STATE-DEPENDENT DOPAMINE SYSTEM REGULATION USING CURRENT AND NOVEL ANTIPSYCHOTIC DRUG MECHANISMS: DEVELOPMENTAL IMPLICATIONS IN A SCHIZOPHRENIA MODEL

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Current antipsychotic drugs act on dopamine (DA) D_2 receptors for their therapeutic effects, but their limitations have driven a search for novel treatments. Pharmaceutical research is generally performed in normal rats, whereas models that account for variables including disease-relevant pathophysiology may improve predictive validity. D_2 antagonists have been shown to reduce DA neuron activity via D_2 autoreceptors to produce over-excitation induced cessation of cell firing (depolarization block). Aripiprazole is a D_2 partial agonist shown to normalize hypodopaminergic and hyperdopaminergic states, but through unclear mechanisms. The methylazoxymethanol acetate (MAM) model was used to observe aripiprazole's effects on hyperdopaminergic activity, compared to control (SAL) rats, using *in vivo*, anesthetized, electrophysiological recordings. Aripiprazole had no effect in controls, but reduced hyperdopaminergic activity in MAM rats, which was not reversed by apomorphine, suggesting a mechanism other than depolarization block. Furthermore, aripiprazole removed D_2 antagonist-induced depolarization block in MAM rats, consistent with autoreceptor agonism, potentially explaining its downregulation of hyperdopaminenrgic activity. These results demonstrate state-dependent neuropharmacological effects. Group II metabotropic glutamate receptors (mGluR2/3) showed promise as a novel target in preclinical research, but the mGluR2/3 agonist, pomaglumetad methionil (POM), showed insufficient efficacy in clinical trials. Although previous studies have shown that mGluR2/3 agonists have no effect on DA in normal rats, MAM rats were used to

determine whether POM normalizes a hyperdopaminergic state. POM dose-dependently reduced DA neuron activity of MAM rats, not observed in SAL rats. Intra-ventral hippocampal (vHPC) infusion of POM was sufficient to reduce dopaminergic activity in MAM rats. POM also increased novel object recognition in MAM rats and blocked stress-induced increases in dopaminergic activity in normal rats. To examine developmental effects of POM on MAM, MAM and SAL rats were treated peripubertally and DA neuron activity and vHPC pyramidal neuron activity were recorded in early or late adulthood. POM-treated MAM rats demonstrated normalized DA neuron activity and vHPC pyramidal neuron activity at both timepoints. Thus, POM indirectly regulates DA neuron activity by reducing increased vHPC activity and can prevent DA system hyperactivity in adult MAM rats following peripubertal administration.

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LIST OF ABBREVIATIONS

APD Antipsychotic Drug

ARI Aripiprazole

BLA Basolateral Amygdala

BOLD Blood Oxygen Level Dependent

CBF Cerebral Blood Flow

CBV Cerebral Blood Volume

CHR Clinical High Risk

DUP Duration Untreated Psychosis

EPS Extrapyramidal Side Effects

FEP First Episode Psychosis

FGA First Generation Antipsychotic

HPA Hypothalamic-Adrenal-Pituitary

HPC Hippocampus

LY34 LY341495

MAM Methylazoxymethanol Acetate

MRI Magnetic Resonance Imaging

NAc Nucleus Accumbens

NVHL Neonatal Ventral Hippocampal Lesion

PAM Positive Allosteric Modulator

PET Positron Emission Tomography

PFC Prefrontal Cortex

PNN Perineuronal Net

POM Pomaglumetad Methionil

PV Parvalbumin

RDoC Research Domain Criteria

SAL Saline

SGA Second Generation Antipsychotic

SPECT Single Photon Emission Computed Tomography

VEH Vehicle

vCA1 Ventral CA1

vHPC Ventral Hippocampus

vSub Ventral Subiculum

VTA Ventral Tegmental Area

PREFACE

This work is dedicated to my brother, Matt, with all of my love.

"I have labored carefully, not to mock, lament, or execrate,
but to understand human actions."

-Benedict de Spinoza, Political Treatise, 1677

"The motive of blind despair can never reasonably have place in the sciences;
since, however unsuccessful former attempts may have proved, there is still room to hope,
that the industry, good fortune, or improved sagacity of succeeding generations
may reach discoveries unknown to former ages."

-David Hume, An Enquiry Concerning Human Understanding, 1748

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1.0 GENERAL INTRODUCTION

Portions of Chapter 1 are adapted from:

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1.1 PATHOPHYSIOLOGY OF SCHIZOPHRENIA

1.1.1 Schizophrenia Within a Systems Neuroscience Perspective of Psychiatric Disorders

Neurons do not generate cognition and behavior in isolation, but through dynamic connections between populations of cells that form complex networks across the nervous system. The formation, elimination, and modulation of these connections can result in long-term changes in function across development and in adaptation to external stimuli. When components of neural communication are dysfunctional, whether from genetic predisposition, environmental insults, or a combination of the two, the resulting maladaptive patterns of behavior may progress to what is referred to as psychiatric disorders (DeFelipe, 2006; Morrison and Murray, 2018).

The schizophrenia spectrum of psychiatric disorders is most characteristically defined by psychotic symptoms, which include hallucinations and delusions. Diagnosis is based on a constellation of factors that also include negative symptoms (such as flattened affect and avolition) and cognitive impairment (Tandon et al., 2013). The psychotic features of schizophrenia typically emerge in late adolescence and early adulthood, indicative of neurodevelopmental influence (Fatemi and Folsom, 2009). The onset of psychosis may be abrupt or insidious, though the majority

of patients undergo a prodromal stage involving the development of sub-threshold symptoms, including attenuated psychotic symptoms, and deterioration in social and cognitive functioning (Larson et al., 2010). Patients diagnosed with schizophrenia face profound functional consequences. A minority of patients experience sustained symptom remittance with treatment and fully recover functional capability following the first episode of psychosis (FEP), but the majority of patients remain chronically ill with exacerbations and remissions of symptoms over time (Hegarty et al., 1994; Harrow et al., 2014; Zipursky et al., 2014).

Patients may demonstrate markedly different symptom profiles and treatment response. Due to issues including heterogeneity and overlap with other disorders, schizophrenia fits the concept of a clinical syndrome better than a unified disease entity (Carpenter Jr, 2007). Though treatments exist, they are not precise and often leave a high disease burden, particularly in the absence of contextual factors, such as a supportive environment and access to resources (Eack and Newhill, 2007; Kennedy et al., 2014). In an effort to accelerate progress in psychiatric research, the National Institute of Mental Health developed the Research Domain Criteria (RDoC). The RDoC initiative aims to produce a more neurobiologically-based framework for psychiatric disorders, compared to their discrete categorization in the Diagnostic and Statistical Manual of Mental Disorders (DSM). RDoC-based research focuses on more "fundamental components" that are thought to be relevant across disorders (e.g. anxiety, social behavior, habit formation) and that can be linked to biological and behavioral measures. Similarly, the development of new treatments within the RDoC framework focuses on the identification of homogeneous subgroups of patients who exhibit a particular symptom rather than a disease-targeted panacea (Insel et al., 2010). This dissertation will focus on the dopamine (DA) system in relationship to the development and

treatment of psychotic symptoms, in the context of schizophrenia, but with potential relevance to related psychotic disorders.

1.1.2 Dopamine Dysfunction in Schizophrenia

The DA hypothesis of schizophrenia postulates that hyperactivity of the DA system drives psychotic symptoms (Van Rossum, 1967). Patients with schizophrenia consistently demonstrate measures of increased presynaptic DA function compared to healthy controls (Howes et al., 2012). There is also evidence for elevated DA activity in psychotic bipolar disorder (Jauhar et al., 2017), suggesting that DA neuron dysfunction may be a final common pathway of transdiagnostic psychotic symptoms.

Pivotal studies that have provided support for the DA hypothesis have used positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging to examine radioligand displacement from DA receptors as a measure of DA activity. Patients with schizophrenia show increased DA release in response to amphetamine, compared to healthy controls (Laruelle et al., 1996; Abi-Dargham et al., 1998; Laruelle et al., 1999), which correlates with transient worsening of psychotic symptoms (Laruelle et al., 1999). Patients also demonstrate increased baseline synaptic DA in the striatum, measured in a DA depletion paradigm (Abi-Dargham et al., 2000), which has been shown to correlate with their amphetamine-induced DA release (Abi-Dargham et al., 2009). Both measures are observed in antipsychotic drug (APD)-naive patients and drug-free patients with prior APD treatment, and both predict treatment response of psychosis to APDs (Abi-Dargham et al., 2000; Abi-Dargham et al., 2009; Demjaha et al., 2012). Elevated striatal DA synthesis capacity, measured by fluorodopa uptake into DA terminals, is also consistently observed in patients and shown to correlate with psychotic severity (Howes et al., 2012). Numerous studies have found increased response capacity of the DA system

in individuals at clinical high risk (CHR) for psychosis, which correlates with greater severity of prodromal symptoms (Howes et al., 2009; Howes et al., 2011a; Howes et al., 2011b; Egerton et al., 2013). Longitudinal studies have further shown that there is a progressive increase in striatal DA function as CHR patients transition to full syndrome expression (Howes et al., 2011a). It has also been shown to predict conversion to psychosis in most (Howes et al., 2009; Egerton et al., 2013), though not all (Howes et al., 2019) studies. The discrepancy may relate to factors including follow-up time and the influence of diagnostic criteria on determining transition rates, which can come down to small differences in severity and duration of symptoms (Howes et al., 2019). Elevated DA synthesis capacity is a less consistent finding in chronic patients in remission, shown to be significantly elevated compared to healthy controls in some studies (Reith et al., 1994; Meyer-Lindenberg et al., 2002; McGowan et al., 2004; Howes et al., 2013), though not all (Dao-Castellana et al., 1997; Elkashef et al., 2000; Shotbolt et al., 2011), suggesting that increased DA function most clearly signals active psychosis.

The observed elevation in DA is limited to subcortical (mesolimbic) projections of DA neurons to the striatum. PET studies have demonstrated that the rostral caudate (associative striatum) has the highest elevation of DA release and strongest correlation to psychotic symptoms in patients with schizophrenia (Kegeles et al., 2010; McCutcheon et al., 2017). In contrast, mesocortical projections display *decreased* DA release compared to healthy controls, which may contribute to impaired prefrontal-dependent cognitive processes. It is currently unknown what accounts for these coexisting differences in DA regulation, which have yet to be demonstrated in an animal model. Taken together, these findings provide strong support for the hypothesis that presynaptic DA dysfunction in subcortical projections, particularly to the associative striatum, is closely linked to the onset and expression of psychotic symptoms.

1.1.3 Hippocampal Dysfunction in Schizophrenia

Elevated DA system activity in schizophrenia results from dysfunction in a larger hippocampal-midbrain-striatal circuit, with a primary locus of pathophysiology that appears to develop in the hippocampus (HPC). Deficits in the structure and function of the HPC are consistently observed in imaging and post-mortem studies of schizophrenia patients (Tamminga et al., 2010). Patients exhibit reduced functional recruitment of the HPC during declarative memory tasks (Heckers et al., 1998; Weiss et al., 2003). Patients also demonstrate increased HPC activity at rest and during cognitive tasks involving processing of salient information, as measured by cerebral blood flow (CBF) (Medoff et al., 2001) and blood oxygen level dependent (BOLD) signal in functional magnetic resonance imaging (fMRI) (Holt et al., 2006; Tregellas et al., 2014). Most studies report increased HPC glutamate levels in both first-episode and chronic patients, independent of medication status (Poels et al., 2014b), and changes in HPC metabolism and blood flow are associated with more severe psychotic symptoms in patients and (Friston et al., 1992; Liddle et al., 1992; Gur et al., 1995) and those at CHR (Allen et al., 2015; Allen et al., 2017). Increased cerebral blood volume (CBV) has specifically been reported in the CA1 and subiculum of the HPC in patients with schizophrenia (Talati et al., 2014). Increased CBV is also present during the prodromal stage and predicts conversion to psychosis (Harrison, 2004; Schobel et al., 2009) and HPC atrophy (Schobel et al., 2013). Cross-sectional comparison of CHR subjects to healthy controls generally does not reveal differences in HPC volume between the two populations (Velakoulis et al., 2006; Wood et al., 2008). However, longitudinal studies have demonstrated that CHR patients that convert to psychosis show a greater progression of grey matter reduction of the anterior CA1 and subiculum compared to those who do not convert to psychosis (Pantelis et al., 2003; Borgwardt et al., 2007; Schobel et al., 2013). Reduced volume of the anterior HPC has also

been associated with illness duration (Velakoulis et al., 2006), although not all studies have found significant reduction over time (Lieberman et al., 2001) and chronic APD treatment or other external factors may contribute to progressive volume loss (Ho et al., 2011; Zipursky et al., 2012).

Multiple lines of evidence have suggested that the increased HPC glutamate levels observed in schizophrenia is due to reduced parvalbumin (PV)+ GABA interneuron regulation of pyramidal neuron activity (Benes, 2000; Benes and Berretta, 2001). Loss of PV+ interneurons may at least in part be due to the vulnerability of their surrounding perineurnal nets (PNNs). PNNs are extracellular matrix structures important for the onset and closure of critical periods for developmental plasticity (Hensch, 2005). Their formation can be disrupted by oxidative stress, such that the PV+ interneurons fail to provide normal inhibitory input to the target pyramidal neurons. Antioxidant agents have been shown to protect PV+ interneurons from the damage caused by oxidative stress and normalize GABAergic inhibition (Cabungcal et al., 2013). Excitotoxicity from sustained oxidative stress and disinhibited pyramidal neuron activity (Schobel et al., 2009; Konradi et al., 2011; Schobel et al., 2013; Heckers and Konradi, 2015), may explain the correlation between increased regional glutamate levels and corresponding reduction in volume found in neuroimaging studies (Benes, 2000; Kraguljac et al., 2013; Do et al., 2015).

1.1.4 The Development of DA Dysfunction via Hippocampal Dysfunction: Insights from Animal Models

The observed dysfunction of the HPC and mesolimbic DA system in patients with schizophrenia can be unified in a pathophysiological model in which altered neurodevelopment via genetic risk combined with environmental stress leads to the disruption of the excitatory/inhibitory balance in regions including the HPC that, in turn, leads to psychotic symptoms via increased DA neurotransmission to the striatum. Many of the details of this theory

have been derived from experiments in animal models of schizophrenia. An inherent challenge in the use of rodent models for schizophrenia research is the inability to reproduce all dimensions of a uniquely human condition. Current models are thus limited to a subset of phenotypes, with no effective means to directly measure subjective mental states (Arguello and Gogos, 2006; Powell and Miyakawa, 2006). Despite their limitations, animal models are necessary tools for generating predictions and confirming causal relationships about the development, pathophysiology, and treatment of psychiatric disorders.

Neurodevelopmental animal models are based on the hypothesis that deviations from normal maturation can produce long-term changes in the brain. The structural and functional abnormalities that result from early-life insults often only fully emerge in adulthood. This makes developmental models valuable for identifying processes through which a triggering event can progress into a pathological state and evaluating preventative interventions with potential use in the prodromal stage (Meyer and Feldon, 2010).

Developmental consequences are commonly observed from environmental manipulations that involve early-life stress, such as social isolation or maternal separation (Harlow et al., 1965; Meaney et al., 1996). Adversity during the juvenile and adolescent period can shape the maturation of circuits that underlie emotional function (Cohen et al., 2013) and the resulting hyperresponsivity to stress is a core component of many psychiatric disorders (Heim and Nemeroff, 2001; Lupien et al., 2009). A large body of work highlights the importance of stress as a risk factor in the development of schizophrenia (Corcoran et al., 2003; van Os et al., 2010; Holtzman et al., 2013; Gomes and Grace, 2017). The correlation between early life stress and severity of positive symptoms (Ruby et al., 2017) may partially be due to the interaction between stress, the HPC, and the DA system (Belujon and Grace, 2015; Howes et al., 2017). Both CHR individuals and

schizophrenia patients demonstrate elevated DA release in response to stress compared to healthy controls (Pruessner et al., 2004; Mizrahi et al., 2012). Furthermore, peripubertal stress exposure in rats has been shown to increase DA neuron activity in adulthood, suggesting that stress before or during puberty is particularly impactful to the responsivity of the DA system (Gomes and Grace, 2016).

The HPC also shows marked vulnerability to stress across many psychiatric conditions. It contains high expression of glucocorticoid receptors to respond to activation of the hypothalamic-adrenal-pituitary (HPA) axis. While an elevation in glucocorticoids is essential to respond to perceived threat, chronic elevation can result in impaired function and atrophy (Sapolsky et al., 1985; Sapolsky et al., 1990). Heightened stress responsivity, such as from insufficient prefrontal inhibition of activity in the amygdala (Rosenkranz and Grace, 2001, 2002; Gomes et al., 2019b), may contribute to HPC dysfunction and the emergent hyperdopaminergic state. It has thus been hypothesized that HPC dysfunction may contribute to the diathesis in prodromal patients that puts them at risk for developing psychosis in response to stress (Corcoran et al., 2003).

The neonatal ventral hippocampal lesion (NVHL) model was the first to test the hypothesis that altered development of the ventral hippocampus (vHPC), the rodent analog of the anterior HPC in humans (Heilbronner et al., 2016), plays a critical role in the pathophysiology of schizophrenia. The model showed that a vHPC lesion in the early postnatal period results in the adult onset of behavioral abnormalities and DA dysfunction relevant to schizophrenia (Lipska et al., 1992; Lipska and Weinberger, 2000). Inspired by findings from the NVHL model and studies demonstrating the importance of immune activation during pregnancy as a risk factor for schizophrenia (Zuckerman et al., 2003; Canetta and Brown, 2012), another prominent neurodevelopmental model of schizophrenia was developed, the methylazoxymethanol acetate

(MAM) model (Johnston et al., 1981; Moore et al., 2006). The MAM model involves administration of the mitotoxin MAM to pregnant dams on gestational day 17, which correlates with the vulnerable timepoint of the 2nd trimester in humans to adverse events such as maternal infection (Brown, 2006). The offspring of MAM-treated dams ("MAM rats") develop region-specific disruption of neuronal maturation that results in adult phenotypes relevant to schizophrenia, in contrast to the offspring of dams that receive a saline injection, ("SAL rats") (Flagstad et al., 2004; Moore et al., 2006; Modinos et al., 2015). Despite their different approaches, both the NVHL model and the MAM model display enhanced responsivity to stress (Lipska et al., 1993; Zimmerman et al., 2013) in addition to similar structural and functional abnormalities in adulthood.

One of the characteristic phenotypes observed in MAM rats is a hyperdopaminergic system. MAM rats demonstrate an increased number of spontaneously active DA neurons in the VTA, referred to as population activity, consistent with the hyperactive presynaptic DA function observed in schizophrenia (Lodge and Grace, 2007). Spontaneously active DA neurons fire in an irregular pattern in their basal state. When exposed to a behaviorally salient signal, DA neurons respond by firing bursts of action potentials, resulting in phasic DA release. Only spontaneously active DA neurons can produce burst firing in response to glutamatergic afferents (Grace and Bunney, 1984; Legault and Wise, 1999; Floresco et al., 2003; Lodge and Grace, 2006b). Regulatory inputs to the VTA normally modulate DA neuron population activity to vary the phasic signal based on environmental context, such as situations that are threatening or rewarding (Floresco et al., 2003).

The MAM model has provided critical insights into how increased HPC drive leads to increased DA neuron activity. Silent DA neurons are held in a hyperpolarized state by the ventral

pallidum, which is controlled by a circuit originating with the vHPC. The subiculum of the HPC extends glutamatergic projections to the nucleus accumbens (NAc), which in turn inhibits the ventral pallidum. (Floresco et al., 2001; Lodge and Grace, 2006b). Similar to findings in schizophrenia patients (Benes and Berretta, 2001), MAM rats show loss of parvalbumin (PV+) interneurons in the vHPC (Lodge et al., 2009), resulting in a baseline hyperactive state from loss of inhibitory control of pyramidal cell activity (Lodge and Grace, 2007). Inhibition of GABAergic neurons in the ventral pallidum releases their inhibitory hold on DA neurons in the VTA (Floresco et al., 2003). This increases the population of spontaneously active DA neurons available for NMDA-mediated phasic burst firing in response to glutamatergic input from the pedunculopontine tegmentum (Floresco et al., 2001), which itself is enabled by the laterodorsal tegmentum (Lodge and Grace, 2006a). Activation of the vHPC in normal rats dramatically increases DA neuron population activity in the VTA, which is correlated with increased DA efflux in the associative areas of the ventral striatum and NAc, whereas inactivation of vHPC in MAM rats can normalize the DA neuron activity and related aberrant behavior (Floresco et al., 2003; Lodge and Grace, 2007).

1.1.5 Dopamine Dysfunction within an Aberrant Salience Framework

The pathologically increased DA neuron population activity observed in MAM rats has been proposed to cause stimuli to produce a maximal DA response independent of context (Fig. 1-1 A-B; Lodge and Grace, 2011), consistent with the "aberrant salience" framework of psychosis (Kapur, 2003; Lisman et al., 2008). It has long been thought that that one of the functional roles of DA is to weight stimuli as attractive or aversive (Berridge and Robinson, 1998). Though traditionally studied for its role in reward prediction error, DA neurons also respond to novel or aversive stimuli in the absence of reward (Matsumoto and Hikosaka, 2009). Thus, it may be more

accurate to say that DA attributes *salience* to relevant stimuli (Kapur, 2003). It also has been suggested that DA projections in more ventral striatal regions produce a more value-related signal, whereas DA transmission in more dorsal striatal regions may carry a more salience-related signal (Lerner et al., 2015; Menegas et al., 2017)

Presentation of novel stimuli (Strange and Dolan, 2001; Fyhn et al., 2002; Jenkins et al., 2004) or exposure to stress (Linden et al., 2004; Valenti et al., 2011a) also produce a robust increase in HPC activity. Cortical areas send projections signaling sensory information to the entorhinal cortex, which acts as a gateway to the HPC via two inputs: the direct pathway (to the CA1) and the indirect pathway via the perforant path (dentate gyrus - CA3 - CA1) (Witter et al., 2000; Lisman and Grace, 2005a). It has been hypothesized that recurrent projections from the dentate gyrus to and from the CA3 are involved in memory recall to predict what is likely to happen next, based on stored memory sequences, which is then sent to the CA1 via Schaffer collaterals. The CA1 is thought to compare the predictions that arrive from CA3 (previously experienced sensory information) with direct pathway input (new sensory information) to determine novelty, that may then go on to influence, and be further weighted by, DA activity to drive behavior (Lisman, 1999; Lisman and Grace, 2005b).

When dysregulated, the abnormally heightened response to neutral stimuli may form the basis of delusions and hallucinations (Kapur, 2003). Furthermore, it has been suggested that dysregulated DA release in schizophrenia includes a reduction in adaptive phasic DA release in response to relevant stimuli (Maia and Frank, 2017). The reduction of relevant signal-to-noise may relate to reductions in functional connectivity of the striatum to cortical brain regions observed in fMRI studies (Fornito et al., 2013; Sarpal et al., 2015; Sarpal et al., 2017; Manivannan et al., 2019) and result in a general disintegration of sensory processing (McCutcheon et al.,

2019a). Alternatively, it has been suggested that hallucinations may represent an extreme form of perceptual bias that incorporate prior beliefs. Dysregulated DA may weight the perception with the bias towards a (false-positive) signal being present (Horga and Abi-Dargham, 2019).

There are current efforts toward trying to integrate current understandings of DA-based salience detection with the "salience network" (comprised of the insula and anterior cingulate), observed in resting state fMRI studies in humans (Seeley et al., 2007; Miyata, 2019). There is early evidence that DA synthesis capacity is related to salience network connectivity, suggesting a relationship between the different domains of salience processing that have been observed in human and rodent research (McCutcheon et al., 2019b). Future work may provide greater insight into how salience is processed across brain networks, its relationship to psychosis, and how best to treat abnormal salience processing.

It has been proposed that APDs create detachment from symptoms by dampening the salience generated from increased striatal DA release, but they do not necessarily remove the mental schemas that attempt to make sense of the unusual experiences (Kapur, 2003; Kapur et al., 2005a). The underlying cognitive processes likely involve complex connections between numerous brain regions that remain dysfunctional. Even within the DA system, current APDs reduce DA neuron hyperactivity, but they do not target or resolve the upstream HPC dysfunction.

1.2 TREATMENTS FOR PSYCHOSIS IN SCHIZOPHRENIA

1.2.1 Current Treatments for Schizophrenia

The success of the first APD chlorpromazine in the 1950s was a breakthrough in the current era of pharmacotherapy and represented a shift in the conceptualization of psychiatric symptoms

as biological constructs that can be targeted and treated (López-Muñoz et al., 2005). In the decade following their established efficacy, it was suggested that APDs may work by acting on DA receptors (Carlsson and Lindqvist, 1963; Van Rossum, 1967). This was later confirmed by the finding that all APDs block D_2 receptors, which is strongly linked to their antipsychotic effect (Seeman and Lee, 1975; Creese et al., 1976). Action at D_2 receptors has since been proposed to be both necessary and sufficient for the therapeutic effect of current APDs on positive symptoms (Kapur and Remington, 2001).

Much of the research on APDs has focused on their mechanisms of action to understand how they achieve their therapeutic effect. APDs are traditionally categorized as either first-generation antipsychotics (FGAs), such as haloperidol, or second-generation antipsychotics (SGAs), such as clozapine, which act on a larger spectrum of receptor types, including serotonergic, cholinergic, and adrenergic receptors (Miyamoto et al., 2005). Due to the high level of heterogeneity within these broad designations, some argue that the current nomenclature is uninformative and should be replaced with a neuropharmacological-based naming system (Zohar et al., 2015). Like many psychoactive drugs, APDs have numerous physiological effects due to their rich pharmacology, although the antipsychotic effect of both FGAs and SGAs remains dependent on the D_2 receptor and are given at doses that match D_2 occupancy (Kapur and Remington, 2001).

Most current APDs act as D_2 receptor antagonists (Seeman, 1987), but to understand how FGAs and SGAs regulate the DA system, it is necessary to look beyond the receptor at downstream consequences on DA neuron activity. D_2 antagonists block both postsynaptic and presynaptic D_2 receptors (autoreceptors). Acute administration of D_2 antagonists in anesthetized rats increases DA neuron firing rate and bursting activity, primarily through blockade of somatodendritic D_2

autoreceptors that normally provide negative feedback for the neuron (Grace and Bunney, 1986; Pucak and Grace, 1994). However, with repeated treatment there is a substantially depolarized membrane potential that culminates in inactivation such that the DA neuron is no longer able to generate action potentials. Inactivation can be reversed by compounds that inhibit DA neurons, such as DA agonists, whereas excitatory compounds are no longer capable of stimulating activity (White and Wang, 1983; Grace and Bunney, 1986). This phenomenon, referred to as depolarization block, is proposed to underlie the regulatory action of D_2 antagonists on DA neuron hyperactivity (Grace et al., 1997; Arnt and Skarsfeldt, 1998).

FGAs and SGAs have similar efficacy on positive symptoms and they are primarily distinguished in clinical use by differing side effect profiles (Leucht et al., 2013). One hypothesis is that differences in specificity of depolarization block between FGAs and SGAs may explain their comparable efficacy and difference in propensity to cause extrapyramidal side effects (EPS; Correll and Schenk, 2008). Acute administration of FGAs activates DA neurons in both the VTA and substantia nigra, whereas SGAs only activate DA neurons in the VTA (Bunney and Grace, 1978; Chiodo and Bunney, 1985; Goldstein et al., 1993; Stockton and Rasmussen, 1996). Accordingly, FGAs produce depolarization block in both the VTA and SNc, whereas SGAs selectively produce depolarization block in the VTA (Fig. 1-2 A-B; Chiodo and Bunney, 1983; White and Wang, 1983; Grace and Bunney, 1986; Lane and Blaha, 1987; Goldstein et al., 1993). Dorsal regions of the striatum that receive DA projections from the SNc in humans are implicated in psychosis (Kegeles et al., 2010; Egerton et al., 2013), though this may relate to depolarization block of lateral regions of the VTA in rodents (related to the dorsomedial substantia nigra in humans). It has been suggested that differing pharmacokinetics may also contribute to their side effect profiles: SGAs typically dissociate from the D_2 receptor more readily than FGAs, which

may make them more accommodating to physiological DA transmission (Kapur and Seeman, 2001). In support of this hypothesis, quetiapine and clozapine, the SGAs with the fastest dissociation rates, show no greater risk of EPS than placebo across their dosage range, whereas risperidone and olanzapine, SGAs with slower dissociation rates from the D_2 receptor, are more likely to cause EPS at high doses (Kapur and Seeman, 2001).

The onset of depolarization block may relate to the range of latencies in clinical improvement of psychosis, which can occur as fast as 24 hours following the initial dose of an APD, with full effect after several weeks of treatment (Agid et al., 2003; Kapur et al., 2005b; Leucht et al., 2005). In normal rats, 21 days of repeated administration of FGAs and SGAs is necessary to reduce the number of spontaneously active DA neurons (Chiodo and Bunney, 1983; Grace and Bunney, 1986), whereas MAM rats undergo rapid onset of depolarization block following acute administration (Valenti et al., 2011b). Enhanced DA system excitability in MAM rats likely underlies the rapid onset of depolarization block, though it is unclear what enables this rapid response.

D_2 partial agonists, such as aripiprazole (ARI), activate the receptor to a lower degree than endogenous DA and are sometimes referred to as third generation APDs (Fig 1-2 C). They are thought to stabilize DA neurotransmission by reducing excessive striatal D_2 stimulation and restoring deficient D_2 stimulation in regions including the prefrontal cortex (PFC; Grace, 1991; Burris et al., 2002). In contrast to D_2 antagonists, which are associated with a therapeutic effect at striatal D_2 receptor occupancy of 65-70% and increased risk of EPS at D_2 receptor occupancy exceeding 80% (Farde et al., 1992; Kapur et al., 2000), ARI has a therapeutic effect with 85-95% striatal D_2 receptor occupation, yet does not produce EPS (Yokoi et al., 2002; Mamo et al., 2007). Furthermore, ARI has been shown to not produce D_2 receptor upregulation or behavioral DA

supersensitivity in rats (Inoue et al., 1997; Tadokoro et al., 2011), and there is preliminary evidence that it may alleviate DA supersensitivity in humans (Tadokoro et al., 2017). D_2 receptor partial agonists may thus be less likely to induce adverse effects related to DA supersensitivity, as observed following chronic D_2 antagonist treatment (Silvestri et al., 2000; Chouinard et al., 2017). These differences between D_2 receptor antagonists and D_2 partial agonists may be related to their distinct methods of DA neuron regulation.

1.2.2 Limitations of Current Antipsychotic Drugs

APDs reduce psychotic symptoms in many patients (Leucht et al., 2009a). However, their therapeutic value is hindered by numerous side effects, limited efficacy across symptom domains, and failure to treat the primary cause of the disease, which can lead to a lifetime of maintenance treatment (Lieberman et al., 2005; Leucht et al., 2012).. These factors negatively impact quality of life and contribute to a high level of treatment nonadherence that typically results in relapse (Robinson et al., 2002; Lieberman et al., 2005). Current APDs are also unable to address heterogeneity, both between individuals with distinct pathologies (Demjaha et al., 2014; Howes and Kapur, 2014) and within an individual through different phases of the disease (Krystal and Anticevic, 2015).

Approximately 30% of patients with schizophrenia display treatment resistance with little or no response to standard medication (Conley and Buchanan, 1997) and a higher rate, up to 60%, only partially respond to treatment (Kane, 1989). In the event of inadequate response, clinicians often resort to the combination of multiple APDs or augmentation with mood stabilizers and SSRIs (Stahl and Grady, 2004; Faries et al., 2005; Fleischhacker and Uchida, 2014). Despite its prevalence as a clinical strategy, there is limited evidence that long-term APD polypharmacy

benefits patients (Stahl and Grady, 2004), and may instead be detrimental due to increased frequency of adverse effects (Correll et al., 2007; De Hert et al., 2012).

There are two overarching categories of treatment resistance. The first category includes patients who once responded to APDs but no longer do. This type of resistance often following relapse (Takeuchi et al., 2019) and may be related to DA supersensitivity following an upregulation in D_2 receptors in response to chronic D_2 antagonist administration (Emsley et al., 2013). There are also patients who never responded to APDs whose psychotic symptoms have been proposed to not involve DA dysregulation (Howes and Kapur, 2014). Studies have shown that schizophrenia patients with low striatal DA synthesis capacity are more likely to be treatment non-responders (Demjaha et al., 2012; Demjaha et al., 2014). This subset of patients may still have a dysfunction involving the HPC-striatal-midbrain circuit, but given that a more active DA system responds faster to D_2 blockade, the low responsivity of their DA system may not be a system conducive to depolarization block or other means of DA neuron downregulation (Grace and Gomes, 2018). However, these patients still show signs of glutamatergic dysfunction, including higher glutamate levels in the anterior cingulate compared to treatment responders (Demjaha et al., 2014; Mouchlianitis et al., 2015), suggesting that targeting the glutamate may alleviate symptoms for both treatment responders and non-responders.

Current APDs exclusively target the DA system, necessitating further development of novel target options for treatment resistant patients. Among both treatment responders and nonresponders, there remain unmet needs in tolerability and safety of current APDs. In recent years, concern has been raised about the risk-to-benefit ratio of maintenance APD treatment, due to issues including DA supersensitivity and potential deleterious effects on brain structure and functioning (Murray et al., 2016; Chouinard et al., 2017). Treating patients with the minimum

necessary dose and using D_2 partial agonists may minimize side effects and the risks associated with long term APD treatment, but targeting upstream brain regions may circumvent the issues associated with D_2-targeting drugs and address additional symptoms.

1.2.3 Novel Antipsychotic Mechanisms

A key goal of APD research is the development of personalized medication, which requires reliable biomarkers to determine which treatment is most suitable for a patient's individual needs. A massive investment has gone into the development of novel target compounds with the hope that they will be more effective in treating a wider range of symptoms in schizophrenia.

One of the most promising novel target candidates was group II metabotropic glutamate receptors (mGluRs), comprised of mGluR2 and mGluR3 (mGluR2/3). Unlike glutamate's rapid excitatory action via ionotropic glutamate receptors, mGluRs modulate glutamate transmission, with the additional advantage of distinct expression patterns (Conn and Pin, 1997). The mGluR2/3s are prominently expressed in limbic brain regions, including the PFC, HPC, and amygdala (Nicoletti et al., 2011). They are primarily localized presynaptically as autoreceptors on glutamatergic terminals to negatively regulate glutamate release (Nicoletti et al., 2011).

Following extensive preclinical support for mGluR2/3 as an APD target (Moghaddam and Adams, 1998; Cartmell et al., 1999, 2000; Lorrain et al., 2003; Greenslade and Mitchell, 2004; Homayoun et al., 2005; Pehrson and Moghaddam, 2010), an mGluR2/3 agonist from Eli Lilly, pomaglumetad methionil (POM), was advanced to clinical trials. It showed promise in preclinical research (Rorick-Kehn et al., 2007; Mezler et al., 2010). It demonstrated potential as a monotherapy in its first double-blind, randomized, placebo-controlled phase 2 clinical trial, and it was found to be well tolerated with no propensity to cause weight gain or EPS (Patil et al., 2007). This success was followed by a long line of disappointments in subsequent phase 2 trials as a

monotherapy or adjunct therapy (Adams et al., 2013; Stauffer et al., 2013; Downing et al., 2014) and failure to meet the primary endpoint as a monotherapy in a large phase 3 trial (Adams et al., 2014; Marek, 2015).

Later analyses of the clinical trials found that certain populations responded better to POM (Kinon et al., 2015), indicating that important questions remained about why those groups responded better and whether glutamatergic-targeting therapies may be best suited for certain populations. For example, early-in-disease patients responded better to POM treatment than chronic patients (Kinon et al., 2015), which could potentially be due to factors including treatment history and disease progression. Additionally, a study in mice showed that SGA administration can downregulate mGluR2 expression, thought to be due to action on 5-HT2A receptors, which may have contributed to the failure (Kurita et al., 2012). There were also efforts to develop mGluR2 positive allosteric modulators (PAMs) that would induce a leftward shift in the dose-response curve for glutamate without directly acting at the glutamate site (Conn et al., 2009; Fraley, 2009; Fell et al., 2012). They similarly showed promising results in APD screening assays (Lavreysen et al., 2013), though a compound tested by AstraZeneca once again did not show efficacy in patients with schizophrenia as a monotherapy (Litman et al., 2016). Despite the failure of the clinical trials, they are suggestive that there may be sub-populations of patients who may benefit most from novel drugs.

1.2.4 Stage Specific Treatments

The neurobiology of schizophrenia evolves over the course of the illness from the prodromal stage, where symptoms and cognitive impairment are present in attenuated form, to the first episode, with full symptom expression, to chronic illness, where symptoms persist at fluctuating levels. Longer duration of untreated psychosis (DUP) is associated with worse

treatment responses and outcomes (Perkins et al., 2005), suggesting that the timing of effective treatment in FEP is a critical factor in prognosis (Lieberman et al., 2019).

No treatment has yet been conclusively proven effective for prevention of transition to psychosis. Prodromal interventions such as the use of SGAs (McGlashan et al., 2006), antidepressants (Cornblatt et al., 2007), and antioxidants, such as omega-3 fatty acids (Amminger and McGorry, 2012) have produced mixed success in reducing transition rates (McGorry et al., 2008; Larson et al., 2010), though several meta-analyses have suggested that preventative treatments may increase time to transition and decrease the rate of conversion (Stafford et al., 2013; van der Gaag et al., 2013).

Consideration of the development of the circuits involved is necessary to enable effective stage-specific treatments. As previously described, there is growing evidence that HPC dysfunction is present at the prodromal stage. Stress, particularly in those who are predisposed to stress vulnerability, may strike at PV+ interneurons prior to the first episode. Protecting PV+ neurons as early as possible in the prodromal period may provide long lasting benefits in alleviating or preventing psychotic symptoms (Gomes et al., 2019b). Targeting excitatory/inhibitory dysfunction in the HPC is a possible intervention strategy in the early stages of psychosis, under the hypothesis that early intervention results in a better long-term outcome.

1.3 PURPOSE OF STUDIES

1.3.1 Rationale

A similar progression of HPC dysfunction, including PV+ interneuron loss, increased glutamatergic drive and atrophy is observed in both patients with schizophrenia and the MAM

model (Moore et al., 2006; Heckers and Konradi, 2010; Modinos et al., 2015). The MAM model has been used to elucidate the role of the HPC in a circuit that leads to increased DA neuron population activity in the VTA (Lodge and Grace, 2007). Compounds that target the vHPC by either increasing function of PV+ interneurons or reducing the activity of pyramidal neurons may be used to normalize HPC output. Several studies have found success with using strategies to target vHPC dysfunction to normalize DA neuron activity and behavior in MAM rats (Gill et al., 2011; Gastambide et al., 2012; Perez and Lodge, 2013; Perez et al., 2013). Targeting upstream dysfunction has the potential to be more effective in treating schizophrenia in patients who have already developed the disease, but an ideal approach would involve intervention prior to the transition to psychosis, which may lead to a better long-term outcome (Perkins et al., 2005; Grace and Gomes, 2018).

Studies in the MAM model have further demonstrated that the peripubertal period is a stress-sensitive window that can be targeted to prevent the development of MAM phenotypes in adulthood (Gomes and Grace, 2016). Previous work has shown that peripubertal administration of the benzodiazepine, diazepam, can prevent the increased anxiety-like behavior and basolateral amygdala hyperactivity, and normalize hyperdopaminergic activity normally present in adult MAM rats (Du and Grace, 2013, 2016a, b). Benzodiazepines are not a realistic prophylactic option clinically due to issues including dependence and tolerance (Longo and Johnson, 2000), but these studies demonstrate that decreasing stress or other means of reducing HPC activity during peripubertal period has the potential to circumvent the pathological processes that leads to DA system dysregulation (Grace and Gomes, 2018). They also highlight the potential for stage-specific pharmacotherapies involving optimal treatment strategies based on the neurobiological course of the disease (Krystal and Anticevic, 2015).

Glutamatergic regulation via mGluR2/3s is an unexplored option as an early intervention treatment for the long-term prevention of DA system hyperactivity. It has been reported that increased HPC activity, local reduction in PV + interneurons, and volume loss following repeated ketamine administration in adult mice can be prevented by coadministration with an mGluR2/3 agonist (Schobel et al., 2013). The potential therapeutic effect of mGluR2/3 agonists has previously been suggested to be DA-independent due absence of mGluR2/3 expression in the VTA (Neki et al., 1996) and their lack of effect on DA levels at baseline (Pehrson and Moghaddam, 2010). However, mGlu2/3 receptors are densely expressed in HPC, including the human anterior HPC (Blümcke et al., 1996) and corresponding vHPC (Neki et al., 1996). Furthermore, previous work has demonstrated APDs can produce different effects on DA neuron electrophysiology in MAM rats compared to SAL rats, such as rapid induction of depolarization block following D_2 antagonist administration (Valenti et al., 2011b). Therefore, it is possible that mGluR2/3 agonists may similarly demonstrate different effects between MAM and control rats. Exploration of DA system state-dependent differences of APD action may provide greater insight into preclinical testing of novel target mechanisms, such as mGluR2/3 agonists, and their potential for early intervention treatments.

1.3.2 Hypothesis and Research Objectives

In this dissertation, we hypothesize that both D_2 partial agonists and mGluR2/3 agonists regulate the DA system in a state-dependent manner and peripubertal mGluR2/3 agonist administration can prevent emergence of vHPC abnormalities in MAM rats that lead to a hyperdopaminergic state in adulthood. In Chapter 2, we investigate whether ARI can modulate DA neuron activity in a state-dependent manner in MAM and SAL rats and whether its reduction of DA neuron activity is dependent on depolarization block. In Chapter 3, we determine the role

of mGluR2/3 receptors in the regulation of DA neuron activity. Finally, in Chapter 4 we examine whether peripubertal treatment with an mGluR2/3 agonist can prevent the DA system hyperactivity observed in adult MAM rats. Taken together, our findings suggest that clinically-relevant models, such as the MAM model, can be used to determine how mGluR2/3 activation affects a system-wide disorder, and may more generally be used to observe the effects of APDs and potential preventative treatments for schizophrenia that may not be present in normal rodents or baseline conditions.

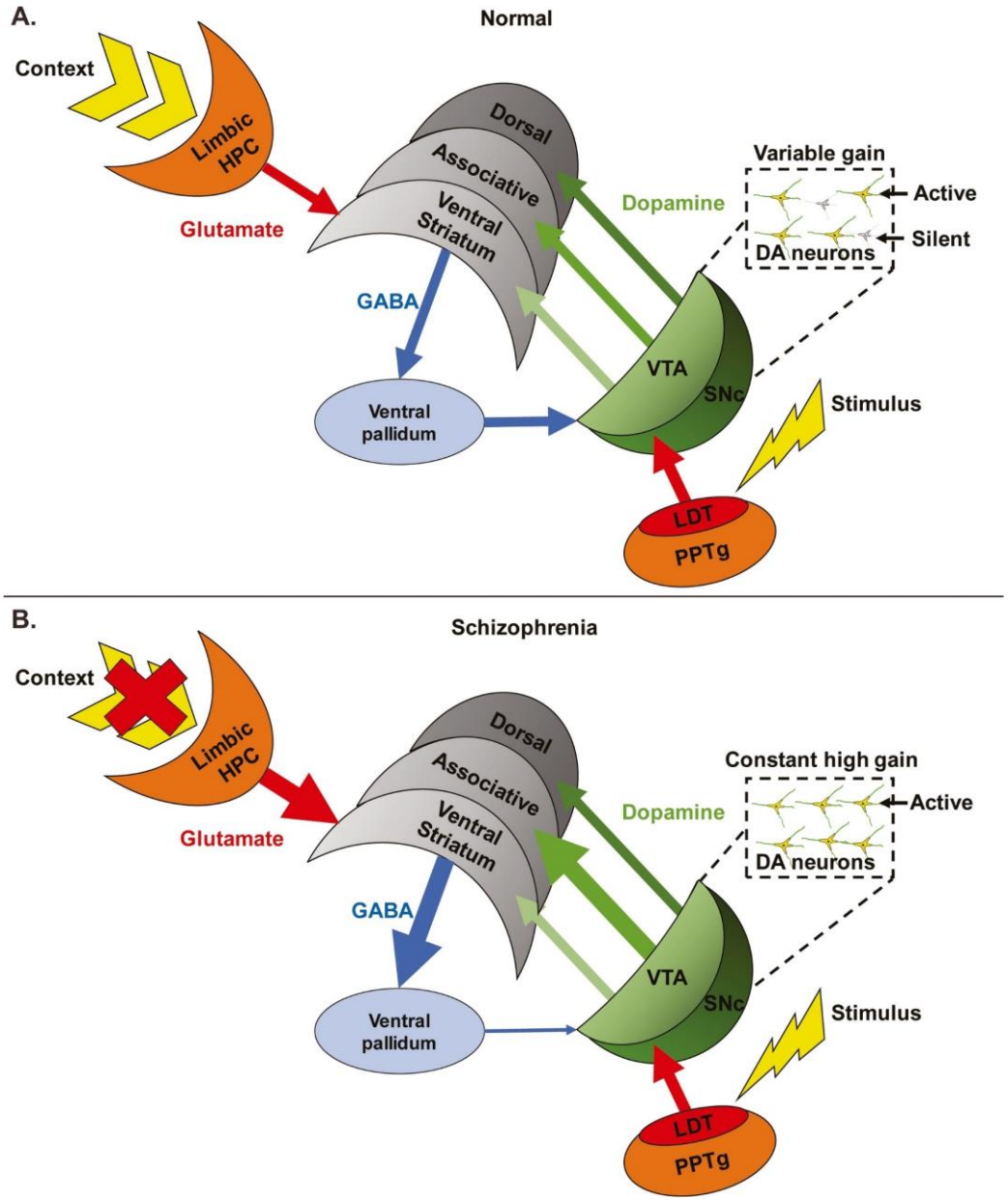

Figure 1-1: Hippocampal-striatal-dopamine dysregulation in schizophrenia

(A) In a normal system, the hippocampus (HPC), regulates dopamine (DA) neuron population activity in the ventral tegmental area (VTA), with variable gain based on context. The HPC sends glutamatergic input to the nucleus accumbens in the ventral striatum, which sends inhibitory projections to the ventral pallidum. The ventral pallidum holds a proportion of DA neurons in an inhibited state. In response to relevant stimuli, only spontaneously active DA neurons can respond to glutamatergic input from the pedunculopontine tegmentum (PPTg), gated by the laterodorsal tegmentum (LDT), with burst firing. The DA signal to the striatum has topographic organization with

DA neurons in the substantia nigra pars compacta (SNc) projections to the dorsal striatum, lateral VTA projections to the associative striatum, and medial VTA projections to the ventral striatum. **(B)** In schizophrenia, the limbic HPC is in a pathologically hyperactive state and no longer responds appropriately to context. Increased excitatory input from HPC to the ventral striatum results in less inhibition of the ventral pallidum and fewer DA neurons held in a silent state. The increased DA signal is most prevalent in the associative striatum.

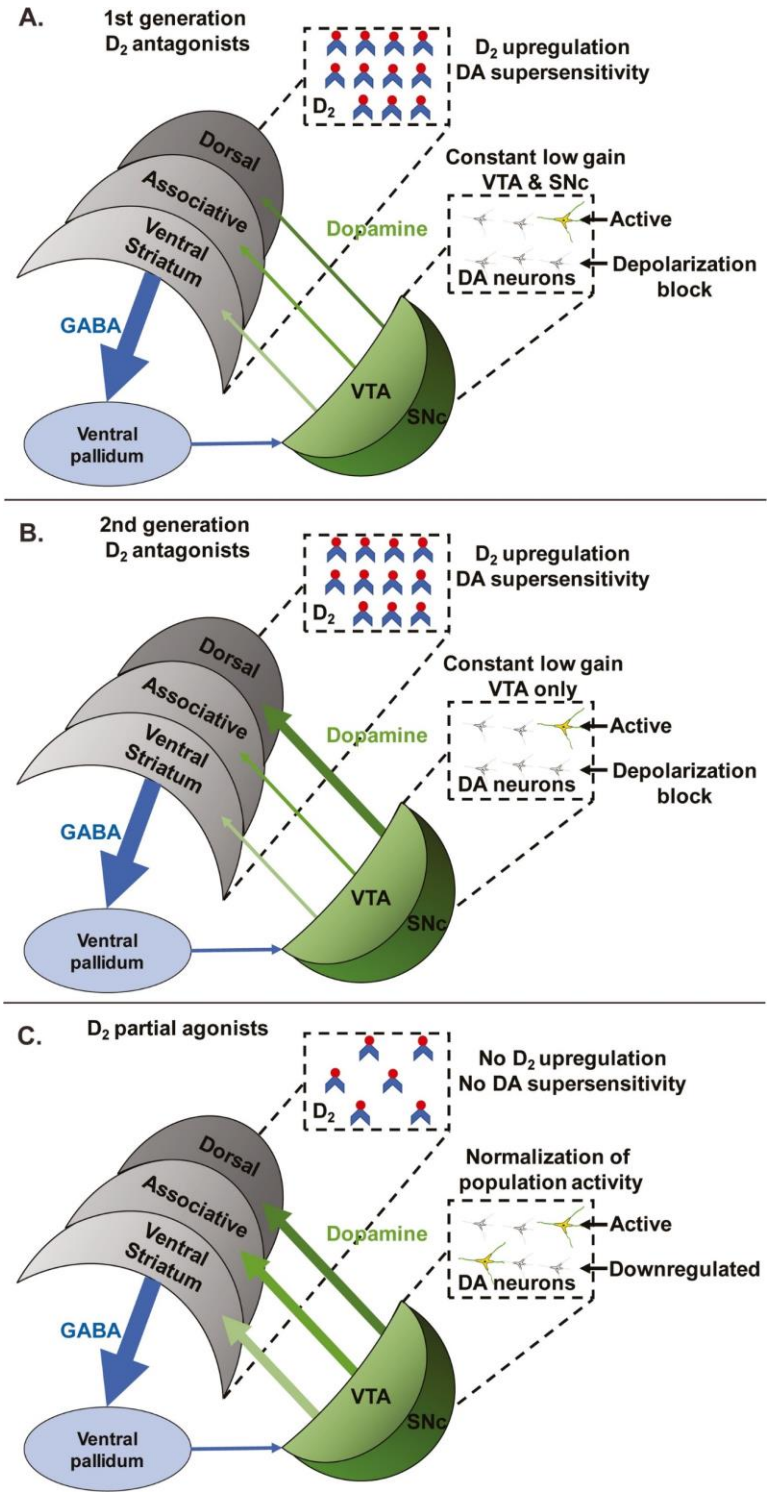

Figure 1-2: Antipsychotic drug modulation of DA neuron activity states

(A) First generation D_2 antagonist antipsychotic drugs (APDs) act on postsynaptic and presynaptic D_2 receptors in the striatum to produce depolarization block of DA neurons in the ventral tegmental area (VTA) and substantia nigra

pars compacta (SNc) and reduces DA neuron population activity in both regions. The DA signal is diminished to all domains of the striatum, resulting in both the antipsychotic effect and extrapyramidal side effects, including motor dysfunction. Following prolonged treatment, the system adapts by upregulating the number of D_2 receptors, which can produce DA supersensitivity. **(B)** Second generation D_2 antagonist APDs only produce depolarization block of DA neurons in the ventral tegmental area (VTA), resulting in a reduced DA signal to the associative and ventral striatum without effect on the DA neurons that project to the dorsal striatum. Thus, SGAs produce an antipsychotic effect without motor side effects. However, like first generation APDs, following prolonged treatment, there is an upregulation of D_2 receptors, which can produce DA supersensitivity. **(C)** D_2 partial agonists normalize DA neuron population activity by reducing hyperdopaminergic activity via DA neuron inhibition and increasing hypodopamineregic activity via postsynaptic stimulation. The downregulation of DA neuron activity does not occur through depolarization block and does not produce an upregulation of D_2 receptors.

2.0 STATE-DEPENDENT EFFECTS OF D2 PARTIAL AGONIST ARIPIPRAZOLE ON DOPAMINE NEURON ACTIVITY IN THE MAM MODEL OF SCHIZOPHRENIA

Chapter 2 is a modified version of:

Sonnenschein, S. F., Gill, K. M., & Grace, A. A. (2019). State-dependent effects of the D 2 partial agonist aripiprazole on dopamine neuron activity in the MAM neurodevelopmental model of schizophrenia. *Neuropsychopharmacology*, *44*(3), 572.

2.1 INTRODUCTION

The majority of current APDs are D_2 receptor antagonists (Seeman and Lee, 1975; Kapur et al., 2000). A mechanism proposed for the therapeutic action of D_2 receptor antagonists on DA dysregulation observed in schizophrenia (Laruelle and Abi-Dargham, 1999) is DA neuron depolarization block (Grace and Bunney, 1986; Grace et al., 1997). In depolarization block, following repeated treatment with D_2 receptor antagonists, a substantially depolarized membrane potential results in over-excitation-induced cessation of spiking (Grace and Bunney, 1986) primarily due to blockade of somatodendritic D_2 autoreceptors that provide local feedback inhibition of spike activity (Pucak and Grace, 1994, 1996). Depolarization block can be reversed by administration of a DA agonist, such as apomorphine. At low doses, apomorphine acts preferentially at D_2 autoreceptors to sufficiently inhibit DA neurons in depolarization block to restore spiking activity, consistent with an APD-induced overexcitation rather than inhibition of DA neuron firing (Bunney and Grace, 1978; Grace and Bunney, 1986). The net result of depolarization block is a reduced number of spontaneously active DA neurons (Bunney and Grace,

1978; Braszko et al., 1981; Chiodo and Bunney, 1983; Grace and Bunney, 1986) available for phasic activation (Floresco et al., 2003) and striatal DA release (Lane and Blaha, 1987).

Following prolonged D_2 antagonist treatment, a compensatory upregulation of D_2 receptors has been observed in humans and animal models that persists after APD discontinuation (O'Dell et al., 1990; See et al., 1990; Lidow and Goldman-Rakic, 1994; Silvestri et al., 2000; Tadokoro et al., 2011). The increase in D_2 receptors is associated with increased behavioral sensitivity to DA. It has been proposed that DA supersensitivity underlies the relapse of psychotic symptoms following discontinuation of treatment or dose reduction, which may include the appearance of new or more severe psychotic symptoms. (Chouinard and Jones, 1980; Chouinard and Chouinard, 2008). In animal models, increased striatal D_2 receptor density is associated with an increased locomotor response to amphetamine and DA agonists following withdrawal from repeated APD administration (Montanaro et al., 1982; Samaha et al., 2007; Samaha et al., 2008; Gill et al., 2014). Although numerous studies have associated the emergence of DA supersensitivity to increased D_2 receptor density, the nature of the relationship is not clearly understood. There have been examples of increased D_2 receptors during ongoing APD treatment without behavioral evidence of DA supersensitivity (Samaha et al., 2007; Samaha et al., 2008) and chronic APD treatment regimens that have not resulted in D_2 receptor upregulation (Wilmot and Szczepanik, 1989), suggesting a complex relationship potentially based on factors including dose and treatment regimen.

In contrast to the D_2 receptor antagonism observed with other APDs, ARI is a D_2 receptor partial agonist. ARI's unique mechanism of action demonstrates properties of a functional agonist or antagonist in animal models of DA hypoactivity and hyperactivity, respectively, thus providing stabilization of the DA system (Grace, 1991; Burris et al., 2002). In contrast to both first-generation APDs and other second-generation APDs, there is evidence that ARI may not induce upregulation

of D_2 receptors or DA supersensitivity (Inoue et al., 1997; Tadokoro et al., 2011). Due to its unique characteristics, including absence of DA supersensitivity following withdrawal from chronic treatment (Tadokoro et al., 2011), we hypothesized that ARI does not induce DA neuron depolarization block.

Animal models provide a useful tool to study the state-dependent action of drugs that may not be readily observed in a normal system. To model the hyperdopamineregic state characteristic of schizophrenia we have utilized MAM rats, born with a developmental disruption induced through administration of this DNA alkylating agent to pregnant dams at gestational day 17 (GD17). Adult offspring of MAM-treated rats demonstrate anatomical, behavioral, pharmacological, and physiological abnormalities consistent with phenotypes observed in patients with schizophrenia (Moore et al., 2006; Lodge and Grace, 2007; Lodge et al., 2009; Modinos et al., 2015) As a consequence of hippocampal hyperactivity, also observed in patients with schizophrenia (Kegeles et al., 2000; Schobel et al., 2009; Schobel et al., 2013), MAM rats display increased DA neuron population activity (Lodge and Grace, 2007) that rapidly results in depolarization block following acute administration of first and second generation APDs (Valenti et al., 2011b). MAM rats thus provide a clinically relevant model to study state-dependent effects of acute and repeated ARI administration on the DA system with the aim of clarifying the range of action of ARI at D_2 receptors.

2.2 METHODS

Subjects

All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals by the United States Public Health Service and approved by the University of

Pittsburgh Institutional Animal Care and Use Committee. Timed pregnant Sprague-Dawley dams (Envigo, Indianapolis, IN) were obtained on GD15 and MAM (20 mg/kg, i.p., Midwest Research Institute, Kansas City, MO) or saline (SAL; 1 ml/kg, i.p.) was administered on GD17. The male pups were weaned on postnatal day 23 and pair-housed with littermates in a temperature (22°C)- and humidity (47%)-controlled facility on a 12 h light/dark cycle (lights on 7 AM to 7 PM) with ad libitum food and water. Rats were used for experiments at 3-6 months of age (≥ 300 g).

Antipsychotic Drug Administration

MAM and SAL rats were assigned randomly to treatment groups of ARI (3 mg/kg or 10 mg/kg; Sigma Aldrich, St Louis, MO), or vehicle (VEH). Treatments were administered orally using palatable vanilla wafers to which the rats were habituated for 2 d prior to treatment. Oral administration was used to circumvent injection stress and better approximate the clinical protocol (Ferguson and Boctor, 2009), as previously described (Du and Grace, 2013; Gill et al., 2014). ARI was placed on a wafer containing 0.2 ml liquid sugar and dissolved into the wafer with an additional 0.2 ml liquid sugar. VEH-treated rats were administered the same preparation without drug. Rats were placed individually into transport tubs to consume the wafers (usually <10 min) before being returned to home cages.

For acute treatments, ARI or VEH wafers were consumed 2 h prior to the start of electrophysiological recording to observe effects at peak brain concentration as previously reported following oral administration in rats (Shimokawa et al., 2005). For repeated treatments, ARI and VEH wafers were administered at approximately 9 AM daily for 21d and rats were recorded either the following day or with 7d withdrawal.

Acute Haloperidol Administration

MAM rats were injected with haloperidol (HAL; 0.6 mg/kg, i.p; Sigma Aldrich) dissolved

in 0.23% glacial acetic acid in distilled H2O and returned to their home cage. Recordings took place 1 h following HAL administration, which was shown to be sufficient time to induce depolarization block in MAM rats (Valenti et al., 2011b).

Electrophysiological Recordings

The activity state of DA neurons in the VTA was measured using in vivo extracellular recordings. Rats were anesthetized with chloral hydrate (400 mg/kg; i.p.) and placed on a stereotaxic frame (Kopf, Tujunga, CA). Supplemental anesthesia was administered i.p. to maintain suppression of the hind limb withdrawal reflex. Body temperature was maintained at 37°C with a temperature-controlled heating pad (CWE Inc., Ardmore, PA). Single-barrel glass electrodes (WPI, Sarasota, FL) were pulled vertically (PE-2, Narasige, Japan), broken under a microscope to an impedance of 6-8 MΩ, and filled with 2 M NaCl containing 2% Chicago Sky Blue dye in 2 M saline. Electrodes were lowered through the VTA from 5.3-5.7 mm posterior, 0.6-1.0 mm lateral, and 6.5-9.0 mm ventral from the top of brain via a hydraulic micropositioner (Kopf). Single-unit activity was obtained using an amplifier (Fintronics, Orange, CT) using a highpass filter at 30 Hz and lowpass at 16 kHz. DA neurons were classified based on established criteria, including a biphasic action potential with duration >2.2 ms, a slow firing rate (1-10 Hz), and irregular and burst firing patterns with the bursts characterized by an interspike interval of 80-160ms (Grace and Bunney, 1983; Grace and Bunney, 1984; Ungless and Grace, 2012). Recordings were performed by making 6-9 vertical electrode passes (“tracks”) in a predetermined grid pattern with each track separated by 0.2 mm. The activity of each DA neuron was recorded for at least 1 minute of stable spontaneous activity with a signal-to-noise ratio greater than 2:1 using LabChart software (AD Instruments, Colorado Springs, CO). Three parameters were analyzed: (1) the average number of

spontaneously active DA neurons encountered per electrode track ("population activity"), (2) average firing rate and (3) the percentage of spikes that occurred in bursts (%SIB).

In a subset of rats, after the first six tracks rats were administered apomorphine (100- 200 μg/kg i.p. or 20 μg/kg i.v., in saline; Sigma-Aldrich) for acute and repeated ARI recordings or ARI (1 mg/kg in saline i.p.; Sigma-Aldrich) for acute HAL recordings. Apomorphine was administered in incrementally increasing doses until approximately a 50% decrease in baseline firing rate or bursting activity was detected in the DA neuron. Immediately following i.v. administration or 10 min following i.p. administration, an additional six tracks were recorded.

Histology

Electrode placement was verified following each experiment via electrophoretic ejection of Chicago Sky Blue dye from the tip of the recording electrode (-20 μA constant current, 20 min). Rats were then overdosed with chloral hydrate and decapitated. The brains were removed and fixed for at least 48 hours (8% paraformaldehyde in PBS), cryoprotected (25% sucrose in PBS) until saturated, and sliced on a cryostat into 60μm sections, which were mounted onto gelatin-coated slides. Slides were stained with a mixture of cresyl violet and neutral red for verification of electrode sites with reference to a stereotaxic atlas (Paxinos and Watson, 2007).

Analysis

Analysis of firing rate and bursting activity was performed using NeuroExplorer (Plexon, Dallas, TX). Population activity ("cells/track") was averaged within each animal and then across animals in each group, whereas the firing rate and burst activity of each neuron was counted as an independent replicate and averaged across animals in a group. Significance for acute and repeated ARI experiments was assessed with a two-way ANOVA (MAM x Treatment) followed by Tukey post-hoc comparisons. Significance for depolarization block experiments was assessed with a two-

tailed, paired t-test and the Wilcoxon signed-rank test was used for nonnormally distributed data. Significance for the haloperidol experiment was assessed with a oneway ANOVA followed by Tukey post-hoc and cells/track comparisons were made with a twotailed paired t-test. All statistics were calculated using SigmaPlot (Systat Software, San Jose, CA) and all data are represented as the mean ± SEM.

2.3 RESULTS

2.3.1 Acute Aripiprazole Administration Reduces VTA DA Neuron Activity of MAM but Not SAL Rats

Electrophysiological recordings were conducted from MAM rats and SAL rats, with each group receiving either VEH or ARI (3 mg/kg or 10 mg/kg, p.o.). VEH-treated MAM rats (n = 6 rats, 63 neurons) exhibited the anticipated elevation in population activity with an average of 1.7 ± 0.1 cells/track compared to VEH-treated SAL rats (n = 8 rats, 61 neurons), which had an average of 1.1 ± 0.1 cells/track (Fig. 2-1 A; 2-way ANOVA main effects: for MAM, $F(2,40) = 3.189$, $p = 0.010$; for ARI, $F(2,40) = 5.049$, $p = 0.011$; MAM-by-ARI interaction: $F(2,40) = 5.882$, $p = 0.005$; post hoc MAM control vs SAL control: $p = 0.001$). Acute ARI treatment significantly reduced DA neuron population activity in MAM rats, both at 3 mg/kg (n = 6 rats, 37 neurons; post hoc MAM control vs MAM 3 mg/kg: $p = 0.007$) and 10 mg/kg (n = 10 rats, 63 neurons; post hoc MAM control vs MAM 10 mg/kg: $p = 0.001$) compared to VEH-treated MAM rats. In contrast, there was no reduction in DA neuron population activity in ARI-treated SAL rats, at 3 mg/kg (n = 6, 41 neurons), or 10 mg/kg (n = 8 rats, 66 neurons), compared to VEH-treated SAL rats (Fig 2-1 A). There was no significant change in firing rate (Fig. 2-1 B) or bursting

(Fig. 2-1 C) with ARI treatment compared to VEH treatment in MAM or SAL rats. There was also no overt shift in the distribution of firing rate or bursting (Fig 2-1 D-E), despite the reduction in population activity observed in MAM rats. In ARI-treated MAM rats, at both 3 mg/kg and 10 mg/kg, a systemic injection of apomorphine (100-200 μg/kg i.p) reduced firing rate and bursting activity of DA neurons held during injection. In an example neuron, apomorphine reduced firing rate of a DA neuron from 5.2 Hz to 3.1 Hz and the percentage of spikes in burst from 22.2% to 0% 5 min following the injection (Fig 2-1 F). An additional six tracks were recorded following apomorphine injection, without significant change in population activity compared to tracks recorded pre-apomorphine (Fig 2-1 G) and a leftward shift in the distribution of the firing rate and bursting activity of recorded DA neurons recorded post-apomorphine compared to preapomorphine (Fig 2-1 H-I).

2.3.2 Repeated Aripiprazole Administration Reduces VTA DA Neuron Activity of MAM but Not SAL Rats

To evaluate the effects of repeated ARI treatment, MAM and SAL rats received 21d of ARI (10 mg/kg) or VEH daily treatment p.o. and their VTA activity was recorded the following day. VEH-treated MAM rats (n = 6 rats, 66 neurons) displayed a significant elevation in DA neuron population activity (average 1.9 ± 0.1 cells/track) compared to VEH-treated SAL rats (Fig. 2-2 A; n = 6 rats, 38 neurons; average 1.0 ± 0.1 cells/track; 2-way ANOVA main effects: for MAM, $F(1,21) = 16.401$, $p < 0.001$; for ARI, $F(1,21) = 11.602$, $p = 0.003$; MAM-by-ARI interaction: $F(1,21) = 9.223$, $p = 0.006$; post hoc MAM control vs SAL control: $p < 0.001$).

Repeated ARI treatment significantly reduced DA neuron population activity in ARI-treated MAM rats (n = 7 rats, 45 cells) compared to VEH treated MAM rats to an average of 1.1 ± 0.1 cells/track (Fig 2-2 A; 2-way ANOVA; post hoc MAM ARI vs MAM control: $p < 0.001$). In

contrast, there was no reduction in DA neuron population activity in ARI-treated SAL rats (n = 6 rats, 37 neurons; average 0.9 ± 0.1 cells per track), compared to VEH-treated SAL rats (Fig 2-2 A). There was no significant change in firing rate with repeated ARI treatment compared to VEH treatment in MAM or SAL rats (Fig. 2-2 B; control baseline: 3.9 ± 0.3 Hz; control ARI: 4.1 ± 0.3 Hz; MAM baseline: 4.0 ± 0.2 Hz; MAM ARI: 4.0 ± 0.3 Hz), and a main effect of ARI treatment on bursting activity in MAM and SAL rats (Fig. 2-2C; control baseline: 26.9 ± 4.2%; control ARI: 37.9 ± 4.3%; MAM baseline: 33.4 ± 3.0%; MAM ARI: 41.4 ± 3.5%; 2-way ANOVA $F(1,193) = 6.016$, $p = 0.015$). In MAM rats that received repeated ARI treatment, a systemic injection of apomorphine (200 μg/kg i.p) resulted in no significant change in DA neuron population activity in an additional six tracks that were recorded 10 min following the injection compared to tracks recorded preapomorphine (Fig 2-2 D); a leftward shift in the firing rate and bursting activity of DA neurons recorded post-apomorphine compared to pre-apomorphine was also observed (Fig 2-2 E-F).

2.3.3 Persistent Reduction of VTA DA Neuron Activity Following Withdrawal From Repeated Aripiprazole Administration

Given our findings that acute and repeated ARI treatment results in a reduction in VTA DA neuron activity in MAM rats that is not restored by apomorphine administration, we examined whether this reduction remained following an extended 7d withdrawal from 21d repeated treatment. VEH-treated MAM rats (n = 5 rats, 66 neurons) displayed a significant elevation in DA neuron population activity with an average of 1.7 ± 0.1 cells/track, compared to VEH-treated SAL rats (n = 8 rats, 62 neurons), with an average of 1.0 ± 0.1 cells/track (Fig. 2-3 A; 2-way ANOVA main effects: for MAM, $F(1,24) = 16.024$, $p < 0.001$; for ARI, $F(1,24) = 35.985$, $p < 0.001$; MAM-by-ARI interaction: $F(1,24) = 25.671$, $p < 0.001$; post hoc MAM control vs SAL control: $p <$

0.001). DA neuron population activity remained reduced in MAM rats following 7d withdrawal from 21d repeated ARI treatment (n = 6 rats, 37 neurons) compared to VEH-treated MAM rats with an average of 0.8 ± 0.1 cells/track and of spikes in bursts (Fig 2-3 A; 2-way ANOVA; post hoc MAM ARI vs MAM control: $p < 0.001$). In contrast, there was no reduction in DA neuron population activity in ARI-treated SAL rats (n = 9 rats, 61 neurons), with an average of 0.9 ± 0.1 cells/track (Fig 2-3 A). MAM rats withdrawn from repeated ARI treatment displayed a significant reduction in firing rate (Fig. 2-3 B; control baseline: 3.7 ± 0.2 Hz; control ARI: 3.5 ± 0.2 Hz; MAM baseline: 3.7 ± 0.2 Hz; MAM ARI: 2.3 ± 0.3 Hz; 2-way ANOVA; main effects: for MAM, $F(1,222) = 6.029$, $p = 0.015$, for ARI, $F(1,222) = 10.019$, $p = 0.002$; MAM-by-ARI interaction: $F(1,222) = 6.268$, $p = 0.013$; post hoc MAM ARI vs MAM control: $p < 0.001$) and there was a main effect of ARI treatment on bursting activity of VTA DA neurons in MAM and SAL rats withdrawn from repeated ARI treatment (Fig. 2-3 C; control baseline: 34.2 ± 3.4; control ARI; 27.6 ± 3.4% MAM baseline: 35.7 ± 3.3%; MAM ARI: 25.0 ± 4.3%; 2-way ANOVA; main effect for ARI, $F(1,222) = 5.772$, $p = 0.017$), which was reflected in the distribution of DA neurons recording in ARI-treated MAM rats compared to VEH-treated MAM rats (Fig. 2-3 D-E). In MAM rats withdrawn from repeated ARI treatment, a systemic injection of apomorphine (200 μg/kg i.p or 20 μg/kg, i.v.) did not cause a significant change in DA neuron population activity in an additional six tracks that were recorded 10 min following the i.p. injection (n = 3 rats) or immediately following an i.v. injection (n = 1 rat) compared to tracks recorded pre-apomorphine (Fig 2-3 F). There was also a leftward shift in the firing rate and, more prominently, bursting activity of DA neurons recorded post-apomorphine compared to pre-apomorphine (Fig. 2-3 G-H).

2.3.4 Acute Aripiprazole Administration Reverses Haloperidol-Induced Depolarization Block in MAM Rats

To determine the effect of acute ARI administration on depolarization block, six tracks were recorded in the VTA of MAM rats 1 h following acute treatment with haloperidol (0.6 mg/kg, i.p.) and an additional six tracks were recorded 10 min following acute ARI administration (1 mg/kg, i.p.). An example DA neuron recorded before ARI administration displayed an average firing rate of 2.5 Hz with 15.9% of spikes fired in bursts. The same neuron displayed a firing rate of 1.9 Hz with 3.9% of spikes fired in bursts following ARI administration (Fig 2-4 A). There was a significant increase in the number of spontaneously active DA neurons recorded after acute ARI administration (1.4 ± 0.1 cells/track) compared to before (0.6 ± 0.1 cells/track; paired t-test; $t(7) = -4.031$, $p = 0.005$). In contrast, there was no change in DA neuron population activity in tracks recorded after VEH administration (1 ml/kg saline, i.p., 0.7 ± 0.2 cells/track) compared to before (0.8 ± 0.2 cells/track; Fig. 2-4 B-D). There was no significant difference in the firing rate (Fig. 2-4 E) or bursting activity (Fig. 2-4 F) between groups.

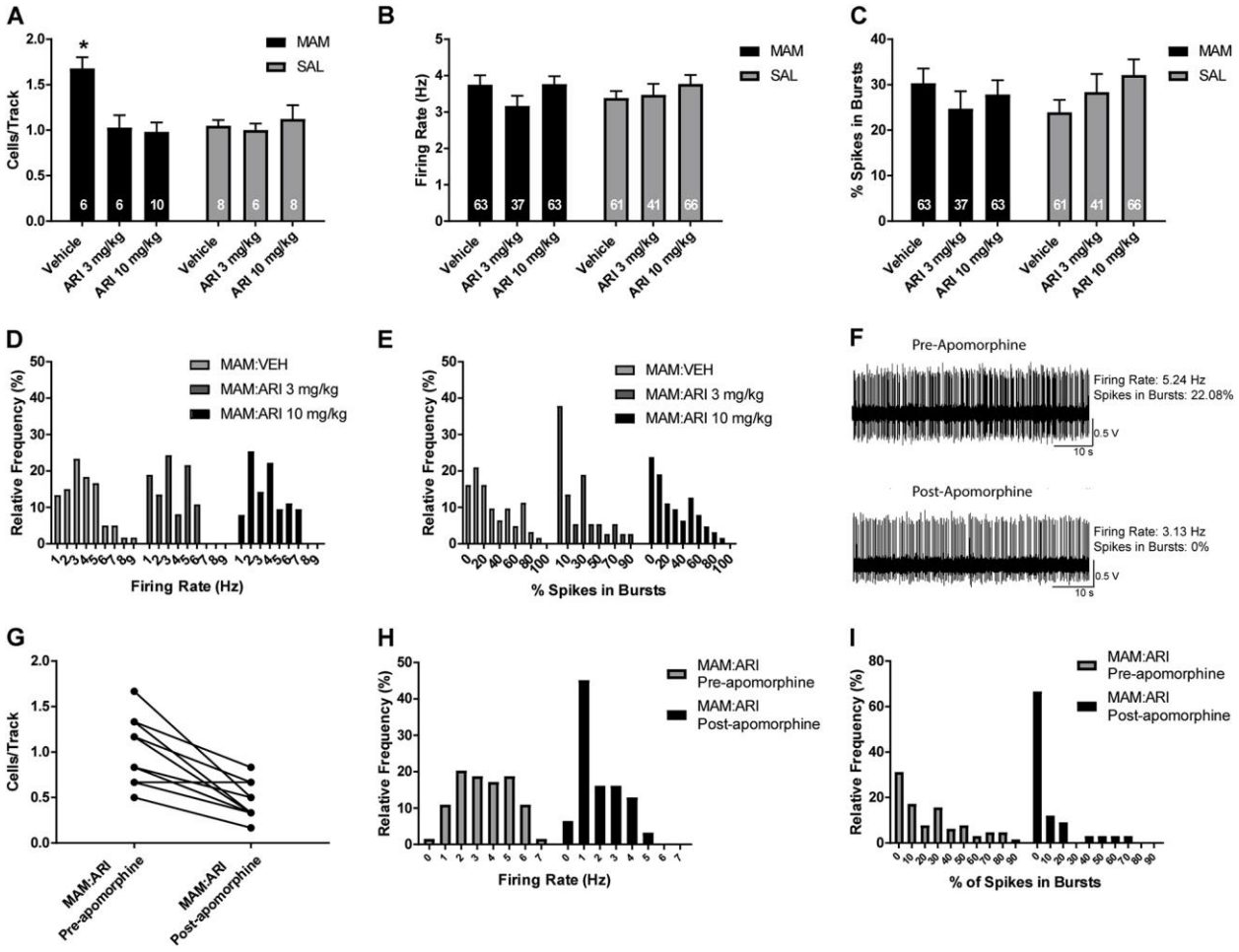

Figure 2-1: Acute administration of ARI reduces DA neuron activity in the VTA in MAM but not SAL rats

(A) 2 h following oral ARI administration, MAM rats displayed a reduced number of spontaneously active DA neurons in the VTA compared to MAM rats that received VEH administration, which was not observed in SAL rats. There were no significant differences in the firing rate **(B-C)** or percentage of spikes in bursts **(D-E)** between ARI and VEH administration in MAM or SAL rats. **(F)** An example trace of a DA neuron recorded before and 5 min after APO, which was administered i.p. following six tracks, 10 min prior to recording an additional six tracks. APO reduced the firing rate and bursting activity of the DA neuron. **(G)** APO administration did not significantly reduce DA neuron population activity in the VTA of ARI-treated MAM rats, although there was a trend toward reduced spontaneous activity and a leftward shift in the distribution of firing rate **(H)** and % of spikes in bursts **(I)** in neurons recorded after APO compared to tracks recorded pre-APO.

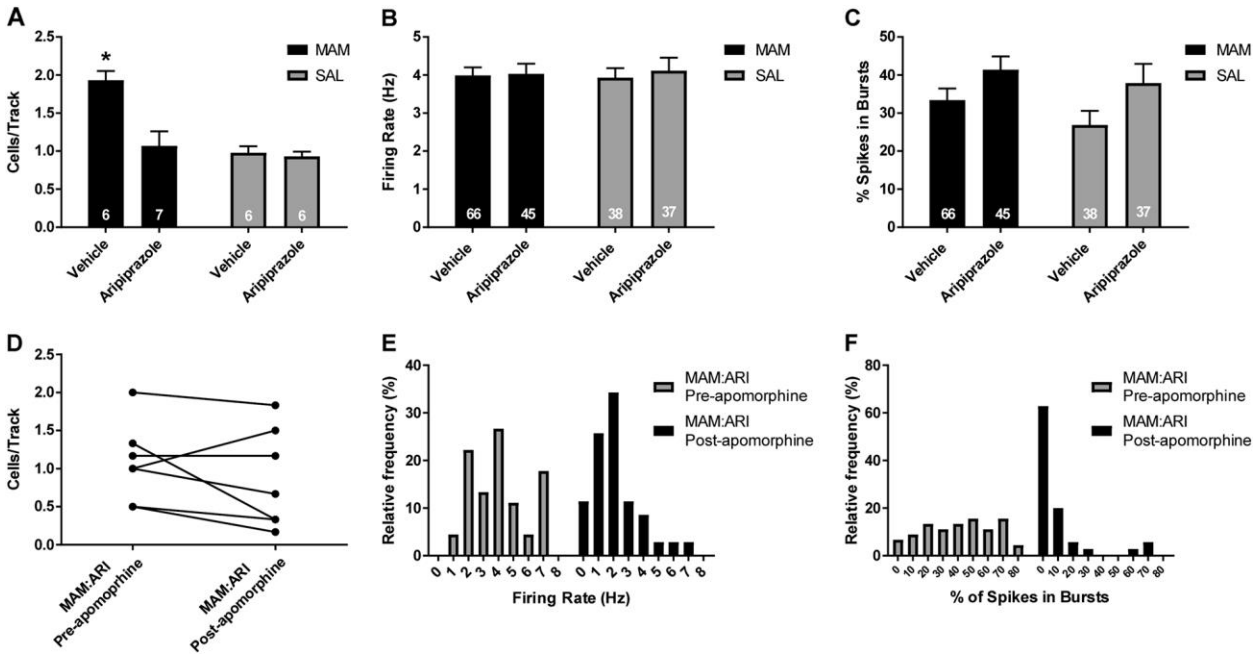

Figure 2-2: Repeated administration of ARI for 21d p.o. reduced DA neuron activity in the VTA in MAM but not SAL rats

(A)24h following 21d repeated ARI treatment, MAM rats displayed a reduced number of spontaneously active DA neurons in the VTA compared to MAM rats that received VEH administration, which was not observed in SAL rats. There was no significant difference in the firing rate **(B)** of DA neurons between ARI and VEH administration in MAM or SAL rats and there was a main effect of ARI treatment on the percentage of spikes in bursts **(C)**. In MAM rats that received ARI, APO was administered i.p. following six tracks, 10 min prior to recording an additional six tracks. **(D)** APO administration did not significantly reduce DA neuron population activity in the VTA of ARI-treated MAM rats and produced a leftward shift in the distribution of firing rate **(E)** and % of spikes in burst **(F)** in DA neurons recorded after APO compared to tracks recorded pre-APO.

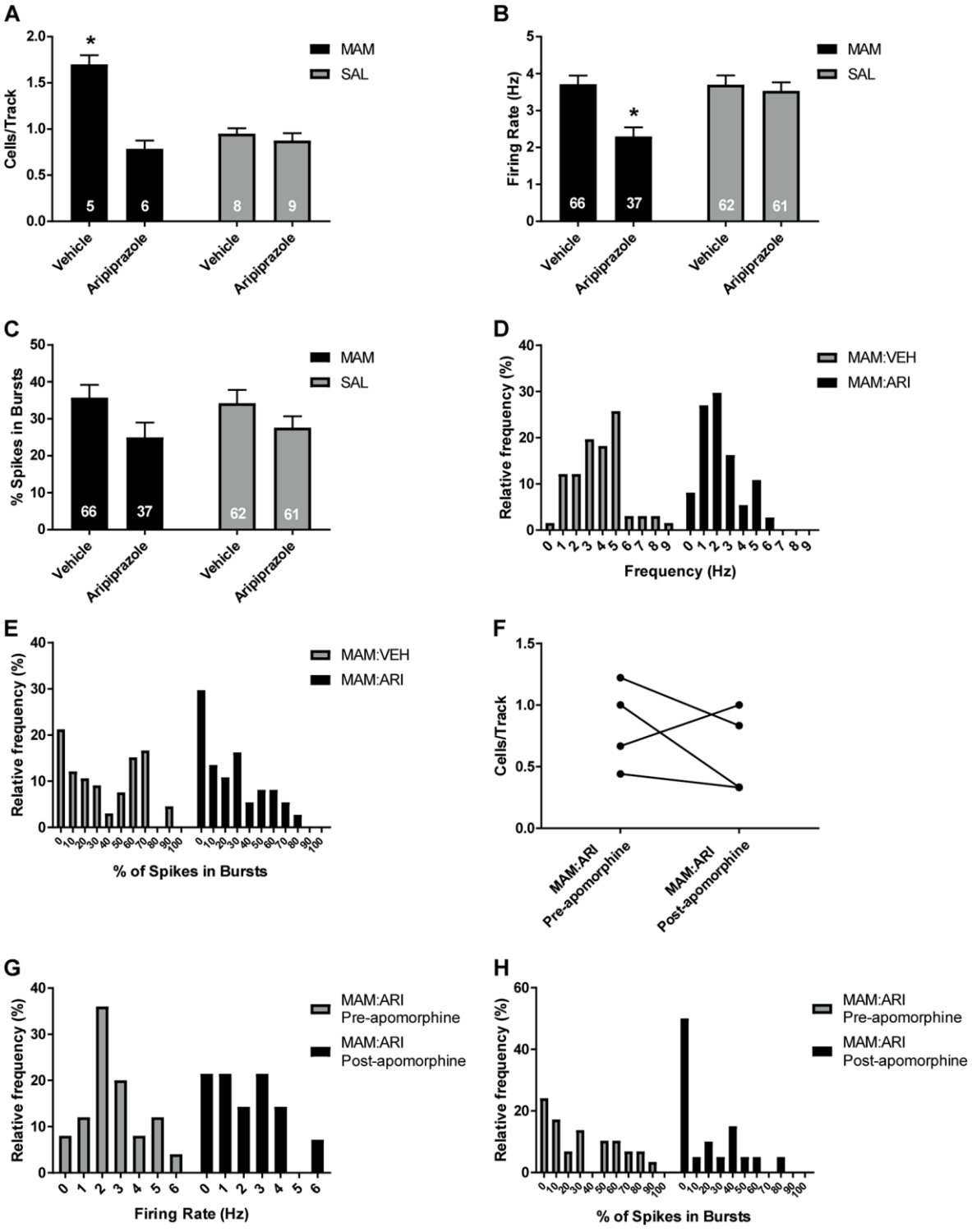

Figure 2-3: Spontaneous DA neuron activity in the VTA of MAM rats remains reduced following 7d withdrawal from 21d ARI treatment in a manner distinct from depolarization block

(A)MAM rats displayed reduced VTA DA neuron following 7d withdrawal from 21d repeated ARI treatment compared to VEH treatment, which was not observed in SAL rats. **(B)** MAM rats withdrawn from repeated ARI treatment displayed a reduced firing rate of DA neurons compared to VEH treated MAM rats, which was not observed in SAL rats. **(C)** ARI treatment reduced the percentage of spikes in bursts in both MAM and SAL rats, compared to VEH-treated rats. This was evident as a leftward shift in the distribution of both firing rate **(D)** and bursting activity **(E)** of DA neurons in ARI-treated MAM rats compared to VEH-treated MAM rats. In MAM rats that received ARI, APO was administered i.p. or i.v. following six tracks, prior to recording an additional six tracks. **(F)** APO administration did not significantly change DA neuron population activity in the VTA of ARI-treated MAM rats and produced a leftward shift in the distribution of firing rate **(G)** and % of spikes in burst **(H)** in DA neurons recorded after APO compared to tracks recorded pre-APO.

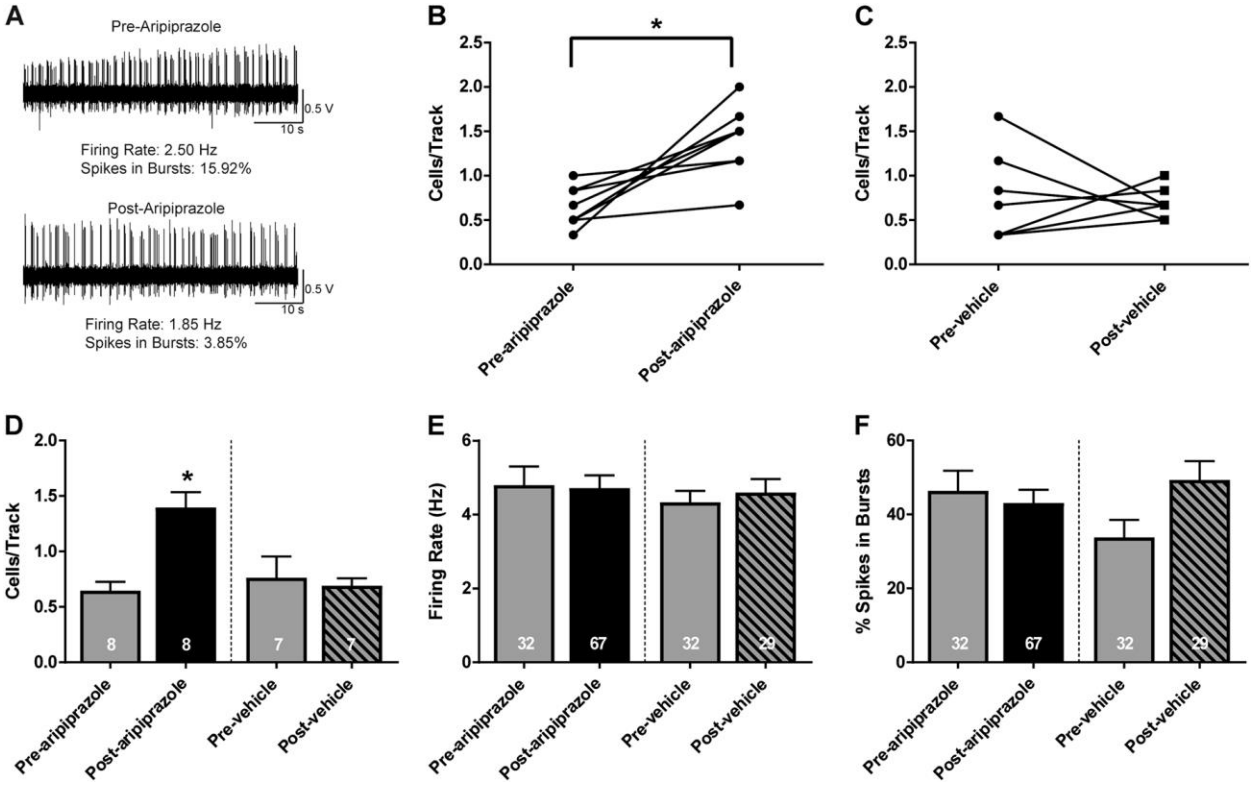

Figure 2-4: Reversal of haloperidol-induced depolarization block in MAM rats via acute ARI administration

(A) 1 h following acute haloperidol (0.6 mg/kg, i.p.) administration in MAM rats, an example trace of a DA neuron demonstrates a reduction in DA neuron firing rate and bursting activity 5 min following acute ARI (1 mg/kg, i.p.) compared to pre-ARI. **(B-C)** Within-subject changes in average DA neuron population activity in the six tracks recorded before ARI or saline administration in HAL-treated MAM rats compared to the six tracks recorded after

ARI or saline administration. **(D)** Acute ARI administration resulted in an increase in the average number of DA cells/track in HAL-treated MAM rats post-ARI administration compared to pre-ARI administration. In contrast, there was no difference in population activity in tracks recorded post-saline administration. There was no significant change in the average firing rate **(E)** or bursting activity **(F)** in DA neurons recorded before and after ARI or VEH administration.

2.4 DISCUSSION

Unlike many other clinically available APDs, which exert their effects through D_2 receptor antagonism, ARI is a D_2 partial agonist thought to stabilize dopaminergic tone (Burris et al., 2002), although the mechanism underlying its state-dependent effect is unclear. The current study examined whether ARI reduces DA neuron activity in vivo in a normal and hyperdopaminergic system via overexcitation-induced depolarization block, as observed following D_2 antagonist administration. We demonstrate that ARI is able to state-dependently decrease the number of spontaneously active DA neurons in the VTA in the MAM rodent model of schizophrenia in a manner distinct from D_2 receptor antagonists. Furthermore, after depolarization block-driven decrease in DA neuron population activity (Grace and Bunney, 1986; Valenti et al., 2011b; Gill et al., 2014), ARI increased DA neuron population activity secondary to reversal of APD-induced depolarization block, as observed with low-dose administration of a DA agonist (Grace and Bunney, 1986; Valenti et al., 2011b). The interpretation of these results is limited to male rats and the chosen treatment and dose regiment. Female rats were excluded from the present study due to differences in DA neuron activity between female MAM and SAL rats across the estrous cycle (Perez et al., 2014). Examination of ARI's action in female rodents across the estrous cycle is a necessary future direction, especially given reported differences in APD response between male

and female patients (Goldstein et al., 2002) and in female patients across the menstrual cycle (Bergemann et al., 2007).

Aripiprazole Produces a Rapid and Long-Lasting Reduction of Hyperdopaminergic Activity Only in MAM Rats

ARI, at both 3 mg/kg and 10 mg/kg, reduced DA neuron population activity in the VTA of MAM rats 2h following oral administration without affecting SAL rats, similar to the rapid reduction of population activity in MAM rats follow D_2 receptor antagonist APD administration (Valenti et al., 2011b). The reduction was also present following 21d repeated ARI treatment and persisted 7d following withdrawal from repeated treatment, suggesting a persistent change in the DA system, as previously observed following withdrawal from repeated HAL treatment (Gill et al., 2014). However, unlike D_2 receptor antagonists (Valenti et al., 2011b; Gill et al., 2014), ARI did not reduce DA neuron activity in SAL rats following repeated treatment or following 7d withdrawal from repeated treatment. Consistent with the lack of observed change in spontaneous DA neuron activity in control rats, an *in vivo* microdialysis study has previously shown that acute ARI administration does not affect cortical or striatal extracellular DA levels in rats following acute and repeated oral administration (Jordan et al., 2004). Repeated oral ARI administration has also been shown to block elevated GTPase activity stimulated by the DA agonist quinpirole, but ARI alone does not stimulate GTPase activity (Inoue et al., 1997). In contrast, (Li et al., 2004) demonstrated a reduction in extracellular DA levels in the PFC and NAc following acute ARI administration at 10 mg/kg, administered subcutaneously. These differences may be explained by differing administration methods, as even 21 d repeated administration of ARI 40 mg/kg p.o. has been shown to not significantly effect striatal DA levels (Jordan et al., 2004). Our results demonstrate that ARI is able to reverse the increased DA neuron activity, as observed in MAM

rats, without effect in a normal system, and that the reduction in DA neuron activity can remain stabilized following withdrawal from repeated treatment.

Aripiprazole Does Not Reduce VTA Dopamine Neuron Activity via Depolarization Block

In contrast to the reduction DA neuron population activity in MAM rats via depolarization block following acute treatment with D_2 antagonists (Valenti et al., 2011b), the present data suggest that ARI administration does not induce depolarization block. The presence of depolarization block has been verified previously by administering low doses of the DA agonist apomorphine, which leads to an inhibition-driven reactivation of neurons previously in a hyperexcitation-induced block (Bunney and Grace, 1978; Grace and Bunney, 1986; Valenti et al., 2011b). In contrast, low doses of apomorphine failed to increase population activity in MAM rats following acute or following 21d repeated ARI treatment. In addition, it did not affect population activity following 7d withdrawal from repeated ARI treatment. However, in all cases, administration of apomorphine produced a leftward shift in the distribution of firing rate and, more prominently, bursting activity of spontaneously active DA neurons recorded post-injection. In some cases, this resulted in a reduction in the number of spontaneously active DA neurons, notably observed following acute ARI administration, possibly because neurons with a previously low firing rate ceased firing. Therefore, the local feedback from the action of apomorphine presumably on D_2 autoreceptors resulted in a net reduction in DA activity of ARI-treated MAM rats, rather than an increase in spontaneous activity which would be observed if the reduction in population activity was due to overexcitation-induced depolarization block. Although ARI displayed antagonist-like reduction of hyperdopaminergic activity in MAM rats, it did not appear to do so through antagonist action at presynaptic D_2 receptors that would result in depolarization block.

The question remains of how ARI reduces DA neuron population activity in MAM rats. It has been hypothesized that ARI simultaneously acts as an antagonist at D_2 receptors postsynaptically and an agonist presynaptically (Kikuchi et al., 1995; Oshiro et al., 1998; Burris et al., 2002) based on differences in receptor reserve (Burris et al., 2002). In the presence of a receptor reserve, ARI behaves like an agonist, and in its absence, ARI predominantly displays antagonist properties (Kikuchi et al., 1995). The greater number of presynaptic spare receptors may underlie ARI's autoreceptor selectivity to inhibit neuron firing (Kikuchi et al., 1995; Burris et al., 2002), which may have contributed to the downregulation of DA neuron population activity. In support of its agonist-like activity at autoreceptors, a reduction in both the average firing rate and bursting activity in MAM rats was observed following 7d withdrawal from repeated treatment, although no significant reduction in firing rate or bursting was observed across the population of DA neurons recorded following acute ARI administration or following 21d repeated ARI treatment compared to VEH-treated rats. Several other in vivo studies have demonstrated ARI's agonist-like activation at D_2 autoreceptors (Kikuchi et al., 1995; Semba et al., 1995), including its ability to block increased DA synthesis in reserpine-treated rats (Kikuchi et al., 1995). No significant differences in average firing rate and bursting activity were observed across the total population of DA neurons recorded between ARI and vehicle-treated MAM rats in this study, but acute i.p. ARI administration has previously been shown to modestly reduce DA neuron firing rate and bursting activity (Bortolozzi et al., 2007; Dahan et al., 2009). Therefore, it may reduce DA neuron population activity through action on presynaptic D_2 receptors to downregulate DA neuron activity. ARI's proposed partial agonism, with lower intrinsic activity at the receptor than a full agonist, may also account for its antagonist-like effect at postsynaptic D_2 receptors in animal

models of DA hyperactivity, such as blockade of apomorphine-induced hyperlocomotion and stereotypy (Kikuchi et al., 1995).

It is difficult to conclude whether this accounts for ARI's actions across a range of systems. It has been suggested from in vitro studies that ARI possesses functional selectivity with intrinsic activity dependent on the cellular environment of the receptor and the signaling pathway activated (Lawler et al., 1999; Shapiro et al., 2003; Mailman and Gay, 2004; Urban et al., 2007), which may contribute to its state-dependent effects. ARI may lack biased signaling in vivo, but it has served as a template for β-arrestin-biased D_2 receptor ligands that have recently been studied as potential APD compounds to more effectively target both cortical and striatal DA dysfunction (Urs et al., 2017). It is also possible that different factors may contribute to the reduction observed following acute and repeated treatment, and/or following withdrawal from repeated treatment. For example, DA neuron population activity also remains reduced following 1 week withdrawal from repeated haloperidol administration, but unlike the acute and repeated effects of haloperidol (Grace and Bunney, 1986; Valenti et al., 2011b), the reduction following withdrawal has been suggested to not due to depolarization block and may instead be due to the modulatory influence of other brain regions (Gill et al., 2014). Finally, ARI has a diverse receptor binding profile, including significant affinity for serotonin (5-HT)1A and 5-HT2A receptors (Jordan et al., 2002; Shapiro et al., 2003), and the potential influence of other neurotransmitter systems on DA neuron activity cannot be excluded. Additional *in vivo* studies are needed to determine the mechanisms underlying ARI's selective downregulation of DA neuron activity in MAM rats, such as whether it is due to action on presynaptic D_2 receptors, and whether they can be generalized to all D_2 receptor partial agonists. Furthermore, it remains to be determined why ARI selectively downregulates DA neuron activity in MAM rats and not in normal rats.

Removal of Haloperidol-Induced Depolarization Block by Aripiprazole

Prevalent problems associated with APD treatment and withdrawal have driven a search for treatments with a more favorable side effect profile and improved efficacy across symptom domains. Novel target compounds for the treatment of schizophrenia have shown promise in preclinical research, but failed to show efficacy in clinical trials. However, preclinical research is typically performed on drug-naïve rats, whereas clinical trials are performed on patients that have received only brief withdrawal from years of prior APD treatment. We previously found that withdrawal from repeated HAL treatment produced persistent DA supersensitivity in MAM rats, interfering with the ability of a novel target compound to reduce amphetamine-induced hyperlocomotion in MAM rats (Gill et al., 2014). It is possible that DA supersensitivity following prior D_2 antagonist treatment in patients with schizophrenia may similarly mask potential effects of novel target compounds in clinical trials, despite their promise in drug screening paradigms performed in normal, drug-naïve rodents. Prior evidence that repeated ARI administration may not upregulate D_2 receptors or produce DA supersensitivity (Inoue et al., 1997; Tadokoro et al., 2011), unlike D_2 receptor antagonists (O'Dell et al., 1990; See et al., 1990; Lidow and Goldman-Rakic, 1994; Silvestri et al., 2000; Tadokoro et al., 2011), indicate that ARI may circumvent this potential confound. However, in patients taking D_2 antagonist APDs, the switch to ARI is reported to result in a temporary worsening of psychotic symptoms, which was suggested to be unmasking of the increase in D_2 receptors from prior treatment (Takase et al., 2015; Tadokoro et al., 2017). Indeed, the ability of ARI to reverse depolarization block in HAL-treated MAM rats demonstrated in the present study is consistent with the reported worsening in psychotic symptoms in patients upon transitioning to ARI from D_2 antagonist drugs. Thus, while ARI may not have superior efficacy in schizophrenia (Leucht et al., 2009a; Leucht et al., 2009b), it may be less likely to induce

persistent deleterious effects that can arise from a persistent elevation in DA sensitivity that occurs with D_2 antagonists (Silvestri et al., 2000; Chouinard et al., 2017).

Overall, this study demonstrates that ARI rapidly normalizes the hyperdopaminergic state observed in MAM rats without effect on DA neuron population activity in a normal system. In contrast to the action of D_2 receptor antagonists, the reduction is unlikely due to depolarization block. Although it is unknown how ARI reduces DA neuron activity in MAM rats, it may act as an agonist on presynaptic receptors to downregulate spontaneous activity.

3.0 THE MGLUR2/3 AGONIST POMAGLUMETAD METHIONIL INDIRECTLY REGULATES DOPAMINE NEURON ACTIVITY VIA ACTION IN THE VENTRAL HIPPOCAMPUS

3.1 INTRODUCTION

APDs that target DA D_2 receptors have remained the primary treatment for schizophrenia since their introduction in the 1950s (Kapur and Mamo, 2003). Their therapeutic effect is attributed to D_2 receptor antagonist or partial agonist action (Creese et al., 1976; Kapur et al., 2000; Burris et al., 2002) that reduces elevated DA neuron activity (Grace and Bunney, 1986; Valenti et al., 2011b; Sonnenschein et al., 2019). Dysregulated presynaptic DA neuron activity (Laruelle and Abi-Dargham, 1999; Howes et al., 2011a) is observed in patients with schizophrenia and correlated to positive symptom severity (i.e. hallucinations, delusions, thought disorder). Accordingly, patients who respond to current APDs primarily display a reduction in positive symptoms (Leucht et al., 2013). However, approximately 30% of patients with schizophrenia display treatment resistance with little or no response to standard treatment (Saha et al., 2005). Even clozapine, the treatment of choice for refractory symptoms, is ineffective in approximately 50% of patients who take it (Kane et al., 1988; Lieberman et al., 1994; Remington et al., 2005). Current APDs are also hindered by a high rate of treatment nonadherence, driven by factors including limited efficacy and poor tolerability (Lieberman et al., 2005). Furthermore, there is evidence of persistent and potentially detrimental changes to brain morphology (Ho et al., 2011) and the DA system (Lidow et al., 1997; Silvestri et al., 2000) with long-term use of D_2 receptor antagonists. These shortcomings highlight the need for alternative strategies, including novel APD mechanisms.

One of the most promising novel target candidates was group II metabotropic glutamate receptors, comprised of mGluR2 and mGluR3 (mGluR2/3). mGluR2/3 are prominently expressed in limbic brain regions, where they are primarily localized presynaptically on glutamatergic terminals to negatively regulate glutamate release (Nicoletti et al., 2011). Aberrant excitatory-inhibitory balance has been well-characterized in schizophrenia as an underlying component of numerous symptoms of schizophrenia (Benes and Berretta, 2001; Lewis et al., 2005), including a primary driver of DA dysregulation (Lodge and Grace, 2011). Despite extensive preclinical support for mGluR2/3 as a novel APD target (Rorick-Kehn et al., 2007; Mezler et al., 2010) and success in an early clinical trial (Adams et al., 2013), the mGluR2/3 agonist pomaglumetad methionil (POM) was pulled from phase III clinical trials for insufficient efficacy in reducing psychotic symptoms compared to current APDs (Marek, 2015). Later analyses found that POM was more effective in certain populations, including early-in-disease patients (Kinon et al., 2015), indicating that further research is necessary to clarify the disconnect between the preclinical research and clinical trial results.

A critical question that needs to be addressed is how, if at all, mGluR2/3 agonists regulate DA neuron activity. Prior research on mGluR2/3 agonists primarily focused on the PFC (Moghaddam and Adams, 1998; Lorrain et al., 2003; Homayoun and Moghaddam, 2007; Pehrson and Moghaddam, 2010). Their potential therapeutic effect was suggested to be DA-independent due absence of mGluR2/3 expression in the VTA (Ohishi et al., 1993a; Ohishi et al., 1993b; Neki et al., 1996) and their lack of effect on DA levels at baseline or following VTA stimulation (Pehrson and Moghaddam, 2010). However, mGluR2/3 is densely expressed in the HPC, including the human anterior HPC (Blümcke et al., 1996) and corresponding rat vHPC (Ohishi et al., 1993a; Ohishi et al., 1993b). Patients with schizophrenia demonstrate a loss of PV+ interneuron regulation

of pyramidal neuron activity in the anterior HPC, associated with the onset of psychosis (Schobel et al., 2013; Heckers and Konradi, 2015). A similar progression of PV+ interneuron loss (Lodge et al., 2009) and vHPC hyperactivity (Lodge and Grace, 2007) is observed in MAM rats. The increased vHPC drive results in an increased number of spontaneously active DA neurons in the VTA, which produces a hyperdopaminergic state consistent with findings in patients (Modinos et al., 2015). MAM rats thus provide a clinically relevant model to determine how mGluR2/3 activation may affect a system-wide disorder. This study aimed to determine the role of mGluR2/3 in the regulation of DA neuron activity and our findings demonstrate that mGluR2/3 agonists act on a hyperactive vHPC to indirectly reduce increased DA neuron population activity in the VTA.

3.2 METHODS

Subjects

All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Timed pregnant Sprague-Dawley dams (Envigo, Indianapolis, IN) were obtained on gestational day (GD)15 and MAM (20 mg/kg, i.p., Midwest Research Institute, Kansas City, MO) or saline (SAL; 1 ml/kg, i.p.) was administered on GD17. Male and female pups were weaned on postnatal day (PD)23. Naïve adult male rats were obtained from Envigo for restraint stress experiments. All rats were housed in groups of 2-3 with littermates in a temperature (22°C) and humidity (47%)-controlled facility with *ad libitum* food and water in a normal 12-h light-dark cycle.

Drug Administration

LY2140023 (pomaglumetad methionil, POM), the prodrug of mGluR2/3 agonist LY404039, was obtained from Selleck Chemicals, Houston, TX. The mGluR2/3 antagonist LY341495 (LY34) was obtained from Tocris Bioscience, Minneapolis, MN. Both drugs were dissolved in 0.9% sterile saline and prepared at a volume of 1 ml/kg, except the 10 mg/kg dose of POM, which was mixed at a volume of 3 ml/kg for improved solubility. Drugs were dissolved with dropwise addition of 1M NaOH to POM (pH ~7) and LY34 (pH ~8) solutions. Vehicle (VEH)-treated rats received 0.9% sterile saline. All systemic treatments were administered intraperitonially.

For electrophysiological experiments, POM or vehicle was administered to adult MAM and SAL rats following anesthetization and 30 minutes prior to electrophysiological recording. Recordings were performed within approximately 3-4 hours of administration. For female rat recordings, vaginal lavages were taken following anesthetization. Estrous stage was determined using a light microscope based on criteria described in (Marcondes et al., 2002; Hubscher et al., 2005). To examine the effects of repeated POM administration on DA neuron activity, adult male MAM and SAL rats were randomly assigned to either acute or repeated administration groups and received either acute or 14d repeated daily i.p. administration of either POM or vehicle. On the 15th day, rats received a final treatment 30 min prior to electrophysiological recording.

For acute, intra-vHPC microinfusions, rats were implanted ipsilateral to the side of the recording with 23-gauge guide cannula 1.0 mm dorsal to the ventral subiculum or ventral CA1 at -5.7 mm posterior and +4.6 mm lateral from bregma, and -6.0 mm ventral from the top of skull. 0.5 µg of POM was gradually infused over 2 minutes at a volume of 0.5 µl through a 33-gauge injection cannula protruding 1.0 mm past the end of the implanted guide cannula. The injection

cannula was left in place for 1 minute to ensure diffusion of drug into the surrounding tissue. The control group consisted of rats that received vehicle infusion into the vHPC. Recordings took place until approximately 2-3 h after infusion.

Electrophysiological Recordings

The activity state of DA neurons in the VTA was measured using in vivo extracellular recordings. Rats were anesthetized with chloral hydrate (400 mg/kg; i.p.) and placed on a stereotaxic frame (Kopf, Tujunga, CA). Supplemental anesthesia was administered i.p. to maintain suppression of the hind limb withdrawal reflex. Body temperature was maintained at 37°C with a temperature-controlled heating pad (CWE Inc., Ardmore, PA). Single-barrel glass electrodes (WPI, Sarasota, FL) were pulled vertically (PE-2, Narasige, Japan), broken under a microscope to an impedance of 6-8 MΩ, and filled with 2 M NaCl containing 2% Chicago Sky Blue dye in 2 M saline. Electrodes were lowered with a hydraulic micropositioner (Kopf) to sample the VTA: AP: -5.3 to -5.7 mm and ML: 0.6 to 1.0 mm from bregma, DV: -6.5 to -9.0 mm from the top of brain). Recordings were performed by making 6-9 vertical electrode passes ("tracks") in a predetermined grid pattern with each track separated by 0.2 mm. Single-unit activity was obtained using an amplifier (Fintronics, Orange, CT) using a highpass filter at 30 Hz and lowpass at 16 kHz. DA neurons were classified based on established criteria, including a biphasic action potential with duration >2.2 ms, 1-10 Hz firing rate, and irregular and burst firing patterns with the burst initiation defined as in interspike interval of ≤ 80 msec and termination as >160 msec (Grace and Bunney, 1983; Grace and Bunney, 1984; Ungless and Grace, 2012). The activity of each DA neuron was recorded for at least 1 minute of stable spontaneous activity using LabChart software (AD Instruments, Colorado Springs, CO).

At the end of each recording, electrode placement was verified following each experiment via electrophoretic ejection of Chicago Sky Blue dye from the tip of the recording electrode (-20 µA constant current, 20 min). Rats were then overdosed with chloral hydrate and decapitated. The brains were removed and fixed for at least 48 hours (8% paraformaldehyde in PBS), cryoprotected (25% sucrose in PBS) until saturated, and sliced on a cryostat into 60µm sections, which were mounted onto gelatin-coated slides. Slides were stained with a mixture of cresyl violet and neutral red for verification of electrode sites with reference to a stereotaxic atlas (Paxinos and Watson, 2007).

Electrophysiological Recording Analysis

Three parameters were analyzed for DA neuron activity: (1) the average number of spontaneously active DA neurons encountered per electrode track ("population activity"), (2) average firing rate and (3) the percentage of spikes that occurred in bursts (%SIB). Analysis of firing rate and bursting activity was performed using NeuroExplorer (Plexon, Dallas, TX). Population activity was averaged within each animal and then across animals in each group, whereas the firing rate and burst activity of each neuron was counted as an independent replicate and averaged across animals in a group. Significance was assessed with a two-way ANOVA (MAM x Treatment or Condition x Treatment) followed by Tukey *post-hoc* comparisons using GraphPad Prism 8 (GraphPad Software, San Diego, CA). Three-way ANOVA (MAM x Treatment x Time) was completed with SAS (SAS Institute, Cary, NC) to examine effects of acute vs repeated treatment.

Novel Object Recognition

The novel object recognition (NOR) test was conducted in a rectangular test box (L70 x W40 x H30 cm). Each rat was habituated to the box for 10 minutes one day prior to the test. The

test day involved two 5 min trials separated by a 1 h intertrial interval. Rats received a randomized injection of either VEH (1 ml/kg) or POM (1 mg/kg or 3 mg/kg), i.p. 30 minutes prior to the first trial. In the first trial (T1), rats were placed in the box containing two identical objects. In the second trial (T2), one of the objects presented in T1 was replaced by a novel object. The familiar and novel objects were too heavy to be displaced by the animals and had different shape, color and texture. The box and the objects were cleaned between each trial. Habituation and behavioral tests were performed during the dark cycle. The behavior was recorded on video and object interaction was scored by an experimenter blinded to treatment group. Interaction time (s) of each object in each trial was recorded manually by the use of stopwatches and defined as time when the rat interacts directly with the object, such as licking, sniffing, or touching it with its forepaws. Recognition memory was assessed using the discrimination index (discrimination index = (novel – familiar / novel + familiar)), corresponding to the difference between the time exploring the novel and the familiar object, corrected for total time exploring both objects. Two-way ANOVA was used to compare the differences between MAM and SAL groups, drug treatment, and the possible interaction between MAM and treatment on behavioral measures. Tukey's *post-hoc* comparisons were conducted for significant main effects. All statistics were calculated using Graphpad Prism 8.

Restraint Stress

Rats were restrained for 2 h in custom made Plexiglas cylinder restraint tubes (internal diameter 6 cm with length adjusted to rat size), placed in a ventilated transport tub. Rats in the control condition remained in their home cage for 2 h. Rats were randomly assigned to receive vehicle (1 ml/kg) or POM (3 mg/kg), i.p., 30 min prior to the stress or control condition. A subset of POM-treated rats received pretreatment with LY34 (1 mg/kg, i.p.) 15 min prior to the POM

injection. In vivo electrophysiological recordings of DA neuron activity were performed in anesthetized rats immediately following the restraint/control condition.

3.3 RESULTS

3.3.1 Pomaglumetad Dose-Dependently Reduces DA Neuron Activity in MAM Rats

Electrophysiological recordings of DA neuron activity in the VTA were conducted from MAM rats and SAL rats that were pretreated with POM or VEH. VEH-treated MAM rats (n = 7 rats, 85 neurons) exhibited the anticipated elevation in population activity with an average of 1.8 ± 0.1 cells/track compared to VEH-treated SAL rats (n = 6 rats, 49 neurons), which had an average of 0.9 ± 0.1 cells/track (Fig. 3-1 A; 2-way ANOVA main effects: for MAM, $F(1,43) = 9.249$, $p = 0.004$; for POM, $F(3,43) = 3.005$, $p = 0.041$; for MAM-by-POM interaction: $F(3,43) = 18.21$, $p < 0.001$; post hoc MAM control vs SAL control: $p < 0.001$). POM significantly reduced DA neuron population activity in MAM rats, at 1 mg/kg (n = 6 rats, 68 neurons; post hoc MAM control vs MAM 1 mg/kg: $p = 0.019$), 3 mg/kg (n = 7 rats, 61 neurons; post hoc MAM control vs MAM 3 mg/kg: $p = 0.007$) and 10 mg/kg (n = 6 rats, 38 neurons; post hoc MAM control vs MAM 10 mg/kg: $p = 0.001$) compared to VEH-treated MAM rats. There was also a significant reduction in DA neuron population activity between 1 mg/kg and 10 mg/kg in MAM rats (post hoc MAM 1 mg/kg vs MAM 10 mg/kg: $p = 0.003$). In contrast, there was no reduction in DA neuron population activity in POM-treated SAL rats, at 1 mg/kg (n = 7 rats, 46 neurons) or 3 mg/kg (n = 6, 48 neurons), with a significant increase in population activity at 10 mg/kg (n = 6 rats, 58 neurons; post hoc SAL control vs SAL 10 mg/kg: $p = 0.010$), compared to VEH-treated SAL rats (Fig 3-1A). There was no significant change in firing rate (Fig. 3-1 B) or bursting (Fig. 3-1 C) with POM treatment compared to VEH treatment in MAM or SAL rats.

A separate cohort of MAM and SAL rats were treated with either acute or repeated POM or VEH (Fig 3-1 D; 3-way ANOVA main effects: for MAM $F(1,52) = 20.38$, $p < 0.001$; for POM, $F(1,52) = 14.82$, $p < 0.001$; for MAM-by-POM interaction: $F(1,52) = 11.82$, $p < 0.001$). Compared to their respective VEH-treated group, POM-treated MAM rats showed a significant reduction in DA neuron population activity for both acute POM (n = 6 rats, 52 neurons; $p = 0.002$) and repeated POM (n = 9 rats, 69 neurons; $p = 0.011$). There was no significant difference between MAM rats treated acutely vs repeatedly with POM ($p > 0.999$) or MAM rats treated acutely vs repeatedly with VEH ($p = 0.950$). There were no significant differences in SAL rat DA neuron population activity across treatment and time groups (Fig 3-1 D). There were also no significant differences in firing rate (Fig 3-1 E) or bursting activity (Fig 3-1 F) of the DA neurons across all groups.

The effect of acute POM administration on DA neuron activity was examined in female MAM (Fig 3-1 G; 2-way ANOVA main effects: for POM $F(1,26) = 12.64$, $p = 0.001$; for Estrous $F(1,26) = 9.213$, $p = 0.005$) and SAL rats split into proestrous/estrous or metestrous/diestrous groups based on cell composition and density from vaginal gavage based on previously described criteria. Female MAM rats in estrous/proestrous treated with VEH (n = 7 rats, 79 neurons) displayed significantly greater DA neuron population activity compared to female MAM rats in metestrous/diestrous (Fig 3-1 H; n = 7 rats, 53 neurons; $p = 0.037$). MAM rats in estrous/proestrous treated with POM (n = 8 rats, 48 neurons) displayed significantly fewer spontaneously active DA neurons compared to MAM rats in estrous/proestrous treated with VEH (Fig 3-1 H; $p = 0.012$). MAM rats in metestrous/diestrous treated with POM (n = 8 rats, 40 neurons) did not display a significant difference in DA neuron population activity compared to VEH (Fig 3-1 H; $p = 0.354$). Female SAL rats displayed a main effect of estrous stage (Fig 3-1 H; 2-way ANOVA main effects: for Estrous $F(1,25) = 8.411$, $p = 0.008$), but no main effect of POM treatment between SAL rats

in estrous/proestrous treated with VEH (n = 7 rats, 67 neurons) or POM (n = 7 rats, 60 neurons) and SAL rats in metestrous/diestrous treated with VEH (n = 7 rats, 30 neurons) or POM (n = 8 rats, 47 neurons). There were no significant differences in firing rate or bursting activity of DA neurons in female rats across conditions.

3.3.2 Intra-Ventral Hippocampal Infusion of Pomaglumetad is Sufficient to Reduce DA Neuron Activity in MAM Rats

Electrophysiological recordings of DA neuron activity in the VTA were conducted following infusion of either POM or VEH in the vHPC of MAM and SAL rats (Fig 3-2 A). MAM rats that received a VEH infusion (n = 7 rats, 75 neurons) displayed significantly higher DA neuron population activity compared to VEH-treated SAL rats (Fig 3-2 B; n = 7 rats, 51 neurons; 2-way ANOVA main effects: for MAM, $F(1,26) = 19.16$, $p = 0.002$; for POM, $F(1,26) = 13.17$, $p = 0.001$; post hoc MAM VEH vs SAL VEH: $p = 0.001$). MAM rats that received a POM infusion (n = 9 rats, 72 neurons) displayed a significant reduction in DA neuron population activity compared to untreated MAM rats ($p = 0.002$). POM treatment did not significantly affect DA neuron activity in SAL rats (Fig 3-2 B). There were no significant differences in the firing rate of DA neurons across groups (Fig 3-2 C) and a main effect of POM on % of spikes in burst (Fig 3-2 D; $F(1,224) = 5.191$, $p = 0.024$).

3.3.3 Pomaglumetad Increases Novel Object Recognition in MAM Rats

MAM rats pretreated with VEH displayed reduced interaction with a novel object compared to a familiar object, as measured by a discrimination index ((novel-familiar)/(novel+familiar)), compared to SAL rats treated with VEH (Fig 3-3 A; 2-way ANOVA main effects: for MAM: $F(2,48) = 8.505$, $p = 0.005$, for MAM-by-POM interaction: $F(2,48) =$

4.188, $p < 0.021$; post hoc MAM VEH vs SAL VEH: $p = 0.003$). MAM rats treated with 1 mg/ kgdid not significantly improve NOR compared to VEH, but MAM rats treated with 3 mg/kg showed a significant increase in discrimination index compared to VEH-treated MAM rats ($p = 0.035$). There was no significant effect of POM on discrimination index in SAL rats (Fig 3-3 A). MAM rats treated with 3 mg/kg SAL displayed a significant reduction in time exploring either object in the test phase compared to VEH-treated SAL rats (Fig 3-3 B; 2-way ANOVA main effect for POM: $F(2,48) = 9.118$, $p < 0.001$, post hoc SAL VEH vs SAL 3mg/kg: $p = 0.003$).

3.3.4 Pomaglumetad Blocks Restraint Stress-Induced Increase in DA Neuron Activity

Rats that were treated with VEH prior to acute restraint stress (n = 6 rats, 88 neurons) displayed a significant increase in DA neuron activity in the VTA in electrophysiological recordings performed immediately following stress (Fig. 3-4 A; 2-way ANOVA main effects: for Treatment, $F(2,30) = 8.799$, $p = 0.001$; for Stress, $F(1,30) = 35.360$, $p < 0.001$; for Treatment-by-Stress interaction: $F(2,30) = 5.765$, $p < 0.008$; post hoc VEH stress vs VEH control: $p < 0.001$), compared to rats that were treated with VEH and left in their home cage (n = 6 rats, 57 neurons). Rats that were treated with POM prior to acute restraint stress (n = 6 rats, 50 neurons) displayed a significant reduction in DA neuron population activity compared to stressed rats that were treated with VEH ($p < 0.001$). The effect of POM on DA neuron population activity in stressed rats was blocked by pretreatment with both POM and the mGluR2/3 antagonist LY34 (n = 6 rats, 64 neurons; $p = 0.02$). Rats that were stressed that received both POM+LY34 did not display a significant difference in DA neuron population activity compared to stressed rats that were treated with VEH ($p = 0.694$). There was no significant effect of treatment on DA neuron activity in rats that were kept in their home cage (Fig 3-4 A). There were also no significant effects in firing rate (Fig 3-4 B) or the % of spikes in burst (Fig 3-4 C).

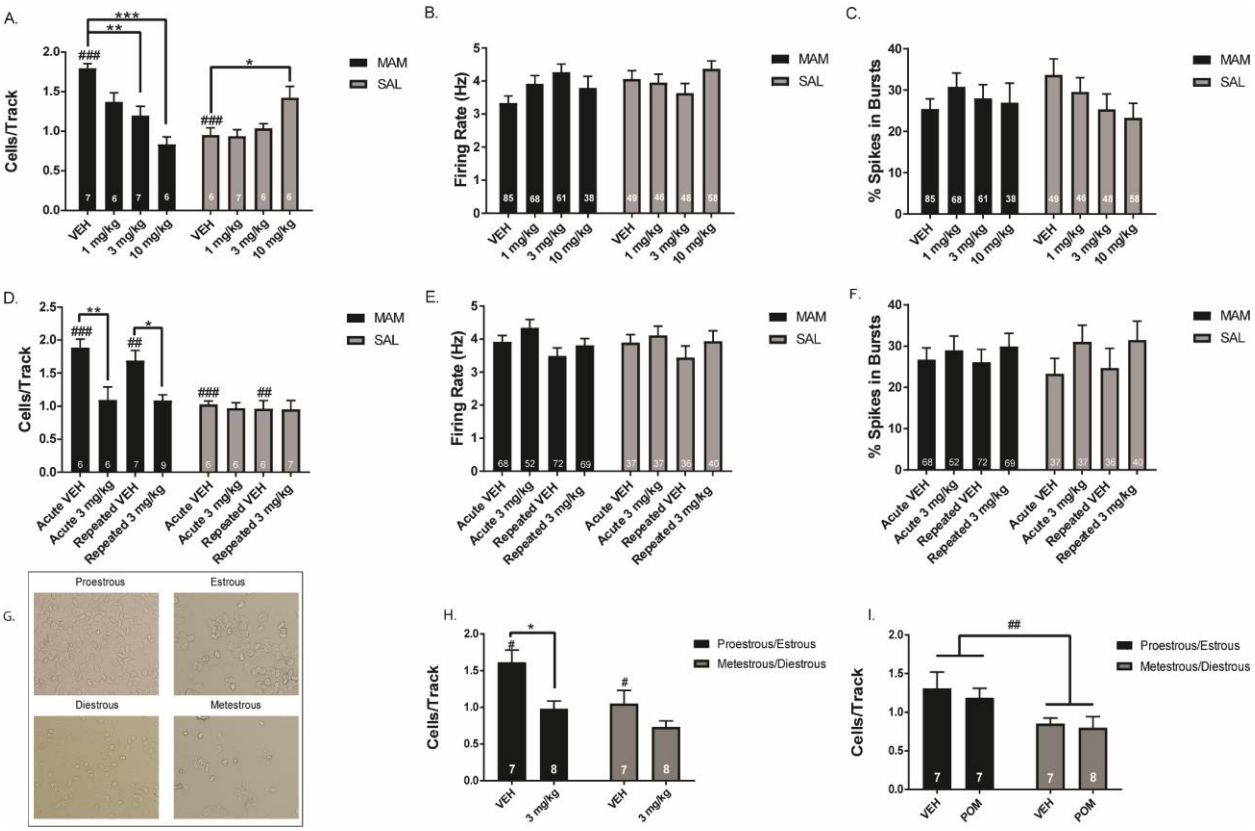

Figure 3-1: Pomaglumetad dose-dependently reduces DA neuron population activity in MAM rats

(A) 30 min following POM i.p., MAM rats displayed a dose depenent reduction in the number of spontaneously active DA neurons in the VTA compared to MAM rats that received VEH, which was not observed in SAL rats. There were no significant differences in the firing rate **(B)** or percentage of spikes in bursts **(C)** between POM and VEH administration in MAM or SAL rats. **(D)** There was no tolerance in the reduction in DA neuron population activity following 14d repeated POM treatment in MAM rats, compared to acute treatment. There were no significant differences in the firing rate **(E)** or percentage of spikes in bursts **(F)** following either acute or repeated treatment in MAM or SAL rats. **(G)** Example pictures of proestrous (high density of nucleated cells and conified cells), estrous (high density of cornified cells with leukocytes), metestrous (low density of cell debris, few cornified cells) and diestrous (low density of leukocytes and nucleated cells) **(H)** MAM rats displayed higher DA neuron population activity in estrous/proestrous compared to diestrous/metestrous. 30 min following POM i.p., MAM rats in estrous/proestrous displayed a reduction in DA neuon population activity **(I))** SAL rats displayed higher DA neuron population activity in estrous/proestrous compared to diestrous/metestrous with no significant effect of POM administration. *p<0.05 **p<0.01 ***p<0.001 within groups #p<0.05 ##p<0.01 ###p<0.001 between groups.

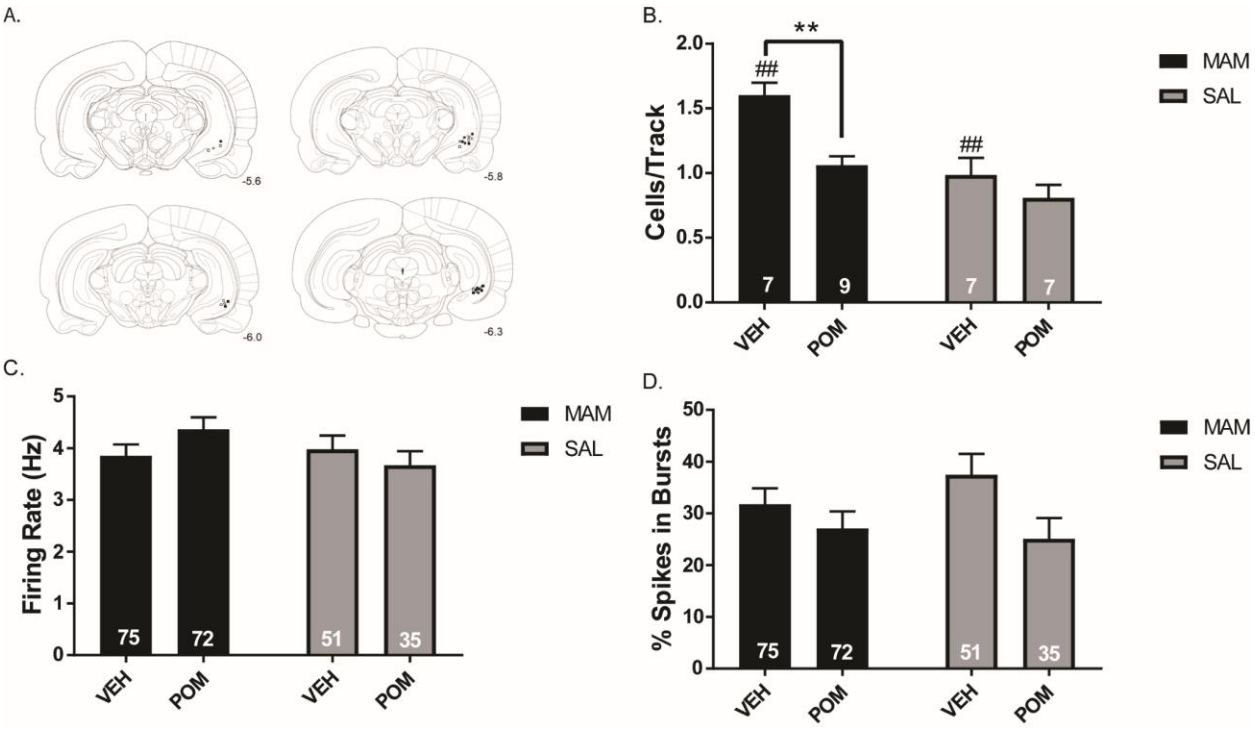

Figure 3-2: Intra-ventral hippocampal infusion of pomaglumetad is sufficient to reduce DA neuron activity in MAM rats

(A) Placements of cannula for vHPC infusion for MAM-VEH ▫ MAM-POM ■ SAL-VEH ○ SAL-POM ● **(B)** Infusion of POM in the vHPC was sufficient to reduce DA neuron population activity in MAM rats, which was not observed in SAL rats **(C-D)** no effect of intra-vHPC POM infusion on firing rate or the percentage of spikes in burst. *p<0.05 **p<0.01 ***p<0.001 within groups #p<0.05 ##p<0.01 ###p<0.001 between groups.

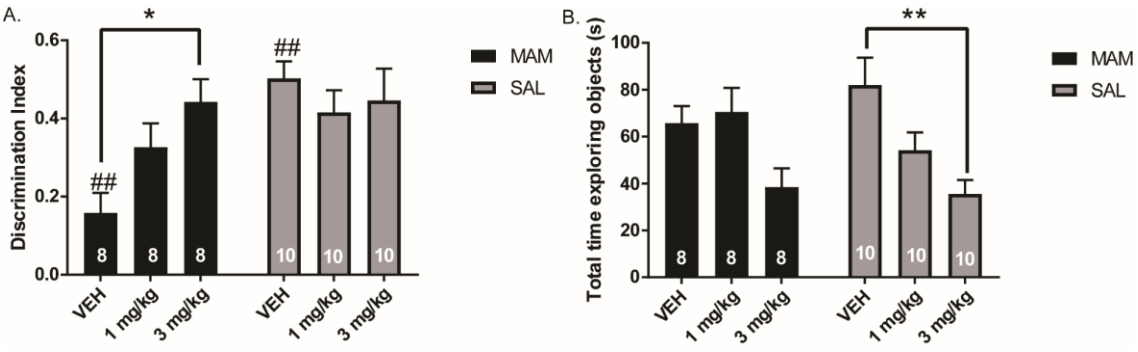

Figure 3-3: Pomaglumetad increases novel object recognition in MAM rats

(A) MAM rats treated with VEH, i.p. spent less time interacting with a novel object compared to a familiar object as measured by a discrimination index ((novel-familiar)/(novel+familiar)) compared to SAL rats. MAM rats treated with 3 mg/kg, i.p. POM spent more time investigating the novel object compared to VEH treated MAM rats. There was no effect of POM on discrimination index in SAL rats. **(B)** POM reduced locomotor activity, measured by total time exploring objects in the test phase, which was significantly reduced in SAL rats treated with 3 mg/kg POM, i.p., compared to VEH-treated SAL rats. *p<0.05 **p<0.01 ***p<0.001 within groups #p<0.05 ##p<0.01 ###p<0.001 between groups.

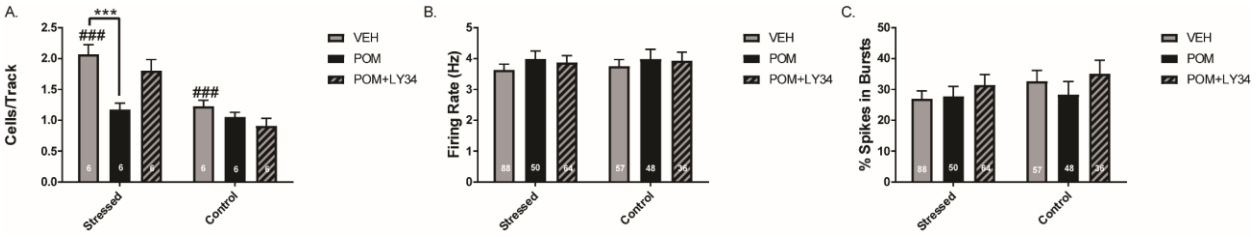

Figure 3-4: Pomaglumetad blocks restraint stress-induced increase in DA neuron activity

(A) Normal rats exposed to 2h acute restraint stress following VEH pretreatment displayed increased DA neuron population activity compared to VEH-treated rats left in their home cage for 2h. POM pretreatment (3 mg/kg, i.p.) normalized DA neuron population activity in rats that received restraint stress to control levels, which was not observed in rats that received both POM and the mGluR2/3 antagonist LY34 prior to restraint stress. **(B-C)** No significant changes were observed in DA neuron firing rate or percentage of spikes in bursts. *p<0.05 **p<0.01 ***p<0.001 within groups #p<0.05 ##p<0.01 ###p<0.001 between groups.

3.4 DISCUSSION

mGluR2/3 Agonists Regulate Dopamine Neuron Population Activity in MAM Rats

Our work demonstrates that the mGluR2/3 agonist, POM, decreases VTA DA neuron population activity in MAM rats via action in the vHPC. Previous studies that have examined the effects of mGluR2/3-targeting drugs on DA release have produced conflicting results. Some have shown that mGluR2/3 agonists do not affect striatal DA release at baseline (Moghaddam and Adams, 1998; Pehrson and Moghaddam, 2010), which is consistent with the lack of effect we observed, in general, on DA neuron population activity in SAL rats and normal rats in baseline conditions. Others have found that mGluR2/3 agonists can reduce striatal DA release (Hu et al., 1999,) and that mGluR2/3 *antagonist* administration can increase striatal DA release at baseline (Karasawa et al., 2006). In contrast, others have found that mGluR2/3 agonist administration produces an *increase* in striatal DA release at basal conditions (Ohno and Watanabe, 1995; Verma and Moghaddam, 1998). Differing effects may relate to differences in microdialysis procedure, the potency and specificity of the drug used, or drug dose. Though mGluR2/3 are primarily located presynaptically on glutamatergic neurons to negatively regulate excess glutamate release, there are also receptors located postsynaptically and on glial cells (Nicoletti et al., 2011), which can make dose an important factor (Jin et al., 2017). This may explain the observed significant increase in DA neuron population activity in SAL rats at the highest dose tested (10 mg/kg). Overall, our results indicate that mGluR2/3 agonists have the potential to reduce DA release through regulation of DA neuron population activity. However, this was only observed in states of increased DA neuron population activity driven indirectly by increased glutamate release from the vHPC.

We also observed a significant reduction in DA neuron population activity in POM-treated female MAM rats, but the effect was dependent on estrous cycle stage. In both MAM and SAL

rats, we observed differences in DA neuron population activity across the estrous cycle. In proestrous/estrous, and particularly in late proestrous/early estrous, we observed a significant increase in DA neuron population activity in MAM and SAL females compared to population activity in metestrous/diestrous. This is consistent with results observed by others in female MAM rats (Perez et al., 2014) and changes in DA release and DA-dependent behavior observed in normal female rats across the estrous cycle (Castner et al., 1993; Xiao and Becker, 1994). Interestingly, POM only significantly reduced the increased DA neuron population activity during proestrous/estrous in MAM rats.

Effects of mGluR2/3 Agonists Beyond the Hippocampus

Intra-vHPC infusion of POM was sufficient to reduce DA neuron population activity. The effects of POM were not tested in the NAc, but due to the glutamatergic projections that the NAc receives from the vSub (Floresco et al., 2001), there is a reasonable chance that an intra-NAc infusion of POM would produce a similar effect. We hypothesize that regulation of excess glutamate anywhere within the circuit that regulates DA neuron population activity would be sufficient to affect DA neuron activity.

Though the present study focused on the action of POM in the vHPC, following systemic treatment, POM would act in all brain regions that express mGluR2/3 receptors. mGluR2/3 receptors are located in numerous cortical and limbic brain regions, including the PFC and the amygdala (Ohishi et al., 1993b; Ohishi et al., 1993a; Neki et al., 1996). POM's regulation of glutamate release in these regions would have widespread effects on related circuits and behavior, and such circuit effects have the potential to affect other symptoms of schizophrenia (Fig. 3-5 A-B).

Behavioral Tests of mGluR2/3 Action

We found that pretreatment with POM dose-dependently improved the performance of MAM rats in NOR. MAM rats have previously been shown to display reduced NOR compared to SAL rats (Gomes and Grace, 2016), which may relate to impairment of HPC function. NOR is considered a working memory task when there is a short delay between the two trials. However, it has been shown to be HPC-dependent when there is a long delay, such as the 1 h used between trials in the present task (Hammond et al., 2004). We observed improvement in NOR despite the acute, dose-dependent decrease in spontaneous locomotor activity following POM administration (Rorick-Kehn et al., 2007). The reduction in locomotor activity, measured by total time exploring objects, only remained significant in the 2nd trial of SAL rats treated with the 3 mg/kg dose. Alterations in spontaneous locomotor behavior must be considered when interpreting locomotor-based behaviors, such as amphetamine induced hyperlocomotion. POM may reduce movement regardless of effects on DA release. Thus, it effects on baseline locomotor activity may confound behavioral results and thus must be accounted for by a normalized measure to baseline.

Prevention of Stress-Induced Increase in DA Neuron Activity

The effects of POM on normalizing increased DA neuron population activity were not specific to the MAM model, but also observed in normal rats exposed to restraint stress. It has previously been shown acute restraint stress increases vHPC activity, which then drives increased DA neuron population activity (Valenti et al., 2011a). Our results replicated this finding and, accordingly, pretreatment with POM prevented the restraint stress-induced increase in DA neuron activity. POM has similarly been found to reduce increased vHPC activity in an elevated plus maze task (Linden et al., 2004). POM's reduction of vHPC activity and DA neuron population activity

following stress may contribute to its anxiolytic-like effects found in other studies (Helton et al., 1998; Kłodzińska et al., 1999; Shekhar and Keim, 2000; Spooren et al., 2002; Linden et al., 2004).

Overall, our results show that POM can normalize DA neuron population activity in conditions of either transient or chronic pathological increased HPC activity and that its direct action in the vHPC is sufficient to produce this effect. However, it is important to note that our results do not indicate that its therapeutic action is identical to D_2-targeting APDs or that its potential therapeutic benefits are limited to treating symptoms related to DA system dysregulation. For example, some patients who are treatment resistant do not show increased striatal DA synthesis capacity or release, but do demonstrate elevated glutamate levels in the anterior cingulate (Demjaha et al., 2014; Mouchlianitis et al., 2015). Therefore, patients who do not respond to D_2-targeting APDs may benefit from a drug that regulates glutamate levels. Better understanding of how mGluR2/3 agonists function mechanistically, particularly in clinically-relevant states, may provide better insight into their potential as a therapeutic target.

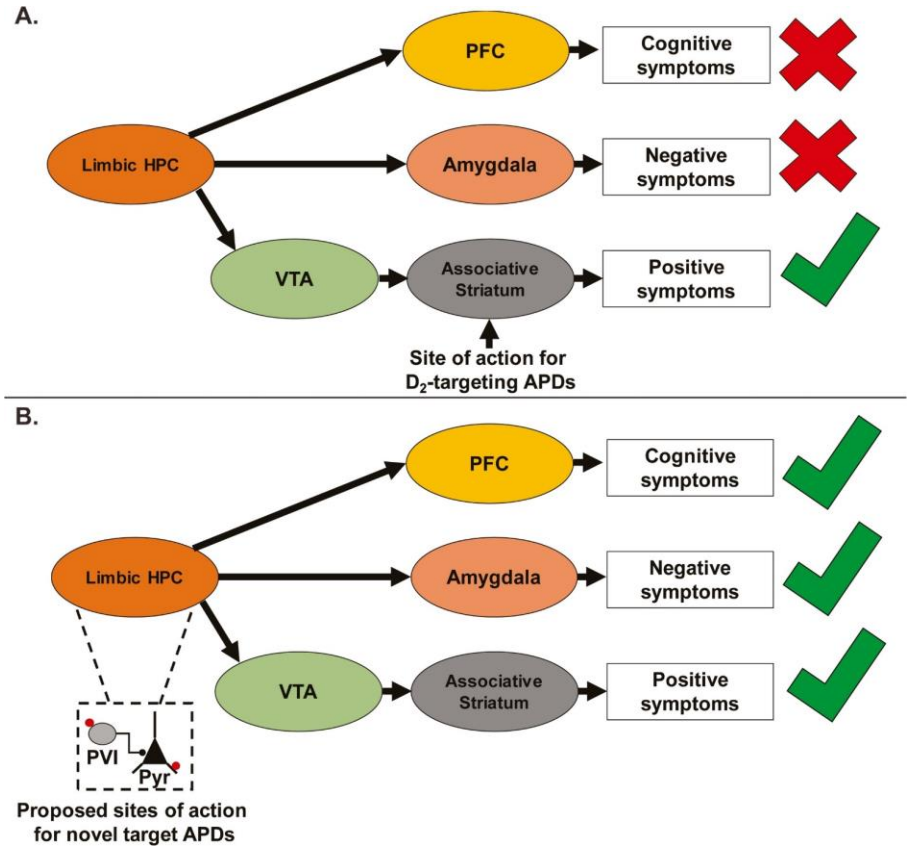

Figure 3-5: A proposed model for antipsychotic drug effects on symptom domains of schizophrenia

(A) In schizophrenia, a hyperactive and dysrhythmic limbic hippocampus (HPC) contributes to positive symptoms through its indirect regulation of DA activity in the ventral tegmental area (VTA), which projects to the associative striatum. It also contributes to negative and cognitive symptoms through its disruption of activity patterns with regions including the prefrontal cortex (PFC) and amygdala. All current antipsychotic drugs (APDs) act on D_2 receptors in the striatum, which results in a reduction in positive symptoms, but produce minimal benefit on cognitive symptoms or negative symptoms. **(B)** Compounds that reduce hypermetabolism in the limbic HPC by either increasing function of parvalbumin positive interneurons (PVI) or reducing the activity of pyramidal neurons (Pyr) should normalize hippocampal output and thereby may be more beneficial in treating all symptom domains of schizophrenia.

4.0 PERIPUBERTAL TREATMENT WITH MGLUR2/3 AGONIST PREVENTS DOPAMINE SYSTEM HYPERACTIVITY IN ADULTHOOD IN THE MAM MODEL OF SCHIZOPHRENIA

4.1 INTRODUCTION

Schizophrenia is a chronic and debilitating disorder that often emerges in late adolescence and early adulthood and is characterized by psychosis and a broad range of other cognitive and behavioral symptoms (Fatemi and Folsom, 2009; Tandon et al., 2013). A longer DUP is associated with a worse functional outcome, including poor response to APD treatment and greater risk of relapse (McGorry et al., 2009). Therefore, it is critical that those experiencing "first-episode psychosis" (FEP) receive rapid and effective treatment (Tandon et al., 2013). Existing treatments are effective in alleviating psychotic symptoms in many patients, but there remains an unmet need for early-intervention treatments that may improve symptoms long term by targeting early neuropathological processes (McGorry et al., 2009; Krystal and Anticevic, 2015)

The prodromal phase is characterized by a period of sub-clinical symptoms in the years prior to the onset of FEP. Based on a clinical staging model (Wood et al., 2011; Fusar-Poli et al., 2012), it consists of nonspecific symptoms such as depression, anxiety, and social withdrawal, followed by the emergence of attenuated psychotic symptoms, cognitive disturbances, and overall functional decline. Psychotic symptoms generally begin after puberty and are progressive in severity (Häfner et al., 1993). Accumulating research has suggested that puberty may serve a critical period surrounding the first episode and targeting pathophysiological processes during this time may provide long-term amelioration of symptoms. The maturational processes involved in puberty can shape brain development to support the alterations in physiological and behavioral

processes across adolescence (Sisk and Foster, 2004). When combined with genetic risk, these dynamic neurobiological changes are thought to contribute to the vulnerability of the developing brain to environmental factors that can lead to the emergence of psychiatric disorders. This may account for why adolescence is a period of peak onset for many psychiatric disorders, including schizophrenia (Kessler et al., 2005). Prior work with the MAM neurodevelopmental model, which demonstrates a number of phenotypes relevant to schizophrenia (Modinos et al., 2015), has shown that pharmacological treatments administered during puberty can prevent pathological phenotypes in adulthoods. Based on the critical role of stress in the development of schizophrenia (Corcoran et al., 2003; Holtzman et al., 2013), MAM rats were treated with daily administration of the anxiolytic drug diazepam peri-pubertally (PD 31–40), which prevented the emergence of the hyperdopaminergic state, anxiety-like behavior and the higher neuronal firing rates within the basolateral amygdala normally present in adult MAM rats (Du and Grace, 2013, 2016b). These findings indicate that alleviating anxiety and abnormal stress responsivity during puberty prevented the transition to the schizophrenia-like phenotype in adult MAM rats. Similarly, prodromal interventions may prevent the progression of psychosis in at-risk individuals (McGorry et al., 2009).

Patients with schizophrenia and animal models have demonstrated a reduction in PV+ GABA interneuron function, which is associated with an imbalance between excitatory and inhibitory neurotransmission (Lodge et al., 2009; Konradi et al., 2011; Lewis et al., 2012; Heckers and Konradi, 2015). Altered glutamatergic transmission in in the HPC, in particular, is prominently observed in the transition to psychosis (Schobel et al., 2013; Poels et al., 2014a). A hyper-metabolic state of the HPC, mimicked in an animal model with chronic ketamine exposure, was associated with PV+ GABAergic interneuron dysfunction, increased glutamate levels, and

reduction in volume in the HPC (Schobel et al., 2013). Based on human and animal model studies, these alterations appear to lead to increased subcortical DA function, especially in the associative striatum, which becomes progressively more pronounced as CHR individuals transition to psychosis, along with further loss of HPC volume (Lodge and Grace, 2007; Kegeles et al., 2010; Howes et al., 2011a). The protection of excitatory-inhibitory circuits within the HPC therefore demonstrates potential for preventative treatment against the emergence of DA system dysfunction.

Group II metabotropic glutamate receptors (mGluR2/3) showed promise in preclinical research as a target to treat the aberrant excitatory-inhibitory balance implicated as a central component of the development and pathophysiology of schizophrenia (Rorick-Kehn et al., 2007; Mezler et al., 2010). The mGluR2/3 pomaglumetad methionil (POM) was pulled from phase III clinical trials (Adams et al., 2014; Marek, 2015), though later analyses found that early-in-disease patients treated with POM demonstrated significant improvement in symptoms, which was not observed in late-in-disease patients (Kinon et al., 2015). This contributed to questions that have been raised in recent years about whether regulating glutamatergic dysfunction early in the disease may be particularly useful in treating symptoms (Krystal and Anticevic, 2015; Lieberman et al., 2019). Based on previous work (see Chapter 3) POM can indirectly regulate DA neuron activity by normalizing increased activity in the vHPC. We therefore aimed to determine whether of peripubertal administration of POM can prevent vHPC dysfunction and increased DA neuron population activity observed in adult MAM rats.

4.2 METHODS

Subjects

All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Timed pregnant Sprague-Dawley dams (Envigo, Indianapolis, IN) were obtained on gestational day (GD)15 and MAM (20 mg/kg, i.p., Midwest Research Institute, Kansas City, MO) or saline (SAL; 1 ml/kg, i.p.) was administered on GD17. Male and female pups were weaned on postnatal day (PD)23. Naïve adult male rats were obtained from Envigo for restraint stress experiments. All rats were housed in groups of 2-3 with littermates in a temperature (22°C) and humidity (47%)-controlled facility with ad libitum food and water in a normal 12-h light-dark cycle.

Drug Administration

LY2140023 (pomaglumetad methionil, POM), the prodrug of mGluR2/3 agonist LY404039, was obtained from Selleck Chemicals, Houston, TX and dissolved in 0.9% sterile saline with dropwise addition of 1M NaOH to POM (pH ~7) at a volume of 1 ml/kg. Vehicle (VEH)-treated rats received 0.9% sterile saline. Peripubertal treatments occurred from PD31-40. All rats were weighed daily and received 3 mg/kg POM (i.p.), 1 ml/kg vehicle (i.p.) or no injection.

Electrophysiological Recordings

In vivo extracellular recordings occurred on either PD 47-56 (“early adulthood”) or PD 83-96 (“late adulthood”). Rats were anesthetized with chloral hydrate (400 mg/kg; i.p.) and placed on a stereotaxic frame (Kopf, Tujunga, CA). Supplemental anesthesia was administered i.p. to maintain suppression of the hind limb withdrawal reflex. Body temperature was maintained at 37°C with a temperature-controlled heating pad (CWE Inc., Ardmore, PA). Single-barrel glass

electrodes (WPI, Sarasota, FL) were pulled vertically (PE-2, Narasige, Japan), broken under a microscope to an impedance of 6-8 MΩ, and filled with 2 M NaCl containing 2% Chicago Sky Blue dye in 2 M saline. Electrodes were lowered with a hydraulic micropositioner (Kopf) to sample neural activity in the region of interest. Single-unit activity was obtained using an amplifier (Fintronics, Orange, CT) using a highpass filter at 30 Hz and lowpass at 16 kHz. Neural activity was recorded for at least 1 minute of stable spontaneous activity using LabChart software (AD Instruments, Colorado Springs, CO). At the end of each recording, electrode placement was verified following each experiment via electrophoretic ejection of Chicago Sky Blue dye from the tip of the recording electrode (-20 µA constant current, 20 min). Rats were then overdosed with chloral hydrate and decapitated. The brains were removed and fixed for at least 48 hours (8% paraformaldehyde in PBS), cryoprotected (25% sucrose in PBS) until saturated, and sliced on a cryostat into 60µm sections, which were mounted onto gelatin-coated slides. Slides were stained with a mixture of cresyl violet and neutral red for verification of electrode sites with reference to a stereotaxic atlas (Paxinos and Watson, 2007).

Ventral Tegmental Area

Recordings were performed by making 6-9 vertical electrode passes ("tracks") in a predetermined grid pattern with each track separated by 0.2 mm (AP: -5.3 to -5.7 mm and ML: 0.6 to 1.0 mm from bregma, DV: -6.5 to -9.0 mm from the top of brain). DA neurons were classified based on established criteria, including a biphasic action potential with duration >2.2 ms, 1-10 Hz firing rate, and irregular and burst firing patterns with the bursts characterized by an interspike interval of 80-160ms (Grace and Bunney, 1983; Grace and Bunney, 1984; Ungless and Grace, 2012).

Ventral Hippocampus

Recordings were performed by making 6 electrode tracks as described above, with coordinates AP: -5.5 to -5.9 mm and ML: 4.6 to 5.0 mm from bregma, DV: -5.5 to -8.5 mm from the top of brain. Neurons with a wide biphasic action potential duration >2.0 ms and ≤2.0 Hz firing rate were classified as putative pyramidal neurons. Burst firing was characterized by at least two spikes with an interspike interval of 80-160ms.

Electrophysiological Recording Analysis

Three parameters were analyzed for DA neuron activity: (1) the average number of spontaneously active DA neurons encountered per electrode track ("population activity"), (2) average firing rate and (3) the percentage of spikes that occurred in bursts (%SIB). Firing rate and %SIB were analyzed for pyramidal neurons. Analysis of firing rate and bursting activity was performed using NeuroExplorer (Plexon, Dallas, TX). Population activity was averaged within each animal and then across animals in each group, whereas the firing rate and burst activity of each neuron was counted as an independent replicate and averaged across animals in a group. Significance was assessed with a two-way ANOVA (MAM x Treatment) followed by Tukey post-hoc comparisons.

Novel Object Recognition

Novel Object Recognition (NOR) was performed on PD 47-48 or PD 83-84. Each rat was habituated to a rectangular box (L70 x W40 x H30 cm) for 10 minutes one day prior to the test. The test day involved two 5 min trials separated by a 1 h intertrial interval. Rats received a randomized injection of either VEH (1 ml/kg) or POM (1 mg/kg or 3 mg/kg), i.p. 30 minutes prior to the first trial. In the first trial (T1), rats were placed in the box containing two identical objects. In the second trial (T2), one of the objects presented in T1 was replaced by a novel object. The familiar and novel objects were too heavy to be displaced by the animals and had different shape,

color and texture. The box and the objects were cleaned between each trial. Habituation and behavioral tests were performed during the dark cycle. The behavior was recorded on video and object interaction was averaged between two experimenters blinded to treatment group. Interaction time (s) of each object in each trial was recorded manually by the use of stopwatches and defined as time when the rat interacts directly with the object, such as licking, sniffing, or touching it with its forepaws. Recognition memory was assessed using the discrimination index (discrimination index = (novel – familiar / novel + familiar)), corresponding to the difference between the time exploring the novel and the familiar object, corrected for total time exploring both objects. Two-way ANOVA was used to compare the differences between MAM and SAL groups, drug treatment, and the possible interaction between MAM and treatment on behavioral measures. Tukey's post-hoc comparisons were conducted for significant main effects.

4.3 RESULTS

4.3.1 Peripubertal Pomaglumetad Treatment Prevents Increased DA Neuron Population Activity in Adult MAM Rats

Peripubertal (PD31-40) POM treatment significantly reduced DA neuron population activity in the VTA of rats recorded from PD 47-56 or "early adulthood" (Fig. 4-1 A; 2-way ANOVA main effects: for Treatment, $F(2,38) = 4.801$, $p = 0.014$; post hoc VEH MAM vs POM MAM: $p = 0.020$) and from PD 83-96 or "late adulthood" (Fig. 4-1 D; 2-way ANOVA main effects: for MAM: $F(1,35) = 17.29$, $p < 0.001$, MAM-by-POM interaction: $F(2,35)=4.518$, $p = 0.018$; post hoc VEH MAM vs POM MAM: $p = 0.022$). There was no significant difference in DA

neuron population activity between VEH and No Injection (NI) in MAM or SAL rats in early (Fig. 4-1 A) or late (Fig. 4-1 D) adulthood. In late adulthood, VEH treated MAM rats displayed significantly greater DA neuron population activity compared to VEH treated SAL rats ($p = 0.0138$) and NI MAM rats displayed significantly greater DA neuron population activity compared to NI SAL rats ($p = 0.020$). There was no significant difference of peripubertal POM treatment in SAL rats in early (Fig 4-1 A) or late (Fig. 4-1 D) adulthood. There was no significant difference in firing rate or percentage of spikes in burst of DA neurons in early adulthood (Fig 4-1 B-C) or late adulthood (Fig. 4-1 E-F).

4.3.2 Reduced Novel Object Recognition Only in Adult MAM Rats That Did Not Receive Injections During Puberty

No significant effects were observed in the NOR task in MAM or SAL rats across treatment groups, both in discrimination index (Fig. 4-2 A) and total time exploring the objects (Fig. 4-2 B). A main effect of MAM was observed in rats that were treated during puberty and tested in the NOR task during late adulthood (Fig 4-2 C; $F(1,59) = 12.67$, $p < 0.001$). Late adulthood MAM rats that received peripubertal POM treatment displayed significantly increased discrimination index compared to MAM rats that received NI during puberty (Fig 4-2 C; $p = 0.031$), though it was not significantly different from MAM rats that received VEH during puberty (Fig 4-2 C). Late adulthood NI-MAM rats displayed significantly lower NOR compared to NI-SAL rats ($p = 0.007$), but VEH-MAM rats did not show a significant difference in NOR compared to VEH-SAL rats (Fig. 4-2 C). A main effect of treatment was observed in total time exploring objects in late adulthood (Fig. 4-2 D; 2-way ANOVA main effect for treatment: $F(2,53) = 3.633$, $p = 0.0332$.

4.3.3 Peripubertal Pomaglumetad Treatment Prevents Increased Firing Rate of Ventral Hippocampal Pyramidal Neurons in Adult MAM Rats

MAM rats that were treated with VEH or NI during puberty and recorded during early adulthood displayed significantly increased firing rate of pyramidal neurons in the vHPC compared to SAL rats that were treated with VEH or NI (Fig. 4-3 A; 2-way ANOVA main effects for: MAM: $F(1,219) = 36.54$, $p < 0.001$, MAM-by-POM interaction: $F(2,219) = 5.782$, $p = 0.004$; post hoc, MAM-VEH vs SAL VEH $p < 0.001$, MAM-NI vs SAL-NI $p < 0.001$). Peripubertal POM treatment in MAM rats significantly reduced the firing rate of pyramidal neurons in the vHPC compared to vHPC of VEH-treated MAM rats ($p = 0.035$) and NI-MAM rats ($p = 0.001$). There was no significant difference in the firing rate of vHPC pyramidal neurons between NI and VEH treatment in MAM rats and no significant difference in firing rate across all treatment groups in SAL rats (Fig 4-3 A). NI-MAM rat vHPC pyramidal neurons demonstrated significantly increased bursting activity compared to POM-MAM rats ($p = 0.015$) and there were no significant differences in bursting activity in SAL rats recorded during early adulthood (Fig 4-3 B).

MAM rats that were treated with VEH or NI during puberty and recorded during late adulthood displayed significantly increased firing rate of pyramidal neurons in the vHPC compared to SAL rats that were treated with VEH or NI (Fig. 4-3 C; 2-way ANOVA main effects for: MAM: $F(1,254) = 23.65$, $p < 0.001$, MAM-by-POM interaction: $F(2,254) = 3.678$, $p = 0.027$; post hoc, MAM-VEH vs SAL VEH $p = 0.002$, MAM-NI vs SAL-NI $p = 0.004$). Peripubertal POM treatment in MAM rats significantly reduced the firing rate of pyramidal neurons in the vHPC compared to vHPC of VEH-treated MAM rats ($p = 0.035$) and NI-MAM rats (Fig. 4-3 C; $p = 0.001$). No significant differences were observed in bursting activity in MAM or SAL rats recorded during late adulthood (Fig 4-3 D).

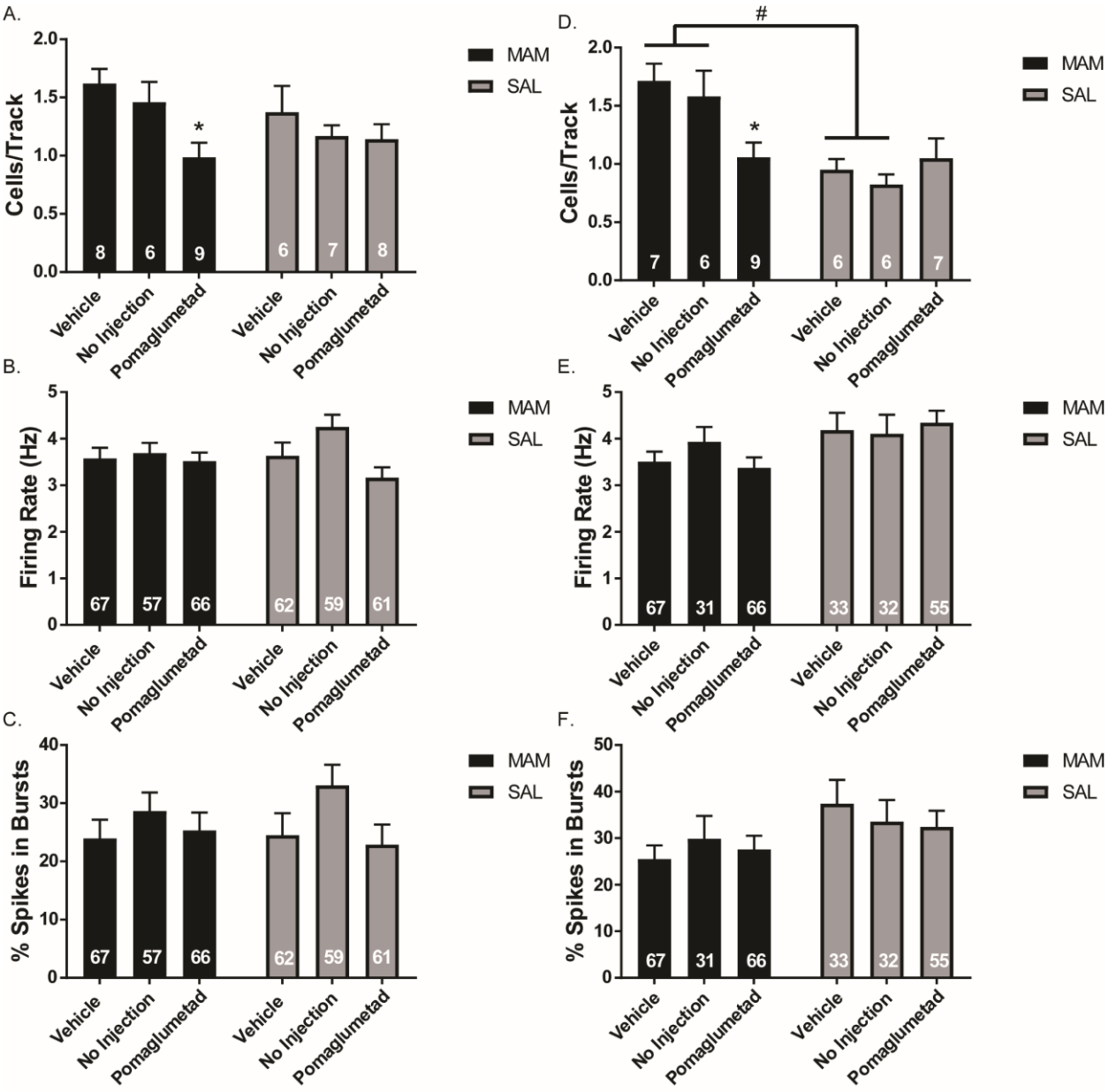

Figure 4-1: Peripubertal pomaglumetad treatment prevents increased DA neuron population activity in adult MAM rats

(A) MAM and SAL rats were treated during puberty (PD 31-40) and electrophysiological recordings of DA neuron activity in the VTA were performed in early adulthood (PD 47-56). POM-treated MAM rats displayed reduced DA neuron population activity in the VTA compared to vehicle and and no injection-treated MAM rats. There were no significant differences in the firing rate **(B)** or bursting activity **(C)** of the DA neurons in MAM or SAL rats in early adulthood. **(D)** In rats that were treated during puberty (PD 31-40) and recorded in late adulthood (PD 83-96), POM-treated MAM rats displayed reduced DA neuron population activity in the VTA compared to vehicle and and no

injection-treated MAM rats. There were no significant differences in the firing rate **(E)** or bursting activity **(F)** of the DA neurons in MAM or SAL rats in late adulthood. *p<0.05 **p<0.01 ***p<0.001 within groups #p<0.05 ##p<0.01 ###p<0.001 between groups.

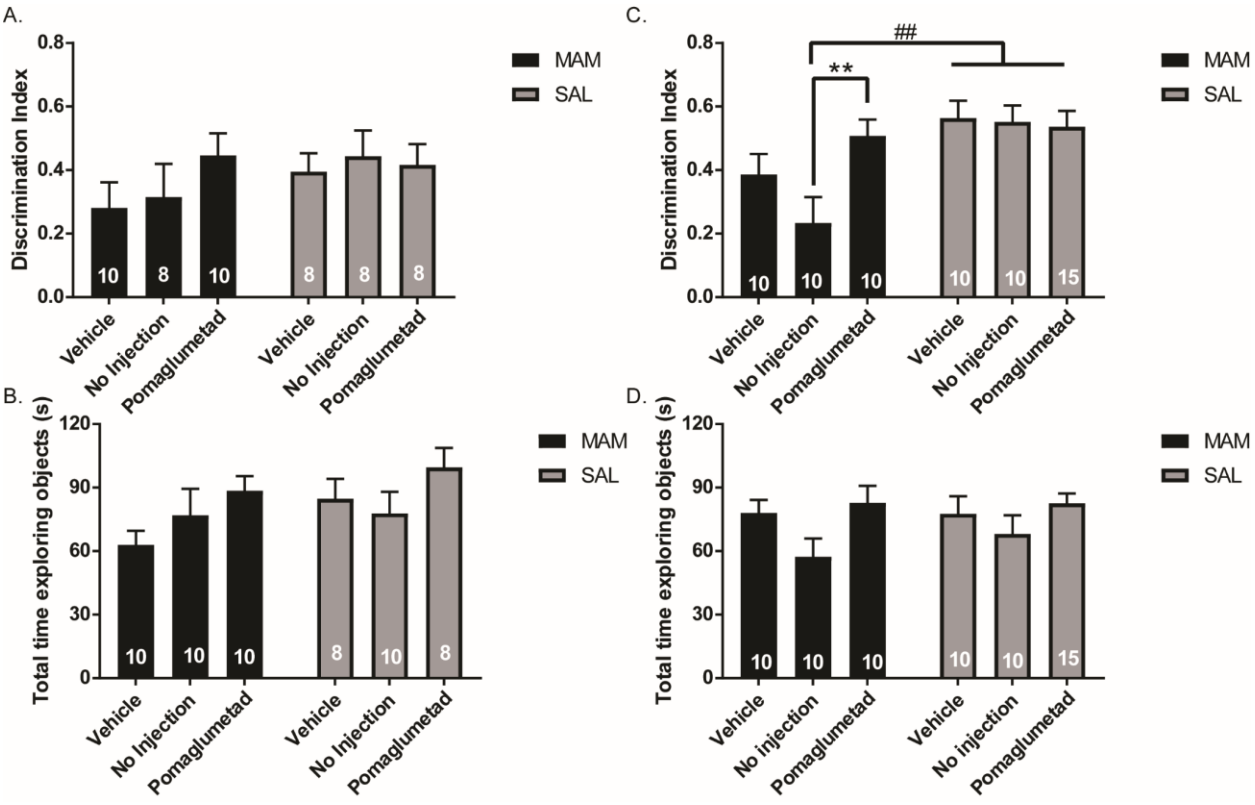

Figure 4-2: Reduced novel object recognition only in adult MAM rats that did not receive injections during puberty

(A) In early adulthood (PD 47-48), MAM and SAL rats that were treated during puberty (PD 31-40) with POM, vehicle, or no injection, displayed no differences in time interacting with a novel object compared to a familiar object as measured by a discrimination index ((novel-familiar)/(novel+familiar)). **(B)** In early adulthood MAM and SAL rats treated during puberty, there were no significant differences in locomotor activity as measured by total time spent exploring the objects. **(C)** In rats that were treated during puberty and tested in late adulthood (PD 83-84), POM-treated MAM rats displayed an increase in time spent exploring the novel object as measured by the discrimination index compared to no injection-treated MAM rats, though not significantly reduced compared to vehicle-treated MAM rats. **(D)** No significant differences were observed in total time spent exploring the objects in

MAM and SAL rats treated during puberty and tested in late adulthood. *p<0.05 **p<0.01 ***p<0.001 within groups #p<0.05 ##p<0.01 ###p<0.001 between groups.

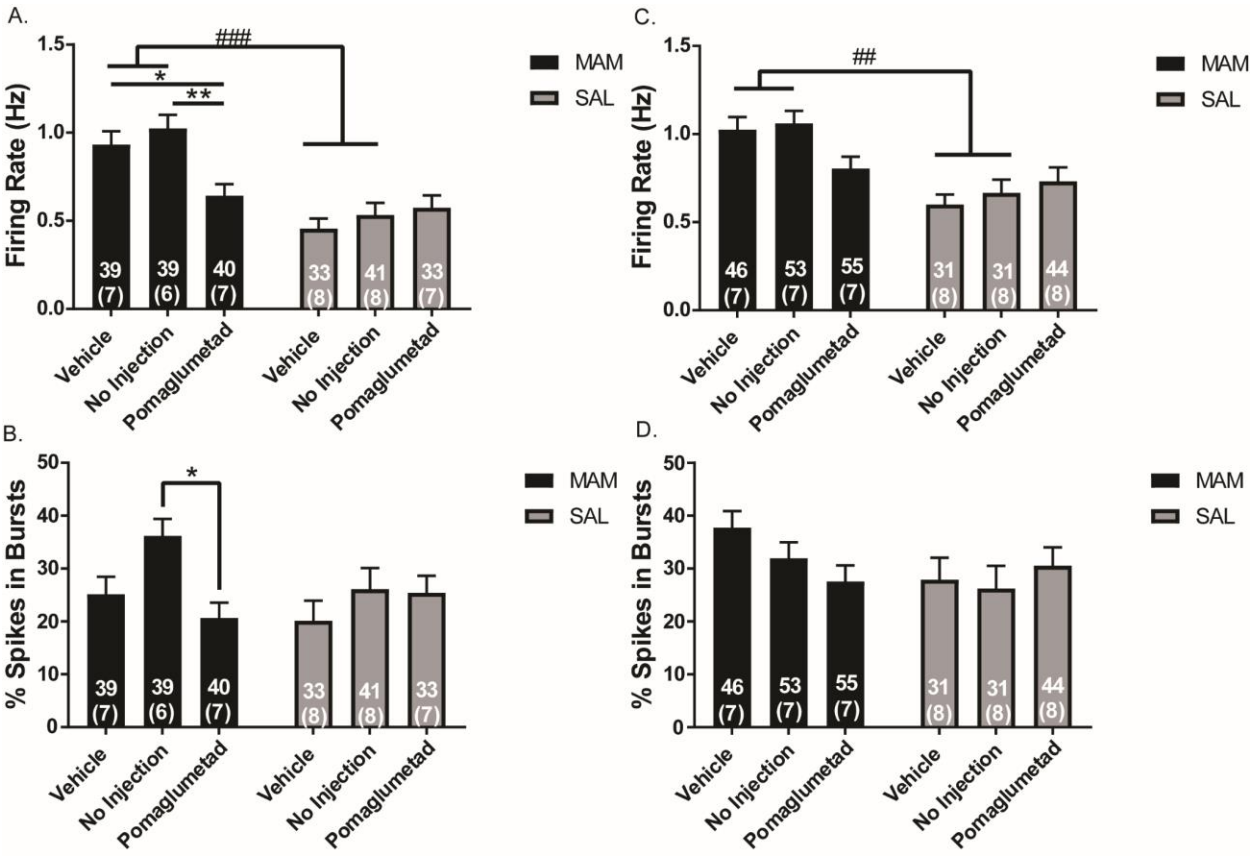

Figure 4-3: Peripubertal pomaglumetad treatment prevents increased firing rate of ventral hippocampal pyramidal neurons in adult MAM rats

(A) MAM and SAL rats were treated during puberty (PD 31-40) and electrophysiological recordings of the vHPC were performed in early adulthood (PD 47-56). Vehicle and no injection-treated MAM rats displayed increased firing rate of putative pyramidal neurons compared to vHPC pyramidal neuron firing rate in SAL rats, which was normalized in MAM rats that received POM treatment during puberty. **(B)** MAM rats that did not receive an injections during puberty showed a significant increase in percentage of spikes in bursts in early adulthood, which was not observed in MAM rats treated with POM during puberty. **(C)** Vehicle and no injection-MAM rats treated during puberty (PD 31-40) and recorded in late adulthood (PD 83-96) also displayed increased vHPC pyramidal neuron firing rate compared to vehicle and no injection-SAL rats. MAM rats treated with POM during puberty had

an average pyramidal neuron firing rate not significantly different from SAL rats. **(D)** No significnat differences in the percentage of spikes in burst in vHPC pyramidal neurons recorded in MAM and SAL rats at late adulthood. *p<0.05 **p<0.01 ***p<0.001 within groups #p<0.05 ##p<0.01 ###p<0.001 between groups.

4.4 DISCUSSION

Although APDs alleviate positive symptoms in many patients with schizophrenia, they are hindered by adverse effects, limited efficacy, and high rates of treatment noncompliance (Kane et al., 1988; Fleischhacker et al., 1994; Conley and Buchanan, 1997; Lieberman et al., 2005). The overall poor functional outcome of many patients (Green et al., 2000) necessitates the development of improved treatment options. All current APDs produce their therapeutic effect by blocking the D_2 receptor (Kapur and Remington, 2001) and there is an unmet need for different mechanisms of action for patients who do not respond to current treatments. One option is to target excitatory-inhibitory dysfunction, which has been proposed to be a more central source of pathophysiology (Lewis et al., 2005; Lodge and Grace, 2011; Moghaddam and Javitt, 2012), upstream of DA dysfunction (Lodge and Grace, 2007). This dysfunction is observed during the prodromal period (Pantelis et al., 2007; Schobel et al., 2013; Allen et al., 2017), and it is also present in patients who are treatment non-responders (Demjaha et al., 2014; Mouchlianitis et al., 2015; Demjaha et al., 2017). DUP is associated with worse functional outcome (Perkins et al., 2005), and so an ideal approach would be to target the neurophysiological processes that lead to psychosis as early as possible.

Previous work in the lab has examined diazepam as a pharmacotherapy during the peripubertal period in the MAM model as a potential preventative treatment. Peripubertal

diazepam administration prevented increased activity in the amygdala, preserved PV+ interneurons in the HPC, and ultimately resulted in long lasting normalization of DA neuron activity in adult MAM rats (Du and Grace, 2013, 2016a, b). Benzodiazepines are generally not a practical option for long-term use as a preventative treatment for psychosis due to issues including tolerance and withdrawal effects (Busto et al., 1986; Longo and Johnson, 2000). However, it is an important proof-of-concept that targeting excitatory-inhibitory processes prior to the onset of DA dysfunction may improve long term outcomes.

A promising drug target that has been shown to regulate glutamate release is the mGluR2/3 (Conn and Pin, 1997). mGluR2/3 agonists have previously been investigated in clinical trials as a novel potential APD. The mGluR2/3 agonist, POM, was shown to be well tolerated and did not possess the side effects associated with current APD treatment, such as weight gain (Adams et al., 2013). However, it was pulled from phase III clinical trials for lack of APD efficacy compared to current APDs (Stauffer et al., 2013; Adams et al., 2014; Marek, 2015). However, later analyses found that it was, in fact, effective in patients early in the disease, though not in patients who had schizophrenia for more than 10 years (Kinon et al., 2015). This finding indicated that further research on this mechanism of action, or other excitatory-inhibitory targets, is necessary. It also emphasized the importance of disease phase as a factor in clinical trials that can play a substantial role in heterogeneity of treatment response, whether due neurophysiological changes in the pathophysiology over time (Krystal and Anticevic, 2015) or other potential confounding variables, such as prior chronic APD treatment (Gill et al., 2014). Our previous work demonstrated that mGluR2/3 agonist POM can act in the vHPC to indirectly regulate DA neuron activity, likely by normalizing increased vHPC activity through its action on presynaptic receptors to negatively

modulate glutamate release (see Chapter 3). In this study, we examined its potential as a preventative treatment against DA system dysfunction in the MAM model.

Peripubertal mGluR2/3 Agonist Administration Prevents HPC-DA Dysfunction in MAM Rats

We found that peripubertal (PD 31-40) administration of POM prevented several of the adult phenotypes of the MAM model, including increased DA neuron population activity in the VTA and increased vHPC pyramidal neuron activity, potentially through preservation of vHPC PV+ interneurons. Previous studies have shown that the increased DA neuron population activity observed in adult MAM rats is indirectly driven by the vHPC, which shows increased pyramidal neuron firing rate in MAM rats, in addition to a loss in PV+ interneurons (Lodge and Grace, 2006b, 2007; Lodge et al., 2009). Accordingly, treatments that reduce vHPC activity have been shown to reduce DA neuron activity in MAM rats (Gill et al., 2011; Gastambide et al., 2012; Perez and Lodge, 2013; Perez et al., 2013).

These findings are consistent with clinical studies that have found increased glutamate, CBF, and CBV to be correlated with severity of psychotic symptoms conversion to psychosis in CHR patients (Harrison, 2004; Schobel et al., 2009; Allen et al., 2017). Severity of psychotic symptoms has been shown in other studies to correlate with measures of increased presynaptic DA function (Laruelle et al., 1999; Howes et al., 2011a), which is also shown to increase in individuals who transition to psychosis (Howes et al., 2011b; Egerton et al., 2013), and correlate with changes in HPC glutamate levels (Stone et al., 2010). Together, these studies fit a model in which increased HPC glutamate levels drive increased presynaptic DA function, leading to increased severity of psychotic symptoms.

This leads to the question of how early intervention of the increased glutamatergic activity may produce long-lasting changes in preventing DA neuron hyperactivity. Puberty is a critical

time in the stabilization of PV+ interneuron connections within the HPC through the formation of PNNs, which is thought to underlie the closure of critical periods in development (Hensch, 2005; Cabungcal et al., 2013). Stress during puberty, particularly in those with a predisposition to increased stress responsivity, may lead to loss of PV+ neurons during this time and subsequent dysregulation of pyramidal neuron activity (Gomes et al., 2019a). MAM offspring display increased stress responsivity, prior to the manifestation of a hyperdopaminergic state (Moore et al., 2006; Zimmerman et al., 2013), which has led to the proposal that treatments aimed at reducing anxiety may prevent the deleterious effects of stress on the DA system during this critical time in development (Gomes et al., 2016). Increased limbic HPC drive, leading to local oxidative stress (Do et al., 2015), excitotoxicity (Plitman et al., 2014), and overall reduced adaptive functioning in related circuits, may create an accumulating pattern of circuit disruption that ultimately leads to psychosis through its downstream effects on the DA system. Intervening in the accumulation of glutamate dysfunction at these early stages (Schobel et al., 2013) may prevent further loss of PV+ interneurons during this period.

The Role of Stress in Early Intervention for Psychosis

mGluR2/3 agonists have been shown to have anxiolytic potential in both animal models and humans. They demonstrate anxiolytic-like activity in rodents in tasks including stress-induced hyperthermia and the elevated plus maze (Helton et al., 1998; Kłodzińska et al., 1999; Shekhar and Keim, 2000; Spooren et al., 2002; Linden et al., 2004). In humans, they have been shown to reduce fear-potentiated startle without sedative effects (Grillon et al., 2003) and demonstrated therapeutic potential for patients with panic disorder (Tizzano et al., 2002) and generalized anxiety disorder (Dunayevich et al., 2008). It is also of note that the effects observed in the present study were present even following the stress of repeated i.p. injections during puberty. Activation of

mGluR2/3 in the amygdala may play a role in its regulation of fear and anxiety states. Infusion of an mGluR2/3 agonist into the BLA disrupts fear-potentiated startle in rats. (Walker and Davis, 2002). These results suggest that mGluR2/3 activation may in part suppress anxiety by inhibiting projection neurons within the BLA. We have previously shown that POM can reduce DA neuron population activity in MAM rats through action in the vHPC. It would need to be confirmed whether it can reduce BLA activity similar to other mGluR2/3 agonists. If so, it would be interesting to determine whether its action in the BLA during peripubertal treatment is necessary for its long-term effects on DA neuron activity in adulthood, or whether reducing vHPC activity alone is sufficient.

Potential Intermediate Phenotypes in the Neurodevelopment of the MAM Model

MAM rats treated during puberty with VEH or NI and tested during early adulthood displayed increased firing rate of pyramidal neurons in the vHPC, but no reduction in NOR, as previously observed in adult MAM rats (Gomes and Grace, 2016). Additionally, their DA neuron population activity was not significantly different from VEH and NI-treated SAL rats recorded in early adulthood. However, MAM rats treated in the same cohorts and tested during late adulthood displayed both increased vHPC pyramidal neuron firing rates and increased DA neuron population activity. Only NI-MAM rats displayed reduced NOR in late adulthood, and all rats were handled equally, suggesting that repeated injections during puberty, regardless of POM or VEH, may improve NOR in adult MAM rats. Stress during puberty can produce mixed results on memory-related behavior (Chaby et al., 2015b)and has been shown to result in greater adaptation to stressful experiences in adulthood, such as increased foraging behavior under threat (Chaby et al., 2015a), consistent with the concept of stress inoculation (Brockhurst et al., 2015; Meichenbaum, 2017). It is therefore possible that the mild stress of i.p. injections during puberty may have improved their

performance in the task. Overall, these results suggest that the late puberty/early adulthood period captured during PD 47-56 may have represented an intermediate phenotype in which the increased vHPC pyramidal neuron activity was present prior to increased DA neuron population activity, which emerged in late adulthood. It is unclear to what extent the transition to postpubertal MAM hyperactive DA system phenotype is sudden or gradual, though based on these results, it appears to follow the onset of increased vHPC pyramidal neuron activity.

Overall, this study demonstrates that peripubertal administration of an mGluR2/3 agonist can produce long lasting effects in the MAM model, including normalizing vHPC pyramidal neuron activity and DA neuron population activity in the VTA. These results provide support for the potential of glutamatergic regulation as an early-stage preventative treatment against the emergence of DA dysfunction in schizophrenia.

5.0 GENERAL DISCUSSION

Portions of Chapter 1 are adapted from:

Sonnenschein, S. F., & Grace, A. A. (2019). Insights on current and novel antipsychotic mechanisms from the MAM model of schizophrenia. *Neuropharmacology*. doi: 10.1016/j.neuropharm.2019.05.009.

5.1 SUMMARY OF FINDINGS

The studies described in this dissertation reveal state-dependent neuropharmacological effects in the regulation of DA neuron activity using the D_2 partial agonist, ARI, and the mGluR2/3 agonist, POM, highlighting the importance of using disease-relevant models in preclinical research. Additionally, we found that peripubertal administration of POM prevented the emergence of increased pyramidal neuron activity in the vHPC and increased DA neuron population activity in adult MAM rats, suggesting that mGluR2/3 may be a promising target as a preventative treatment against psychosis in patients.

In Chapter 2, we presented evidence that ARI administration reduces the number of spontaneously active DA neurons in MAM rats, which remained stable following 1-week withdrawal from repeated ARI administration, similar to previous observations following withdrawal from repeated D_2 receptor antagonist treatment (Gill et al., 2014). However, unlike D_2 receptor antagonists (Valenti et al., 2011; Gill et al., 2014), neither acute nor repeated ARI administration showed an effect on DA neuron population activity in normal rats. Additionally, low doses of apomorphine either failed to restore, or further reduced, DA neuron population activity in MAM rats, suggesting that it did not act via depolarization block. ARI was also shown

to reverse depolarization block in haloperidol-treated MAM rats, resembling depolarization block reversal following low-dose DA agonist administration (Valenti et al., 2011), and consistent with distinct effects that are dependent on the state of the DA system.

In Chapter 3, we demonstrated that POM could dose-dependently reduce DA neuron population activity in MAM rats and that intra-vHPC infusion of POM was sufficient to produce this effect. POM also increased novel object recognition in MAM rats and blocked stress-induced increases in DA neuron population activity in normal rats. However, these effects were only observed in conditions with increased vHPC activity, such as in MAM rats, or following restraint stress, but they were not observed in normal rats or baseline conditions, consistent with previous studies demonstrating no effect of mGluR2/3 agonists on DA levels in normal rats (Verma and Moghaddam, 1998; Pehrson and Moghaddam, 2010). This study reveals a novel role of mGluR2/3 agonists in the regulation of DA neuron activity that may contribute to its potential therapeutic effects. Once again, distinct effects were observed in a hyperdopaminergic vs baseline state; however, in contrast to ARI, the state-dependent effects of POM on DA system regulation appeared to require the presence of increased vHPC activity.

In Chapter 4, we found that MAM rats treated with POM during puberty (PD 31-40) demonstrated normalized DA neuron activity and vHPC pyramidal neuron activity when examined at early and late adulthood timepoints. In early adulthood, control MAM rats demonstrated significantly increased pyramidal neuron activity in the vHPC, but no main effect of MAM on DA neuron activity or novel object recognition. This may represent an intermediate-phenotype in neurodevelopment of the MAM model; however, even at this timepoint, the pyramidal neurons in the vHPC showed increased firing rate that was normalized by peripubertal POM treatment. No

significant effects of treatment were observed in SAL rats at any time point or experimental assay, consistent with previous observations that the effects of POM are state-dependent.

5.2 TECHNICAL CONSIDERATIONS

5.2.1 Anesthesia

All of the electrophysiological recordings described were performed with chloral hydrate anesthesia. Anesthesia inevitably alters neural activity compared to an awake/behaving state, particularly in brain regions related to arousal and cognition (Brown et al., 2011). Chloral hydrate was used in these experiments due to its minimal effects on DA neuron firing properties compared to the awake state (Bunney and Grace, 1978; Chiodo and Bunney, 1985). Its extensive use in similar studies of DA neuron activity in the VTA , in addition to its use in the recordings of other brain regions, including the BLA (Rosenkranz and Grace, 2001, 2002; Du and Grace, 2016b), mPFC (Lavin et al., 2005), thalamus (Lavin and Grace, 1998; Zimmerman and Grace, 2018), ventral pallidum (Lavin and Grace, 1998), NAc (Belujon and Grace, 2014), and HPC (Lodge and Grace, 2007), allows for comparison between studies. Nonetheless, it cannot be assumed to be equivalent to neural activity in the awake state and results must be interpreted with this in mind.

5.2.2 Depolarization Block

Depolarization block has been observed across numerous studies in rats, but questions remain about the consistency and consequences of the phenomenon. Some have argued that it may be an artifact of anesthesia (Mereu et al., 1995; Melis et al., 1998). However, chloral hydrate, the anesthesia used for DA neuron recordings, normally blunts the excitatory response of DA neurons

to APDs (Bunney et al., 1973) and depolarization block has been observed in nonanesthetized, paralyzed rats (Bunney and Grace, 1978; Chiodo and Bunney, 1985). There have also been inconsistencies in the effect of depolarization block on striatal DA release. Some have reported a reduction in basal DA release that corresponds with depolarization block (Blaha and Lane, 1987; Lane and Blaha, 1987; Moore et al., 1998), whereas others have not found a difference in striatal DA levels (Ichikawa and Meltzer, 1991; Moghaddam and Bunney, 1993). However, the lesion produced from microdialysis probe insertion was later found to disrupt depolarization block, and the issue can be avoided by implanting the microdialysis probe prior to APD administration (Moore et al., 1998). The long-term changes to DA neuron activity following APD withdrawal are also unclear and require further investigation. DA neuron activity remains reduced for at least a week following repeated D_2 antagonist administration in both MAM and SAL rats. However, population activity is no longer restored with administration of a DA agonist in MAM rats (Gill et al., 2014). This finding requires replication, but suggests that the reduction following withdrawal may no longer be caused by depolarization block and may be due to other compensatory mechanisms.

5.2.3 Neuronal Criteria for Identification

The cells identified as DA neurons were based on established criteria involving factors such as waveform and firing rate (Grace and Bunney, 1983; Grace and Bunney, 1984; Ungless and Grace, 2012). The VTA contains approximately 70% DA neurons, 30% GABA interneurons, and a small percentage of glutamatergic neurons (Yamaguchi et al., 2007; Nair-Roberts et al., 2008). There is heterogeneity even within the tyrosine hydroxylase+ neurons in the VTA, such as neurons that corelease DA and glutamate (Stuber et al., 2010). However, the vast majority of DA neurons can be reliably identified using the established electrophysiological criteria, and the large number

of DA neurons sampled across the 6-9 tracks limits the impact of any potential misidentifications (Ungless and Grace, 2012).

We observed greater electrophysiological diversity in the vHPC compared to the VTA, consistent with the wide variety of cell subtypes and wide range of electrophysiological properties previously identified (Scharfman, 1992; Parra et al., 1998). The lack of established, specific criteria for pyramidal neurons in the vSub under anesthesia was a limitation in the electrophysiological analysis. However, putative pyramidal neurons were identified strictly by the criteria described in Chapter 4, which was based on previous vHPC recording performed in the lab (Lodge and Grace, 2007). Further research is necessary to confirm specific electrophysiological criteria for the identification of neuronal subpopulations in the HPC.

5.2.4 Drug Administration

Drug dosage and administration methods are critical factors in demonstrating consistent effects, both when comparing between animal studies and translating between animal and human research. Metabolic differences must be considered to determine corresponding receptor occupancy (Kapur et al., 2003) in addition to the administration method, particularly when looking at the effects of repeated treatment. For example, transient APD delivery has been found to be more effective than continuous D_2 blockade, which was more likely to result in D_2 receptor upregulation and behavioral tolerance. It has been reported that continuous administration may be more likely to result in DA supersensitivity (Samaha et al., 2008), whereas intermittent dosing with "drug holidays" is more likely to maintain efficacy (Remington and Kapur, 2010). Receptor expression was not investigated in these studies, though it could be included in future studies to examine potential receptor upregulation or downregulation. Ideally, drug doses should be based on *in vivo* receptor occupancy levels, which can improve translation between human and animal

studies (Kapur et al., 2003; Remington and Kapur, 2010). The drug doses used in these experiments were based on dosages used in prior research, though future research should examine changes in receptor expression and receptor occupancy for improved translatability.

5.3 FUTURE DIRECTIONS AND IMPLICATIONS FOR THE TREATMENT OF SCHIZOPHRENIA

5.3.1 Animal Model Selection and Interpretation in Preclinical Research

Normal animals are useful as a blank slate to examine the effects of a drug, but they are not well-suited for APD screening compared to models that incorporate disease-relevant abnormalities. For example, D_2 antagonists and D_2 partial agonists function differently in a hyperdopaminergic state, as observed in the MAM model of schizophrenia, which may be more representative of their actions in a patient (Valenti et al., 2011b; Sonnenschein et al., 2019). State-dependent differences in electrophysiological and behavioral measures were also observed with mGluR2/3 administration. Effects were observed in the presence of vHPC hyperactivity, but not under normal conditions. Similarly, state-dependent differences on DA neuron population activity have been observed with the administration of a PAM of the α5 subunit of $GABA_A$ receptors (Gill et al., 2011) and may be relevant for other compounds, particularly those that act as modulators to target excitatory-inhibitory processes.

The choice of proper animal models and assays to study APDs is particularly important when testing novel mechanisms. There has been much debate over whether current preclinical assays of APD efficacy are useful for screening novel mechanisms of action (Carpenter and Koenig, 2008; Nestler and Hyman, 2010). For example, blockade of amphetamine-induced

hyperlocomotion is a behavioral task in rodents commonly used to predict APD efficacy to treatpositive symptoms. However, amphetamine-induced hyperlocomotion is believed to arise from a ventromedial limbic striatal action (Creese and Iversen, 1975), whereas imaging studies show that increased raclopride displacement and increased f-DOPA uptake that correlate with psychosis is present primarily in the associative striatum (Kegeles et al., 2010; Kesby et al., 2018). A more effective screen might selectively target DA release in the associative striatum, such as a task that addresses the role of DA function in salience attribution and selective attention, which may be a more reliable measure of APD efficacy.

5.3.2 Treatment History

It has long been suggested that neuroadaptations to chronically administered psychiatric drugs can produce discontinuation syndromes and facilitate relapse, with rapid withdrawal inducing more severe relapse (Viguera et al., 1997). Given that patients typically receive long-term APD treatment, animal research would benefit from implementing more chronic treatment studies to examine long-term responses and identify any compensatory mechanisms, including changes in receptor response and more wide scale changes in circuit plasticity. One of the consequences of chronic D_2 antagonist treatment is D_2 receptor upregulation (Silvestri et al., 2000; Samaha et al., 2007), which can produce a state referred to as DA supersensitivity (Chouinard et al., 2017). Development of DA supersensitivity has been attributed to breakthrough symptoms and the rapid rate of relapse following APD discontinuation or dose reduction, which often results in new and more severe symptoms (Chouinard and Jones, 1980; Chouinard et al., 2017). Studies in rats have demonstrated that DA supersensitivity occurs both during and after treatment, potentially accounting for breakthrough symptoms, although the consequences are more apparent following withdrawal (Samaha et al., 2007). Some have argued

that breakthrough symptoms are more likely due to treatment noncompliance and can be circumvented by continuous drug blood levels usinglong acting injectables (Correll et al., 2018). Long-term treatment with long acting injectable APDs has been shown to reduce the rate of relapse compared to oral administration in randomized controlled trials (Kishimoto et al., 2012), but still roughly 20% of patients treated with long acting injectable APDs relapse within a year (Leucht et al., 2011). (Murray et al., 2016). Despite the increase in D_2 receptors, less therapeutic tolerance occurs to APDs in a clinical setting than what would be expected from antagonism of a receptor, which may be due to the indirect method of DA neuron regulation (i.e. depolarization block); however, the reduction of DA neuron activity is far from returning the system to a normal state. Further research on the interrelated topics of APD tolerance, withdrawal, and relapse is critical for clear guidelines on switching between APDs and determining which patients would benefit from dose reduction following stabilization.

Under current clinical trial protocols, switching from one D_2 antagonist to another may not appear to be a critical issue. However, when testing novel target compounds, prior treatment history may significantly impact the results. While the washout period typically used in clinical studies may be adequate to decrease blood levels of the APD, a short withdrawal period is not sufficient to reverse the effects of the drug on the DA system (Gill et al., 2014). It also raises the possibility that DA supersensitivity following prior D_2 antagonist treatment in patients with schizophrenia may mask potential effects of novel target compounds in clinical trials, despite their promise in screening assays performed in normal, drug-naïve rodents. The results of the trial could, therefore, be potentially confounded by the long-lasting changes in the system made by the presence of the APD. Such a confound may contribute to why POM performed better in early-in-disease patients (Kinon et al., 2015). In the presence of D_2 receptor upregulation following chronic D_2 receptor antagonist treatment (Silvestri et al., 2000; Samaha

et al., 2007), it is possible that only another D_2 receptor-targeting drug may be effective in reversing hyperresponsivity to DA. However, evidence suggests that D_2 partial agonists, such as ARI, do not induce DA supersensitivity (Tadokoro et al., 2011; Amada et al., 2019). Thus, testing novel compounds on patients who are withdrawn from ARI, drug-naïve, or confirmed to be non-compliant with their prior medication may be more effective strategies in identifying therapeutic value. The effects of prior APD treatment, and other changes to the brain that are not represented in normal or drug-naïve rats, must be considered in the development of novel drugs.

5.3.3 Improved Treatments for Psychosis

Future development of APDs should aim to provide normal regulation of DA neuron activity rather than general suppression of DA neurotransmission. Given the accumulating evidence that loss of PV + interneuron regulation of pyramidal cell activity underlies many aspects of schizophrenia, including its role in DA system hyperactivity (Benes and Berretta, 2001; Lodge and Grace, 2007; Lodge et al., 2009; Lewis et al., 2012), GABAergic and glutamatergic targets still hold promise as an effective therapy. Moreover, targeting excitatory-inhibitory processes may avoid consequences associated with D_2 antagonist treatment and may also alleviate more symptoms of the disorder, including negative and cognitive symptoms (Fig. 5-1 A-B), which often precede FEP and persist after treatment of psychotic symptoms (Harvey et al., 2005). In particular, compounds that can regulate HPC activity, such as mGluR2/3 agonists, have the potential to treat dysfunction upstream of DA dysregulation. Additionally, it is important to determine whether novel target compounds are effective in patients that are treatment resistant to current APDs. Compounds such as mGluR2/3 agonists may be effective in treating symptoms in patients who do not

respond to current D2 targeting APDs, but still show increased glutamate levels (Demjaha et al., 2012; Demjaha et al., 2014).

Interventions that directly target the pathophysiological processes that cause psychosis also have the potential to modify its progression. APD treatment does not normally begin until FEP at the earliest. However, given that longer DUP is associated with worse outcome and greater structural and functional deficits (Perkins et al., 2005; Lappin et al., 2006; Sarpal et al., 2017; Goff et al., 2018), it suggests that treatment/prevention of psychotic symptoms may be able to influence the course of the disease. Preventative interventions may provide greater and/or more long-lasting benefits than treating psychotic symptoms once they progressed beyond the CHR state.

In support of preventative treatments, interventions administered during adolescence can reduce the expression of schizophrenia-related anomalies in adult rodents (Du and Grace, 2013, 2016a, b). We propose that MAM rats are not "doomed from the womb", but instead fit a "two hit model" where the early disruption in brain development leads to heightened vulnerability to stress, with the potential for intervention prior to the second hit. The HPC is of particular interest as a treatment target because PV+ interneurons in the HPC continue to mature through late adolescence (Caballero et al., 2013) and the HPC is highly susceptible to stress (Sapolsky, 1985; Sapolsky et al., 1990). Therefore, severe stress or heightened stress responsivity during adolescence may damage the development of the vulnerable PV+ interneurons, leading to impaired DA system function that may reciprocally impact stress (Gomes and Grace, 2016; Gomes et al., 2019a). Interestingly, it is specifically *distressing* psychotic-like experiences in adolescence that are most indicative of CHR state, greater risk of developing a psychotic disorder (Kline et al., 2014), and reduced functional connectivity in cognitive networks (Karcher et al., 2019). Therefore, treatments that reduce stress, or more specifically, protect PV+ interneurons, may be particularly effective when administered during adolescence.

Safe and effective interventions in at-risk populations require more reliable strategies for estimating the risk of conversion in CHR individuals, which may also provide insight into potential targets for course-altering medication. Multi-modal strategies have been the focus of a number of recent studies, such as the incorporation of different types of neuroimaging techniques, neurocognitive batteries, neurophysiological assessments, and polygenetic risk models (Koike et al., 2013; Cooper et al., 2014). There is also potential in the use of machine learning to process patterns of data from multi-modal techniques (Pettersson-Yeo et al., 2013). Many of these studies aim to find sub-categories of CHR individuals based on neurobiological biomarkers to reduce heterogeneity and improve clinical trial targeting. In the case of FEP, it is also crucial to develop more reliable tools to predict whether a patient will or will not respond to DA blockade, so that alternative approaches (e.g. mGluR2/3-targeting drugs) may be implemented as early as possible. Neuromelanin-sensitive MRI, which provides a noninvasive measure of neuromelanin as a measure of DA metabolism, is a method that has shown early promise as one such assay that could be useful for identification of potential treatment-resistant patients (Wengler et al., 2019). Further research is needed to accurately differentiate and predict the varying trajectories of CHR individuals.

The uncertainty and ethical concerns of a prodromal diagnosis limits the confidence in early intervention (Patel et al., 2014). Responsible implementation of preventative treatment requires accurate prediction of the risk of transition with as few false positives as possible to prevent stigma, unnecessary treatment, and related adverse effects. Additionally, not all psychotic patients progress to a chronic psychotic disorder and some will convert to an affective disorder (Bromet et al., 2011). Future research directions include: strategies to better predict risk of conversion in CHR individuals, finding reliable biomarkers to predict disease course and treatment

response, and determining which therapeutic strategies would be most effective for preventing/minimizing psychotic symptoms. Through the pursuit of these questions, it may eventually be possible to prevent the progression of psychosis with early intervention in schizophrenia and related disorders.

5.4 CONCLUDING REMARKS

The therapeutic value of current APDs is hindered by numerous side effects and limited efficacy across symptom domains, which can lead to a lifetime of maintenance treatment (Lieberman et al., 2005; Leucht et al., 2012). Current APDs are also unable to address heterogeneity, both between individuals with distinct pathologies (Demjaha et al., 2014; Howes and Kapur, 2014) and within an individual through different phases of the disease (Krystal and Anticevic, 2015). Novel pharmacological targets have been investigated based on theories largely generated from animal research, yet clinical trial results have been less than promising (Dunlop and Brandon, 2015). The apparent lack of predictive validity, as evidenced by the failed clinical trials, necessitates reevaluation of the rigor and design of preclinical research for the future development of novel therapies. Similarly, clinical trial design should be re-evaluated based on data emerging from preclinical studies. Current efforts must be aimed at investigating potential variables that contribute to this disconnect to improve the translation of animal models and the therapeutic value of pharmaceutical treatments.

The mechanisms of action tested in the clinical trials of mGluR2/3 agonists were derived from decades of human and animal model research showing excitatory-inhibitory imbalance as a core component of schizophrenia and a promising target across symptom domains. Their failure created uncertainty in the current theoretical framework of schizophrenia regarding the potential

to treat glutamatergic dysfunction. Moving forward, one option is to leave these mechanisms ofaction in the "pharmaceutical graveyard" and forgo the pursuit of similar novel target compounds for the treatment of schizophrenia. Alternatively, we propose that these drugs, or others based on similar theoretical concepts, still hold promise, but require additional preclinical screening to better conduct clinical trials.

Animal research has provided critical insight into the mechanisms of action of current APDs and a framework to test hypotheses about the neurobiology underlying schizophrenia. Studies performed in the MAM model have demonstrated the importance of studying the effects of APDs on a system representative of patients. The hyperdopaminergic system in MAM rats shows rapid onset of depolarization block to D_2 antagonists and responds to D_2 partial agonists through a manner distinct from depolarization block (Valenti et al., 2011b; Sonnenschein et al., 2019), as discussed in Chapter 2. These findings are consistent with clinical results and not observed in normal rats. We also propose that therapeutic strategies should be directed on upstream regulation of DA neurons, such as the indirect regulation of DA neuron activity through the use of mGluR2/3 agonists, as discussed in Chapter 3. The MAM model also has shown that pharmacological methods can be used to prevent full emergence the HPC-VTA dysfunction in adulthood (Du and Grace, 2013, 2016a, b), as observed following peripubertal mGluR2/3 agonist administration, discussed in Chapter 4. These findings support the potential of targeting glutamatergic dysfunction early in the disease to alleviate symptoms and potentially prevent conversion of psychosis.

The failure of recent novel target drugs does not invalidate the theory behind their development, but highlights the gaps that exist in their translation. Animal models are best applied in collaboration with studies performed in patients to find parallels and test predictions. Representative animal models, informed by clinical studies, can then be used to investigate the

contribution of variables that underlie patient heterogeneity. These findings must then be incorporated into the design of clinical trials of novel target compounds, or else promising targets may be abandoned despite great potential to improve treatment options for schizophrenia.

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