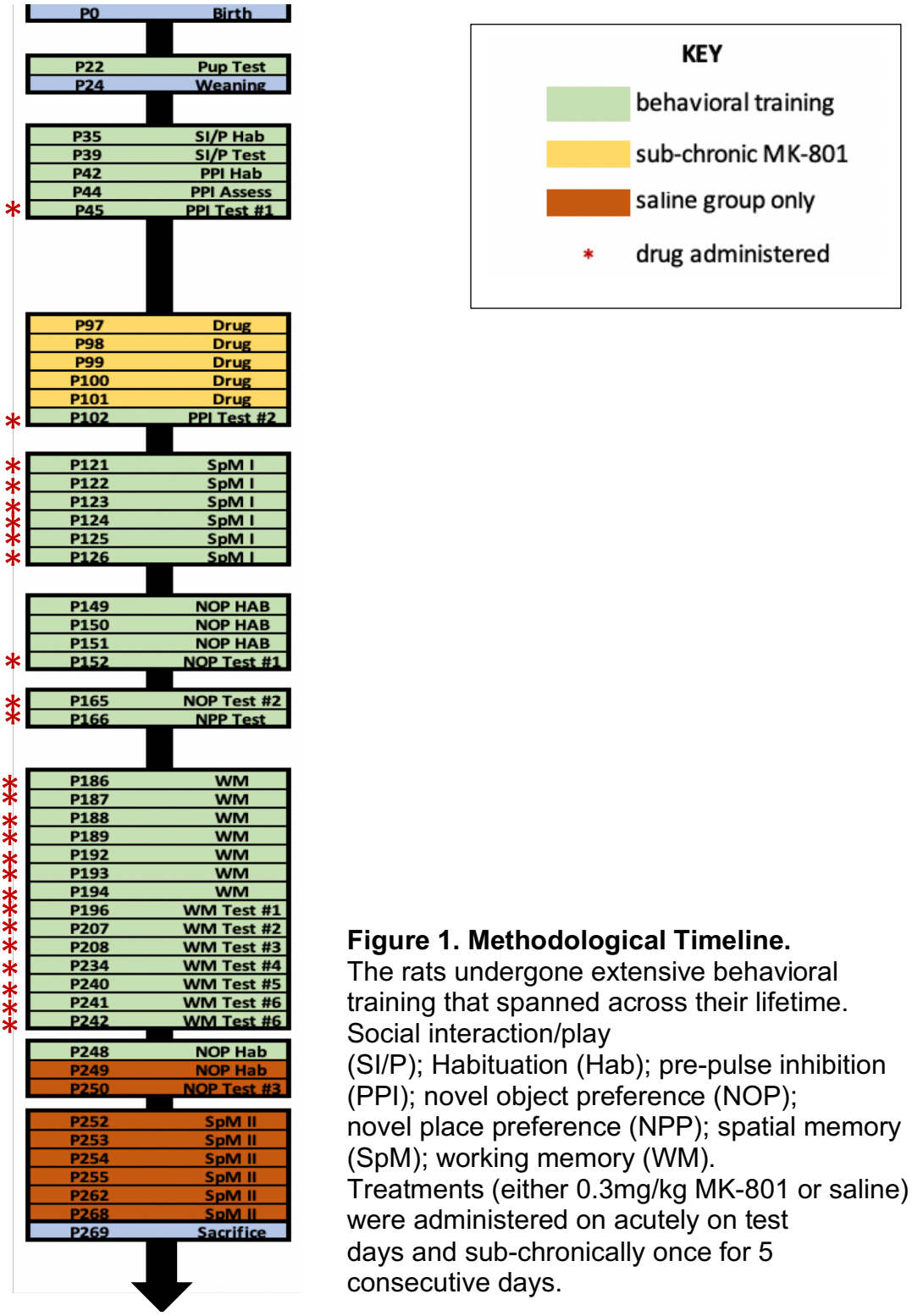

Figure 1. Methodological Timeline.
The rats undergone extensive behavioral training that spanned across their lifetime. Social interaction/play (SI/P); Habituation (Hab); pre-pulse inhibition (PPI); novel object preference (NOP); novel place preference (NPP); spatial memory (SpM); working memory (WM). Treatments (either 0.3mg/kg MK-801 or saline) were administered on acutely on test days and sub-chronically once for 5 consecutive days.

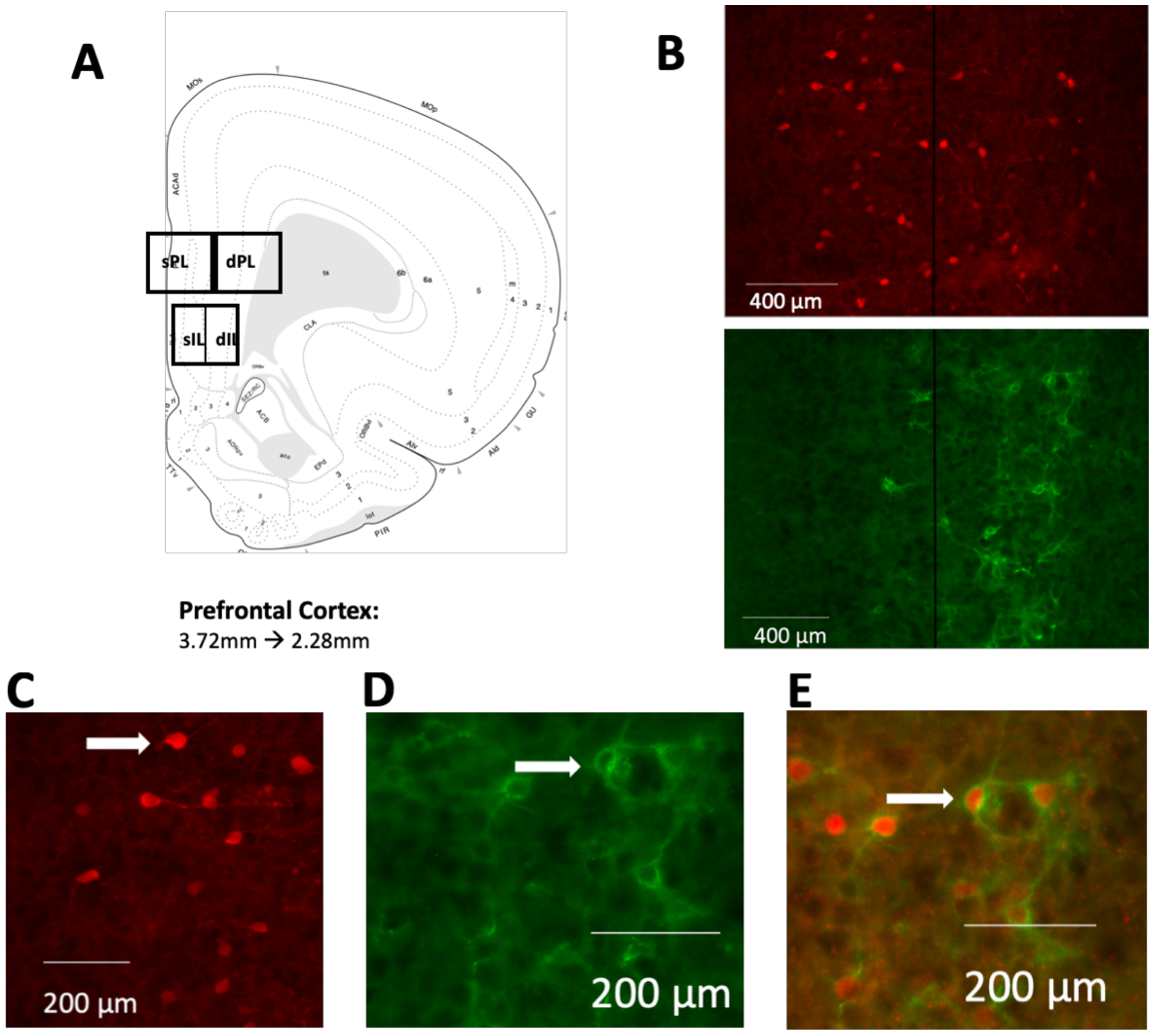

Figure 2. Experimental Methods: (A) Regions that were sampled included the superficial PL(sPL), deep PL (dPL), superficial IL (sIL), and deep IL (dIL). The anatomical measurement is relative to Bregma. An example of the division of superficial and deep layers of the IL in a 20x photomicrograph is shown in (B). The two regions were divided by drawing a line at the midline. A representative example of a counted PV cell is shown in (C). A representative example of a counted PNN is shown in (D). A representative example of a counted PV cell surrounded by a PNN is shown in (E).

Statistical Analyses

To look for differences in the seven measures listed below within the regions of interest, we took the average number of PV cells and PNNs in the superficial, deep and total (superficial + deep for each region) regions of the PL and IL across all the slices for each brain and calculated group means. A Grubb's outlier test (Grubbs, 1969) was conducted to remove any outstanding outliers. A two-way ANOVA [Treatment (Saline, MK-801) x Genotype (Con, DISC1-KO)] was conducted for each of the variables of interest-- number of PV cells without PNNs, total number of PV cells, total number of PNNs, % of PNNs that surrounds a PV cell, and % of PV cells surrounded by PNNs--to determine if there were any differences due to genotype and/or treatment. In the event of a main effect or interaction, Holm-Sidak's post-hoc analyses were conducted with corrections for multiple comparisons.

RESULTS

Prelimbic Cortex Findings

Treatment by gene interaction influences total number of PNNs within the PL. There was a trending interaction observed (F (1,28) = 3.274; p = 0.081) across the total PL. This is evidenced in that within saline treatment group, DISC1-KO saw a modest decrease in the number of PNNs compared to WT. In the DISC1-KO group, MK-801 treatment appeared to increase PNN count compared to the WT group with the same treatment. No observable difference in PNN counts were seen specifically within the superficial or deep regions of PL, regardless of treatment or gene. See figure 3 for graphical data and figure 4 for representative photomicrographs.

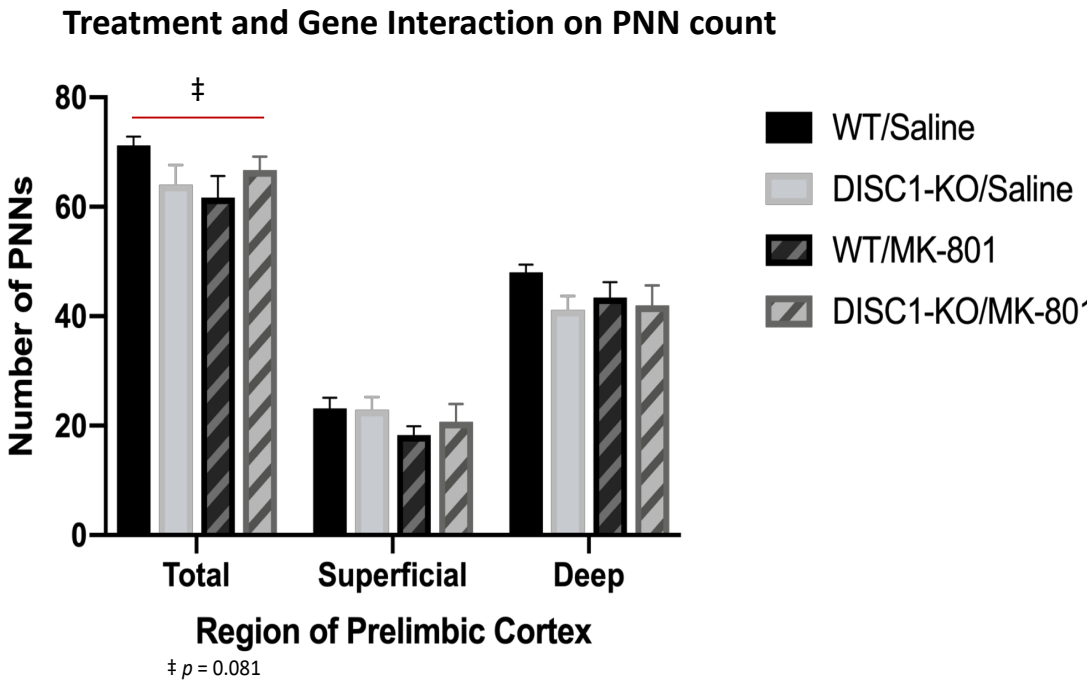

Figure 3. A trending interaction of treatment and gene on the number of PNNs was seen across the total PL (‡ p =0.081). DISC1-KO group saw a modest

decrease in PNNs compared to WT when given saline. Likewise, there is a slight, but appreciable decrease in number of PNNs in WT group when given MK-801 compared to saline. However, there is a subtle yet observable increase in PNNs when given MK-801 in the DISC1-KO group.

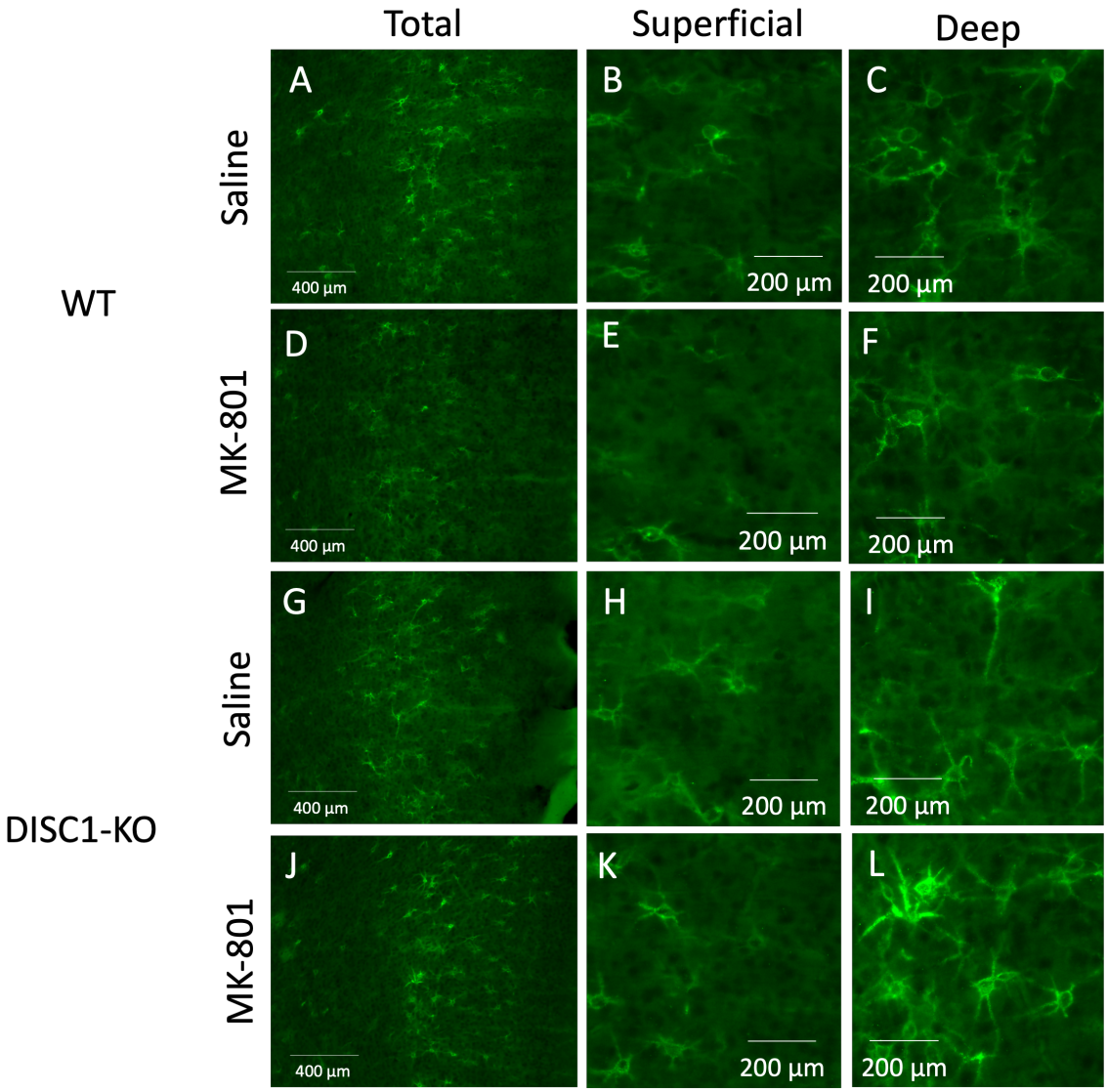

Figure 4. Representative photomicrographs of sampled regions in the PL that were analyzed for PNNs: superficial PL, deep PL, and total (superficial + deep combined) PL. The total PL photos were taken at a lower magnification (10x) to capture the entire region (A,D,G,J). A slight decrease in number of PNNs is seen in WT when treated with MK-801.

Gene dependent increase in the number of PV cells without PNNs in superficial PL. While genotype did not significantly alter the number of PV cells without PNNs across all regions of the PL ($p > 0.05$), there was an observable trend towards an increase in the count of PV cells without PNNs in the superficial layers ($F(1,29) = 3.463$; $p = 0.072$). DISC1-KO group saw an appreciable, though non-significant, decrease from the WT group. Interestingly, while not significant, Mk-801 seemed to increase the number of unsheathed PV cells in both WT and DISC1-KO groups. See figure 5 and figure 6.

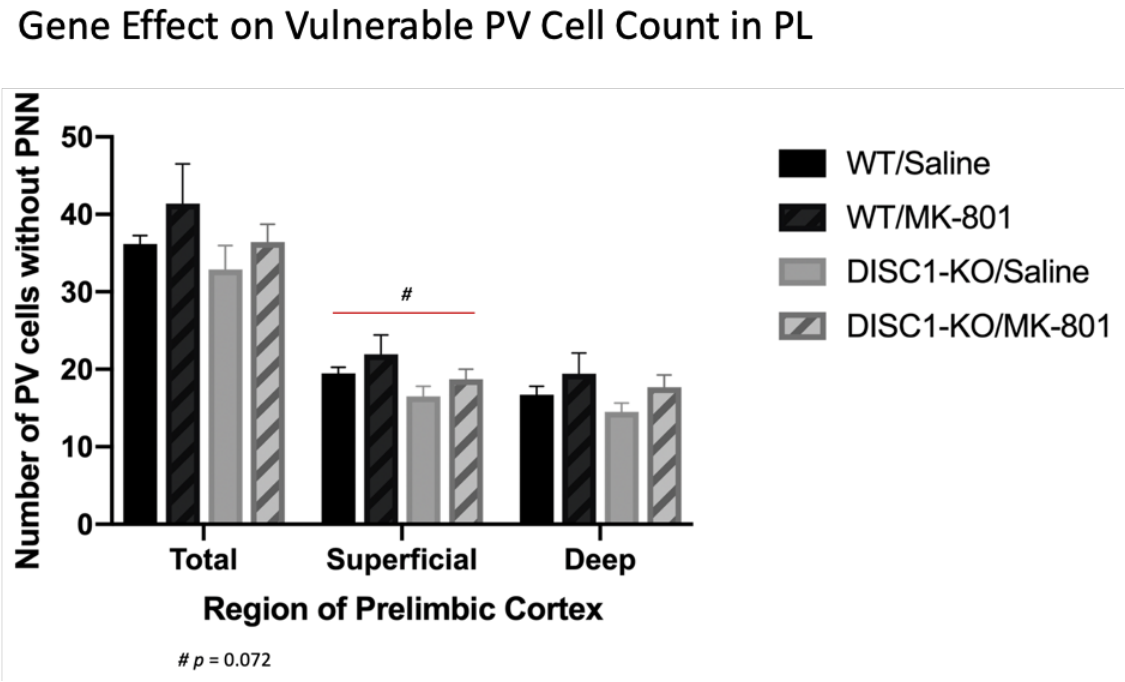

Figure 5. A trending effect of gene on the number of vulnerable PV cells is observed in the superficial PL (# $p = 0.072$). DISC1-KO has a smaller number of PV cells without PNNs compared to WT when treated with saline. Likewise, DISC1-KO treated with MK-801 also had a smaller number of PV cells without PNN when compared to saline. MK-801 also seems to increase the number of unsheathed PV cells modestly.

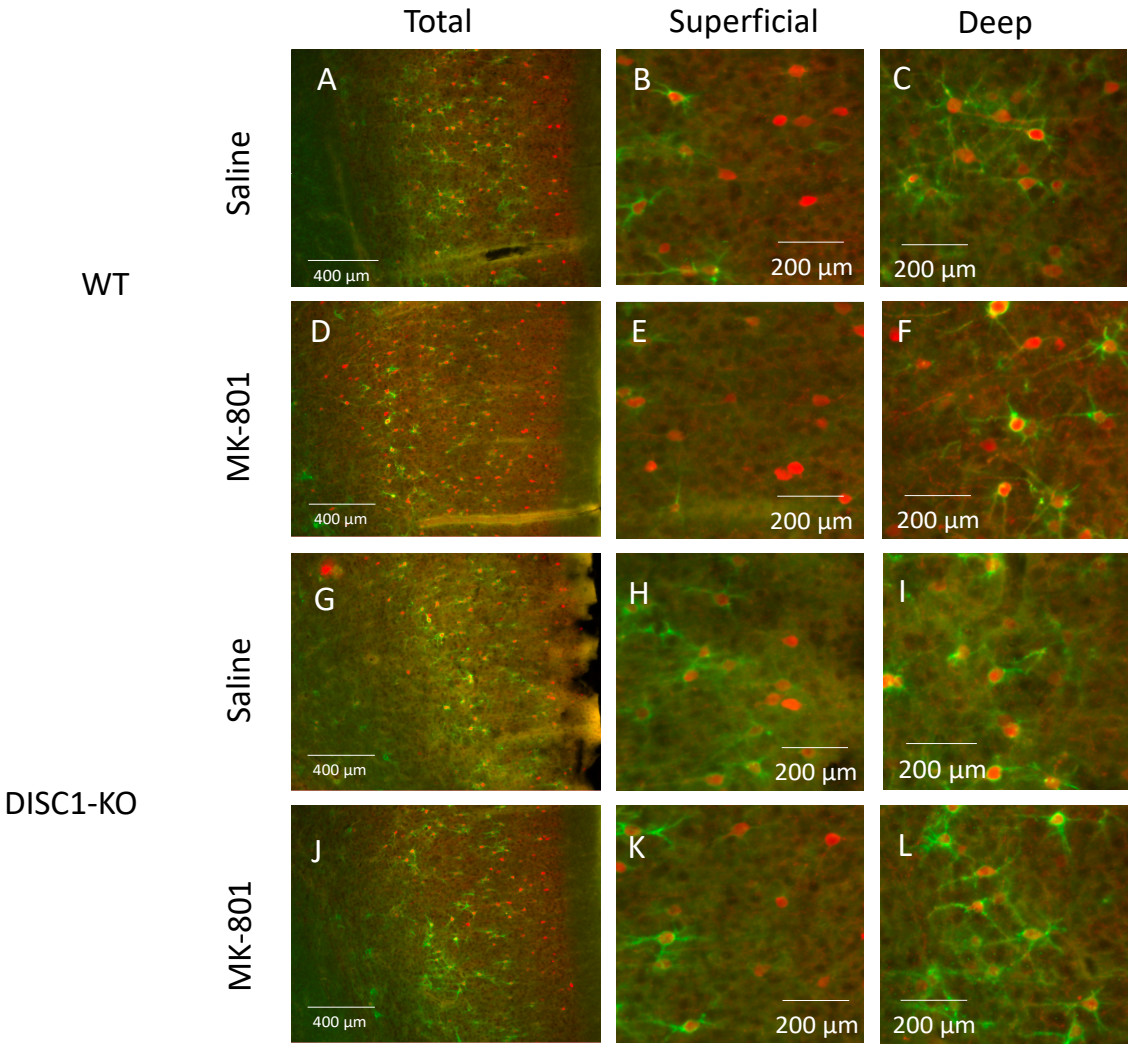

Figure 6. Representative photomicrographs of sampled regions in the PL used for analysis of PV (red) and PNNs (green): superficial PL, deep PL and total (superficial + deep) PL. Total PL photos were captured at a lower magnification (10x) to fit the entire region in frame (A,D,G,J). MK-801 seems to increase, though not significantly, the number of unsheathed PV cells regardless of genotype. No appreciable differences were seen elsewhere in the PL.

Infralimbic Cortex Findings

Drug-induced changes in non-PNN surrounded PV cell counts in superficial, but not deep infralimbic layers. In the IL, there was a significant main

effect of treatment (saline vs. MK-801; $F(1,29) = 5.054$, $p < 0.05$) such that the number non-PNN sheathed PV cells showed a modest increase in MK-801 groups, regardless of genotype. This main effect of drug treatment was observed only within the superficial layers of the IL. However, post-hoc multiple comparisons of the groups did not reveal any significant differences. See Figure 7 for graphical data and Figure 8 for representative photomicrographs.

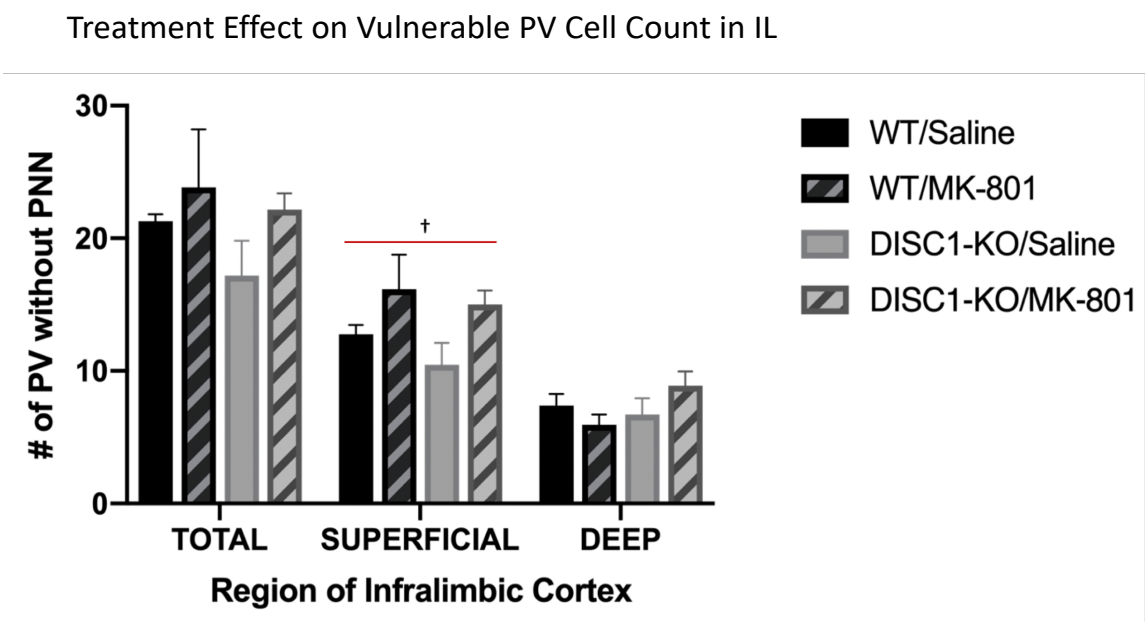

Figure 7. A significant main effect of MK-801 treatment was seen within the superficial layers of the IL, evidenced by an increase in the mean number of PV cells without PNN ensheathment († $p < 0.05$). MK-801 treatment or genotype did not yield any significant differences in the number of ensheathed PV cells in the deep or total region of the IL.

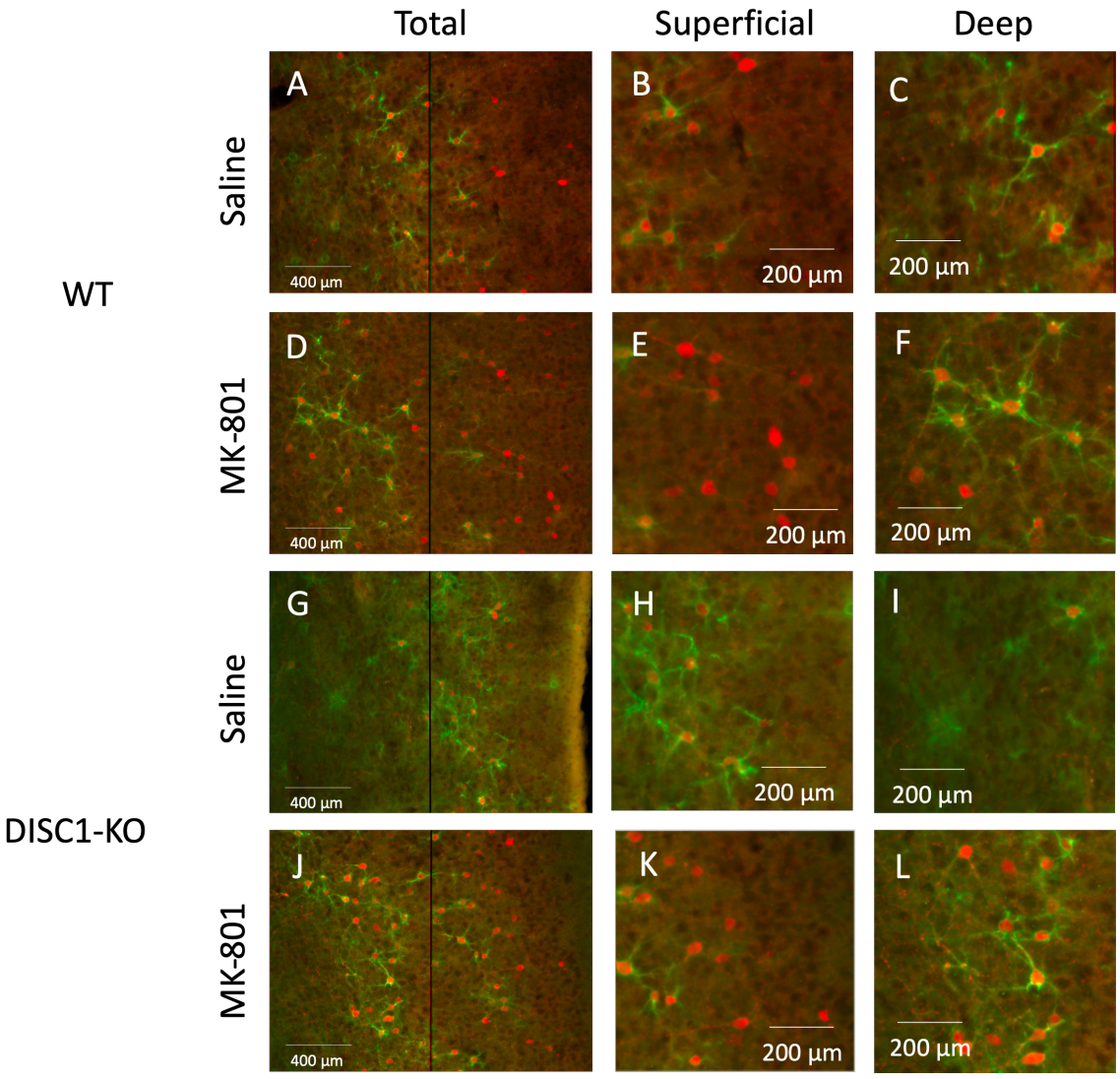

Figure 8. Representative overlaid (PV= red; PNNs = green) photomicrographs of IL regions that were analyzed: superficial IL, deep IL, and total IL (superficial and deep combined) in saline and MK-801 groups. Here, a modest increase in unsheathed PV cells in groups exposed to chronic MK-801 can be appreciated within the superficial IL, but not within the deep or total IL regions in both WT and DISC1 groups.

PV cell count within the deep layers of appear to be mediated by a genotype by drug interaction. There was a trending Gene x Treatment interaction (F (1,28) = 3.022, p = 0.093). Though there were no significant differences in the

PV cell count between groups across the IL, there was an appreciable difference between WT/Saline and DISC1-KO/Saline groups in the deep layers. DISC1-KO/Saline group saw a moderate decrease in PV cell count compared to WT counterparts. There were no significant differences seen between the groups in the total (p = 0.23) or superficial (p = 0.677) regions. See Figure 9 for graphical data and Figure 10 for representative photomicrographs of the regions of the IL.

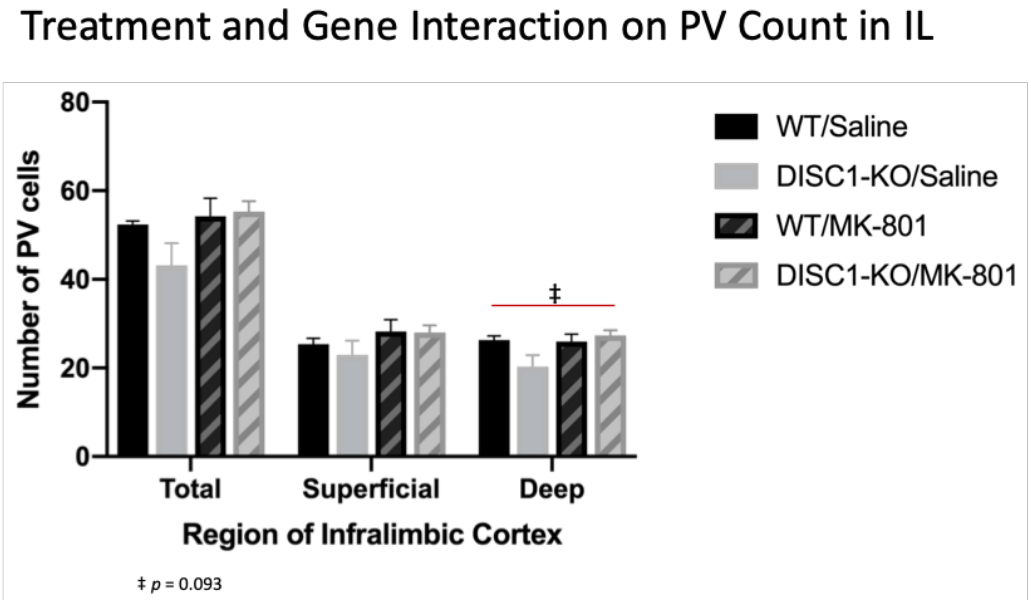

Figure 9. A trending interaction between treatment and genotype was observed in the deep layers of the IL. In saline treated groups, the DISC1-KO group saw a modest decrease in average count of PV cells compared to WT. Conversely, DISC1-KO group treated with MK-801 saw an appreciable increase in average count compared to saline. The decrease in DISC1-KO/Saline group appears to be driving the interaction effect seen. No other appreciable or significant differences were seen in the total or superficial regions of the IL.

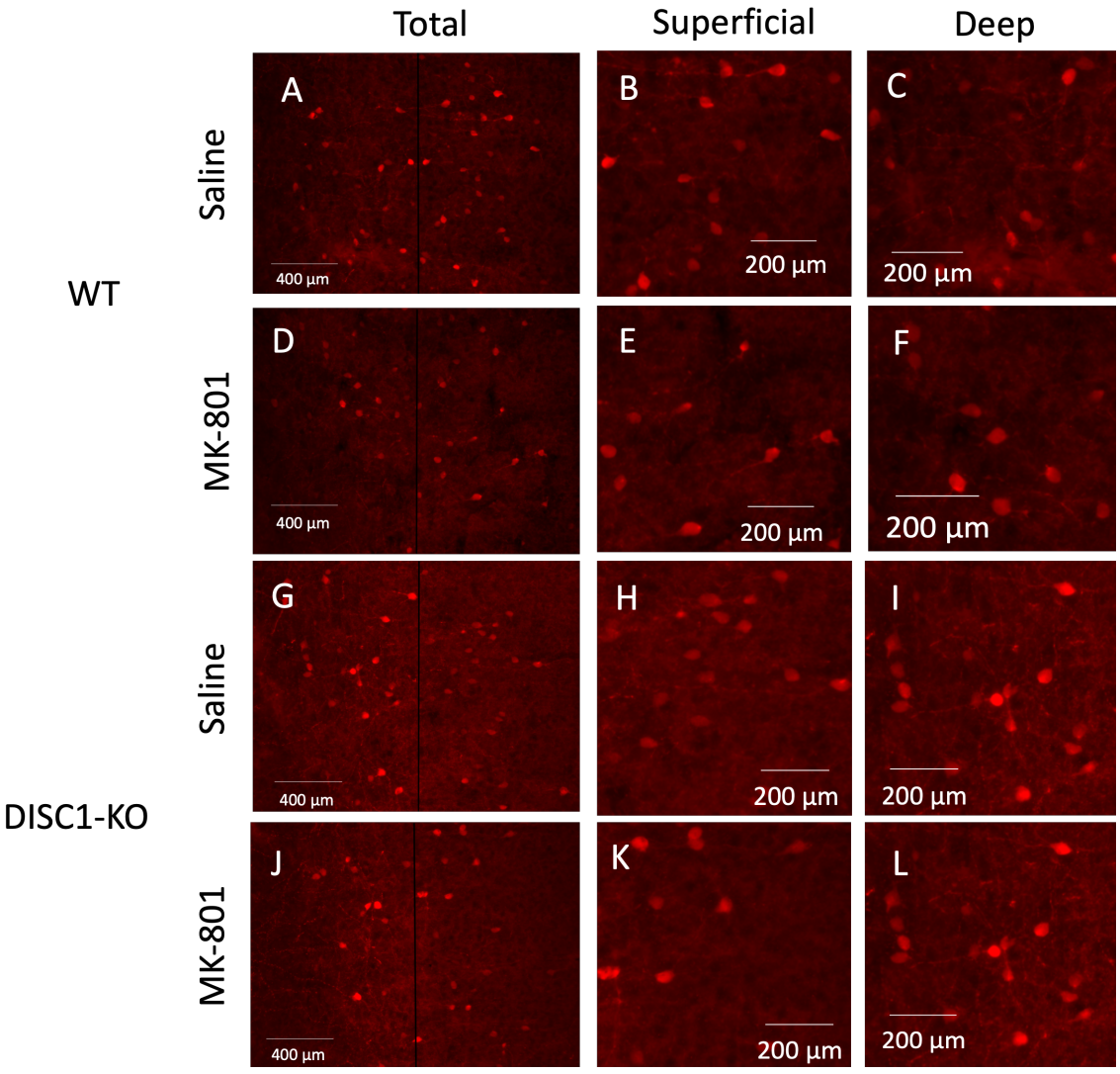

Figure 10. Representative photomicrographs of sampled regions in the IL that were analyzed: superficial IL, deep IL, and total (superficial and deep combined) IL in saline and MK-801 groups. There was a modest, though non-significant decrease in the average number of PV cells in the deep IL in DISC1-KO group compared to WT when treated with saline. There was a modest increase in PV count in the DISC1-KO group when treated with MK-801 compared to saline. No significant differences were observed across all groups in superficial or total IL.

Genotype-driven decrease in the % of PV cells surrounded by PNNs in deep layers of the IL. There was a trend towards a main effect of gene (F (1, 27) = 3.939, p = 0.057) evidenced by a noticeable decrease in the % of PV cells

surrounded by PNNs in the DISC1-KO/saline group compared to the WT/saline group. No additional notable changes were observed elsewhere in the IL. See figure 11 for graphical data and figure 12 for representative photomicrographs of IL regions.

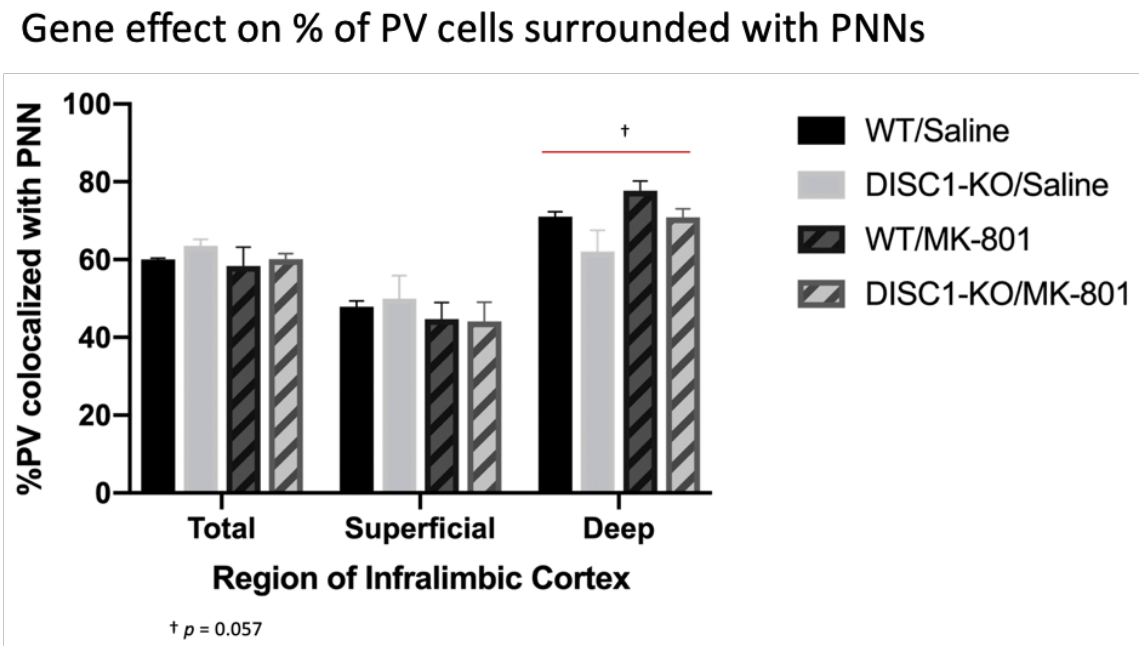

Figure 11. There is a trending, though not significant, effect of gene knockout on the % of PV cells surrounded by/colocalized with PNNs in the deep IL († = 0.057). WT group saw a greater % of PV surrounded with PNN than DISC1-KO group. The DISC1-KO group treated with MK-801 appears to show an increase in the % of PV colocalized with PNN when viewed against DISC1-KO group that was treated with saline. Similar percentages of PV with PNNs are seen in the WT/Saline and DISC1-KO/MK-801. No other significant or discernable differences were detected in any of the groups across both total and superficial IL.

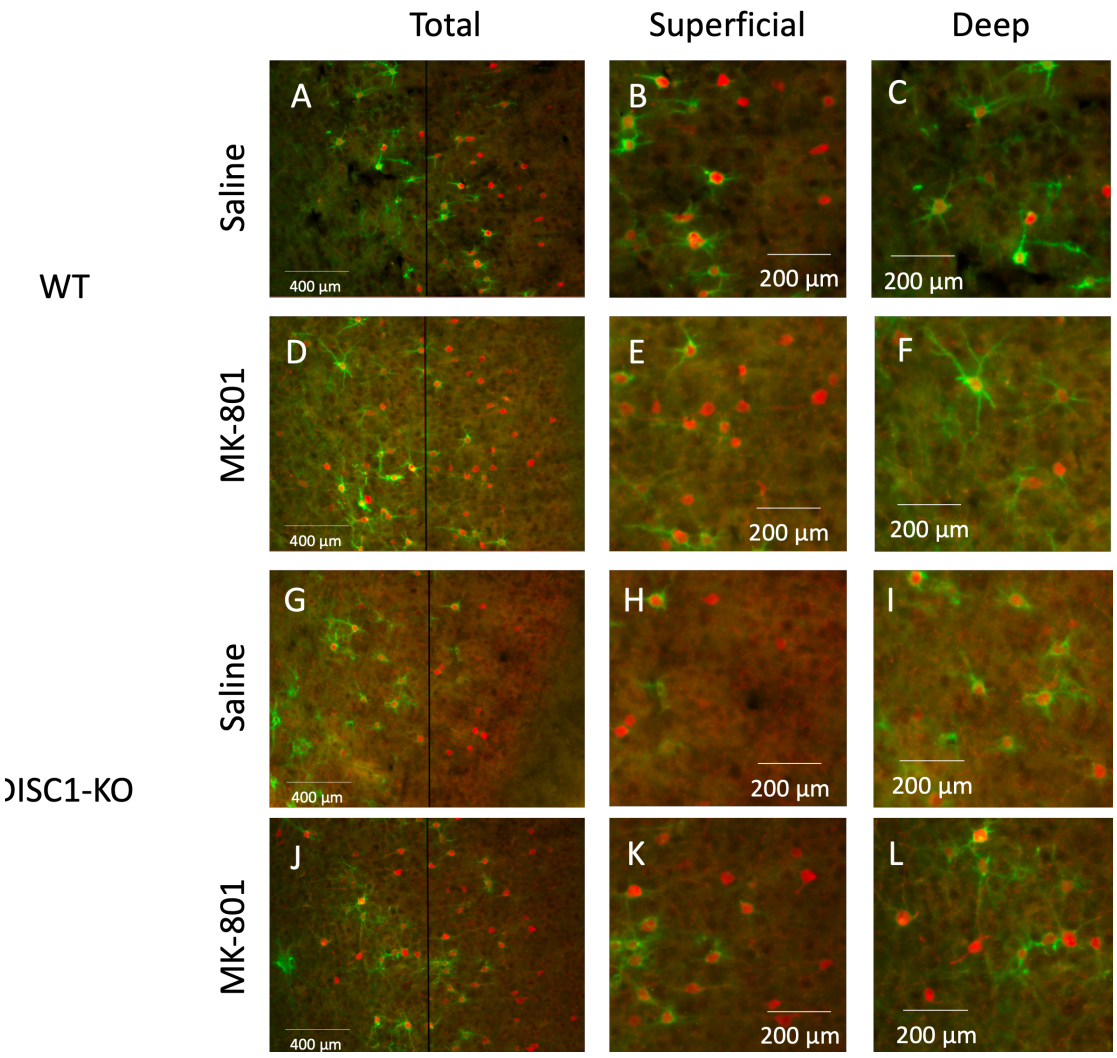

Figure 12. Representative photomicrographs of sampled regions in IL used for analysis: superficial IL, deep IL, and total (superficial and deep combined) IL. There was a trending decrease in the percentage of PV surrounded by PNN in DISC1-KO compared to WT regardless of treatment in the deep IL. A noticeable but nonsignificant increase in DISC1-KO was noticed when given MK-801 treatment compared to saline.

DISCUSSION

The present study investigated the neuroanatomical consequences of a complete knock out of the DISC1 gene in male rats on changes in the characteristics/relationship of PV containing neurons and PNNs. This work further expanded on what is known regarding this particular risk gene by additionally employing a diathesis-stress model of schizophrenia, with the DISC1 genetic predisposition compounded by a later adult NMDA antagonist challenge through both acute and sub-chronic administration of MK-801 (S. R. Jones & Fernyhough, 2007). Specifically, we looked for any genotype-dependent and/or drug treatment-dependent effects on the PV cell and PNN populations within the prefrontal cortex of rats. We observed that a knockout of DISC1 modestly decreases the number of PV cells and PNNs in saline control groups, while treatment with MK-801 had regionally- and layer-specific effects on PV and PNNs. Importantly, this work is the first to characterize neural changes associated with this novel DISC1 rat knockout model, and the results closely mirror those seen in postmortem schizophrenic prefrontal cortex (Lewis et al., 2005).While the present results are trending in nature, they provide exciting evidence for a likely role of DISC1 in the contributions to the well-described changes seen in human PV and PNN changes in schizophrenic patient populations.

Regionally-Specific Effects of DISC1-KO in Rat Prefrontal Cortex

DISC1-KO lead to a modest decrease in the average number of PV cells compared to WT rats within the deep layers of the IL, a finding that is in general agreement with previous findings in other DISC1 condition and partial knock-down rodent models (Ibi et al., 2010; Niwa et al., 2010; Umeda et al., 2016). The observed modest reduction in average PV cell count may be due to a decrease in protein expression and/or intensity, possibly leading to PV levels being difficult to detected in cells that may actually contain sub-threshold levels of PV protein. This interpretation is consistent with postmortem findings in the PFC of schizophrenic patients (Takanori Hashimoto et al., 2003; Lewis et al., 2005; Volk et al., 2000; Woo, Kim, & Viscidi, 2008), in that there is a selective decrease in both GAD67 expression specific to PV expressing neurons (Akbarian et al., 1995; Volk et al., 2000) and PV expression in schizophrenia (Lewis et al., 2005; Zhang & Reynolds, 2002) . Additionally, the DISC1-KO rats in this study exhibited a decreased number of PNNs in the PL compared to WT. These results are comparable to recent work describing a decreased labeling intensity of PNNs seen in schizophrenic postmortem tissue samples (Enwright et al., 2016; Mauney et al., 2013; Pantazopoulos et al., 2010). Since we did not specifically evaluate the staining intensity or mRNA levels in our present samples, we can reasonably assume that the decrease seen may be due to an inability to detect PNNs under 40x magnification due to the decreased protein expression. Differences in PNN count previously seen under low magnification

were eliminated when looking under a higher magnification and higher brightness index (Enwright et al., 2016).

MK-801 Decreases PNNs, and Leads to an Increase in PV Expressing Cells.

Here, we observed that MK-801 administration modestly increased the average number of PV cells that are not surrounded by PNNs in the superficial layers of both the IL and the PL. It is unclear if this increase is reflecting an overall decrease in PNNs, in PV cells, or a combination of both. However, we also saw that MK-801 treatment moderately decreased the number of PNNs in the PL of WT rats, suggesting that NMDA antagonist treatment might primarily affect PNNs, which supports previous findings of a reduction of PNN colocalized PV cells and reduction of cells that are positive for PNNs (Matuszko, Curreli, Kaushik, Becker, & Dityatev, 2017). This also replicates what has been seen in postmortem schizophrenic brains, in which there was a reduction of PNNs surrounding interneurons in the mPFC (Mauney et al., 2013). The regionally-dependent effects are in line with the postmortem findings of decreased densities of PNNs largely specific to layer 3, which is considered a superficial layer (Mauney et al., 2013). Interestingly, MK-801 seemed to increase the number of PV cells in the deep IL, which was contradictory to our initial hypothesis that a two-hit (gene + later NMDA antagonist challenge) would lead to an exaggerated decrease in PV cells comparable to that seen in postmortem human patient populations. Despite this observation, these findings are highly consistent with previous findings from our group describing an age-dependent increases in PV

following chronic ketamine administration. Indeed, prior work has characterized a decrease in PV cell count when ketamine was administered at a young age, with a concomitant increase when administered in adulthood (Abdul-Monim, Neill, & Reynolds, 2007; Honeycutt & Chrobak, 2018; Sabbagh et al., 2013). In the present study, all rats received their first injection of MK-801 in early adulthood at P45, and received chronic administrations starting at P100, which is well into adulthood, therefore making it very plausible that the increases described herein are likely driven by the age at which the MK-801 was administered.

Functional consequences of increasing trend of unsheathed PV cells.

We observed trends of increased number of unsheathed PV cells with MK-801 treatment in the superficial layers of both IL and PL compared to saline. The lack of PNNs surrounding the PV cells suggests these cells are vulnerable to damage, which can lead to abnormalities or decreases in PV cells. This is consistent with previous evidence of PNN disruption resulting in a decrease in PV expression (Yamada, Ohgomori, & Jinno, 2015). The implications of decreased PV interneurons could be cortical dysfunction due to the role of PV in generating gamma frequency oscillations. This gamma oscillatory activity has been found to be critical in synchronizing cortical activity during cognitive tasks (Volman, Behrens, & Sejnowski, 2011). Therefore, abnormal oscillatory activity secondary to PV dysfunction may translate into impaired executive function capabilities that has been characterized frequently in schizophrenia.

There were a number of limitations in this study as the rats were sacrificed at a very old age, introducing potential age-related changes that could confound the results. Likewise, the rats utilized in the present work had undergone extensive behavioral training throughout their life, which has been noted to increase PV expression, and could strengthen the synapses (Patz, Grabert, Gorba, Wirth, & Wahle, 2004). These confounding factors could muddle the interpretations and potentially be the reason why we did not see many robust changes. Future work should aim to elucidate these findings in the absence of these extraneous variables in order to isolate the differential effects of DISC1-KO genotype and MK-801 treatment on both neural and behavioral outcomes.

Despite the limitations, there were some distinct advantages to the design of this study. While there are other studies that have measured the changes in PV utilizing a DISC1 model, this is the first rat model of schizophrenia with a full knock out of DISC1 in addition to an NMDA antagonist (MK-801) treatment challenge in adulthood. A full knock out allows a clearer interpretation of the relationship between DISC1 and the abnormalities seen in schizophrenia. This model reduces the ambiguity inherent in knockdown and/or transient disruption models where location of the changes may confer differential results which may not translate readily to patient populations. Furthermore, this is the first model to our knowledge that also looked at the relationship between DISC1 and alterations in the expression of PNNs. This model allowed us to address the decreases in PNNs seen in post mortem brains of schizophrenia and further

elucidate if the dysfunction may be driven by DISC1 in a novel and effective manner.

Future directions would be to look at the neuroanatomical changes of a DISC1-KO rat model in the hippocampus, as this gene is highly expressed in this brain region (Austin, Ky, Ma, Morris, & Shughrue, 2004; Ma et al., 2002). Since decreases in hippocampal PV expression has been well-established in schizophrenic postmortem brain tissue, it is likely that more robust effects of the knockout will be observed here (Konradi et al., 2011; Torrey et al., 2005; Zhang & Reynolds, 2002). Indeed, the hippocampal findings in schizophrenia research are replicated more consistently compared to the prefrontal cortex (Lieberman et al., 2018). Therefore, such findings may provide greater evidence that supports the use of a full knockout of DISC1 as a translational rat model of schizophrenic neural and behavioral deficits. To determine if a second insult is required (as proposed in the diathesis-stress model of schizophrenia), future studies should administer the NMDA antagonist treatment during adolescence, since psychosis typically emerges during early adulthood and this period has been shown to be particularly vulnerable to insult (Gomes, Rincón-Cortés, & Grace, 2016).

REFERENCES

Abdolmaleky, H. M., Cheng, K., Faraone, S. V., Wilcox, M., Glatt, S. J., Gao, F., … Thiagalingam, S. (2006). Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Human Molecular Genetics*, *15*(21), 3132–3145. https://doi.org/10.1093/hmg/ddl253

Abdul-Monim, Z., Neill, J. C., & Reynolds, G. P. (2007). Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbumin-immunoreactive expression in the rat. *Journal of Psychopharmacology (Oxford, England)*, *21*(2), 198–205. https://doi.org/10.1177/0269881107067097

Akbarian, S., Kim, J. J., Potkin, S. G., Hagman, J. O., Tafazzoli, A., Bunney, W. E., & Jones, E. G. (1995). Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Archives of General Psychiatry*, *52*(4), 258–266.

Albus, M., Hubmann, W., Mohr, F., Hecht, S., Hinterberger-Weber, P., Seitz, N.-N., & Küchenhoff, H. (2006). Neurocognitive functioning in patients with first-episode schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience*, *256*(7), 442. https://doi.org/10.1007/s00406-006-0667-1

Alkuraya, F. S., Cai, X., Emery, C., Mochida, G. H., Al-Dosari, M. S., Felie, J. M., … Walsh, C. A. (2011). Human Mutations in NDE1 Cause Extreme Microcephaly with Lissencephaly. *The American Journal of Human Genetics*, *88*(5), 536–547. https://doi.org/10.1016/j.ajhg.2011.04.003

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA.

Andreasen, N. C., Rezai, K., Alliger, R., Swayze, V. W., Flaum, M., Kirchner, P., … O'Leary, D. S. (1992). Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia. Assessment with xenon 133 single-photon emission computed tomography and the Tower of London. *Archives of General Psychiatry*, *49*(12), 943–958.

Austin, C. P., Ky, B., Ma, L., Morris, J. A., & Shughrue, P. J. (2004). Expression of Disrupted-In-Schizophrenia-1, a schizophrenia-associated gene, is prominent in the mouse hippocampus throughout brain development. *Neuroscience*, *124*(1), 3–10. https://doi.org/10.1016/j.neuroscience.2003.11.010

Balmer, T. S. (2016). Perineuronal Nets Enhance the Excitability of Fast-Spiking Neurons. *ENeuro*, *3*(4). https://doi.org/10.1523/ENEURO.0112-16.2016

Bayer, T. A., Falkai, P., & Maier, W. (1999). Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the "Two hit hypothesis." *Journal of Psychiatric Research*, *33*(6), 543–548. https://doi.org/10.1016/S0022-3956(99)00039-4

Belforte, J. E., Zsiros, V., Sklar, E. R., Jiang, Z., Yu, G., Li, Y., … Nakazawa, K. (2010). Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nature Neuroscience*, *13*(1), 76–83. https://doi.org/10.1038/nn.2447

Benes, F. M., & Berretta, S. (2001). GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *25*(1), 1–27. https://doi.org/10.1016/S0893-133X(01)00225-1

Benes, Francine M., McSparren, J., Bird, E. D., SanGiovanni, J. P., & Vincent, S. L. (1991). Deficits in Small Interneurons in Prefrontal and Cingulate Cortices of Schizophrenic and Schizoaffective Patients. *Archives of General Psychiatry*, *48*(11), 996–1001. https://doi.org/10.1001/archpsyc.1991.01810350036005

Beraldi, G. H., Prado, K. S., Amann, B. L., Radua, J., Friedman, L., & Elkis, H. (2018). Meta-analyses of cavum septum pellucidum in mood disorders in comparison with healthy controls or schizophrenia. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, *28*(12), 1325–1338. https://doi.org/10.1016/j.euroneuro.2018.10.001

Bernard, C., & Prochiantz, A. (2016). Otx2-PNN Interaction to Regulate Cortical Plasticity [Research article]. https://doi.org/10.1155/2016/7931693

Berretta, S. (2012). Extracellular matrix abnormalities in schizophrenia. *Neuropharmacology*, *62*(3), 1584–1597. https://doi.org/10.1016/j.neuropharm.2011.08.010

Berretta, S., Pantazopoulos, H., Markota, M., Brown, C., & Batzianouli, E. T. (2015). LOSING THE SUGAR COATING: POTENTIAL IMPACT OF PERINEURONAL NET ABNORMALITIES ON INTERNEURONS IN SCHIZOPHRENIA. *Schizophrenia Research*, *167*(0), 18–27. https://doi.org/10.1016/j.schres.2014.12.040

Bilder, R. M., Goldman, R. S., Robinson, D., Reiter, G., Bell, L., Bates, J. A., … Lieberman, J. A. (2000). Neuropsychology of First-Episode Schizophrenia: Initial Characterization and Clinical Correlates. *American Journal of Psychiatry*, *157*(4), 549–559. https://doi.org/10.1176/appi.ajp.157.4.549

Bitanihirwe, B. K. Y., & Woo, T.-U. W. (2014). Perineuronal Nets and Schizophrenia: The Importance of Neuronal Coatings. *Neuroscience and Biobehavioral Reviews*, *45*, 85–99. https://doi.org/10.1016/j.neubiorev.2014.03.018

Bowie, C. R., & Harvey, P. D. (2006). Cognitive deficits and functional outcome in schizophrenia. *Neuropsychiatric Disease and Treatment*, *2*(4), 531–536.

Boydell, J., van Os, J., McKenzie, K., Allardyce, J., Goel, R., McCreadie, R. G., & Murray, R. M. (2001). Incidence of schizophrenia in ethnic minorities in London: ecological study into interactions with environment. *BMJ : British Medical Journal*, *323*(7325), 1336.

Brandon, N. J., Millar, J. K., Korth, C., Sive, H., Singh, K. K., & Sawa, A. (2009). Understanding the Role of DISC1 in Psychiatric Disease and during

Normal Development. *The Journal of Neuroscience*, *29*(41), 12768. https://doi.org/10.1523/JNEUROSCI.3355-09.2009

Brown, A. S., Schaefer, C. A., Wyatt, R. J., Goetz, R., Begg, M. D., Gorman, J. M., & Susser, E. S. (2000). Maternal exposure to respiratory infections and adult schizophrenia spectrum disorders: a prospective birth cohort study. *Schizophrenia Bulletin*, *26*(2), 287–295.

Brown, Alan S. (2011). The environment and susceptibility to schizophrenia. *Progress in Neurobiology*, *93*(1), 23–58. https://doi.org/10.1016/j.pneurobio.2010.09.003

Brown, Alan S., Begg, M. D., Gravenstein, S., Schaefer, C. A., Wyatt, R. J., Bresnahan, M., … Susser, E. S. (2004). Serologic Evidence of Prenatal Influenza in the Etiology of Schizophrenia. *Archives of General Psychiatry*, *61*(8), 774–780. https://doi.org/10.1001/archpsyc.61.8.774

Brown, Alan S., & Derkits, E. J. (2010). Prenatal Infection and Schizophrenia: A Review of Epidemiologic and Translational Studies. *The American Journal of Psychiatry*, *167*(3), 261–280. https://doi.org/10.1176/appi.ajp.2009.09030361

Buckley, P. F. (2005). Neuroimaging of schizophrenia: structural abnormalities and pathophysiological implications. *Neuropsychiatric Disease and Treatment*, *1*(3), 193–204.

Burdick, K. E., Kamiya, A., Hodgkinson, C. A., Lencz, T., DeRosse, P., Ishizuka, K., … Malhotra, A. K. (2008). Elucidating the relationship between DISC1,

NDEL1 and NDE1 and the risk for schizophrenia: Evidence of epistasis and competitive binding. *Human Molecular Genetics*, *17*(16), 2462–2473. https://doi.org/10.1093/hmg/ddn146

Buxbaum, J., Georgieva, L., Young, J., Plescia, C., Kajiwara, Y., Jiang, Y., … O'Donovan, M. (2008). Molecular dissection of NRG1-ERBB4 signaling implicates PTPRZ1 as a potential schizophrenia susceptibility gene. *Molecular Psychiatry*, *13*(2), 162–172. https://doi.org/10.1038/sj.mp.4001991

Cabungcal, J.-H., Steullet, P., Morishita, H., Kraftsik, R., Cuenod, M., Hensch, T. K., & Do, K. Q. (2013). Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(22), 9130–9135. https://doi.org/10.1073/pnas.1300454110

Caroni, P. (2015). Regulation of Parvalbumin Basket cell plasticity in rule learning. *Biochemical and Biophysical Research Communications*, *460*(1), 100–103. https://doi.org/10.1016/j.bbrc.2015.02.023

Coba, M. P., Komiyama, N. H., Nithianantharajah, J., Kopanitsa, M. V., Indersmitten, T., Skene, N. G., … Grant, S. G. N. (2012). TNIK is required for postsynaptic and nuclear signalling pathways and cognitive function. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(40), 13987–13999. https://doi.org/10.1523/JNEUROSCI.2433-12.2012

Cochran, S. M., Kennedy, M., McKerchar, C. E., Steward, L. J., Pratt, J. A., & Morris, B. J. (2003). Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *28*(2), 265–275. https://doi.org/10.1038/sj.npp.1300031

Dalman, C., Allebeck, P., Cullberg, J., Grunewald, C., & Köster, M. (1999). Obstetric Complications and the Risk of Schizophrenia: A Longitudinal Study of a National Birth Cohort. *Archives of General Psychiatry*, *56*(3), 234–240. https://doi.org/10.1001/archpsyc.56.3.234

Daskalakis, Z. J., Christensen, B. K., Chen, R., Fitzgerald, P. B., Zipursky, R. B., & Kapur, S. (2002). Evidence for impaired cortical inhibition in schizophrenia using transcranial magnetic stimulation. *Archives of General Psychiatry*, *59*(4), 347–354.

Degreef, G., Bogerts, B., Falkai, P., Greve, B., Lantos, G., Ashtari, M., & Lieberman, J. (1992). Increased prevalence of the cavum septum pellucidum in magnetic resonance scans and post-mortem brains of schizophrenic patients. *Psychiatry Research*, *45*(1), 1–13.

Deng, D., Jian, C., Lei, L., Zhou, Y., McSweeney, C., Dong, F., … Mao, Y. (2017). A prenatal interruption of DISC1 function in the brain exhibits a lasting impact on adult behaviors, brain metabolism, and interneuron

development. *Oncotarget*, *8*(49), 84798–84817. https://doi.org/10.18632/oncotarget.21381

Dienel, S. J., & Lewis, D. A. (2018). Alterations in cortical interneurons and cognitive function in schizophrenia. *Neurobiology of Disease*. https://doi.org/10.1016/j.nbd.2018.06.020

Dityatev, A., Brückner, G., Dityateva, G., Grosche, J., Kleene, R., & Schachner, M. (2007). Activity-dependent formation and functions of chondroitin sulfate-rich extracellular matrix of perineuronal nets. *Developmental Neurobiology*, *67*(5), 570–588. https://doi.org/10.1002/dneu.20361

Do, K. Q., Trabesinger, A. H., Kirsten-Krüger, M., Lauer, C. J., Dydak, U., Hell, D., … Cuénod, M. (2000). Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *The European Journal of Neuroscience*, *12*(10), 3721–3728.

Duncan, G. E., Moy, S. S., Perez, A., Eddy, D. M., Zinzow, W. M., Lieberman, J. A., … Koller, B. H. (2004). Deficits in sensorimotor gating and tests of social behavior in a genetic model of reduced NMDA receptor function. *Behavioural Brain Research*, *153*(2), 507–519. https://doi.org/10.1016/j.bbr.2004.01.008

Egerton, A., Modinos, G., Ferrera, D., & McGuire, P. (2017). Neuroimaging studies of GABA in schizophrenia: a systematic review with meta-analysis. *Translational Psychiatry*, *7*(6), e1147. https://doi.org/10.1038/tp.2017.124

Enwright, J. F., Sanapala, S., Foglio, A., Berry, R., Fish, K. N., & Lewis, D. A. (2016). Reduced Labeling of Parvalbumin Neurons and Perineuronal Nets in the Dorsolateral Prefrontal Cortex of Subjects with Schizophrenia. *Neuropsychopharmacology*, *41*(9), 2206–2214. https://doi.org/10.1038/npp.2016.24

Erlenmeyer-Kimling, L., Rock, D., Roberts, S. A., Janal, M., Kestenbaum, C., Cornblatt, B., … Gottesman, I. I. (2000). Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: the New York High-Risk Project. *The American Journal of Psychiatry*, *157*(9), 1416–1422. https://doi.org/10.1176/appi.ajp.157.9.1416

Feigenson, K. A., Kusnecov, A. W., & Silverstein, S. M. (2014). Inflammation and the Two-Hit Hypothesis of Schizophrenia. *Neuroscience and Biobehavioral Reviews*, *38*, 72–93. https://doi.org/10.1016/j.neubiorev.2013.11.006

Flatow, J., Buckley, P., & Miller, B. J. (2013). Meta-analysis of oxidative stress in schizophrenia. *Biological Psychiatry*, *74*(6), 400–409. https://doi.org/10.1016/j.biopsych.2013.03.018

Galarreta, M., & Hestrin, S. (2002). Electrical and chemical synapses among parvalbumin fast-spiking GABAergic interneurons in adult mouse neocortex. *Proceedings of the National Academy of Sciences*, *99*(19), 12438–12443. https://doi.org/10.1073/pnas.192159599

Garey, L., Ong, W., Patel, T., Kanani, M., Davis, A., Mortimer, A., … Hirsch, S. (1998). Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *Journal of Neurology, Neurosurgery, and Psychiatry*, *65*(4), 446–453.

Gejman, P., Sanders, A., & Duan, J. (2010). The Role of Genetics in the Etiology of Schizophrenia. *The Psychiatric Clinics of North America*, *33*(1), 35–66. https://doi.org/10.1016/j.psc.2009.12.003

Giedd, J. N. (2004). Structural Magnetic Resonance Imaging of the Adolescent Brain. *Annals of the New York Academy of Sciences*, *1021*(1), 77–85. https://doi.org/10.1196/annals.1308.009

Glantz, L. A., & Lewis, D. A. (2000). Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Archives of General Psychiatry*, *57*(1), 65–73.

Glausier, J. R., & Lewis, D. A. (2013). Dendritic Spine Pathology in Schizophrenia. *Neuroscience*, *251*, 90–107. https://doi.org/10.1016/j.neuroscience.2012.04.044

Glotzbach, E., Mühlberger, A., Gschwendtner, K., Fallgatter, A. J., Pauli, P., & Herrmann, M. J. (2011). Prefrontal Brain Activation During Emotional Processing: A Functional Near Infrared Spectroscopy Study (fNIRS). *The Open Neuroimaging Journal*, *5*, 33–39. https://doi.org/10.2174/1874440001105010033

Goff, D. C., Hill, M., & Barch, D. (2011). The treatment of cognitive impairment in schizophrenia. *Pharmacology, Biochemistry, and Behavior*, *99*(2), 245–253. https://doi.org/10.1016/j.pbb.2010.11.009

Goghari, V. M., MacDonald, A. W., & Sponheim, S. R. (2014). Relationship between Prefrontal Grey Matter Volumes and Working Memory Performance in Schizophrenia: A Family Study. *Schizophrenia Research*, *153*(0), 113–121. https://doi.org/10.1016/j.schres.2014.01.032

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., … Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(21), 8174–8179. https://doi.org/10.1073/pnas.0402680101

Goldman-Rakic, P. S., & Selemon, L. D. (1997). Functional and Anatomical Aspects of Prefrontal Pathology in Schizophrenia. *Schizophrenia Bulletin*, *23*(3), 437–458. https://doi.org/10.1093/schbul/23.3.437

Gomes, F. V., Rincón-Cortés, M., & Grace, A. A. (2016). Adolescence as a period of vulnerability and intervention in schizophrenia: Insights from the MAM Model. *Neuroscience and Biobehavioral Reviews*, *70*, 260–270. https://doi.org/10.1016/j.neubiorev.2016.05.030

Green, M. F., Kern, R. S., Braff, D. L., & Mintz, J. (2000). Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the “right stuff”? *Schizophrenia Bulletin*, *26*(1), 119–136.

Green, Michael F. (2016). Impact of Cognitive and Social Cognitive Impairment on Functional Outcomes in Patients With Schizophrenia. *The Journal of Clinical Psychiatry*, *77*(suppl 2), 8–11. https://doi.org/10.4088/JCP.14074su1c.02

Haenschel, C., Bittner, R. A., Waltz, J., Haertling, F., Wibral, M., Singer, W., … Rodriguez, E. (2009). Cortical oscillatory activity is critical for working memory as revealed by deficits in early-onset schizophrenia. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*(30), 9481–9489. https://doi.org/10.1523/JNEUROSCI.1428-09.2009

Hasenstaub, A., Otte, S., Callaway, E., & Sejnowski, T. J. (2010). Metabolic cost as a unifying principle governing neuronal biophysics. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(27), 12329–12334. https://doi.org/10.1073/pnas.0914886107

Hashimoto, T., Arion, D., Unger, T., Maldonado-Avilés, J. G., Morris, H. M., Volk, D. W., … Lewis, D. A. (2008). Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Molecular Psychiatry*, *13*(2), 147–161. https://doi.org/10.1038/sj.mp.4002011

Hashimoto, Takanori, Volk, D. W., Eggan, S. M., Mirnics, K., Pierri, J. N., Sun, Z., … Lewis, D. A. (2003). Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *The*

Journal of Neuroscience: The Official Journal of the Society for Neuroscience, *23*(15), 6315–6326.

Heinrichs, R. W. (2005). The Primacy of Cognition in Schizophrenia. *American Psychologist*, *60*(3), 229–242. https://doi.org/10.1037/0003-066X.60.3.229

Hensch, T. K. (2005). Critical period plasticity in local cortical circuits. *Nature Reviews. Neuroscience*, *6*(11), 877–888. https://doi.org/10.1038/nrn1787

Hill, S. K., Schuepbach, D., Herbener, E. S., Keshavan, M. S., & Sweeney, J. A. (2004). Pretreatment and longitudinal studies of neuropsychological deficits in antipsychotic-naïve patients with schizophrenia. *Schizophrenia Research*, *68*(1), 49–63. https://doi.org/10.1016/S0920-9964(03)00213-5

Hoff, A. L., Sakuma, M., Wieneke, M., Horon, R., Kushner, M., & DeLisi, L. E. (1999). Longitudinal neuropsychological follow-up study of patients with first-episode schizophrenia. *The American Journal of Psychiatry*, *156*(9), 1336–1341. https://doi.org/10.1176/ajp.156.9.1336

Honeycutt, J. A., & Chrobak, J. J. (2018). Parvalbumin Loss Following Chronic Sub-Anesthetic NMDA Antagonist Treatment is Age-Dependent in the Hippocampus: Implications for Modeling NMDA Hypofunction. *Neuroscience*, *393*, 73–82. https://doi.org/10.1016/j.neuroscience.2018.09.031

Huang, H.-S., Matevossian, A., Whittle, C., Kim, S. Y., Schumacher, A., Baker, S. P., & Akbarian, S. (2007). Prefrontal Dysfunction in Schizophrenia Involves Mixed-Lineage Leukemia 1-Regulated Histone Methylation at

GABAergic Gene Promoters. *Journal of Neuroscience*, *27*(42), 11254–11262. https://doi.org/10.1523/JNEUROSCI.3272-07.2007

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology*, *387*(2), 167–178. https://doi.org/10.1002/(SICI)1096-9861(19971020)387:2<167::AID-CNE1>3.0.CO;2-Z

Ibi, D., Nagai, T., Koike, H., Kitahara, Y., Mizoguchi, H., Niwa, M., … Yamada, K. (2010). Combined effect of neonatal immune activation and mutant DISC1 on phenotypic changes in adulthood. *Behavioural Brain Research*, *206*(1), 32–37. https://doi.org/10.1016/j.bbr.2009.08.027

Inan, M., Zhao, M., Manuszak, M., Karakaya, C., Rajadhyaksha, A. M., Pickel, V. M., … Manfredi, G. (2016). Energy deficit in parvalbumin neurons leads to circuit dysfunction, impaired sensory gating and social disability. *Neurobiology of Disease*, *93*, 35–46. https://doi.org/10.1016/j.nbd.2016.04.004

Jaaro-Peled, H. (2009). Chapter 9 - Gene models of schizophrenia: DISC1 mouse models. In S. Akira (Ed.), *Progress in Brain Research* (pp. 75–86). https://doi.org/10.1016/S0079-6123(09)17909-8

Jackson, M. E., Homayoun, H., & Moghaddam, B. (2004). NMDA receptor hypofunction produces concomitant firing rate potentiation and burst activity reduction in the prefrontal cortex. *Proceedings of the National*

Academy of Sciences of the United States of America, *101*(22), 8467–8472. https://doi.org/10.1073/pnas.0308455101

Jones, E. G. (1997). Cortical Development and Thalamic Pathology in Schizophrenia. *Schizophrenia Bulletin*, *23*(3), 483–501. https://doi.org/10.1093/schbul/23.3.483

Jones, P. B., Rantakallio, P., Hartikainen, A.-L., Isohanni, M., & Sipila, P. (1998). Schizophrenia as a Long-Term Outcome of Pregnancy, Delivery, and Perinatal Complications: A 28-Year Follow-Up of the 1966 North Finland General Population Birth Cohort. *American Journal of Psychiatry*, *155*(3), 355–364. https://doi.org/10.1176/ajp.155.3.355

Jones, S. R., & Fernyhough, C. (2007). A New Look at the Neural Diathesis–Stress Model of Schizophrenia: The Primacy of Social-Evaluative and Uncontrollable Situations. *Schizophrenia Bulletin*, *33*(5), 1171–1177. https://doi.org/10.1093/schbul/sbl058

Kahn, R. S., & Keefe, R. S. E. (2013). Schizophrenia Is a Cognitive Illness: Time for a Change in Focus. *JAMA Psychiatry*, *70*(10), 1107–1112. https://doi.org/10.1001/jamapsychiatry.2013.155

Kamiya, A., Kubo, K., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., … Sawa, A. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nature Cell Biology*, *7*(12), 1167–1178. https://doi.org/10.1038/ncb1328

Kamiya, A., Tomoda, T., Chang, J., Takaki, M., Zhan, C., Morita, M., … Sawa, A. (2006). DISC1–NDEL1/NUDEL protein interaction, an essential component for neurite outgrowth, is modulated by genetic variations of DISC1. *Human Molecular Genetics*, *15*(22), 3313–3323. https://doi.org/10.1093/hmg/ddl407

KANE, J. M., & LENCZ, T. (2008). Cognitive deficits in schizophrenia: short-term and long-term. *World Psychiatry*, *7*(1), 29–30.

Kann, O., Papageorgiou, I. E., & Draguhn, A. (2014). Highly energized inhibitory interneurons are a central element for information processing in cortical networks. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *34*(8), 1270–1282. https://doi.org/10.1038/jcbfm.2014.104

Karlsgodt, K. H., Sun, D., & Cannon, T. D. (2010). Structural and Functional Brain Abnormalities in Schizophrenia. *Current Directions in Psychological Science*, *19*(4), 226–231. https://doi.org/10.1177/0963721410377601

Kawasaki, Y., Maeda, Y., Suzuki, M., Urata, K., Higashima, M., Kiba, K., … Hisada, K. (1993). SPECT analysis of regional cerebral blood flow changes in patients with schizophrenia during the Wisconsin Card Sorting Test. *Schizophrenia Research*, *10*(2), 109–116.

Keefe, R. S. E., Eesley, C. E., & Poe, M. P. (2005). Defining a cognitive function decrement in schizophrenia. *Biological Psychiatry*, *57*(6), 688–691. https://doi.org/10.1016/j.biopsych.2005.01.003

Keilhoff, G., Becker, A., Grecksch, G., Wolf, G., & Bernstein, H.-G. (2004). Repeated application of ketamine to rats induces changes in the hippocampal expression of parvalbumin, neuronal nitric oxide synthase and cFOS similar to those found in human schizophrenia. *Neuroscience*, *126*(3), 591–598. https://doi.org/10.1016/j.neuroscience.2004.03.039

Kendler, K. S., & Robinette, C. D. (1983). Schizophrenia in the National Academy of Sciences-National Research Council Twin Registry: a 16-year update. *American Journal of Psychiatry*, *140*(12), 1551–1563. https://doi.org/10.1176/ajp.140.12.1551

Kim, D., Jeong, H., Lee, J., Ghim, J.-W., Her, E. S., Lee, S.-H., & Jung, M. W. (2016). Distinct Roles of Parvalbumin- and Somatostatin-Expressing Interneurons in Working Memory. *Neuron*, *92*(4), 902–915. https://doi.org/10.1016/j.neuron.2016.09.023

Kim, J. Y., Duan, X., Liu, C. Y., Jang, M.-H., Guo, J. U., Pow-anpongkul, N., … Ming, G. (2009). DISC1 regulates new neuron development in the adult brain via modulation of AKT-mTOR signaling through KIAA1212. *Neuron*, *63*(6), 761–773. https://doi.org/10.1016/j.neuron.2009.08.008

Konradi, C., Yang, C. K., Zimmerman, E. I., Lohmann, K. M., Gresch, P., Pantazopoulos, H., … Heckers, S. (2011). Hippocampal interneurons are abnormal in schizophrenia. *Schizophrenia Research*, *131*(1–3), 165–173. https://doi.org/10.1016/j.schres.2011.06.007

Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., Bremner, J. D., … Charney, D. S. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Archives of General Psychiatry*, *51*(3), 199–214.

Kucewicz, M. T., Cimbalnik, J., Matsumoto, J. Y., Brinkmann, B. H., Bower, M. R., Vasoli, V., … Worrell, G. A. (2014). High frequency oscillations are associated with cognitive processing in human recognition memory. *Brain: A Journal of Neurology*, *137*(Pt 8), 2231–2244. https://doi.org/10.1093/brain/awu149

Kundakovic, M., & Jaric, I. (2017). The Epigenetic Link between Prenatal Adverse Environments and Neurodevelopmental Disorders. *Genes*, *8*(3). https://doi.org/10.3390/genes8030104

Lahti, A. C., Koffel, B., LaPorte, D., & Tamminga, C. A. (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *13*(1), 9–19. https://doi.org/10.1016/0893-133X(94)00131-I

Lensjø, K. K., Lepperød, M. E., Dick, G., Hafting, T., & Fyhn, M. (2017). Removal of Perineuronal Nets Unlocks Juvenile Plasticity Through Network Mechanisms of Decreased Inhibition and Increased Gamma Activity. *The*

Journal of Neuroscience, *37*(5), 1269–1283. https://doi.org/10.1523/JNEUROSCI.2504-16.2016

Lewis, D. A., Curley, A. A., Glausier, J., & Volk, D. W. (2012). Cortical Parvalbumin Interneurons and Cognitive Dysfunction in Schizophrenia. *Trends in Neurosciences*, *35*(1), 57–67. https://doi.org/10.1016/j.tins.2011.10.004

Lewis, D. A., Hashimoto, T., & Volk, D. W. (2005). Cortical inhibitory neurons and schizophrenia. *Nature Reviews Neuroscience*, *6*(4), 312–324. https://doi.org/10.1038/nrn1648

Lewis, D. A., & Levitt, P. (2002). Schizophrenia as a Disorder of Neurodevelopment. *Annual Review of Neuroscience*, *25*(1), 409–432. https://doi.org/10.1146/annurev.neuro.25.112701.142754

Li, Y., Sansgiri, R., Estroff, J., Mehta, T., Poussaint, T., Robertson, R., … Levine, D. (2011). Outcome of Fetuses with Cerebral Ventriculomegaly and Septum Pellucidum Leaflet Abnormalities. *AJR. American Journal of Roentgenology*, *196*(1), W83–W92. https://doi.org/10.2214/AJR.10.4434

Liao, Q., Wang, Y., Cheng, J., Dai, D., Zhou, X., Zhang, Y., … Duan, S. (2015). DNA methylation patterns of protein coding genes and long noncoding RNAs in female schizophrenic patients. *European Journal of Medical Genetics*, *58*(2), 95–104. https://doi.org/10.1016/j.ejmg.2014.12.001

Liddle, P. F., Friston, K. J., Frith, C. D., Hirsch, S. R., Jones, T., & Frackowiak, R. S. J. (1992). Patterns of Cerebral Blood Flow in Schizophrenia. *The British*

Journal of Psychiatry, *160*(2), 179–186. https://doi.org/10.1192/bjp.160.2.179

Lieberman, J. A., Girgis, R. R., Brucato, G., Moore, H., Provenzano, F., Kegeles, L., … Small, S. A. (2018). Hippocampal dysfunction in the pathophysiology of schizophrenia: a selective review and hypothesis for early detection and intervention. *Molecular Psychiatry*, *23*(8), 1764. https://doi.org/10.1038/mp.2017.249

Lipska, B. K., Peters, T., Hyde, T. M., Halim, N., Horowitz, C., Mitkus, S., … Kleinman, J. E. (2006). Expression of DISC1 binding partners is reduced in schizophrenia and associated with DISC1 SNPs. *Human Molecular Genetics*, *15*(8), 1245–1258. https://doi.org/10.1093/hmg/ddl040

Liu, H., Gao, P.-F., Xu, H.-W., Liu, M.-M., Yu, T., Yao, J.-P., & Yin, Z.-Q. (2013). Perineuronal nets increase inhibitory GABAergic currents during the critical period in rats. *International Journal of Ophthalmology*, *6*(2), 120–125. https://doi.org/10.3980/j.issn.2222-3959.2013.02.02

Ma, L., Liu, Y., Ky, B., Shughrue, P. J., Austin, C. P., & Morris, J. A. (2002). Cloning and characterization of Disc1, the mouse ortholog of DISC1 (Disrupted-in-Schizophrenia 1). *Genomics*, *80*(6), 662–672.

MacLaren, E. J., Charlesworth, P., Coba, M. P., & Grant, S. G. N. (2011). Knockdown of mental disorder susceptibility genes disrupts neuronal network physiology in vitro. *Molecular and Cellular Neurosciences*, *47*(2), 93–99. https://doi.org/10.1016/j.mcn.2010.12.014

Mao, Y., Ge, X., Frank, C. L., Madison, J. M., Koehler, A. N., Doud, M. K., … Tsai, L.-H. (2009). DISC1 regulates neural progenitor proliferation via modulation of GSK3β/β-catenin signaling. *Cell*, *136*(6), 1017–1031. https://doi.org/10.1016/j.cell.2008.12.044

Marenco, S., & Weinberger, D. R. (2000). The neurodevelopmental hypothesis of schizophrenia: following a trail of evidence from cradle to grave. *Development and Psychopathology*, *12*(3), 501–527.

Mathew, S. S., & Hablitz, J. J. (2011). Presynaptic NMDA Receptors Mediate IPSC Potentiation at GABAergic Synapses in Developing Rat Neocortex. *PLoS ONE*, *6*(2). https://doi.org/10.1371/journal.pone.0017311

Matuszko, G., Curreli, S., Kaushik, R., Becker, A., & Dityatev, A. (2017). Extracellular matrix alterations in the ketamine model of schizophrenia. *Neuroscience*, *350*, 13–22. https://doi.org/10.1016/j.neuroscience.2017.03.010

Mauney, S. A., Athanas, K. M., Pantazopoulos, H., Shaskan, N., Passeri, E., Berretta, S., & Woo, T.-U. W. (2013). Developmental Pattern of Perineuronal Nets in the Human Prefrontal Cortex and Their Deficit in Schizophrenia. *Biological Psychiatry*, *74*(6), 427–435. https://doi.org/10.1016/j.biopsych.2013.05.007

Maynard, T. M., Sikich, L., Lieberman, J. A., & LaMantia, A. S. (2001). Neural development, cell-cell signaling, and the “two-hit” hypothesis of schizophrenia. *Schizophrenia Bulletin*, *27*(3), 457–476.

McCartney, D. L., Walker, R. M., Morris, S. W., Anderson, S. M., Duff, B. J., Marioni, R. E., … Evans, K. L. (2018). Altered DNA methylation associated with a translocation linked to major mental illness. *NPJ Schizophrenia*, *4*. https://doi.org/10.1038/s41537-018-0047-7

Meyer, U., & Feldon, J. (2010). Epidemiology-driven neurodevelopmental animal models of schizophrenia. *Progress in Neurobiology*, *90*(3), 285–326. https://doi.org/10.1016/j.pneurobio.2009.10.018

Meyer-Lindenberg, A., & Tost, H. (2014). Neuroimaging and plasticity in schizophrenia. *Restorative Neurology and Neuroscience*, *32*(1), 119–127. https://doi.org/10.3233/RNN-139014

Miyamoto, S., Leipzig, J. N., Lieberman, J. A., & Duncan, G. E. (2000). Effects of ketamine, MK-801, and amphetamine on regional brain 2-deoxyglucose uptake in freely moving mice. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *22*(4), 400–412. https://doi.org/10.1016/S0893-133X(99)00127-X

Morishita, H., Cabungcal, J.-H., Chen, Y., Do, K. Q., & Hensch, T. K. (2015). Prolonged Period of Cortical Plasticity upon Redox Dysregulation in Fast-Spiking Interneurons. *Biological Psychiatry*, *78*(6), 396–402. https://doi.org/10.1016/j.biopsych.2014.12.026

Morris, J. A., Kandpal, G., Ma, L., & Austin, C. P. (2003). DISC1 (Disrupted-In-Schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5 and NUDEL: regulation and loss of interaction

with mutation. *Human Molecular Genetics*, *12*(13), 1591–1608. https://doi.org/10.1093/hmg/ddg162

Morris, N. P., & Henderson, Z. (2000). Perineuronal nets ensheath fast spiking, parvalbumin-immunoreactive neurons in the medial septum/diagonal band complex. *The European Journal of Neuroscience*, *12*(3), 828–838.

Morrow, B. A., Elsworth, J. D., & Roth, R. H. (2007). Repeated phencyclidine in monkeys results in loss of parvalbumin-containing axo-axonic projections in the prefrontal cortex. *Psychopharmacology*, *192*(2), 283–290. https://doi.org/10.1007/s00213-007-0708-0

Mueser, K. T., & Jeste, D. V. (2008). *Clinical Handbook of Schizophrenia*. New York, NY: Guilford Press.

Murase, S., Lantz, C. L., & Quinlan, E. M. (2017). Light reintroduction after dark exposure reactivates plasticity in adults via perisynaptic activation of MMP-9. *ELife*, *6*, e27345. https://doi.org/10.7554/eLife.27345

Murray, A. J., Woloszynowska-Fraser, M. U., Ansel-Bollepalli, L., Cole, K. L. H., Foggetti, A., Crouch, B., … Wulff, P. (2015). Parvalbumin-positive interneurons of the prefrontal cortex support working memory and cognitive flexibility. *Scientific Reports*, *5*. https://doi.org/10.1038/srep16778

Najjar, S., & Pearlman, D. M. (2015). Neuroinflammation and white matter pathology in schizophrenia: systematic review. *Schizophrenia Research*, *161*(1), 102–112. https://doi.org/10.1016/j.schres.2014.04.041

Nakazawa, K., Zsiros, V., Jiang, Z., Nakao, K., Kolata, S., Zhang, S., & Belforte, J. E. (2012). GABAergic interneuron origin of schizophrenia pathophysiology. *Neuropharmacology*, *62*(3), 1574–1583. https://doi.org/10.1016/j.neuropharm.2011.01.022

Nestler, E. J., Peña, C. J., Kundakovic, M., Mitchell, A., & Akbarian, S. (2016). Epigenetic Basis of Mental Illness. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *22*(5), 447–463. https://doi.org/10.1177/1073858415608147

Niendam, T. A., Ray, K. L., Iosif, A.-M., Lesh, T. A., Ashby, S. R., Patel, P. K., … Carter, C. S. (2018). Association of Age at Onset and Longitudinal Course of Prefrontal Function in Youth With Schizophrenia. *JAMA Psychiatry*, *75*(12), 1252–1260. https://doi.org/10.1001/jamapsychiatry.2018.2538

Nishijo, H., Rafal, R., & Tamietto, M. (2018). Editorial: Limbic-Brainstem Roles in Perception, Cognition, Emotion, and Behavior. *Frontiers in Neuroscience*, *12*. https://doi.org/10.3389/fnins.2018.00395

Niwa, M., Kamiya, A., Murai, R., Kubo, K., Gruber, A. J., Tomita, K., … Nabeshima, T. (2010). Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. *Neuron*, *65*(4), 480–489. https://doi.org/10.1016/j.neuron.2010.01.019

Norkett, R., Modi, S., & Kittler, J. T. (2017). Mitochondrial roles of the psychiatric disease risk factor DISC1. *Schizophrenia Research*, *187*, 47–54. https://doi.org/10.1016/j.schres.2016.12.025

Onstad, S., Skre, I., Torgersen, S., & Kringlen, E. (1991). Twin concordance for DSM-III-R schizophrenia. *Acta Psychiatrica Scandinavica*, *83*(5), 395–401. https://doi.org/10.1111/j.1600-0447.1991.tb05563.x

Pantazopoulos, H., Woo, T.-U. W., Lim, M. P., Lange, N., & Berretta, S. (2010). Extracellular Matrix-Glial Abnormalities in the Amygdala and Entorhinal Cortex of Subjects Diagnosed With Schizophrenia. *Archives of General Psychiatry*, *67*(2), 155–166. https://doi.org/10.1001/archgenpsychiatry.2009.196

Patel, K. R., Cherian, J., Gohil, K., & Atkinson, D. (2014). Schizophrenia: Overview and Treatment Options. *Pharmacy and Therapeutics*, *39*(9), 638–645.

Patz, S., Grabert, J., Gorba, T., Wirth, M. J., & Wahle, P. (2004). Parvalbumin Expression in Visual Cortical Interneurons Depends on Neuronal Activity and TrkB Ligands during an Early Period of Postnatal Development. *Cerebral Cortex*, *14*(3), 342–351. https://doi.org/10.1093/cercor/bhg132

Paylor, J. W., Lins, B. R., Greba, Q., Moen, N., de Moraes, R. S., Howland, J. G., & Winship, I. R. (2016). Developmental disruption of perineuronal nets in the medial prefrontal cortex after maternal immune activation. *Scientific Reports*, *6*, 37580. https://doi.org/10.1038/srep37580

Paylor, J. W., Wendlandt, E., Freeman, T. S., Greba, Q., Marks, W. N., Howland, J. G., & Winship, I. R. (2018). Impaired Cognitive Function after Perineuronal Net Degradation in the Medial Prefrontal Cortex. *ENeuro*, *5*(6). https://doi.org/10.1523/ENEURO.0253-18.2018

Pepper, E. J., Pathmanathan, S., McIlrae, S., Rehman, F., & Cardno, A. G. (2018). Associations between risk factors for schizophrenia and concordance in four monozygotic twin samples. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *177*(5), 503–510. https://doi.org/10.1002/ajmg.b.32640

Picchioni, M. M., & Murray, R. M. (2007). Schizophrenia. *BMJ : British Medical Journal*, *335*(7610), 91–95. https://doi.org/10.1136/bmj.39227.616447.BE

Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J. W., & Maffei, L. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. *Science (New York, N.Y.)*, *298*(5596), 1248–1251. https://doi.org/10.1126/science.1072699

Pletnikov, M. V., Ayhan, Y., Nikolskaia, O., Xu, Y., Ovanesov, M. V., Huang, H., … Ross, C. A. (2008). Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia. *Molecular Psychiatry*, *13*(2), 173–186. https://doi.org/10.1038/sj.mp.4002079

Pollock, E., Everest, M., Brown, A., & Poulter, M. O. (2014). Metalloproteinase inhibition prevents inhibitory synapse reorganization and seizure genesis.

Neurobiology of Disease, *70*, 21–31. https://doi.org/10.1016/j.nbd.2014.06.003

Rolls, E. T. (2015). Limbic systems for emotion and for memory, but no single limbic system. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *62*, 119–157. https://doi.org/10.1016/j.cortex.2013.12.005

Rosoklija, G., Toomayan, G., Ellis, S. P., Keilp, J., Mann, J. J., Latov, N., … Dwork, A. J. (2000). Structural abnormalities of subicular dendrites in subjects with schizophrenia and mood disorders: preliminary findings. *Archives of General Psychiatry*, *57*(4), 349–356.

Roth, T. L., Lubin, F. D., Sodhi, M., & Kleinman, J. E. (2009). Epigenetic mechanisms in schizophrenia. *Biochimica et Biophysica Acta*, *1790*(9), 869–877. https://doi.org/10.1016/j.bbagen.2009.06.009

Rozycki, M., Satterthwaite, T. D., Koutsouleris, N., Erus, G., Doshi, J., Wolf, D. H., … Davatzikos, C. (2018). Multisite Machine Learning Analysis Provides a Robust Structural Imaging Signature of Schizophrenia Detectable Across Diverse Patient Populations and Within Individuals. *Schizophrenia Bulletin*, *44*(5), 1035–1044. https://doi.org/10.1093/schbul/sbx137

Sabbagh, J. J., Murtishaw, A. S., Bolton, M. M., Heaney, C. F., Langhardt, M., & Kinney, J. W. (2013). Chronic ketamine produces altered distribution of

parvalbumin-positive cells in the hippocampus of adult rats. *Neuroscience Letters*, *550*, 69–74. https://doi.org/10.1016/j.neulet.2013.06.040

Saetre, P., Agartz, I., De Franciscis, A., Lundmark, P., Djurovic, S., Kähler, A., … Jönsson, E. G. (2008). Association between a disrupted-in-schizophrenia 1 (DISC1) single nucleotide polymorphism and schizophrenia in a combined Scandinavian case–control sample. *Schizophrenia Research*, *106*(2), 237–241. https://doi.org/10.1016/j.schres.2008.08.024

Sanders, A. R., Duan, J., Levinson, D. F., Shi, J., He, D., Hou, C., … Gejman, P. V. (2008). No Significant Association of 14 Candidate Genes With Schizophrenia in a Large European Ancestry Sample: Implications for Psychiatric Genetics. *American Journal of Psychiatry*, *165*(4), 497–506. https://doi.org/10.1176/appi.ajp.2007.07101573

Schumacher, J., Laje, G., Jamra, R. A., Becker, T., Mühleisen, T. W., Vasilescu, C., … Cichon, S. (2009). The DISC locus and schizophrenia: evidence from an association study in a central European sample and from a meta-analysis across different European populations. *Human Molecular Genetics*, *18*(14), 2719–2727. https://doi.org/10.1093/hmg/ddp204

Selemon, L. D. (2004). Increased Cortical Neuronal Density in Schizophrenia. *American Journal of Psychiatry*, *161*(9), 1564–1564. https://doi.org/10.1176/appi.ajp.161.9.1564

Selemon, L. D., Rajkowska, G., & Goldman-Rakic, P. S. (1998). Elevated neuronal density in prefrontal area 46 in brains from schizophrenic

patients: Application of a three-dimensional, stereologic counting method. *Journal of Comparative Neurology*, *392*(3), 402–412. https://doi.org/10.1002/(SICI)1096-9861(19980316)392:3<402::AID-CNE9>3.0.CO;2-5

Sharma, R. P., Grayson, D. R., & Gavin, D. P. (2008). Histone Deactylase 1 expression is increased in the prefrontal cortex of Schizophrenia subjects; analysis of the National Brain Databank microarray collection. *Schizophrenia Research*, *98*(1–3), 111–117. https://doi.org/10.1016/j.schres.2007.09.020

Steullet, P., Cabungcal, J.-H., Coyle, J., Didriksen, M., Gill, K., Grace, A. A., … Do, K. Q. (2017). Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. *Molecular Psychiatry*, *22*(7), 936–943. https://doi.org/10.1038/mp.2017.47

Steullet, Pascal, Cabungcal, J.-H., Cuénod, M., & Do, K. Q. (2014). Fast oscillatory activity in the anterior cingulate cortex: dopaminergic modulation and effect of perineuronal net loss. *Frontiers in Cellular Neuroscience*, *8*. https://doi.org/10.3389/fncel.2014.00244

Supprian, T., Heils, A., Hofmann, E., Warmuth-Metz, M., Stöber, G., & Franzek, E. (1999). [Agenesis septum pellucidum in a schizophrenic patient]. *Der Nervenarzt*, *70*(3), 269–275.

Supprian, T., Sian, J., Heils, A., Hofmann, E., Warmuth-Metz, M., & Solymosi, L. (1999). Isolated absence of the septum pellucidum. *Neuroradiology*, *41*(8), 563–566.

Suzuki, Y., Jodo, E., Takeuchi, S., Niwa, S., & Kayama, Y. (2002). Acute administration of phencyclidine induces tonic activation of medial prefrontal cortex neurons in freely moving rats. *Neuroscience*, *114*(3), 769–779.

Teroganova, N., Girshkin, L., Suter, C. M., & Green, M. J. (2016). DNA methylation in peripheral tissue of schizophrenia and bipolar disorder: a systematic review. *BMC Genetics*, *17*(1), 27. https://doi.org/10.1186/s12863-016-0332-2

Thompson, E. H., Lensjø, K. K., Wigestrand, M. B., Malthe-Sørenssen, A., Hafting, T., & Fyhn, M. (2018). Removal of perineuronal nets disrupts recall of a remote fear memory. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(3), 607–612. https://doi.org/10.1073/pnas.1713530115

Torrey, E. F., Barci, B. M., Webster, M. J., Bartko, J. J., Meador-Woodruff, J. H., & Knable, M. B. (2005). Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. *Biological Psychiatry*, *57*(3), 252–260. https://doi.org/10.1016/j.biopsych.2004.10.019

Tripathi, A., Kar, S. K., & Shukla, R. (2018). Cognitive Deficits in Schizophrenia: Understanding the Biological Correlates and Remediation Strategies. *Clinical Psychopharmacology and Neuroscience*, *16*(1), 7–17. https://doi.org/10.9758/cpn.2018.16.1.7

Tropea, D., Hardingham, N., Millar, K., & Fox, K. (2018). Mechanisms underlying the role of DISC1 in synaptic plasticity. *The Journal of Physiology*, *596*(14), 2747–2771. https://doi.org/10.1113/JP274330

Tsien, R. Y. (2013). Very long-term memories may be stored in the pattern of holes in the perineuronal net. *Proceedings of the National Academy of Sciences*, *110*(30), 12456–12461. https://doi.org/10.1073/pnas.1310158110

Umeda, K., Iritani, S., Fujishiro, H., Sekiguchi, H., Torii, Y., Habuchi, C., … Ozaki, N. (2016). Immunohistochemical evaluation of the GABAergic neuronal system in the prefrontal cortex of a DISC1 knockout mouse model of schizophrenia. *Synapse (New York, N.Y.)*, *70*(12), 508–518. https://doi.org/10.1002/syn.21924

Veling, W., Selten, J.-P., Susser, E., Laan, W., Mackenbach, J. P., & Hoek, H. W. (2007). Discrimination and the incidence of psychotic disorders among ethnic minorities in The Netherlands. *International Journal of Epidemiology*, *36*(4), 761–768. https://doi.org/10.1093/ije/dym085

Veres, J. M., Nagy, G. A., & Hájos, N. (2017). Perisomatic GABAergic synapses of basket cells effectively control principal neuron activity in amygdala networks. *ELife*, *6*. https://doi.org/10.7554/eLife.20721

Voineskos, D., Rogasch, N. C., Rajji, T. K., Fitzgerald, P. B., & Daskalakis, Z. J. (2013). A review of evidence linking disrupted neural plasticity to schizophrenia. *Canadian Journal of Psychiatry. Revue Canadienne De Psychiatrie*, *58*(2), 86–92. https://doi.org/10.1177/070674371305800205

Volk, D. W., Austin, M. C., Pierri, J. N., Sampson, A. R., & Lewis, D. A. (2000). Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Archives of General Psychiatry*, *57*(3), 237–245.

Volman, V., Behrens, M. M., & Sejnowski, T. J. (2011). Downregulation of Parvalbumin at Cortical GABA Synapses Reduces Network Gamma Oscillatory Activity. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(49), 18137–18148. https://doi.org/10.1523/JNEUROSCI.3041-11.2011

Wen, T. H., Afroz, S., Reinhard, S. M., Palacios, A. R., Tapia, K., Binder, D. K., … Ethell, I. M. (2018). Genetic Reduction of Matrix Metalloproteinase-9 Promotes Formation of Perineuronal Nets Around Parvalbumin-Expressing Interneurons and Normalizes Auditory Cortex Responses in

Developing Fmr1 Knock-Out Mice. *Cerebral Cortex (New York, N.Y.: 1991)*, *28*(11), 3951–3964. https://doi.org/10.1093/cercor/bhx258

Wen, T. H., Binder, D. K., Ethell, I. M., & Razak, K. A. (2018). The Perineuronal "Safety" Net? Perineuronal Net Abnormalities in Neurological Disorders. *Frontiers in Molecular Neuroscience*, *11*, 270. https://doi.org/10.3389/fnmol.2018.00270

Wender, P. H., Rosenthal, D., Kety, S. S., Schulsinger, F., & Welner, J. (1974). Crossfostering: A Research Strategy for Clarifying the Role of Genetic and Experiential Factors in the Etiology of Schizophrenia. *Archives of General Psychiatry*, *30*(1), 121–128. https://doi.org/10.1001/archpsyc.1974.01760070097016

Williams, S., & Boksa, P. (2010). Gamma oscillations and schizophrenia. *Journal of Psychiatry & Neuroscience : JPN*, *35*(2), 75–77. https://doi.org/10.1503/jpn.100021

Wobrock, T., Schneider, M., Kadovic, D., Schneider-Axmann, T., Ecker, U. K. H., Retz, W., … Falkai, P. (2008). Reduced cortical inhibition in first-episode schizophrenia. *Schizophrenia Research*, *105*(1–3), 252–261. https://doi.org/10.1016/j.schres.2008.06.001

Woloszynowska-Fraser, M. U., Wulff, P., & Riedel, G. (2017). Parvalbumin-containing GABA cells and schizophrenia: experimental model based on targeted gene delivery through adeno-associated viruses. *Behavioural*

Pharmacology, *28*(8), 630–641. https://doi.org/10.1097/FBP.0000000000000360

Woo, T.-U. W. (2014). Neurobiology of Schizophrenia Onset. *Current Topics in Behavioral Neurosciences*, *16*, 267–295. https://doi.org/10.1007/7854_2013_243

Woo, T.-U. W., Kim, A. M., & Viscidi, E. (2008). Disease-specific alterations in glutamatergic neurotransmission on inhibitory interneurons in the prefrontal cortex in schizophrenia. *Brain Research*, *1218*, 267–277. https://doi.org/10.1016/j.brainres.2008.03.092

Wu, Q., Tang, W., Luo, Z., Li, Y., Shu, Y., Yue, Z., … Feng, L. (2017). DISC1 Regulates the Proliferation and Migration of Mouse Neural Stem/Progenitor Cells through Pax5, Sox2, Dll1 and Neurog2. *Frontiers in Cellular Neuroscience*, *11*. https://doi.org/10.3389/fncel.2017.00261

Yamada, J., Ohgomori, T., & Jinno, S. (2015). Perineuronal nets affect parvalbumin expression in GABAergic neurons of the mouse hippocampus. *The European Journal of Neuroscience*, *41*(3), 368–378. https://doi.org/10.1111/ejn.12792

Yao, J. K., Leonard, S., & Reddy, R. (2006). Altered glutathione redox state in schizophrenia. *Disease Markers*, *22*(1–2), 83–93.

Ye, F., Kang, E., Yu, C., Qian, X., Jacob, F., Yu, C., … Zhang, M. (2017). DISC1 regulates neurogenesis via modulating kinetochore attachment of

Ndel1/Nde1 during mitosis. *Neuron*, *96*(5), 1041-1054.e5. https://doi.org/10.1016/j.neuron.2017.10.010

Yuste, R., & Bonhoeffer, T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annual Review of Neuroscience*, *24*, 1071–1089. https://doi.org/10.1146/annurev.neuro.24.1.1071

Zhang, Z. J., & Reynolds, G. P. (2002). A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophrenia Research*, *55*(1), 1–10. https://doi.org/10.1016/S0920-9964(01)00188-8

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