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Attentional Dysfunction in Schizophrenia: The Effects of Dual Orexin Receptor Blockade on an NMDA Receptor Hypofunction Model

Paige Little

Attentional Dysfunction in Schizophrenia: The Effects of Dual Orexin Receptor Blockade on an NMDA Receptor Hypofunction Model

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by

Paige Little

Accepted for Honors:

Joshua Burk

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Joshua Burk, Director

Randolph Coleman

_____

Randolph Coleman

Meghan Quinn

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Meghan Quinn

Williamsburg, VA
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Abstract

Hypofunctionality at the N-Methyl-D-aspartic acid receptor (NMDAR) is a commonly used model of the neurodevelopmental disorder schizophrenia due to the complex circuitry changes that follow NMDAR blockade. While these animal models are very popular for modeling the cognitive deficits seen in schizophrenia, actual treatments for this disorder remain sparse. Orexins (hypocretins) are neuropeptides that are capable of modulating activity along pathways relevant to attention, but are rarely tested for their efficacy in attenuating attentional dysfunction. This study was conducted to determine if systemic administration of the dual orexin receptor antagonist filorexant (MK-6096) was able to attenuate sustained attentional dysfunction induced by administration of the NMDAR antagonist dizocilpine (MK-801). Results from this study demonstrate that administration of dizocilpine worsened task performance through an increase in response omissions; however, systemic administration of filorexant was not able to significantly attenuate attentional dysfunction. Future research should continue to investigate the orexinergic system as a potential modulator of the complex symptomatology present in attentional dysfunction associated with schizophrenia.

Attentional Dysfunction in Schizophrenia: The Effects of Dual Orexin Receptor Blockade on an NMDA Receptor Hypofunction Model

Schizophrenia is a complex psychiatric disorder that affects roughly 1% of the global population and is thought to arise from developmental abnormalities. While medications for schizophrenia have been present since the early 1950s (Kane & Correll, 2010), current pharmacological treatments are ineffective for many of the associated symptoms (Jones et al., 2011) with only 20% of patients with schizophrenia responding well to treatment (Patel et al., 2014). Symptoms of schizophrenia fall into three main categories: positive symptoms, which include psychotic symptoms like delusions and hallucinations (Jones et al., 2011); negative symptoms, which include an absence of emotion or goal-oriented behavior (Patel et al., 2014); and cognitive deficits, which include problems in areas of attention, executive function, and working memory (Brisch et al., 2014). While current antipsychotic medications have found success in the treatment of positive symptoms, little progress has been made in the treatment of negative symptoms and cognitive deficits.

Theories of Schizophrenia

The complex symptomatology presented in schizophrenia has historically been thought to result from a dysfunctionality of the neurotransmitter dopamine (DA) (Brisch et al., 2014; Thompson et al., 2004). The DA hyperactivity hypothesis states that overactivity of DA in the brain leads to many of the symptoms of schizophrenia. This theory is supported by the evidence that amphetamines (stimulants that increase DA release) increase psychotic symptoms, and drugs that block DA receptors (neuroleptics) are effective at decreasing positive symptoms (Thompson et al., 2004). However, the pathways that lead to symptomatology in schizophrenia are much

more complicated than a simple excess of DA. The current preferred method of treatment of schizophrenia, neuroleptics, are antagonists that block DA D2 receptors in an effort to combat DA hyperactivity (Patel et al., 2014; Stahl, 2013). Neuroleptics can be effective in the treatment of positive symptoms but have little to no effect on negative or cognitive symptoms (Jones et al., 2011). While research has struggled to prove a higher basal level of DA or its metabolites in patients with schizophrenia compared with controls (Thompson et al., 2004), stimulated DA release is greater in patients with schizophrenia (Laruelle, 2014). Thus, while it is clear there is a marked DA dysfunctionality in the presentation of schizophrenia, the exact mechanism is more complicated than solely a hyperdopaminergic state. Modern theories of schizophrenia focus on specific DA pathways: the mesolimbic pathway (reward pathway) and the mesocortical pathway (Stahl, 2013). The mesolimbic pathway projects mainly from DA cell bodies in the ventral tegmental area (VTA) to the nucleus accumbens (NA) (Arias-Carrión et al., 2010; Cachope & Cheer, 2014; Stahl, 2013) whereas the mesocortical pathway projects from the VTA to areas like the prefrontal cortex (PFC) (Arias-Carrión et al., 2010; Stahl, 2013). An oversimplification yields the idea that hyperactivity in the mesolimbic pathway is hypothesized to be the source of the positive symptoms of schizophrenia, whereas hypoactivity in the mesocortical pathway could be responsible for the negative and cognitive symptoms (Brisch et al., 2014; Rao et al., 2019; Stahl, 2013).

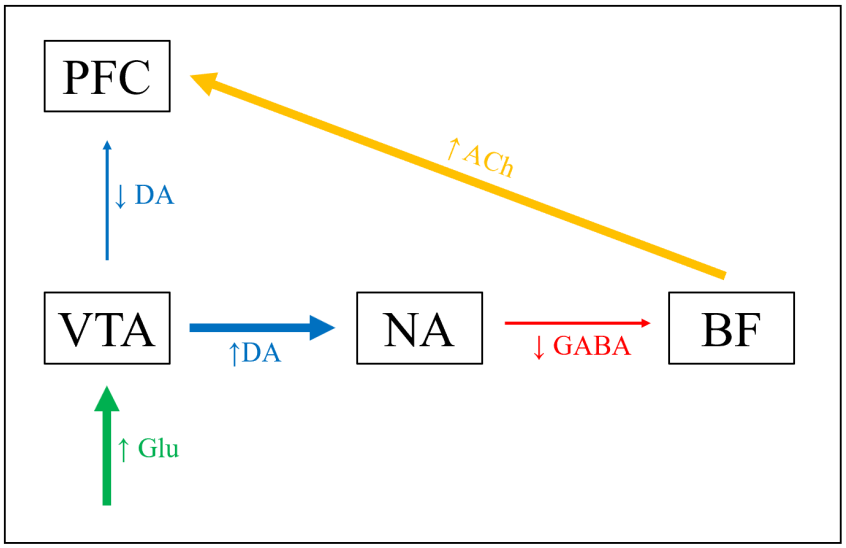
The combination of a simultaneous insufficient and excessive DA functionality is most likely due to abnormally functioning regulation pathways, which are dependent on the neurotransmitter glutamate and one of the receptors it binds to, the N-Methyl-D-aspartic acid receptor (NMDAR). Glutamate is the main excitatory neurotransmitter in the brain and is

involved in much of the neurocircuitry outside of the main pathways relevant to schizophrenia, one of which is the cortico-brainstem glutamate pathway (Stahl, 2013). While glutamate binds to other receptors as well (AMPA and kainate receptors) the expression of schizophrenia seems to result from the downstream effects of hypofunctionality at NMDARs (Howes et al., 2015; Stahl, 2013). In order to function, the NMDAR requires binding at its modulatory site by either glycine or D-serine (Howes et al., 2015; Laruelle, 2014; Moghaddam & Javitt, 2012). The NMDAR hypofunction theory of schizophrenia states that underactivity of glutamate at NMDARs on specific GABA interneurons leads to the expression of all three types of symptoms associated with schizophrenia (Moghaddam & Javitt, 2012; Stahl, 2013). This underactivity could be due to developmental abnormalities in the receptors themselves (Kawabe & Miyamoto, 2019; Lim et al., 2012) which gives schizophrenia the classification of a "neurodevelopmental disorder." This theory is supported by noncompetitive antagonists of the NMDARs, such as phencyclidine (PCP), dizocilpine (MK-801), and ketamine, producing positive, negative, and cognitive symptoms (Howes et al., 2015; Laruelle, 2014; Moghaddam & Javitt, 2012).

The two main DA pathways relevant to schizophrenia (mesolimbic and mesocortical) are controlled both directly and indirectly by GABA interneurons (Howes et al., 2015; Stahl, 2013). NMDARs on GABA interneurons (either in the hippocampus or the cortex) provide inhibition of excitatory glutamate released in the VTA and thus DA release in the NA (mesolimbic DA pathway) (Brisch et al., 2014; Stahl, 2013). Glutamate hypofunctionality at NMDARs on these GABA interneurons will lead to less inhibition of excitatory glutamate on the VTA, which means more activation of the mesolimbic system and more release of DA in the NA (positive symptoms) (Brisch et al., 2014; Stahl, 2013; Figure 1).

Figure 1

Depiction of the neural pathways affected by hypofunctionality at NMDARs



Note. VTA = ventral tegmental area; PFC = prefrontal cortex; NA = nucleus accumbens; BF = basal forebrain; Glu = glutamate; DA = dopamine; GABA = γ-Aminobutyric acid; ACh = acetylcholine

Furthermore, an overactive NA may contribute to the increased cholinergic activity associated with attentional impairments due to a dysregulation of the basal forebrain (BF) (Sarter et al., 2005). On the other hand, overactivation of the VTA by excess glutamate will also follow the mesocortical DA pathway and eventually lead to inhibition of DA release in the PFC (negative symptoms and cognitive deficits) (Laruelle, 2014; Stahl, 2013; Figure 1).

Overall, this means that hypoactive NMDARs on specific GABA interneurons may be the cause of the DA dysregulation seen for the positive, negative, and cognitive symptoms associated with schizophrenia.

The Attentional System in Schizophrenia

Cognitive deficits are present in roughly 75-85% of schizophrenic patients (Kraguljac et al., 2013) and are still not currently treated. Common cognitive deficits associated with schizophrenia include problems with sustained attention and filtering out of distractors (Sarter et al., 2012), problems with memory (Kraguljac et al., 2013), and inability to shift attentional sets (Ceaser et al., 2008; Elliott et al., 1995). Furthermore, both the prognosis and outcome of patients diagnosed with schizophrenia increase with enhanced cognitive function (Sakurai et al., 2015). The cognitive deficits and attentional problems associated with schizophrenia are hypothesized to be a result of dysregulation of the acetylcholine (ACh) cholinergic system (Sarter et al., 2012). In fact, inhibitors of acetylcholinesterase (AChE), the primary enzyme responsible for ACh breakdown, produces psychotic symptoms (Hyde & Crook, 2001; Sarter et al., 2012) suggesting a higher basal level of ACh in patients with schizophrenia. This higher basal level of ACh may prevent the additional cognitive recruitment required for cognitive and attention-based tasks (Sarter et al., 2012). ACh is a neurotransmitter consistently associated with attention, due to observed increases in ACh levels during sustained attention tasks (Burk et al., 2018). Higher levels of ACh are associated with more cognitively demanding tasks involving distractors (Lustig & Sarter, 2016). Furthermore, antagonism of both ACh receptors also leads to decreases in various task performances (Klinkenberg et al., 2011). ACh binds to both G-protein coupled muscarinic receptors, as well as ionotropic ligand gated nicotinic receptors (Hyde & Crook, 2001). Both muscarinic and nicotinic ACh receptors seem important in attentional processing (Burk et al., 2018), and both seem to show altered expression in schizophrenia (Hyde & Crook, 2001). However, attentional problems in schizophrenia may also arise from downstream effects of the midbrain hyperdopaminergic state (Hyde & Crook, 2001). As

previously discussed, negative symptoms and cognitive deficits associated with schizophrenia might result from an overactive VTA leading to an inhibition of DA release in the PFC (Laruelle, 2014; Stahl, 2013; Figure 1). Underactive PFC DA might be directly responsible for some attentional problems, as DA has been shown to have a vital role in working memory, and DA receptor agonists administered in the mPFC are able to reduce symptoms associated with attentional dysfunction (Burk et al., 2018). However, an overactive NA may also contribute to ACh efflux through the projections from the NA to the basal forebrain (BF) (Hyde & Crook, 2001; Sarter et al., 2005, 2012; Figure 1). The BF cholinergic system is of vital importance to attentional processing, as it innervates many areas implicated in attention, including the PFC and hippocampus (Scarr et al., 2013). The BF cholinergic system is, in part, regulated by GABA projections from the NA; thus, theoretically, one downstream effect of the overly active mesolimbic system seen in schizophrenia is a dysfunctional NA leading to a dysregulation of GABA onto the BF and thus an alteration in the BF cholinergic projections to the PFC (Hyde & Crook, 2001; Sarter et al., 2005, 2012; Figure 1). DA within the NA may inhibit the GABA projections from the NA to BF (Sarter et al., 2005), meaning that an overactive DA inhibition of a GABA inhibitor could lead to an excess of ACh release in the PFC. While this is clearly not the only method by which the BF is affected due to an overly active mesolimbic DA system (Sarter et al., 2005), this theory has been supported in animal research with the addition of GABA agonists to the BF attenuating behavioral changes due to DA infusion in the NA (Hyde & Crook, 2001). Furthermore, problems with ACh projections to the hippocampus may also be impaired in schizophrenia (Hyde & Crook, 2001) which may explain some of the complex difficulties seen in memory utilization (Brisch et al., 2014).

Attention can be measured in a variety of ways, from the five-choice serial reaction time task, to set-shifting abilities, to sustained attention measures (Burk et al., 2018). The inability to properly sustain attention is considered a crucial characteristic of schizophrenia (Hoonakker et al., 2017). In fact, a recent clinical study found greater deficits in sustained attention in patients with higher treatment resistance for schizophrenia (Lin et al., 2019). Sustained attention tasks are commonly used to test models of pathology and novel treatment forms, and the BF cholinergic system is commonly tested in an operant-box version of the sustained attention task (Callahan & Terry, 2015). The dysfunctional PFC seen in schizophrenia due to NMDAR hypofunction leading to an overactive mesolimbic pathway, underactive mesocortical pathway, and excess ACh release in the PFC can be tested with sustained attention tasks (Howe & Burk, 2007).

The Orexin System

Orexins (also known as hypocretins) are neuropeptides implicated in many physiological functions including sleep, hunger, reward, and arousal (Arrigoni et al., 2010; Burk et al., 2019; Zajo et al., 2016). Orexin cell bodies originate in the lateral hypothalamus and project to a variety of locations within the central nervous system (Ebrahim et al., 2002), including to areas of importance to arousal, learning, and memory (Burk et al., 2019). Orexins exist in two different forms (OxA and OxB) with two G-protein coupled receptors (Ox1R and Ox2R); Ox1R binds OxA with high affinity and OxB with low affinity, whereas Ox2R binds both OxA and OxB with a high affinity (Scammell & Winrow, 2011). Patients with narcolepsy have been found to have reductions in cerebrospinal fluid levels of orexins, as well as reductions in orexin neurons in post-mortem analysis (Ebrahim et al., 2002). Furthermore, mice lacking orexins or their receptors behave in a resemblance similar to human narcolepsy, strengthening the idea that

orexins are important for wakefulness and arousal (Arrigoni et al., 2010). Arousal and attention seem to be linked by orexins, with attention requiring arousal, creating an important connection between the orexinergic system and attention (Burk et al., 2019). Orexins can stimulate the BF to release ACh in the cortex, and ACh efflux as well as attentional performance is disrupted following Ox1R antagonism in the BF (Boschen et al., 2009; Burk et al., 2019). This shows a very important connection between attentional performance, orexin function, and ACh release, and may explain why patients with narcolepsy also show problems with attention (Burk et al., 2019).

Orexins and Schizophrenia

Orexins have the potential to mediate the overactivity seen in the midbrain in schizophrenia via multiple pathways. The mesolimbic DA pathway beginning with the VTA is already overly excited in schizophrenia, leading to all three types of symptoms (see above). Orexins, via both Ox1R and Ox2R, also directly excite the VTA and thus the NA (Scammell & Winrow, 2011). Furthermore, intracranial OxA administration activates the VTA and leads to elevated DA activity in both the VTA and PFC (Burk et al., 2019). A Ox1R antagonist administered in the VTA has been found to reduce DA levels in the NA (Calipari & España, 2012), suggesting that administration of an orexin receptor antagonist could be beneficial in mitigating the overactivity of DA in the VTA.

Interestingly, orexins also function to modulate overexcitation of glutamate in the midbrain via NMDAR expression. Orexins are able to increase the synaptic expression of NMDARs in the VTA, thus increasing sensitivity to glutamate (Calipari & España, 2012; Scammell & Winrow, 2011). Ox1R antagonism prevents this increased NMDAR expression and

VTA excitation (Calipari & España, 2012), suggesting a second method by which orexinergic antagonism can mediate overactivity within the VTA and NA.

Lastly, orexins can stimulate the release of ACh in the cortex by activating neurons within the BF cholinergic system (Burk et al., 2019). The BF cholinergic projections to the cortex in schizophrenia are also overactivated (see above). This means that orexin receptor antagonism could be beneficial in attenuating the hypercholinergic state by reducing additional ACh release in the cortex.

Modelling Schizophrenia

In order to try and discover new treatment options, schizophrenia is commonly modelled in animals. Popular models of schizophrenia fall into categories like developmental models (gestational MAM, neonatal lesions) and pharmacological models (DA agonism and NMDAR antagonism) (Jones et al., 2011). Due to the capacity for NMDAR blockade to mimic all three symptoms of schizophrenia, NMDAR antagonists are one of the most commonly used models of schizophrenia in animals. Dizocilpine (MK-801) is a highly potent NMDAR antagonist (McKay et al., 2013) that produces both the cognitive deficits (Rapanelli et al., 2013) and some of the dysfunctionality in neural pathways seen in schizophrenia (Howe & Burk, 2007). Furthermore, administration of NMDAR antagonists like dizocilpine is associated with increases in both DA and ACh in the NA, as expected in this model of schizophrenia (Del Arco et al., 2008). Dizocilpine can be administered at different periods in development to mimic hypofunctionality at NMDARs; is most commonly administered in early postnatal days (Kawabe & Miyamoto, 2019; Rapanelli et al., 2013; Stefani & Moghaddam, 2005) or shortly before task performance (Rapanelli et al., 2013). Early postnatal administration of dizocilpine halts the proper

development of NMDARs and produces severe deficits in learning (Rapanelli et al., 2013). The more common administration of dizocilpine shortly before task performance still mimics the hypofunctionality induced deficits of this model, while also providing behavioral and attentional strain reminiscent of schizophrenia (Rapanelli et al., 2013)**.**

Previous research in our lab has validated the acute use of dizocilpine shortly before task performance as a model of schizophrenia (Maness et al., *unpublished*). In fact, acute dizocilpine administration (i.p. injections of 0.1 mg/kg) decreased accuracy in the sustained attention task, especially once a distractor was present. Interestingly, accuracy was improved following the intracerebroventricular (ICV) administration of filorexant (MK-6096), a dual orexin-receptor antagonist.

The Present Study

The effort to more deeply understand and treat cognitive and attentional problems associated with schizophrenia and dysregulated cholinergic neurotransmission remains of great importance. This study will use acute administration of dizocilpine as a model of schizophrenia in rats. These models are associated with a dysfunctional PFC due to DA overstimulation in the VTA, and excessive ACh activity in the PFC. The neuropeptides orexins have been seen to increase both DA levels in the VTA and ACh levels in the cortex, thus an orexin receptor antagonist may be able to reduce levels of both. Previous research in our lab has supported this hypothesis through ICV administration of filorexant; however, a more clinically applicable study that does not involve direct drug-to-brain administration is needed. This study will serve the purpose of analyzing systemic administration of filorexant on a dizocilpine model of schizophrenia. The deficits in sustained attention associated with sole dizocilpine injections are

hypothesized to be attenuated following the addition of the dual orexin receptor antagonist filorexant.

Materials and Methods

Subjects

A total of six Sprague Dawley rats (three female, three male) were used in this study. Rats were either singly or group housed in plastic tubs in the temperature and humidity-controlled vivarium at the College of William and Mary. Rats were kept on a 14-hour light/10-hour dark cycle, with food available ad libitum and restricted water access. Rats were tested daily, and received 10 minutes of water after testing. If a rat was not tested, it received 20 minutes of water that day. This study was approved by the Institutional Animal Care and Use Committee at the College of William and Mary.

Apparatus and Training

Rats were trained and tested in one of five Med Associates Inc Operant boxes. Each box was held inside of a sound-attenuating cubicle and contained two retractable levers surrounding a water dipper that holds 0.01 ml of water. There were three panel lights above the levers, with only the centermost light being used in this study. A house light on the opposite side of the box was also used.

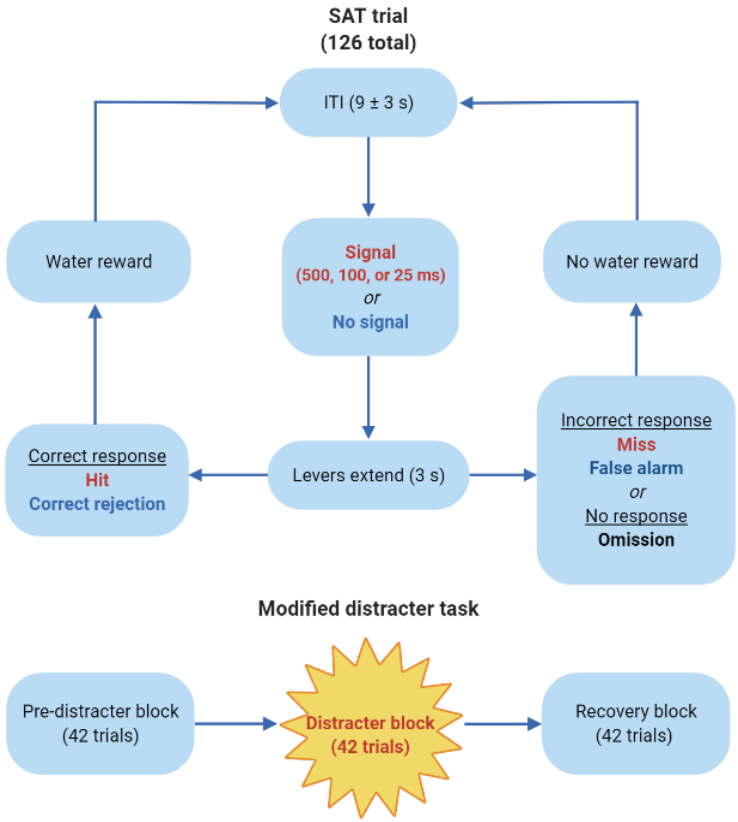
Rats were trained in three training stages prior to drug administration. During the first stage of training, only the levers were used, and were constantly extended to reinforce lever presses, with a lever press resulting in the water dipper delivering 0.01 ml of water. Five consecutive presses of one lever halted water delivery until the other lever was pressed to prevent a lever bias. Continuation to the next stage of training occurred once 120 lever presses

and water rewards were delivered for three consecutive training days. The second stage of training brought in the use of the panel lights, with the addition of signal trials and non-signal trials. Signal trials consisted of the central panel light being on for one second, whereas non-signal trials consisted of no signal. Correct responses consisted of hits or correct rejections, with water access provided; incorrect responses consisted of misses, false alarms, or omissions, for which water was not provided. For half of the subjects, following a signal, a hit was recorded, and water access was provided following a press on the right lever, whereas a press on the left lever was recorded as a miss and water was not provided. Following no signal, a correct rejection was recorded, and water access was provided following a press on the left lever, whereas a press on the right lever was recorded as a false alarm and water was not provided. The correct responses were switched for the other half of subjects. After no response for three seconds, an omission was recorded, and water was not provided. Following an incorrect response or an omission, a correction trial occurred where only the correct lever was extended. If the correct lever went unpressed for three correction trials, a forced trial occurred where only the correct lever was extended for 90s (or until pressed). During this second stage, the time in-between each trial, or the inter-trial interval (ITI) was 12 seconds. Continuation to the final stage occurred once 70% accuracy was recorded for three consecutive training days (70% hits and 70% correct rejections).

The final stage of the task, the sustained attention task (SAT), consisted of 126 trials. Trials included either signals of 500, 100, or 25 ms, or no signal, and a shortened ITI of 9 +/- 3 seconds was used for increased attentional demand (Figure 2).

Figure 2

Depiction of the Sustained Attention Task (SAT)



Note. ITI = Inter-trial interval; Maness et al., *unpublished*

Reinforcement rules followed the same as the previous trial, and at least 70% hits at the 500ms signal and 70% correct rejections were required to move on to drug administration and be included in the experiment. During drug administration, rats were tested on a modified version of the SAT (Figure 2). This modified version included the same SAT but was broken into three testing blocks of 42 trials. The pre-distractor block is the same as the SAT, but the distractor block includes flickering of the house light (0.5 seconds on/0.5 seconds off) for increased attentional demand (Burk et al., 2018; Callahan & Terry, 2015). The final block, the recovery

block, is the same as the first block and helps measure recovery from the increased attentional strain.

Drug Administration

This within-groups study involved four days of intraperitoneal (i.p.) injections for all six rats. On all injection days, rats received injections at a minimum of 20 minutes prior to testing. Drug administration days were spaced at least one day apart, with the first injection day being a simple practice saline injection day to allow the rats exposure to injections and the modified SAT. Dizocilpine (MK-801) was dissolved in saline to create a solution of 0.075 mg/ml, which was used for injections of 0.075mg/kg per rat. Filorexant (MK-6096) was dissolved in dimethyl sulfoxide (DMSO) to create a solution of 1.0 mg/ml, which was used for injections of 1.0 mg/kg per rat. Both drugs were stored in a freezer and used within one week of preparation.

A vehicle administration day was used as the control and consisted of one injection of saline and one injection of DMSO. On the dizocilpine day, rats were administered with one injection of 0.075mg/kg of dizocilpine and one injection of DMSO. On the dizocilpine and filorexant co-administration day, rats were administered with one injection of 0.075 mg/kg of dizocilpine and one injection of 1.0 mg/kg of filorexant.

Behavioral Analysis

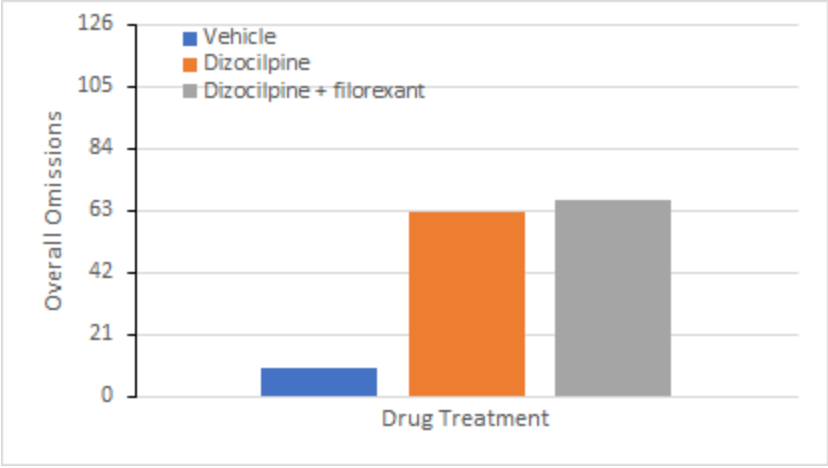
The number of hits, misses, correct rejections, false alarms, and omissions were collected during each block. The number of correct hits per signal trial (h500, h100, h25), correct rejections, and omissions were analyzed in repeated measures ANOVAs and paired samples t-tests. Data was analyzed in SPSS with a significance level of 0.05.

Results

To assess the effects of drug treatment on omissions, a drug (3 levels: vehicle, dizocilpine, dizocilpine + filorexant) x block (3 levels: trial blocks 1, 2, and 3) ANOVA was conducted. This analysis yielded a main effect of drug ($F(2,10) = 7.987$, $p = .023$) and of block ($F(2,10) = 8.298$, $p = .021$). A paired samples t-test was conducted to determine the difference between means, which yielded a significant difference between omissions during dizocilpine administration and vehicle administration ($t(5) = 4.150$, $p = .009$; Figure 3). There was also a significant difference between omissions during dizocilpine + filorexant administration as compared to vehicle administration ($t(5) = 4.477$, $p = .007$; Figure 3). However, filorexant was not able to significantly lower the rates of omissions increased by dizocilpine, as could be seen by comparing dizocilpine administration with dizocilpine + filorexant administration ($t(5) = 0.202$, $p = .848$; Figure 3).

Figure 3

Omissions across all testing blocks

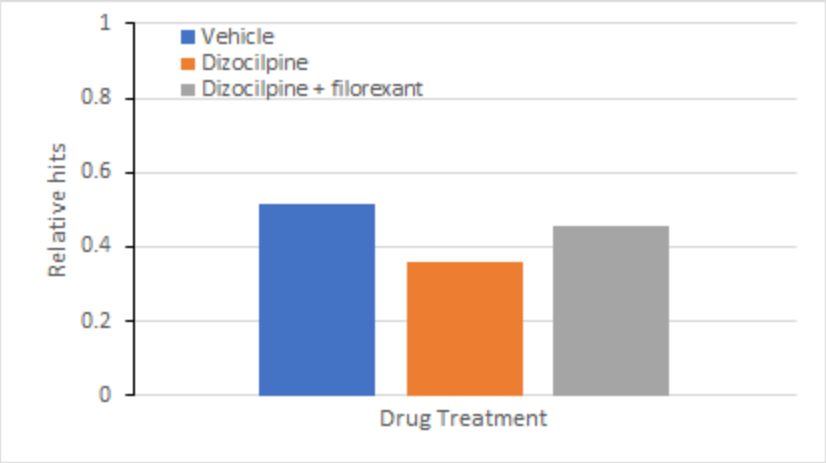


To assess the effects of drug treatment on correct rejections, a drug (3 levels: vehicle, dizocilpine, dizocilpine + filorexant) x block (3 levels: trial blocks 1, 2, and 3) ANOVA was

conducted. There was no main effect of drug ($F(2,10) = 1.479$, $p = .276$) or block ($F(2,10) = .091$, $p = .891$) on correct rejections. For hits, a drug x block x signal duration ANOVA was conducted. There was no main effect of drug ($F(2,10) = 2.306$, $p = .165$) or block ($F(2,10) = 2.459$, $p = .147$) on correct hits. Interestingly, while it was not found to be significant, filorexant administration resulted in around a 10% increase in correct hit rate across all testing blocks and signal durations as compared to dizocilpine administration alone (Figure 4).

Figure 4

Correct hits across all testing blocks and signal durations



Discussion

The purpose of this experiment was to determine if the addition of the dual orexin receptor antagonist filorexant could attenuate the attentional problems associated with the NMDAR antagonist model of schizophrenia induced by dizocilpine administration. We hypothesized that administration of dizocilpine would produce sustained attentional deficits due to the effects that hypofunctionality at NMDARs has on dopaminergic and cholinergic pathways in the brain. This hypothesis was supported insofar as dizocilpine administration resulted in an

increase in omissions that could reflect attentional deficits. We also hypothesized that administration of filorexant following NMDAR antagonism by dizocilpine would result in an attenuation of attentional deficits; this hypothesis was not supported. While filorexant was not able to significantly improve task performance, it was able to produce an increase in overall hit rate that was trending towards significance, suggesting the potential for some therapeutic effect of orexin antagonism.

The main finding in this study was the capacity of dizocilpine administration to reduce attentional performance through an increase in response omissions. Interpreting an increase in omissions during the sustained attention task is complicated, as an increase in omissions could mean one of three things: an increase in attentional deficits, a change in motivation, or an increase in motor deficits. In a previous study, a smaller dose of dizocilpine (0.05 mg/kg) was associated with increased false alarms, without an effect on omissions, suggesting no decrease in motivation (Howe & Burk, 2007). The slightly larger dose used in this study (.075 mg/kg) may have produced the more robust changes in task performance, like the increase in omissions. Unfortunately, it is not possible to simply state that an increase in omissions is equivalent to sustained attentional deficits; however, given that the subjects did continue to perform and not simply omit every trial suggests that motor deficits are not the primary issue. Furthermore, it is possible that the circuitry affected by dizocilpine affects a wider range than areas involved in attention, and may have resulted in a change in motivation. Thus, while an increase in omissions cannot be entirely attributed to attentional deficits, it is clear that dizocilpine administration affected the capacity to perform well in the task. Outside of omissions, there were no significant results of drug administration on signal detection accuracy. This may have to do with the small

sample size utilized in this study; an increase in sample size in a future study may further elucidate these drugs' effects on signal detection.

The behavioral deficits seen in this study may be due to NMDAR antagonism leading to a variety of chemical changes relevant to schizophrenia. While it cannot be stated that the results here generated an explicit deficit in attention and not motivation, attentional deficits are considered common in these models of schizophrenia. Attentional deficits in schizophrenia may arise from an excess release of ACh in the PFC, which is demonstrated following acute ketamine administration (Nelson et al., 2002). This abnormally high ACh release in the PFC could prevent additional cognitive recruitment necessary for attentional tasks (Sarter et al., 2012). These circuitry changes seem to arise from parvalbumin (PV) containing GABA interneurons in the hippocampus and cortex, insofar as hypofunctionality at these GABA interneurons leads to all three symptoms of schizophrenia (see above). Sub-chronic use of the NMDAR antagonist PCP leads to a reduction in PV in the PFC and hippocampus (Cadinu et al., 2018). Acute dizocilpine administration leads to mRNA level alterations of PV in the PFC and hippocampus (Lee & Zhou, 2019). This means that the dizocilpine used in this study may have led to task-related deficits due to its capacity to disrupt proper circuitry. Because dizocilpine works at all NMDAR receptors and not solely these PV-containing GABA interneurons that are relevant to schizophrenia, this is not a perfect model. However, the fact that dizocilpine typically acts at NMDARs on GABA interneurons (Lee & Zhou, 2019), especially on interneurons in the PFC (Nakazawa et al., 2017), may explain why this model is associated more with cognitive deficits and attentional problems rather than an inability to perform the task.

Acute use of dual orexin receptor antagonists for the treatment of attentional problems is not very common. In fact, to our knowledge, this is the first study to examine systemic, subhypnotic administration of filorexant in an attempt to attenuate attentional deficits. Some research has examined dual orexin receptor antagonists for the control of impulsivity (Gentile et al., 2018), but they are rarely analyzed at subhypnotic doses; in fact, filorexant is more commonly investigated as a treatment for insomnia (Janto et al., 2018; Winrow et al., 2012). Thus, finding the correct subhypnotic dose to attenuate attentional dysfunction without inducing somnolence is difficult. While previous work in our lab supports direct ventricular administration of filorexant to attenuate attentional dysfunction induced by dizocilpine (Maness et al., *unpublished*), comparing dosing between intracerebroventricular administration and i.p. injection is difficult. Considering filorexant in this study had no significant effect on omissions may demonstrate the dose could have either been too low or too high; too low of a dose of filorexant may not attenuate the cholinergic and dopaminergic systems in this model, whereas too high of a dose could have produced somnolence. Future research should investigate if a different subhypnotic dose of filorexant is capable of reducing omissions and increasing signal detection accuracy.

Treatments for schizophrenia are still extremely limited, and more research needs to focus on treating the cognitive deficits and attentional problems associated with this complex neurodevelopmental disorder. Future research should continue to support the NMDAR antagonistic models of schizophrenia, and should continue to manipulate the orexinergic system in an attempt to attenuate these attentional problems.

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