

The effects of intranasal orexin-A on MK-801-induced attentional deficits: addressing cognitive impairment in an NMDA receptor hypofunction model of schizophrenia

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## APPROVAL PAGE

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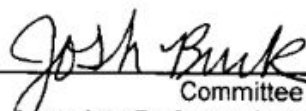
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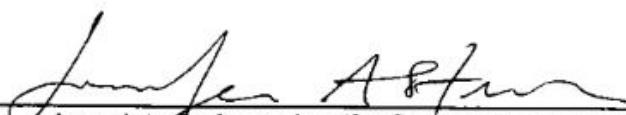
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## COMPLIANCE PAGE

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## ABSTRACT

Schizophrenia (SZ) is a debilitating condition wherein those afflicted experience positive symptoms, including hallucinations and delusions, as well as negative symptoms, including alterations of processing affecting cognition and social interactions. The NMDA receptor hypofunction model of SZ asserts that a reduction in hippocampal NMDA receptor input produces the pathology of this disorder, promoting excessive frontocortical excitatory neurotransmission – particularly overstimulation of basal forebrain cholinergic neurons – that ultimately impairs cognitive and sensorimotor processes. Orexin-A (OxA), a neuropeptide principally involved in wakefulness and appetitive behaviors, has been shown to demonstrate cognitive-enhancing qualities in models of psychiatric and neurodegenerative illness. In the present study, the effects of OxA on attentional performance were examined in a NMDA receptor antagonist model of SZ. Male Fischer 344 Brown Norway F1 Hybrid rats (N = 12) received both intraperitoneal injections of MK-801 and intranasal administration of OxA prior to placement in a sustained attention task requiring differentiation between signal trials (500, 100, and 25ms illumination of a central panel light) and non-signal trials (no light illumination). Overall, it was shown that the highest dose of OxA exacerbated MK-801-induced attentional deficits. While the small OxA concentration slightly protected against impairments in the correct rejection of the signal and increased omission rates at the low dose of MK-801, this excitatory neuromodulator was largely unable to improve performance in the attention task. These findings suggest that, in a state of cortical hyperexcitation like what is observed both in SZ and following NMDA receptor antagonism, the introduction of pharmacotherapies augmenting activity at the orexinergic system further exacerbates existing cognitive dysfunction in lieu of alleviating these symptoms.

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This thesis is dedicated to my mother, Wendy, my father, Michael, and my fellow graduate students in the psychology master's program at W&M. We did it!

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## **Chapter 1: Introduction and Research Hypotheses**

### *Overview*

The debilitating and complex condition known as schizophrenia (SZ) is a chronic disorder of the nervous system. As described in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), SZ is comprised of five primary symptoms: delusions, hallucinations, muddled speech, disorganized or catatonic behavior, and negative symptoms. Despite a low incidence of diagnosis – with only 1% of the world’s population being affected at a given time – those with SZ can experience a tremendously reduced quality of life due to the severity of the symptoms (Faris & Dunham, 1939). The experiences and behaviors associated with schizophrenia have historically been dichotomized into “positive” and “negative” symptoms. Positive symptoms reflect experiences or behaviors that should not normally be present. This class of symptoms includes hallucinations, delusions, and disorganize thoughts and have been classically treated using antipsychotic medications in tandem with counseling. The severity of affective, cognitive, and social impairments – together called negative symptoms because they denote a deficit in normal behaviors – are notoriously difficult to ameliorate and are more strongly associated with poor treatment outcomes than are the positive symptoms (Tamminga, Buchanan & Gold, 1998). While classically categorized as negative symptoms, cognitive impairments including attention, learning, and memory deficits are occasionally organized separately from the positive and negative symptoms and are considered their own distinct group (Bowie & Harvey, 2006). The Positive and

Negative Syndrome Scale (PANSS), first described by Stanley Kay, Abraham Fiszbein, and Lewis Opler in 1987, allows clinicians to measure the severity of the two distinct categories of SZ symptoms in the context of the global psychopathology.

### *Schizophrenia and the dopamine hypothesis*

The pathology of SZ is a result of the dysregulation of multiple brain networks, and parsing out the underlying neurological mechanisms inciting these imbalances has been a challenge. For the last 50 years, the dopamine (DA) hypothesis has provided informative insight into the investigation of the etiology of the condition. The premise of this model proposes that the development and symptoms of the disorder emerges from both subcortical hyperdopaminergia and cortical hypodopaminergia (Howes & Kapur, 2009; Davis et al., 1991). Previous findings have shown that there is an increase in expression and density of the presynaptic dopamine-2 receptor (D2R) subtype, which enhances DA neurotransmission upon stimulation, in extrastriatal regions of the SZ-afflicted brain, including the substantia nigra, ventral putamen, and nucleus accumbens (Wong et al., 1986; Joyce et al., 1988; Seeman & Kapur, 2000). Radioligand binding and positron emission studies have also revealed a substantial loss of cortical D1Rs in the prefrontal cortices of SZ patients, and the extent of the loss is correlated to the severity of the cognitive impairment (Okubo et al., 1997; Abi-Dargham et al., 2002; Guo et al., 2003). Augmented DA activity in the striatum and dampened DAergic neurotransmission in the cortex induces the positive and negative symptomologies, respectively (Brozoski et al., 1979; Davis et al., 1991).

Both typical and atypical antipsychotics inhibit the activity of D2-like receptors in the mesolimbic pathway, including the D2R as well as D3 and D4 receptor subtypes (Kapur & Seeman, 2001; Burstein et al., 2005). In support of this model, the degree to which a given neuroleptic occupies presynaptic D2Rs in subcortical regions tends to predict its therapeutic efficacy (Seeman & Lee, 1975; Creese, Burt & Snyder, 1976). Many second-generation antipsychotics assuage DAergic hyperexcitation by antagonizing serotonin-2 receptors (5-HT<sub>2</sub>R) in addition to D2Rs; by de-emphasizing their affinities to DA receptors and acting more potently in the 5-HT<sub>2</sub> system, these drugs are able to alleviate SZ symptoms with similar efficacy while minimizing extrapyramidal side effects (Kuroki, Nagau & Nakahara, 2008; Leucht et al., 2009).

Despite having prevailed as the chief paradigm in the study of SZ of the last several decades, the DA hypothesis does not encompass all facets of the disorder; namely, there is not sufficient evidence to suggest that targeting DA dysregulation effectually ameliorates the pathology in its entirety. While antipsychotic medications tend to effectively normalize DA concentrations and adequately assuage the severity of psychosis, they take several weeks to exert their therapeutic effects while failing to improve the cognitive and social deficits (Kapur et al., 2006). In addition to this, around 30 percent of those diagnosed are classified as treatment-resistant, determined by little to no responsiveness to at least two neuroleptics (Meltzer, 1997). Those who demonstrate a reduction in positive symptomology resulting from antipsychotic therapy may still experience the brunt of the negative syndrome. This inability of antipsychotic therapies

primarily targeting DAergic neurons in reducing the catatonia, anhedonia, and withdrawal associated with SZ necessitates the exploration of other major neuromodulatory networks in its etiology and treatment.

#### *N-methyl-D-aspartate receptors in SZ*

In response to newly-available data as well as the widespread shortcomings of the DA hypothesis, a new model centered around glutamatergic dysregulation was posited. Following the discovery of markedly reduced N-methyl-D-aspartate receptor (NMDAR) signaling in individuals with SZ by Kim and colleagues in 1980, and later supported by findings demonstrating reduced NR1 subunit mRNA in postmortem hippocampal tissue from SZ-afflicted individuals when compared to healthy controls, an alternate hypothesis emerged that attempted to incorporate this network into understanding the origins of the widespread corticolimbic neurotransmitter imbalances (Gao et al., 2000; Clinton & Meador-Woodruff, 2004). This model suggests that schizophrenic symptomology is a byproduct of an underactive NMDAR system, disabling the excitatory-inhibitory feedback loop and inciting extensive aberrations in glutamate release. NMDAR hypofunction also disrupts other neurotransmitter systems, particularly DAergic, 5-HTergic, and cholinergic networks (Kapur & Seeman, 2002). In large part, this NMDAR hypofunction model attempts to offer an alternative to the typical neuroleptic medications that fail to remedy the cognitive, social, and affective deficits. The NMDA hypofunction model suggests that therapeutics targeting the glutamatergic system, which may also normalize DA levels, could further improve treatment outcomes for these individuals.

NMDARs are ionotropic glutamate receptors (iGluRs) that are expressed in numerous brain regions, including the neocortex, ventral striatum, hippocampus, amygdala, and, to a lesser extent, the thalamus (Meador-Woodruff & Healy, 2000). Glutamate, being the primary endogenous ligand that depolarizes these receptors, is the main excitatory neurotransmitter in the brain and facilitates the majority of all excitatory neurotransmission in the mammalian nervous system (Traynelis et al., 2010). NMDARs themselves are either di- or tri-heteromeric in nature, being comprised of an NR1 subunit – to which glutamate has an affinity – as well as an NR2 glutamate-binding subunit and/or an NR3 glycine-binding subunit (Schüler et al., 2008). With cell bodies originating in the ventral hippocampus, the glutamatergic system is composed of NMDARs as well as  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), kainate receptors, and eight metabotropic glutamate receptor (mGluR) subtypes (Nakanishi, 1992; Ferraguti & Shigemoto, 2006). The stimulation of these glutamatergic receptor subtypes incites excitatory post-synaptic potentials (EPSPs) and is vital for mediating various aspects of neural plasticity and cognition (Li & Tsien, 2009). In particular, the CA1 region of the hippocampus is densely packed with NMDARs and is necessary for long-term potentiation (LTP), the synapse-strengthening process by which a wide array of cognitive processes are facilitated (Collingridge & Bliss, 1987).

In general, the suppression of hippocampal NMDAR signaling significantly reduces synaptic plasticity, producing cognitive deficits ranging from attentional processing to long-term memory storage in both human and non-human

paradigms (Tsien, Huerta & Tonegawa, 1996). Interestingly, the blockade of NMDA receptors induces a state of cortical hyperexcitation. NMDAR hypofunction boosts the extracellular release of endogenous excitatory neurotransmitters, including glutamate and aspartate, which greatly augment the stimulation of post-synaptic non-NMDA glutamatergic receptors, particularly in the prefrontal cortex (PFC) (Moghaddam & Javitt, 2011). This general hyperexcitation, in turn, diminishes the signal-to-noise ratio in cortical regions associated with cognition (Bustos et al., 1992; Liu & Moghaddam, 1995; Moghaddam et al., 1997). This conclusion has been supported by research suggesting that there is an increased quantity of cortical glycine-binding sites resulting from impaired glutamate neurotransmission in the brains of SZ patients (Ishimaru, Kurumaji & Toru, 1992; Ishimaru, Kurumaji & Toru, 1994). As a consequence of the overstimulation of non-NMDA glutamate receptors, the release of other excitatory monoamines – particularly dopamine (DA) and serotonin (5-HT) – is greatly augmented in the PFC and, to a lesser extent, the striatum. Therefore, excessive glutamatergic activity increases frontocortical noise itself as well as through the promotion of the release of other excitatory ligands.

One potential explanation for the excessive cortical activation observed following NMDAR hypoactivation involves the impairment of fast-spiking cortical interneurons (Wilson et al., 1994; Markram et al., 2004; Homayoun & Moghaddam, 2007). In the neurotypical brain, the interaction of glutamatergic and GABAergic systems acts as an effective “acceleration” and “brake” system,

respectively, by which excitatory neurotransmission is regulated, helping to maintain homeostatic signal transduction in cortical regions through a continuous feedback loop (Carlsson et al., 2001). The overstimulation of NMDARs elicits the GABAergic response, dampening excitatory neurotransmitter input to the cortex; similarly, the underactivation of the glutamatergic system decreases the inhibitory effects of the GABA interneurons. The suppressant function of GABA receptors (GABARs) on the activity of glutamate and other excitatory neuropeptides is dependent on adequate NMDAR input. Studies using microdialysis to examine GABAergic outflow after the pharmacological blockade of NMDARs have demonstrated that a hypofunctioning NMDAR system induces dysfunction in the regulatory actions and spontaneous firing rate of these putative GABA interneurons. GABAergic suppression disrupts the ability of these neurons to lessen excitatory outflow to the cortex, leading to excessive stimulation of prefrontal pyramidal neurons (Homayoun & Moghaddam, 2007). The failure of these inhibitory mechanisms impairs a variety of cognitive processes through the malfunction of excitatory neuromodulator activity and excessive frontocortical activation.

Findings from studies using an assortment of cognitive and behavioral assays have illustrated a relationship between NMDAR inhibition and deficits in vigilance, learning, and memory. For example, Sakimura et al. (1995) showed that mice with NR2A subunit gene disruption demonstrated substantially reduced hippocampal LTP, negatively impacting spatial memory performance when compared to wild-type mice. In a similar fashion, Bannerman and colleagues

(2013), while failing to discern disparities in spatial memory performance following deletion of CA1 NMDARs in the dentate gyrus and dorsal hippocampus, observed deficits in the utilization of spatial information to make navigation-related decisions in a water maze. In contrast to the previous experiment, Tsien, Huerta, and Tonegawa (1996), using CA1 NMDAR knockout mice, confirmed that the lack of this gene leads to a disruption in learning in the Morris water maze as well as impaired spatial memory as measured by increased escape latencies in the task.

The pharmacological inhibition of NMDARs yields similar behavioral outcomes when compared to the genetic deletion of these receptors. Ketamine, a dissociative anesthetic, is a non-competitive NMDAR antagonist known to reduce calcium influx by binding inside of the ion channel, suppressing its overall firing rate (Malhotra et al., 1996). Phencyclidine (PCP), similarly classified as a non-competitive NMDAR channel blocker, also produced dysfunctions in frontocortical excitatory neurotransmission and disturbed cognitive performance during tasks dependent on balanced catecholamine concentrations in the PFC (Zukin & Zukin, 1979; Morris, Cochran & Pratt, 2005). NMDAR antagonism particularly exacerbates prefrontal DAergic activity, as the excessive stimulation of non-NMDA glutamate receptors induces endogenous DA release (Moghaddam et al., 1997). The glutamatergic-mediated augmentation of DA and other monoamine concentrations in brain areas implicated in attention, learning, and both working and long-term memory suggests that excitatory transmission promotes cognition to a certain extent, but excessive excitatory activity becomes

counterproductive in this regard, and NMDAR blockade incites hyperexcitation-related deficits.

It has been speculated that impairment in NMDAR and non-NMDA glutamate receptor function in the SZ brain contributes to profound cognitive deficits. As has been confirmed by evidence from transcranial magnetic stimulation (Rogasch, Daskalakis & Fitzgerald, 2014) and ligand binding (Deakin et al., 1989) studies, there is overstimulation of glutamatergic afferents and an increase in glutamatergic binding in cortical regions of the schizophrenic brain, largely due to systematic failure of GABA interneuron-mediated cortical inhibition. Rogasch, Daskalakis and Fitzgerald (2014) also revealed diminished long-range corticolimbic connectivity in these individuals associated with aberration in small populations of neurons that interact with these connections. General prefrontal function is markedly reduced in SZ as well, and Beneyto et al. (2007) suggest this may be largely due to reduced function of NMDAR afferents and their contribution to numerous cognitive processes. Following this logic, drugs that are able to ameliorate frontocortical hyperexcitation induced by dampened cortical NMDAR inputs may be able to lessen SZ symptomology, particularly when considering the negative cognitive, affective, and social tendencies.

#### *The cholinergic system and schizophrenia*

Cholinergic neurons, which release the neurotransmitter acetylcholine (ACh) throughout the nervous system, are required for a wide variety of psychological and behavioral phenomena. Being comprised of both ionotropic nicotinic ACh receptors (nAChRs) and metabotropic muscarinic ACh receptors (mAChRs),

activation of the basal forebrain cholinergic system (BFCS) in the typically-functioning brain enhances cognition (Drachman, 1977). ACh efflux is particularly elevated during attentionally-demanding tasks, with cortical cholinergic projections playing a major role in mediating sustained attentional processing and allocating vigilance-related resources (Sarter, Gehring & Kozak, 2006; Dumas & Newhouse, 2011). The cholinergic network is especially involved in the top-down, knowledge-based processing of relevant environmental stimuli as well as the deemphasis of irrelevant information, and this process interacts with the bottom-up input of sensory information to improve attentional performance (Sarter et al., 2001). The degree to which a particular task is attentionally-demanding dictates the extent of basal forebrain cholinergic activation. The ability of this system to filter out extraneous and distracting information while simultaneously enhancing the detection of relevant stimuli makes the integrity of corticopetal cholinergic projections required for attention and the processes that depend on it, such as learning and working memory.

In the same vein, compromised cholinergic neurotransmission has been linked to impairments in basic cognitive functioning, and dampened function of cortical cholinergic innervation is a chief contributor to both age-related cognitive impairments and those seen in individuals with neurodegenerative conditions such as Alzheimer's and Parkinson's diseases (Coyle, Price & DeLong, 1983; Perry et al., 1985; Everitt & Robbins, 1997). Similarly, suppression of basal forebrain corticopetal neurons with cholinergic antagonists or the immunotoxin 192 IgG-saporin, for example, produces marked deficits in a number of measures

relating to cognition, including visual attention and memory formation (McGaughy, Kaiser & Sarter, 1995; Chudasama et al., 2004; Burk, Lowder & Altamose, 2008; Hasselmo & Sarter, 2011).

Overactivation of these receptors impairs cognition as well, adhering to the “inverted-U” phenomenon of neuronal stimulation. When considering the dose-response association between BFCS activation and behavior, this parabolic relationship suggests that both sub-threshold and elevated ACh levels have a counterproductive impact on a variety of physiological and psychological processes while moderate levels of activation demonstrate beneficial qualities (Picciotto, 2003). In the typically-functioning brain, reduced concentrations of a cholinergic ligand do not tend to elicit effects on behavior, but too high of a concentration can overstimulate these receptors and induce a hypercholinergic state. Excessive output from basal forebrain neurons has been shown to diminish the cortical signal-to-noise ratio and bolster sensory stimuli-evoked discharge, inciting disturbances in both cognitive and sensory processes (Donoghue & Carroll, 1987; Perry & Perry, 1995). The capacity for potent cholinergic agents to dampen both bottom-up and top-down processing of cognitive and sensory information further validates the necessity of the BFCS in modulating intellectual and perceptual systems.

Because of the crucial role this system plays in cognitive functioning, dysregulation of basal forebrain projections to the cortex is suggested to contribute to the pathology of SZ. Previous research examining cholinergic receptors in the etiology of SZ support the notion that cholinergic output from the

basal forebrain is elevated in the SZ brain, likely contributing to both the positive and negative symptoms of the disorder (Tandon & Greden, 1989). Glutamate receptors are located on basal forebrain corticopetal cholinergic neurons. Thus, these cortically projecting cholinergic neurons show an elevated firing rate in the presence of augmented glutamate release resulting from NMDAR regulatory failure (Fadel, Sarter & Bruno, 2001). In a state of cortical hyperexcitation induced by NMDAR and GABAR hypofunction, amplified ACh release has been shown to reduce performance in a wide variety of cognitive measures. As discussed by Tandon and Greden (1989), for individuals with SZ, excessive frontocortical cholinergic activity – believed to stem predominantly from mAChR overactivation – diminish attentional performance by increasing the perception and processing of irrelevant stimuli. In congruence with this idea, the atypical antipsychotic medication clozapine is believed to elicit some of its therapeutic effects through mAChR antagonism (Bolden, Cusack & Richelson, 1991; Meltzer & McGurk, 1999). Finally, both the density of and the binding at nAChRs and mAChRs are significantly decreased throughout cortical and striatal structures of the schizophrenic brain (Durany et al., 2000; Crook et al., 2001; Marutle et al., 2001; Terry, 2008; Scarr et al., 2009). This reduction in receptor reactivity is likely a product of their desensitization, as is known to occur following chronic cholinergic overstimulation (Nastuk & Wolfson, 1976).

One very common and accessible way by which individuals with SZ are speculated to self-medicate is by smoking cigarettes. While 25 to 30 percent of the general population smokes on a regular basis, anywhere from 80 to 90% of

those afflicted with SZ are habitual cigarette smokers (Ripoll, Bronnec & Bourin, 2004). Nicotine, the primary psychoactive ingredient in cigarettes, is known to demonstrate cognition-enhancing characteristics for those with and without a diagnosis of SZ; interestingly, however, the mechanisms underlying nicotine's beneficial qualities for people with SZ differ from those with a neurotypical brain. In particular, nicotine is considerably more reinforcing for those with SZ due to its capacity to temporarily relieve sensory and cognitive disturbances exacerbated by superfluous cholinergic activity. Nicotine administration has been shown to normalize deficits in inhibitory sensorimotor gating, including P50 auditory gating and prepulse inhibition (Siegel et al., 1984; Adler et al., 1993). When considering measures of cognition, following a period of forced abstinence in a study conducted by Sacco and colleagues (2005), habitual smokers with SZ performed more poorly on sustained attention and spatial working memory tasks than non-schizophrenic smoking controls. These impairments in performance were alleviated following reinstatement of cigarette-smoking; if those with SZ were pre-treated with mecamylamine hydrochloride, a non-selective nAChR antagonist, nicotine failed to alleviate these cognitive deficits.

There are a number of ways through which nicotine administration is able to normalize dysregulated excitatory neurotransmission in the brains of persons with SZ. One method involves targeting low-affinity alpha-7 ( $\alpha 7$ ) nAChRs, which have been shown to augment NMDAR firing. Nicotine exerts its effects presynaptically, thereby releasing glutamate onto postsynaptic NMDARs. A synergistic effect has been observed when  $\alpha 7$  nAChRs and NMDARs are

simultaneously stimulated (Aramakis & Metherate, 1998). Many hippocampal  $\alpha 7$  nAChRs both terminate onto and share synapses with voltage-gated NMDARs, suggesting that activity at this nAChR subtype modulates glutamate-induced excitatory post-synaptic potentials to a degree (Zappetini et al., 2014). Armakis and Metherate go on to suggest that, in effect,  $\alpha 7$  nAChR stimulation may be required for these particular NMDARs to activate. Zappetini et al. (2014), by utilizing a non-metabolizable tracer, calcium imaging, and immunocytochemical assays to quantify the impact of nicotine pre-exposure on NMDAR activation, revealed that the increase in NMDAR-mediated glutamatergic outflow is present in the nucleus accumbens (NAcc) and, to a slightly lesser extent, hippocampal nerve terminals. This effect was not present following stimulation of alpha-4 beta-2 ( $\alpha 4\beta 2$ ) nAChRs, as this receptor subtype was found to reduce presynaptic GluN2B-containing NMDAR activation when agonized. In the context of the NMDAR hypofunction model of SZ, pharmacotherapies that stimulate normally underactive NMDARs, such as  $\alpha 7$  nAChR agonists, may help to normalize frontocortical excitatory neurotransmission.

The crucial interaction between NMDARs and nAChRs is further validated by studies examining the behavioral effects of nicotine and similar compounds following NMDAR blockade. For instance, nicotine administration ameliorates deficits in prepulse inhibition induced by PCP, denoting its therapeutic potential for alleviating sensorimotor dysregulation (Spielewoy & Markou, 2004). Mice given the  $\alpha 7$  nAChR agonist SSR180711 for two weeks following a ten-day period of sub-chronic PCP administration demonstrated significantly improved

performance in a novel object recognition test compared to those given saline after PCP exposure (Hashimoto et al., 2008). Mice that were systematically administered the potent NMDA receptor antagonist MK-801 prior to placement in a fear conditioning task were able overcome NMDAR blockade-induced learning deficits when also receiving bilateral nicotine infusions (André, Leach & Gould, 2011). Yang et al. (2013) antagonized  $\alpha 7$  nAChRs in the dorsolateral PFC and discovered that, largely through the suppression of NMDAR activity, there was a marked reduction in the attentional modulation of the visual cortex and, therefore, representation of space in the primate. Low doses of nicotine, however, enhanced these visual representations. Thus, due to the interactions between these two networks,  $\alpha 7$  nAChR stimulation is able to remediate cognitive deficits resulting from suppression of NMDAR activation.

Another potentially beneficial aspect of nicotine that helps to alleviate frontocortical hyperexcitation involves reducing elevated ACh levels via GABAergic stimulation. Cholinergic neurons from the basal forebrain innervate the hippocampus through the septo-hippocampal pathway, which is a bi-directional network that modulates ACh release primarily through the activation of GABAR projections to the BFCS (Dutar et al., 1995; Khakpai et al., 2013). Stimulation of  $\alpha 7$  nAChRs has shown to inhibit basal forebrain cholinergic activity through the promotion of GABAergic input from these hippocampal innervations (Alkondon et al., 2000; Maggi, Sher & Cherubini, 2001). Through the mild suppression of corticopetal cholinergic activity, cortical overstimulation in SZ can be quelled and some aspects of cognition can be temporarily restored. Agonists

that target the  $\alpha 7$  nAChR subtype demonstrate similar cognition-boosting properties as well, and such drugs have been suggested to offer promise as both monotherapy and adjunct therapy for SZ (Martin, Kem & Freedman, 2004). Nicotine is also well-known to boost DA release in the NAcc, ventral tegmental area (VTA), and PFC, helping to address the problem of hypofrontality in SZ patients (Corrigall & Coen, 1991; Corrigall, Coen & Adamson, 1994; Dalack, Healy & Meador-Woodruff, 1998).

One issue with nicotine, however, is that it more effectively stimulates  $\alpha 4\beta 2$  nAChRs compared to  $\alpha 7$  nAChRs. Because activity at  $\alpha 4\beta 2$  nAChRs promotes cholinergic neurotransmission, symptoms related to psychosis can be worsened by cigarette smoking and nicotine self-administration. Not only is the  $\alpha 4\beta 2$ R subtype more commonly expressed than its  $\alpha 7$  counterpart, but both nicotine and the endogenous ligand bind more efficiently to these receptors (De Luca et al., 2006). The  $\alpha 4$  subunit of the  $\alpha 4\beta 2$  nAChR is especially implicated in striatal basal DA release, largely contributing to nicotine's rewarding qualities for smokers both with and without SZ. While activity at  $\alpha 7$  nAChRs may alleviate some of the symptoms of SZ – namely those related to cognition – it may also increase the likelihood of a psychotic episode, mediated primarily through the exacerbation of already-elevated mesolimbic DA concentrations. This idea is also supported by the capacity for nicotine to diminish the antagonist effects of antipsychotics at D2Rs by augmenting cortical DA release, occasionally necessitating an increase in dosage of these drugs (Pierre, 2005). Measurements of DA efflux resulting from nAChR activation following systematic

intracranial infusions of nicotine revealed that, over time, less cortical DA was being released following nicotine due to the desensitization of both nAChRs and DARs (Marks, Grady & Collins, 1993). Thus, while demonstrating some transient cognition-enhancing properties, nicotine as a method of self-medication is unsuitable as a long-term treatment option.

### *The orexinergic system and SZ*

The orexinergic network is believed to play a crucial role in cognition. Also called hypocretins, orexins are considered the primary wakefulness neuromodulators in the brain, acting as a regulator of both circadian and appetitive processes (Sakurai et al., 1998; Saper, Scammell & Lu, 2005). This neuronal network, which is comprised of approximately 70,000 neurons in humans and originates in the lateral hypothalamus and adjacent perifornical area (PFA), was first discovered in 1996 by Gautvik, de Lecea and colleagues and later classified by both de Lecea and Sakurai in separate studies in 1998. The orexinergic system consists of orexin-A (OxA) and orexin-B (OxB) endogenous isoforms (collectively referred to as orexins), derived from the same amino acid precursor prepro-orexin, as well as G-protein coupled orexin-1 (Ox1R) and orexin-2 (Ox2R) receptors. While OxB has low affinity to Ox1Rs and elicits most of its effects through Ox2R stimulation, OxA displays equally potent activation of both Ox1Rs and Ox2Rs (Hagan et al., 1999). OxA and OxB induce a biphasic calcium response when binding at orexinergic receptors, both releasing intracellular calcium via phospholipase C as well as promoting an influx of calcium from the extracellular space (Smart et al., 1999). Unlike other

hypothalamic neuropeptides, OxA and OxB have been shown to facilitate the release of both GABA and glutamate when acting at axon terminals, which, together, regulate virtually all hippocampal excitatory neurotransmission (van den Pol et al. 1998; Chemelli et al., 1999).

As has been established in both *in vitro* and *in vivo* recording studies, C-fos expression in orexin neurons is highest during the day hours in diurnal animals, increasing feelings of wakefulness and promoting hunger, whereas without exposure to daylight cues, the activity of the orexinergic network is suppressed and both arousal and food-seeking behaviors are reduced (Willie et al., 2001). In fact, these cells must be effectively “switched off” to maintain both consolidated NREM stages and the muscular atonia associated with REM sleep (Sakurai, 2007). Activity of these cells is transiently augmented following sensory stimulation and emotional perturbation, suggesting that the system is markedly sensitive to sensorimotor processes and psychological drives (Mileykovskiy, Kiyashchenko & Siegel, 2005). For those with narcolepsy, hypofunctioning Ox1Rs and especially Ox2Rs generate abnormal patterns of arousal, inducing daytime somnolence in addition to frequent bouts of cataplexy and unconsciousness (Lin et al., 1999). Those that suffer from narcolepsy and related sleep disorders also tend to demonstrate deficits in cognitive performance (Fulda & Schulz, 2001). The necessity of the orexinergic activation in regulating circadian rhythmicity and maintaining an enduring state of wakefulness suggests that OxA and OxB are two significant neurological correlates of consciousness; thus, it can be postulated that this system likely contributes in some capacity to

the cognitive processes that are enhanced in a state of increased arousal and alertness.

In support of this proposition, stimulation of Ox1Rs and Ox2Rs have been found to improve attention, learning, and working memory, primarily by instigating the release of wakefulness-promoting and cognition-enhancing neuropeptides. The orexinergic system projects all throughout the brain and interacts with not only structures related to consciousness, but many midbrain and forebrain structures implicated in cognitive processes, such as the locus coeruleus, amygdala, paraventricular nucleus of the thalamus, VTA, and infralimbic and prelimbic prefrontal cortical areas (Peyron et al., 1998; Ebrahim et al., 2002; Fadel & Deutch, 2002). In neurotypical, well-rested organisms, targeting OxRs has negligible effects on attentional and cognitive performance, likely due to the normal activation of this network. However, in sleep-deprived individuals, administration of OxA alleviates cognitive impairment induced by lack of sleep (Deadwyler et al., 2007). Similarly, the inhibition of Ox1Rs has been demonstrated to impair cognition. Rats given both systematic and intrabasalis SB-334867, an Ox1R antagonist, and placed in a sustained attention task revealed substantial reductions in the ability to correctly identify the occurrence of a brief visual stimulus (Boschen, Fadel & Burk, 2009; Fadel & Burk, 2010). The suppression of sustained attentional capacity elicited by Ox1R antagonism is similar to that observed follow loss of basal forebrain corticopetal cholinergic neurons. The inactivation of these neurons with SB-334867, too, precludes acquisition, consolidation and retrieval of spatial memories related to

performance in the Morris water maze, highlighting the importance of the orexinergic system at multiple stages of cognition (Akbari, Naghdi & Motamedi, 2006).

Hypothalamic orexinergic neurons are thought to play an important modulatory role in cognition enhancement, in part, because they augment excitatory neurotransmitter levels, including norepinephrine (NE), histamine, and especially DA in the mesocorticolimbic DA circuit (Peyron et al., 1998). The BFCS is innervated by lateral hypothalamic orexinergic fibers (Nambu et al., 1999). Findings from ultrastructural and light microscopy studies have found that around 70% of medial septal cholinergic neurons contain orexin immunoreactive appositions on their dendrites or cell bodies (Wu et al., 2004; Fadel, Pasumarthi & Reznikov, 2005; Fadel & Frederick-Duus, 2008). While both Ox1Rs and Ox2Rs are found throughout the basal forebrain, Eggermann and colleagues (2001) speculate that, because OxB administration more effectively depolarizes cholinergic neurons when compared to OxA, Ox2Rs are more highly expressed in this region and are primarily responsible for the cholinergic effects of orexinergic activation. Fadel and Burk (2010) propose that projections of orexin neurons to the basal forebrain create an anatomical substrate for the relationship between arousal and attention, augmenting the release of ACh to the cortex and promoting a state of alertness and vigilance. The activation of the cortex via orexin-mediated cholinergic release is believed to underlie the cognition-promoting characteristics of compounds that boost activity at this system.

OxA has also demonstrated nAChR-stimulating properties. By using two-photon imaging and examining slices from rat PFCs, Lambe et al. (2005) were able to reveal a sizeable amount of shared thalamocortical synaptic activity in the PFC following both OxB and nicotine administration. Due to the measurably similar actions of these two receptor systems, it may be the case that orexins would have similar stimulatory and cognition-enhancing effects as nicotine. To test this possibility, Lambe and colleagues bilaterally infused nicotine and OxB into the medial PFC of rats engaged in an attention task which assessed detection of a 0.3 s or 1 s visual stimulus. Task accuracy was significantly increased by both nicotine and the larger of two OxA doses, compared to saline, during trials with the shorter signal duration. As such, by examining both neural activity and attentional processing, it has been confirmed that, to a large extent, OxA is able to activate the thalamocortical arousal pathway in a similar manner as nAChR stimulation. Additionally, nicotine consumption increases *Fos* expression in orexin-containing neurons of the hypothalamus, potentially underlying some of the therapeutic benefits of cigarette smoking in SZ (Pasumarthi, Reznikov & Fadel, 2006).

Despite the propensity for this network to promote cognition when activated, limited research has been conducted regarding the contribution of these neurons in SZ symptomology, particularly the negative symptoms. Chien et al. (2015) measured plasma concentrations of OxA of individuals with and without a diagnosis of SZ and discovered that OxA levels were substantially higher in a small subset of SZ-afflicted persons. Compared to SZ patients with

typical OxA levels, those in the group with elevated OxA plasma concentrations – comprising around 20 percent of the sample – displayed less cognitive and disorganized symptoms as determined by a substantial reduction in preservation errors on the Wisconsin Card Sorting Test (WCST), suggesting that increased orexinergic activity may be able to attenuate the negative syndrome. While the behavioral effects of orexins or orexin-like analogs have not been examined in models of SZ, it has been shown that modafinil, a wakefulness-promoting agent that augments the release of hypothalamic histamine through orexinergic stimulation, alleviates cognitive and attentional deficits in individuals with SZ (Ishizuka et al., 2003; Turner et al., 2004; Hunter et al., 2006). However, as modafinil acts in a variety of ways, including blocking the reuptake of DA, its cognition-enhancing properties cannot be attributed solely to the activation of this system, necessitating the exploration of pharmacotherapies acting primarily at Ox1Rs and Ox2Rs in the context of SZ.

### *Research hypotheses*

Since the discovery of orexins in the 1990's, a wealth of useful information has been uncovered regarding the capacity of orexins to induce wakefulness and increase appetite. More recently, the role of OxA in cognition was detailed, and due to its cognition-enhancing qualities, it has been suggested to potentially alleviate the cognitive deficits associated with SZ. Because the negative syndrome is notoriously treatment-resistant and correlates strongly with poor life outcomes, there is an impetus to explore the potential of this cognition-enhancing neuropeptide in alleviating these pervasive symptoms. As was previously

detailed, the NMDAR hypofunction hypothesis of SZ suggests that cognitive and perceptual abnormalities emerge from a marked reduction in hippocampal NMDAR input, inducing GABAergic dysfunction and significantly augmenting frontocortical excitatory neurotransmission. A commonly-utilized method to reproduce this effect in rodents is with the administration of NMDAR antagonists, as these drugs have demonstrated the ability to emulate many of the neurological and behavioral abnormalities that are present in those with SZ (Mohn et al., 1999; Coyle, 2012).

While dopaminergic agonists such as amphetamines are effective in emulating the positive syndrome of SZ, NMDAR antagonists have demonstrated the capacity to encompass both positive and negative aspects of this disorder (Luby et al., 1959; Snyder, 1980). By binding to a hydrophobic site within the ion channel, these drugs effectively preclude the depolarization of NMDARs. Compounds frequently utilized in pharmacological models of SZ include ketamine, PCP, and MK-801 (Kapur & Seeman, 2002). MK-801, also referred to as dizocilpine, is the most potent NMDAR antagonist of the three and has demonstrated the capacity to elicit widespread social, cognitive, and perceptual dysregulation associated with SZ. At sufficient doses, typically at or exceeding 0.1 mg/kg, MK-801 administration produces social withdrawal (Rung et al., 2005), attentional deficits (Howe & Burk, 2007), learning and memory impairment (Butelman, 1989; de Lima et al., 2005), and motor aberrations including hyperactivity (Hargreaves & Cain, 1992) in rodents. Due to the robustness of this powerful NMDAR antagonist in accurately emulating the psychological and

behavioral irregularities frequently observed in patients with SZ, MK-801 was selected to reproduce the NMDAR hypofunction established in SZ for the present study.

One goal of this project is to increase the translational potential of any treatments. Intranasal administration offers a relatively easy, painless method of drug delivery. Because OxA – but not OxB – is easily able to diffuse through the blood brain barrier, and because OxA has been shown to enhance attentional performance, OxA was administered intranasally in the current experiment (Kastin & Akerstrom, 1999; Dhuria, Hanson & Frey, 2009; Zajo et al., 2016). When compared to oral and intravenous routes of administration, mist or liquid introduced through the nose also allows for more rapid onset of a drug's effects due to rapid uptake through the nasal membrane (Thorne et al., 2004). In support of this, the efficacy of OxA facilitated intranasally surpassed that of OxA injections in reversing the effects of sleep deprivation on cognitive performance (Deadwyler et al., 2007). By utilizing OxA in this fashion, not only is its role as a potential cognition-enhancer in SZ able to be elucidated, but the clinical efficacy of intranasal OxA administration can be further explored.

Because OxA has previously demonstrated the capacity to alleviate cognitive impairment in certain conditions, such as in sleep-deprived organisms, it might be hypothesized to ameliorate the negative syndrome of SZ more effectively than modern antipsychotic medications. Alternatively, levels of ACh, a neurotransmitter critical for attention, are elevated in the MK-801 SZ model; because of this, it may be that further stimulation of the ACh system with OxA

may be detrimental to performance on a number of cognitive measures. To further elucidate OxA's role as a cognitive-enhancing pharmacotherapy in the context of SZ, following training on a previously-validated sustained attention task, the effects of various doses of intranasal OxA were examined in tandem with MK-801 injections (McGaughy & Sarter, 1995). In this attentional paradigm, subjects were required to correctly differentiate between signal and non-signal trials (wherein a brief flash of a visual stimulus either does or does not occur) during varying periods of time in order to attain reinforcement. We anticipated that, in the absence of OxA, attentional performance would be significantly impaired for rats given MK-801. By intranasally administering OxA following MK-801 injections, OxA's capacity to ameliorate the treatment-resistant cognitive deficits stemming from NMDAR hypofunction was explored in a rodent model of SZ.

## Chapter 2. Methods and Results

### Methods

#### Subjects

12 male Fischer 344 Brown Norway F1 hybrid rats, bred at Charles River Laboratories (Wilmington, MA) and weighing between 250 and 350 g at the beginning of behavioral training, were utilized throughout the duration of the experiment. All behavioral testing occurred at least six days per week between the hours of 9AM and 4PM. Rats were housed in pairs throughout the entirety of the protocol and were allotted *ad libitum* access to rodent chow, but they were placed on a water restriction schedule to establish tap water as a reinforcer for accurate performance in the task. On testing days, rats were given access to a water bottle for ten minutes following their time in the task and they received 20 minutes of water access on days when they were not tested.

#### Apparatus

Each rat was trained in one of 12 operant boxes from Med Associates Inc., with all task procedures and data collection controlled by MedPC IV software (Georgia, VT). Each box consisted of an intelligence panel with left and right retractable levers, access to a water dipper with a 0.01 ml cup, and a stimulus light centralized above the water port. A house light was situated on the opposite wall facing the intelligence panel and remained illuminated throughout the testing session. Each box was located inside of a sound-attenuating chamber.

## Procedure

### *Animal training*

Rats trained in three phases to reach criterion performance in the sustained attention task. In the initial stage, rats were shaped to press the right and left levers approximately equally to maximize reinforcement. In order to protect against the development of side bias, responses were reinforced on a fixed ratio (FR1) schedule, with the stipulation that if the rat pressed one lever five consecutive times, it needed to press the other lever to receive reward access. A lever press that met this criterion led to the 0.01 ml water dipper being raised for three seconds. Once they attained 120 total rewards over a span of three days, the rats were moved to the attention task with correction trials.

In this second phase of training, rats were shaped to discriminate between signals (illumination of the central panel light for one second) and non-signals (the central panel light did not illuminate). The inter-trial interval (ITI) was  $12 \pm 3$  s, randomized to prevent possible anticipation of the commencement of a new trial. After the ITI, a signal was presented or there was no illumination of the central panel light and then both levers were extended for three seconds. The rules of the task were counterbalanced such that half of the rats were trained to press the right lever to indicate detection of the 1 s signal and press the left lever in the absence of this light; conversely, for the other half of the rats, a left lever press was rewarded in signal trials and a right lever press was reinforced in non-signal trials. If the rats responded incorrectly – that is, pressing the signal lever when no signal is presented (false alarm), pressing the non-signal lever when a signal is

presented (miss), or by failing to press altogether within 3 sec of when the levers were inserted into the chamber (omission) – a correction trial ensued. The correction trial was identical to the previous trial. If the rat made three consecutive incorrect lever presses during correction trials, a forced trial occurred wherein only the correct lever was extended into the chamber. The central panel light remained illuminated during forced trials that resulted from errors on signal trials. These trials only ended once the correct lever was pressed or 90 seconds had passed. After the rat correctly identified the presence (hit) and absence (correct rejection) of the signal light at a rate of 70% or higher for three consecutive days, they progressed to the final version of the task.

In the last phase of training, rats were trained to detect signals of varying durations – 500, 100, and 25ms – without the inclusion of correction trials. While the 500 ms is not difficult to detect for rats, correctly identifying the shorter signal durations, especially the 25 ms signal, increases the attentional demands of the task. Also, variable signal durations increase attentional demands relative to sessions with a single signal (Koelga, 1987). Each testing session lasted between 35 and 40 minutes and contained 162 trials that were evenly split between signal and non-signal trials, with an equal number of trials at each signal duration. In order to further augment the degree to which the task taxes attentional resources, the ITI was reduced to  $9 \pm 3$  s (McGaughy & Sarter, 1995), in order to increase the rate at which rats attended to the presence or absence of the visual signal. When rats answered incorrectly in this task, they were not allowed access to water at the conclusion of the trial. The behavioral criteria were

70% or higher accuracy in identifying both 500 ms signal trials and non-signal trials for three successive days. Once rats achieved these criteria, they were considered eligible for inclusion in the present experiment.

#### *MK-801 and OxA preparation*

MK-801 was purchased from Tocris Bioscience (Bristol, United Kingdom). A stock solution of 0.125 mg/kg MK-801 maleate in sterile saline was prepared and then pipetted into aliquots and stored in a freezer. The prepared solution was utilized within one week of its initial freezing. On a testing day, an aliquot was diluted, as needed and only used on that one testing day.

OxA was also purchased from Tocris Bioscience. Stock OxA concentrations of 5nM and 10 nM were prepared and then frozen and stored for the duration of the experiment. All aliquots were thawed immediately before use.

#### *Drug administration*

After rats reached criterion task performance, they began a week-long period to acclimate to intranasal administration wherein 25  $\mu$ L saline was administered per nare daily. To accomplish this, the saline was pipetted and, with the rat's nostrils oriented upwards, administered in small droplets that were then inhaled through the nose. Once the animals had received seven days of intranasal saline, drug administration commenced.

Drug administration sessions were conducted in a completely within-subject design, with every subject receiving a total of nine distinct combinations of MK-801 (0 mg/kg, 0.075 mg/kg, and 0.125 mg/kg) and OxA (0 nM, 5 nM, and

10 nM). During a day of testing, a randomized MK-801-OxA dose pairing was selected for each rat to protect against order effects, and at least one day separated drug administration sessions to minimize potential interactions from prior drug administration. Because the full range of effects induced by MK-801 are present in less than half an hour after its administration, rats were given intraperitoneal injections of one of the three concentrations of MK-801 20 minutes before testing (Pinault, 2008). After 15 minutes had passed following MK-801 injections, 50  $\mu$ L of either 0 nM, 5 nM, or 10 nM of OxA was administered intranasally at a volume of 25  $\mu$ L per nare. Following a one minute acclimation period, rats were then placed in the attention task. This procedure was repeated on each day of drug administration until all possible combined doses of MK-801 and OxA had been given prior to task performance.

### *Data analyses*

Data analysis entailed calculating the total number of hits and misses at the 500, 100, and 25 ms levels as well as correct rejections, false alarms, and omissions per testing session. Signal detection accuracy in the task was determined by dividing total number of hits at each signal duration by the sum of the hits and misses at that specific signal length ( $h/(h+m)$ ). These calculations, considered relative hits, were generated for each MK-801-OxA dose combination and signal duration combination per animal. Likewise, the proportion of relative correct rejections for non-signal events was determined by dividing total number of correct rejections by number of correct rejections and number of false alarms ( $cr/(cr+fa)$ ) for each combination of drug treatment. Repeated-measures

ANOVAs and paired-sample t-tests, which were corrected with the Bonferroni procedure, were conducted using SPSS (version 23.0, Chicago, IL).

### Results

In a 3 (MK-801; 0, 0.075, and 0.125 mg/kg)  $\times$  3 (OxA; 0, 5, and 10 nM)  $\times$  3 (signal duration; 500, 100, and 25 ms) repeated measures ANOVA for relative hits, MK-801 elicited a main effect on performance in the sustained attention task,  $F(2,22) = 8.231$ ,  $p = .002$  (*Figure 1*). Paired samples t-tests averaging across OxA concentration revealed that, while relative hits at the two lower MK-801 doses did not differ, signal detection accuracy at 0.125 mg/kg ( $M = 0.444$ ,  $SD = 0.321$ ) was significantly lower than both 0 mg/kg ( $M = 0.706$ ,  $SD = 0.109$ ;  $t(11) = 2.985$ ,  $p = .012$ ) and 0.075 mg/kg ( $M = 0.673$ ,  $SD = 0.259$ ;  $t(11) = 2.440$ ,  $p = .033$ ). In addition to this main effect, an interaction between OxA dose and signal duration was discovered,  $F(4,44) = 2.712$ ,  $p = .042$  (*Figure 2*). To further elucidate this, one-way ANOVAs averaging across MK-801 dose and examining OxA dose as a factor at each signal duration were conducted, but none of these analyses yielded significant results.

Due to the high omission rates at 0.125 mg/kg of MK-801, examining the highest dose with this measure of accuracy may not be appropriate due to skewed hit percentages. To address this issue, an additional 2 (MK-801)  $\times$  3 (OxA)  $\times$  3 (signal duration) repeated measures ANOVA excluding the largest concentration of MK-801 was conducted. The main effect of MK-801 yielded by the previous analysis was no longer significant after the removal of the highest dose, as 0.075 mg/kg did not impair attentional performance. Despite this failure

of MK-801 to induce deficits in signal detection, an MK-801  $\times$  OxA  $\times$  signal duration interaction was revealed,  $F(4,44) = 2.630$ ,  $p = .047$ . To assess the basis for this interaction, 2 (MK-801)  $\times$  3 (signal duration) repeated measures ANOVAs were conducted at each of the three OxA concentrations to further elucidate this relationship. An MK-801  $\times$  signal duration interaction was found after intranasal administration of saline,  $F(2,22) = 6.805$ ,  $p = .005$  (*Figure 3*). Despite this, when determining if performance at any of the three signal durations had significantly changed from 0 to 0.075 mg/kg of MK-801, paired samples t-tests exploring this relationship yielded no significant outcomes; only during 100 ms trials was signal detection accuracy almost significantly decreased from 0 to 0.075 mg/kg following intranasal saline exposure,  $t(11) = 1.971$ ,  $p = .074$ . No MK-801  $\times$  OxA interactions were uncovered at 5 nM or 10 nM of OxA.

A 3 (MK-801)  $\times$  3 (OxA) repeated measures ANOVA was utilized to examine the effects of MK-801 and OxA on the rats' accuracy on non-signal trials. This analysis yielded a main effect of MK-801,  $F(2,22) = 6.099$ ,  $p = .008$  (*Figure 4*). Paired samples t-tests revealed that correct rejections were lower following injections of 0.125 mg/kg MK-801 compared to vehicle,  $t(11) = 2.859$ ,  $p = .016$ , or to 0.075 mg/kg MK-801,  $t(11) = 2.382$ ,  $p = .036$ . In addition to this main effect, there was a significant interaction between MK-801 and OxA,  $F(4,44) = 3.063$ ,  $p = .026$ . To further elucidate this finding, three repeated measures ANOVAs with OxA dose as a factor were conducted for each of the MK-801 concentrations. These analyses revealed that there was a main effect of OxA in the absence of MK-801 (vehicle administration),  $F(2,22) = 4.350$ ,  $p = .026$ .

Subsequent paired samples t-tests revealed that relative correct rejections for either OxA dose were not significantly different compared with intranasal saline administration. However, correct rejection accuracy following 5 nM OxA was significantly lower than following 10 nM OxA,  $t(11) = 2.262$ ,  $p = .045$ . One potential concern is that the MK-801  $\times$  OxA interaction for correct rejections may be influenced by completion of relatively few trials at the highest MK-801 dose. However, a 2 (MK-801)  $\times$  3 (OxA) repeated measures ANOVA, excluding the highest MK-801 concentration still yielded a significant MK-801  $\times$  OxA interaction,  $F(2,22) = 5.296$ ,  $p = .013$ .

For omissions, a 3 (MK-801)  $\times$  3 (OxA) repeated measures ANOVA revealed a main effect of MK-801 concentration,  $F(2,22) = 5.296$ ,  $p = .013$  (*Figure 5*). Omissions after injections of saline were lower than following injections of 0.075 mg/kg of MK-801,  $t(11) = 4.781$ ,  $p = .001$ , omissions at 0.075 mg/kg were less than 0.125 mg/kg,  $t(11) = 3.138$ ,  $p = .009$ , and, lastly, omissions after saline injections were substantially less than following 0.125 mg/kg,  $t(11) = 5.772$ ,  $p < .001$ . The main effect of OxA approached significance ( $F(2,22) = 3.116$ ,  $p = .064$ ), but there was no interaction between the two drugs. Paired-samples t-tests examining the impact of each dose of OxA on omission rates averaging across MK-801 concentration unveiled that trial omissions were almost significantly higher after intranasal saline when compared to 5 nM of OxA ( $t(11) = 2.176$ ,  $p = .052$ ) and at 10 nM when compared to 5 nM,  $t(11) = 2.023$ ,  $p = .068$  (*Figure 6*).

## Chapter 3. Discussion and Conclusion

### Discussion

The present experiment was designed to test two separate drugs' effects on sustained attentional processing. Firstly, it aimed to reproduce the robust finding that MK-801 is a cognition-impairing agent and worsens performance in this task. Secondly, this experiment tested whether intranasal OxA administration could reduce attentional deficits following NMDAR blockade. In the context of psychiatric illness, the current research employed a previously-utilized model of SZ, and the potential for OxA – which has been found to demonstrate cognitive-enhancing qualities – to remediate the negative cognitive symptoms. Overall, it was found that the large dose of MK-801 had negative implications for attentional performance across all measures of accuracy in the task and that OxA administration had no effect or tended to worsen these impairments. Therefore, based on the results of this experiment, it is likely that OxA would not be an appropriate pharmacotherapy in this SZ model.

#### *OxA on measures of attentional performance following MK-801 administration*

The highest MK-801 dose had a profoundly negative impact on hit accuracy. OxA itself did not significantly affect signal detection in the task following MK-801 administration. Thus, the hypothesis that intranasal OxA could reverse attentional impairments following MK-801 administration was not supported. Similarly to relative hit performance, MK-801, at the highest concentration, significantly reduced the ability of the rats to accurately respond

on non-signal trials. Again, once the 0.125 mg/kg concentration was removed from the analyses, the ability of MK-801 to induce these attentional deficits disappeared. The most interesting findings were the effects following 0, 5, and 10 nM OxA in the absence of MK-801. After an injection of only saline, correct rejections at 5 nM were reduced when compared to 10 nM OxA, but neither dose was markedly different when compared to intranasal administration of vehicle. The basis for this effect is unclear, but further experiments assessing the spread of different intranasal OxA doses among brain regions may be useful in clarifying why performance was worse following the lower OxA dose compared with the higher OxA dose. There was no notable effect of OxA concentration following exposure to MK-801. Finally, MK-801 also elevated omission rates.

A likely reason that OxA was unable to alleviate NMDAR blockade-associated attentional deficits is due to its capacity to promote frontocortical ACh release. Lateral hypothalamic orexinergic cells stimulate basal forebrain cholinergic neurons, and augmenting activity at these receptors appears to further exacerbate the already-overactive cholinergic system present in SZ-afflicted individuals. As was previously detailed, a surplus of cholinergic input in the cortex leads to the overstimulation of various sensory and perceptual systems by weakening the brain's ability to filter irrelevant environmental stimuli, and it is likely that elevated frontocortical ACh, in congruence with overstimulated PFC pyramidal cells resulting from NMDAR hypofunction, worsens cognitive performance (Donoghue & Carroll, 1987; Perry & Perry, 1995; Homayoun & Moghaddam, 2007). As a consequence of excessive excitatory

neurotransmission in both cortical and subcortical regions, it may be that the rats' visual system was stimulated to such an extent that there was an impairment in filtering whether or not a visual cue was presented. This notion is supported by the finding that, while relative hits were not impaired by combinations of MK-801 and OxA, high concentrations of exogenous OxA, in congruence with NMDAR blockade, appeared to somewhat worsen relative correct rejections, leading them to err on the side of signal detection. Drugs that primarily act at  $\alpha 7$ -nAChRs, which facilitate the activity of inhibitory septo-hippocampal GABAergic innervations of the basal forebrain, demonstrate promise in alleviating SZ symptoms; however, perhaps because OxA promotes ACh release at other cholinergic receptor sites, it does not possess this therapeutic effect.

As MK-801 concentration increased, omission rates also increased in a dose-dependent manner. Omissions were elevated at 0.075 mg/kg of MK-801 when compared to injections of saline, and omissions were higher at 0.125 mg/kg than at 0.075 mg/kg. A near-significant main effect of OxA revealed that rats tended to omit slightly more often after intranasal saline and 10 nM of OxA when compared to 5 nM, suggesting that perhaps small concentrations of OxA help to protect against NMDA antagonism-induced elevations in trial omissions. While no noteworthy motor aberrations were observed before or shortly after engagement in the attention task, it is possible that increased concentrations of MK-801 disrupted motor functioning and interfered with movements related to lever-pressing (Tricklebank et al., 1989).

*Implications of OxA treatment in SZ*

In a SZ model using NMDA receptor blockade, the introduction of pharmacotherapies enhancing OxA activity do not improve measures of sustained attentional performance. In fact, OxA sometimes further exacerbated the adverse impact of MK-801. In neurological conditions wherein frontocortical neurotransmission is substantially reduced, such as in Alzheimer's, ADHD, or Parkinson's disease, OxA and other compounds agonizing these orexin-releasing neurons have been speculated to alleviate cognitive deficits by bolstering the release of monoamines such as DA, ACh, and histamine throughout pertinent cortical regions (Fadel, Pasumarthi & Reznikov, 2005; Vittoz & Berridge, 2006). The present findings suggest that OxA, while having previously been found to either boost cognition or alleviate related deficits in following some forms of dysfunctional CNS activity, is not a viable pharmacotherapy when attempting to treat the negative symptoms of SZ. Thus, although OxA can still be considered a cognition-boosting agent, this beneficial quality is not reflected across all disorders and may be, in large part, reliant on dampened cholinergic neurotransmission (Zajo et al., 2016). With disorders such as SZ, where cortical excitation is elevated, it appears that OxA has little or possibly even a negative effect on attention and cognitive processing.

One potential shortcoming of this study is the effectiveness of the chosen doses of MK-801. With 0 nM of OxA, while the high dose of 0.125 mg/kg had an extremely profound impact in terms of both impairing accuracy and increasing omissions in the attention task, the 0.075 mg/kg concentration failed to induce deficits in both of those regards. Previous studies utilizing 0.1 mg/kg or higher

have consistently provided findings similar to those generated by this experiment in that many facets of cognition, such as attention, learning, and working memory, are impaired; however, lower doses, such as 0.05 and 0.075 mg/kg, have offered mixed results in their cognition-impairing abilities (Hlíňáka & Krejčíb, 2002; Howe & Burk, 2007; van der Staay et al., 2011). Therefore, it may be that 0.075 mg/kg on its own was too low of a concentration to sufficiently block NMDAR function to the extent that it would produce the full impairment in attentional performance that is seen quite drastically with the 0.125 mg/kg dose. While this may affect the interpretation of the current study in terms of its applicability to emulating SZ symptomology, the elevated dose of intranasal OxA still reduced attentional capacity, perhaps indicative of OxA's tendency to further worsen the effects of NMDAR inhibition in the cortex.

#### *Future directions*

If OxR activation worsens frontocortical hypexcitation induced by NMDAR antagonism, it may be the case that reducing the input from these receptors could enhance cognition in this and other conditions demonstrating similar abnormalities in cortical excitatory neurotransmission. Drugs that target and reduce the activation of Ox1Rs and Ox2Rs, for example, could temporarily lessen the consequences of hyperfrontality and alleviate some of the cognitive deficits seen in SZ and similar disorders. Preliminary findings from our lab have shown that, at small concentrations, the Ox2R antagonist TCS-OX2-29 is able to transiently improve performance in the sustained attention task in neurotypical rats when infused directly into the medial PFC (Tapp, Maness, & Burk, in

preparation). These findings, taken with the results from the present experiment, provide an impetus to explore the implications of Ox1R or Ox2R blockade on attentional and cognitive performance in a similar NMDAR hypofunction model of SZ. Additionally, the orexin system is critical for sleep and circadian rhythmicity, so the suppression of these neurons may have unforeseen impacts on sleep quality and drowsiness that may assuage its beneficial effects on cognitive performance.

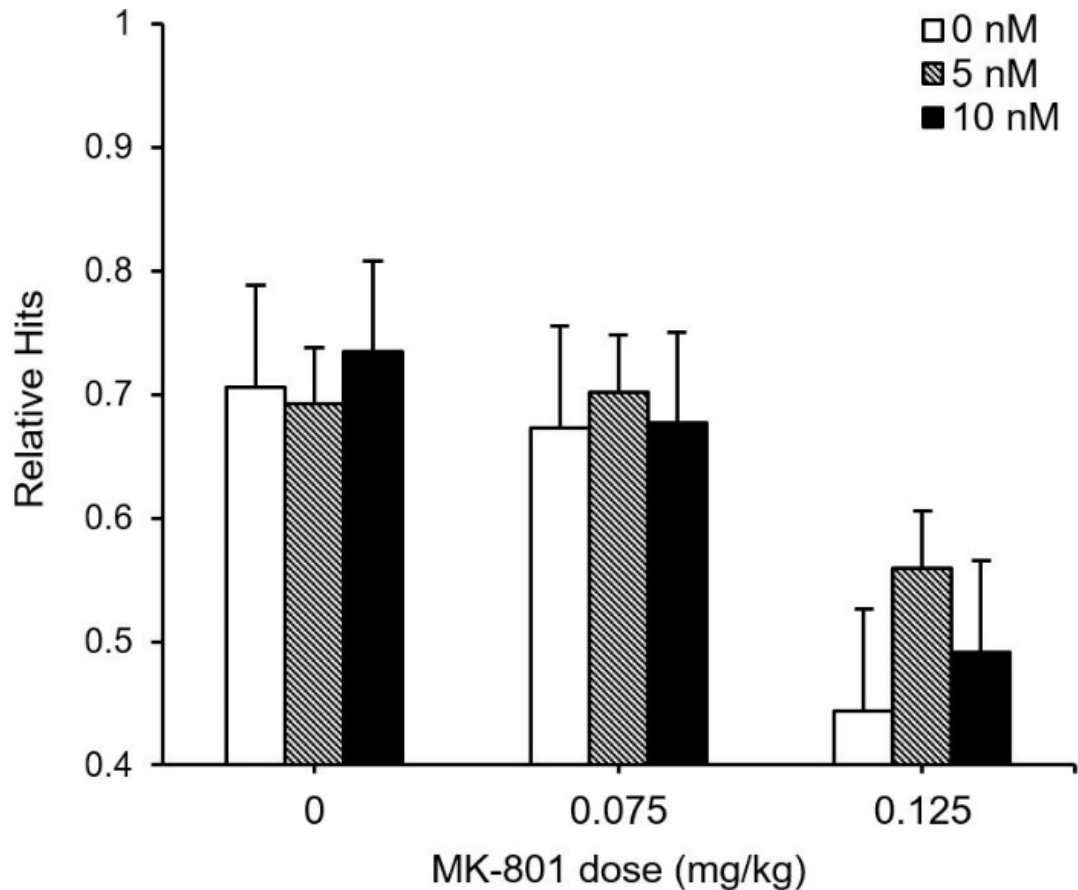
Because the cognition-boosting qualities of OxA are worth further elucidation in the context of intranasal administration due to ease of self-administration and how quickly it elicits its effects, it may be useful to examine its therapeutic effects following widespread loss of cholinergic neurons. The tendency for OxA to worsen measures of accuracy in the attention task in rats treated with MK-801, particularly with correct rejections, indicates that OxA may be exerting much of its influence on basal forebrain cholinergic inputs to the cortex and subcortex. One method that could verify this synergistic orexinergic-cholinergic interaction, similarly to a study by Zajo, Fadel and Burk (2016) wherein intracerebroventricular infusions of OxA were able to alleviate attentional deficits following basal forebrain corticopetal lesions, would involve disrupting BFCS functionality – either with a cholinergic antagonist or the cholinergic immunotoxin 192 IgG-saporin – and administering OxA intranasally preceding placement in the operant task. In doing so, the influence of lateral hypothalamic orexinergic efferents on the basal forebrain would be better understood, and the therapeutic potential of nasal OxA administration would be further established in

the context of Alzheimer's and Parkinson's diseases, wherein cholinergic neurotransmission is compromised.

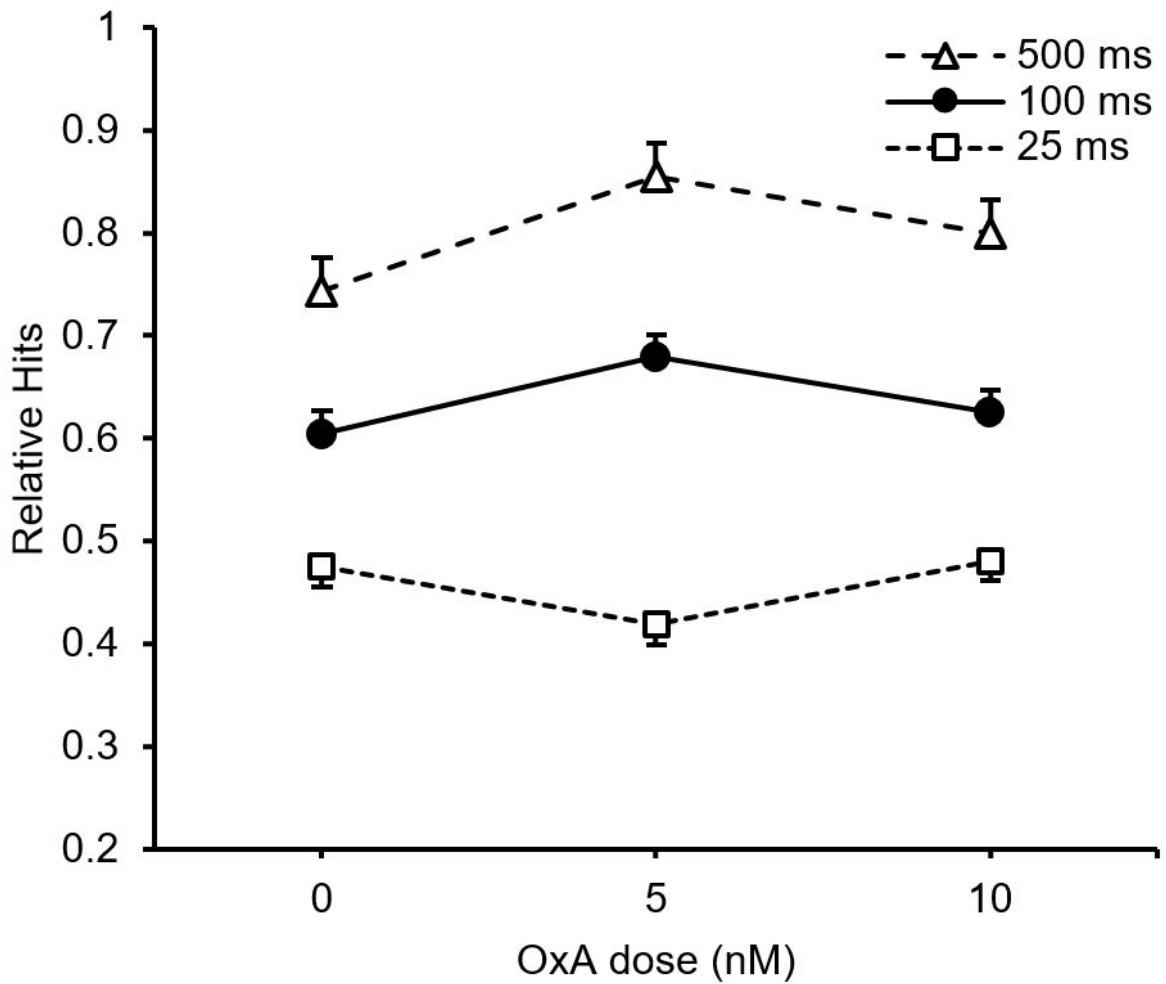
### *Conclusion*

In an MK-801 model of SZ, increased activation of orexin receptors through the introduction of intranasal OxA did not alleviate MK-801-induced attentional deficits. This failure of OxR stimulation to enhance cognition in this paradigm is likely due to its tendency to worsen the cognitive and perhaps perceptual disturbances resulting from a failure of NMDAR and GABAR regulation of excitatory neurotransmitter release in the PFC and other cortical regions. Additionally, because of orexinergic innervation onto basal forebrain cholinergic neurons, hypercholinergia was likely aggravated after OxA exposure, failing to improve measures of attentional accuracy. These findings further discern the role of this neuropeptide as a "cognition-enhancer" in certain circumstances and not others, and they also suggest that alleviating the abnormal cognition and behaviors seen in SZ may lie in the reduction, rather than promotion, of frontal lobe stimulation. In this regard, Ox1R and/or Ox2R antagonists may provide a unique advantage in this model by assuaging cortical hyperexcitation. In a similar vein, further attempts to explore the potential for drugs that strengthen OxR activity as a form of pharmacotherapy to treat disorders with a cognitive component would likely yield more successful results in the framework of dampened cortical excitation. In particular, cognitive decline stemming from loss of cholinergic fibers, which occurs during neurodegeneration and is especially aggravated in dementing states, may benefit from intranasal

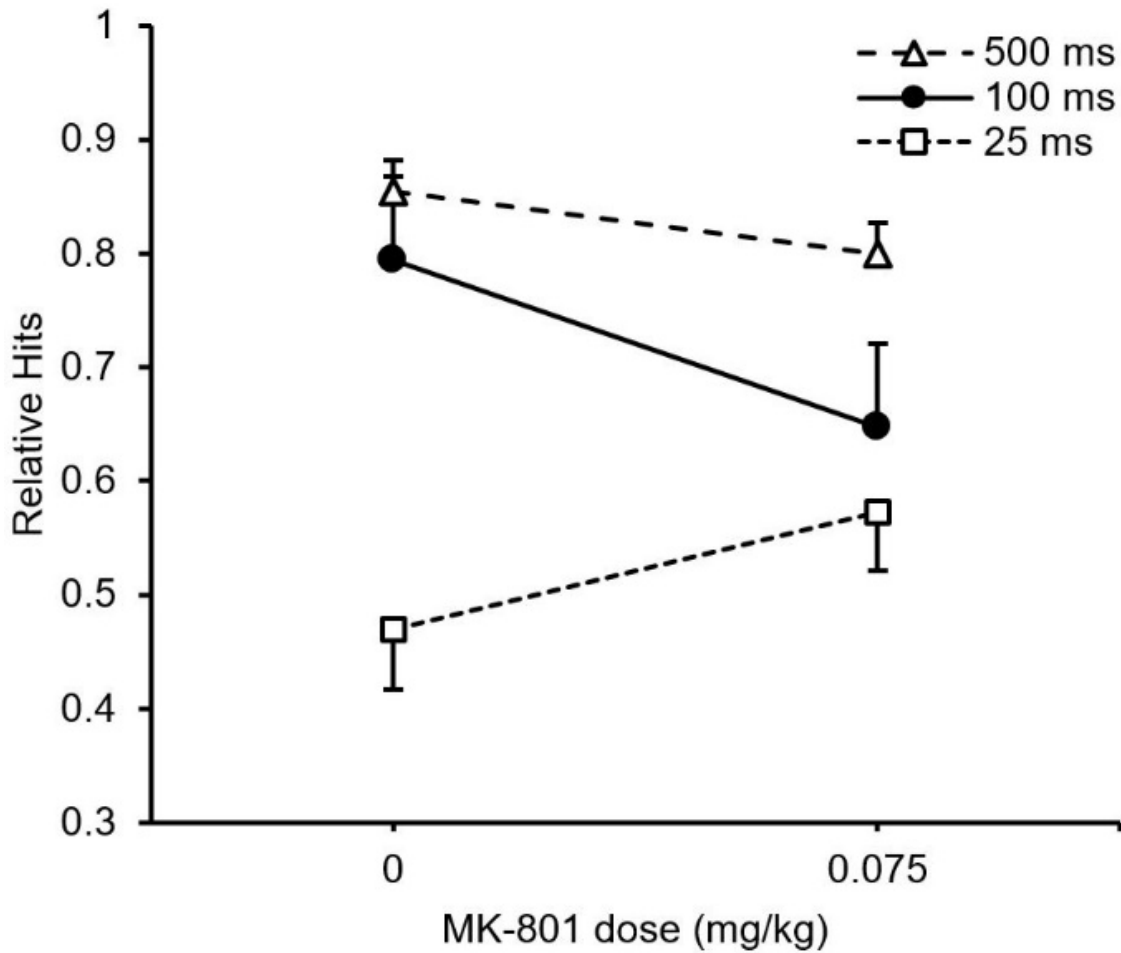
OxA administration. Therefore, while not advantageous in a rodent model of SZ, OxA can still be classified as a cognition-boosting agent in the appropriate neurological context.

**Appendix**

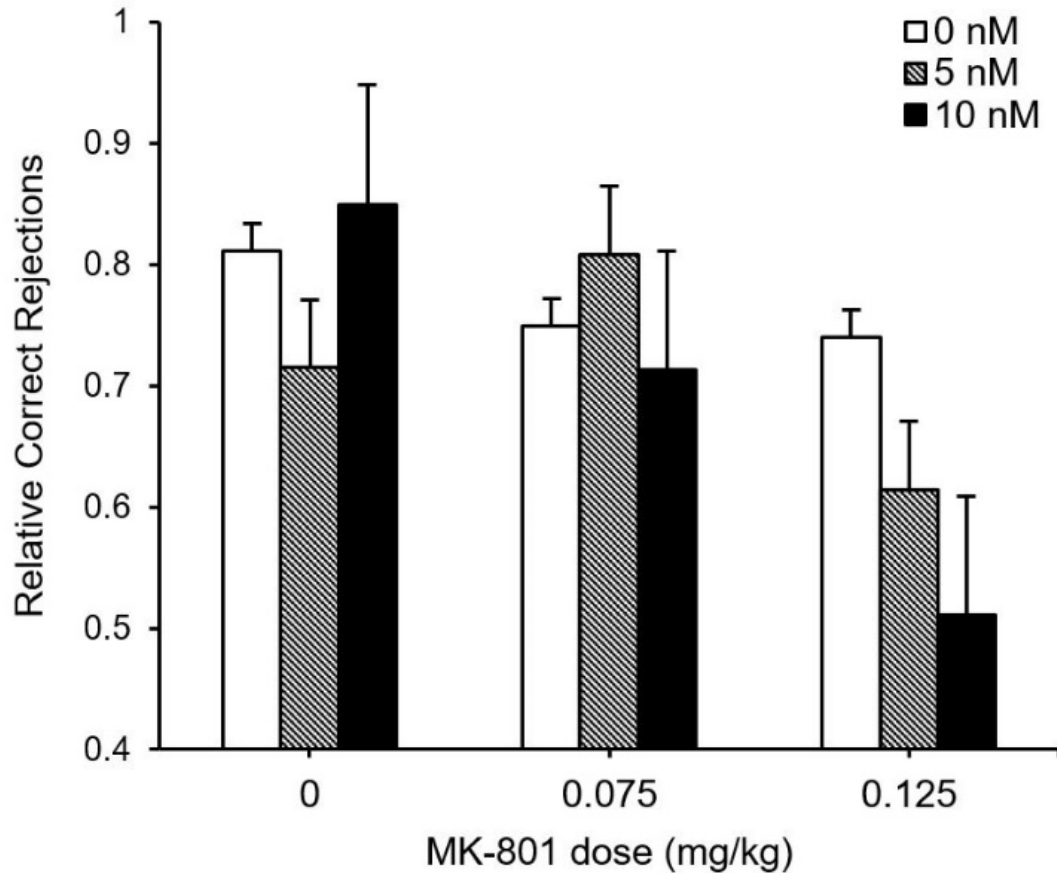
*Figure 1.* Hit percentages at each dose of OxA (0, 5, and 10 nM) across the three concentrations of MK-801 (0, 0.075, and 0.125 mg/kg). MK-801 impaired overall performance on this measure at 0.125 mg/kg, but not at 0.075 mg/kg. While there was a significant interaction between MK801, OxA, and signal duration when excluding the largest MK-801 dose, there were no differences in signal detection accuracy between the three OxA concentrations at 0 and 0.075 mg/kg.



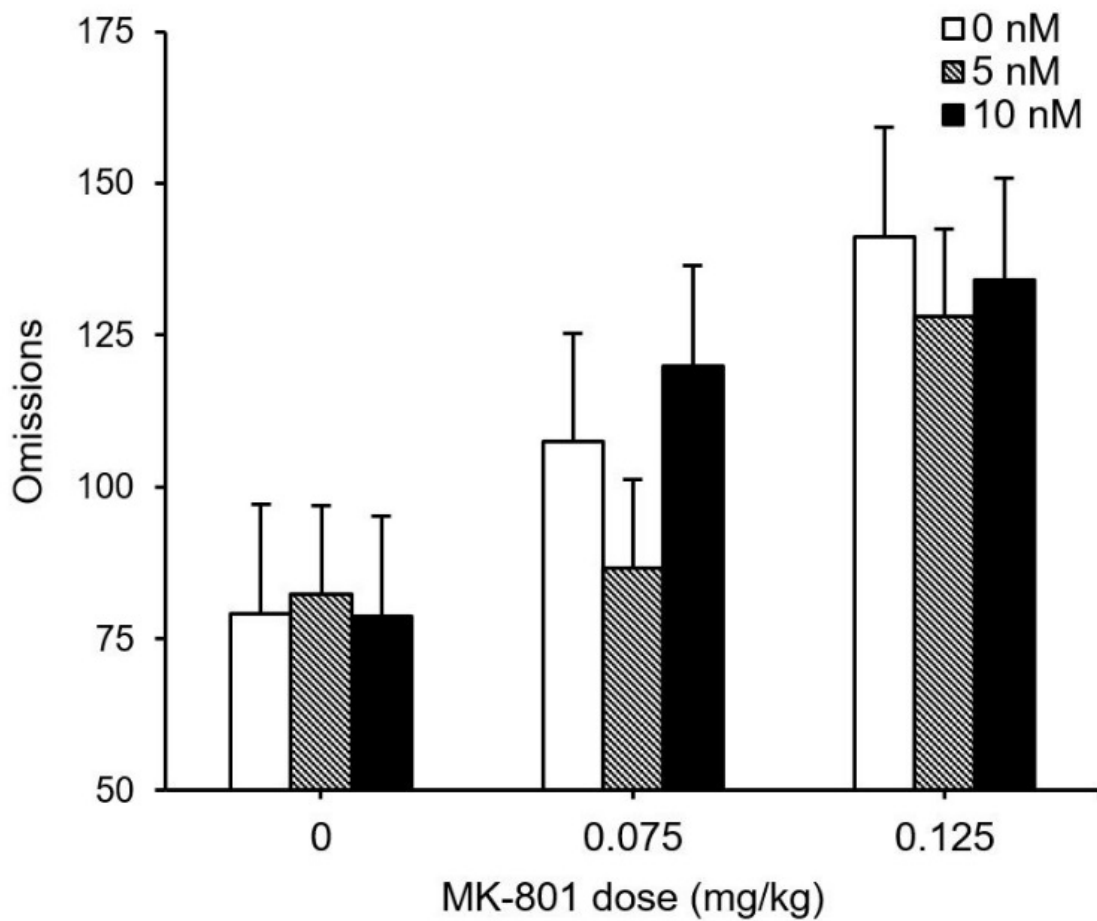
*Figure 2.* Hit percentages at each signal duration (500, 100, and 25 ms) at the three doses of OxA averaged across MK-801 concentration. There was a statistically significant interaction between OxA and signal duration, which was further examined in figure 3.



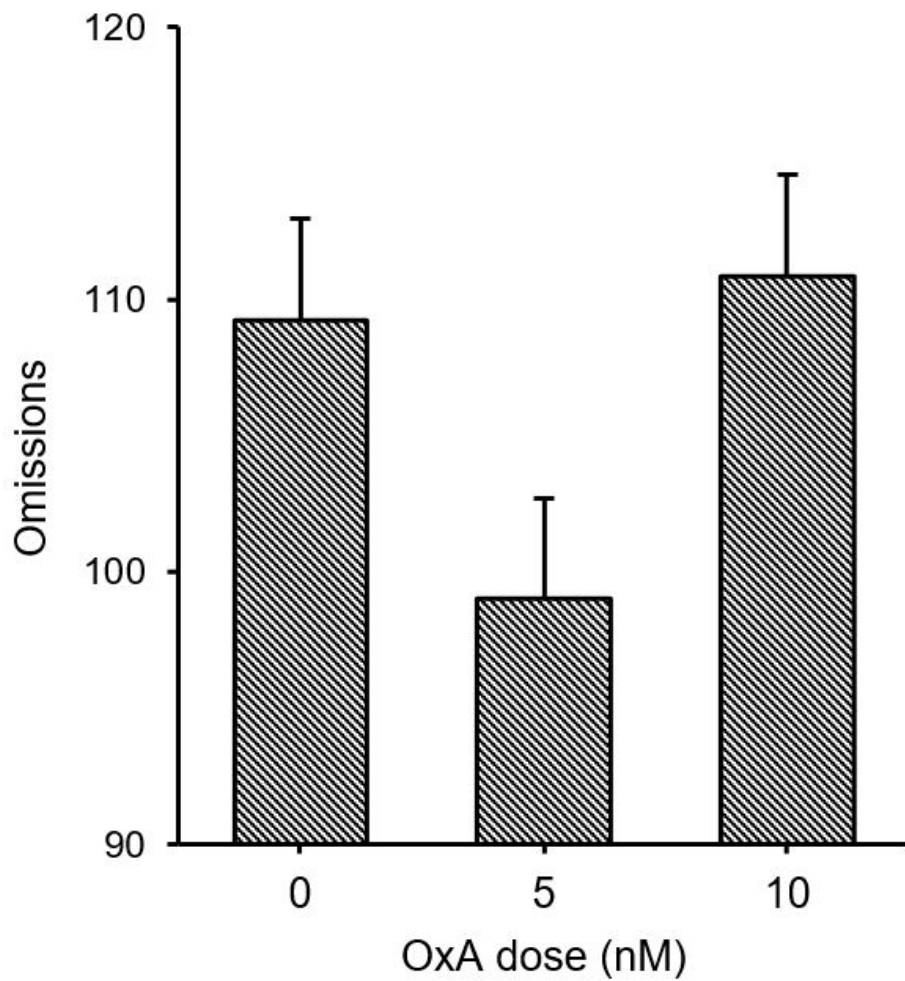
*Figure 3.* Hit percentages at each signal duration (500, 100, and 25 ms) at 0 mg/kg and 0.075 mg/kg of MK-801 following intranasal saline administration. There was a statistically significant interaction between MK-801 and signal duration. Despite this, there were no substantial changes in signal detection accuracy within the three signal durations from saline injections to the low dose of MK-801.



*Figure 4.* Correct rejection percentages at each of the three OxA doses across the three concentrations of MK-801. Similarly to relative hits, MK-801 impaired overall performance on this measure at 0.125 mg/kg, but not at 0.075 mg/kg. At 0 mg/kg of MK-801, the identification of non-signal trials following 5 nM of OxA was impaired when compared to performance at 10 nM. From 0 mg/kg to 0.075 mg/kg, correct rejections after exposure to 10 nM of OxA decreased while 5 nM prevented a significant impairment in performance. At 0.075 mg/kg of MK-801, correct rejection accuracy at 5 nM of OxA was enhanced when compared to 10 nM.



*Figure 5.* Omission rates at each of the three OxA doses across the three concentrations of MK-801. As the dose of MK-801 was augmented, trial omissions dose-dependently increased despite OxA administration. While omissions for 0 nM and 10 nM of OxA were elevated from 0 to 0.075 mg/kg of MK-801, 5 nM of OxA protected against this significant increase, but trial omissions increased dramatically following 0.125 mg/kg regardless of OxA dose.



*Figure 6.* Omission rates following administration of 0, 5, and 10 nM of OxA averaged across MK-801 concentration. When considering trial omissions, the main effect of OxA approached significance, and follow up tests revealed that rats almost omitted less after 5 nM of OxA than both saline and 10 nM.

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