Positive allosteric modulation of cannabinoid type-1 receptors:

Effects of GAT211 on MK-801-induced behaviors in rats

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ABSTRACT

Antipsychotics help alleviate the positive symptoms associated with schizophrenia; however, their debilitating side effects spur the search for better treatment options. Acute N-methyl-D-aspartate receptor (NMDAR) blockade with noncompetitive antagonists such as MK-801 has been used to screen novel compounds for their antipsychotic potential in rodent models. Given interactions between NMDAR and cannabinoid type 1 receptors (CB1R), we tested the ability of GAT211, a CB1R positive allosteric modulator, to reverse two behavioural effects of acute MK-801 treatment, including: (1) increased locomotor activity; and (2) reduced prepulse inhibition (PPI) of the acoustic startle response. Male, Long Evans rats were treated with MK-801 (0.15 mg/kg) and/or GAT211 (0.3-3.0 mg/kg) and locomotor activity or PPI were assessed 15 min later. As expected, acute MK-801 produced a profound increase in locomotor activity and impaired PPI. GAT211 treatment alone dose-dependently reduced locomotor activity and the acoustic startle response. GAT211 (3.0 mg/kg) also blocked the exaggerated locomotor activity caused by MK-801 and showed some modest ability to normalize MK-801-induced PPI impairments. These findings support continued preclinical research regarding the usefulness of CB1R positive allosteric modulators as novel antipsychotic medications.

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DEDICATIONS

My wife. There is no overstating the toll that graduate work has on friends and family. I owe a significant portion of my success to my wife who has always been patient, supportive and encouraging throughout long hours at the lab and buried in textbooks. She never complained when I rambled incessantly about neuroscience and always showed enthusiasm about what I was excited about; whether it was working on my clay brain or debating the ethics of humans merging with artificial intelligence. I couldn't do what I do without her and I am grateful for her support. Thanks Jess.

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The rats. I had the opportunity to work with hundreds of rodents during my research and I immensely enjoyed my interactions with them. Each had their own personality and uniquely contributed to the data in this thesis. Their sacrifice is worthy of acknowledgment, not only for their contribution to science, but to reinforce that animal research is carried out with dignity and respect for the animals.

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LIST OF ABBREVIATIONS

2-AG	2-arachidonoylglycerol (endogenous cannabinoid)
ABHD6	α-β-hydrolase domain 6 (2-AG enzyme)
AC	adenylyl cyclase
AEA	anandamide (endogenous cannabinoid)
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor(s)
cAMP	cyclic adenosine monophosphate
CB1R	cannabinoid type-1 receptor(s)
CBD	cannabidiol
CCK	cholecystokinin
CSPP	cortex, striatum, pallidum or pontine tegmentum [circuit]
CNV	[genetic] copy-number variant
D_2	dopamine receptor 2
DAGL	diacylglycerol lipase (2-AG synthesizing enzyme)
eCB	endocannabinoid(s)
GABA	gamma-amino-butyric acid
GPCR	G-protein-coupled receptor(s)
HIPP	hippocampus
vHIPP	ventral hippocampus
MAGL	monoacylglycerol lipase [enzyme that breaks down 2-AG]
MAPK	mitogen-activated protein kinase
MK-801	non-competitive NMDAR antagonist (i.e., dizocilpine)

NAc	nucleus accumbens
NAM	negative allosteric modulator
NMDAR	N-methyl-D-aspartate receptor(s)
NOR	novel object recognition
PAM	positive allosteric modulator
PFC	prefrontal cortex
dlPFC	dorsolateral PFC
PI3K	Phosphoinositide 3-kinases
PPI	prepulse inhibition
RNA	ribonucleic acid
mRNA	messenger RNA
SGA	second generation antipsychotic
THC	tetrahydrocannabinol

1.0 GENERAL INTRODUCTION

This thesis is arranged in three chapters: (1) General Introduction; (2) Manuscript summarizing the research conducted; (3) General Discussion. The aim of this thesis is to extend the available research regarding behavioral symptoms associated with psychiatric illness, specifically in relation to the positive symptoms associated with schizophrenia. Chapter 1.0 begins by highlighting the need for better antipsychotics with fewer side effects, then discusses relevant hypotheses that attempt to explain the observed neurocircuitry purportedly affected in the brain. Chapter 2.0 of this thesis includes pre-clinical research investigating whether the endocannabinoid (eCB) system may serve as a unique neuromodulator of positive behavioral symptoms in rodents. Chapter 3.0 concludes with a discussion of our research findings in context with the literature.

1.1 Schizophrenia: setting the stage

Schizophrenia is a severe mental illness accompanied by debilitating psychiatric symptoms and continues to rank among the top 15 causes of disability in the world today (Moreno-Küstner, Martín, & Pastor, 2018). Although estimates range, systematic reviews suggest a lifetime prevalence of 0.4 - 0.75 % (Nicholl, Akhras, Diels, & Schadrack, 2010; Simeone, Ward, Rotella, Collins, & Windisch, 2015). Symptoms are often grouped into positive (e.g., hallucinations, delusions, disorganized speech and behavior), negative (e.g., avolition, affective flattening) and cognitive (e.g., memory, executive function) domains, and significant heterogeneity is present among individuals with the disorder (Manseau & Goff, 2015; Tandon et al., 2013). The research described in this thesis focuses on behaviors specifically associated with positive symptoms of schizophrenia, although implications overlap with a range of psychotic symptoms.

Current treatment approaches are less than ideal for many patients. Virtually all clinical antipsychotics are D_2 (type-2 dopamine receptor) antagonists that aim to alleviate the positive symptoms associated with schizophrenia (Collins et al., 2011; Kapur & Mamo, 2003). Unfortunately, side effects include cognitive impairment, sedation, weight gain, tremors, spasticity, severe extrapyramidal symptoms (e.g., tardive dyskinesia, Parkinsonism), and other dyskinesias resulting from impaired or depleted dopaminergic signaling in nigrostriatal efferent neurons projecting to the basal ganglia (Blair & Dauner, 1992; Fakhoury, 2017; Seeman, 2002). This range of side effects contributes to low compliance rates and poor patient outcomes and exemplifies the persistent need for better treatment options (Goff et al., 2017; Li, Snyder, & Vanover, 2016; Sendt, Tracy, & Bhattacharyya, 2015). Despite decades of research, pharmacological innovation has remained largely stalemated since the advent of clozapine in the 1950s (Lally & MacCabe, 2015). Although second generation antipsychotics (SGAs) are accompanied by significantly fewer extrapyramidal side-effects (purportedly via 5-HT inhibition), some side effects do persist (e.g., cardiometabolic dysfunction) and there remains significant interest in pharmacological approaches with enhanced specificity and symptom reduction. SGAs are associated with reduced side effects compared to traditional antipsychotics, largely due to their ability to transiently antagonize D_2 receptor activity and dissociate (non-competitive) in the presence of dopamine (Seeman, 2002). This subtlety allows for dopaminergic signaling to continue, while providing a transient antagonistic effect that helps alleviate positive symptoms, spare cognitive deficits, prevent long-term side effects like tardive dyskinesia, and helps improve individual treatment strategies (Seeman, 2002). These observations highlight the continued exigency to investigate novel antipsychotic treatment methods to improve our

understanding of psychotic behavior and to provide new pharmacology tools to assist in individualized treatment strategies.

1.2 Hypotheses of schizophrenia

The previous section discussed difficulties in untangling dopamine signaling from positive behavioral symptoms; however, dopamine signaling is only one factor implicated in these phenotypes. In fact, measurements of dopamine were not made in the present work and thus, only general inferences may be made regarding its involvement. Although dopamine was beyond the scope of our research, it is an essential neurotransmitter involved with positive symptoms and must therefore, be discussed. The current section discusses three hypotheses of schizophrenia that will provide a foundation to make inferences and contextualize our findings.

Various hypotheses have been developed to attempt to explain the circuitry and signaling mechanisms involved in the positive symptoms of schizophrenia. The complexity of neural circuitry that underlies psychosis is wide reaching throughout the brain, resulting in an evolution of hypotheses in previous decades. This section has three primary aims: (a) describe the neurocircuitry of psychosis through the lens of three leading hypotheses ([*]footnote) of schizophrenia; (b) elaborate on how these observations are relevant to rodent models of psychosis; and (c) describe the relevant neurocircuitry underlying positive schizophrenia symptoms and their relevance to animal models.

[*] When using these theories to guide research, a distinction must be drawn between *acute* versus *chronic* development of disorders. Schizophrenia is a neurodevelopmental disorder that often expresses through behavioral symptoms years or decades after disease progression begins. The following hypotheses are in line with a wide range of acute and chronic observations, however; there is an inherent experimental confound when using acute models to mimic developmental (or chronic) disease states like the positive symptoms associated with schizophrenia. These limitations are discussed further in sections 1.3.2-1.3.4 and a distinction between acutely induced deficits and neurodevelopmental disease states are again discussed in Chapter 2.0. Meanwhile, a distinction between acute animal models and developmental human disorders should be maintained in the forefront throughout this thesis. Figure 1 provides a schematic of how the following hypotheses may interact.

1.2.1 Dopamine hypothesis

Dysfunctional dopaminergic signaling has been a central theory of the neurobiology of schizophrenia for decades (Coyle, Balu, Benneyworth, Basu, & Roseman, 2010; Kapur & Mamo, 2003). The dopamine hypothesis arose in the mid-19th century and continues to inform the development of antipsychotics, although clinical prescribing today is largely based on a trial-and-error strategy (Lally & MacCabe, 2015). It is supported by two primary categories of observations, both of which have robust support across decades of research. First, traditional antipsychotics successfully reduce the positive symptoms of schizophrenia via D_2 (dopamine receptor 2) antagonism. Second, amphetamines (dopaminergic agonists) increase the positive symptoms of schizophrenia by increasing excitatory postsynaptic current (EPSC) in the ventral basal ganglia that modulate and tune kinetic movement (Aguilar, Chen, & Lodge, 2015; Kapur & Seeman, 2001; Kapur & Mamo, 2003; Seeman, 2002; Seeman, 2013). [Section 1.3.3 discusses these behaviors further; sections 1.3.5 & 1.3.6 discuss paradigms used to measure similar behaviors in rodents.]

The basal ganglia is essential for finetuning multiple behaviors and a distinction is necessary between two active circuits that exist between the midbrain and the striatum. First, in context with the present research, spontaneous motor movements associated with the positive symptoms of schizophrenia are hypothesized to be regulated by ventral tegmental area (VTA) dopaminergic efferents to the ventral striatum and limbic regions (e.g., nucleus accumbens (NAc), hippocampus) and together encompass the mesolimbic dopamine system (Aguilar et al., 2015; Kapur & Seeman, 2001; Kapur & Mamo, 2003). The mesolimbic dopamine system aides in assigning salience (via dopamine) to events in which conditioning has occurred (e.g., reward,

punishment). Antipsychotics that target the D2 receptor are thought to exert a preferential modulation of the mesostriatal circuit as well as positive symptoms that underscore hyperdopaminergia (e.g., in the NAc) (Kapur & Seeman, 2001; Kapur & Mamo, 2003). These observations provide justification for using some behavioral assays (e.g., open field test used in rodent research) in which behaviors are reliably induced with D2 agonists and reduced using antipsychotics (Kapur & Mamo, 2003). These observations are also applicable to phenomena such as sensorimotor gating and prepulse inhibition (discussed at length later), which have relevance to the positive symptoms of schizophrenia and also appear alongside mesolimbic dopamine disturbances (Braff & Geyer, 1990; Swerdlow & Light, 2018). The second common midbrain-striatal circuit, which is less applicable to the current thesis (although certainly involved), includes projections from the substantia nigra to the *dorsal* striatum and pallidum. Evidence suggests that the mesostriatal dopamine pathway (substantia nigra – dorsal striatum) underlies sensory and pain processing, motor planning and may also be involved in modulating sensorimotor gating as well (Rodrigues, Salum, & Ferreira, 2017; Takeda et al., 2005). This pathway is often implicated in basal ganglia related dyskinesias. In summary, the *mesolimbic* dopamine pathway projects to the ventral striatum and has behavioral effects on spontaneous and conditioned locomotor activity as well as sensorimotor gating deficits relevant to positive psychiatric symptoms (Aguilar et al., 2015; Jones & Robbins, 1992; Swerdlow & Light, 2018).

The dopamine hypothesis came about following the serendipitous discovery that chlorpromazine (D_2 receptor antagonist) induced a state of indifference in animal studies; shortly after, it showed efficacy at improving manic and psychotic symptoms in human patients (Kapur & Mamo, 2003). These discoveries led to the widespread use of reserpine and chlorpromazine to treat psychosis, with haloperidol entering the scene shortly after (Kapur & Mamo, 2003). As

these drugs became increasingly prescribed and studied, it was eventually hypothesized that monoamine receptors may be involved and ultimately, linked to reduced dopaminergic activity in mesolimbic pathways (Kapur & Mamo, 2003; Seeman, Chau Wong, Tedesco, & Wong, 1975; Seeman, 2013; Stahl, 2018). First generation antipsychotics competitively block postsynaptic D_2 receptors, while SGAs (*atypical*) are more commonly used today and help mitigate against some long-term side effects (Coyle et al., 2010; Seeman, 2002; Seeman, 2013; Stahl, 2018). Atypical antipsychotics may exert efficacy and reduce side effects (e.g., dyskinesias) by promoting serotonergic activity throughout mesolimbic and mesostriatal circuits, supported by evidence showing that the serotonergic system may exert tonic inhibition (persistent, low-amplitude) throughout basal ganglia circuitry (Di Giovanni et al., 1999; Geyer, Puerto, Menkes, Segal, & Mandell, 1976).

It is clear today that multiple pathways and neurotransmitters are implicated with the positive symptoms of schizophrenia. Due to the dopamine hypothesis; however, a series of experiments have aimed to characterize the role of dopamine in schizophrenia and two consistent observations are worth highlighting: (1) increased dopaminergic efflux in key brain regions is associated with positive symptoms; (2) D_2 antagonism using antipsychotic drugs reduces positive symptoms. These findings suggest that increased D_2 activity may be involved in the pathology of schizophrenia; yet, there is a limit to which dopaminergic antagonism may be used before unacceptable side effects arise.

1.2.2 Glutamate hypothesis

The glutamate hypothesis evolved from the dopamine hypothesis, but integrates research suggesting that dysfunctional glutamatergic activity also plays a role in positive schizophrenia symptoms. Both, human and rodent studies provide evidence that glutamatergic dysregulation

may be causal in the pathophysiology of schizophrenia and glutamate receptors may be a viable pharmacological target to improve some psychiatric symptoms (Fakhoury, 2017; Uno & Coyle, 2019). Schizophrenia is associated with hypofunctioning N-methyl-D-aspartate receptors (NMDAR) that are highly active in the corticolimbic system (e.g., hippocampus, PFC, nucleus accumbens (NAc)). NMDAR binding sites were discovered during human studies in the 1980s and administration of NMDAR antagonists (e.g., PCP, ketamine) induced symptoms in healthy controls that were remarkably comparable to the positive deficits observed in schizophrenia (Jackson, Homayoun, & Moghaddam, 2004; Moghaddam & Javitt, 2012; Uno & Coyle, 2019; Vollenweider, Leenders, Øye, Hell, & Angst, 1997). This propelled a large amount of research surrounding pharmacological manipulation of glutamate receptors and their ligands.

Glutamate is the most abundant neurotransmitter in the brain and it works in concert with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to excite multiple areas throughout the brain (Moghaddam & Javitt, 2012). To activate NMDAR requires a complex series of events referred to as a "triple gate," which requires three concurrent events for activation: (a) ligand binding with AMPA receptors that contributes to cell depolarization and the expulsion of a magnesium block in NMDAR; (b) glutamate binds to NMDAR; (c) a co-agonist (e.g., serine or glycine) binds to NMDAR (Coyle et al., 2010; Uno & Coyle, 2019). NMDAR hypofunction is thought to decrease the rate of activity of parvalbumin GABAergic (inhibitory) interneurons (Deutsch et al., 2010; Kawaguchi, 2001; Markram et al., 2004; Povysheva et al., 2006), which normally exert an important inhibitory effect on cortical pyramidal neurons (reference Fig. 1) which in turn, influence ventral tegmental area (VTA) efferent dopaminergic neurons (Coyle et al., 2010; Homayoun & Moghaddam, 2007). Put more simply, NMDAR hypofunction results in aberrant neurotransmitter signaling that resembles

some phenotypes observed in schizophrenia. Therefore, drugs that restore NMDAR function may help restore some dysfunction. This can be tremendously valuable in the drug discovery process. For example, if it is discovered that a novel drug can restore behavioral deficits of interest following NMDAR antagonism, we gain insight into potential therapeutics for future human trials in addition to enhancing understanding of how networks in the central nervous system interact. In summary, glutamate is prolific in the brain, relies on a complicated sequence to activate NMDAR, and antagonizing NMDAR replicates some behavioral and physiological phenotypes of interest within behavioral research.

The glutamate hypothesis arose from research suggesting that NMDAR hypofunction may be involved in the pathophysiology of schizophrenia and led to novel pharmacological approaches (e.g., ketamine, PCP, MK-801) to induce behavioral deficits such as locomotor activity and prepulse inhibition (PPI; described further in 1.3.5). Presently, it is worth noting that these deficits are associated with dysfunctional glutamatergic and dopaminergic signaling (Fakhoury, 2017; Uno & Coyle, 2019).

1.2.3 Cannabinoid hypothesis

Evidence has emerged in recent decades suggesting an association between the endocannabinoid (eCB) system and the pathophysiology of schizophrenia (Fakhoury, 2017; Volk & Lewis, 2016). The eCB system will be discussed at length in section 1.4; however, some context is briefly described here. The eCB system includes two primary ligands, anandamide (AEA; Devane et al., 1992) and 2-arachidonoylglycerol (2-AG; Stella, Schweitzer, & Plomelli, 1997) that are post-synaptically synthesized and serve as retrograde inhibitors of presynaptic neurons (Lu & MacKie, 2016; reference Fig. 4). It is also worth noting that eCBs are synthesized, expressed and modulated in part by astrocytes (Smith, Bekar, & Nedergaard, 2020).

The primary receptor that modulates activity in the central nervous system is the cannabinoid type-1 receptor (CB1R), due to its prolific expression through a number of brain regions implicated in positive symptoms, including the ventral basal ganglia, nucleus accumbens, PFC, cerebellum, limbic areas, and others (Kucera et al., 2018; Lu & MacKie, 2016; Mechoulam & Parker, 2013; Tsou, Brown, Sañudo-Peña, Mackie, & Walker, 1998). There is an abundance of evidence that suggests dysregulated eCB signaling may underlie dopaminergic hyperactivity associated with psychiatric symptoms (Aguilar et al., 2015; D'Souza et al., 2005; Hudson, Renard, Norris, Rushlow, & Laviolette, 2019; Peres et al., 2016; Silveira et al., 2017). Interestingly, CB1R is widely expressed on GABAergic interneurons and glutamatergic pyramidal neurons (Fig. 1), both of which influence the release of dopamine in the brain (Galve-Roperh, Palazuelos, Aguado, & Guzmán, 2009; Sherif, Cortes-Briones, Ranganathan, & Skosnik, 2018). Other studies report AEA is elevated in the cerebrospinal fluid of schizophrenia patients and inversely associated with psychotic symptoms, suggesting a possible compensatory upregulation of the eCB system in response to dysregulated signaling pathways (Fakhoury, 2017; Giuffrida et al., 2004; Vigano et al., 2009). Although causality is still difficult to assess, the evidence suggests dysregulation of the eCB system is likely involved in the etiology and symptoms of schizophrenia (Bioque et al., 2013; Fakhoury, 2017; Vigano et al., 2009; Volk & Lewis, 2016). Due to the eCB system's unique expression in the brain, combined with exerting an inhibitory effect on presynaptic neurons, targeting CB1R may have potential to attenuate positive symptoms associated with a hypoglutamate-hyperdopaminergic state (Pertwee & Ross, 2002).

The eCB hypothesis suggests that eCB dysregulation (purportedly hyperactivity) may underscore some pathology of schizophrenia and may have a causal role in some of the positive

symptoms. In relation to NMDAR antagonism and systemic pharmacological methods used in behavioral research, the eCB hypothesis aims to provide a developmental model of schizophrenia. The eCB hypothesis works in conjunction with glutamate and dopamine hypotheses to begin to paint a more holistic view of schizophrenia and helps identify additional novel pharmacological targets (e.g., CB1R). GABAergic and glutamatergic neurons in the cortico-striatal-pallidum or pontine tegmentum (CSPP) circuit, play essential roles in modulating and finetuning behaviors that that involve movement or startle reflexivity (Bakshi & Geyer, 1995; Fendt, Li, & Yeomans, 2001; Geyer et al., 1976; Rodrigues et al., 2017). Interestingly, many of those same neurons express CB1R, suggesting that increasing the propensity of successful endocannabinoid ligand binding may influence dopaminergic firing in the mesolimbic circuit and CSPP circuit. The eCB hypothesis claims that aberrant eCB activity is implicated in some positive symptoms; however, it remains to be seen how the eCB system interacts with glutamatergic, GABAergic and dopaminergic signaling pathways.

In conclusion, the three aforementioned hypotheses complement one another and help inform treatments aimed at alleviating some aspects of psychiatric symptoms. The complexity suggests that a single treatment approach is unlikely to mitigate all schizophrenia symptoms; however, multiple approaches may help in tailoring individualized treatment strategies as well as improving current ones. Many signaling pathways are involved in the etiology of schizophrenia and patients experiencing a large range of symptoms and treatment responses. Additionally, changes in one signaling system have far-reaching effects on the efficiency of other systems. For example, the eCB system may be targeted to attenuate excessive dopaminergic release, to the effect that some glutamatergic dysregulation is normalized; however, unintended co-effects may occur in other regions. Lastly, the eCB system is related to pathophysiological aspects of

schizophrenia. Studies showing that phytocannabinoids and cannabinoid agonists influence GABAergic and glutamatergic signaling in the brain in a manner consistent with dysfunctional dopaminergic signaling, as well as the "phenomenology of schizophrenia" (Sherif, Radhakrishnan, D'Souza, & Ranganathan, 2016) and may be uniquely positioned to modulate behavioral phenotypes associated with the positive symptoms of schizophrenia (Fakhoury, 2017).

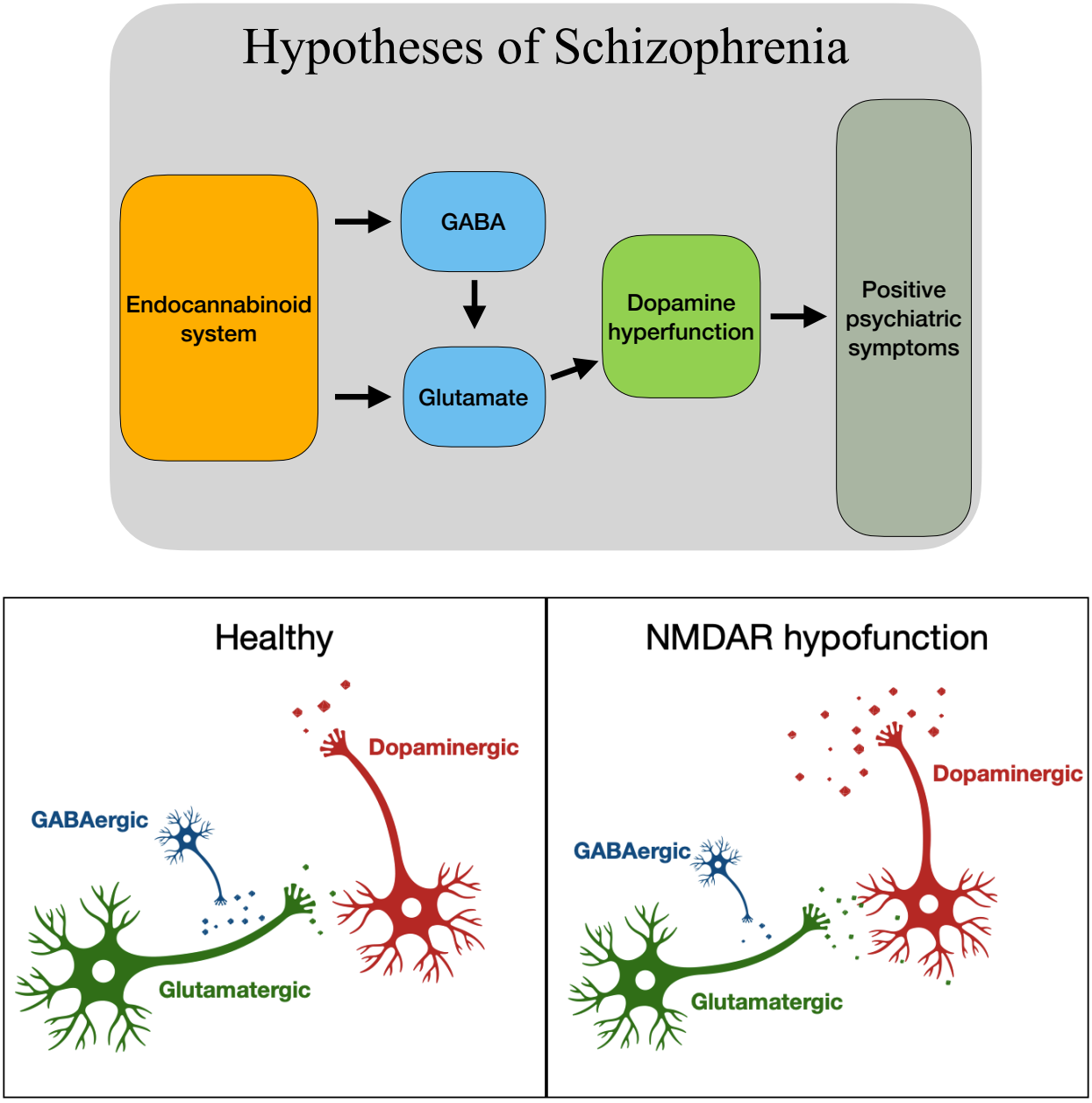

Fig 1. Hypotheses of schizophrenia as they apply to positive psychiatric symptoms associated with hypoglutamate-hyperdopaminergic phenotype. **(top)** Hyperdopaminergia is associated with positive psychiatric symptoms and this is regulated by a complex interplay between glutamatergic and GABAergic neurons. CB1R is highly expressed on glutamatergic and GABAergic neurons and may be implicated in the pathophysiology of schizophrenia. Therefore, the eCB system may be a viable pharmacological target to restore some dysregulation. **(bottom)** On the left, GABA release helps attenuate glutamate release and indirectly modulating dopamine. The right shows effects of NMDAR antagonists such as MK-801 which preferentially inhibit GABAergic interneurons resulting in disinhibition of dopamine release associated with schizophrenia. GABergic and glutamatergic cell bodies typically project from the cortex, while dopamine neurons are present in the mesolimbic pathway. (Adapted from Lins, 2019).

1.2.4 Genetic findings

Although genetics are not directly investigated in this thesis, there are highly relevant genetic findings that lend support to the hypotheses previously described. Early genetic studies of schizophrenia supported the dopamine hypothesis, but unfortunately many of those studies lacked significant power and were riddled with biases (Uno & Coyle, 2019). Previous authors go on to report that modern studies that highlight 11 rare genetic copy-number variants (CNVs) associated with increased risk of developing schizophrenia, including a CNV responsible for encoding the NMDAR (Uno & Coyle, 2019). There is also evidence that a subset of schizophrenia patients have higher levels of α-β-hydrolase domain 6 (ABHD6; 2-AG degrading enzyme) mRNA, which is co-localized with diacylglycerol lipase (DAGL; 2-AG synthesizing enzyme) on pyramidal neurons in the dorsolateral PFC (dlPFC) (Volk & Lewis, 2016). These findings suggest that 2-AG metabolism may be increased in the same pyramidal neurons responsible for 2-AG synthesis, suggesting that enzymatic degradation by ABHD6 may counter 2-AG synthesis by DAGL directly at the source of 2-AG production (Volk & Lewis, 2016). These findings further support the hypotheses previously described and begin to provide an infrastructure to build a more comprehensive understanding of neurotransmitters and receptors implicated in positive psychiatric phenotypes. In light of these observations, a handful of potential research questions arise regarding the role the eCB system plays in the positive symptoms of schizophrenia. The following section addresses how behavioral researchers can test these hypotheses using animal models.

1.3 Animal models

Modeling symptoms of human disorders in animals is challenging and often relies on preclinical behavioral research to guide future human studies. As such, it is important to not overextend

results of behavioral research regarding human phenotypes. Although new technologies and innovative brain scanning techniques continue to advance, there are still significant ethical and practical limitations of investigating some neurophysiological mechanisms in humans. There continues to be a need for animal research in order to make inferences and assess neural mechanisms associated with relevant behavioral phenotypes (Nestler & Hyman, 2010). When using an animal model to mimic human disease states, researchers often use *reliability* and *validity* to determine the degree to which a given model informs human disease states.

In general, a model is said to have high *reliability* if the experimental model continuously produces similar results (i.e., between researchers, laboratories and assays that measure the same behavior). Conversely, behavioral assays have high *validity* if they accurately measure the behavior they intend to measure. For example, the elevated plus maze (EPM) is a behavioral rodent assay and has high reliability and validity due, in part, to its reliance on innate rodent behaviors (i.e., approach-avoidance) which are reliably altered in response to pharmacological manipulation (Korte & De Boer, 2003).

Predictive validity is particularly important when inferring drug efficacy in rodent studies (Nestler & Hyman, 2010). Predictive validity describes how much predictive power pharmacological manipulation in animal models has in replicating the human response to therapeutics and should predict current and future therapeutic drug effects (Nestler & Hyman, 2010). As discussed previously, the NMDAR antagonist MK-801, has high predictive validity because it causes impairments in animal studies (e.g., induces hyperlocomotion), and impairments are reliably reversed with antipsychotics (Bakshi & Geyer, 1995; Neill et al., 2010; Sebban, Tesolin-Decros, Ciprian-Ollivier, Perret, & Spedding, 2002). (Predictive validity is discussed in more detail in section 1.3.4 in consideration of our findings).

1.3.1 Research domain criteria (RDoC)

This thesis has some relevance to a large range of psychiatric behaviors that share phenotypes similar to schizophrenia; however, considerable overlap occurs amongst the wide range of symptoms experienced in psychiatric disorders. The National Institute of Mental Health (NIMH) began an initiative in 2009 called the Research Domain Criteria (RDoC) (Cuthbert & Insel, 2013). RDoC aims to move away from heterogenous diagnosing of mental illnesses and rather, focuses on specific mechanistic deficits applicable to multiple disease states (Cuthbert & Insel, 2013). This has particular relevance to the current study because positive symptoms are expressed, treated and experienced very differently amongst schizophrenia patients. Therefore, in the interest of precision and RDoC standards; this thesis focuses on two behavioral phenotypes in rodents that have some relevance to positive symptoms in schizophrenia; however, that is not to say these behaviors apply specifically to schizophrenia patients, nor does it imply that the subjective experience human patients experience is induced in rodents. Alternatively, this thesis aims to enhance mechanistic insight and further characterize eCB influence on behaviors as they apply to preclinical basic science. Results from animal studies should only be used as a metric to assess biobehavioral dimensions and in no way do they inform about the subjective experience of mental illness. For example, impaired prepulse inhibition (PPI) is considered a positive symptom of schizophrenia [investigated in Chapter 2.0]; impaired PPI is also reported in obsessive-compulsive disorder (Ahmari, Risbrough, Geyer, & Simpson, 2012; Kohl, Heekeren, Klosterkötter, & Kuhn, 2013) and Giles de la Tourette's syndrome and there is modest evidence that it may be dysregulated in bipolar disorder (Kohl et al., 2013). This belabors the point that, although we are interested in behaviors with relevance to the positive symptoms of

schizophrenia, our results may inform a variety of psychiatric disorders and have some limitations.

1.3.2 Modelling psychiatric illness

Using animal models gives researchers increased experimental control when analyzing the etiology of mental illness and allows for the identification of new drug targets when human clinical studies are not otherwise possible (Geyer, 2008; Howland, Greenshaw, & Winship, 2019; Kaiser & Feng, 2015). Researchers often use acute pharmacological methods to resemble symptoms in disease states, allowing for a mechanistic assessment of behavioral and pathophysiological deficits (Geyer, 2008; Howland et al., 2019; Kaiser & Feng, 2015). Using acute rodent models allows researchers to investigate the neurobiology of mammalian deficits; however, results should be interpreted with caution as they do not directly inform the disease state itself (Howland et al., 2019). Animal models allow researchers to investigate specific biological aspects of psychiatric illnesses and discover novel approaches to alleviate translatable behaviors (Bale et al., 2019).

1.3.3 Acute MK-801 model of psychiatric illness

In consideration of the research investigating NMDAR antagonism, acute administration with MK-801 is a reliable method to induce behavioral deficits in rodents (Bakshi & Geyer, 1995; Brosda et al., 2011; Cadinu et al., 2018; Moghaddam & Javitt, 2012; Sebban et al., 2002; Trujillo & Akil, 1991). This thesis investigates two specific behaviors in rats resulting from acute MK-801 administration: (1) hyperlocomotor activity (Suryavanshi, Ugale, Yilmazer-Hanke, Stairs, & Dravid, 2014); (2) impaired prepulse inhibition (PPI; Brosda et al., 2011; Howland, Cazakoff, & Zhang, 2012). Acute NMDA receptor blockade with noncompetitive antagonists like MK-801 have been used to screen novel compounds for their antipsychotic potential in

rodents for decades (Moghaddam & Javitt, 2012). MK-801 gets trapped in NMDAR channel pores upon opening and blocks NMDAR activity, which induces psychotic-like symptoms in humans and rodents (Moghaddam & Javitt, 2012; Traynelis et al., 2010).

Multiple considerations are necessary when using acute MK-801 administration to induce hyperlocomotion and PPI impairment. MK-801 has divergent effects depending on neuronal subtypes and active neurotransmitters. Figure 1 shows how NMDAR antagonism may inhibit GABA release which usually serves as a braking mechanism modulating glutamatergic pyramidal neurons; the end result is a disinhibitory effect (or increase) of downstream dopamine release (Homayoun & Moghaddam, 2007). MK-801 recapitulates these dysregulations and allows for assessment of novel compounds to determine efficacy at modulating resulting behaviors. In contrast to the complex biopsychosocial influences that result in the development of schizophrenia, MK-801 produces an acute state that mimics aspects of positive symptoms, including: increased dopamine, NMDAR hypofunction, preferential binding to GABAergic interneurons and glutamatergic dysregulation (Cadinu et al., 2018; Coyle, Tsai, & Goff, 2003; Howland et al., 2012; Jackson et al., 2004; Suryavanshi et al., 2014; Trullas & Skolnick, 1990; Van Den Buuse, Ruimschotel, Martin, Risbrough, & Halberstadt, 2011). One suggestion as to why this effect is preferential to GABAergic interneurons is because these neurons are often Fast Spiking (FS) to allow for quick modulation and reaction to glutamate signaling (Homayoun & Moghaddam, 2007; Markram et al., 2004; Povysheva et al., 2006). Interestingly, GABAergic dysregulations are reliably observed in schizophrenia patients as well and evidence suggests they are due to NMDAR abnormalities (Cadinu et al., 2018). Other research shows that altered NMDAR activity at interneurons contributes to altered gamma oscillations associated with schizophrenia (Cadinu et al., 2018; Hudson, Sokolenko, O'Brien, & Jones, 2020; Sherif et al.,

2018), while further evidence implicates dysregulated NMDAR co-agonists like d-serine or glycine (Karasawa, Hashimoto, & Chaki, 2008; Suryavanshi et al., 2014). Although the exact mechanisms are unclear (Bubeníková-Valešová, Horáček, Vrajová, & Höschl, 2008), two influential observations result from NMDAR hypofunction: (1) an increase in synaptic glutamate which leads to enhanced binding at neighboring AMPA receptors as well as downstream targets on glutamatergic and dopaminergic cell bodies; and (2) reliable behavioral alterations are associated with this 'NMDAR antagonism – increased downstream signaling' *state* (Brosda et al., 2011; Bubeníková-Valešová et al., 2008; Cadinu et al., 2018; Carlsson & Carlsson, 1989; Meyer & Feldon, 2009). These observations provide support for the glutamate hypothesis of schizophrenia.

In summary, MK-801 has a large influence on brain-wide signaling and undoubtedly produces a large range of side effects beyond the scope of schizophrenia and positive symptomology. Acute MK-801 administration does not recapitulate human developmental disorders, nor does it recapitulate NMDAR expression and function observed in schizophrenia. We were interested in using MK-801 because it reliably produces an acute state in rodents; albeit, lacking construct validity when compared to human disease states. By assessing MK-801 induced behaviors and physiological changes (e.g., glutamate hypofunction-dopamine hyperfunction state) in rodents, we gain valuable preclinical insight regarding therapeutic potential of novel compounds. Specifically, MK-801 creates an acute state in rodents promoting: (a) disinhibition of dopamine release in the cortex, striatum, and nucleus accumbens (NAc) (Homayoun & Moghaddam, 2007; Kokkinou, Ashok, & Howes, 2018; Moghaddam & Javitt, 2012); (b) a glutamate hypofunction-dopaminergic hyperfunction state (Moghaddam & Krystal,

2012); (c) hyperlocomotor behavior (Kruk-Slomka, Budzynska, Slomka, Banaszkiewicz, & Biala, 2016; Suryavanshi et al., 2014); and (d) impaired PPI (Howland et al., 2012).

1.3.4 Validity of using MK-801 models

There is robust support for using NMDAR antagonists to study the pathophysiology of schizophrenia (Cadinu et al., 2018). MK-801 was chosen due to its reliability and usefulness in evaluating novel pharmacological approaches and to enhance our understanding of the neural correlates of PPI impairment and locomotor hyperactivity. Nestler and Hyman (2010), highlighted *etiological*, *face* and *predictive validity* as particularly important considerations when determining how translatable animal models are. This section discusses the acute MK-801 model in the context of the three types of validity.

a. *Etiological validity.* Indicates the degree to which MK-801 mimics the developmental state consisting of positive psychiatric symptoms. Unfortunately, MK-801 does not replicate the development of positive symptoms, which are thought to progress over many years (or decades) and are influenced by a complex interplay of genetics and environmental factors. Therefore, etiological validity in these studies was low. Construct validity of the MK-801 model is also poor.

b. *Face validity.* Indicates the degree to which a model is consistent with pathophysiological and behavioral changes observed in human disease states. In reference to experiments described in Chapter 2.0, acute (i.p.) administration of MK-801 had high face validity because hyperlocomotor activity reliably increased and startle habituation plus PPI reliably decreased. These observations support the MK-801 model as having high phenomenological validity and an effective method towards

testing and identifying novel antipsychotic drug approaches (Bubeníková-Valešová, Horáček, Vrajová, & Höschl, 2008; Geyer et al., 2001; Swerdlow & Light, 2018).

c. *Predictive validity.* This indicates how accurate a model is at predicting the human response to drug treatments. As discussed previously, predictive validity is important in the context of this thesis because one of our aims was to conduct pre-clinical research determining the efficacy of targeting the eCB system to modulate MK-801 induced behaviors. Therefore, it was important to begin with a model such as acute NMDAR antagonism, due to reliable reversal of behaviors using antipsychotics. Acute MK-801 models maintain high predictive validity making it an ideal preclinical approach to explore novel antipsychotic therapeutics.

There are some fundamental challenges associated with animal research: (a) there is insufficient evidence that behaviors can even be studied through the lens of a single neurotransmitter system, (b) there is still much we do not know about the degree to which NMDAR is involved in the pathogenesis of schizophrenia; (c) acute models do not inform about the developmental nature of symptoms; and (d) there is a large degree in heterogeneity in the degree and experience of symptoms, as well as variability in response to different pharmacological approaches (Nestler & Hyman, 2010). This serves as a reminder to not overinterpret our findings by making assumptions about the human experiences of mental illness. Nestler and Hyman (2010) encourage researchers to provide greater conceptual clarity by stating precise research goals up front, in order to prevent overgeneralization of results and protect against unfounded claims regarding human disease states. As such, transparency and operational definitions go far in preventing misinterpretation of results.

1.3.5 Measuring locomotor activity using open field test (OFT)

Locomotor hyperactivity is commonly used as a metric to assess the behavioral cluster of symptoms associated with positive psychiatric symptoms (Van Den Buuse, 2010). The open field test (OFT) is used to measure activity levels in rodents and informs a large variety of experimental designs (Fig. 2a). MK-801 induces locomotor hyperactivity in rodents and this has been attributed, in part, to increased dopaminergic activity in the mesolimbic dopamine circuit (Jones & Robbins, 1992; Kapur & Mamo, 2003). Therefore, measuring locomotor activity following MK-801 administration has some face validity in modeling psychotic agitation and stereotypic and hyperactive movement impairments observed in schizophrenia (Breier, Malhotra, Pinals, Weisenfeld, & Pickar, 1997; Van Den Buuse, 2010; Van Den Buuse et al., 2011). This thesis was interested in whether pharmacological manipulation of the eCB system may be used to reduce hyperlocomotion in rodents.

1.3.6 Measuring sensorimotor gating with prepulse inhibition (PPI) startle chambers

Much like locomotor activity allows for general inferences about hyperkinetic movement impairments, basic scientists use PPI startle chambers to quantify and infer sensorimotor gating function (Brosda et al., 2011; Fendt et al., 2001). Figure 2b (top) is a schematic showing how PPI is measured. Briefly, a rodent is placed in a startle chamber and a loud audible pulse is administered inducing a startle response. Interestingly, when a loud pulse is preceded by a prepulse, startle reflex is attenuated. This phenomenon is referred to as prepulse inhibition (PPI) and allows researchers to infer functionality of circuits underlying sensorimotor gating (specifically the CSPP circuit). PPI deficits are commonly observed in psychiatric patients and reliably induced with MK-801 in rodent studies (Suryavanshi et al., 2014). By using PPI as an

indicator of sensorimotor gating in rodents, researchers gain valuable insight into some behavioral deficits associated with positive psychotic symptoms.

Researchers gain multiple behavioral measures from running PPI experiments. Figures 2, 3 and 5 provide context for the following measures that were quantified following a 22 min PPI protocol. The measures looked at in this thesis include: (a) *startle amplitude.* This is the startle amplitude induced by a 120 dB pulse alone and it is measured as arbitrary units providing a relative quantification of motor responses by reflex force. This force is calculated using a motion sensor and potentiometer that are calibrated based on animal size (Curzon, Zhang, Radek, & Fox, 2009). Startle amplitude is reliably enhanced with MK-801 (Fig. 3 shows how startle amplitude is assessed during P120 Before, During and After blocks); (b) *reactivity.* This is a metric of the startle reflex and is measured as a unitless force in response to the prepulse alone and it is reliably increased by MK-801. This is often measured to show that inhibition of startle is not due to the prepulse alone; (c) *PPI long.* This is a measure of the percentage reduction in startle amplitude attributed to the presence of 50, 80 and 140 ms prepulse intensities and MK-801 reliably reduces PPI long; and (d) *PPI short.* This is a measure of PPI as it relates specifically to short prepulse interval (30 ms) trials and is considered separately due to observations suggesting PPI is decreased during prepulse short trials. MK-801 impairs PPI short. PPI allows researchers to consider all of these metrics of behavior in a single rodent, with each measure providing additional insight into drug interactions, circuitry deficits and behavioral abnormalities relating to psychiatry.

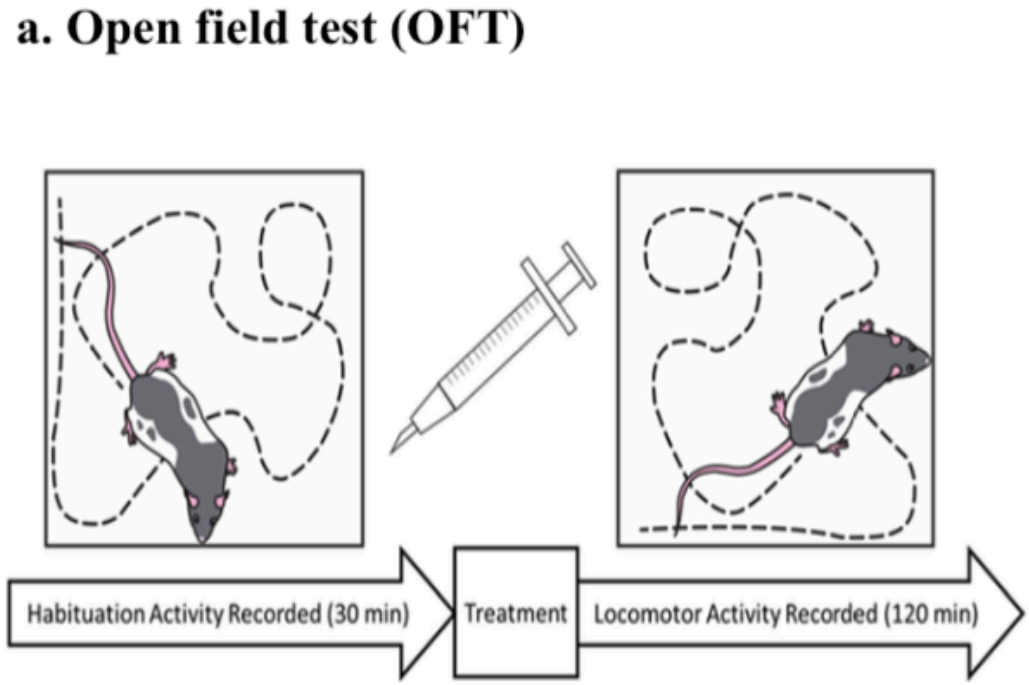

Fig 2. Schematics portraying the two behavioral paradigms used to measure rodent behavior: (a) The OFT was used before (30 min) and after (120 min) treatment administration (adapted from: Lins, Marks, Zabder, Greba, & Howland, 2019). (b) Commercially available startle chambers were used to deliver a series of pulses and prepulses which induce a startle response in rodents. The force of the startle reflex is then calculated and may be used to compare sensorimotor gating function.

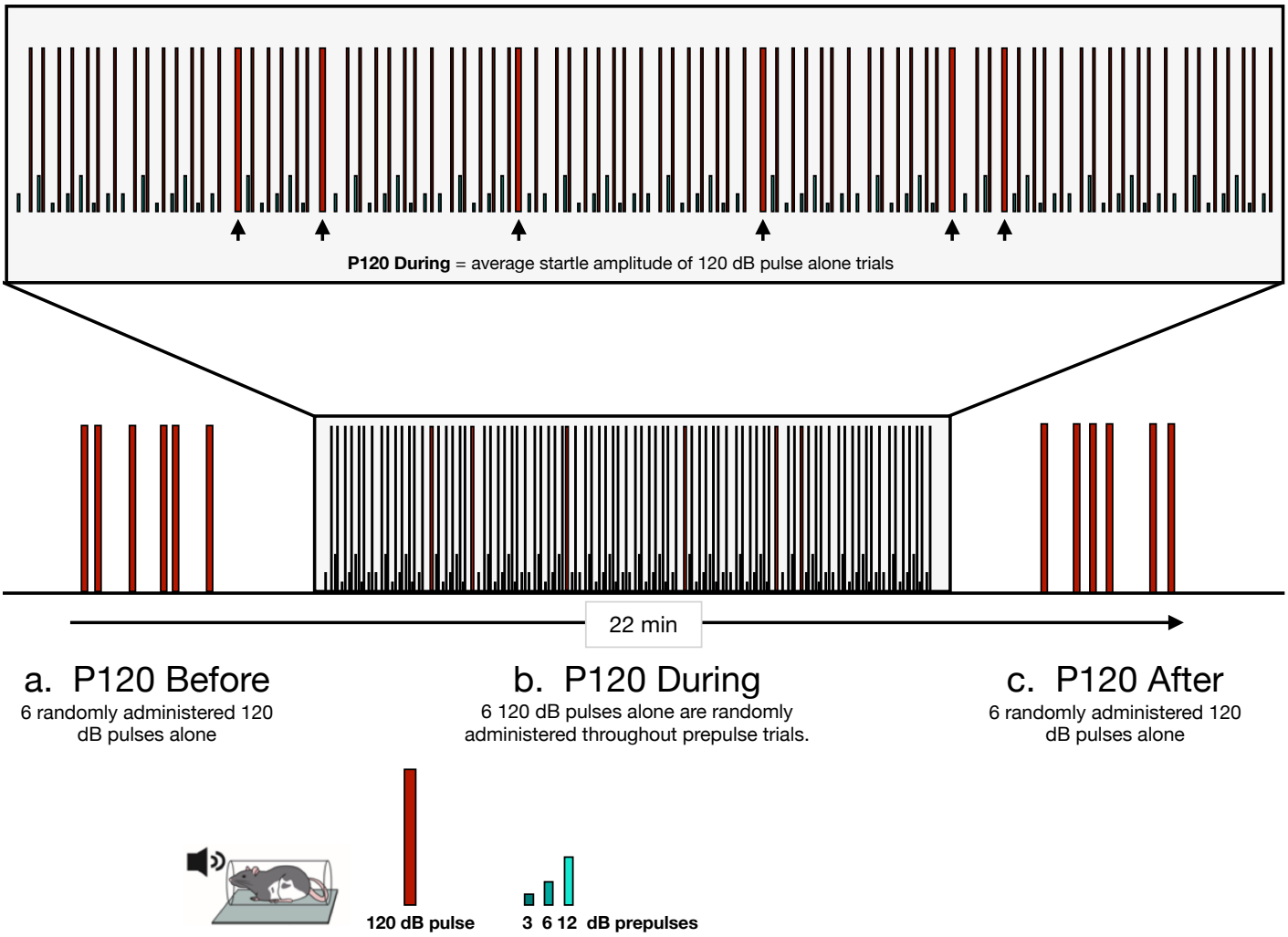

Fig 3. Schematic shows how P120 Before, During and After are calculated. The PPI protocol includes placing the rodent in a startle attenuation chamber and then beginning a 22 min program that administers a series of pulses and prepulses. The startle amplitude is then recorded following *pulse alone*, *prepulse alone* (called Reactivity), and *prepulse + pulse* (prepulse inhibition; PPI). Moving from left to right depicts an example of how pulses and prepulses are administered. **(a) P120 Before.** This phase during the PPI protocol (~5 min) randomly administers six 120 dB pulses alone. P120 Before is then calculated by averaging the 6 startle amplitudes. **(b) P120 During.** In addition to 84 *prepulse + pulse* trials, there are also 6 random 120 dB pulses administered alone. The evoked startle amplitudes are averaged to determine P120 During. **(c) P120 After.** This is the final ~ 5 min of the PPI protocol and is conducted the exact same way as P120 Before. By averaging startle amplitudes during these phases we are able to compare startle response over time.

1.4 The endocannabinoid system (eCB)

Targeting the eCB system has gained increased support as a potentially novel therapeutic approach to treat some aspects of schizophrenia (Galve-Roperh et al., 2009; Harkany et al., 2007; Mechoulam & Parker, 2013). The eCB system is comprised of type 1 cannabinoid receptors (CB1R) that are highly expressed on presynaptic neurons throughout the brain (Mechoulam & Parker, 2013). Endogenous cannabinoids (e.g., anandamide, 2-AG) are synthesized post-synaptically and retroactively inhibit presynaptic neurons, primarily via CB1R in the central nervous system (Mechoulam & Parker, 2013; Murray, Morrison, Henquet, & Forti, 2007). CB1R are G-protein coupled 7-transmembrane receptors (GPCR) that are considered the most prolific receptors in the brain (Di Marzo, Bifulco, & De Petrocellis, 2004). Although CB1R are not expressed on dopaminergic neurons, they are widely expressed on GABAergic and glutamatergic neurons, both of which modulate dopaminergic activity (Galve-Roperh et al., 2009). Due to the inhibitory effect of CB1R activation on presynaptic neurons, the eCB system has potential to modulate dysfunctional neurotransmitter signaling in the brain (Ross, 2007). With this in mind, the next section discusses CB1R molecular signaling in the context of determining the best pharmacological approach to manipulate CB1R.

1.4.1 CB1R molecular signaling

CB1R is a GPCR and successful ligand binding activates the $G_{i/o}$ subunit causes the alpha (a) and Beta-gamma (B_y) subunits to separate, resulting in: (a) inhibition of adenylate cyclase (AC) causing a decreases in cyclic adenosine monophosphate (cAMP) and subsequent downstream signaling; (b) stimulation of potassium channels leading to an influx of potassium and hyperpolarization of neurons (i.e., reducing the likelihood of an action potential occurring) (Chevaleyre et al., 2007; De Oliveira, Ramos, Amaro, Dias, & Vieira, 2019; Howlett et al., 2002;

Kellogg, Mackie, & Straiker, 2009; Lu & MacKie, 2016; Tsetsenis et al., 2011). Put more simply, CB1R activation leads to AC inhibition resulting in activation of inwardly rectifying potassium channels. Figure 4 is a schematic showing how different pharmacological approaches may target CB1R.

Tetrahydrocannabinol (THC), a partial CB1R agonist and the psychoactive ingredient in cannabis, is capable of *inducing* psychosis in some patients, rather than alleviating it (Manseau & Goff, 2015). A plethora of studies have shown that cannabis use increases the risk of experiencing psychotic symptoms as well as increasing the risk of developing schizophrenia (review: Manseau & Goff, 2015; Murray et al., 2007). Although THC does not appear to cause schizophrenia, it certainly worsens symptoms and progresses aspects of the disease. Therefore, THC does not appear to have therapeutic efficacy with respect to locomotor activity and PPI. Although CB1R agonism worsens some positive psychiatric symptoms, other CB1R allosteric modulators (PAMs) may have potential to exert a more subtle effect on CB1R activity by binding at allosteric sites, in contrast to agonists binding at orthosteric sites (Ross, 2007).

Allosteric CB1R sites (Fig. 4c-d) are distinct from orthosteric sites and provide an additional pharmacological target to modulate CB1R activation. CB1R partial agonists like THC bind to orthosteric sites, while CB1R allosteric modulators enhance the efficacy of successful ligand binding at orthosteric sites (Conn, Christopoulos, & Lindsley, 2009). This may provide a unique opportunity to attenuate neuronal activity using "biased agonism" or second messenger pathways (Ahn, Mahmoud, & Kendall, 2012). The next section discusses the preclinical efficacy of CB1R positive allosteric modulators (PAM; reference 1.4.2) as a more subtle approach to selectively enhance CB1R activity (i.e., in the presence of eCBs). Given the unique distribution of CB1R on GABAergic and glutamatergic neurons, CB1R PAMs may have therapeutic

potential by indirectly modulating dopamine release, while producing potentially fewer side effects compared to current antipsychotics (Conn et al., 2009). Therefore, CB1R PAMs may increase the propensity for on-hand eCBs (specifically 2-AG) and have potential to enhance inhibitory valence in active synapses.

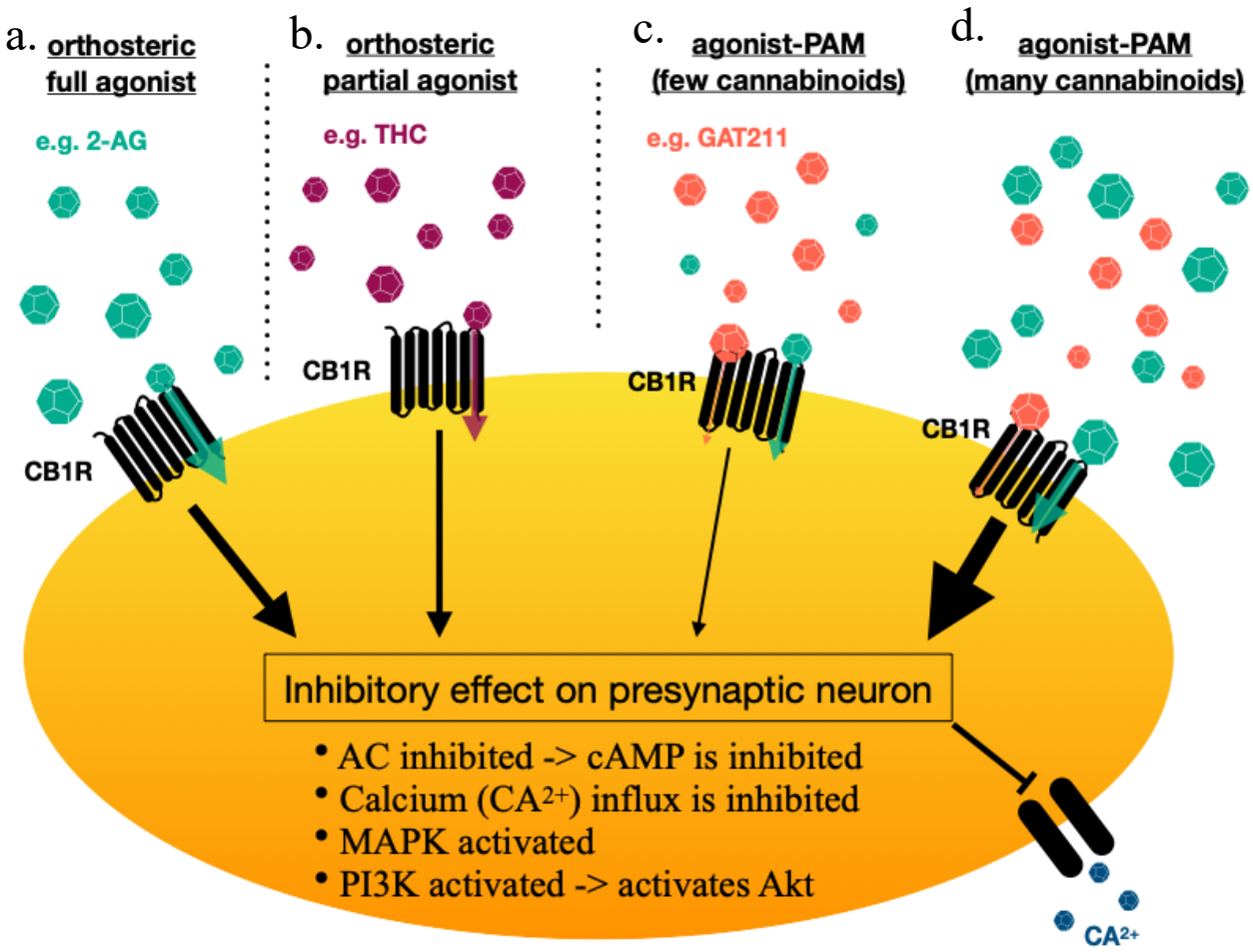

Fig 4. Schematic depicts CB1R molecular signaling and primary signaling pathways. CB1 receptors are seven-transmembrane receptors coupled to a $G_{i/o}$ protein. Activation of CB1R contributes to a series of intracellular signalling cascades including: cyclic adenosine monophosphate (cAMP) inhibition, CA^{2+} suppression via voltage-gated calcium channels (VGCC) and activation of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinases (PI3K) signaling pathways. **(a)** Full CB1R agonists such as 2-AG activate CB1R resulting in substantial presynaptic inhibition. **(b)** partial agonists provide less CB1R activation than full agonists; although, research shows THC reliably worsens some behavioral phenotypes. **(c-d)** Agonist-positive allosteric modulators (PAMs) like GAT211 bind at allosteric CB1R sites and provides some agonistic properties in addition to increasing the propensity of successful ligand binding in the presence of orthosteric ligands (e.g., 2-AG). Panel c and d show the difference of PAM activity in the presence of few versus many synaptic endocannabinoids, highlighting how PAMs may exert differential effects at different synapses.
(Adapted from: Petrucci et al., 2017; Zou & Kumar, 2018)

1.4.2 Positive allosteric modulation of CB1R using GAT211

CB1R PAMs have significant advantages over CB1R agonists and partial agonists such as THC because they act to enhance endogenous cannabinoid tone rather than overriding it, thereby limiting potential for on-target adverse effects associated with supraphysiological activation; and limiting desensitizing and tolerance-inducing effects associated with direct activation (Alaverdashvili & Laprairie, 2018; Laprairie et al., 2017; Mallipeddi, Janero, Zvonok, & Makriyannis, 2017). GAT211 is an agonist-PAM of CB1R and is a racemic compound with two enantiomers: GAT-228 (R) and GAT-229 (S) that bind to CB1R allosteric sites (primary agonistic properties come from GAT-228, while PAM properties come from GAT-229) (Laprairie et al., 2017). In the absence of on-hand cannabinoids in the synaptic cleft (shown in Fig. 4c-d), GAT211 has minimal pharmacological effect on presynaptic neurons making it ideally suited to enhance inhibitory feedback communication in circuits actively releasing 2-AG and AEA (Laprairie et al., 2017; Ross, 2007).

The next chapter describes my masters research in an attempt to determine whether CB1R PAMs have any preclinical efficacy as novel therapeutics to alleviate some positive symptoms associated with schizophrenia. To that end, we tested the effects of GAT211 on MK-801 induced hyperlocomotion and PPI impairment in rodents.

1.5 Hypotheses

Our general hypothesis was that CB1R PAMs will attenuate some positive psychiatric symptoms. More specifically, we hypothesized that GAT211 would reverse two MK-801 induced behaviors in rodents: (1) hyperlocomotion; and (2) PPI impairments. In addition to these two primary behaviors, we also predicted that GAT211 would reduce deficits associated with startle amplitude and reactivity.

1.6 Objectives

During the following experiments, our aim was to further characterize the relationship and interactions between the eCB system and MK801-induced behavioral phenotypes. Additionally, we investigated whether CB1R PAMs have preclinical potential for improving the positive symptoms of schizophrenia.

2.0 EFFECTS OF MK-801 AND GAT211, A TYPE 1 CANNABINOID RECEPTOR POSITIVE ALLOSTERIC MODULATOR, ON LOCOMOTOR ACTIVITY AND PREPULSE INHIBITION IN MALE, LONG EVANS RATS*

* This data chapter has been submitted to the journal Psychopharmacology.

2.1 Abstract

Rationale. Antipsychotics help alleviate the positive symptoms associated with schizophrenia; however, their debilitating side effects spur the search for better treatment options. Novel compounds can be screened for antipsychotic potential following acute N-methyl-D-aspartate receptor (NMDAR) blockade with noncompetitive antagonists such as MK-801 in rodent behavioral models. Given the known interactions between NMDAR and type 1 cannabinoid receptors (CB1R)(Li, Yan, Wilson, & Swartzwelder, 2010; Rodríguez-Muñoz, Sánchez-Blázquez, Merlos, & Garzón-Niño, 2016), compounds that modulate CB1Rs may have therapeutic potential for schizophrenia. *Objectives.* This study assessed whether the CB1R positive allosteric modulator GAT211, in contrast to Δ^9-tetrahydrocannabinol (THC), has the potential to reduce psychiatric behavioral phenotypes following acute MK-801 treatment in rats. *Methods.* The effects of GAT211 and THC were compared following acute MK-801 administration in addition to GAT211 effects on prepulse inhibition of the acoustic startle response. *Results.* As expected, acute MK-801 (0.15 mg/kg) produced a significant increase in locomotor activity and impaired PPI. GAT211 treatment alone (0.3-3.0 mg/kg) dose-dependently reduced locomotor activity and the acoustic startle response. GAT211 (3.0 mg/kg) also prevented hyperlocomotion caused by MK-801 and showed some modest ability to normalize MK-801-induced PPI impairments. *Conclusion.* These findings support continued preclinical research regarding the usefulness of CB1R positive allosteric modulators as antipsychotics.

Keywords: schizophrenia; antipsychotic; cannabis; THC; NMDAR; open field; acoustic startle

2.2 Introduction

Schizophrenia is a severe mental illness that continues to rank among the top 15 causes of disability in the world today (Moreno-Küstner et al., 2018; Nicholl et al., 2010; Simeone et al., 2015). All clinically utilized antipsychotics are either typical, high-affinity dopamine 2 receptor (D2R) antagonists that mainly reduce positive symptoms associated with psychoses; or atypical antipsychotics with actions on serotonin, norepinephrine, and dopamine receptors that may be efficacious at treating both positive and negative symptoms of psychoses (Kapur & Mamo, 2003). Unfortunately, severe side effects including extrapyramidal motor effects, cognitive impairment, weight gain, and metabolic disturbances warrant the need for better treatments (Goff et al., 2017; Li et al., 2016; Sendt et al., 2015). In an effort to reduce the signs and symptoms of psychoses via novel mechanisms, we investigated how pharmacological manipulation of the endocannabinoid (eCB) system influences behavioral deficits in the MK-801 rodent model of schizophrenia.

The eCB system has gained attention as a potential therapeutic target for schizophrenia (Bolognini & Ross, 2015; Galve-Roperh et al., 2009; Harkany et al., 2007; Lu & MacKie, 2016; Mechoulam & Parker, 2013). Type 1 cannabinoid type receptors (CB1R) are highly expressed pre-synaptically on GABAergic interneurons and glutamatergic pyramidal neurons throughout the brain, including areas of the cortico-striatal-pallidum or pontine tegmentum (CSPP) circuitry (Lu & MacKie, 2016; Mackie, 2005; Mechoulam & Parker, 2013; Tsou et al., 1998). In particular, considerable evidence suggests that dysregulated eCB signaling in the prefrontal cortex (PFC), ventral hippocampus (vHIPP) and ventral pallidum may underlie dopaminergic hyperactivity implicated in the pathophysiology of schizophrenia (Aguilar et al., 2015; Hudson et

al., 2019; Peres et al., 2016; Silveira et al., 2017). Interestingly, there is also evidence that eCB signaling in astrocytes may play a large role in modulating brain signaling (Smith et al., 2020). The 2 most-abundant endogenous cannabinoids are anandamide (AEA; Devane et al., 1992) and 2-arachidonoylglycerol (2-AG; Stella et al., 1997). These neurotransmitters are synthesized post-synaptically and inhibit pre-synaptic neurons that express CB1R (Mechoulam & Parker, 2013; Murray et al., 2007; Volk & Lewis, 2016). Due to its inhibitory effects, the eCB system is uniquely positioned to attenuate some behavioral deficits resulting from hyperactive neurotransmission (Ross, 2007).

The cannabinoid hypothesis of schizophrenia proposes that excessive activation of the eCB system – for example, by chronic THC consumption during adolescence – may lead to a hyperdopaminergic state and dysregulated glutamatergic signaling in the brain (Fakhoury, 2017; Pickel et al., 2020). Therefore, glutamate hypofunction-dopamine hyperfunction theories and the cannabinoid hypothesis complement one another and address the fact that prolonged treatment approaches for schizophrenia will likely require a *subtle* modulation of glutamate release (Moghaddam & Krystal, 2012), one that allosteric modulation of CB1R has potential to influence.

GAT211 is a racemic mixture containing equal parts of the *R*-enantiomer GAT228 (a CB1R allosteric agonist) and the *S*-enantiomer GAT229 (a CB1R positive allosteric modulator [PAM]), and it is therefore described as an agonist-PAM (*i.e.* ago-PAM) of CB1R (Laprairie et al., 2017). Unlike orthosteric agonists that bind an identical site on their receptor to the endogenous ligand, PAMs binds to a separate site(s), which increases that receptor's affinity for orthosteric ligands as well as the potency and/or efficacy of that receptor's signaling (Laprairie et al., 2017; Leweke, Mueller, Lange, & Rohleder, 2016). PAMs have significant advantages over

CB1R agonists and partial agonists such as THC because they act to enhance endogenous cannabinoid tone rather than overriding it, thereby limiting potential for on-target adverse effects associated with supraphysiological activation; and limiting desensitizing and tolerance-inducing effects associated with direct activation (Seibenhener & Wooten, 2015).

In the present study, we measured 2 rodent behaviors associated with dysfunctional dopaminergic and glutamatergic signaling, both of which are highly implicated in the neuropathology of schizophrenia: (1) locomotor activity and (2) prepulse inhibition (PPI) of the acoustic startle response (Fakhoury 2017; Uno and Coyle 2019) in rats treated with or without the THC or the CB1R ago-PAM GAT211. Locomotor activity was measured in an open field (Seibenhener & Wooten, 2015) and considered as a metric for psychomotor agitation and stereotypic movement impairments observed in schizophrenia (Van Den Buuse et al., 2011). PPI is the inhibition of the startle response by a low intensity stimulus or 'prepulse' and is considered an indicator of sensorimotor gating (Ahmari et al., 2012; Fakhoury, 2017; Geyer et al., 2001). PPI depends on CSPP function and is impaired in patients with neuropsychiatric disorders as well as in rats following CSPP manipulation (Swerdlow, Geyer, & Braff, 2001; Swerdlow & Light, 2018). We used acute treatment with dizocilpine (MK-801), a potent anticonvulsant and selective, non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist, in male rats to produce changes in locomotor activity and PPI reminiscent of schizophrenia (Cadinu et al., 2018; Homayoun & Moghaddam, 2007).

Our general hypothesis was that GAT211 would have the potential to attenuate hyperlocomotion, startle response and PPI deficits in rodents. To that effect, we tested the efficacy of GAT211 at attenuating MK-801-induced alterations in (a) locomotor activity and (b) PPI; using THC as a comparator compound during locomotor testing.

2.3 Methods

2.3.1 Subjects

One hundred and fifty-three adult male Long-Evans rats were used for locomotor testing (n = 144) and for PPI (n = 12) (Charles River Laboratories, Kingston, NY, USA). Two rats were removed due to mistakes during injections and a third animal was identified as an outlier (> 2 standard deviations from the mean). Animals were pair housed and left undisturbed for 1 week upon arrival at our facility. Food (Purina Rat Chow) and water were provided *ad libitum* and animals were housed 2/cage in ventilated plastic home cages. The vivarium was temperature-controlled, and a 12:12-h lighting cycle was maintained, with lights on at 0700 each day. Each home cage included enrichment in the form of a plastic tube and all experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and the University of Saskatchewan Research Ethics Board.

Three subsets of animals were used for experiments (reference Table 1). First, we compared GAT211 and MK-801 (0.15 mg/kg) effects during locomotor trials (n = 93). Each rodent received 2 *i.p*. injections 5 min apart and were randomly assigned to 1 of 8 groups: (a) ethanol vehicle and saline (n = 20); (b) GAT211 (0.3 mg/kg) and saline (n = 8); (c) GAT211 (1.0 mg/kg) and saline (n = 9); (d) GAT211 (3.0 mg/kg) and saline (n = 12); (e) ethanol vehicle and MK-801 (n = 17); (f) GAT211 (0.3 mg/kg) and MK-801 (n = 8); (g) GAT211 (1.0 mg/kg) and MK-801 (n = 9); (h) GAT211 (3.0 mg/kg) and MK-801 (n = 10). Second, we compared THC and MK-801 (0.15 mg/kg) effects during locomotor trials (n = 48). Each rodent received 2 *i.p*. injections 5 min apart in 4 groups: (a) methanol vehicle and saline (n = 12); (b) THC (3.0 mg/kg) and saline (n = 12); (c) methanol vehicle MK-801 (n = 12); (d) THC (3.0 mg/kg) and MK-801 (n = 12). Third, a within subjects design (n = 12) was used and rats were pseudo-randomly assigned

to receive all 6 treatments over the course of 6 trials with 3-4 day washout periods: (a) ethanol vehicle and saline; (b) GAT211 (1.0 mg/kg) and saline; (c) GAT211 (3.0 mg/kg) and saline (n = 9); (d) ethanol vehicle and MK-801; (e) GAT211 (1.0 mg/kg) and MK-801; (f) GAT211 (3.0 mg/kg) and MK-801. Experiments were conducted by multiple researchers arranged in the following manner: (a) THC x MK-801 locomotor effects: researcher A (n = 48); (b) GAT211 x MK-801 locomotor effects: researcher B (n = 20), C (n = 20) and D (n = 56); (c) GAT211 x MK-801 effects on PPI: researcher D (n = 12). Scoring of behavior was automated with Noldus EthoVision software allowing researchers to remain blind to treatment throughout analysis.

2.3.2 Compound preparation

THC was dissolved in a vehicle of methanol, kolliphor, and saline at a ratio of 1:1:6 and injected at a volume of 5 mL/kg. Concentration was mixed at 0.60 mg/mL and injected at a 3.0 mg/kg dose. GAT211 was dissolved in a vehicle of ethanol, kolliphor, and saline at a ratio of 1:1:6 and injected at a volume of 5 mL/kg. Concentrations were 0.06 mg/mL for the 0.3 mg/kg dose, 0.20 mg/mL for 1 mg/kg dose, and 0.60 mg/mL for the 3 mg/kg dose. MK-801 was dissolved in saline at a concentration of 0.15 mg/mL and injected at 1 mL/kg. After mixing, MK-801 was aliquoted and stored at -20ºC. THC and GAT211 were prepared weekly, aliquoted and refrigerated prior to experiments.

2.3.3 Locomotor activity

Locomotor activity testing was performed in a separate room than injections and all rats were naïve to the procedure. Activity was measured in a 40 (w) x 40 (l) x 60 (h) cm arena made from white corrugated plastic (Fig. 5a). A ceiling camera recorded activity from above and simultaneously captured activity of 4 rodents in 4 separate arenas. Analysis of distance traveled

was assessed using EthoVision software (Noldus Information Technology, Wageningen, The Netherlands). Spontaneous baseline activity was measured for 30 min in the open fields prior to injections. Then, rodents were randomly assigned to a treatment condition and administered 2 *i.p.* injections 5 min apart. After a 15 min waiting period, rats were returned to their respective open fields and distance traveled (m) was assessed for 120 min (Fig. 1a).

2.3.4 prepulse inhibition (PPI)

Based on findings from locomotor experiments the 1.0 and 3.0 mg/kg doses of GAT211 were retained for PPI trials. Startle response and PPI were assessed using a within-subjects design over 6 trials, including a washout period of at least 3 days between trials. We used a single SR-LAB Startle Response System (San Diego Instruments, San Diego, California, USA) and calibrated the chamber to 70 dB background noise (±2 dB). Trials lasted for 22 min and administered a range of pulses alongside prepulses (Fig. 1b and 5b). A wide range of parameters were assessed, including: (a) startle response to a 120 dB pulse; (b) reactivity to prepulse (3, 6, 12 dB); (c) PPI short interval (30 ms); (d) PPI long intervals (50, 80, 140 ms); (e) PPI intensities (3, 6, 12 dB).

2.3.5 Data analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 21 for Windows (IBM, Chicago, IL). A 2x4 analysis of variance (ANOVA) was used to assess locomotor activity (Fig. 6) with 2 factors: MK-801 (saline, 0.15 mg/kg) and GAT211 (vehicle, 0.3, 1.0, 3.0 mg/kg); while a 2x2 ANOVA was used to assess THC (saline, 3.0 mg/kg) and MK-801 (saline, 0.15 mg/kg). For startle and PPI data, data was collected using a within-subjects design in which each rat (n = 12) was exposed to six treatments. A 2x3x3 RM ANOVA was used to assess startle (Fig. 7) across three factors: MK-801 (saline, 0.15 mg/kg), GAT211 (vehicle,

1.0, 3.0 mg/kg), block* (P120 Before, During, After; * blocks described further in Figure 3). A 2x3 ANOVA was used to assess reactivity (Fig. 8) using 2 factors: MK-801 (saline, 0.15 mg/kg) and GAT211 (vehicle, 1.0, 3.0 mg/kg); in addition to a 2x3x4 ANOVA across 3 factors: MK-801 (saline, 0.15 mg/kg), GAT211 (vehicle, 1.0, 3.0 mg/kg) and Prepulse Intensity (no stim, 3, 6, 12 dB). A 2x3x3x3 RM ANOVA was used to assess PPI long (Fig. 9) data across four factors: MK-801 (saline, 0.15 mg/kg), GAT211 (vehicle, 1.0, 3.0 mg/kg), Prepulse Intensity (3, 6, 12 dB), and Prepulse Interval (50, 80, 140 ms). An additional 2x3x3 ANOVA was used to compare PPI short data (Fig. 10) at only the 30 ms interval: MK-801 (saline, 0.15 mg/kg), GAT211 (vehicle, 1.0, 3.0 mg/kg) and Prepulse Intensity (3, 6, 12 dB). Significance between group means was determined using Tukey and Bonferroni *post-hoc* tests depending on the circumstances (Tukey tests were used for repeated measures [RM] analyses). Statistical significance for comparisons was considered $p \leq 0.05$. PPI calculations are described (Lins, Marks, Phillips, & Howland, 2017) and in line with a previously published protocol (Howland et al., 2012). PPI short was analyzed separately as it produces prepulse facilitation (Ballendine et al., 2015; Howland et al., 2012). Non-significant main effects and interactions were not included in the Results.

2.4 Results

2.4.1 GAT211 reduces MK-801-induced locomotor hyperactivity

Figure 6 shows dose-dependent effects of GAT211 or THC on MK-801-induced locomotor activity. First, we assessed baseline locomotor data (30 min trials prior to treatment) to ensure no baseline differences occurred between treatment groups (data not shown): (a) 2x4 ANOVA was used to assess baseline activity for the GAT211 experiment. No significant main effects of MK-801 ($F(1,85) = 1.66$, $p = 0.20$) or GAT211 ($F(3,85) = 1.78$, $p = 0.16$) or an

interaction ($F(3,85) = 1.07$, $p = 0.37$) were noted; (b) for the THC experiment, 2x2 ANOVA revealed no main effects of MK-801 ($F(1,44) = 0.01$, $p = 0.92$) or THC ($F(1,44) = 0.39$, $p = 0.54$) and no interaction ($F(1,44) = 0.09$, $p = 0.76$), demonstrating there were no locomotor differences between pre-treatment groups.

Figure 6a and 6b show the effects of GAT211 or THC on MK-801 induced locomotor activity. First, a 2x4 ANOVA [(saline, 0.15 mg/kg MK-801) x (vehicle, 0.3, 1.0 and 3.0 mg/kg GAT211)] revealed significant main effects of MK-801 ($F(1,85) = 50.04$, $p < 0.0001$) and GAT211 ($F(3,85) = 50.04$, $p < 0.01$) and no interaction effect ($F(3,85) = 1.76$, $p = 0.16$). Tukey's *post-hoc* analysis revealed that GAT211 (3.0 mg/kg) reduced locomotor activity when compared to vehicle ($p < .05$), 0.3 mg/kg ($p < .05$), and 1.0 mg/kg ($p < .05$) doses (shown in Fig 6a inset). In contrast, a 2x2 ANOVA [(saline, 0.15 mg/kg MK-801) x (3.0 mg/kg THC)] assessed THC effects and revealed a similar main effect of MK-801 ($F(1,44) = 35.39$, $p < 0.0001$); however, no main effect of THC ($F(1,44) = 1.70$, $p = 0.20$) and no MK-801 x THC interaction effect ($F(1,44) = 0.34$, $p = 0.56$).

In addition to total distance traveled, GAT211 (3.0 mg/kg) and THC (3.0 mg/kg) groups were compared by distance traveled in 15 min bins; Fig. 6c and 6d). A 2 x 2 x 8 RM ANOVA [(saline, MK-801) x (vehicle, GAT211) x (8 x Time blocks)] revealed significant main effects of MK-801 ($F(1,33) = 18.9$, $p = 0.001$), GAT211 ($F(1,33) = 12.5$, $p < 0.001$) and Time ($F(3.9,129.6) = 51.8$, $p < 0.0001$). In addition, interaction effects were observed for GAT211 x Time ($F(7,231) = 4.38$, $p = 0.0001$) and GAT211 x MK-801 ($F(1,33) = 5.8$, $p < 0.05$). Tukey's *post-hoc* analysis revealed that locomotor activity was significantly higher in the 0-15 min Time block and GAT211 groups moved significantly less in the 0-15 and 15-30 min Time blocks compared to controls ($p < 0.05$). *Post-hoc* analysis of the GAT211 x MK-801 interaction

revealed that MK-801 increased locomotor activity compared to saline groups and GAT211 significantly reduced that hyperactivity ($p < 0.05$). A 2 x 2 x 8 RM ANOVA [(saline, MK-801) x (vehicle, THC) x (8 x Time blocks)] assessed THC (Fig. 6d) and revealed main effects of Time ($F(1.8,79.5) = 45.5$, $p < 0.0001$) and MK-801 ($F(1,44) = 35.4$, $p < 0.0001$), plus an interaction effect of Time x MK-801 ($F(7,308) = 12.3$, $p < 0.0001$). *Post-hoc* analyses revealed that the main effect of Time occurred between 15 and 30 min ($p < 0.05$) and MK-801 was significantly increased in the first 5 Time blocks (T+90 min) compared to controls ($p < 0.05$).

2.4.2 GAT211 and MK-801 reduce and increase startle, respectively

Figure 7 illustrates the effects of MK-801 or GAT211 on startle amplitude. A 2 x 3 x 3 RM ANOVA [(saline, MK-801) x (vehicle, 1.0 and 3.0 mg/kg GAT211) x (P120 Before, During and After)] revealed main effects of P120 ($F(1.0,11.5) = 71.52$, $p < 0.001$), MK-801 ($F(1,11) = 11.00$, $p < 0.01$) and GAT211 ($F(2,22) = 11.09$, $p < 0.001$) and all interactions were insignificant. *Post-hoc* analyses of the P120 main effect revealed startle amplitude was higher in P120 Before compared to P120 During and After blocks (Fig. 7a), demonstrating an expected habituation to a 120 dB pulse ($p < .05$). *Post-hoc* analyses revealed the main effect of GAT211 was due to the 3.0 mg/kg dose significantly decreasing startle ($p < 0.05$; Fig. 7c). Overall startle results are shown in Figure 7d, highlighting that MK-801 increased startle amplitude and GAT211 (3.0 mg/kg) significantly reduced the enhanced startle in P120 Before ($p < 0.05$).

2.4.3 MK-801 increases, while GAT211 does not affect, startle reactivity

Figure 8 shows the effects of MK-801, GAT211, and prepulse intensity on reactivity (startle response to the prepulse alone). A 2 x 3 x 4 RM ANOVA [(saline, MK-801) x (vehicle, 1.0 and 3.0 mg/kg GAT211) x (No stim, 3, 6 and 12 dB)] revealed MK-801 significantly increased startle reactivity ($F(1,11) = 6.20$, $p < 0.05$). There were no main effects of GAT211

(F(2,22) = 2.46; $p = 0.11$) or prepulse intensity (F(3,33) = 0.36; $p = 0.78$) and all interactions were insignificant (statistics not reported). Figure 8a shows the effect of GAT211 and MK-801 on reactivity and Figure 8b shows treatment effects at different prepulse intensities.

2.4.4 GAT211 fails to block the MK-801-induced PPI impairment

Figure 9 shows the effects of GAT211 and MK-801 on PPI for long interval trials. A 2 x 3 x 3 x 3 RM ANOVA [(saline, MK-801) x (vehicle, 1.0 and 3.0 mg/kg GAT211) x (50, 80 and 140 ms prepulse interval) x (3, 6 and 12 dB prepulse intensity)] revealed main effects of MK-801 (F(1,11) = 9.69, $p < 0.05$) and prepulse intensity (F(2,22) = 162.7, $p < 0.001$), but no main effects of GAT211 (F(2,22) = 1.02, $p = 0.38$) nor prepulse interval (F(1.4,15.1) = 3.68, $p = 0.06$); all interactions were insignificant (statistics not reported). As expected, louder prepulses increased PPI and MK-801 significantly decreased PPI overall. Although GAT211 failed to significantly affect PPI, inspection of data reveals that for pp12 trials GAT211 may reverse the MK-801 impairment (Figure 9d), although the inconsistency of this effect for other prepulse intensities raises concerns about its reliability. This is discussed further in the General Discussion and provides compelling justification for a future experiment (see sections: 3.2.2).

Figure 10 shows results comparing the effects of GAT211, MK-801 and Prepulse Intensity on PPI short (30 ms prepulse interval). A 2 x 3 x 3 RM ANOVA [(saline, MK-801) x (vehicle, 1.0 and 3.0 mg/kg GAT211) x (3, 6 and 12 dB prepulse intensity)] revealed a main effect of prepulse intensity (F(2,22) = 15.05, $p < 0.001$), but no main effects of MK-801 (F(1,11) = 4.70; $p = 0.053$) nor GAT211 (F(2,22) = 0.14, $p = 0.87$). However, a MK-801 x Intensity interaction effect (F(2,22) = 7.69, $p < 0.05$) confirmed that MK-801 did vary by prepulse intensity (Figure 10b and c). *Post-hoc* analyses revealed that MK-801 significantly impaired short interval PPI for trials with 12 dB prepulses.

2.5 Discussion

This study assessed the effect of the CB1R ago-PAM GAT211 on hyperlocomotion and PPI impairment in rodents induced with MK-801. *In vivo*, MK-801: (a) increased locomotor activity; (b) increased startle amplitude; (c) increased prepulse startle reactivity; and (d) impaired PPI. When administered alone, GAT211 (3.0 mg/kg): (a) reduced locomotor activity; (b) reduced startle amplitude during P120 before trials; (c) had no effect on reactivity; and (d) no effect on PPI. When GAT211 was co-administered with MK-801, we observed: (a) GAT211 (3.0 mg/kg) reduced MK-801 induced hyperactivity in the early 15 min time blocks of locomotor testing; and (b) although insignificant, a subtle dose dependent effect of GAT211 on MK-801 induced startle and PPI warrants further research, specifically during early P120 trials and increased prepulse Intervals and Intensities, in which sensitivity to pulse is highest. These results provide evidence that GAT211 has some efficacy to reduce hyperlocomotor activity, startle amplitude and habituation to a 120 dB pulse. In contrast to our hypothesis, GAT211 had minimal effects on reactivity and PPI, however, additional research is necessary to tease apart these effects under new experimental designs. Additionally, we showed that THC (3.0 mg/kg) had no effect on locomotor activity, neither alone nor when co-administered with MK-801.

2.5.1 MK-801 and GAT211 effects on locomotor activity

Locomotor activity was measured using a 120 min open field protocol. Results from the locomotor experiments show that MK-801 increased distance traveled and GAT211 reduced distance traveled. These results are in line with the significant body of evidence suggesting NMDAR antagonism induces hyperlocomotor activity in rats (Moghaddam & Javitt, 2012). Acute administration of GAT211 reduced locomotor activity in rats. One explanation is that GAT211 exerts a net inhibitory influence on CSPP and mesolimbic circuits that mediate

hyperlocomotion via CB1R on dysregulated glutamatergic and GABAergic neurons involved with modulating motor activity (Carlsson & Carlsson, 1989; Moghaddam & Krystal, 2012). Seminal research suggests corticostriatal circuitry modulates hyperarousal via glutamatergic neurons that provide negative feedback in response to hyperarousal (Carlsson & Carlsson, 1989). NMDAR antagonists like MK-801 disrupt this circuit producing the characteristic glutamate hypofunction-dopamine hyperfunction state associated with hyperlocomotor activity (Carlsson & Carlsson, 1989; Moghaddam & Krystal, 2012). We predicted that GAT211 would restore CSPP dysregulation and enhance negative feedback mechanisms that normally inhibit hyperlocomotion (Carlsson & Carlsson, 1989; Moghaddam & Krystal, 2012). In summary, these experiments show systemic administration of GAT211 reduces locomotor activity in rodents, possibly by increasing CB1R activity on GABAergic interneurons and indirectly disinhibiting glutamatergic neurons that modulate ascending sensorimotor communication.

2.5.2 MK-801 and GAT211 effects on PPI

The PPI protocol used in these studies consisted of a 22 min PPI program that produced a wide range of prepulse intensity (e.g., 3, 6 and 12 dB) and interval (e.g., 30, 50, 80 and 140 ms) trials, followed by a loud 120 dB pulse that induced a startle response. Results from the PPI experiments are consistent with previous studies, showing that NMDAR antagonism (e.g., MK-801): (a) increased startle amplitude (Lins et al., 2017; Varty, Bakshi, & Geyer, 1999; Wiley, Harvey, Balster, & Nicholson, 2003); (b) increased reactivity (Lins et al., 2017); and (c) impaired PPI assessed with long (Swerdlow et al., 2001) and short intervals (Howland et al., 2012; Lins et al., 2017). We hypothesized that GAT211 would interact with MK-801 to improve the aforementioned behavioral deficits; however, results suggest that while GAT211 reduced

startle amplitude and habituation to a 120 dB pulse it had no significant effect on reactivity or PPI.

We used a range of prepulse intervals to assess PPI because interval-specific effects have been reported in the literature and in our lab (Fendt et al., 2001; Lins et al., 2017). Our results indicate that during long interval trials (50, 80 and 140 ms), MK-801 reliably impaired PPI; however, prepulse *facilitation* occurred during short interval trials, particularly for lower intensity prepulses. These findings are consistent with previous studies on prepulse facilitation during short prepulse interval trials (Brosda et al., 2011; Howland et al., 2012). Interestingly, prepulse facilitation is consistently observed in MK-801 models as well as in some patients with psychiatric disorders such as autism (Perry, Minassian, Lopez, Maron, & Lincoln, 2007) and attention deficit hyperactivity disorder (Feifel, Minassian, & Perry, 2009; Howland et al., 2012). It remains to be seen whether prepulse facilitation accompanies other mental illnesses (e.g., schizophrenia), but these indicators may serve as valuable biomarkers to improve individualized treatment strategies (Howland et al., 2012).

2.5.3 Future directions

Taken together, effects of GAT211 on PPI and its impairment by MK-801 were modest, although the compound did affect startle amplitude. Further research is required to disentangle these relationships and clarify the therapeutic potential of cannabinoid allosteric modulators. Furthermore, these data could be advanced using more potent GAT211 derivatives (Garai et al., 2020), different protocols and models of the behavioral symptoms of schizophrenia (e.g., sociability and cognitive assays, neurodevelopmental animal models, genetic knock-in/out and lesion models), and chronic treatment paradigms. In conclusion, our observations support the

continued investigation of CB1 PAMs as novel pharmacological approaches to attenuate hyperkinetic phenotypes.

Conflict of Interest Statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

2.6 Figures

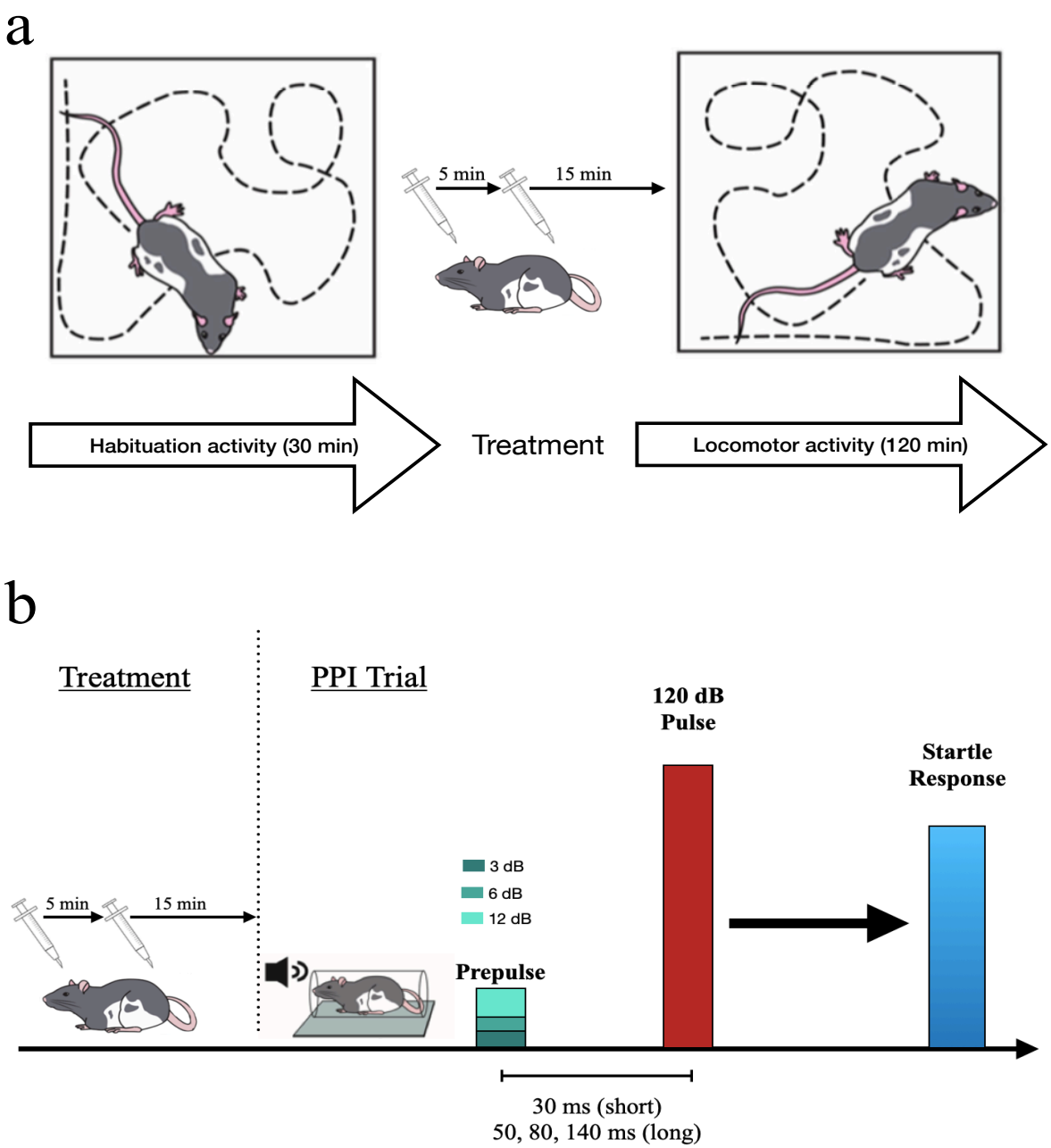

Fig 5. Schematics depicting the methods and timeline used to assess locomotor activity and prepulse inhibition (PPI). **(a)** Using the open field test (OFT), locomotor activity was first assessed at baseline (30 min). Next, treatments were randomly assigned and administered *i.p.* 5 min apart and given a 15 min break following injections. Rats were then returned to the OFT for a 120 min trial to assess the effect of treatment on distance traveled. **(b)** Prepulse inhibition (PPI) is a 22 min program that administers a series of pulses to assess startle reflex and PPI (the attenuating of startle response by a prepulse. Pulses are administered pseudo-randomly and vary based on prepulse intensity (3, 6 and 12 dB) and prepulse interval (30, 50, 80 and 140 ms) preceding a 120 dB pulse. Injection procedures remained similar and PPI began 15 mins after the second injection.

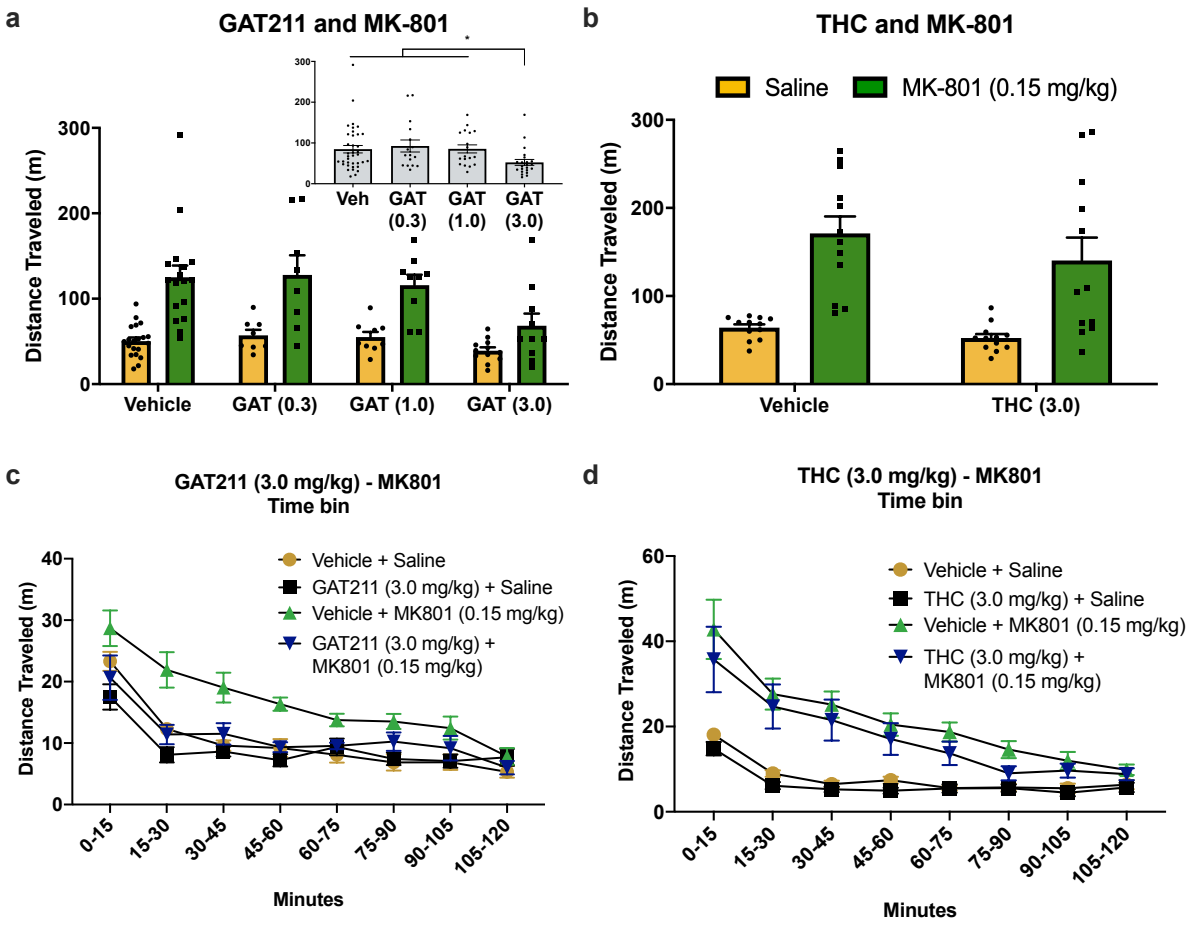

Fig 6. Panels show dose-dependent effects of GAT211 or THC on MK-801-induced locomotor activity in the open field test (OFT). Baseline measures were assessed prior to treatments with no observed differences between groups ($p < 0.05$; data not shown), while post-treatment locomotor activity was recorded for 120 mins (above) and GAT211 versus THC effects are contrasted. **(a)** GAT211 (3.0 mg/kg) significantly reduced distance traveled compared to lower doses (inset), while MK-801 increased distance traveled. **(b)** THC was assessed similarly, revealing the same effect of MK-801; however, no effect of THC was observed. *$p < 0.05$ compared to saline within either Vehicle of THC (3.0 mg/kg). **(c)** A 2 x 2 x 8 RM ANOVA [MK-801 x GAT211 x Time block] assessed treatment effects by Time (15 min bins). Data highlights main effects of MK-801, GAT211 and Time, with *post-hoc* analyses revealing that distance traveled was reduced in the first 15 min Time block. Additionally, GAT211 x MK-801 and GAT-211 x Time interactions were observed with the greatest locomotor changes occurring in the first two Time blocks. **(d)** There was no main effect of THC on MK-801 induced hyperactivity. Data are displayed as mean ± S.E.M. for GAT211 ($n = 93$) and THC ($n = 48$).

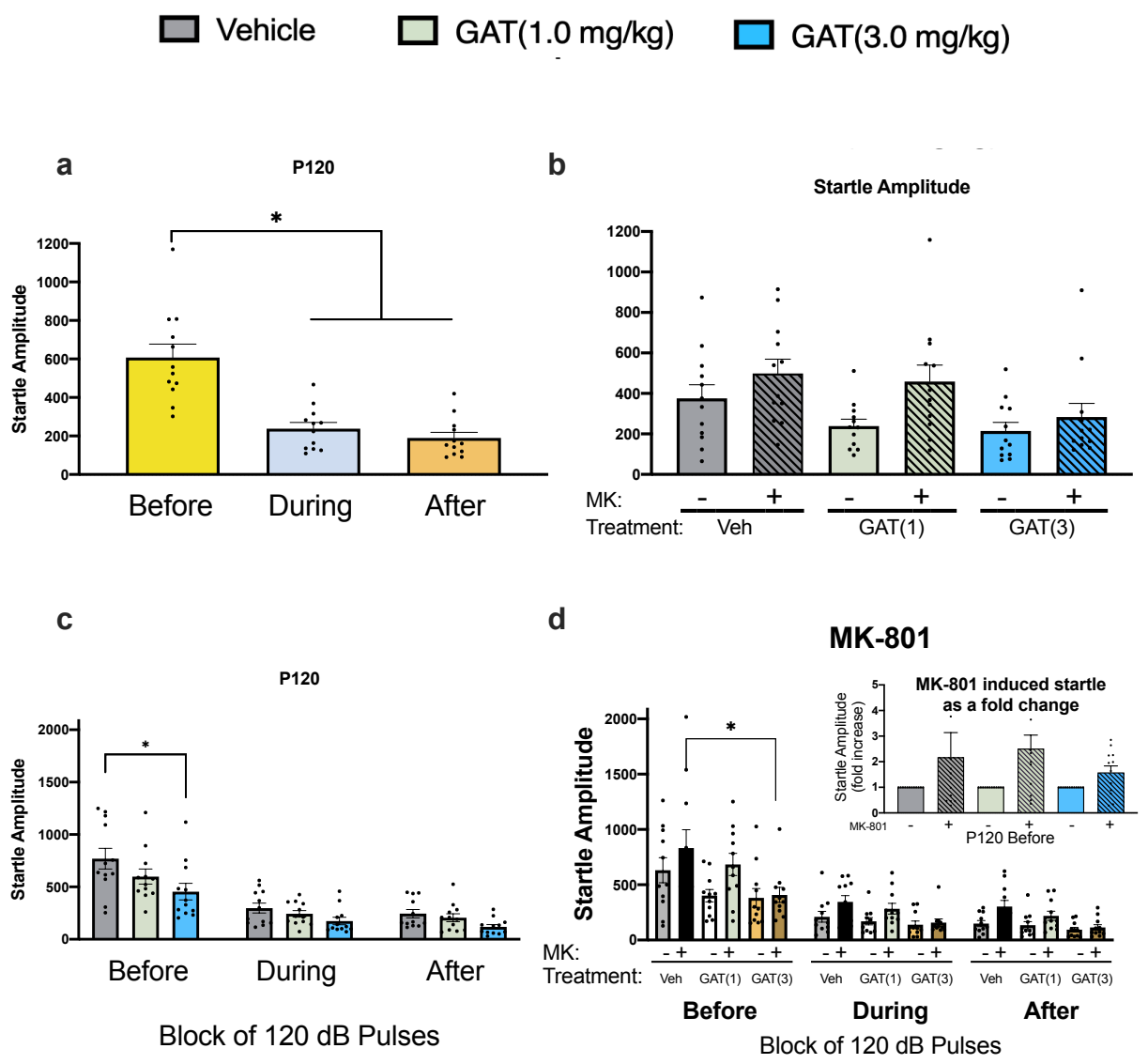

Fig 7. Startle amplitudes were calculated in response to a 120 dB pulse and Tukey's *post-hoc* analyses assessed significant differences in groups ($p < 0.05$). Block data represents the average startle amplitude in response to six 120 dB pulses in the first 5 min (Before block), randomly interspersed during prepulse trials (During block) and in the final 5 min (After block). **(a)** Shows a habituation to a 120 dB pulse by P120 groups. **(b)** the effects of MK-801 and GAT211 on startle amplitude for the P120 trials during the test session. **(c)** MK-801 trials were removed to show the effect of GAT211 at P120 levels. GAT211 (3.0 mg/kg) significantly reduced startle amplitude during P120 Before trials. **(d)** Inset shows MK-801 induced increase in startle as a fold change compared to saline conditions (baseline: 1 fold). The inset also highlights behavior during P120 Before trials, due to increased sensitivity to pulse in early trials (no significant differences between groups, possibly due to a large variability in response to MK-801; 3 data points not shown (adjusted y-axis down from 15 for clarity)). All data are displayed as mean ± S.E.M. $n = 12$. GAT(1) = 1.0 mg/kg; GAT(3) = 3.0 mg/kg.

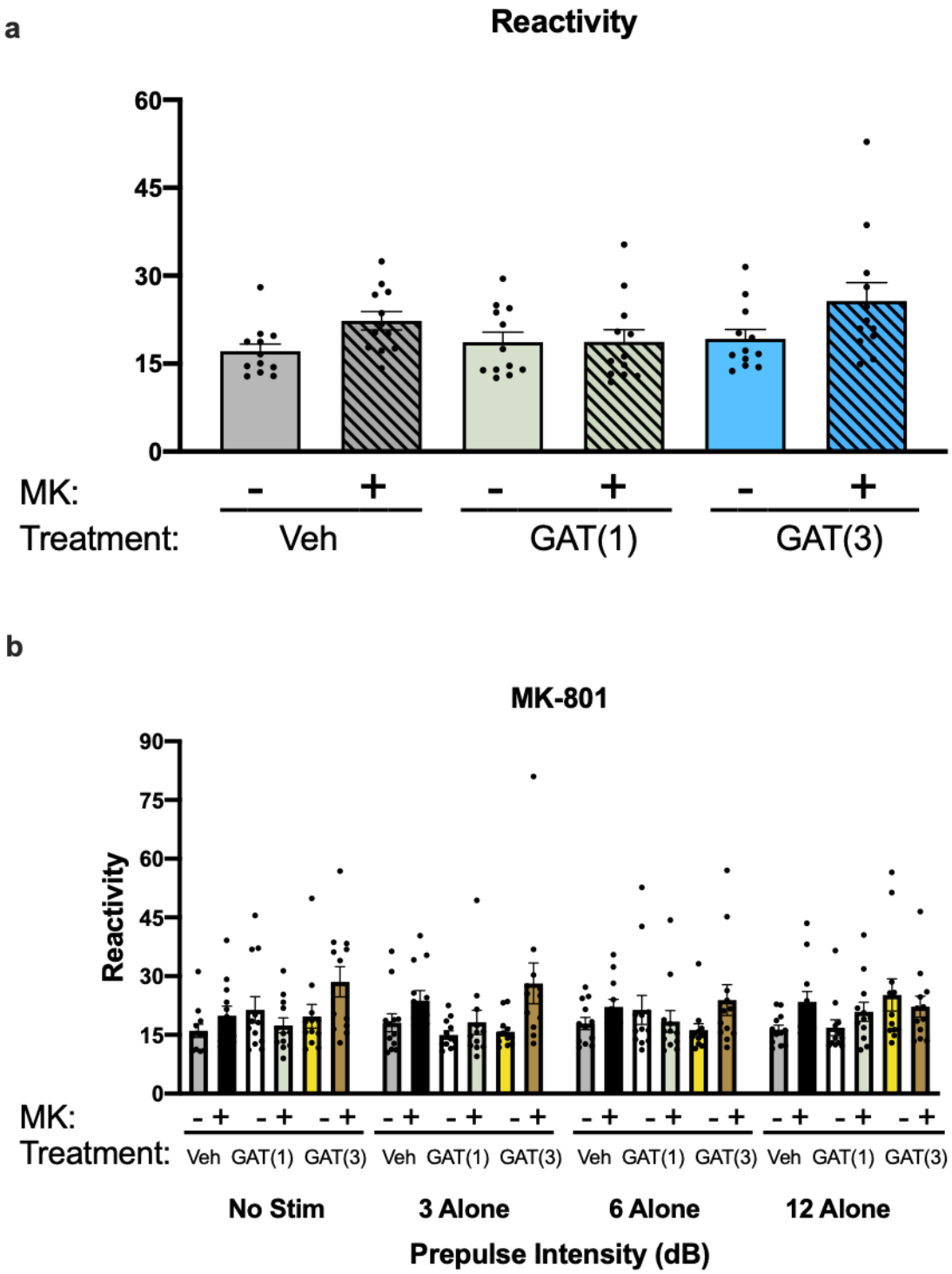

Fig 8. Graphs show the effects of MK-801, GAT211 and prepulse intensity on reactivity (acoustic startle response to the prepulse). **(a)** Prepulse intensity and GAT211 had no significant effect on reactivity; although, MK-801 significantly increased reactivity. **(b)** No additional main effects were observed; however, GAT211 (3.0 mg/kg) displays a modest ability to selectively increase reactivity during low-intensity trials. GAT(1) = 1.0 mg/kg; GAT(3) = 3.0 mg/kg.

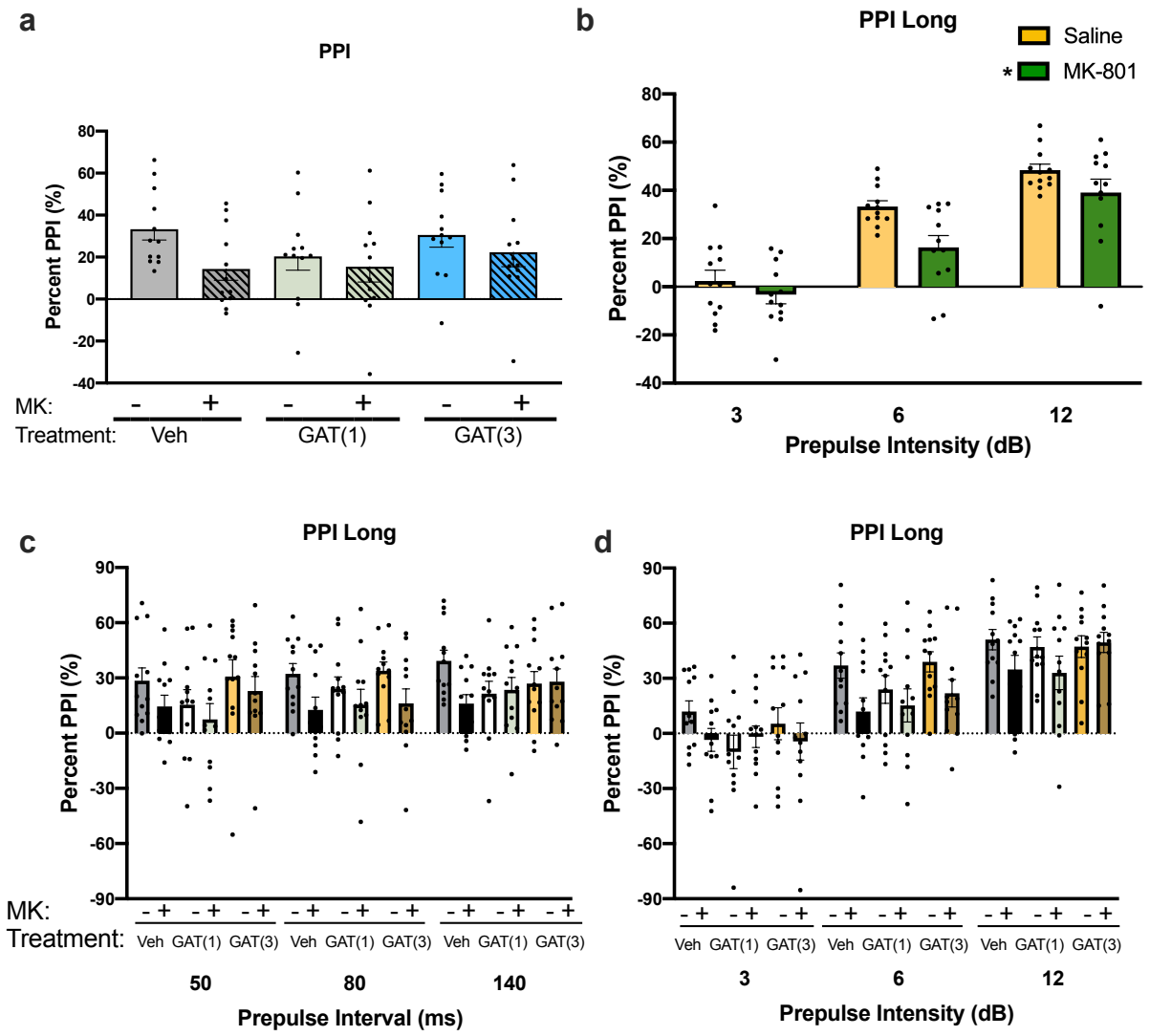

Fig 9. Prepulse Inhibition (PPI) long was assessed based on average PPI by prepulse interval (50, 80, 140 ms) and intensity (3, 6 and 12 dB) trials. **(a)** MK-801 significantly reduced PPI; however, no main effect of GAT211 was observed. Data shows the effect of treatment on PPI, regardless of prepulse intensity or interval (e.g., prepulse trials are combined) **(b)** Highlights the main effects of MK-801 and intensity. **(c)** There was no main effect of Interval ($p = 0.06$). Of note, GAT211 (3.0 mg/kg) appears to have a modest (non-significant) effect on recovering PPI during the 140 ms prepulse Interval. **(d)** There was a main effect of Intensity, with increased PPI recovery occurring at higher dB prepulses. Similar to panel c, MK-801 induced PPI deficits appear to modestly recover at 12 dB Intensities, when GAT211(3.0 mg/kg) is co-administered. GAT(1) = 1.0 mg/kg; GAT(3) = 3.0 mg/kg.

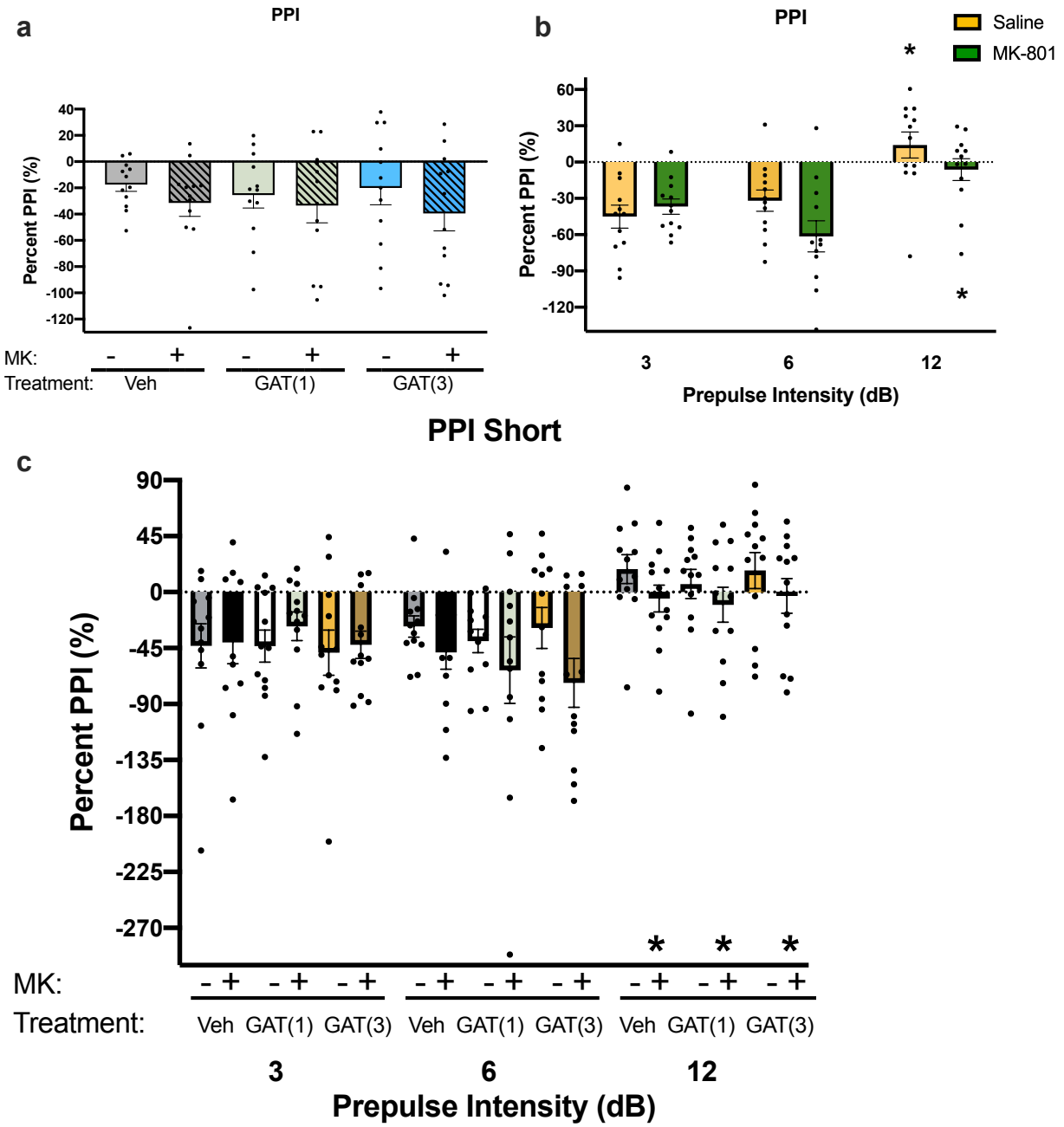

Fig 10. Prepulse Inhibition (PPI) short was assessed by 30 ms prepulse Interval trials. **(a)** GAT211 had no significant effect on PPI short, nor any interaction effects. **(b)** There was a main effect of prepulse Intensity with significance occurring during 12 dB prepulse Intensities. **(c)** In addition to a MK-801 main effect, a MK-801 x Intensity interaction effect was observed. Prepulse facilitation is also observed at low Intensities which is recovered at the 12 dB prepulse Intensity in addition to enhanced prepulse facilitation in MK-801 groups, as prepulse Intensity increases. GAT(1) = 1.0 mg/kg; GAT(3) = 3.0 mg/kg.

2.7 Table

Table 1. An overview of the experimental methods and sample sizes used. All rodents were male Long Evans rats.

Locomotor Testing		**MK-801**	
		Saline	MK-801
GAT-211	Vehicle	*n* = 20	*n* = 17
	GAT-211 (0.3 mg/kg)	*n* = 8	*n* = 8
	GAT-211 (1.0 mg/kg)	*n* = 9	*n* = 9
	GAT-211 (3.0 mg/kg)	*n* = 12	*n* = 10
		MK-801	
		Saline	MK-801
THC	Vehicle	*n* = 12	*n* = 12
	THC (3.0 mg/kg)	*n* = 12	*n* = 12

PPI Testing (within subjects)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Vehicle - Saline	*n* = 12					
GAT(1.0) - Saline	*n* = 12					
GAT(3.0) - Saline	*n* = 12					
Vehicle - MK-801	*n* = 12					
GAT(1.0) - MK-801	*n* = 12					
GAT(3.0) - MK-801	*n* = 12					

3.0 GENERAL DISCUSSION

3.1 Findings

The experiments discussed in Chapter 2.0 used pharmacological manipulation to promote eCB activity in rodents in order to assess effects on behavioral symptoms induced by the NMDAR antagonist MK-801. We showed that systemic MK-801 administration reliably increases locomotor activity in rats and if co-administered with GAT211, hyperlocomotor activity is blocked. Similarly, THC was co-administered with MK-801 to compare an alternative approach to manipulating the eCB system and show THC had no effect on exaggerated locomotion. These results show that allosteric modulation results in heterogenous behavioral outcomes compared to partial CB1R agonists such as THC. In addition to assessing locomotor activity, we assessed whether GAT211 has efficacy at reducing startle reflex, reactivity to a prepulse and PPI impairments following MK-801 administration. Furthermore, systemic co-administration of GAT211 dose-dependently enhanced habituation and reduced startle amplitude in response to a 120 dB pulse (Chapter 2.0, Fig. 7). Finally, we co-administered GAT211 and MK-801 to assess whether GAT211 had any efficacy at modulating PPI deficits. Results indicate that GAT211 has minimal efficacy at recovering MK-801-induced PPI deficits; although, a modest (non-significant) increase in PPI was observed during pp12 dB trials, regardless of MK-801 administration (discussed further in 3.2.2). This has some preclinical relevance as it provides insight into how the eCB system modulates mesolimbic and mesocortical pathways associated with positive psychiatric symptoms. These findings suggest GAT211 has efficacy to reduce hyperlocomotor activity and attenuate startle amplitude; however, continued research is necessary to determine the full range of behavioral effects that GAT211 and other CB1R allosteric modulators have on sensorimotor gating and PPI.

3.2 Methodological considerations

3.2.1 Locomotor activity

Testing for locomotor hyperactivity was based upon the premise that enhanced dopaminergic activity induced by psychotomimetic drugs (i.e., PCP, Ketamine, MK801) results in increased locomotor activity (Breier et al., 1997; Jackson et al., 2004; Van Den Buuse, 2010; Vollenweider et al., 1997). MK-801 induced hyperactivity mimics some aspects of psychomotor agitation seen in a subset of schizophrenia patients, which is reliably blocked with antipsychotics giving the MK-801 induced state predictive validity in rodents. This approach is commonly used in behavioral research as a metric resembling hyperdopaminergia and resulting psychotic agitation observed in schizophrenia (Van Den Buuse, 2010). Results suggest that GAT211 administered alone at 3.0 mg/kg, reduces locomotor activity and the greatest reductions occurred in early time bins (observed in 15 and 30 min time bins). This is expected behavior in rodents as they reliably display enhanced activity at the beginning of a trial. Additionally, early time bin data captures behavior in closer proximity to injection times, suggesting that drug effects may be peaking then. Taken together, these observations provided some justification to further assess data by time bins.

The effect of GAT211 on MK-801 induced activity was expectedly subtle and most activity occurred towards the beginning of the 120 min locomotor experiments, which is in accordance with previous findings in our lab. Additionally, there was a large variation in response to MK-801 which prompted us to assess results by time bin. Figure 6c highlights these results and shows that GAT211 and MK-801 interacted in early time bins whereas THC did not, contrasting the heterogeneity between different pharmacological approaches to modulate CB1R.

3.2.2 Prepulse inhibition (PPI)

Sensorimotor gating deficits are commonly observed in patients with psychiatric symptoms and has particular relevance to the positive symptoms of schizophrenia. A large body of evidence suggests that these deficits are the result of increased dopamine tone, as seen in animal MK-801 models as well as human patients with sensorimotor gating deficits (Geyer et al., 2001; Swerdlow et al., 2001). To measure sensorimotor gating deficits, researchers often rely on the quantification of PPI to assess functionality of the CSPP circuit which underscores a large amount of motor and movement control, including sensorimotor gating (Ahmari et al., 2012).

The 22 min protocol in these experiments was used as a metric for drug effects on sensorimotor gating, and conveniently gave us the opportunity to assess startle amplitude, reactivity and PPI using a large range of prepulse intensities and intervals. Startle amplitude serves as a measure of motor activity and compliments results showing reduced locomotor activity. Results presented in Figure 7, suggest that GAT211 dose dependently reduces startle amplitude. Taken together, these observations suggest that CB1R PAMs have some efficacy at attenuating hyperkinetic behaviors. In contrast to startle amplitude to a loud 120 dB pulse, *reactivity* is a measure of startle response to the quieter prepulse. Some research suggests that if there are differences in reactivity between treatment groups, some PPI affects may be due to the prepulse and therefore, may be a confounded indicator of sensorimotor gating function. Regardless, our results showed no sign of changes in reactivity between groups.

We predicted that GAT211 would exert a restorative effect on MK-801 induced PPI deficits due to the unique expression of CB1R in implicated brain regions responsible for modulating sensorimotor gating (Li et al., 2010; Marsicano & Lutz, 1999; Pertwee & Ross, 2002). Aside from the interesting trend of GAT211 to recover deficits in 12 dB prepulse trials

(Fig. 9d), GAT211 showed no significant ability in these experiments to restore MK-801 induced PPI impairments. Despite the lack of statistical significance, PPI results during 12dB prepulse trials are certainly worth noting and following up on. Figure 9c and d provide an interesting trend that justify further hypotheses-driven experiments in which GAT211 is explored at different doses and prepulse intensities. Based on observations in Figure 9d, the threshold for prepulse intensity appears to include 6 and 12 dB prepulses (e.g., no PPI effect is observed at 3 dB, while PPI percentage begins increasing above baseline [PPI = 0] in the 6 to 12 dB prepulses. Therefore, it would be very interesting to test whether GAT-211 recovers MK-801 induced PPI impairments at 6 and 12 dB prepulses. Furthermore, the results reported throughout locomotor and PPI experiments, suggest that GAT-211 0.3 mg/kg and 1.0 mg/kg doses may be a little low. Thus, it would be additionally interesting to assess the higher doses of GAT211 (e.g., 3.0 and 10.0 mg/kg doses). When taken together, these data provide strong support to test the hypothesis that GAT-211 at 3.0 and 10.0 mg/kg doses will improve PPI deficits during ≥ 6 dB prepulse trials. The alternative, would be to isolate the data we have collected thus far; however there are concerns with this approach that encourage a separate experiment, including: (a) the MK-801 effect persists at all prepulse intensity trials; (b) GAT211 doses may be slightly low; (c) enhanced likelihood of committing a type 1 error. Therefore, this provides compelling evidence to support future research investigating the effects of ≥ 3.0 mg/kg doses of CB1R PAMs on PPI during ≥ 6 dB prepulse intensity trials. This will also allow for the opportunity to investigate newly developed GAT compounds that have improved situational relevance (e.g., full PAM compounds).

3.2.3 GAT211

This thesis has centered around the hypothesis that positive allosteric modulation of CB1R with GAT211 may be a viable way to attenuate hyperactive neurocircuitry associated with positive psychiatric symptoms. This was due to the observations that CB1 receptors are uniquely expressed on GABAergic and glutamatergic receptors in the brain in addition to having novel retrograde inhibitory effects that provide a unique and subtle approach to alter brain wide signalling. GAT211 targets allosteric CB1R sites and increases the propensity for successful ligand binding (primarily with 2-AG), promoting enhanced CB1R activation. As previously discussed, GAT228 provides the agonistic tendencies of GAT211, while GAT229 provides the PAM attributes (Laprairie et al., 2017). In consideration of the pure PAM properties of GAT229, our lab has begun piloting PPI studies using GAT229 in addition to investigating other CB1R-targeting compounds continually being developed (e.g. GAT591). Currently, we predicted that the ago-PAM properties of GAT211 would be sufficient to provide a uniquely subtle influence on CB1R, such that inhibitory feedback networks in the brain (reliant on postsynaptic release of eCBs and presynaptic expression of CB1R) would be enhanced, having some efficacy towards alleviating two behaviors with relevance to the positive symptoms of schizophrenia.

3.2.4 Effects of repeated dosing with GAT211

PPI data collected in these experiments made use of a within subjects design in which 12 rodents were each given all six treatments (Chapter 2.0: Figs 7-10; Table 1) in a pseudo-random order, with at least a three-day washout period in between treatments. This approach was favorable because it capitalizes on *Recycle*, *Reduce* and *Reusing* principles and limited the number of rodents required. However, there is some uncertainty regarding the degree to which repeated dosing contributed to the findings. Furthermore, we don't know what effect MK-801

administration (versus saline) had on remaining PPI trials. There is little to no research on the effects of repeated GAT211 dosing and these uncertainties identify a viable area for future research. Ideally, this could be tested using a between-subjects design in which rodents receive GAT211 at varied doses and frequency.

3.2.5 Systemic administration

The experiments in Chapter 2.0 used a systemic approach (i.p. injection) to administer GAT211. This is often used as an early strategy to ascertain drug interaction effects and provides insight for future studies using more localized approaches (e.g., intracanulla administration directly into specific brain regions; transgenic knock-out/in mice lines that allow for precise control of receptor subtype expression, etc.). Systemic approaches also have relevance to clinical psychiatry, as many drugs used to treat mental illness are given systemically (e.g., oral administration). However, by using systemic approaches we sacrifice some specificity when interpreting responses to drug treatments. In the case of GAT211, CB1R is expressed throughout many regions and different neuronal subtypes; therefore, behavioral changes only allow for limited inferences about activities and interactions occurring at the synaptic level. One hypothesis in this thesis was that CB1R influence on GABAergic and glutamatergic neurons in the cortex, striatum, VTA and CSPP circuit (e.g., regions underscoring motor movement and gating), which may exert a global inhibitory influence to counter hypoglutamate-hyperdopaminergic phenotypes. CB1R is also expressed on neurons in the nucleus accumbens (NAc) of rodents and non-primates as well as in the hippocampus and hypothalamus of the mammalian brain (Kucera et al., 2018; Markov et al., 2009). Therefore, findings from this thesis are most accurately interpreted within the context of systemic (global) administration, and the

effects of GAT211 are experienced across a plethora of important brain regions beyond the scope of this thesis.

We hypothesized that positive allosteric modulation may improve inhibitory valence in the brain, meaning that the complex arrangement of excitatory and inhibitory signals modulating brain activity would undergo a net inhibitory effect on gross circuit activity. This subtle modulation is essential in consideration that CB1R partial agonists (e.g. THC) lead to the worsening and provocation of positive schizophrenia symptoms (Müiller-Vahl & Emrich, 2008; Vigano et al., 2009). GAT211 and other PAMs are different because they primarily interact with on-hand eCBs (e.g., 2-AG; AEA), yet remain relatively inactive otherwise.

3.3 Making connections

3.3.1 Basal ganglia circuitry

One of the primary purposes of the basal ganglia is to finetune sensory and motor signals traveling through the thalamus and to help with precision and coordination of movement (Blumenfeld, 2010, Chapter 16). GAT211 exerts an inhibitory effect via densely expressed CB1 receptors throughout the basal ganglia, which have complex interactions with multiple neurotransmitter systems. Due to the basal ganglia's dependence on excitatory *and* inhibitory signals, in addition to the presence of CB1R throughout CSPP circuits (including the basal ganglia); it is plausible that GAT211 exerted a net inhibitory effect on striatal circuits while remaining relatively inert in others.

Additionally, thalamic nuclei send projections to limbic, prefrontal, oculomotor, premotor, supplementary motor, and primary motor cortices, suggesting that modulating these systems is likely to influence motor behaviors (Blumenfeld, 2010, p. 753). Interestingly, CB1R also highly expressed amongst thalamic nuclei; therefore, the inherent dysfunction of dyskinesias

(including sensorimotor gating deficits) lies in dysfunctional finetuning that often relies on excitatory and inhibitory mechanisms to modulate neurotransmitter release (Swerdlow et al., 2001). Therefore, increasing the propensity of inhibitory neurotransmitters may reliably reduce hyperlocomotor activity resulting from hyperactive circuits, but may do little to *recover* motor deficits resulting from dysregulated finetuning of striatal signaling. Therefore, the subtle inhibitory valence of GAT211 may lack sufficient specificity to recover complex circuitry deficits and have greater propensity to downregulate hyperactive circuits. This is one possibility that may account for increased GAT211 efficacy in *hyperactive* circuits; however, limited efficacy at recovering *dysregulated* PPI circuits.

3.3.2 Endocannabinoid signaling in astrocytes

Astrocytes express CB1 receptors and may provide an additional mechanism of action from which cannabinoids exert modulatory influence (Smith et al., 2020). Astrocytes maintain a broad reach throughout the brain and are important for mediating multiple synapses; therefore, it is highly probable that GAT211 increased binding affinity at CB1 receptors on astrocytes. For example, transient heterosynaptic depression (fast synaptic activity, < 1s) in the hippocampus relies on eCB signaling to suppress glutamate-mediated astrocyte activity (Smith et al., 2020). This highlights an important role that eCBs play in modulating glutamatergic activity in astrocytes and this effect, likely occurs in other circuits. Thus, some of the behavioral phenotypes observed in GAT211-treated rodents may be due to physiological alteration of glutamate in astrocytes and, although we did not directly investigate astrocytes in this thesis, they undoubtedly play a role in modulating behavioral phenotypes and warrant future research.

3.3.3 Final considerations

Today, antipsychotics reduce positive symptoms of schizophrenia, but do very little to improve cognitive and negative symptoms (Uno & Coyle, 2019). Although this thesis targeted behaviors with relevance to the positive symptoms of psychiatric illness, future research is essential to characterize the role the eCB system has in modulating the wide range of psychiatric symptoms. Our lab plans to continue characterizing the effects GAT211 with the aim of providing pre-clinical data that may inform individualized treatment approaches and aide in developing unique symptom management strategies for mental health patients. The present research centered around behaviors in rodents, however, the end hope is that novel therapeutics may benefit humans in the future.

These data provide support for CB1R PAMs as having therapeutic benefit for a subset of positive behavioral phenotypes. Moving forward, it will also be interesting to explore the efficacy of newly available GAT compounds, which are currently being developed and released for basic research. These variants may facilitate enhanced understanding of neural interactions and allow for increased specificity over modulating behavior. Finally, there is a need to continue these projects within a repeated dosing experimental design to begin ascertaining efficacy and relationship between the eCB system and the pathogenesis of novel behaviors. As one final note, there is no data that explores the relationship of CB1R agonists and NMDAR antagonists on neural oscillations (Sherif et al., 2018). Neural oscillations are influenced by a complex interaction between NMDAR- and CB1R-signalling on glutamatergic pyramidal neurons and GABAergic interneurons; both of which are implicated in schizophrenia (Sherif et al., 2018). Furthermore, CB1R is highly expressed in similar brain regions with altered gamma band frequencies associated with psychiatric symptoms. In light of these findings alongside our own,

it will be interesting to determine how CB1R PAMs influence dysregulated neural oscillations associated with psychiatric disorders.

3.4 Conclusion

This thesis has demonstrated that CB1R PAMs attenuate some behaviors associated with the positive symptoms of schizophrenia and firmly suggests that the eCB system may be targeted for therapeutic benefit. Antipsychotics are far from perfect and side effects have profound affects that contribute to day-to-day dysfunction and affect nearly every patient (Harvey & Bowie, 2012; Kitchen, Rofail, Heron, & Sacco, 2012; Roebuck et al., 2018). Moving forward, it is essential to continue characterizing the neurophysiological mechanisms of psychosis and to conduct preclinical research to investigate how CB1R PAMs may help reduce the burden of psychiatric symptoms.

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