CIRCADIAN DISRUPTION CAUSES DEFICITS IN ATTENTION AND RESPONSE INHIBITION IN ADULT LONG-EVANS RATS

BY

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DISSERTATION

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Abstract

Circadian rhythms are endogenous rhythms governing behavior and physiology. Circadian disruption is an environmental factor that impacts cognition and increases the risk of neurodegenerative disease by altering the circadian clock at a molecular level. Conventional sources of circadian disruption in human populations include working beyond the regular hours of '9 to 5' (shift work) and untimely exposure to light (light-at-night, LAN). Our study investigated the effects of 2 models of circadian disruption on response inhibition, which has previously been unaddressed, and attention using a 5-choice serial reaction time task (5-CSRTT). Adult Long-Evans rats of both sexes were maintained on a 12h:12h light:dark cycle and tested under 3 conditions: 4 h into the dark phase with no exposure to ambient light at the time of testing (control), 4 h into the dark phase with exposure to ambient light during testing (a model of LAN), and 4 h into the light phase (a model of shift work). Our hypothesis that rats tested under both models of circadian disruption would have reduced response inhibition and attention versus controls was confirmed. We also established that changes in expression occur in *Per2* in light phase models of circadian disruption. *Chat* and *Drd1* showed rhythmic expression with peak expression during the dark phase.

Because acetylcholine (ACh) governs circadian rhythms and attention, and DA modulates response inhibition, we performed drug challenges to examine for an interaction between the 2 neurotransmitter systems in our models. We combined an ACh agonist (nicotine) with antagonists for DA receptor 1 (SCH 23390) and DA receptor 2 (eticlopride) under the 3 circadian conditions to identify differential drug responses between treatment groups. The 2 circadian disruption models showed increased

sensitivity to nicotine compared to control. SCH 23390 ameliorated the effect of nicotine in both models. This response to the combination of drugs confirms an interaction between cholinergic and dopaminergic neurotransmitters and identifies novel effects of circadian disruption on response to drugs. These results could potentially hold the key to better understanding altered cognitive functioning in real-world scenarios caused by conventional sources of circadian disruption.

Dedicated to Appa and Amma

For always believing in me!

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Chapter 1 Behavior

1.1 Five Choice Serial Reaction Time Task

The 5-choice serial reaction time task (5-CSRTT) has become one of the standard methods to quantify attention and impulsive behavior. There are various versions of this task that have been developed as per the needs of the lab and the endpoints that are being analyzed (Beaudin et al., 2017; Robbins, 2002; Spinelli et al., 2006). Carli et al. adapted the 5-CSRTT in 1983 (Carli et al., 1983) from a task that was used to test attention in human subjects. In addition to attention, this test can also measure impulsivity (van Gaalen et al., 2006; Hahn et al., 2002; Robbins, 2002). This task is used to test pharmacological responses to drugs and toxicants and neurological manipulations. It also measures attention deficits caused by chronic drug exposure (Dalley et al., 2005a, 2005b), and effects of discrete brain lesions and neurotransmitter depletions (Chudasama et al., 2003; Harrison et al., 1997). When coupled with microdialysis the 5-CSRTT can measure changes in neurotransmitter release or metabolic activity during the task (Dalley et al., 2001). With all the different parameters that can be measured by one test, the 5-CSRTT is valuable for understanding disrupted mechanisms in preclinical models of conditions such as ADHD (Puumala et al., 1996), Alzheimer's, Parkinson's (Carli et al., 1985), and schizophrenia (Amitai et al., 2007).

In this task, the rat needs to sustain spatial attention across the 5 cue holes over many trials. The rat faces 5 horizontal openings on a wall of the test chamber and on the opposite side is the port for receiving positive reinforcement, i.e., food pellets as shown in Figure 1.1. Since the rats are on a restricted diet, food serves as motivation to perform the tasks. There is also a house light and a light above the food magazine that illuminates when the pellet is dispensed. The rat triggers a trial by taking the food

already dispensed in the food tray. After the rat initiates the trial, it has a few seconds to turn around and face the 5 cue lights. This time duration is referred to as the cue delay and often serves as a parameter that can be changed to alter the difficulty of the task. The cue light in one of the 5 ports illuminates at this point, and the rat is supposed to nose poke in the illuminated cue port for it to register as a correct response. If this happens then the palatable food reinforcer is delivered. If the rat pokes in an incorrect hole, then it is registered as an incorrect response and gets a timeout, where all lights are switched off for a period. If the rat does not respond in the stipulated time (*limited hold*) allotted for it make a response, then the trial is considered as omitted. Here again, the rat gets a timeout identical to that of the incorrect response. If it responds before the cue illuminates, it is deemed a premature response and also results in a timeout for 5 s (Bari et al., 2008; P.J and Strupp). Reinforcers are delivered only for correct responses.

A task like this can test both sustained attention (vigilance) and select attention (alertness). The rat needs to be vigilant to see which cue hole is illuminated and must be alert enough to make a quick enough response to not miss the trial. The 5-CSRTT can be modified as per experimental requirements (Bushnell, 1998). The difficulty of this task can be easily altered by making a few variations such as changing the cue delay by either lengthening or shortening it, changing the duration of the stimulus (cue light), or by adding a distractor which would be an auditory input or an olfactory input. The automated data acquisition allows for accurate measurements of latencies to perform actions and reduces chances of errors that would occur with hand-scoring. The data points measured by the software can be used to calculate different measures. Percent accuracy, calculated based on percent correct and percent incorrect responses, is a

direct measure of attention. Causes for percent omissions include a subject omitting trials because of inattentiveness, lack of motivation or motor deficits. A premature response reflects deficits in inhibitory control processes of response preparation, thus indicating impulsive behavior. Perseverative responses are repeated nose pokes in the cue hole after receiving reinforcement, and are an indicator of a lack of response control or a compulsive action (Robbins, 2002). This chapter will focus on attention and response inhibition aspects of behavior in association with this task.

1.2 Attention

Attention is a multidimensional construct, which is broadly defined as the facilitated processing of one piece of information over others (Stefanatos and Baron, 2007). Attention is comprised of several components, of which sustained attention and select attention are the two most quantifiable aspects. Sustained attention or vigilance is the ability to maintain attention on a task for a period of time. Select attention or alertness is the ability to enter the state of focusing rapidly on additional information or stimuli while ignoring extraneous information. This is often measured as the time or latency taken to respond to a stimulus (Nigg and Nikolas, 2008). Attention often works in conjunction with *executive functions* like working memory, response inhibition, and cognitive flexibility. The prefrontal cortex is heavily implicated in higher order executive functions which include attention (Euston et al., 2012; Miller and Cohen, 2001). It has been previously established that the medial region of the PFC (mPFC) is important for working memory and attention (Euston et al., 2012; Muir et al., 1996; Passetti et al., 2002) in rodents. The mPFC roughly corresponds to the dorsolateral PFC in primates and humans (Farovik et al., 2008; Uylings et al., 2010; Vertes, 2004, 2006). Lesions of

the mPFC give rise to severe attentional deficits (Kahn et al., 2012; Muir et al., 1996; Passetti et al., 2003). Neuroimaging and electrophysiological studies have also implicated the mPFC in tasks requiring sustained attention (Bentley et al., 2011; Gill et al., 2000; Totah et al., 2009). An increase in attentional load is often associated with increases in neuronal activity in the mPFC (Gill et al., 2000). Even though the mPFC receives innervation from different neurotransmitters, acetylcholine (ACh) is crucial for optimal attention.

1.3 Cholinergic Neurotransmission

Animal studies have helped establish neuroanatomical circuitry underlying attention. The PFC is densely innervated by cholinergic neurons which play a crucial role in PFC functioning (Bloem et al., 2014). Acetylcholine (ACh) is a neurotransmitter produced by a relatively small number of neurons but which affects the entire brain (Woolf and Butcher, 2011). The basal forebrain cholinergic system includes the medial septum, substantia inominata, and the nucleus basalis as shown in Figure 1.2. The neurons from the nucleus basalis of Maynert (NBM) project to the cerebral cortex and amygdala (Woolf and Butcher, 2011). The neurons from the medial septum project to the hippocampus and cingulate cortex. Acetylcholine is also produced in the midbrain region, pedunculopontine nucleus and laterodorsal tegmental area (Mesulam et al., 1983), and exerts its effects via receptors present on different neurons and glial cells (Picciotto et al., 2012; Van der Zee and Keijser, 2011). This section outlines the evidence for cholinergic modulation of attentional systems in animals, focusing primarily on rodent behavioral neuropharmacology assessed with the 5-CSRTT (Robbins, 2002).

The role of cholinergic neurotransmitters in sustained attention is vital to the accuracy of responding in the 5-CSRTT and is highly contingent on cortical ACh levels. Involvement of ACh in attention was established by performing local cholinergic lesioning using specific immunotoxin 192 immunoglobulin G (IgG)-saporin, which resulted in severely compromised performance in sustained attention tasks (Chudasama et al., 2004; Dalley, 2004; Gill et al., 2000). Gill et al. also showed that the neuronal activity associated with attention is absent after cholinergic lesions (Gill et al., 2000). Microdialysis studies have shown that ACh efflux is more significant during tasks involving sustained attention like the 5-CSRTT (Passetti F. et al., 2008). The efflux increases with increasing demands of the tasks implicating ACh in the maintenance of optimal attention (Dalley et al., 2001; Himmelheber et al., 2001). Acetylcholine has also been implicated in stimulus detection and response selection (Milstein et al., 2005). Recent studies have shown that there is both tonic and phasic cholinergic release of ACh in the mPFC, where the phasic release of ACh in the mPFC is caused by attended cues, and tonic increases in cholinergic activity are correlated with higher cue detection rate (Parikh et al., 2007).

Types of Cholinergic Receptors

There are two types of cholinergic receptors, muscarinic ACh receptors and nicotinic ACh receptors, that allow ACh to change the electrical activity of the target cells and to affect other processes through intracellular signaling cascades. Despite that both these receptors initiate post synaptic signaling cascades, they work in fundamentally different pathways.

1. Muscarinic Acetylcholine Receptors (mAChRs) are G-protein coupled receptors and function through an intracellular signaling cascade (Bubser et al., 2012). There are 5 different types of mAChRs, where M1, M3, and M5 interact with Gq protein whereas M2 and M4 interact with Gi/o proteins (Brown, 2010). M1, M2, and M4 are present in the cortex with a higher expression of M1 and M2 than M4 (Levey et al., 1991). The conductance of ion channels, mainly potassium and calcium channels, is altered by these receptors via a variety of intracellular signaling cascades (Thiele, 2013). Acetylcholine and muscarine are examples of agonists that can be used to stimulate these receptors.
2. Nicotinic Acetylcholine Receptors (nAChRs) are ionotropic receptors constituted predominantly of α (α2- α10) and β (β2 - β4) subunits in the brain (Gotti et al., 2006). There are two main subfamilies of nAChRs, homopentameric receptors formed by 5 α subunits and heteropentameric receptors constituted of both α and β subunits (Alkondon and Albuquerque, 2004). In the cerebral cortex, there are only two main types of nAChRs present, a homopentamer of α7 subunits and heteromeric receptors with two α4, two β2 and a fifth subunit that could be α4, α5 or β2 (Albuquerque et al., 2009). These receptors are selective cationic channels, permitting flow of Na^+, K^+, or Ca^{2+} thereby depolarizing the membrane. Nicotine acts as an agonist at these receptors, thus the name nicotinic receptors. Antagonists like mecamylamine and dihydro-β-erythroidine (DHBE) bind to the receptor and inhibit the action of ACh. The work described in this dissertation focuses on the effects of nAChRs.

Role of Nicotinic Receptors in Attention

During attention tasks, there is a release of ACh in the mPFC that is linked to attention and cue detection (Parikh et al., 2007; Passetti F. et al., 2008). Mice lacking β2 nAChR subunits were tested on 5-CSRTT. These subunits in the prelimbic cortex were essential to respond to the cue lights that were presented, and re-expression of the β2 subunits was able to rescue the behavior (Guillem et al., 2011). Another study failed to see the same effects with β2 subunits but reported that α7 subunit knock-out mice had attentional deficits shown by a decrease in accuracy and an increase in omissions on the 5-CSRTT (Hoyle et al., 2006; Young et al., 2004, 2007). Systemic administration and local infusion of nicotine into mPFC showed an increase in attention on the 5-CSRTT (Hahn et al., 2003; Stolerman et al., 2000). Mecamylamine, a non-specific nAChR antagonist, reduced accuracy and increased omissions (Grottick and Higgins, 2000). To further investigate the roles of heteromeric and homomeric nAChRs in the effects of nicotine on 5-CSRTT, Hahn et al. administered the β2* subunit-specific antagonist DHBE and the α7 subunit-specific antagonist, methyllycaconitine (MLA) (Hahn et al., 2011). They established that effects of nicotine are mediated via α7 nAChRs. Young et al. on the other hand showed that effects of nicotine are mediated via β2* subunits instead of α7 subunits (Young and Geyer, 2013). These are a few of the studies identifying the role nAChRs play in modulating attention. The difference in the effects of β2* subunits instead of α7 subunits of nAChRs in these studies could be due to the use of different species and strains. Guillem et al. and Young et al. used a mouse model in their study while Hahn et al. used a rat model. While ACh may not exclusively modulate attention, it is activated by behavioral situations that tax the

attentional capabilities in an animal. Disruption in the cholinergic system is seen with an array of neurodegenerative disorders like Alzheimer's (Ferreira-Vieira et al., 2016; Mufson et al., 2008) and neuropsychiatric disorders like attention deficit hyperactivity disorder (ADHD) and schizophrenia (English et al., 2009). Cholinergic neurotransmission is critical for attention in healthy individuals, while alterations to this neurotransmission are a component of many neuropsychiatric disorders.

1.4 Response Inhibition

Impulsivity is caused by disruption of the inhibitory control of behavior and is a complex behavioral construct. Impulsive responses include decisions and actions that are poorly conceived, prematurely expressed, and may involve increased risk-taking or the inability to accept delayed gratification (Robbins, 2002; Winstanley et al., 2006). Such a behavioral pattern is a component of numerous psychiatric disorders, including addiction disorders, and may also result from exposure to environmental contaminants. Animal and human studies have helped elucidate the neuroanatomy and neurobiology that give rise to such behavior (Dalley et al., 2011). Two commonly used **behavioral paradigms** to analyze impulsivity are the 5-CSRTT and the delay discounting task (Robbins, 2002; Winstanley et al., 2006). As previously discussed, 5-CSRTT measures visuospatial attention. Over the course of the task, the subjects also learn to inhibit their responses until the next visual cue is presented. Any response made before the visual cue illuminates is regarded as a premature response and is a measure of impulsivity (Robbins, 2002). In the delay discounting paradigm, individuals who tend to be more impulsive choose options associated with smaller rewards delivered more quickly, indicating intolerance to waiting for gratification (Winstanley et al., 2006). This task can

be operant-based or questionnaire-based depending on the species being tested. Our study uses premature responding on 5-CSRTT as a measure of impulsive behavior.

1.5 Dopaminergic Neurotransmission

Animal studies have helped map the neuroanatomical circuitry involved in impulsivity. The critical regions involved in response inhibition are PFC, the nucleus accumbens (NAc) and the striatum. Damage to the PFC results in deficits in the ability to inhibit an inappropriate response (Brass, 2002; Dove et al., 2000). Human patients with damage to ventromedial frontal cortex, which includes the orbitofrontal cortex, have exhibited deficits in decision making and impulsive social behavior as determined by the Iowa gambling task (Bechara et al., 1994). Lesions to the orbitofrontal cortex in rodent brains cause the rats to choose less impulsively, i.e., they prefer larger and more delayed rewards. Even though the human and rodent studies seem to contradict each other, both species are choosing the response associated with the larger reward regardless of the unfavorable consequences, i.e., the monetary loss in the human studies and longer delay in the rodent study (Bechara et al., 1994; Winstanley et al., 2006). These studies indicate that the orbitofrontal cortex plays a crucial role in modulating impulsive choice on delay discounting. Lesions to the anterior cingulate cortex in rats caused an increase in premature responses on 5-CSRTT, and the rats chose smaller, more immediate rewards on delay discounting paradigms.

The frontal cortex, hippocampus, and amygdala project to the NAc, which then projects to caudate putamen. Excitotoxic lesions to the core of the NAc increased impulsive action on the 5-CSRTT and impulsive choice on the delay discounting paradigm (Christakou, 2004). The NAc has two distinct parts, core, and shell, with

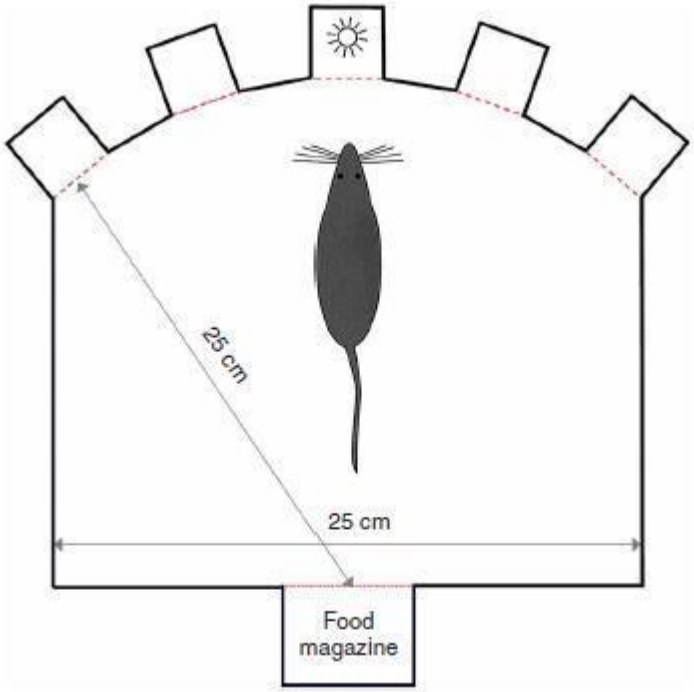
different innervations. Excitotoxic lesions to the amygdala also increase impulsive responses via the connection to NAc (Winstanley et al., 2004). There is evidence that the infralimbic (IL) cortex is involved in modulating impulsive action. Excitotoxic lesioning the IL, which is a part of the PFC, induced premature responding on 5-CSRTT (Chudasama et al., 2003).

Dopamine (DA) plays a crucial role in maintaining optimal response inhibition (Colzato et al., 2009). Figure 1.3 shows the dopaminergic projections in the rodent brain. The mesolimbic pathway transmits DA from the ventral tegmental area (VTA) in the midbrain to the NAc; the mesocortical pathway transmits DA from VTA to PFC; and the nigrostriatal pathway transmits DA from the substantia nigra pars compacta to the dorsal striatum (Dahlström and Fuxe, 1964). The mesolimbic and mesocortical pathways are jointly termed as the mesocorticolimbic projections. Changes in the levels of DA lead to altered behavioral responses, including altered response inhibition. Dopamine neurons from the substantia nigra and the VTA project to the forebrain, a region associated with response inhibition (Robbins, 2002). Changes in the optimal levels of DA neurotransmission correlate with an increase in impulsive behavior (Arnsten and Pliszka, 2011; Cools and D'Esposito, 2011; D'Amour-Horvat and Leyton, 2014). Studies show that manipulating the dopaminergic system has severe consequences on the performance of the 5-CSRTT. Studies also used various dopaminergic agonists and antagonists to observe the effects on the 5-CSRTT. Drugs like amphetamine and GBR12909 that increased the levels of synaptic DA enhanced impulsive behavior on 5-CSRTT, whereas DA antagonists predominantly decreased premature responding (Baarendse and Vanderschuren, 2012; van Gaalen et al., 2006).

Dopamine depletions in the medial PFC affected the accuracy when the cue delays are made unpredictable (Granon et al., 2000). Dopamine receptors (DRs) play an important modulatory role in the expression of impulsive behavior. SCH 23390, a DR1 antagonist, blocks impulsive responses caused by amphetamine or by chemical lesions using 5,7-DHT creatinine sulphate (van Gaalen et al., 2006; Harrison et al., 1997). These studies demonstrated that blocking DR1 receptors affected premature responding by preventing DA from binding to the receptor and eliciting a signaling cascade. Antagonists of D2/3s block the impulsive response caused by amphetamine when infused in the core of NAc (Pattij et al., 2007).

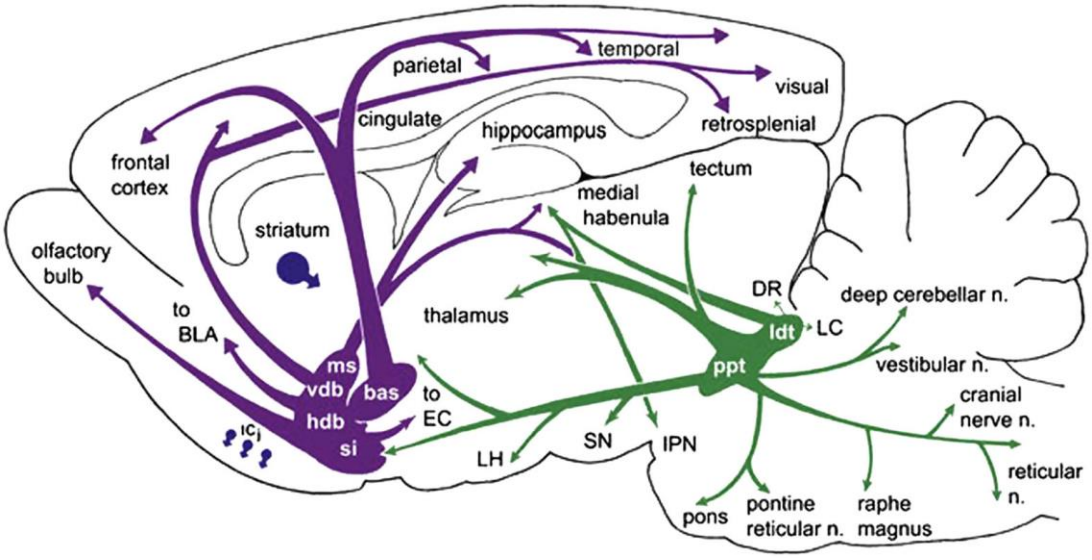
The literature indicates that there is an optimal amount of DA needed for appropriate behavioral responses, and this amount is affected by genetic polymorphisms and environmental factors. Polymorphisms in the DR2 receptor gene (*Drd2*) in humans caused individuals to perform less impulsively after being exposed to amphetamine (Hamidovic et al., 2009). Abnormal transmission at D2/D3 receptors also appear to be an underlying factor for impulsivity in a study by Lee et al. Lower D2/D3 receptor availability in the striatal area of methamphetamine-dependent subjects correlated with an increase in impulsive behavior (Lee et al., 2009). There are other neurotransmitters like serotonin and noradrenaline that play crucial roles in impulsive behavior, but often dopaminergic neurotransmission is concurrently involved in modulating this impulsivity (Pattij and Vanderschuren, 2008).

1.6 Figures



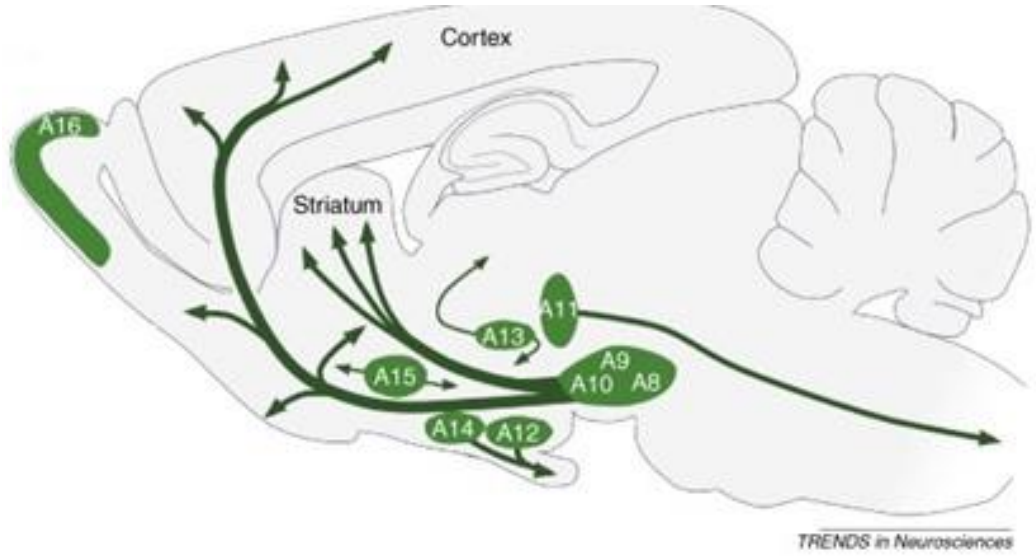
Bari et al. 2008

Figure 1.1. Schematic of the test chamber where the 5 nose-poke holes are on one wall and the food magazine is on the opposite wall (Bari et al., 2008).



Woolf et al. 2011

Figure 1.2. Cholinergic projections from the nucleus basalis of Maynert (bas) project to the prefrontal cortex (Woolf and Butcher, 2011).



Björklund et al. 2007

Figure 1.3. Sagittal section of an adult rat brain showing dopamine neurons localized in 9 cell groups. Dahlström and Fuxe introduced the numbering of the cell groups, from A8 to A16 in 1964 (Fuxe, 1964), which are still valid at present.

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Chapter 2 Circadian Rhythms

2.1 Circadian Rhythms

Circadian rhythms are 24-hour endogenous rhythms that span the daily light-dark cycle and govern both behavior and physiology (Reppert and Weaver, 2002; Vitaterna et al., 2001). The suprachiasmatic nucleus (SCN), the master clock, is in the anterior hypothalamus directly above the optic chiasm on either side of the third ventricle. The SCN has two anatomic subdivisions: a ventral “core” which is adjacent to the optic chiasm and receives retinal input and a dorsal “shell” that envelopes the core and receives dense projections from the core (Abrahamson and Moore, 2001; Leak et al., 1999). Neurons in the core and shell regions have different neurochemistry. The neuronal cells in the core contain vasoactive intestinal polypeptide (VIP), calretinin, neurotensin (NT), and gastrin releasing peptide (GRP), and the neurons in shell region contain arginine vasopressin (AVP), angiotensin II, and met-enkephalin (Welsh et al., 2010).

The neurons in the SCN generate endogenous rhythms that readily align to the light-dark (LD) cycles in the environment (entrainment), establishing that the neurons in the SCN can sense environmental timing cues (Bernard et al., 2007). These environmental cues are called zeitgebers (“timegiver,” ZT) (Aschoff, 1960; Golombek and Rosenstein, 2010). These rhythms entrain both external and internal zeitgebers. Examples of internal cues are hormones (Rawashdeh and Maronde, 2012) while external cues include light (Duffy and Wright, 2005), food (Stephan, 2002), social cues (Ehlers et al., 1988) and cognitively-demanding tasks (Gritton et al., 2013a). All animals under normal circadian conditions have their behavior and feeding entrained to their sleep-wake cycles. These rhythms govern rhythmicity in body temperature, blood pressure,

circulating hormones and metabolism within an organism (Aschoff, 1960; Buhr and Takahashi, 2013; Eckel-Mahan and Storm, 2009; Green et al., 2008).

In addition to the master clock in the brain there are clocks present in the peripheral organs which also demonstrate rhythms (Mohawk et al., 2012; Yoo et al., 2004). Like the SCN, these peripheral clocks also align to zeitgebers like light and food. Albrecht et al. developed a model for the relationship between the master clock and the peripheral clock called the '*orchestra model.'* In this model, the central clock coordinates with each of the peripheral clocks and the peripheral clocks can adapt to their own perceived external stimuli. The liver and kidney may entrain to feeding cues, but they are also aligned with the light-dark cues perceived by the SCN in the brain (Dibner et al., 2010). Recent studies have provided a better understanding of the role of peripheral clocks in circadian physiology (Mohawk et al., 2012; Richards and Gumz, 2012).

Food also acts as a potent zeitgeber for both master clock and peripheral clocks (Challet et al., 2009; Richards and Gumz, 2012). In nocturnal rodents, food restriction to one or two meals during the light phase results in increased locomotor activity in anticipation of the meal. (Mistlberger, 1994; Stephan, 2002). The rhythms in the peripheral clocks also align with the mealtime (Boulos and Terman, 1980). The increased locomotor activity persists even if the food is withheld for several days (Coleman et al., 1982). This activity displayed in anticipation of food is known as food-anticipatory activity, and food-entrainable oscillators located outside the SCN drive this activity (Verwey and Amir, 2009).

2.2 Components of the Circadian Clock

The molecular clock mechanism is a transcriptional feedback loop involving the following genes: *Clock* gene, three period genes (*Per1, Per2, Per3*), 2 cryptochrome genes (*Cry1, Cry2*), *Bmal1*, and *Rev-erba*, all of which are expressed in the SCN (Rosenwasser and Turek, 2015). *Clock* and *Bmal1* genes form the positive end of the transcriptional loop which heterodimerizes and binds to specific DNA elements, E-boxes (5'- CACGTG-3') and (5'-CACGTT-3') in the promoter regions of the target genes (Gekakis et al., 1998; Ohno et al., 2007). These targets include other clock genes including period (*Per1, Per2, Per3*) and cryptochrome (*Cry1, Cry2*), which form the negative limb of the feedback loop. PER and CRY proteins dimerize and translocate through the nucleus where they inhibit transcription of CLOCK/BMAL1 (Buhr and Takahashi, 2013; Gekakis et al., 1998). The negative limb of this loop, PER and CRY, must be degraded to terminate the repression and start a new cycle of transcription. The stability of these proteins is crucial for establishing the period of circadian rhythms (Buhr and Takahashi, 2013). Studies have shown that there is rhythmic histone acetylation that aids chromatin remodeling, giving rise to cyclic transcription (Etchegaray et al., 2003). Doi et al. established that CLOCK by itself has histone acetyltransferase (HAT) activity (Doi et al., 2006). CLOCK/BMAL1 complex enhances the HAT function that facilitates transcriptional activity whereas Per1 recruits histone deacetylase (HDAC) to the DNA that has the CLOCK/BMAL1 complex to initiate deacetylation (Duong et al., 2011). The rhythmic deacetylation of histone at the promoter region in this gene is further regulated by SIRT1 which is sensitive to the NAD+ levels (Asher et al., 2008; Nakahata et al., 2008). NAD+/NADH ratio has been shown to regulate the ability of CLOCK/BMAL1 to bind DNA in vitro (Rutter et al., 2001).

This ratio of NAD+/NADH indicates that cellular metabolism is crucial in regulating the transcription of these genes thus having a direct effect on the clock gene (Buhr and Takahashi, 2013). Additionally, CLOCK/BMAL1 also drive the transcription of the orphan nuclear receptors REV-ERB and ROR. REV-ERB and ROR factors, in turn, inhibit and activate the transcription of *Bmal1* respectively (Guillaumond et al., 2005; Preitner et al., 2002).

Mutations or deletions in any of these genes manifests as a modification in the circadian phenotype. Mice with *Bmal1* knockout show no rhythms in the absence of an external light stimulus (Allada et al., 2001; Ko and Takahashi, 2006). A mutation in *Clock* increases the free-running period and leads to a gradual loss of rhythms under long term free-running conditions (Allada et al., 2001; Ko and Takahashi, 2006). *Clock*-null mice, on the other hand, express normal circadian rhythms with a slight shortening of the circadian period (DeBruyne et al., 2006). DeBruyne et al. eventually provided evidence to show that NPAS2, a transcription factor, can substitute for CLOCK as a dimerization partner in the SCN, thus helping maintain the rhythms (DeBruyne et al., 2007). Disruption of *Per1* and *Per2* genes shortens the circadian period and reduces the free-running rhythms (Shearman et al., 1997; Zheng et al., 2001). *Cry* mutants also express alterations in free-running, but *Cry1/Cry2* double mutants are arrhythmic (Horst et al., 1999; Miyamoto and Sancar, 1999). Some clock gene mutations also have a significant effect on sleep-wake homeostasis, cognition and locomotor behavior indicating a potential molecular link between circadian and motivational systems in the brain (Naylor et al., 2000; Rosenwasser, 2010; Tafti and Franken, 2002).

2.3 Pathways and Neurotransmitters in Circadian Rhythms

Light input to SCN

Photic input from the retina is relayed to the SCN by the retinohypothalamic tract (RHT) (Ebling, 1996; Reghunandanan and Reghunandanan, 2006). The RHT is crucial for entrainment as Johnson et al. showed that in a light-dark cycle, the RHT-lesioned animals exhibited free-running rhythms (Johnson et al., 1988). The primary neurotransmitters involved in relaying photic cues from the retina to the SCN are glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP). Stimulation by light causes direct secretion of glutamate by the neurons from RHT that project to the SCN (Mikkelsen et al., 1995). Gannon et al. showed that there was high mRNA expression for the AMPA subunits GluR1, GluR2, and GluR4, and the NMDA receptor subtype NMDAR1 in the SCN and surrounding areas (Gannon and Rea, 1994). PACAP is co-localized in a sub-population of glutamate-containing retinal ganglion cells which are also involved in transmitting the photic cues. Thus, PACAP can potentiate the action of glutamate on the SCN. Studies have shown that at low doses PACAP can reset the clock in the photic pattern during the subjective night (Harrington et al., 1999; Minami et al., 2002).

Acetylcholine (ACh)

Cholinergic projections from the nucleus basalis magnocellularis (NBM) project both to the prefrontal cortex (PFC) (Robbins, 2002), which regulates attention, and to the SCN (Gritton et al., 2013b). A study by Bina et al. located the cholinergic neurons that project to the suprachiasmatic nucleus by retrograde and anterograde tract-tracing and immunohistochemistry for choline acetyltransferase in the rat (Bina et al., 1993). They

identified the absence of cholinergic neuronal bodies in the SCN, and that the cholinergic terminals in the SCN are afferents from the basal forebrain and mesopontine tegmentum, a region where cholinergic neuronal cell bodies are located (Bina et al., 1993). Earnest et al. established that carbachol, a choline carbamate that stimulates both muscarinic and nicotinic receptors, induced a phase-dependent shift in free-running activity resembling one caused by brief exposure to light. This study suggests a role for ACh in the mechanism by which light regulates circadian rhythms (Earnest and Turek, 1985). It appears that ACh only modulates the photic information that reaches the SCN instead of being involved in the light-input pathway (Reghunandanan and Reghunandanan, 2006). Van der Zee et al. showed that muscarinic and nicotinic receptors are co-localized in the SCN area. The endogenous neurotransmitter ACh is an agonist to both receptors that are co-localized and differentially contributes to the SCN time-keeping system (van der Zee et al., 1991). Evidence for this includes that mecamylamine, a general nicotinic antagonist, blocks light-induced phase shifts in circadian activity rhythm in the golden hamster (Keefe et al., 1987) and intravenous administration of nicotine has an excitatory effect on SCN cells (Brownstein et al., 1975). Also, Yamakawa et al. applied electrical stimulation in the basal forebrain to demonstrate that cholinergic input from the basal forebrain is both necessary and sufficient for eliciting this arousal-induced resetting of the circadian clock. These results establish the crucial connection between the forebrain arousal center and the circadian system (Yamakawa et al., 2016). Shibata et al. demonstrated that ACh excited 7% but inhibited 26% of SCN neurons in both immature and adult rats, thus establishing that ACh regulates circadian rhythms through a direct effect on SCN neuronal activity

(Shibata et al., 1983). Gritton et al. established that cholinergic projections in the basal forebrain provide signaling for cognitive entrainment in light-phase trained rats (Gritton et al., 2013a).

Dopamine

In addition to playing a pivotal role in optimal response inhibition (Harrison et al., 1997; Pattij and Vanderschuren, 2008), dopamine (DA) neurotransmission also plays a role in behaviors that have periodic rhythms such as locomotion and wakefulness (Mendoza and Challet, 2014). Dopaminergic signaling is known to have a rhythmic expression in the striatum and varies across the 24 h cycle (Hood et al., 2010). Mutations in clock genes can produce phenotypes that have deficits in normal dopaminergic signaling or a higher inclination to drug addiction or psychiatric phenotypes (Hampp et al., 2008; McClung et al., 2005; Roybal et al., 2007). *Rev-erbα* mutant mice had higher production of the protein tyrosine hydroxylase (i.e., TH, the rate-limiting enzyme for DA synthesis). The results from this study reveal that clock gene expression can modulate behavior through the rhythmic regulation of molecular components involved in DA neurotransmission (Jager et al., 2014).

The SCN projects to different regions of the brain to regulate the sleep-wake cycle, and it innervates the paraventricular thalamus, which in turn projects to the cortex, striatum and the midbrain (Abrahamson and Moore, 2001; Moga et al., 1995). Alternatively, SCN projections to the lateral habenula (LHb) in the mid-posterior thalamus and the orexinergic (ORX) system in the lateral hypothalamus could also indirectly modulate extracellular DA levels (Bourdy and Barrot, 2012; Moorman and

Aston-Jones, 2010). Studies have shown that DA links the maternal SCN to the fetus and D1 dopamine receptor (*Drd1*) plays a crucial role in this process (Reppert and Weaver, 2002; Viswanathan and Davis, 1997).

Extracellular DA levels and behaviors that can affect DA levels also have a feedback effect on the circadian activity of the master clock (Mendoza and Challet, 2014). *Per2* mutant mice are more sensitive to cocaine injections (Abarca et al., 2002) and *Clock* mutant mice have been shown to be hyperdopaminergic and have increased behavioral responses to cocaine and sucrose administration (McClung et al., 2005; Roybal et al., 2007). Also, the genes involved in dopaminergic neurotransmission are clock-controlled as they have E-boxes in their promoter region which are bound by CLOCK and BMAL1 (Hampp et al., 2008; Sleipness et al., 2007). Studies have shown that there are diurnal variations in VTA in the expression of DA receptors, tyrosine hydroxylase, and monoamine oxidase. Alterations to circadian machinery could potentially affect the dopaminergic neurotransmission, in turn affecting behavior and decreasing inhibitory control (Parekh et al., 2015; Sleipness et al., 2007; Sleipness Evan P. et al., 2008)

Additionally, psychostimulants like methamphetamine, which increase DA signaling, are incredibly disruptive to circadian rhythms (Honma et al., 1986) and affect daily rhythms of animals in constant dark conditions (Honma et al., 1989). Reward stimuli elicited in response to methamphetamine cause phase shifts in rhythms of behavior in hamsters (Cain et al., 2004). These psychostimulant drugs alter the expression of genes in the striatum and other brain regions implicated in DA neurotransmission (Lynch et al., 2008). In addition to the food-entrainable oscillators discussed earlier in the chapter, there is also a methamphetamine-sensitive circadian oscillator that restores

circadian rhythms in the absence of the master clock when methamphetamine is available (Honma and Honma, 2009). Clock gene expression has been shown to be altered in animals who are exposed to methamphetamine in drinking water; they showed desynchrony in locomotor rhythms and reversal in the phase of clock gene expression in the striatum but not the SCN (Masubuchi et al., 2000). Methamphetamine has also been shown to shift the rhythms of *Per2* in the striatum (Natsubori et al., 2013). Thus, drugs that potently alter DA signaling are also capable of exerting strong effects on circadian rhythmicity.

Dopamine receptors are expressed in the SCN throughout adulthood and play a crucial role in inducing phase shifts (Grippo et al., 2017). Grippo et al. identified a direct neuronal connection from DA neurons of the VTA to the SCN. Activation of these midbrain DA neurons accelerated the entrainment to light cycle shift. They also showed that D1 dopamine receptor (*Drd1*) knockout mice exhibit a slower rate of entrainment in response to a shift in light cycle. Their study establishes that Drd1-dependent DA signaling within the SCN governs the rate at which endogenous rhythms synchronize with environmental conditions (Grippo et al., 2017).

2.4 Circadian Disruption

Circadian rhythms are the 24-hour daily rhythms seen in all organisms that regulate both physiology and behavior and align to a variety of factors including light, food, and social cues (Arendt, 2010). When these innate rhythms are perturbed either due to internal or external factors, the disruption can give rise to numerous behavioral disorders and disease states. Misalignment of the rhythms can be caused by working beyond the hours of ‘9 to 5’ or by being exposed to light when there is supposed to be

none (Evans and Davidson, 2013). The literature suggests that there is a connection between altered states of circadian rhythms and impacts on cognition as seen in numerous disease states including attention deficit hyperactivity disorder (ADHD), schizophrenia, depressive and bipolar disorders, and Alzheimer's (Coogan et al., 2013; Landgraf et al., 2014). Additionally, disruptions in sleep and circadian patterns are seen in neurobehavioral disorders like ADHD and autism (Coogan et al., 2016; Singh and Zimmerman, 2015). Two models of circadian disruption that are used in this study are

1. Shift Work- Working beyond the regular hours of '9-5' is termed shift work. According to the Bureau of Labor nearly 15% of workers in the U.S work on shifts beyond the traditional daytime hours. There a disconnect between the endogenous rhythms and perceived external cues with shift work, which can cause detrimental effects on both behavior and physiology (Costa, 2010). This type of disruption has detrimental effects on the gastrointestinal system (Knutsson and Bøggild, 2010; Segawa et al., 1987; Zober et al., 1998) and increases risks of metabolic disorders like high blood pressure and cholesterol and obesity (Eckel et al., 2005). It also increases the risk of coronary diseases (Axelsson et al., 2006; Virkkunen et al., 2006). A large number of studies have shown detrimental effects of shift work on reproductive function (Axelsson et al., 2005; Nurminen, 1998). Shift work also has detrimental effects on cognition. A study done by Marquié et al. shows that the association between shift work and deficits in cognition increased with the duration of how long people performed shift work (Marquié et al., 2015). Selvi et al. also showed that shift workers had greater deficits in attention and increased impulsivity compared to daytime

workers (Selvi et al., 2015). This type of circadian disruption also increases risks to neurodegenerative diseases like Alzheimer's and Parkinson's (Abbott and Videnovic, 2016).

2. Light at Night (LAN)- Untimely exposure to light can impair the endogenous circadian rhythms necessary for maintaining optimal biological function, including cognition, endocrine and immune function (Cissé et al., 2017; Fonken and Nelson, 2014). Fonken et al. established that LAN disrupts the timing of food intake and other metabolic signals leading to weight gain in a mouse model (Fonken et al., 2010). LAN is also known to have detrimental effects on the immune system (Bedrosian et al., 2011). LeGates showed that a LAN model of disruption caused an increase in depression-like behavior and impaired hippocampal potentiation and learning (LeGates et al., 2014). Exposure to chronic low levels of light at night altered circadian clock genes in both the SCN and in peripheral tissues (Fonken et al., 2013; Shuboni and Yan, 2010). Wright et al. studied the exposure of electric light on the human circadian system and compared that to natural lighting (outdoor camping). They showed that people exposed to natural light were more accurately synchronized to solar time, and showed lower variability in melatonin and sleep rhythms (Wright et al., 2012). There is a need for addressing the effects of LAN on other aspects of cognition like attention and response inhibition.

2.5 Impact of Circadian Disruption on Cognition

Similar to the findings from human studies of circadian disruption, animal studies also have shown that disruption to circadian rhythms affects cognition mainly in terms of

memory formation (Gerstner and Yin, 2010) and sustained attention (Bruce, 1960). However, in our preliminary studies, we determined that circadian disruption was affecting impulsive behavior as well. Response inhibition is defined as the suppression of unwanted action that may interfere with goal-driven behavior (Mostofsky and Simmonds, 2008). Deficient response inhibition causes impulsive behavior, which is defined as the inability to prevent detrimental actions that would interfere with achieving goals. Impulsive responding includes actions that are inappropriately timed and can involve poor decision making, increased risk taking, and the inability to delay gratification (Dalley et al., 2011). Impulsive behavior is seen in association with various neurobehavioral disorders like ADHD (Winstanley et al., 2006) and can also be a result of exposure to xenobiotics and neurotoxicants (Eubig et al., 2010). These risk factors for impulsive behavior have received much focus and have been studied to identify mechanisms (Aguiar et al., 2010; Dingemans et al., 2011; Stewart et al., 2006). However, the effects of environmental factors such as circadian disruption on impulsive behavior have been largely unaddressed. By seeking to understand how the environmental factor of circadian disruption results in impulsive behavior, we can, in turn, better define the relationship between circadian rhythmicity and impulse control, and the roles of the endogenous neurotransmitters involved in both maintaining circadian rhythms and response inhibition. The cholinergic neurotransmitter system plays a crucial role in modulating circadian rhythms and attention, and can indirectly regulate impulsive behavior (Faure et al., 2014; Landgraf et al., 2014; Robbins, 2002). The prefrontal cortex (PFC) is important for optimal performance on the 5-choice serial reaction time task (5-CSRTT), which is regulated by dopaminergic and cholinergic

neurotransmitter systems (Bloem et al., 2014; Dalley et al., 2004). Dopamine neurons in the PFC, have an abundance of α4β2* nicotinic ACh receptors (nAChRs) on their synaptic terminals (Faure et al., 2014) and any increase in ACh or its agonists in this region can increase the basal levels of dopamine, thus modulating impulsive behavior. Since circadian rhythms are also regulated by the cholinergic neurotransmitter system (Hut and Van der Zee, 2011), any alterations in those rhythms can lead to changes in the basal levels of DA expression (Sleipness et al., 2007). Additionally, it has been shown that the circadian clock also has direct control over genes that are pertinent to DA synthesis and degradation (Huang et al., 2015). Based on all these factors it can be concluded that DA expression in PFC can potentially be influenced directly, or via interactions with cholinergic agonists, when the innate circadian rhythms are disrupted.

2.6 Gaps in Knowledge

Many people who are subject to circadian disruption are often staff in health and protective services where is no room for an erroneous decision (Alterman et al., 2013; Park and Kim, 2013). Previous studies have largely focused on attention, a cognitive aspect that is affected due to circadian disruption (Gritton et al., 2012, 2013b) but there are no studies that investigate the effects of these disruptions on impulsive behavior. Additionally, there are no studies that identify the effect of a light at night model of circadian disruption on behavior. By understanding how the disruption of circadian rhythms affects cognition, behavior and the underlying neurochemistry, we ultimately may be able to better understand which individuals would be more at risk for detrimental effects from circadian disruption, and possibly develop interventions which could

improve the quality of life in those affected by either circadian disruption or disorders of which circadian disruption is a component.

To address these gaps in knowledge, we designed experiments that aim at studying the effects of two different models of circadian disruption on attention and response inhibition and the changes associated with the underlying neurochemistry. We hope to learn more about the interactions between the underlying dopaminergic and cholinergic neurotransmitter systems that alter behavior under conditions of circadian disruption. Chapter III details these aims.

2.7 References

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Chapter 3 Specific Aims

To address the gaps in knowledge detailed in Chapter II, I developed two main hypotheses:

Specific Aim 1: Circadian disruption will give rise to deficits in attention and response inhibition and will also alter the underlying neurochemistry that governs these two aspects of behavior.

We tested rats on the 5-CSRTT, a task used to assess attention and impulsive behavior, under two different models of circadian disruption

1. Light at Night model (LAN): Rats were tested in the dark-phase with exposure to ambient light in the room, 4 h after the lights were switched off.
2. Shift work model: Rats were tested in their light-phase (rest phase) 4 h after the lights were switched on.

In the control condition the rats were tested in their dark-phase testing with no ambient light exposure. Once asymptotic performance was reached, the brains from the rats in all 3 light conditions were harvested at 4 different time points to study the mRNA transcripts of cholinergic and dopaminergic proteins in brain regions relevant to attention and impulsive behavior on the 5-CSRTT. I isolated tissue from the infralimbic cortex (IL) and dorsomedial striatum (DmSTR) as these regions are important for impulsive behavior and attention. The genes examined in these regions included choline acetyltransferase (ChAT) *Chat*, β2 subunit of nicotinic acetylcholine receptors (β2 nAChR) *Chrnb2*, dopamine transporter (DAT) *Slc6a3*, norepinephrine transporter (NET) *Slc6a2*, tyrosine hydroxylase *Th*, dopamine 1 receptor (DR1) *Drd1*, and dopamine 2 receptor (DR2) *drd2*, which code for cholinergic and dopaminergic synaptic

proteins. Additionally, I also examined the expression of Period 2 *Per2* in DmSTR as the literature supports a bidirectional relationship between expression of *Per2* and dopaminergic proteins. I wanted to test if there was an effect of the two models of circadian disruption used in this study on the expression of *Per2*. For my dissertation project, I analyzed the expression of these genes at two-time points of ZT 4 (4 h after lights on) and ZT 16 (4 h after lights off).

I saw significant deficits with respect to attention and response inhibition in both models of circadian disruption compared to the control group. *Per2* expression in DmSTR in the light phase group was altered compared to the expression in LAN and control group, which showed a rhythmic expression. *Chat* and *Drd1* expression in DmSTR showed a rhythmic expression. I did not see any change in expression in any of the genes in IL. Taken together these results provide evidence that distinct types of circadian disruption affect attention and impulsive behavior and the underlying neurochemical systems.

Specific Aim 2: Pharmacological challenges used to understand the interaction between cholinergic and dopaminergic neurotransmitters will have differential responses in both models of circadian disruption.

To examine this potential interaction, we used cholinergic and dopaminergic agonists and antagonists, alone and in combination with each other, to study their effects on impulsive behavior on 5-CSRTT under the two conditions of circadian disruption. I predicted that in both cases of circadian disruption there would be increased impulsivity compared to the control animals. In an initial study, I optimized doses of nicotine and dopaminergic antagonists to be used in our models of circadian disruption. All the

testing in this initial part of the project was conducted under the light at night condition where we had seen an effect on impulsive behavior in our preliminary studies. Important doses were identified for the cholinergic agonist nicotine (NIC), DR1 specific antagonist SCH 23390 (SCH), and DR2 specific antagonist eticlopride (ETI) on attention and impulsive behavior. I also studied the effect of these antagonists in combination with NIC and AMPH to understand the effects on attention and impulsive behavior. I found that NIC and AMPH increased impulsive behavior while the dopamine receptor antagonists decreased impulsive behavior. Both had minimal effects on attention. In combination the antagonists reduced the effects of NIC and AMPH, which established an interaction between cholinergic and dopaminergic neurotransmitters in our model.

Then, I examined the effects of NIC, SCH and ETI on attention and impulsive behavior under conditions of circadian disruption previously established. I administered the drugs by themselves and the combined NIC with SCH and NIC with ETI. Light condition modulated drug response. Our most prominent finding was that nicotine increased impulsive behavior and the combination with antagonists decreased impulsive behavior in both models, but these drugs had minimal effect in the circadian control group. The pharmacological challenges in this aim established how each of these neurotransmitter systems, individually and in combination, affected impulsive behavior in an incremental or decremental manner, and were affected by circadian disruption.

Chapter 4 Effect of Two Models of Circadian Disruption on Attention and Response Inhibition and mRNA Expression of Cholinergic and Dopaminergic Proteins that Modulate Response Inhibition

4.1 Abstract

Circadian rhythms are endogenous rhythms that govern behavior and physiology. Like many toxicants, circadian disruption is an environmental factor that impacts cognition and increases the risk of neurodegenerative disease and other disorders by altering the circadian clock at a molecular level. Common sources of circadian disruption in human populations include working beyond the regular hours of '9 to 5' (shift work) and untimely exposure to light (light-at-night, LAN). Previous studies have identified that circadian disruption affects attention and memory. Our study investigated the effect of 2 models of circadian disruption on response inhibition, which has previously been unaddressed, and attention using a 5-choice serial reaction time task (5-CSRTT). Adult Long-Evans rats of both sexes were maintained on a 12h:12h light: dark cycle and tested under 3 conditions: 4 h into the dark phase with no exposure to ambient light at the time of testing (control), 4 h into the dark phase with exposure to ambient light during testing (a model of LAN), and 4 h into the light phase (a model of shift work). Our hypothesis that rats tested under the two models of circadian disruption would have reduced response inhibition and be less attentive than controls was confirmed. We also established that changes in expression occur in *Per2* in the light phase-tested model of circadian disruption. Choline acetyltransferase (*Chat*) and Dopamine receptor1 (*Drd1*) showed rhythmic expression with peak expression during dark phase. Our study is the first of its kind to identify the detrimental effects of both light at night and shiftwork models of circadian disruption on impulsive behavior. These results could potentially hold the key to better understanding altered cognitive functioning in real-world scenarios caused by conventional sources of circadian disruption.

4.2 Introduction

Industrialization has led to the development of modern technology that has been beneficial to prosperity and health of the general population. This progress is accompanied by novel, unintended effects on human and wildlife behavior and physiology. The daily light-dark cycles produced by the rotation of the earth span approximately 24 hours and have major influence over both behavior and physiology of all organisms. Circadian rhythms are 24 hour-long endogenous rhythms that modulate behavior and physiology. These rhythms are governed by the suprachiasmatic nucleus (SCN), a master clock located in the hypothalamus, Environmental factors like light have a direct effect on SCN which in turn affects the modulation of behavior and physiology (Silver and Kriegsfeld, 2014). Untimely exposure to light, termed circadian disruption, can cause a conflict with intrinsically entrained rhythms and has detrimental effects on both behavior and physiology (Arble et al., 2010; Karatsoreos, 2012; Potter et al., 2016). There are two types of circadian disruption discussed in this chapter:

1. Light phase group which mimics shift work, where work-time schedules fall beyond the regular working hours of ‘9 am – 5 pm’ and external cues conflict with the internal rhythms (Wright et al., 2013) and
2. Light at night (LAN) which refers to untimely exposure to light at night (Dominoni et al., 2016; Fonken and Nelson, 2014)

Under such circumstances, the sleep-wake cycle is disrupted and time-inappropriate cues such as exposure to light at night and eating during the normal sleep phase give further conflicting signals to the SCN. This misalignment of rhythms affects both behavior and physiology (Bedrosian et al., 2016; Golombek and Rosenstein, 2010; Gritton et al., 2009a, 2012, 2013; Russart and Nelson, 2017). This chapter focuses on

the neurobehavioral deficits that are caused by these two models of disruption. This desynchrony caused by circadian disruption has effects on aspects of cognition like attention, working memory, and cognitive flexibility (Frank and Ovens, 2002). Some studies have shown that attention is affected by disrupting these innate circadian rhythms (Gritton et al., 2009a) but very little has been done to establish the effects of circadian disruption on response inhibition. As stated earlier circadian disruption can be caused by factors such as working beyond the regular hours or by being exposed to light inappropriately (Evans and Davidson, 2013), and circadian disruption affects cognitive functioning (Gritton et al., 2012, 2013) making this an issue with extensive societal effects. The primary motivation for this study is to better understand how circadian disruption affects impulsive behavior and its underlying neurochemistry. People who are engaged in around-the-clock work include staff in protective and health services who have no room for erroneous decisions (Alterman et al., 2013; Park and Kim, 2013). By better understanding how circadian disruption affects cognition, behavior and the underlying neurochemistry, we ultimately will be able to better identify those at increased risk and develop interventions. Effective interventions could drastically improve the quality of life for those affected by circadian disruption, and for those who suffer from neurological disorders involving circadian disruption.

Attention is a multidimensional construct, which is broadly defined as the prioritized processing of one piece of information over others (Stefanatos and Baron, 2007). Response inhibition is the suppression of unwanted actions that could interfere with goal-driven behavior (Mostofsky and Simmonds, 2008). Deficiency in response inhibition gives rise to impulsive behavior, the inability to prevent detrimental actions

that interfere with achieving goals. This kind of behavior includes inappropriately-timed actions, poor decision making, increased risk-taking and the inability to delay gratification (Dalley et al., 2011). Furthermore, lack of attention and impulsive behavior are often seen in conjunction with neurological disorders such as attention deficit hyperactivity disorder (ADHD), autism, schizophrenia, depressive and bipolar disorders, and Alzheimer's disease (Coogan et al., 2013; Landgraf et al., 2014). In addition to attentional deficits and impulsive behavior, disorders like ADHD and autism are also associated with disrupted sleep and circadian patterns (Coogan et al., 2016; Singh and Zimmerman, 2015), and altered states of circadian rhythms are correlated with cognitive deficits in several neurological disorders. The altered behavior and disrupted circadian rhythms seen in these neurobehavioral disorders indicate a potential interaction between the two.

The SCN regulates the circadian rhythms via acetylcholine (ACh) signaling (Wright et al., 2012). Studies have shown that there can be a bidirectional relationship between attention and circadian rhythms, both modulated by ACh (Gritton et al., 2012; Landgraf et al., 2014). Preliminary studies in our lab showed that animals subjected to untimely exposure to light (LAN) were more impulsive. Impulsive behavior is regulated by dopamine (DA) via binding to the dopamine-1 or -2 receptors (DR1s or DR2s) (Dalley and Roiser, 2012). In several brain regions including the prefrontal cortex (PFC), a brain region critical for both attention and impulsivity, and nucleus accumbens and striatum, cholinergic functioning interacts with dopaminergic signaling. In these regions, ACh release stimulates DA neurons to release DA via binding of nicotinic subtypes of ACh

receptors (nAChRs) by ACh (van Gaalen et al., 2006; Livingstone and Wonnacott, 2009).

To better understand the effects of circadian disruption on behavior and cognition, we used rodent models of two different types of circadian disruption. The rats were tested on the 5-choice serial reaction time task (5-CSRTT) which measures attention and increased impulsive behavior. We hypothesized that both models of circadian disruption would show poor attention and impulsive behavior compared to the control group. We also postulated that circadian disruption would affect the underlying neurochemistry which would manifest as altered behavioral responses. To address the changes in the underlying neurochemistry, we investigated the expression of both cholinergic and dopaminergic endpoints using qPCR in brain regions relevant to attention and impulsive behavior. We analyzed the expression of relevant genes in dorsomedial striatum and infralimbic cortex as these two brain regions play a crucial role in modulating attention and impulsive behavior (Christakou, 2004; Tsutsui-Kimura et al., 2016)

The control group of rats were tested in their dark-phase, the active time for nocturnal rats, with no exposure to ambient light. The LAN group was also tested during dark-phase, but with exposure to ambient light in the testing room and chambers, which served as the untimely exposure to light. Lastly, we tested rats in their light phase, the rest phase for nocturnal animals, which modeled shift work. The data showed deficits in attention and impulsive behavior in both models, light phase and LAN group of rats, compared to control group. We also observed circadian disruption-associated changes in expression of *Per2* in the dorsomedial striatum, and effects of zeitgeber time on *Chat*

and *Drd1*. We conclude from the data presented here that both models of circadian disruption influence behavior, and that the effect on light-phase tested rats is subtler than that on dark-phase tested rats.

4.3 Materials and Methods

Subjects

Three cohorts of 40 Long-Evans rats, 20 of each sex (120 total rats), approximately 70 days of age, were purchased from Envigo (Indianapolis, IN). Rats were single-housed in polycarbonate shoebox cages with wood-chip bedding (Beta Chip, Northeastern Products Corp., Warrensburg, NY) in a temperature- and humidity-controlled room (targeted 22^{o} C, 40-55% humidity). 2020X Teklad Rodent Diet (Envigo) was fed to the rats. Food restriction was initiated after a one-week acclimation period to reduce rats' body weights over 2 weeks to target weights of 85% of their free-feed body weights. After that, target weights were incrementally increased by 5-10 g every 2 weeks, with a maximum of 250 ± 10 g for female rats and 350 ± 10 g for male rats, to allow for growth. Food restriction was intended to maintain motivation for performing operant-based behavioral tasks. Tap water was provided *ad libitum*. TestDiet sucrose pellets (AIN-76A 5TUL, 45 mg pellets, St. Louis, MO) were used for food-based reinforcement during behavior testing. At the time the food restriction began, the rats were randomly assigned to a treatment group and were housed in chambers where the light cycle was regulated. Figure 4.1 shows the schematic representation of the experimental plan.

The two conditions of circadian disruption include LAN and light phase (shift work) groups. The rats in LAN were tested 4 h after the lights were switched off (zeitgeber time 16, ZT 16), during their active period. They were subjected to ambient light during

testing and transportation to the testing room. The light phase group of rats were tested 4 h after the lights were switched on during their rest phase (ZT 4). The control group of rats was tested 4 h after the lights were switched off during their active phase (ZT 16) with no exposure to ambient light. Handling of the control group of rats during the daily testing period occurred under red lights exclusively. In the testing chambers, control rats were exposed to a red LED stimulus cue light as well as low-intensity, yellow LEDs from 5 nose-poke holes.

During the daily testing sessions for both circadian disruption groups, the overhead fluorescent, white lights were on in the testing room. Rats were exposed to the overhead lights in the testing room. While in the testing chambers, rats were exposed to the house light (2.8 watt bulb) in each chamber, as well as a 2.8 watt stimulus cue light above the pellet trough during behavior tasks. All rats performing the 5-CSRTT were also exposed to low-intensity, yellow LEDs from the 5 nose-poke holes. The light intensity in the cages ranged from 220 to 360 lux (average 290 lux) for the circadian disruption groups. Rats were behavior tested at the appropriate ZT 4 or 16. Timers that controlled the lights for the home cages were staggered to allow light-dark transitions for 12 rats at a time, counterbalanced across sexes and treatment groups, to permit adequate time for daily testing of all rats. This schedule was maintained across cohorts.

Apparatus and 5-Choice Serial Reaction Time Task (5-CSRTT)

Behavioral testing began 3 weeks after the rats arrived when rats were approximately 90 days of age. Training and testing sessions, 1 per day, were performed 6 days each week in 12 5-choice operant behavior-conditioning chambers housed in sound-insulated

and ventilated cubicles (Med Associates Inc., St. Albans, VT). Each chamber consisted of 5 evenly-spaced nose-poke cue holes (2.5 x 2.5 x 2.5 cm and 2 cm above the floor) on one wall. Each aperture had a yellow LED light centered in the back and an infrared photocell to detect head entries. The opposite wall had a pellet trough with a head-entry detector in the center panel, a stimulus cue light directly above it, and then a house light mounted 6 cm above the cue light. Experimental contingencies were programmed using MedState Notation programming language, and data acquisition was performed using MED-PC IV software (version 4.38, Med Associates). Behavioral-testing programs were modified from those used by Beaudin et al. (Beaudin et al., 2017).

During the seven initial training phases of the 5-CSRTT, rats learned to associate nose pokes in the pellet trough and the 5 nose-poke holes with food reinforcement. The house light remained on during all these phases. The criterion to advance from one phase to the next was 99 or 100 successful nose pokes. Each session during the 7 initial phases lasted until 100 pellets were earned or 60 min elapsed. Rats took an average of 7.5 days, ranging from 7 to 12 days, to complete initial training. The next phase of training was Visual Discrimination 1, during which cue lights were introduced. Cue lights were illuminated for 15 s during each trial. Nose pokes in the illuminated cue hole resulted in reinforcer delivery. Poking in any other cue hole, or not poking during the 15 s period, resulted in a time-out (see next section). Rats were tested on this phase until criteria of making 75% or more correct responses for 2 out of 3 consecutive days were met within 15 sessions. All rats in this study met the criteria, taking an average of 3.8 days, ranging from 3 to 7 days, to progress to next phase. In the next phase, Visual Discrimination 2, the duration of cue light illumination was shortened to 1 s, requiring

rats to be more attentive to optimize performance. Rats still had a 15 s limited-hold period to nose poke. Rats were tested on this phase for 5 days.

Sustained Attention Task

Following the training sessions, rats were tested on the Sustained Attention phase for 21 days. During daily sessions in each phase, the yellow LED cue lights in the 5 nose-poke holes would randomly illuminate in one hole per trial, in a counterbalanced manner, so that each hole was illuminated during an equal number of trials per session. The house light was illuminated except during time-out periods. Each trial began with a nose-poke in the pellet trough. If it was the first trial, or if the previous trial resulted in sucrose pellet delivery, the rat was given 3 s to consume the pellet (reinforcer duration). After the 3 s reinforcer duration, the rat was given a 3 s turn-around time to allow the rat time to orient toward the wall with the 5 nose-poke holes. If the previous trial had resulted in a time-out, there was no reinforcer duration after a nose poke in the trough. In this phase of the test, there were variable delays of 3, 5 or 7 s until a cue light would illuminate in a nose-poke hole. These delays consisted of the 3 s turn-around time plus an additional delay of 0, 2 or 4 s. The cue light in the nose-poke holes would illuminate for a maximum of 1 s and, concurrently, a 15 s limited-hold period would commence during which a nose poke could be registered.

One of 4 trial outcomes could result. A **correct** trial was when the rat poked in the hole in which the cue light was illuminated during the 15 s limited-hold period. An **incorrect** trial was when the rat poked in one of the other 4 holes. A **premature** trial was when the rat poked during the delay before the cue light illuminated. Premature

nose-pokes were not recorded during the 3 s turn-around time, so premature trials only occurred when the cue delay was 5 or 7 s. An **omission** occurred when the rat did not poke during the limited-hold period. Correct trials resulted in a food pellet being dispensed, the cue light over the feeder illuminating, and the beginning of a new trial when the rat retrieved it. If the rat continued to nose poke in any of the 5 cue holes after making a correct response, those nose pokes were recorded as a **perseverative** response but had no consequence. Perseverative responses were recorded only when there was a correct trial. Incorrect, premature, and omission responses triggered an immediate 5 s time-out, during which all cue lights and the house light extinguished. Poking in any of the 5 nose-poke holes during a time-out reset the time-out timer. When 5 s elapsed without any nose pokes, the cue light above the pellet trough illuminated until the rat poked in the trough, thus beginning the next trial. Each daily session lasted until 150 trials or 60 min elapsed. For the control group of rats, the house light was turned off, and the cue light over the feeder was replaced with a red LED light.

Tissue Collection

After the completion of the Sustained Attention task, the rats within each group were randomly assigned to a ZT, zeitgebers (“timegiver”). The four time points included ZT 4, ZT 8, ZT 16, and ZT 20 to collect brains to determine the effects of circadian disruption on the rhythmicity of various genes (N=4 of each sex per time point). Samples from ZT 4 and ZT 16 only were analyzed for this chapter. Females were collected during the diestrus phase of their estrous cyclicity and for each female, a male was collected at the same ZT. The collection was done under red lights if they were being collected during

their dark phase, depending on the circadian disruption group to which they belonged. Rats were euthanized using carbon dioxide with a flow rate of 2.4-7.2 LPM. The brain was extracted, quickly frozen by immersion in liquid nitrogen, and stored at -80^{o} C. Brains were sliced using a microtome and kept at -20^{o} C until each region of interest was in the cutting plane. Coronal sections of the brains were then made using the microtome. Slices were made at bregma 3.20 mm for taking punches from the infralimbic cortex (IL) and bregma 1.70 mm for taking punches from dorsomedial striatum (Paxinos and Watson, 1998). Bilateral punches were taken from the infralimbic cortex (1.5 mm diameter) and the dorsomedial striatum (2 mm diameter) and stored in RNAlater (ThermoFisher) at -20 ^{o} C (See Figure 4.2).

RNA extraction and Quantitative RT-PCR

The tissue was removed from RNAlater, homogenized, and total RNA was isolated using RNeasy mini kits (Qiagen) following the manufacturer's protocol. RNA was eluted into 50 µl elution solutions. RNA concentrations and purity were determined using a NanoQuant spectrophotometer (Thermo Scientific, Waltham, MA USA), measuring absorbance at 260 nm and the ratio 260 nm/280 nm. RNA samples were used if the 260/280 ratio was greater than 1.9. ABI High Capacity cDNA Reverse Transcription kit was used (Applied Biosystems) to convert 10 ng RNA to cDNA. 100 ng cDNA was further used to quantify the differential gene expression using the Power SYBR Green PCR Master Mix (ThermoFisher) to analyze the transcript levels of the genes in Table 1 on a 96-well plate with a total volume 10 µl on Quantstudio 3 Real-Time PCR system. *Gapdh* was used as the housekeeping gene. *Per2* was analyzed in the dorsomedial

striatum as previous literature had established that DA regulates the expression of clock proteins in the dorsal striatum in rodents (Hood et al., 2010). Norepinephrine transporter (NET) plays a crucial role in regulating extracellular DA in the medial prefrontal cortex so expression of NET was analyzed only in IL (Yamamoto and Novotney, 1998). While NET clears DA in IL, dopamine transporter (DAT) performs this function in the striatum (Hoffman et al., 1998). The genes and primers are listed in Table 4.1.

Data Analysis

R program for statistical computing and graphics (R Core Team, 2016) was used to calculate **percent correct** (number of correct responses/total trials *100), **percent incorrect** (number of incorrect responses/total trials *100), **percent accuracy** (percent correct/(percent correct + percent incorrect)), **percent premature** (number of premature responses/total trials *100), **percent omissions** (number of omitted responses/total trials *100), and **average perseverative responses** (sum of perseverative responses in all 5 nose-poke holes/number of correct responses). **Average latency to correct responses** (sum of latencies to all correct responses/number of correct responses), **average latency to incorrect responses** (sum of latencies to all incorrect responses/number of correct responses), and **average latency to collect reinforcers** (sum of latencies to collect reinforcers/number of correct responses) were also calculated. Percent accuracy indicates the ability of the subject to sustain attention. Percent premature responses indicate deficits in response inhibition. Percent omissions is a measure of inattentiveness, a lack of motivation, or both (Robbins, 2002b). Data are reported as mean ± SEM.

All statistical analyses were conducted using SPSS for Windows (version 24, SPSS Inc., Chicago, IL). Mixed model ANOVAs were used to analyze all data, with statistical significance set at $p \leq 0.05$. The data from the 21 days of Sustained Attention phase were evaluated to characterize learning of the 5-CSRTT. Experimental factors of block (7 3-day blocks) and cue delay (3 cue delays, except for 2 for percent premature) were within-subjects factors, and treatment (3 light conditions) and sex were between-subjects factors. Post-hoc testing (Bonferroni) was performed when appropriate using SPSS.

The data from quantitative RT-PCR were tested for normality. Multivariate ANOVA was used to analyze normal data where light condition, ZT, and sex were fixed factors and the gene expression was the dependent variable. Tukey test was used to perform the post-hoc analysis when appropriate. A non-parametric test, Kruskal-Wallis was used if the data were not normal. Differences with $p \leq 0.05$ were considered statistically significant.

4.4 Results

All the data in detail here are included for the two primary measures of interest, accuracy and premature responding. Numerical data on all measures including average perseverative responses and latencies are indicated in Table 4.2.

Experiment 1- Behavior Testing with Three Light Conditions

Across the 7 blocks of testing, accuracy increased and premature responding decreased until asymptotic performance was reached by block 6 where performance did not differ significantly from block 7, for all 3 treatment groups. Figure 4.3 shows %

accuracy and Figure 4.4 shows data for % premature across the 7 blocks the rats were tested on sustained attention. Across the 7 blocks control group ($F_{2, 120} = 59.1$, $p < 0.001$) had significantly higher accuracy compared to both LAN and light phase groups ($p< 0.001$ for both). Cue delay had an effect ($F_{2, 2400} = 73.1$, $p < 0.001$) where the rats showed better accuracy at the shorter cue delays of 3 s and 5 s ($p < 0.001$). The cue delay by block interaction was significant ($F_{12, 2400} = 2.4$, $p = 0.004$) where the rats at the longer cue delays of 5 s ($p = 0.036$) and 7 s ($p < 0.001$) had lower % accuracy. Between the two longer cue delays the rats at the 7 s cue delay ($p = 0.014$) were less accurate than the rats at the 5 s delay. Similar effects were observed at block 2 where the rats performed poorly at the longest cue delay compared to the two shorter cue delays of 3 s ($p < 0.001$) and 5 s ($p = 0.010$). At block 7, rats performed poorly at 7 s cue delay compared to the longest cue delay 5 s ($p = 0.002$).

Premature responding was affected by light condition ($F_{2, 120} = 22.1$, $p < 0.001$) where across the 7 blocks the two circadian disruption conditions also showed higher premature responding ($p < 0.001$). The light condition by cue delay interaction was significant ($F_{2, 1560} = 108.9$, $p < 0.001$). During both 5 s and 7 s cue delays, the control group of rats made fewer premature responses compared to the two conditions of disruption (5 s $p_{LAN} = 0.012$ and $p_{lightphase} = 0.017$ versus controls; 7 s $p < 0.001$ for both versus controls). The effect of light condition ($F_{20, 808} = 33.8$, $p < 0.001$) altered the premature responding from block 2 onward.

Because the rats reached asymptotic performance by block 6 for both % accuracy and % premature, further statistical analysis focused on block 7 alone. Figure 4.5 shows the main effect of light condition ($F_{2, 114} = 77.1$, $p < 0.001$) where the control group had

significantly higher % accuracy compared to both disruption groups of LAN and light phase testing ($p < 0.001$ for both). The main factor of cue delay also was significant ($F_{2, 228} = 20.3$, $p < 0.001$) with % accuracy at 7s decreased compared to 5 s ($p < 0.001$) and 3 s ($p < 0.001$). There were no other significant measures or interactions for % accuracy.

For % premature, main effects of light condition ($F_{2, 114} = 32.2$, $p < 0.001$) and cue delay ($F_{1, 114} = 471.1$, $p < 0.001$) were both significant as was the interaction between the two ($F_{2, 114} = 42.5$, $p < 0.001$). These effects on premature responding are shown in Figure 4.6. Post hoc analysis revealed that at 5 s cue delay both light phase ($p =0.002$) and LAN ($p = 0.003$) made more premature responses than control. Comparable results were observed at 7 s cue delay ($p < 0.001$ for both versus control). The rats in each light condition group made more premature response at 7 s compared to 5 s ($p < 0.001$). There was an effect of sex on premature responding in block 7 ($F_{1, 114} = 4.0$, $p = 0.046$) where males made more premature responses than females in all 3 light condition groups. This data is shown in Figure 4.7.

Rats made more % omissions at 3 s ($F_{2, 228} = 13.9$, $p < 0.001$) compared to both the longer cue delays of 5 s ($p < 0.001$) and 7s ($p < 0.001$) but the main factor of light condition was not significant. Females made more omissions compared to males ($F_{1, 114} = 7.5$, $p < 0.007$) (not shown). Rats in both LAN and light phase took longer to make a correct response to compared control group ($F_{2, 114} = 15.1$, $p < 0.001$; post hoc for both compared to control $p < 0.001$). Rats also took longer to make a correct response at the shortest cue delay of 3 s ($F_{2, 228} = 14.7$, $p < 0.001$) compared to 5 s ($p < 0.001$) and 7s ($p < 0.001$), but the light condition by cue delay interaction was not significant.

Rats were slower to make an incorrect response at the lowest cue delay of 3 s ($F_{2, 228} = 14.7$, $p < 0.001$) compared to 5 s ($p < 0.001$) and 7 s ($p < 0.001$). There were no other effects observed in this measure. There were no effects of light condition or cue delay seen in average perseverative responding or reinforcement latency. The dependent measures for all cue delays and light conditions are shown in Table 4.2

Experiment 2- Effect of Circadian Disruption on Cholinergic and Dopaminergic Gene Expression in Dorsomedial Striatum and Infralimbic Cortex

We analyzed the effects of light condition, time (ZT) and sex on the genes listed in Table 4.1 on infralimbic cortex (IL) and dorsomedial striatum (DmSTR). There was no effect of light condition, time (ZT) or sex on any gene in the IL. *Per2* in the DmSTR showed a differential expression at ZT 4 and 16 ($F_{1, 40} = 22.8$, $p < 0.001$) shown in Figure 4.8. There was a light condition and ZT interaction ($F_{2, 40} = 4.7$, $p = 0.038$) with a higher expression at ZT 16 in both control ($p = 0.002$) and light at night ($p = 0.004$) groups compared to ZT 4. There was no significant difference between ZT 4 and ZT 16 for the light phase rats. The expression of *Per2* at ZT 16 in light phase trended towards showing a difference in expression compared to ZT 16 in control rats ($p = 0.055$).

The expression of *Chat* in DmSTR was significantly affected by ZT ($F_{1, 43} = 4.3$, $p = 0.043$). Figure 4.9 shows that expression of *Chat* was greater at ZT 16 compared to ZT 4. No other factors were significant for *Chat.* Expression of dopamine 1 receptor (*Drd1*) was also affected by ZT only ($F_{1, 56} = 5.1$, $p = 0.029$). Figure 4.10 shows that expression of *Drd1* was higher at ZT 16 compared to ZT 4.

4.5 Discussion

The purpose of this study was to examine the effects of two models of circadian disruption on attention and impulsive action using % accuracy and % premature in the 5-CSRTT as proxy measures (Robbins, 2002a). By analyzing gene expression, we aimed to identify the changes in the underlying neurochemistry brought about by both the models of circadian disruption. The overall results of the study indicate that both light at night (LAN) and shift work (light phase) models of circadian disruption were detrimental to cognition.

In our first experiment, we showed that circadian disruption caused significant deficits in attention and response inhibition. Rats in all three treatment groups learned the task and demonstrated better attention and response inhibition as the task progressed, but throughout the 21 days of sustained attention phase there remained significant deficits in both behaviors for the treatment groups. The deficits on attention using other cognitively-demanding tasks have been previously shown by Gritton et al. (Gritton et al., 2009b, 2012, 2013), but this is the first time the 5-CSRTT has been used to establish deficits in attention caused by models of light at night and shiftwork. Cordova et al. used this task to study the attentional impairments caused by sleep deprivation, another model of circadian disruption (Córdova et al., 2006). Another unique aspect of this study is that we established effects of circadian disruption on response inhibition for the first time. This study also identified changes in the expression of *Per2*, *Chat*, and *Drd1* in the dorsomedial striatum.

Experiment 1 established that rats in both groups of circadian disruption, LAN and light phase, showed significant deficits in impulsive behavior, where all the rats started at approximately the same level of impulsive behavior, but as the task progressed the %

premature responses, a measure for impulsive action, made by the control group decreased to a greater extent compared to the corresponding decrease across blocks in the two circadian disruption groups. This effect of circadian disruption on impulsive behavior is unique and has been identified in this study for the first time.

Experiment 2 established that circadian disruption can cause differential alteration in gene expression at a molecular level. The lack of changes in expression of *Per2* in the light phase group as compared to changes between ZT 4 and ZT 16 in the other two groups could potentially be one reason behind the observed behavioral deficits. Our results from this experiment demonstrated a regional difference in the expression of the cholinergic and dopaminergic genes.

A study done by Gritton et al. showed that rats learned a task requiring attentional effort faster when trained during their dark phase (active phase) compared to rats trained in their light phase (rest phase) (Gritton et al., 2012). The dark phase testing conditions in the Gritton study were identical to ours, but the difference in light-phase dependent learning of the task was absent in our study between the rats tested at in the dark phase (LAN and control) and light phase (shift work). There was no difference in the time taken to reach criterion (Visual Discrimination 1 phase) between the 3 light condition groups. The differences in attention and response inhibition that manifested between the control and the two treatment groups could be because of effects circadian disruption exerted on the underlying neural networks that modulate these aspects of cognition. The 5-CSRTT requires focused attentional effort throughout the period of testing, resulting in increased synaptic ACh levels (Arnold et al., 2002; Kozak et al., 2006; Peters et al., 2011). Based on the studies by Arnold et al., sustained attention

tasks increased the cortical ACh efflux by ~140% (Arnold et al., 2002). Kawamura et al. demonstrated that there is evidence that cortical ACh peaks during the dark phase, the active phase for the rats. This study by Kawamura established that there are innate rhythms for ACh release, and any disruption to these innate rhythms could have a direct ramification on attention (Jiménez-Capdeville and Dykes, 1993; Kametani and Kawamura, 1991). The light phase group, which mimics effects of shift work, show deficits in attention, potentially due to disruption in these cholinergic rhythms. Murakami et al. showed that there was spike in the levels of ACh 30-60 minutes after exposure to light (Murakami et al., 1984). It is possible that there is a similar spike in the levels of ACh above that already present during the dark phase in our model of LAN, where the rats are exposed to the ambient light during their dark phase for cognitive testing. This supra-normal spike in levels of ACh may have caused deficits in attention, as any deviation from optimal ACh levels can impair cognition (Newman and Gold, 2016).

Both our models showed deficits in response inhibition, a behavior primarily governed by DA (Dalley and Roiser, 2012; Pattij and Vanderschuren, 2008; Robbins, 2002a). Any changes in the innate levels of DA could potentially manifest as changes in behavior. Diurnal variations in dopaminergic transmission have been well established in the literature. Casteñada et al. showed an overall decrease in the levels of DA in rat brains when rats transitioned to the light phase and a subsequent increase in the levels of DA when they transitioned to the dark phase, indicating that DA levels are higher during the dark phase. These changes in DA levels were absent in their group that was exposed to 24 h of light continuously (Castañeda et al., 2004). Hampp et al. established that monoamine oxidase (Maoa), an enzyme that degrades DA, is also regulated by the

clock components (Hampp et al., 2008), which was not examined in our study. Core clock genes that govern these rhythms include *Clock, Bmal1, Cry,* and *Per* genes to name a few (Buhr and Takahashi, 2013; Takahashi, 2017). These genes have direct effects on dopaminergic neurotransmission (Hampp et al., 2008; Hood et al., 2010; Sleipness et al., 2007a; Verwey et al., 2016). Studies by Hampp and Sleipness indicate that the promoter regions for monoamine oxidase gene (*Maoa*) contain canonical E- box sites which serve as a binding region for heterodimers of CLOCK/BMAL1. These promoter regions are conserved across mouse, rat, and human species suggesting a comparable regulation (Hampp et al., 2008; Sleipness et al., 2007b). Despite the SCN being the master pacemaker, circadian genes and proteins are expressed throughout the brain which constitute SCN- independent pacemakers that entrain to both photic and non-photic stimuli, including food and drug administration (Iijima et al., 2005; Stephan, 1984).

The literature establishes that there are diurnal variations in both cholinergic and dopaminergic neurotransmission. We can infer that the subjects in our study were also subject to same variations, where the control group of rats would follow normal variations in rhythmicity, thus enabling those rats to optimize attention and response inhibition. In both treatment groups with altered light conditions, LAN and light phase, there were deviations from the normal diurnal variation (Mahoney Lab manuscript in preparation), thus giving rise to the deficits in attention and response inhibition. This study is the first to identify deficits in response inhibition in both models of circadian disruption. Nelson et al. have identified physiological deficits in a LAN model of circadian disruption, such as disruption of circadian regulation and melatonin signaling,

metabolic dysregulation, increased frequency of cancer, and disruption of other hormonally-driven systems, but our study is the first to identify cognitive deficits in a LAN model (Russart and Nelson, 2017).

Experiment 2 in this study was aimed at identifying changes at the molecular level caused by both models of circadian disruption. The results of this study indicate deficits in the expression of *Per2* in the light phase group. The expression of *Per2* in control and LAN group showed higher expression at ZT 16 compared to ZT 4, consistent with the literature (Hood et al., 2010). This difference was absent in light phase group of rats.

Circadian clock genes expressed in DmSTR can modulate dopaminergic neurotransmission, and these daily rhythms in DA release could potentially influence attention and impulsive behavior (Verwey et al., 2016; Webb et al., 2015). While we did not find rhythmic differences in *Drd2* or *Th*, previous studies indicate that *Per2* mutant animals show reduced expression of the monoamine oxidase (*Maoa)* gene resulting in elevated levels of dopamine (Hampp et al., 2008). Expression of *Maoa* was not investigated in this study, but the deficits we observed in the expression of *Per2* could be giving rise to similar effects. Alterations in the bidirectional relationship between DA and clock-component genes could also have contributed to the observed effects. The absence of difference in *Per2* clock gene expression in the LAN model indicates a possibility of an alternative mechanistic pathway. Release of ACh is high during the active phase and is accompanied by high ChAT activity (Hut and Van der Zee, 2011) similar to our results. *Drd1* also showed a higher expression during the active phase. It is possible that in both models of circadian disruption the higher expression of *Drd1* contributed to the increase in impulsive behavior. We hypothesize that DR1 is bound by

elevated DA levels in these rats, thus initiating a post-synaptic signalling cascade at a higher rate, causing an increase in impulsivity.

Future studies will parse out more effects of these two models of circadian disruption by quantifying mRNA levels for *Th*, *DAT* and the *DR*s in the ventral tegmentum and substantia nigra, regions where the dopaminergic neuronal bodies that project to the IL and DmSTR are located. Additionally, quantifying the protein levels corresponding to the genes tested in this study in both IL and DmSTR could provide us more information regarding the underlying neurochemistry in both the models of circadian disruption. Our current experiment establishes that both models of circadian disruption have profound consequences on brain function regulated by cholinergic and dopaminergic neurotransmission.

4.6 Figures and Tables

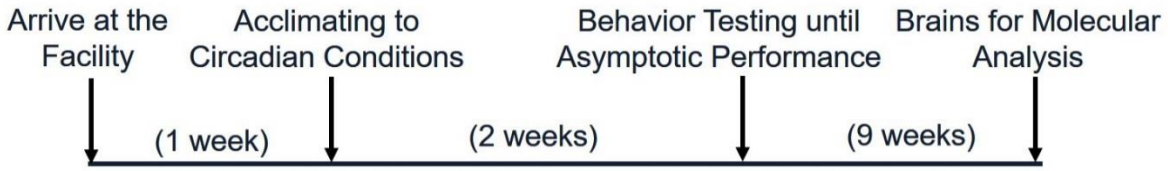


Figure 4.1. Schematic of the experimental plan that was followed for three cohorts of 40 rats each.

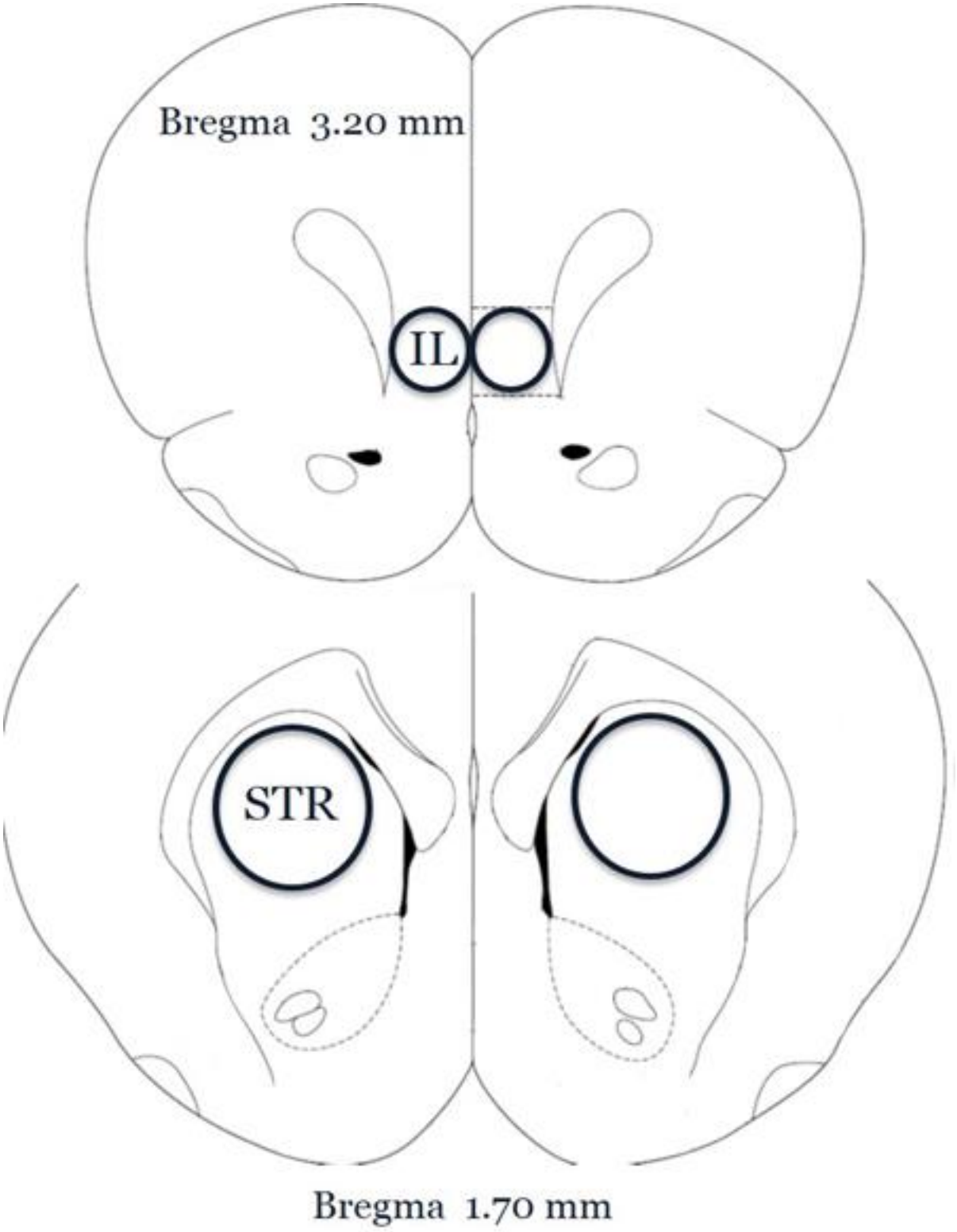
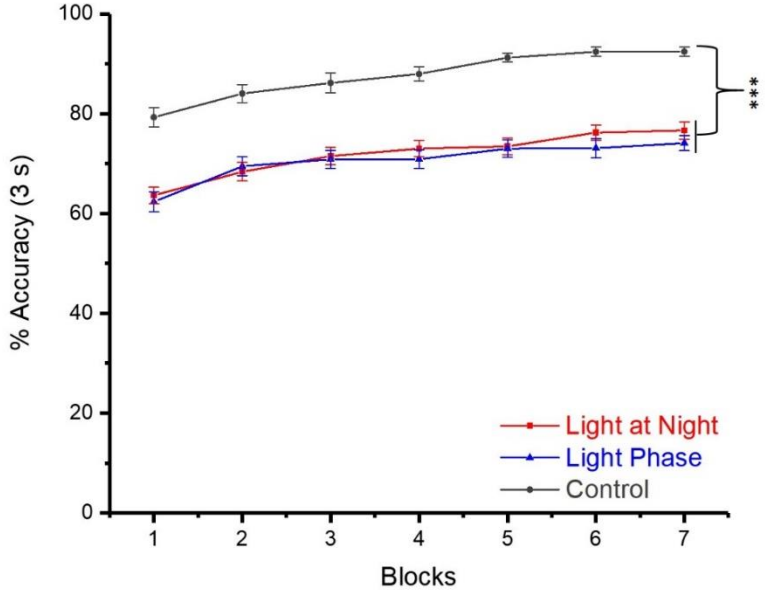


Figure 4.2. The circled area in the image identifies the brain regions the punches were taken from to isolate mRNA to further quantify gene expression from (Paxinos and Watson, 1998).

Gene of Interest	Brain Regions	Primer Sequence		Accession number
Choline Acetyltransferase	STR, IL	fwd chat	CCTCGTCTGTGGAGTTTGCG	NM_001170593.1
		rev chat	AGATTGCTTGGCTTGGTTGG	
β2 nAChR	STR, IL	fwd chrnb2	TGCGAAGTGAGGATGATGAC	NM_019297.1
		rev chrnb2	ACGGTCCCAAAGACACAGAC	
Dopamine Receptor 1	STR, IL	fwd drd1	GGCCCTTTGGGTCCTTTTGT	NM_012546.3
		rev drd1	ATCACGCAGAGGTTCAGAATGG	
Dopamine Receptor 2	STR, IL	fwd drd2	AAGCGCCGAGTTACTGTCAT	NM_012547.1
		rev drd2	GACCACAAAGGCAGGGTTG	
Tyrosine Hydroxylase	STR, IL	fwd th	CCTTCCAGTACAAGCACGGT	NM_012740.3
		rev th	TGGGTAGCATAGAGGCCCTT	
Norepinephrine Transporter	IL	fwd net	TAAGAAGTCAGGTCCAGCACC	NM_031343.1
		rev net	AGTAGAGCAAGGAAGGCACC	
Dopamine Transporter	STR	fwd dat	TGCTGGTCATTGTTCTGCTC	NM_012694.2
		rev dat	GCTCCAGGAAGGGTAACTCC	
Period 2	STR	fwd per2	CACCCTGAAAAGAAAGTGCGA	NM_031678.1
		rev per2	CAACGCCAAGGAGCTCAAGT	
Glyceraldehyde 3-phosphate dehydrogenase	STR, IL	fwd gapdh	GGTGGACCTCATGGCCTACA	NM_017008.4
		rev gapdh	GGCCTCTCTCTTGCTCTCAGTATC	

Table 4.1: Genes that were analyzed using quantitative RT-PCR to understand how circadian disruption altered the expression in the dorsomedial striatum (STR) and the infralimbic cortex (IL).

(a.)



(b.)

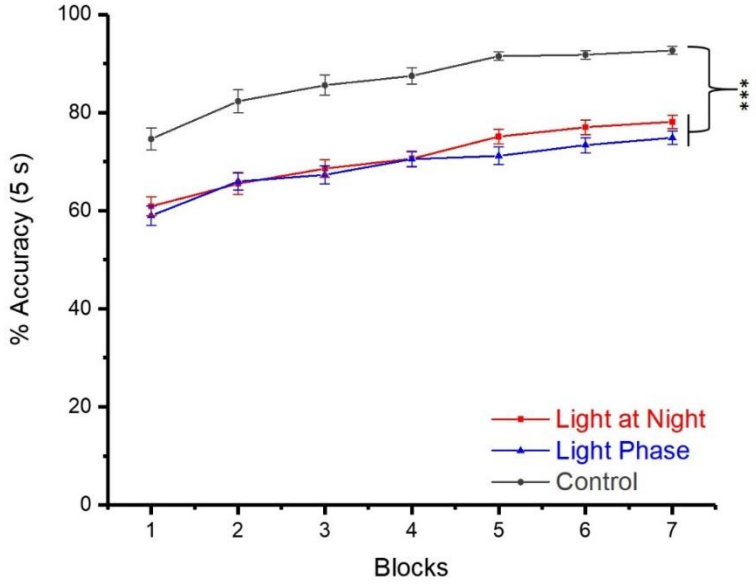


Figure 4.3. Percent accuracy, a measure of attention was siginificantly diminished in rats that had been subjected to circadian disruption. The grey line indicates that the control rats performed significantly better from block 1 compared to the treated groups (blue and red line). This difference was maintained through the 7 blocks of the task. The rats in all three light condition groups reached asymptotic performance by block 6. The effect of circadian disruption was consistent across 3 cue delays (a.) 3 s, (b.) 5 s, (c.) 7 s. *** $p < 0.001$ compared to control.

(c.)

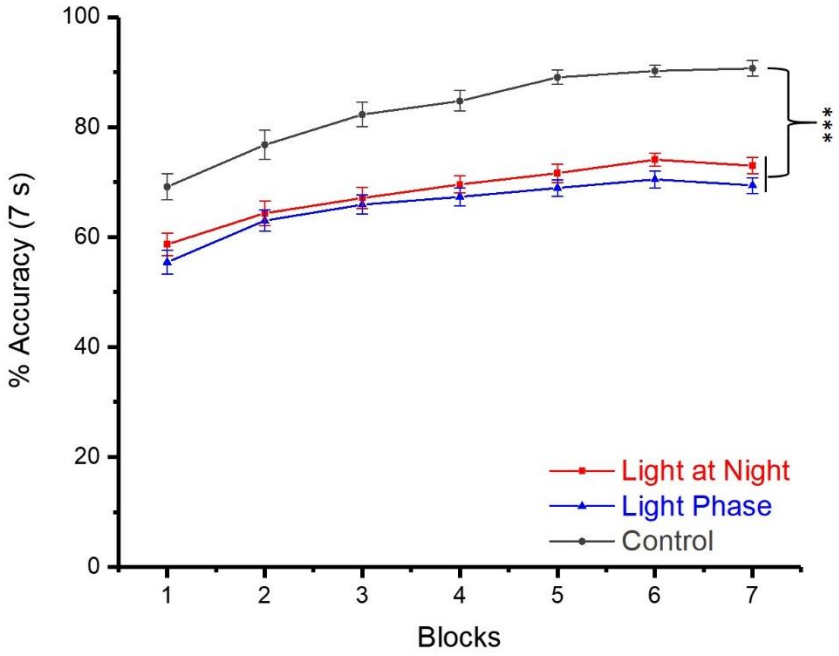
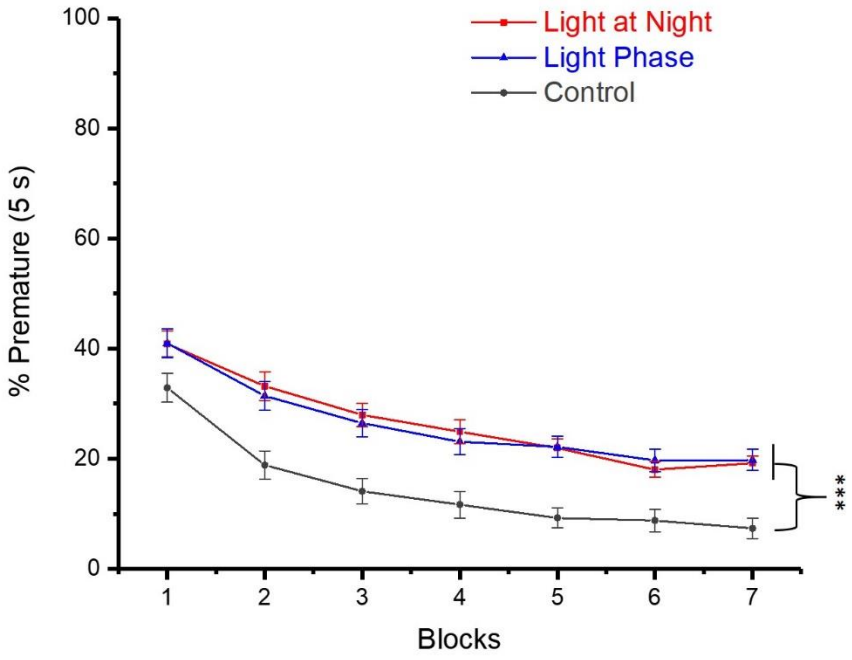


Figure 4.3 (continued). Percent accuracy, a measure of attention was siginificantly diminished in rats that had been subjected to circadian disruption. The grey line indicates that the control rats performed significantly better from block 1 compared to the treated groups (blue and red line). This difference was maintained through the 7 blocks of the task. The rats in all three light condition groups reached asymptotic performance by block 6. The effect of circadian disruption was consistent across 3 cue delays (a.) 3 s, (b.) 5 s, (c.) 7 s. *** $p < 0.001$ compared to control.

(a.)



(b.)

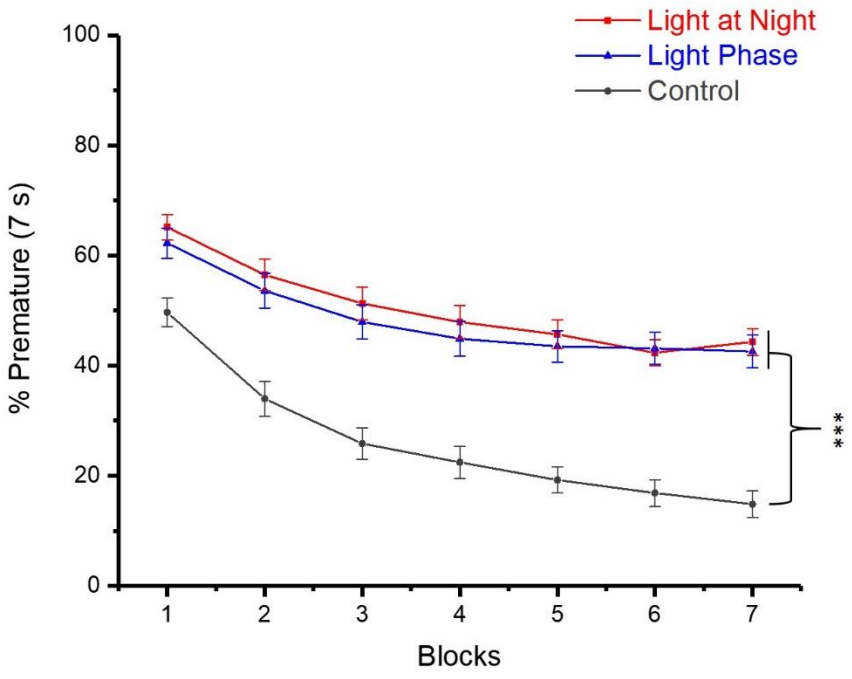


Figure 4.4. Percent premature, a measure of impulsive behavior was significantly increased in rats that had been subjected to circadian disruption. Even though the difference in block 1 was not significant, the

Figure 4.4 (Continued) control rats, represented by the grey line began performing significantly better from block 2 onwards compared to the circadian disruption groups (red and blue lines). This difference was maintained through block 7 of the task. The rats in all three light condition groups reached asymptotic performance by block 6. The effect of circadian disruption was consistent across both cue delays (a.) 5 s, (b.) 7 s. *** $p < 0.001$ compared to control.

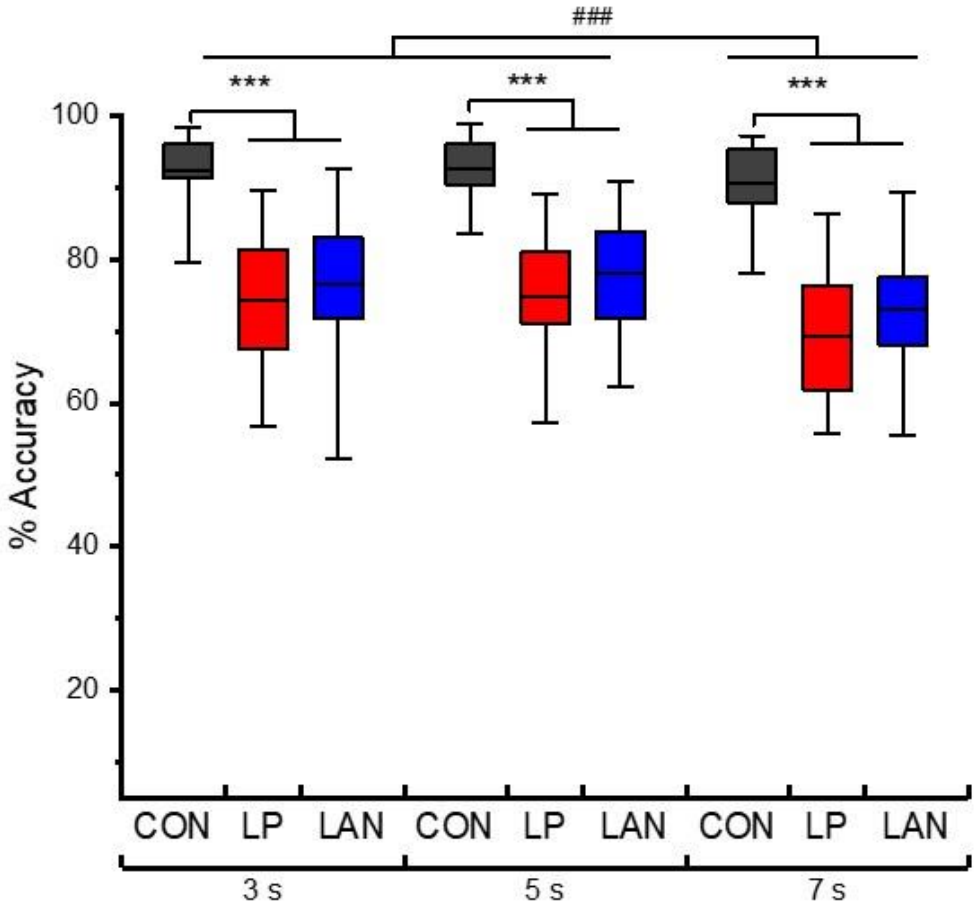


Figure 4.5. Percent accuracy in block 7 for the three light conditions Control (CON), Light at Night (LAN) and Light Phase (LP) showing deficits in both LAN and LP compared to control (*). The longer cue delay of 7 s also affected % accuracy (# compared to 7 s). ***/### $p < 0.001$

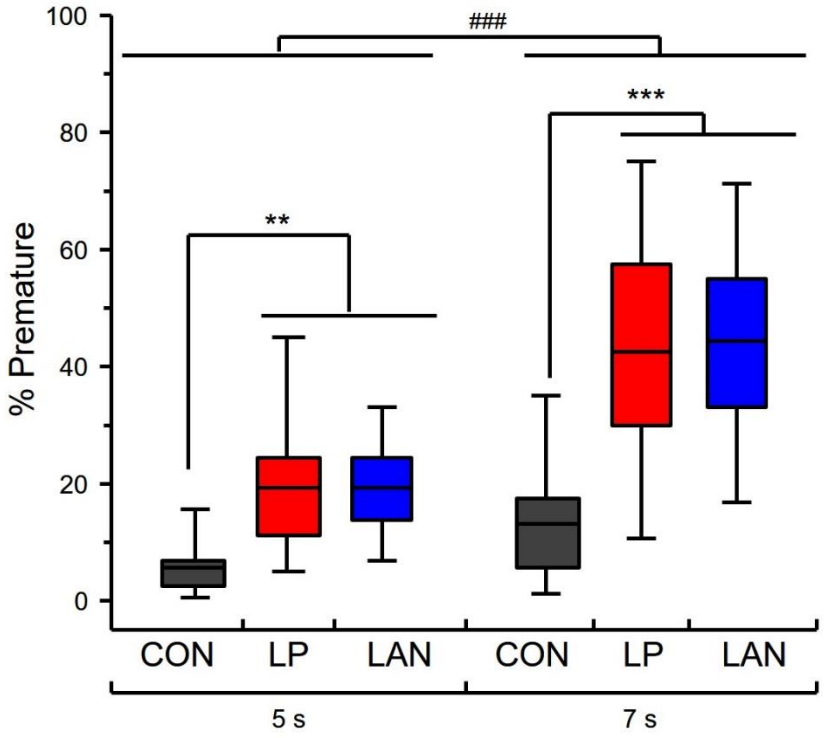


Figure 4.6. Percent premature in block 7 for the three light conditions Control (CON), Light at Night (LAN) and Light Phase (LP) showing increased impulsivity in both LAN and LP compared to control (*). The longer cue delay of 7 s also affected % premature (# compared to 7 s). ***/### $p < 0.001$, **/## $p < 0.01$.

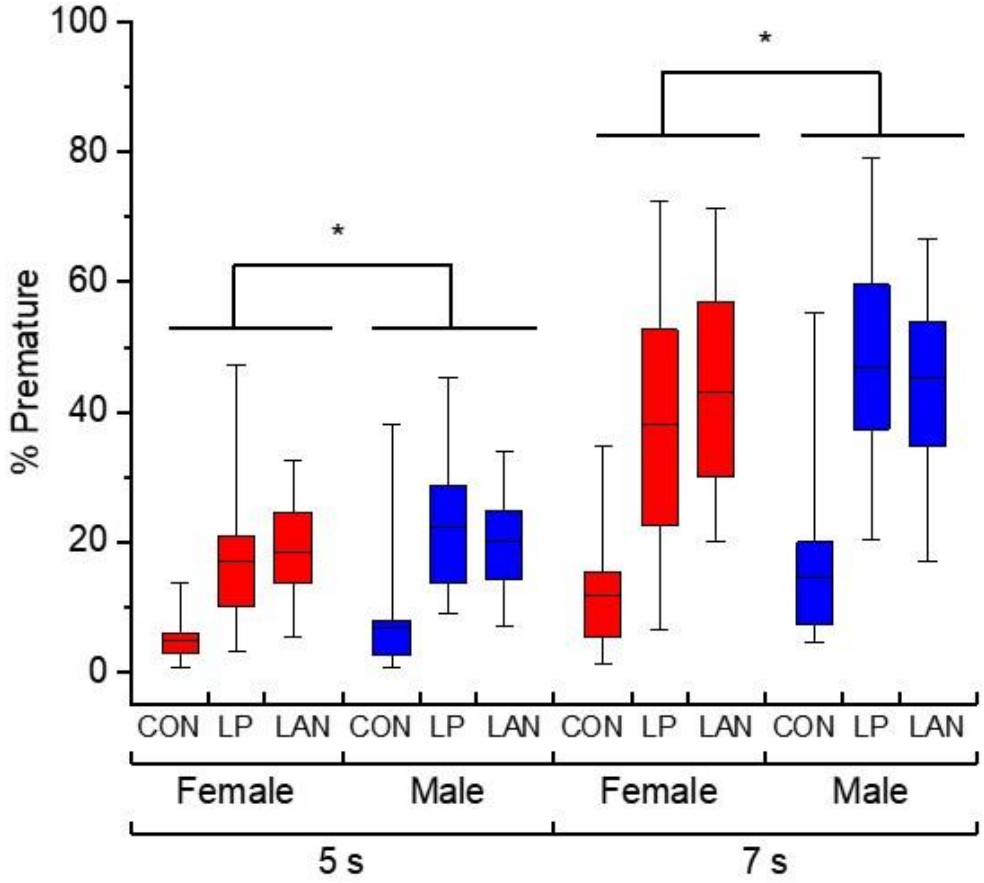


Figure 4.7. Males made more premature responses in block 7 for all three light conditions Control (CON), Light at Night (LAN) and Light Phase (LP) at both cue delays. * p = 0.046

Dependent Measures	Control			LAN			Light Phase		
	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s
% Accuracy	92.4 ± 0.9	92.6 ± 0.7 ###	**90.6 ± 1.3** ###	76.6 ± 1.7 ***	78.1 ± 1.3 ***	**72.9 ± 1.4** *** ###	74.1 ± 1.5 *** ###	74.8 ± 1.3 ***	**69.3 ± 1.4** *** ###
% Premature	N/A	7.3 ± 1.8 ###	**14.8 ± 2.3** ###	N/A	19.1 ± 1.2 ***	**44.3 ± 2.4** *** ###	N/A	19.7 ± 1.9 *** ###	**42.5 ± 3** *** ###
% Omission	5.9 ± 1	4.2 ± 0.8 ^^^	3.9 ± 0.5 ^^^	3.6 ± 0.8	2.6 ± 0.6 ^^^	2.1 ± 0.5 ^^^	4.7 ± 1	4.3 ± 0.9 ^^^	4.2 ± 0.8 ^^^
Average Perseverative Responses	0.09 ± 0.01	0.07 ± 0	0.08 ± 0.01	0.15 ± 0.1	0.16 ± 0.09	0.15 ± 0.09	0.1 ± 0.02	0.08 ± 0.02	0.08 ± 0.01
Correct Response Latency	0.85 ± 0.03	0.81 ± 0.02 ^^^	0.81 ± 0.03 ^^^	1.17 ± 0.05 ***	0.97 ± 0.04 *** ^^^	1.02 ± 0.06 *** ^^^	1.21 ± 0.05 ***	1.08 ± 0.04 *** ^^^	1.09 ± 0.04 *** ^^^
Incorrect Response Latency	4.02 ± 0.3	3.24 ± 0.35 ^^^	3.05 ± 0.35 ^^^	4.36 ± 0.23	3.57 ± 0.2 ^^^	3.2 ± 0.2 ^^^	4.36 ± 0.2	3.61 ± 0.18 ^^^	3.28 ± 0.17 ^^^
Reinforcement latency	1.64 ± 0.1	1.84 ± 0.19	1.95 ± 0.3	1.56 ± 0.1	1.57 ± 0.09	1.57 ± 0.08	1.6 ± 0.07	1.68 ± 0.09	1.7 ± 0.1

Table 4.2. Dependent measures from 5-CSRTT at each light condition and cue delay. * Compared to control group of rats. # Compared to 3 s and 5 s. ^ Compared to 3 s. ###/^^^/*** $p < 0.001$ ##/^^/** $p < 0.01$ #/^/* $p < 0.05$.

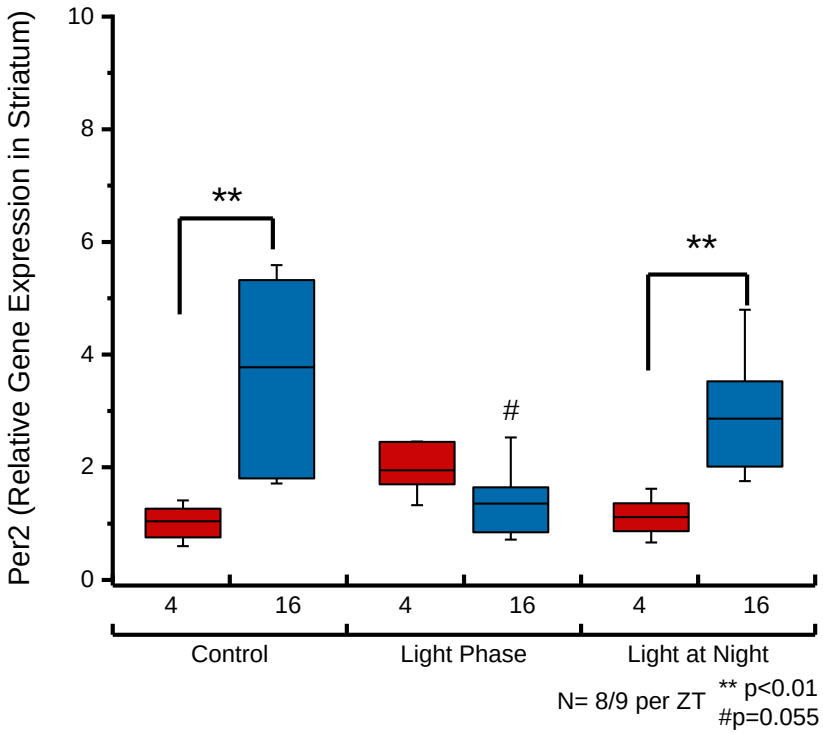


Figure 4.8. Expression of *Per2* is quantified at ZT 4 (4 hours after lights turn on, rest phase) and ZT 16 (4 hours after light turn off, active phase) for control rats showing a significant increase at ZT 16 compared to ZT 4. The LAN group shows a similar pattern of expression despite deficits in attention and impulsive behavior. The expression of *Per2* in light phase tested rats seems to have an inverted expression because of the circadian disruption. ** $p < 0.01$, # $p = 0.055$.

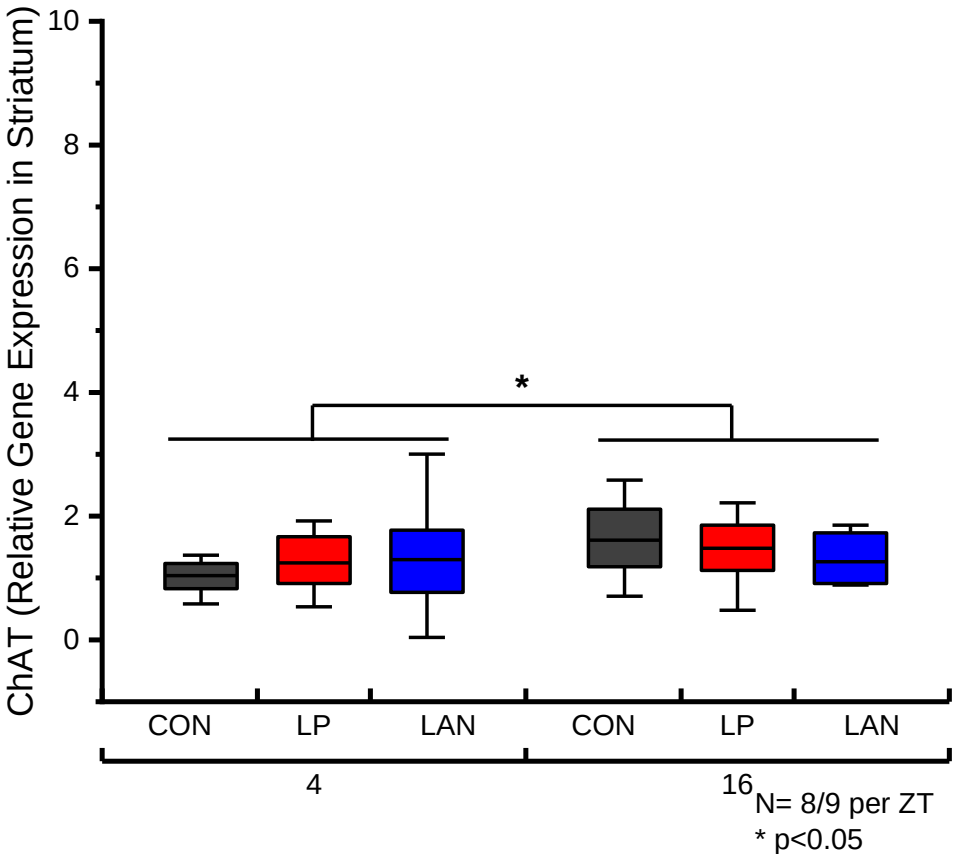


Figure 4.9. *Chat* showed a higher expression at ZT 16, active phase for the rats, across all light conditions compared to ZT 4. Control (CON), Light at Night (LAN) and Light Phase (LP).
* $p < 0.05$

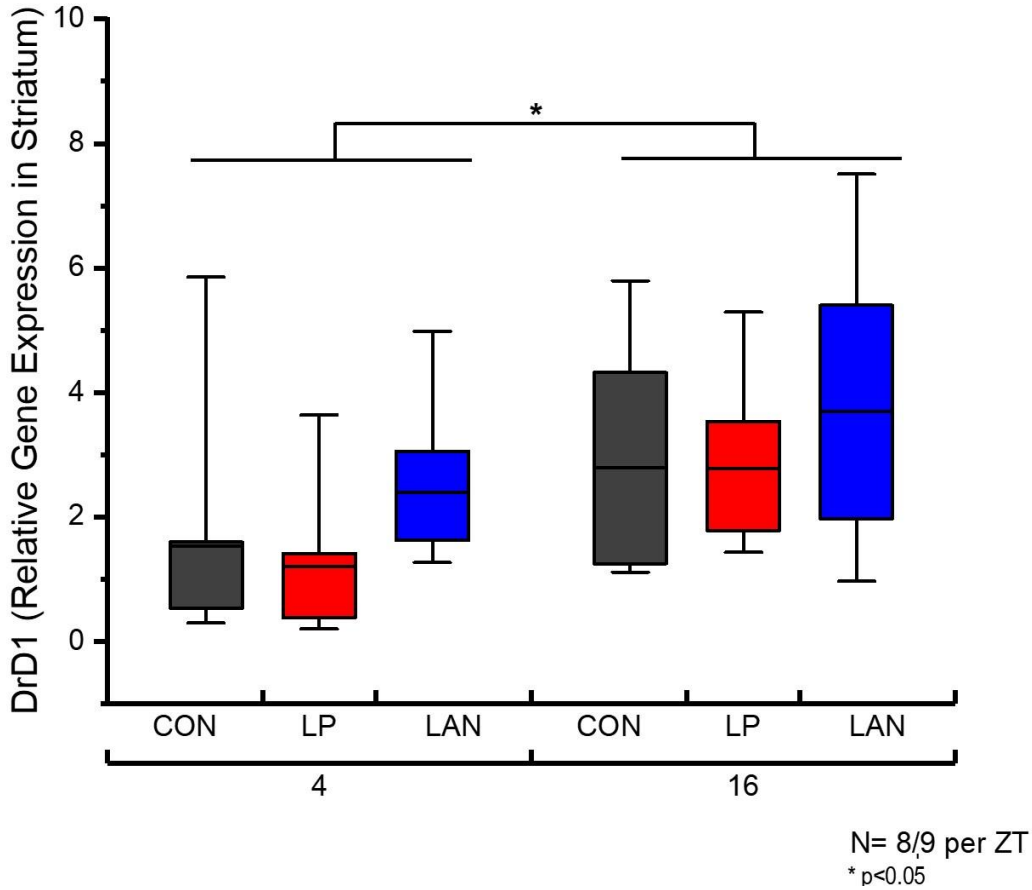


Figure 4.10. *Drd1* expression was higher at ZT 16, active phase for the rats, across all light conditions compared to ZT 4 (*). Control (CON), Light at Night (LAN) and Light Phase (LP) * $p < 0.05$

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Chapter 5 Cholinergic and Dopaminergic Interactions Alter Attention and Response Inhibition in Long-Evans Rats Performing the 5-Choice Serial Response Time Task (5-CSRTT)

5.1 Abstract

Acetylcholine (ACh) neurotransmission is important for attention, while dopamine (DA) signaling modulates impulsive behavior. Prior studies have established an existing relationship between ACh and DA that mediates dopamine release in the prefrontal cortex of the brain in rats performing the 5-choice serial response time task (5-CSRTT). This study is aimed to identify cholinergic and dopaminergic interactions that govern attention and impulsive behavior using adult Long-Evans rats of both sexes tested on the 5-CSRTT. In Experiment 1, the effects of single cholinergic and dopaminergic drugs on 5-CSRTT performance were evaluated. Drugs like nicotinic ACh receptor (nAChR) agonist nicotine, amphetamine, and GBR12909 that increase the synaptic levels of ACh and DA respectively increased impulsive behavior. Amphetamine and GBR12909 decreased attention while there was no effect of nicotine. The antagonists mecamylamine, a general nAChR antagonist, flupenthixol a DA 1/2 receptor antagonist, and SCH 23390 a DA 1 receptor antagonist, decreased impulsive behavior. These antagonists had a mixed effect on attention. Dihydro-β-erythroidine hydrobromide (DHBE), an α4β2* subunit-specific nAChR antagonist, did not have significant effects on attention or impulsivity at the doses administered. Eticlopride, a DA 2 receptor antagonist, decreased attention at the shortest cue delay but did not affect impulsivity. The acetylcholinesterase inhibitor donepezil decreased both attention and impulsive behavior. Subsequently, effects of nicotine and amphetamine were tested after pretreatment with SCH 23390 and eticlopride. SCH 23390 attenuated the effects of

nicotine and amphetamine on impulsivity, while eticlopride only attenuated the effect of nicotine on impulsivity. Minimal effects on attention were seen in the combination trials. This data establishes that response inhibition, is enhanced by interactions between cholinergic and dopaminergic neurotransmission. Additionally, our study confirms that dopamine D1 receptor plays an essential role in modulation of response inhibition.

5.2 Introduction

Sustained attention is the ability to focus on a stimulus over a period of time and respond accordingly to a signal (P.J and Strupp). Response inhibition is the ability to withhold a prepotent response, with deficits in response inhibition being characteristic of impulsive behavior (Pattij et al., 2007). Attention and impulsive behavior are modulated by cholinergic and dopaminergic signaling in the prefrontal cortex (PFC) in rodents (Bloem et al., 2014; Granon et al., 2000; Muir et al., 1994; Robbins, 2002b). The PFC receives dense cholinergic and dopaminergic innervation involved in maintaining optimal cognitive performance in the 5-choice serial reaction time task (5-CSRTT), a versatile task used to measure both attention and impulsive behavior (Robbins, 2002b). While acetylcholine (ACh) neurotransmission modulates attention, there exists an interaction between the ACh and dopaminergic systems that plays a role in maintaining appropriate response inhibition (Livingstone and Wonnacott, 2009a; Wonnacott, 1997). Reward pathways which are stimulated by dopamine are also indirectly stimulated by acetylcholine (ACh) and exogenous cholinergic agonists such as nicotine (van Gaalen et al., 2006b; Gardner, 2011; Livingstone and Wonnacott, 2009a). The nicotinic acetylcholine receptors (nAChRs) that interact with dopamine are ligand-gated, pentameric cation channels comprised of α and β subunits. Different nAChR subtypes

give rise to differential sensitivities to nicotine and ACh. Dopaminergic pathways that arise in the midbrain and project to the PFC express nicotinic receptors on the membranes of presynaptic dopamine axonal terminals so that changes in the levels of endogenous ACh alter dopamine release (Faure et al., 2014; Wonnacott, 1997). Effects of dopamine on 5-CSRTT performance are mediated through binding of dopamine receptors 1 and 2 (DR 1, DR 2) at the synaptic cleft to elicit post-synaptic signal transduction (van Gaalen et al., 2006c; Pattij et al., 2007), but the functional role of DR 2 is less clear (Floresco and Magyar, 2006). Evidence indicates a fast phasic release of ACh is involved in optimizing sustained attention on the 5-CSRTT (Bloem et al., 2014). However, the roles of ACh and dopamine neurotransmission in optimizing response inhibition have received less scrutiny.

The focus of this study was to examine the role of cholinergic neurotransmission, and its interaction with dopaminergic neurotransmission, in optimal response inhibition on the 5-CSRTT. Many studies have evaluated the effects of single cholinergic or dopaminergic drugs on 5-CSRTT performance, typically at a single cue delay until the signal(Baarendse and Vanderschuren, 2012; van Gaalen et al., 2006c; Hahn and Stolerman, 2002; Hahn et al., 2002a). There are very few studies that have evaluated combinations of drugs at a single cue delay, and showed that dopaminergic antagonists reduce the impulsive behavior induced by drugs like nicotine and amphetamine (van Gaalen et al., 2006c; Hahn et al., 2002a). Given our emphasis on impulsivity, we chose a version of the 5-CSRTT that appears to result in a robust difference in impulsive responding between cue delays, while also allowing attention to be evaluated (Beaudin et al., 2017). In Experiment 1, we evaluated the effects of single cholinergic or

dopaminergic drugs at different cue delays. In addition to evaluating the effects of nicotine and nAChR antagonists, we also explored the use of the acetylcholinesterase inhibitor donepezil to understand its effects on attention and response inhibition. Donepezil is approved for treatment of Alzheimer's disease (Kosasa et al., 2000). In rodent models, acute exposure to donepezil enhances learning and memory by increasing cholinergic signaling (Cutuli et al., 2009; Kosasa et al., 2000). However, the effects of donepezil on 5-CSRTT performance in normal rats have not been reported; Alzheimer's models typically employ hypocholinergic rats as controls in assessing the effects of drugs. We hypothesized that we would see an increase in attention and impulsive behavior with nicotine, an increase in attention and impulsive behavior with cholinergic antagonists, and an improvement in attention and decrease in impulsive behavior with donepezil. With respect to the dopaminergic drugs, we expected to observe deficits in attention and an increase in impulsive behavior with the administration of amphetamine and GBR 12909, both of which increase synaptic dopamine levels. Additionally, we hypothesized that dopamine antagonists would improve attention and reduce impulsive behavior.

After trials involving single drugs, we performed Experiment 2 on separate rats. We planned to administer nicotine or amphetamine to animals pre-treated with dopamine antagonists SCH 23390 or eticlopride to evaluate how the combined effects of these drugs altered attention and impulsive behavior. We hypothesized that the dopamine antagonists would attenuate the effects of nicotine or amphetamine on attention and response inhibition in rats performing the 5-CSRTT.

The results showed that, in our experimental model, nicotine did not affect attention, and amphetamine and GBR 12909 decreased attention, whereas all 3 of these drugs increased impulsive behavior. In general, nAChR and DR antagonists decreased impulsive behavior but had minimal effects on attention. Donepezil decreased both attention and impulsive behavior. The combination drug studies demonstrated that DR antagonists ameliorated the detrimental effects of nicotine and amphetamine on impulsive behavior, although there were differences based on the specific DRs targeted by the different antagonists. Effects on attention were minimal in the combination studies. Our findings establish the modulatory role of nicotine on response inhibition, potentially via the nAChRs that are present in the presynaptic terminal in the PFC and the striatal regions (Livingstone and Wonnacott, 2009a).

5.3 Materials and Methods

Subjects

Two separate cohorts of 28 Long-Evans rats, 14 of each sex, approximately 70 days of age, were purchased from Envigo (Indianapolis, IN.) Rats were single-housed in polycarbonate shoebox cages with wood-chip bedding (Beta Chip, Northeastern Products Corp., Warrensburg, NY) in a temperature- and humidity-controlled room (targeted 22^{o} C, 40-55% humidity). 2020X Teklad Rodent Diet (Envigo) was fed to the rats. Food restriction was initiated after a one-week acclimation period to reduce rats' body weights over 2 weeks to target weights of 85% of their free-feed body weights. After that, target weights were incrementally increased by 5-10 g every 2 weeks, with a maximum of 250 ± 10 g for female rats and 350 ± 10 g for male rats, to allow for growth. Food restriction was intended to maintain motivation for performing operant-based

behavioral tasks. Tap water was provided *ad libitum*. TestDiet sucrose pellets (AIN-76A 5TUL, 45 mg pellets, St. Louis, MO) were used for food-based reinforcement during behavior testing.

Rats were maintained on a 12:12 h light:dark cycle and were tested 3 to 5 h after the onset of the dark phase. During the daily testing sessions, rats were exposed to overhead lights during transport and in the testing room. In the testing chambers, rats were exposed to 2.8-watt house light and a stimulus cue light as well as low-intensity, yellow LEDs from 5 nose-poke holes.

Apparatus and 5-Choice Serial Reaction Time Task (5-CSRTT)

Behavioral testing began 3 weeks after the rats arrived when rats were approximately 90 days of age. Training and testing sessions, 1 per day, were performed 6 days each week in 12 5-choice operant behavior-conditioning chambers housed in sound-insulated and ventilated cubicles (Med Associates Inc., St. Albans, VT). Each chamber was configured with 5 evenly-spaced nose-poke cue holes (2.5 x 2.5 x 2.5 cm and 2 cm above the floor) on one wall. Each aperture had a yellow LED light centered in the back and an infrared photocell to detect head entries. The opposite wall had a pellet trough with a head-entry detector in the center panel, a stimulus cue light directly above it, and then a house light mounted 6 cm above the cue light. Experimental contingencies were programmed using MedState Notation programming language, and data acquisition was performed using MED-PC IV software (version 4.38, Med Associates). Behavioral-testing programs were modified from those used by Beaudin et al. (Beaudin et al., 2017).

During the 7 initial training phases of the 5-CSRTT, rats learned to associate nose pokes in the pellet trough and the 5 nose-poke holes with food reinforcement. The house light remained on during all of these phases. Criterion to advance from one phase to the next was 99 or 100 successful nose pokes. Each session during the 7 initial phases lasted until 100 pellets were earned or 60 min elapsed. Rats took an average of 7.4 days, ranging from 7 to 12 days, to complete initial training. The next phase of training was Visual Discrimination 1, during which cue lights were introduced. One cue light was illuminated for 15 s during each trial. Nose pokes in the illuminated cue hole resulted in reinforcer delivery. Poking in any other cue hole, or not poking during the 15 s period, resulted in a time-out (see next section). See Supplemental Figure 1 and Supplemental Table 1 for a schematic and accompanying information for this and subsequent phases. Rats were tested on this phase until criteria of making 75% or more correct responses for 2 out of three consecutive days were met within 15 sessions. All rats in this study met the criteria, taking an average of 3.8 days, ranging from 3 to 7 days to progress to next phase. In the next phase, Visual Discrimination 2, the duration of cue light illumination was shortened to 1 s, requiring rats to be more attentive to optimize performance. Rats still had a 15 s limited-hold period to nose poke. Rats were tested on this phase for 5 days.

Sustained Attention Task

Following the training sessions, rats were tested on the Sustained Attention phase for 21 days. During daily sessions in each phase, the yellow LED cue lights in the 5 nose-pokes holes would randomly illuminate in one hole per trial, in a counterbalanced

manner, so that each hole was illuminated during an equal number of trials per session. The house light was illuminated except during time-out periods. Each trial began with a nose-poke in the pellet trough. If it was the first trial, or if the previous trial resulted in sucrose pellet delivery, the rat was given 3 s to consume the pellet (reinforcer duration). After the 3 s reinforcer duration, the rat was given a 3 s turn-around time to allow the rat time to orient toward the wall with the 5 nose-poke holes. If the previous trial had resulted in a time-out, there was no reinforcer duration after a nose poke in the trough. In this phase of the test, there were variable cue delays of 3, 5 or 7 s until a cue light would illuminate in a nose-poke hole. These delays consisted of the 3 s turn-around time plus an additional delay of 0, 2 or 4 s. The cue light in the nose-poke holes would illuminate for a maximum of 1 s and, concurrently, a 15 s limited-hold period would commence during which a nose poke could be registered.

One of 4 trial outcomes could result. A **correct** trial was when the rat poked in the hole in which the cue light was illuminated during the 15 s limited-hold period. An **incorrect** trial was when the rat poked in one of the other 4 holes. A **premature** trial was when the rat poked during the delay before the cue light illuminated. Premature nose-pokes were not recorded during the 3 s turn-around time, so premature trials only occurred when the cue delay was 5 or 7 s. An **omission** occurred when the rat did not poke during the limited-hold period. Correct trials resulted in a food pellet being dispensed, the cue light. If the rat continued to make nose poke in any of the 5 cue holes after making a correct response, those nose pokes were recorded as a **perseverative** response but had no consequence. Perseverative responses were recorded only when there was a correct trial. Incorrect, premature, and omission

responses triggered an immediate 5 s time-out, during which cue and house lights extinguished. Poking in any of the 5 nose-poke holes during a time-out reset the time-out timer. When 5 s elapsed without any nose pokes, the cue light above the pellet trough illuminated until the rat poked in the trough, thus beginning the next trial. Each daily session lasted until 150 trials or 60 min elapsed.

Drug Challenges

After rats completed 21 sessions of Sustained Attention, they continued testing on that phase until all rats in the experiment completed 21 sessions. Subsequently, rats in Experiment 1 tested for an average of 28 extra days (range 24 to 29), and those in Experiment 2 tested for an average of 4 extra days (range 1 to 5), prior to drug challenges commencing. Rats in Experiment 1 tested longer due to a delay in implementing drug trials. Between the Sustained Attention phase and drug testing, rats were tested with only one cue delay of 5 s to maintain what was termed a baseline level of performance. Drug testing occurred daily Monday through Friday. Mondays and Wednesdays were baseline performance days with only the 5 s cue delay, Tuesdays and Fridays were drug administration days, and all rats received a saline injection on Thursdays. During drug and saline days, all 3 cue delays (3, 5 and 7 s) were present during testing sessions.

Rats in Experiment 1 were tested with a series of single drugs (see Table 5.1), while those in Experiment 2 received single and then paired drug combinations (see Table 5.2). Drugs were administered in the order listed in each table. For each drug, all doses were administered using a Latin square design, with a minimum of 3 days between

each dose, before moving to the next drug. All of the drugs were dissolved in sterile 0.9% normal saline (Baxter Healthcare Corp., Deerfield, IL) except GBR 12909, which did not dissolve well in saline and so was dissolved in 25% sterile DMSO (Tocris Bioscience, Minneapolis, MN). All drugs were administered intraperitoneally. Concentrations of each dosing solution were adjusted so that 1 µL/g body weight was always administered. The drugs used and times of administration before beginning testing sessions were (-)-nicotine ditartrate (NIC) 20 min (Tocris), donepezil hydrochloride 30 min (EMD Millipore Corp., Billerica, MA), mecamylamine hydrochloride (MEC) 30 min (USP, Rockville, MD), dihydro-β-erythroidine hydrobromide (DHBE) 20 min (Tocris), d-amphetamine hemisulfate salt (AMPH) 10 min (Sigma-Aldrich, St. Louis, MO), GBR 12909 dihydrochloride 20 min (Sigma-Aldrich), *cis*-(Z)-flupenthixol dihydrochloride (FLU) 30 min (Sigma-Aldrich), SCH 23390 hydrochloride 30 min (Tocris), S-(-)-eticlopride hydrochloride 20 min (Sigma-Aldrich), and saline vehicle 20 min, based on available pharmacokinetic information.

Experiment 1: Single Drugs

Rats (14 males, 14 females) were administered single drugs in the order listed in Table 1. During the first week, all rats were administered 0.5 mg/kg NIC for 3 consecutive days to acclimate rats to the undesirable physiologic effects of NIC that might impair performance (Bizarro and Stolerman, 2003). One female died during the DHBE administration phase and thus was only included in analyses of drugs through that point. Testing lasted 18 weeks.

Experiment 2: Drug Combinations

A new set of rats (14 males, 14 females) were administered single drugs followed by combinations in the order listed in Table 5.2. Results from Experiment 1 were used to select or modify doses for Experiment 2. Like Experiment 1, all rats were administered 0.5 mg/kg NIC for 3 days during the first week. One female died during the NIC and SCH 23390 administration phase and thus was only included in analyses of drugs through that point. Testing lasted 12 weeks.

Data Analysis

R program for statistical computing and graphics (R Core Team, 2016) was used to calculate **percent correct** (number of correct responses/total trials *100), **percent incorrect** (number of incorrect responses/total trials *100), **percent accuracy** (percent correct/(percent correct + percent incorrect)), **percent premature** (number of premature responses/total trials *100), **percent omissions** (number of omitted responses/total trials *100), and **average perseverative responses** (sum of perseverative responses in all 5 nose-poke holes/ number of correct responses). **Latency to correct responses** (sum of latencies to all correct responses/number of correct responses), **latency to incorrect responses** (sum of latencies to all incorrect responses/number of correct responses), and **latency to collect reinforcers** (sum of latencies to collect reinforcers/number of correct responses) were also calculated. Percent accuracy indicates the ability of the subject to sustain attention; percent premature responses indicate a deficit in response inhibition; percent omissions is a measure of

inattentiveness, a lack of motivation, or both (Robbins, 2002b). Data are reported as mean ± SEM.

For some drugs, omissions increased as dose increased. If an individual rat had > 50% omissions for a drug dose, the rat's data for that dose were excluded from analysis. If, for any drug, a dose caused > 50% omissions in > 50% of rats, the entire dose was excluded from analysis. We omitted entire doses for the following drugs: 4 mg/kg donepezil, and 0.04 and 0.06 mg/kg SCH 23390 in Experiment 1; 0.06 mg/kg eticlopride in the AMPH-eticlopride trials of Experiment 2.

All statistical analyses were conducted using SPSS for Windows (version 24, SPSS Inc., Chicago, IL). Mixed model ANOVAs were used to analyze all data, with statistical significance set at $p \leq 0.05$. The data from the 21 days of Sustained Attention phase were evaluated to characterize learning of the 5-CSRTT before drug challenges. Experimental factors of block (7 3-day blocks) and cue delay (3 cue delays, except for 2 for percent premature) were within-subjects factors, and sex was a between-subjects factor. Then data from the last 3 days of the Sustained Attention phase (block 7), when asymptotic performance had been reached, was evaluated with cue delay as a within-subjects factor and sex as a between-subjects factor. For data from drug trials, dose (2-4 doses in Experiment 1 and 2-6 doses in Experiment 2, including controls) and cue delay were within-subjects factors and sex was a between-subjects factor. Doses of each drug were compared to control for Experiment 1. Two sets of comparisons were made for Experiment 2: 1) individual or combination doses were compared with control, and 2) combination doses were compared with NIC or AMPH alone to gauge the

effectiveness in attenuating the effects caused by NIC or AMPH. Post-hoc testing (Sidak) was performed when appropriate using SPSS.

5.4 Results

For all data on initial learning and drug challenges, significant sex-related differences were infrequent and thus are only included, when present, for the 2 primary measures of interest, accuracy and premature responding. Data on other measures including perseverative responding, which was rarely affected, omissions, and latencies during drug trials are presented in tabular form in the Supplemental Material to assist with interpretation of drug effects. Due to substantial significant differences in premature responding and latency to incorrect responses between cue delays, data for the drug trials were separately analyzed for each cue delay (3, 5, 7 s for accuracy; 5, 7 s for premature responses). Full F test results are reported for accuracy and premature responding, but not for other measures in the interest of space.

Cholinergic Drug Effects

Data on accuracy and premature responding for NIC, MEC, DHBE, and donepezil are presented in Figure 5.1.1 and Table 5.1.2. Other dependent measures are in Table 5.3.

Nicotine did not have statistically significant effects on accuracy, but NIC increased premature responding at cue delays of 5 s ($F_{3, 84} = 3.9$, $p = 0.012$) and 7 s ($F_{3, 84} = 7.2$, $p < 0.001$). Post-hoc analysis determined that the 0.1 mg/kg dose was ineffective, the 0.3 mg/kg dose was effective only at the longer 7 s delay ($p = 0.001$), and 0.5 mg/kg increased premature responding at both 5 s ($p = 0.01$) and 7 s ($p = 0.003$) delays. Other aspects of performance were not affected by NIC.

The non-selective nAChR antagonist MEC significantly decreased accuracy at both 3 s ($F_{3, 84} = 23.2$, $p < 0.001$) and 5 s ($F_{3, 84} = 8.6$, $p < 0.001$) cue delays, but only at the highest dose of 3 mg/kg ($p < 0.001$ for both delays). Similarly, only the 3 mg/kg dose of MEC significantly decreased premature responding at both cue delays of 5 s ($F_{3, 84} = 7.6$, $p < 0.001$) and 7 s ($F_{3, 84} = 18.3$, $p < 0.001$) when compared to saline control ($p = 0.011$ for 5 s, $p < 0.001$ for 7 s). However, the 3 mg/kg dose also significantly increased omissions (although not enough to meet criteria for exclusion of the dose), and significantly increased latencies for both correct and incorrect responses.

The selective nAChR antagonist DHBE did not alter accuracy or premature responding at either dose. Other aspects of performance were not altered by DHBE.

Donepezil, which inhibits acetylcholinesterase to increases synaptic ACh concentrations, reduced accuracy at the higher dose of 2 mg/kg at all three cue delays: 3 s ($F_{2, 55} = 14.4$, $p < 0.001$, post-hoc $p < 0.001$), 5 s ($F_{2, 54} = 9.2$, $p < 0.001$, post-hoc $p < 0.001$) and 7 s ($F_{2, 55} = 4.7$, $p = 0.013$, post-hoc $p = 0.013$). Donepezil also had a significant effect on premature responding at cue delays 5 s ($F_{2, 54} = 3.2$, $p = 0.047$) and 7 s ($F_{2, 53} = 9.6$, $p < 0.001$), but on post-hoc analysis only the higher dose of 2 mg/kg remained significant at 7 s ($p = 0.026$). For donepezil trials, the main effect of sex was significant at 7 s cue delay ($F_{1, 28} = 3.5$, $p = 0.02$), but the sex by dose interaction was not. At 7 s, females made less percent premature responses than males (25.1 ± 3.7% vs. 37.7 ± 3.5%). Like MEC, 2 mg/kg donepezil significantly increased omissions, increased perseverative responding, and increased latencies for both correct and incorrect responses. The 4 mg/kg donepezil dose was removed from analysis due to the excessive number of omissions it caused.

Dopaminergic Drug Effects

Data on accuracy and premature responding for AMPH, GBR 12909, FLU, SCH 23390, and eticlopride are presented in Figures 5.2 and 5.3 and Supplemental Table 5.4. Other dependent measures are in Supplemental Table 5.5.

Amphetamine, which increases synaptic dopamine concentrations, significantly decreased accuracy at all 3 cue delays: 3 s ($F_{3, 80} = 3.8$, $p = 0.014$), 5 s ($F_{3, 80} = 7.9$, $p < 0.001$), and 7 s ($F_{3, 79} = 12.9$, $p < 0.001$). Post-hoc analyses were not significantly different between doses at 3 s, but 0.8 mg/kg at 5 s, and 0.4 and 0.8 mg/kg at 7 s significantly decreased accuracy compared to saline (all $p \leq 0.001$). The sex by dose interaction was significant at 3 s ($F_{3, 80} = 3.5$, $p = 0.02$), but there were not differences between sexes at each dose upon post-hoc evaluation. AMPH also significantly increased premature responding compared to saline at both cue delays of 5 s ($F_{3, 79} = 18.0$, $p < 0.001$) and 7 s ($F_{3, 79} = 12.7$, $p < 0.001$) at 0.4 mg/kg ($p = 0.009$ for 5 s, $p < 0.001$ for 7 s) and 0.8 mg/kg ($p < 0.001$ at both cue delays). AMPH did not significantly alter other aspects of performance at the significant doses and cue delays.

The effects of the dopamine transporter inhibitor GBR 12909 were essentially the same as AMPH. The high dose, 10 mg/kg, decreased accuracy at both 5 s ($F_{3, 71} = 11.9$, $p < 0.001$) and 7 s ($F_{3, 71}. = 8.3$, $p < 0.001$) ($p < 0.001$ for both on post-hoc). GBR 12909 also increased premature responding at both cue delay 5 s ($F_{3, 71} = 13.4$, $p < 0.001$) and 7 s ($F_{3, 71} = 8.4$, $p < 0.001$). Post-hoc analysis revealed that 5 mg/kg ($p = 0.039$ at 5 s, $p = 0.021$ at 7 s) and 10 mg/kg ($p < 0.001$ at both 5 and 7 s) were effective. GBR 12909 did not significantly alter other aspects of performance.

The effects of the non-selective DR 1/DR 2 antagonist FLU on accuracy at shorter delays were like those of GBR 12909 and AMPH at longer delays but differed on premature responding. FLU decreased accuracy at 3 s ($F_{3, 79} = 17.5$, $p < 0.001$) and 5 s ($F_{3, 79} = 5.6$, $p = 0.002$) cue delays, but not at 7 s. The 0.15 mg/kg dose decreased accuracy at 3 s ($p = 0.007$), while 0.3 mg/kg decreased accuracy at both 3 s and 5 s ($p \leq 0.001$ for both). The main effect of sex was significant at the 7 s cue delay ($F_{1, 27} = 4.5$, $p = 0.044$). At 7 s, females had greater accuracy than males (78.7 ± 1.3% vs. 74.7 ± 1.3%). Both 0.15 and 0.3 mg/kg decreased premature responding at both 5 s and 7 s ($p < 0.001$ for all). Both 0.15 ($F_{3, 77} = 13.0$, $p < 0.001$) and 0.3 mg/kg ($F_{3, 78} = 23.3$, $p < 0.001$) FLU increased correct and incorrect response latencies, and 0.3 mg/kg also increased omissions and premature responding.

The DR 1-selective antagonist SCH 23390 had a mixed effect on accuracy, depending on cue delay, and decreased premature responding, similar to FLU. At 3 s cue delay, 0.02 mg/kg significantly decreased accuracy ($F_{1, 27} = 5.4$, $p = 0.028$). There was no dose-related effect on accuracy at 5 s. At 7 s, 0.02 mg/kg significantly increased accuracy ($F_{1, 25} = 7.0$, $p = 0.014$). SCH 23390 at 0.02 mg/kg significantly increased premature responding at both 5 s ($F_{1, 24} = 12.5$, $p = 0.002$) and 7 s ($F_{1, 24} = 11.2$, $p = 0.003$). SCH 23390 increased omissions above those seen with saline and increased both correct response and incorrect response latencies. The 0.04 and 0.06 mg/kg doses were excluded from analysis due to the high number of omissions both doses caused.

The DR 2-selective antagonist eticlopride also decreased accuracy, but only at the 3 s cue delay ($F_{3, 76} = 5.0$, $p = 0.003$). The 0.04 mg/kg ($p = 0.025$) and 0.06 mg/kg ($p = 0.03$) doses were both significant on post-hoc analysis. The main effect of sex was significant at

both 3 s ($F_{1, 27}$ = 5.0, p = 0.034) and 5 s ($F_{1, 26}$ = 6.8, p = 0.015). At both delays, females had higher accuracy than males (3 s: 72.9 ± 2.3% vs. 65.6 ± 2.3%; 5 s: 80.8 ± 1.7% vs. 47.6 ± 1.7%). Eticlopride did not significantly affect premature responding. The 0.06 mg/kg dose increased perseverative responding at the 3 s delay and increased correct response latency across all cue delays.

Experiment 2 - Baseline 5-CSRTT Performance

Like the rats in Experiment 1 during the Sustained Attention phase, accuracy increased, and premature responding decreased over the 7 blocks of training. Asymptotic performance, when performance in a block was not statistically different from that in subsequent blocks, was attained by block 4 for attention and block 6 for premature responding (not shown).

During block 7, there was a significant difference in accuracy between cue delays ($F_{2, 56}$ = 6.4, p = 0.003). Accuracy at 3 s and 5 s were not significantly different, but accuracy was significantly higher at 3 s than at 7 s (p = 0.003) and marginally higher at 5 s than at 7 s (p = 0.06). See Table 3 for dependent measures for Experiment 2, block 7. There was also a significant difference between cue delays for premature responding ($F_{1, 28}$ = 330.2, p < 0.001), with there being over twice as many premature responses at 7 s than 5 s, as was seen in Experiment 1. The main effect of sex was not significant for either measure. The latency to incorrect responses was significantly different between cue delays, as seen in Experiment 1, with the average latency being significantly longer at 3 s versus 5 s and 7 s (both $p \leq 0.001$). Other measures were not significantly different between cue delays.

Nicotine and Dopamine Antagonists Combined

Data on accuracy and premature responding for both NIC-dopamine antagonist trials, and AMPH-dopamine antagonist trials, are presented in Figure 5.4 and Table 5.6. Other dependent measures are listed in Supplemental Tables 5.1.7- 5.1.10.

For NIC combined with SCH 23390, the main factor of drug dose did not have a significant effect on accuracy at 3 or 5 s cue delays. There was a significant effect at 7 s ($F_{5, 126} = 3.1$, $p = 0.011$) but significant differences between the drugs were not found on post-hoc analysis (not shown).

Premature responding was significantly affected by dose at both 5 s ($F_{5, 126} = 23.5$, $p < 0.001$) and 7 s ($F_{5, 126} = 33.5$, $p < 0.001$). At both cue delays, NIC increased premature responding above levels seen with saline, while only at 7 s did both SCH 23390 doses and the NIC + 0.02 mg/kg SCH 23390 dose reduce premature responding significantly below levels seen with saline alone (all $p < 0.001$). At both cue delays, both SCH 23390 doses significantly attenuated the effects of NIC on premature responding when given in combination with NIC (all $p < 0.001$). The main effect of sex was significant at 7 s for the NIC-SCH 23390 trials ($F_{1, 26} = 4.9$, $p = 0.036$). Females had less percent premature responses than males (24.5 ± 2.5% vs. 32.4 ± 2.5%). Regarding other measures, 0.02 mg/kg SCH 23390 increased omissions alone and in combination with NIC and increased incorrect response latencies at the shorter cue delays, compared to saline.

In the eticlopride trials, there was a significant main effect of drug dose on accuracy at 3 s cue delay ($F_{5, 123} = 9.0$, $p < 0.001$), but not at 5 s or 7 s. Post-hoc analysis revealed that at 3 s, the 0.06 mg/kg dose of eticlopride, alone and in combination with

NIC, significantly decreased accuracy as compared to saline (p = 0.002 for eticlopride, p < 0.001 for combination) (not shown). The NIC + 0.06 mg/kg eticlopride combination also reduced accuracy (p < 0.001) as compared to NIC alone, which did not affect accuracy (not shown).

For premature responding, the main effect of eticlopride dose was significant at both 5 s ($F_{5, 123}$ = 12.6, p < 0.001) and 7 s ($F_{5, 123}$ = 16.3, p < 0.001) cue delays. Post-hoc analysis revealed that NIC increased premature responding at both delays (both p < 0.001), while only 0.06 mg/kg eticlopride at the 7 s cue delay (p = 0.003) significantly decreased premature responding compared to controls. When combined with NIC, eticlopride significantly attenuated the effects of NIC: 0.03 mg/kg eticlopride at 5 s (p = 0.018) and 0.06 mg/kg eticlopride at both cue delays (p ≤ 0.001 for both). The higher dose of eticlopride also increased omissions across cue delays and inconsistently increased correct and incorrect response latencies at shorter cue delays, as compared to saline.

Amphetamine and Dopamine Antagonists Combined

The effects of AMPH and SCH 23390 on accuracy in the combination trials were limited. The main effect of dose was significant at 3 s ($F_{5, 124}$ = 5.1, p < 0.001) and 7 s ($F_{5, 123}$ = 4.5, p = 0.001) cue delays, but not at 5 s. The post-hoc analysis determined that none of the drugs alone or in combination significantly affected accuracy compared to saline, except for a decrease in accuracy caused by 0.02 mg/kg SCH 23390, which was only at the 3 s cue delay (p = 0.008) (not shown). At 7 s, accuracy was greater when

administered the combination of AMPH + 0.02 mg/kg SCH 23390 versus AMPH alone (p = 0.046) (not shown).

For premature responding, the main effect of dose was significant at both 5 s ($F_{5, 122}$ = 20.4, $p < 0.001$) and 7 s ($F_{5, 123}$ = 25.9, $p < 0.001$). At both cue delays, AMPH significantly increased premature responding ($p < 0.001$ for both), and the AMPH + 0.01 mg/kg SCH 23390 combination was significantly greater than control ($p = 0.001$ at 5 s, $p = 0.002$ at 7 s), while both doses of SCH 23390 significantly attenuated the effect of AMPH on premature responding, 0.02 mg/kg to a greater extent ($p < 0.001$ at 5 s, $p = 0.002$ at 7 s) than 0.01 mg/kg SCH 23390 ($p = 0.005$ at 5 s, $p = 0.027$ at 7 s). For other dependent measures, 0.02 mg/kg SCH 23390 increased omissions and correct response latencies, while 0.01 mg/kg and AMPH had minimal effects.

Eticlopride also had limited effects on accuracy. Only at 5 s was the main effect of dose significant ($F_{3, 76}$ = 5.6, $p = 0.002$), with 0.03 mg/kg eticlopride decreasing accuracy ($p = 0.001$), while AMPH had no significant effect (not shown). The 0.06 mg/kg eticlopride dose was excluded from analysis due to high percent omissions. The 0.03 mg/kg eticlopride dose also increased omissions, but not to the extent that met the criteria for exclusion.

In the eticlopride combination trials, the main effect of dose was significant for premature responding at both 5 s ($F_{3, 73}$ = 17.0, $p < 0.001$) and 7 s ($F_{3, 75}$ = 14.9, $p < 0.001$). AMPH increased premature responding at both cue delays ($p < 0.001$ for both), while AMPH + 0.03 mg/kg eticlopride also significantly differed from control ($p = 0.002$ for 5 s, $p = 0.007$ for 7 s). Eticlopride marginally attenuated the effects of AMPH at 5 s ($p = 0.068$).

5.5 Discussion

The present study examined the effects of cholinergic and dopaminergic drugs on accuracy and premature responding in the 5-CSRTT, where the behavioral measures correspond to attention and impulsive behavior respectively (Robbins, 2002a). Our interest in this project arose when we observed in a separate, unpublished study that a cholinergic manipulation significantly altered premature responding. Strong roles have been established for dopaminergic signaling in the type of motor or action-related impulsivity assessed by the 5-CSRTT (Winstanley, 2011), but less emphasis has been placed on the role of cholinergic signaling. The PFC, nucleus accumbens, and striatum are key regions affecting premature responding and accuracy on the 5-CSRTT. The nicotinic ACh system has critical roles in modulating cognitive functioning in these regions(Havekes et al., 2011; Wallace and Bertrand, 2013) , and nAChRs containing β2 subunits are important for attentional performance(Poorthuis and Mansvelder, 2013), with less known about nAChRs important for response inhibition.

Regarding attention in Experiment 1, NIC did not have an effect at any of the doses (0.1 to 0.5 mg/kg) or cue delays, whereas AMPH and GBR 12909, both of which increase the concentration of dopamine in the synaptic cleft, decreased accuracy at the longer delays (5 and 7 s). Donepezil, which increases the synaptic ACh concentration, reduced accuracy across all cue delays. In contrast, the dopamine antagonists and MEC, which is a general nAChR antagonist, also decreased accuracy, but at the shorter cue delays, particularly 3 s. The DR 1 antagonist SCH 23390 had a biphasic effect, decreasing accuracy at 3 s delay, but increasing it at the 7 s cue delay.

For impulsivity in Experiment 1, NIC, AMPH, and GBR 12909 all increased premature responding at both cue delays (5 and 7 s). The DR 1/DR 2 antagonist FLU, the DR 1 antagonist SCH 23390, and MEC, but not the DR 2 antagonist eticlopride, all decreased premature responding at both cue delays. Donepezil decreased premature responding at the longer cue delay. In Experiment 2, the results of the single drug trials recapitulated the findings in Experiment 1, but not as strongly, and the effects of single doses and combinations on attention were quite limited. However, the effects on premature responding were more robust. Both DR 1 and DR 2 antagonists attenuated the effect of NIC, while only the DR 1 antagonist attenuated the effect of AMPH.

Dopamine-Related Findings

Our findings confirm physiologic roles for dopamine in both attention and response inhibition. In considering the role of dopamine, previous studies have demonstrated that systemic administration of drugs such as AMPH and GBR 12909, which enhance dopaminergic signaling, often have no effect on accuracy as assessed by the 5-CSRTT (Cole and Robbins, 1987; van Gaalen et al., 2006c; Harrison et al., 1997; Koskinen and Sirviö, 2001; Paterson et al., 2011), but when an effect is seen, it is to reduce accuracy (Baarendse and Vanderschuren, 2012). In the few studies where dopamine antagonists affect accuracy, the response is usually limited to DR 1 agonists improving accuracy (van Gaalen et al., 2006c; Hahn et al., 2002a), as opposed to our results. The current findings suggest that there are optimal levels of synaptic dopamine for peak attentional performance. The optimal levels of dopamine vary by cue delay, with decreased accuracy resulting from too little or too much dopaminergic signaling. The influence of

catecholamine signaling resulting in an inverted U-shaped effect on PFC functioning has been well characterized by the Arnsten group (Arnsten and Li, 2005). At the 3 s delay, FLU, SCH 23390, and eticlopride, which impede signaling via antagonism of DR 1, DR 2, or both, all impaired attention, suggesting that the level of dopaminergic signaling was reduced below the optimal point. At the longer cue delays, both AMPH and GBR 12909 also impaired attention, suggesting that these drugs increased the level of dopaminergic signaling above the point optimal for attentional performance. SCH 23390 increased accuracy at 7 s, suggesting that the baseline level of dopaminergic signaling at 7 s was supra-optimal and was reduced to a more optimal level by DR 1 antagonism. In line with this, at 7 s delay, the relative attentional load was highest, so phasic catecholamine release in the PFC was heightened (Arnsten and Pliszka, 2011). A model developed by Dreyer et al. shows that phasic bursts of dopamine release are particularly associated with higher DR 1 occupancy (Dreyer et al., 2010). This suggests why the DR 1 antagonist, SCH 23390, was more efficacious than the DR 2 antagonist at improving attention at the longest cue delay.

Consistent with prior studies, systemic AMPH and GBR 12909 both increased impulsivity (Baarendse and Vanderschuren, 2012; Cole and Robbins, 1987; van Gaalen et al., 2006c; Harrison et al., 1997; Paterson et al., 2011), which aligns with what is known about the role of dopaminergic signaling in facilitating impulsive action in rodent models (Jupp and Dalley, 2014). Also consistent with most of prior studies was the decrease in premature responding caused by DR 1 antagonists in both experiments (Fleckenstein et al., 2007; van Gaalen et al., 2006c; Hahn et al., 2002a; Harrison et al., 1997) and the ability of systemic SCH 23390 to attenuate the effects of AMPH (van

Gaalen et al., 2006c). Prior studies report an equivocal effect of DR 2 antagonists on premature responding (van Gaalen et al., 2006c; Hahn et al., 2002a; Harrison et al., 1997; Koskinen and Sirviö, 2001), while our findings of a minimal effect of eticlopride alone, or on the effects of AMPH, match the findings of a comparable study (van Gaalen et al., 2006c). The effects of AMPH and GBR 12909 are unlikely to solely be due to indirect DR agonism alone because systemic administration of either a DR 1 agonist or a DR 2 agonist decreased impulsivity, rather than increased it, in a study by Winstanley et al. (Winstanley et al., 2010). Rather, systemic effects of drugs such as AMPH, which work through broader mechanisms than specifically targeting receptors (Fleckenstein et al., 2007), are due to a combination of site-specific effects (e.g., PFC, striatum), receptor-mediated effects, and effects on additional neurotransmitter systems including noradrenergic and serotonergic systems (Jupp and Dalley, 2014; Winstanley, 2011).

Our findings for the AMPH-DR antagonist trials in Experiment 2 argue for a stronger role of DR 1 signaling in mediating the effects of AMPH in our model, given that that both SCH 23390 doses effectively attenuated the effect of AMPH to increase premature responding. However, visual examination of Figure 4 suggests that 0.03 mg/kg eticlopride also attenuated the effect of AMPH, but not to the extent that there was a significant difference from AMPH alone. It is possible that a significant effect would have been seen with 0.06 mg/kg eticlopride, but that dose was omitted from analysis due to a high omission rate. These findings parallel those of van Gaalen et al (van Gaalen et al., 2006c). In that study, which used DR antagonist doses identical to ours and 0.5 mg/kg AMPH (as opposed to 0.6 mg/kg), 0.02 mg/kg SCH 23390 and 0.06 mg/kg eticlopride both significantly attenuated the effect of AMPH, but eticlopride did so to a lesser extent

than SCH 23390. This further serves to confirm that although differential responses to DR 1 and DR 2 agonists and antagonists can occur in targeted brain regions (e.g., SCH 23390 but not eticlopride decreased premature responding when infused into the nucleus accumbens (Pattij et al., 2007), the sum of effects of systemically-administered dopaminergic agents on impulsivity in the 5-CSRTT is modulated by multiple DR subtypes.

Nicotinic Acetylcholine-Related Findings

We determined that NIC did not affect attention in our model. Some prior studies have demonstrated that NIC improves accuracy (Hahn and Stolerman, 2002; Hahn et al., 2011; Stolerman et al., 2000), while others found no effect (Blondel et al., 1999, 2000; Day et al., 2007; van Gaalen et al., 2006c; Ruotsalainen et al., 2000). There are methodological aspects of the version of the 5-CSRTT we used that may have contributed to a lack of effect on accuracy by NIC. Blondel et al. showed that a single NIC dose increased accuracy, but that the effect on accuracy was lost with as little as 2 additional NIC doses within one week's time (Blondel et al., 1999). The loss of effect on accuracy persisted with subsequent NIC injections over multiple weeks (Blondel et al., 1999; Stolerman et al., 2009). We chose to begin both experiments by injecting NIC for 3 consecutive days to avoid generalized disruptions in performance reported when NIC is first administered to naïve animals (Stolerman et al., 2009), which may have contributed to the lack of effect of NIC on accuracy in our study. Another potential factor is that we used variable cue delays within the same session on training and drug injection days. In a study by Hahn et al., NIC administered with fixed cue delays

resulted in a dose-dependent increase in accuracy, but NIC with variable due delays had minimal effect on accuracy (Hahn and Stolerman, 2002).

An additional contributing factor is that the strain we used, Long-Evans rats, may be relatively insensitive to the effects of NIC on attention as assessed by the 5-CSRTT. While acquisition and baseline performance of the 5-CSRTT can vary between strains (Auclair et al., 2009; Didriksen Michael and Christensen Anne Vibeke, 2009), strain-related differences in response to NIC have also been reported (Hahn et al., 2016; Mirza and Bright, 2001). A recent study by Hahn et al. compared the responses of Long-Evans, Sprague-Dawley, and Wistar rats to NIC on 5-CSRTT performance. At doses up to 0.2 mg/kg NIC, and under conditions of variable cue delay, NIC had a minimal effect on correct responses in the Long-Evans and Sprague-Dawley strains, while NIC improved attention in the Wistar strain, like our findings in Long-Evans rats.

Despite absence of an effect of NIC, the general nAChR antagonist MEC reduced accuracy at the shorter cue delays of 3 and 5 s, like the findings with the dopamine antagonists. While the majority of prior 5-CSRTT studies report no effect of MEC on accuracy (Blondel et al., 2000; Hahn et al., 2016; Ruotsalainen et al., 2000; Stolerman et al., 2000), at least 2 other studies also reported that MEC reduced accuracy (Grottick and Higgins, 2000; Jones et al., 1995). At the same time, we determined that the subtype-selective nAChR antagonist DHBE did not affect accuracy, which aligns with the findings of other prior studies (Blondel et al., 2000; Grottick and Higgins, 2000; Hahn et al., 2011).

In contrast, NIC increased premature responding, which was a finding in several other studies (Blondel et al., 1999, 2000; van Gaalen et al., 2006c; Hahn et al., 2002b;

Stolerman et al., 2000), including in the Long-Evans strain (Hahn et al., 2016), but not in all studies (Grottick et al., 2003; Hahn and Stolerman, 2002; Hahn et al., 2011; Mirza and Stolerman, 2000; Ruotsalainen et al., 2000). Our 5-CSRTT methodology may have enhanced our ability to detect a NIC response. The study by Hahn et al. demonstrated that a variable cue delay, which we employed in the current study, greatly enhanced premature responding in response to NIC as compared to a fixed cue delay (Hahn and Stolerman, 2002). Additionally, Mirza and Stolerman reported that as cue delay was increased from 1 s to 20 s, the ability of NIC to increase premature responding became more prominent (Mirza and Stolerman, 1998). This suggests that although our cue delays of 5 and 7 s were not exceedingly long, they were long enough to make it more likely NIC would elicit an effect.

Opposite to the effect of NIC, a decrease in premature responding, was seen with MEC, similar to several prior studies (Grottick and Higgins, 2000; Mirza and Stolerman, 2000; Ruotsalainen et al., 2000; Stolerman et al., 2000), but again not all (Blondel et al., 2000; Jones et al., 1995). The specific nAChR antagonist DHBE did not influence impulsivity, which parallels other studies (Blondel et al., 2000; Grottick and Higgins, 2000; Hahn et al., 2011).

The concordance of our NIC results with those of AMPH and GBR 12909, and the MEC results with those of the DR antagonists, leads to consideration of the interaction between the nicotinic and dopaminergic systems in 5-CSRTT performance. Nicotine interacts with the dopaminergic system via nAChRs, a family of ligand-gated, pentameric cation channels constituted from combinations of α and β subunits expressed in the mammalian brain (Gotti et al., 2006). It has been shown that nAChRs

are present on midbrain dopaminergic cell bodies in the ventral tegmental area (VTA) and substantia nigra (SN), and on the axonal terminals of the dopamine neuronal projections to the dorsal striatum, nucleus accumbens, and PFC (Livingstone and Wonnacott, 2009b). Thus, systemic administration of NIC stimulates nAChRs on dopaminergic cell bodies in the midbrain, and on dopaminergic terminals in the PFC, nucleus accumbens, and striatum, leading to an increase in dopamine release in these target regions, and a subsequent increase in impulsive behavior (Livingstone and Wonnacott, 2009b; Marshall et al., 1997; Ohmura et al., 2012).

Of the different nAChRs that mediate these effects, receptors containing α4β2 subunits have important roles in modulating dopamine-release in all of the aforementioned brain regions, while nAChRs containing other subunits such as α7 or β5 have roles in some, but not all of these regions (Cao et al., 2005; Gotti et al., 2006; Livingstone and Wonnacott, 2009b; Livingstone Phil D. et al., 2009; Pistillo et al., 2015). The current literature points to ACh in the PFC, particularly the medial PFC (mPFC), as being critical for attentional processes, including those assessed by the 5-CSRTT. Acetylcholine is released in the mPFC during 5-CSRTT performance (Dalley et al., 2001), with phasic ACh release being associated with signal detection (Sarter et al., 2016). In the PFC, α4β2-containing NAChRs facilitate increased cholinergic tone, and thus enhance attention, while nAChRs with α7 subunits control the duration of ACh release (Wallace and Bertrand, 2013). However, evidence for a relationship between cholinergic and dopaminergic signaling in the mPFC driving attentional processes is not as strong as it is for a similar relationship in striatal regions. A recent study by Boekhoudt et al. demonstrated that chemogenetic activation of dopamine neurons in the

SN, which project to the striatum and nucleus accumbens, impaired accuracy on the 5-CSRTT; activation of dopamine neurons in the VTA, which project to the PFC, did not impair accuracy, although omissions were increased, demonstrating more subtle effects on attention (Boekhoudt et al., 2017). Furthermore, Marshall et al. demonstrated that direct application of NIC to the PFC, nucleus accumbens, and striatum increased dopamine release in each of the 3 regions, but to a much lesser extent in the PFC (Marshall et al., 1997).

Thus, although nAChRs modulate dopamine release in the mPFC (Livingstone and Wonnacott, 2009b), effects of drugs on cholinergic or dopaminergic signaling alone more strongly mediate effects on attention then an interaction between the cholinergic and dopaminergic systems. As discussed above, methodological aspects of our 5-CSRTT experiments likely created a situation where the effects of NIC on attention were weak, but the effects of MEC and the DR antagonists either on dopamine release alone, or due to ACh-mediated dopamine release, were strong enough to negatively impact attentional processes. Regarding the lack of effect of the specific nAChR antagonist DHBE as compared to an effect by the general antagonist MEC, both drugs overlap in targeting several nAChRs including those with β2 subunits, while a primary difference between the two drugs is that MEC targets α7 nAChRs and DHBE does not (Chavez-Noriega et al., 1997) . Thus, under our experimental circumstances, we suspect that the complementary effects of both α4β2 and α7 nAChRs needed to be inhibited for an effect on attention to be evident.

The relationship between cholinergic and dopaminergic signaling for influencing impulsive responding on the 5-CSRTT is better established. Both mPFC and

dorsomedial striatum are important regions in the circuit for premature responding, while the nucleus accumbens is an even more critical component (Cassaday et al., 2014; Eagle and Baunez, 2010; Pattij et al., 2007). There are regional differences in the influence of DR 1 or DR 2 on premature responding: DR 1 more strongly mediates premature responding in the nucleus accumbens (Pattij et al., 2007; Pezze et al., 2007), while DR 2 plays a more prominent role in the dorsal striatum (Agnoli et al., 2013). As discussed above, nAChRs modulate dopamine release in all of these regions (Livingstone and Wonnacott, 2009b), and NIC causes dose-dependent dopamine release in the same regions, although to a greater extent in the nucleus accumbens and striatum mPFC (Marshall et al., 1997).

Considering the above, our findings support the role of an ACh-dopamine relationship in mediating impulsive behavior in our model of the 5-CSRTT: NIC increased premature responding (Experiments 1 and 2), MEC decreased premature responding (Experiment 1), and both DR 1 and DR 2 antagonists attenuated the effects of NIC on premature responding, with SCH 23390 appearing to have a stronger effect than eticlopride at the doses we used (Experiments 1 and 2). Our results suggest that at both cue delays of 5 and 7 s, NIC caused sufficient dopamine release to increase impulsivity above baseline levels. Concurrently, a decrease in nAChR-mediated dopamine release by MEC limited the effect of baseline levels of dopamine on impulsivity, and direct antagonism of DRs by SCH 23390 or eticlopride, limited the effect of NIC-elevated dopamine.

Donepezil-Related Findings

In our model, 2 mg/kg donepezil, but not 0.5 mg/kg, decreased attention across all cue delays, although more strongly at 3 and 5 s delays, and decreased impulsive responding at 7 s. Two prior studies employing the 5-CSRTT did not demonstrate that donepezil affected accuracy or premature responding, but the highest donepezil dose in both studies was 1 mg/kg, which may not have been high enough to elicit effects (Balducci et al., 2003; Kirkby et al., 1996).

The pattern of effects resembles those caused by the nAChR antagonist MEC and the DR antagonist FLU. This was unexpected given that donepezil has been shown at the same 2 mg/kg dose to inhibit acetylcholinesterase by close to 100%, and to substantially increase ACh (2100%) and dopamine (80%) concentration in dialysate from the frontal cortex (Giacobini et al., 1996). One possible explanation is that excessive release of ACh, dopamine, or both, resulted in neurotransmitter levels that were higher than required for optimal attention or response inhibition. As discussed above, behavioral responses often follow an inverted U-shaped curve, initially improving and then deteriorating as synaptic ACh (Newman and Gold, 2016) or dopamine (Arnsten and Li, 2005) levels increase. A study by Zhang et al. reports a potential mechanism that might mediate the effect of increased synaptic ACh by donepezil (Zhang et al., 2004). The authors discuss how cholinergic neurons in the striatum fire tonically, which enhances striatal dopamine release via nAChRs on the post-synaptic dopamine terminals and optimizes dopamine-related behavioral performance. Normally acetylcholinesterase quickly hydrolyzes synaptic ACh and prevents nAChRs from becoming desensitized. However, if the dose of donepezil is high enough, AChE is fully

inhibited resulting in continuously elevated synaptic ACh. This, in turn, desensitizes striatal nAChRs and causes diminished dopamine release. Given the higher dose of donepezil that was required to see effects in our study, as compared to the lower therapeutic doses used for human patients, the above explanation is plausible. Additionally, 5 mg/kg donepezil has been shown to decrease dopaminergic neuronal firing rate in the VTA, which was mediated by an effect on muscarinic AChRs (Schilström et al., 2007). A separate study reported that donepezil facilitated nAChR desensitization in dopaminergic neurons in the SN via allosteric modulation of nAChRs (Angelantonio et al., 2004). These additional off-target effects of donepezil may have also contributed to our findings. In retrospect, our expectations that donepezil would improve attention and response inhibition may have been unfounded given that in rodent models the cognitive-enhancing effects of acetylcholinesterase inhibitors, such as donepezil, have mainly been reported in subjects with experimentally-induced hypocholinergic states (Braida et al., 1996; Flood et al., 1983).

Based on our findings, we propose that our effects on impulsive behavior were due to an increase in synaptic dopamine levels via nAChR binding by NIC. An interaction between the cholinergic and dopaminergic systems via nACHRs would also explain why the dopamine antagonists ameliorated the effects of NIC and AMPH on premature responding. The consistent effects seen with SCH 23390 in our study indicate that DR 1 played a more significant role in modulating impulsive behavior, but that DR 2 also had roles in impulsivity and attention.

Our methodology for the 5-CSRTT appears to be effective at eliciting drug effects on premature responding, while it is less effective for evaluating effects on attention. This

may be in part due to the use of varied cue delays during testing sessions, as opposed to the practice of many other research groups to use only one cue delay per session. Our choice of the Long-Evans strain, which is infrequently used in 5-CSRTT studies, may have also contributed to our ability to readily discern changes in premature responding. Regardless of the reason, the drugs used in this study establish the effective doses at which they elicit differences in behavior in our model, and thus will be used in our future studies.

In conclusion, we have shown that both D1 and D2 receptors are involved in regulating impulsive behavior when there is an enhancement of DA neurotransmission. The present data demonstrate that an interaction between cholinergic and dopaminergic neurotransmission is critical to mediate inhibitory control, but we need to explore further the underlying neural and psychological mechanisms to identify targets for treatment strategies when there are deficits in response inhibition.

5.6 Figures and Tables

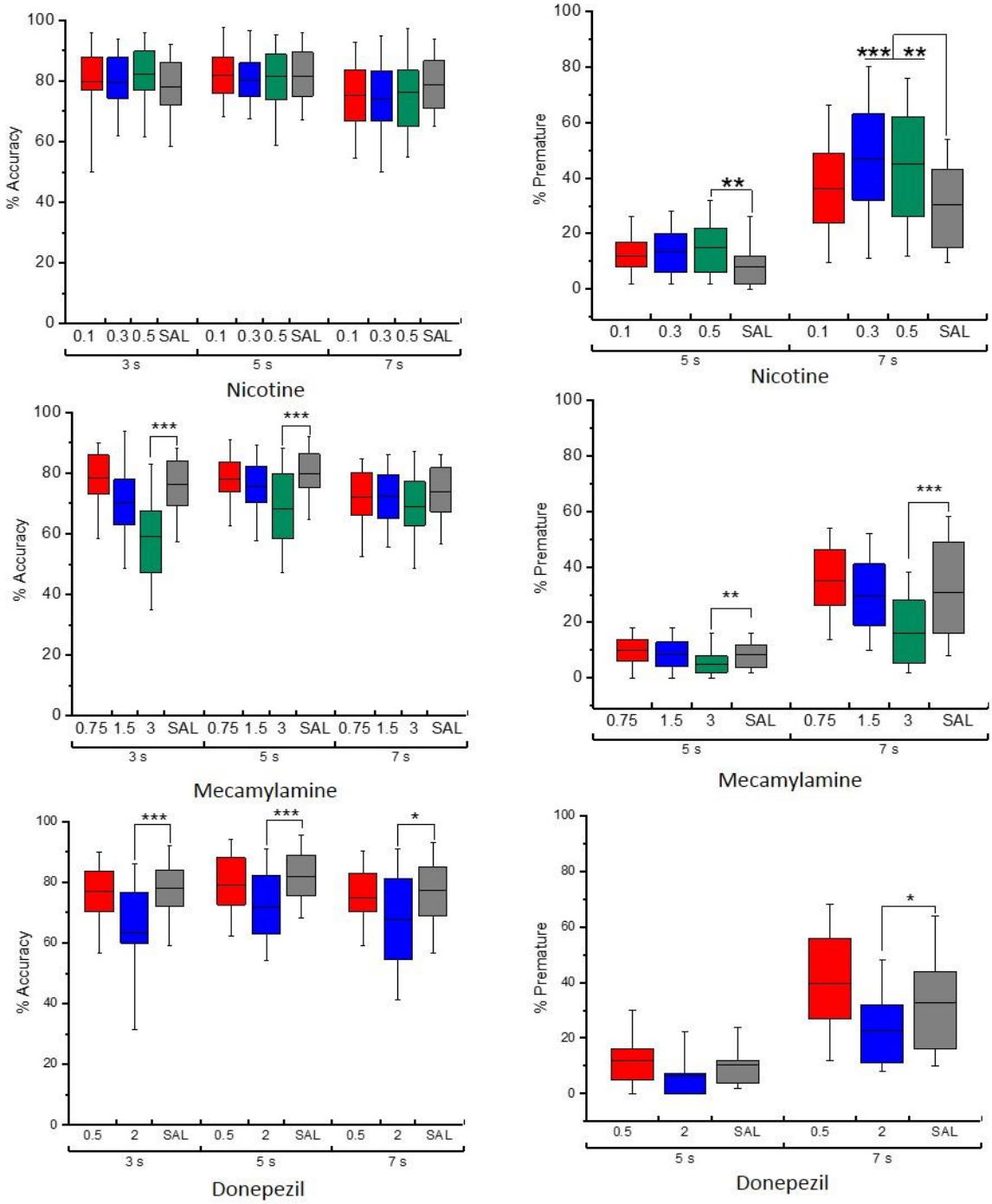

Figure 5.1. Performance on 5-CSRTT at different cue delays after administering cholinergic drugs. Nicotine did not alter accuracy but increased premature responding at both cue delays. Mecamylamine decreased accuracy and premature responding at the highest dose. Donepezil decreased accuracy at all three cue delays at the higher dose of 2mg/kg. Results for DHBE are not shown as it did not affect accuracy and premature responding. All doses are in mg/kg. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 compared to saline control

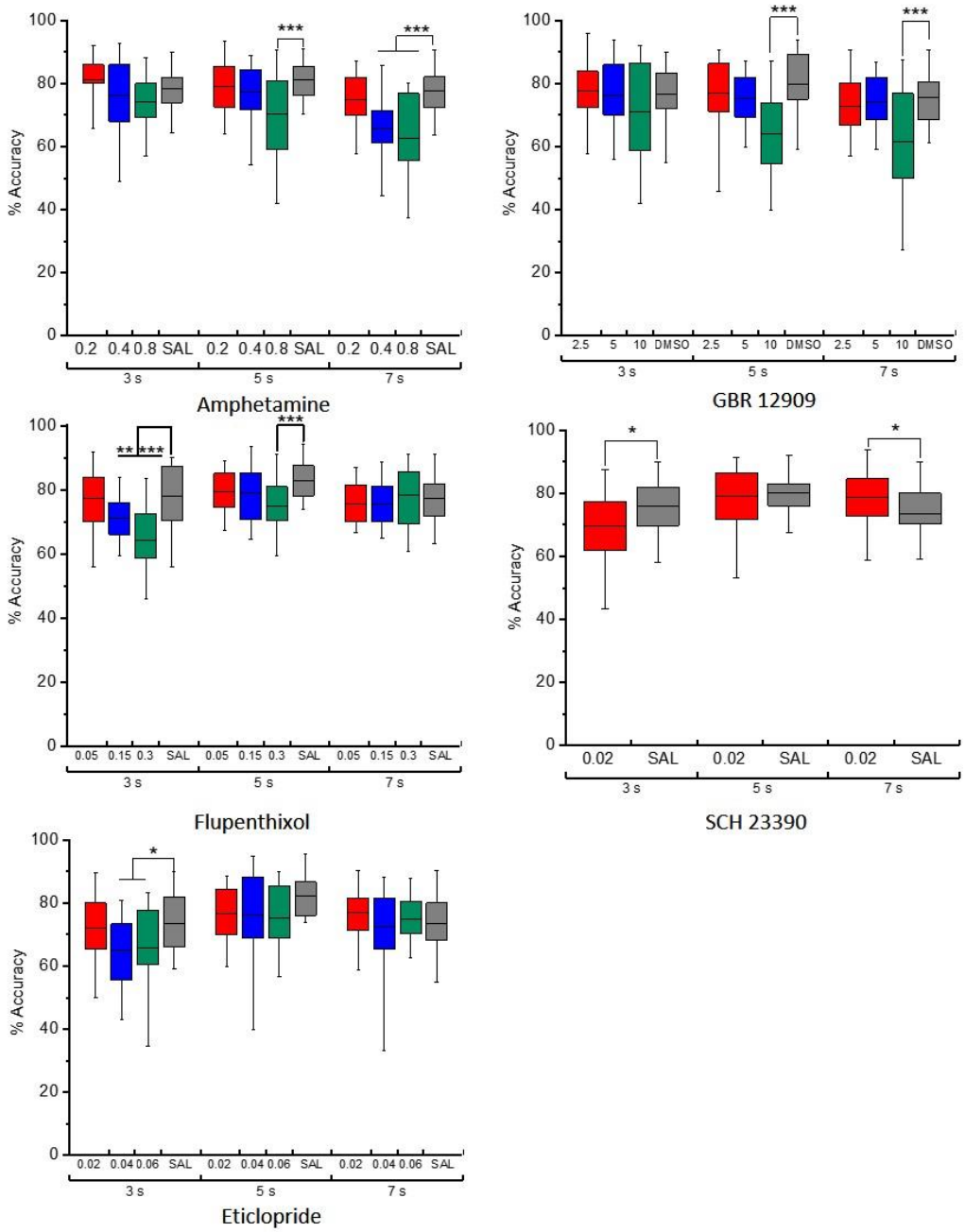

Figure 5.2. Accuracy after administering dopaminergic drugs. Amphetamine and GBR 12909 decreased accuracy at the higher cue delays of 5 s and 7 s. Flupenthixol decreased accuracy at cue delays of 5 and 7 s. SCH 23390 decreased accuracy at the lower cue delay of 3 s and increased accuracy at 7 s. Eticlopride had a minimal effect on accuracy.

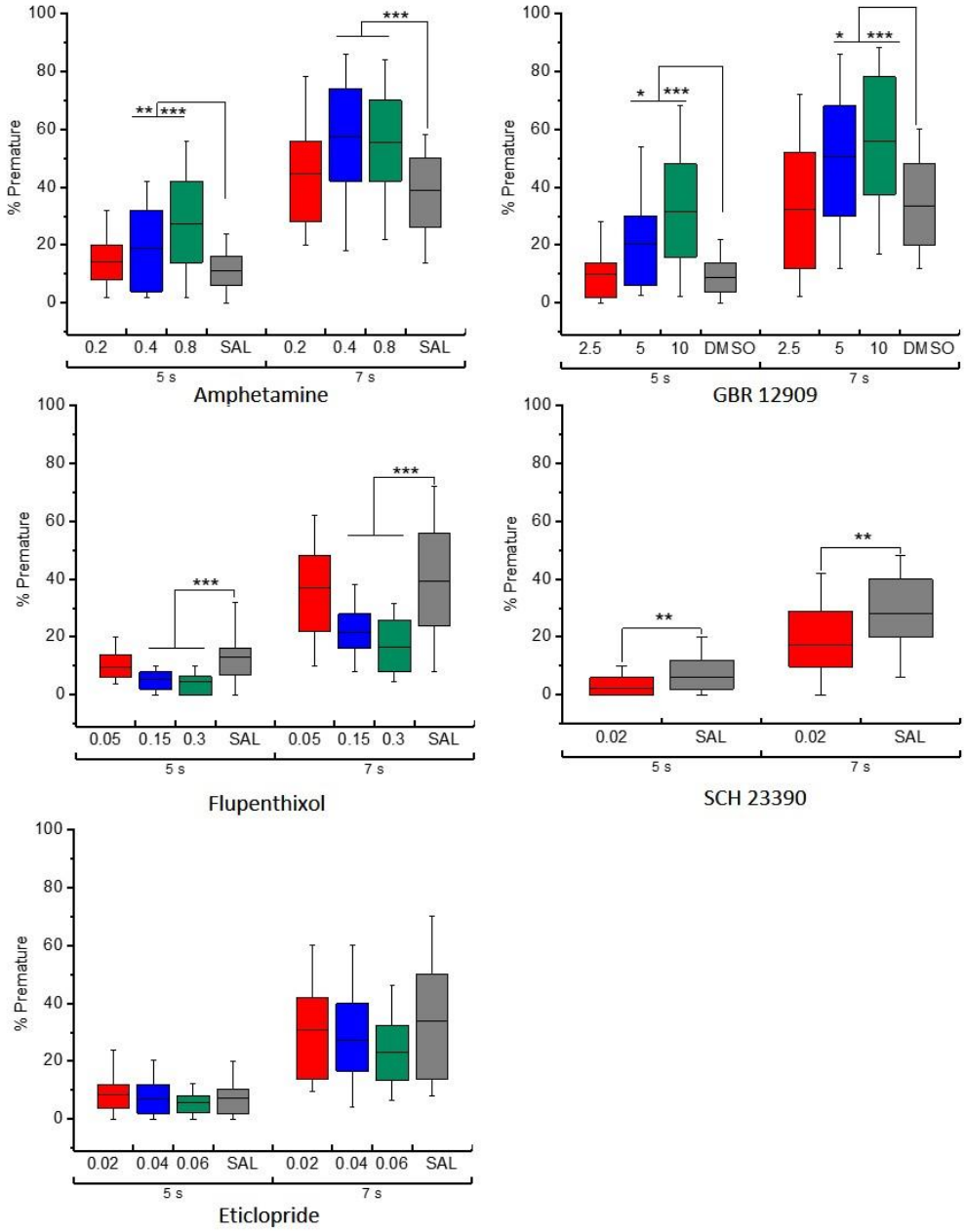

Figure 5.3. Premature responding after administering dopaminergic drugs. Amphetamine and GBR 12909 increased premature responding at both delays of 5 and 7 s, while flupenthixol and SCH 23390 decreased premature responding at both delays. Eticlopride did not alter premature responding. All doses are in mg/kg. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ compared to saline control.

Drug	Mechanism	Doses (mg/kg)
Nicotine	nAChR agonist	0.5 x 3 sessions
Nicotine	nAChR agonist	0.1, 0.3, 0.5
Donepezil	AChE inhibitor	0.5, 2, 4
Mecamylamine	Non-selective nAChR antagonist	0.75, 1.5, 3
Dihydro-β-erythroidine	Subunit-selective[a] nAChR antagonist	6, 9
Amphetamine	Increases synaptic dopamine levels	0.2, 0.4, 0.8
GBR 12909	Dopamine transporter inhibitor	2.5, 5, 10
Flupenthixol	Non-selective DR antagonist	0.05, 0.15, 0.3
SCH 23390	DR 1 antagonist	0.02, 0.04, 0.06
Eticlopride	DR 2 antagonist	0.02, 0.04, 0.06

Table 5.1. Drugs, mechanisms of action, and doses administered in Experiment 1

Drugs	Doses (mg/kg)
Nicotine	0.5 x 3 sessions
Nicotine/SCH 23390	0.5/0.01, 0.02
Nicotine/Eticlopride	0.5/0.03, 0.06
Amphetamine/SCH 23390	0.6/0.01, 0.02
Amphetamine/Eticlopride	0.6/0.03, 0.06

Table 5.2. Drug combinations and doses administered in Experiment 2.

	% Accuracy			% Premature		% Omission			Average Perseverative Response			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
	Cue Delay			Cue Delay		Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
	3 s	5 s	7 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s
Block 7, Experiment 1	77.5 ± 1.7	76.9 ± 1.9	75.7 ± 2.1	16.6 ± 1.8	**41.4 ± 3.2 †††**	3.9 ± 0.7	3.2 ± 0.5	3.7 ± 0.6	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	1.12 ± 0.05	0.97 ± 0.03	0.99 ± 0.05	4.51 ± 0.22	4.13 ± 0.25	**3.31 ± 0.2 † ^^^**	1.84 ± 0.14	1.93 ± 0.2	1.81 ± 0.1
Block 7, Experiment 2	77.6 ± 1.9	75.9 ± 1.9	**72.2 ± 1.8 ^^**	14.5 ± 1.7	**36.3 ± 2.1 †††**	3.0 ± 0.7	2.7 ± 0.7	3.3 ± 0.8	0.09 ± 0.03	0.06 ± 0.01	0.08 ± 0.03	1.08 ± 0.05	1 ± 0.05	1.03 ± 0.05	5 ± 0.21	**4.16 ± 0.19 *****	**3.66 ± 0.23 ^^^**	1.46 ± 0.08	1.41 ± 0.06	1.51 ± 0.11

Table 5.3. Dependent measures from Block 7 of the 21 days of the sustained attention phase for both experiments, with cue delay as a main factor. Premature responding was significantly greater at the longest cue delay of 7 s in both experiments. Latency to make incorrect responses was significantly lower at the 7 s delay. *Compares cue delay 3 s to 5 s. ^Compares 3 s to 7 s. †Compares 5 s to 7 s. * ^ †p ≤ 0.05, ** ^^ ††p ≤ 0.01, *** ^^^ †††p ≤ 0.001

Drugs	Dose (mg/kg)	Time before testing	% Accuracy			% Premature	
			Cue Delay			Cue Delay	
			3 s	5 s	7 s	5 s	7 s
Nicotine	0.1	20	79.9 ± 2.2	81.8 ± 1.82	75.0 ± 2.3	11.8 ± 1.4	37.1 ± 3.5
	0.3		79.3 ± 2.1	80.2 ± 2.2	74.0 ± 2.5	13.27 ± 1.51	**46.9 ± 3.9 *** **
	0.5		81.7 ± 1.9	81.4 ± 1.9	76.5 ± 2.4	**14.6 ± 1.8 ****	**44.2 ± 4.0 ****
Vehicle	0	20	78.1 ± 2.2	81.4 ± 1.9	78.54 ± 1.79	8.1 ± 1.3	30.4 ± 2.9
Mecamylamine	0.75	30	78.3 ± 1.7	77.9 ± 1.5	72.1 ± 2.0	9.9 ± 1.1	35.1 ± 2.7
	1.5		70.2 ± 2.3	75.7 ± 1.7	72.5 ± 1.8	8.6 ± 1	29.5 ± 2.7
	3		**58.9 ± 2.8 *** **	**68.2 ± 2.5 *** **	68.9 ± 2.3	**5.1 ± 0.9 ***	**16.3 ± 2.2 *** **
Vehicle	0	20	76.3 ± 1.9	79.9 ± 1.5	73.9 ± 1.9	8.5 ± 0.9	30.9 ± 3.2
DHBE	6	10	78.9 ± 1.9	80.9 ± 1.6	73.4 ± 1.6	10.7 ± 1.5	36.0 ± 2.9
	9		79.1 ± 1.7	80.9 ± 1.3	77.2 ± 1.4	9.1 ± 1.3	34.6 ± 3.7
Vehicle	0	20	76.8 ± 1.7	79.05 ± 1.5	73.7 ± 1.9	8.9 ± 1.4	29.5 ± 3.1
Donepezil	0.5	30	77 ± 1.8	79.1 ± 1.9	74.7 ± 1.8	12.0 ± 1.6	39.6 ± 3.8
	2		**62.7 ± 3.47*** **	**71.4 ± 2.7 *** **	**65.5 ± 4.1 ***	5.9 ± 1.9	**20.2 ± 3.5 ***
Vehicle	0	20	76.24 ± 2.6	81.3 ± 1.6	76.9 ± 2.14	10 ± 1.5	31.6 ± 3.5

Table 5.4. Effects of cholinergic drugs on attention and impulsive behavior in Experiment 1. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ compared to vehicle control. The highest dose of donepezil of 4 mg/kg was not included in the analysis due to omissions > 50%.

Drugs	Dose (mg/kg)	Time before testing (min)	% Omissions			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
			Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
			3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s
Nicotine	0.1	20	2.8 ± 1.1	2.2 ± 0.8	1.7 ± 0.6	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0	1.13 ± 0.07	0.91 ± 0.05	0.86 ± 0.05	4.52 ± 0.23	3.3 ± 0.39	2.78 ± 0.31	2.1 ± 0.33	1.9 ± 0.27	2.51 ± 0.84
	0.3		4.0 ± 1.4	3.3 ± 1.3	3.5 ± 1.6	0.03 ± 0	0.03 ± 0.01	0.09 ± 0.05	1.07 ± 0.06	1.02 ± 0.18	0.94 ± 0.2	4.23 ± 0.35	2.69 ± 0.39	2.58 ± 0.35	2.38 ± 0.39	2.38 ± 0.44	2.24 ± 0.34
	0.5		3.3 ± 1.3	2.2 ± 0.9	2.7 ± 1.1	0.02 ± 0	0.02 ± 0	0.05 ± 0.01	**1 ± 0.04 ***	0.78 ± 0.03	0.75 ± 0.05	4.34 ± 0.29	3.7 ± 0.48	2.29 ± 0.31	1.86 ± 0.17	1.94 ± 0.29	2.2 ± 0.41
Veh.	0	20	2.4 ± 0.9	2.2 ± 0.7	2.0 ± 0.7	0.02 ± 0	0.02 ± 0	0.02 ± 0	1.26 ± 0.11	0.9 ± 0.05	0.83 ± 0.05	4.91 ± 0.26	3.66 ± 0.31	2.46 ± 0.31	1.67 ± 0.08	1.9 ± 0.23	1.85 ± 0.14
Mecamylamine	0.75	30	3.5 ± 1.5	3.0 ± 1.0	1.7 ± 0.5	0.05 ± 0.01	0.04 ± 0	0.04 ± 0	1.47 ± 0.13	0.97 ± 0.05	0.95 ± 0.08	4.59 ± 0.17	3.41 ± 0.31	2.5 ± 0.25	1.59 ± 0.07	1.74 ± 0.15	1.82 ± 0.13
	1.5		5.4 ± 1.2	3.6 ± 0.6	3.2 ± 0.7	0.04 ± 0	0.04 ± 0	0.04 ± 0	1.61 ± 0.13	1.07 ± 0.07	1.09 ± 0.09	5.77 ± 0.32	4.42 ± 0.37	3.52 ± 0.37	2.04 ± 0.24	2.43 ± 0.46	2.18 ± 0.4
	3		**18.7 ± 2.5 *** **	**15.7 ± 2.5 *** **	**12.6 ± 2.1 *** **	0.07 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	**2 ± 0.14 ** **	**1.55 ± 0.12 *** **	**1.34 ± 0.11 *** **	**7.15 ± 0.29 *** **	**6.59 ± 0.39 *** **	**5.09 ± 0.34 *** **	1.85 ± 0.11	2.22 ± 0.21	2.82 ± 0.89
Veh.	0	20	3.5 ± 1.1	2.0 ± 0.7	2.1 ± 0.6	0.06 ± 0.01	0.05 ± 0.01	0.03 ± 0	1.4 ± 0.09	1.01 ± 0.06	0.84 ± 0.04	5.35 ± 0.24	3.92 ± 0.32	2.6 ± 0.3	2.55 ± 0.57	2.07 ± 0.24	2.18 ± 0.25
DHBE	6	10	2.5 ± 0.9	1.2 ± 0.5	1.5 ± 0.6	0.04 ± 0	0.04 ± 0.01	0.04 ± 0.01	1.25 ± 0.09	0.92 ± 0.04	0.82 ± 0.06	4.79 ± 0.22	3.61 ± 0.41	2.88 ± 0.33	1.81 ± 0.14	2.01 ± 0.2	2.16 ± 0.32
	9		2.7 ± 1.0	2.8 ± 0.8	2.8 ± 1.1	0.03 ± 0	0.05 ± 0.01	0.04 ± 0	1.26 ± 0.07	0.91 ± 0.05	0.78 ± 0.04	4.6 ± 0.23	3.84 ± 0.32	2.27 ± 0.29	1.76 ± 0.2	1.66 ± 0.09	**1.68 ± 0.1 ***
Veh.	0	20	2.4 ± 0.8	1.8 ± 0.6	2.9 ± 0.9	0.04 ± 0	0.04 ± 0	0.03 ± 0	1.36 ± 0.07	1 ± 0.05	0.9 ± 0.06	5.15 ± 0.33	3.22 ± 0.23	2.24 ± 0.3	1.65 ± 0.1	1.65 ± 0.09	1.8 ± 0.23
Donepezil	0.5	30	4.0 ± 0.8	3.3 ± 1.0	2.3 ± 0.9	0.03 ± 0	0.04 ± 0.01	0.02 ± 0	1.26 ± 0.09	0.95 ± 0.06	0.83 ± 0.05	4.95 ± 0.28	3.97 ± 0.33	2.83 ± 0.28	1.69 ± 0.11	1.93 ± 0.17	1.82 ± 0.2
	2		**19.9 ± 5.2 *** **	**16.7 ± 4.9 *** **	**14.1 ± 4.5 ** **	**0.13 ± 0.04 ***	**0.12 ± 0.04 ***	0.07 ± 0.04	**1.94 ± 0.22 *** **	**1.44 ± 0.18 ** **	**1.03 ± 0.06 ** **	6.38 ± 0.4	4.76 ± 0.42	4.28 ± 0.42	**7.22 ± 3.81 ***	2.86 ± 0.53	**4.4 ± 1.61 ** **
Veh.	0	20	3.9 ± 1.2	2.1 ± 0.8	2.9 ± 0.8	0.03 ± 0	0.03 ± 0	0.02 ± 0	1.42 ± 0.24	1.03 ± 0.1	0.79 ± 0.04	5.37 ± 0.4	3.73 ± 0.36	2.89 ± 0.37	1.78 ± 0.13	1.78 ± 0.13	1.8 ± 0.16

Table 5.5. Effects of cholinergic drugs on other dependent measures of 5-CSRTT performance in Experiment 1. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ compared to vehicle (Veh.) control.

Drugs	Dose (mg/kg)	Time before testing	% Accuracy			% Premature	
			Cue Delay			Cue Delay	
			3 s	5 s	7 s	5 s	7 s
	0.2		81.1 ± 1.7	79.0 ± 1.6	74.9 ± 1.8	14.1 ± 1.7	44.6 ± 3.4
Amphetamine	0.4	10	76.4 ± 2.4	77.4 ± 2.1	**65.8 ± 2.3 *****	**18.8 ± 2.7 ****	**57.3 ± 4.0 *****
	0.8		74.1 ± 2.1	**70.5 ± 2.7 *****	**62.8 ± 3.2 *****	**27.4 ± 3.2*****	**55.3 ± 3.8*****
Vehicle	0	20	78.2 ± 1.5	81.0 ± 1.3	77.6 ± 1.5	11.2 ± 1.3	38.7 ± 3.0
	2.5		77.6 ± 2.4	78.3 ± 2.2	73.1 ± 2.3	10.5 ± 1.87	34.7 ± 4.7
GBR 12909	5	20	76.2 ± 2.5	75.2 ± 1.8	74.1 ± 2.1	**20.3 ± 3.3 ***	**50.2 ± 4.9 ***
	10		70.9 ± 3.1	**63.8 ± 2.8 *****	**61.7 ± 3.5 *****	**31.5 ± 4.1 *****	**55.9 ± 4.4 *****
Vehicle	0	20	76.7 ± 2.0	79.8 ± 2.0	75.5 ± 1.8	9.0 ± 1.3	33.8 ± 3.1
	0.05		77.2 ± 2.1	79.4 ± 1.4	75.5 ± 1.4	9.6 ± 1.1	37.0 ± 3.3
Flupenthixol	0.15	30	**71.1 ± 1.6 ****	79.1 ± 1.9	75.4 ± 1.5	**5.3 ± 0.7 *****	**21.4 ± 1.7 *****
	0.3		**64.4 ± 2.1 *****	**75.0 ± 1.7 *****	78.3 ± 2	**4.5 ± 0.8 *****	**16.4 ± 1.9 *****
Vehicle	0	20	78.1 ± 1.9	82.7 ± 1.2	77.1 ± 1.6	12.9 ± 1.8	39.1 ± 3.8
SCH 23390	0.02	30	**69.4 ± 2.3 ***	77.6 ± 2.1	**78.1 ± 1.7 ***	**3.2 ± 0.6 ****	**18.6 ± 2.5 ****
Vehicle	0	20	74.8 ± 2.0	80.0 ± 1.4	74.8 ± 1.4	6.8 ± 1	28.6 ± 2.4
	0.02		72.0 ± 2.2	76.5 ± 2.1	76.8 ± 1.8	8.4 ± 1.4	30.9 ± 3.4
Eticlopride	0.04	20	**64.9 ± 2.5 ***	76.3 ± 3.1	72.4 ± 3.0	6.8 ± 1.3	27.3 ± 3.3
	0.06		**65.8 ± 3.0 ***	75.1 ± 2.2	74.9 ± 1.5	5.5 ± 0.7	22.9 ± 2.6
Vehicle	0	20	73.3 ± 2.1	82.0 ± 1.5	73.3 ± 2.2	7.4 ± 1.2	33.6 ± 4.1

Table 5.6. Effect of dopaminergic drugs on attention and impulsive behavior in Experiment 1. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ compared to vehicle control. The higher SCH 23390 doses of 0.04 and 0.06 mg/kg were not included in the analysis due to omissions > 50%.

Drugs	Dose (mg/kg)	Time before testing	% Omissions			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
			Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
			3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s
Amphetamine	0.2	10	4.2 ± 1.3	3.9 ± 1.1	2.8 ± 0.7	0.04 ± 0.01	0.03 ± 0.01	0.17 ± 0.14	1.07 ± 0.06	0.91 ± 0.04	1.04 ± 0.1	4.32 ± 0.27	3.23 ± 0.35	2.18 ± 0.26	2.37 ± 0.38	1.86 ± 0.16	1.78 ± 0.17
	0.4		5.9 ± 3.7	4.8 ± 3.6	1.8 ± 0.7	0.06 ± 0.01	0.09 ± 0.02	0.05 ± 0.01	0.97 ± 0.04	0.84 ± 0.04	0.78 ± 0.07	4 ± 0.37	2.93 ± 0.31	2.63 ± 0.3	1.84 ± 0.15	2.25 ± 0.36	1.85 ± 0.19
	0.8		4.6 ± 1.4	4.8 ± 1.5	5.8 ± 2.6	0.05 ± 0.01	0.05 ± 0.01	0.61 ± 0.59	**0.89 ± 0.03 ***	0.88 ± 0.06	0.89 ± 0.08	**3.63 ± 0.3 ***	3.14 ± 0.33	2.75 ± 0.34	1.79 ± 0.17	2.03 ± 0.32	1.97 ± 0.37
Vehicle	0	20	2.5 ± 1.0	2.4 ± 0.7	2.6 ± 1.1	0.04 ± 0.01	0.05 ± 0.01	0.33 ± 0.29	1.1 ± 0.07	0.92 ± 0.06	0.78 ± 0.04	4.74 ± 0.29	3.11 ± 0.21	1.94 ± 0.24	1.8 ± 0.25	1.79 ± 0.19	1.82 ± 0.18
GBR 12909	2.5	20	3.7 ± 1.4	3.6 ± 1.7	2.5 ± 0.7	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	1.35 ± 0.16	0.99 ± 0.1	0.89 ± 0.08	4.74 ± 0.3	3.97 ± 0.4	2.92 ± 0.34	2.31 ± 0.21	2.7 ± 0.34	2.23 ± 0.39
	5		3.0 ± 1.5	3.6 ± 1.6	3.2 ± 1.4	0.04 ± 0	0.06 ± 0.01	0.06 ± 0.01	1.2 ± 0.11	0.83 ± 0.03	0.89 ± 0.13	**3.89 ± 0.27 ***	2.66 ± 0.27	2 ± 0.39	1.96 ± 0.26	1.7 ± 0.09	1.92 ± 0.18
	10		5.1 ± 1.4	5.4 ± 1.5	6.0 ± 1.7	0.07 ± 0.01	0.09 ± 0.02	0.07 ± 0.02	1.1 ± 0.13	1.05 ± 0.11	1.12 ± 0.13	**3.71 ± 0.36 ****	3.11 ± 0.29	3.11 ± 0.4	1.97 ± 0.18	2.3 ± 0.29	2.24 ± 0.45
Vehicle	0	20	3.2 ± 1.0	3.4 ± 0.9	2.7 ± 0.9	0.07 ± 0.02	0.04 ± 0.01	0.08 ± 0.02	1.25 ± 0.07	0.94 ± 0.05	0.79 ± 0.03	5.12 ± 0.31	3.73 ± 0.36	2.31 ± 0.24	2.66 ± 0.53	2.75 ± 0.51	2.94 ± 0.6

Table 5.7. Effects of dopaminergic drugs on other dependent measure in 5-CSRTT performance in Experiment 1. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 compared to vehicle control

Flupenthixol	0.05		2.5 ± 0.8	2.2 ± 0.7	1.8 ± 0.6	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0	1.21 ± 0.09	0.91 ± 0.04	0.77 ± 0.04	5.05 ± 0.28	3.52 ± 0.27	2.25 ± 0.25	1.69 ± 0.13	1.82 ± 0.19	1.59 ± 0.07
	0.15	30	6.7 ± 2.0	5.7 ± 2	4.3 ± 1.1	0.04 ± 0	0.05 ± 0.01	0.05 ± 0.01	**1.51 ± 0.09 ***	**1.09 ± 0.05 ***	0.98 ± 0.05	**5.86 ± 0.24 ****	**4.63 ± 0.41 ****	2.97 ± 0.35	1.95 ± 0.17	1.92 ± 0.14	2.3 ± 0.28
	0.3		**13.8 ± 1.9 *****	**17.9 ± 3.1 *****	**13.3 ± 2.6 *****	**0.1 ± 0.03**	0.05 ± 0.03	0.07 ± 0.02	**1.84 ± 0.12 *****	**1.39 ± 0.1 *****	**1.15 ± 0.09 *****	**6.85 ± 0.3 *****	**5.53 ± 0.38 *****	**4.48 ± 0.43 *****	8.68 ± 5.45	3.02 ± 0.95	4.45 ± 1.58
Vehicle	0	20	2.8 ± 1.1	1.5 ± 0.7	2.3 ± 0.8	0.04 ± 0.01	0.03 ± 0	0.03 ± 0.01	1.15 ± 0.07	0.86 ± 0.04	0.78 ± 0.05	4.49 ± 0.23	3.1 ± 0.34	2.18 ± 0.31	1.68 ± 0.1	1.78 ± 0.16	1.94 ± 0.21
SCH 23390	0.02	30	**10.7 ± 2.1 *****	**7.7 ± 1.9 *****	**5.8 ± 1.4 ****	0.07 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	**1.62 ± 0.13 ****	**1.27 ± 0.09 ****	1 ± 0.11	**6.11 ± 0.31 ***	**4.91 ± 0.43 ****	**3.52 ± 0.42 ***	3.23 ± 1.01	5.23 ± 2.94	2.52 ± 0.34
Vehicle	0	20	2.8 ± 0.9	1.79 ± 0.5	1.5 ± 0.4	0.04 ± 0.01	0.03 ± 0	0.03 ± 0.01	1.22 ± 0.07	0.92 ± 0.05	0.8 ± 0.04	5.05 ± 0.29	3.43 ± 0.29	2.39 ± 0.31	1.82 ± 0.12	2.34 ± 0.54	1.92 ± 0.16
Eticlopride	0.02		4.3 ± 1.6	3.7 ± 1.5	3.9 ± 1.7	0.03 ± 0	0.05 ± 0.01	0.04 ± 0.01	1.32 ± 0.08	0.99 ± 0.07	0.8 ± 0.05	5.13 ± 0.23	3.35 ± 0.29	2.46 ± 0.28	2.2 ± 0.3	1.84 ± 0.15	1.89 ± 0.17
	0.04	20	7.8 ± 2.1	8.3 ± 2.5	7.9 ± 2.1	0.04 ± 0.01	0.03 ± 0	0.07 ± 0.02	1.53 ± 0.14	1.12 ± 0.07	1.04 ± 0.08	5.18 ± 0.25	**4.62 ± 0.28 ***	2.95 ± 0.29	1.84 ± 0.16	2.04 ± 0.27	1.98 ± 0.18
	0.06		8.4 ± 2.3	7.9 ± 2.4	8.5 ± 2.4	**0.07 ± 0.01 ***	0.03 ± 0.01	0.05 ± 0.01	**1.68 ± 0.13 ****	**1.27 ± 0.11 ****	**1.11 ± 0.09 ***	5.95 ± 0.26	4.09 ± 0.29	**3.27 ± 0.25***	2.86 ± 0.95	2.09 ± 0.23	3.5 ± 1.3
Vehicle	0	20	2.8 ± 0.6	1.8 ± 0.5	2 ± 0.7	0.03 ± 0	0.04 ± 0.01	0.04 ± 0	1.2 ± 0.07	0.96 ± 0.06	0.81 ± 0.04	5.05 ± 0.36	3.36 ± 0.42	2.2 ± 0.29	2.25 ± 0.31	2.05 ± 0.32	2.33 ± 0.4

Table 5.7 (Continued). Effects of dopaminergic drugs on other dependent measure in 5-CSRTT performance in Experiment 1. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 compared to vehicle control.

Drugs	Dose (mg/kg)	% Accuracy			% Premature	
		Cue Delay			Cue Delay	
		3 s	5 s	7 s	5 s	7 s
Nicotine	0.5	78.8 ± 2.1	78.1 ± 2.0	75.3 ± 2.0	**20.5 ± 1.8 *****	**48.6 ± 3.1 *****
SCH 23390	0.01	78.7 ± 1.7	79.6 ± 1.9	80.9 ± 2.2	7.5 ± 1.4	**22.2 ± 2.2 *****
	0.02	74.6 ± 2.4	79.9 ± 2.3	79.5 ± 1.9	6.3 ± 1.0	**17.8 ± 2.3 *****
Nicotine + SCH 23390	0.5 + 0.01	76.9 ± 2.3	81.5 ± 2.3	74.7 ± 2.2	**10.7 ± 1.2 ^^^**	**27.3 ± 2.3 ^^^**
	0.5 + 0.02	77.1 ± 2.2	80.2 ± 2.0	79.5 ± 1.9	**8.7 ± 1.6 ^^^**	**21.5 ± 3.1 *** ^^^**
Vehicle	0	79.1 ±2.19	78.2 ±2.3	74.1 ± 2.8	9.9 ± 1.6	33.7 ± 2.3
Nicotine	0.5	77.8 ± 1.9	79.4 ± 1.8	74.5 ± 2.6	**20.4 ± 2.4 *****	**46 ± 3.3 *****
Eticlopride	0.03	71.5 ± 2.9	74.9 ± 2.2	71.6 ± 1.9	8.4 ± 1.1	26.4 ± 2.4
	0.06	**67.3 ± 2.6 ****	74.2 ± 2.7	74.2 ± 2.8	7.5 ± 1.1	**19.9 ± 2.3 ****
Nicotine + Eticlopride	0.5 + 0.03	76.9 ± 2.3	76.7 ± 3.1	71.9 ± 2.8	**14.1 ± 1.7 ^**	38.5 ± 3.5
	0.5 + 0.06	**64.6 ± 3.6 *** ^^^**	74.4 ± 2.5	71.4 ± 3.3	**12.1 ± 1.0 ^^^**	**25.6 ± 2.0 ^^^**
Vehicle	0	77.9 ± 1.5	79.1 ± 1.9	76.2 ± 2.0	10.4 ± 1.4	32.7 ± 2.6
AMPH	0.6	77.1 ± 2.3	73.7 ± 2.3	67.9 ± 3.05	**27.9 ± 3.9 *****	**53.4 ± 4.5 *****
SCH 23390	0.01	71.8 ± 3.4	79.0 ± 2.3	79.4 ± 1.9	4.4 ± 0.9	15.3 ± 2.3
	0.02	**68.9 ± 3.7 ****	77.2 ± 2.5	75.9 ± 2.8	5.3 ± 1.3	15.2 ± 2.8
AMPH + SCH 23390	0.6 + 0.01	79.3 ± 2.6	78.2 ± 1.6	70.2 ± 2.1	**17.8 ± 2.6 *** ^^**	**40.7 ± 4.2 ** ^**
	0.6 + 0.02	81.3 ± 1.6	79. ± 1.9	**76.9 ± 2.3 ^**	**13.3 ± 2.5 ^^^**	**36.2 ± 4.4 ^^**
Vehicle	0	79.4 ± 1.9	80.5 ± 2.0	75.8 ± 1.7	6.07 ± 1.2	24.5 ± 2.3
Amphetamine	0.6	81.0 ± 2.1	76.7 ± 2.2	72.2 ± 2.4	**27.5 ± 3.2 *****	**55.9 ± 3.5 *****
Eticlopride	0.03	74.5 ± 3.6	**71.6 ± 3.4 *****	71.2 ± 4.0	10.7 ± 2.1	31.3 ± 3.0
Amphetamine + Eticlopride	0.6 + 0.03	78.2 ± 2.6	78.1 ± 1.9	72.4 ± 2.9	**18.6 ± 2.8 ****	**44.4 ± 4.1 ****
Vehicle	0	82.1 ± 1.6	83.3 ± 1.7	77.5 ± 1.9	7.7 ± 1.4	30.8 ± 3.0

Table 5.8. Effects of nicotine or amphetamine combined with dopaminergic antagonists. *Compared to saline. ^Compared to nicotine or amphetamine. * ^$p \leq 0.05$, ** ^^$p \leq 0.01$, *** ^^^$p \leq 0.001$. The higher eticlopride dose of 0.06 mg/kg was not included in the AMPH-eticlopride analysis due to omissions > 50%.

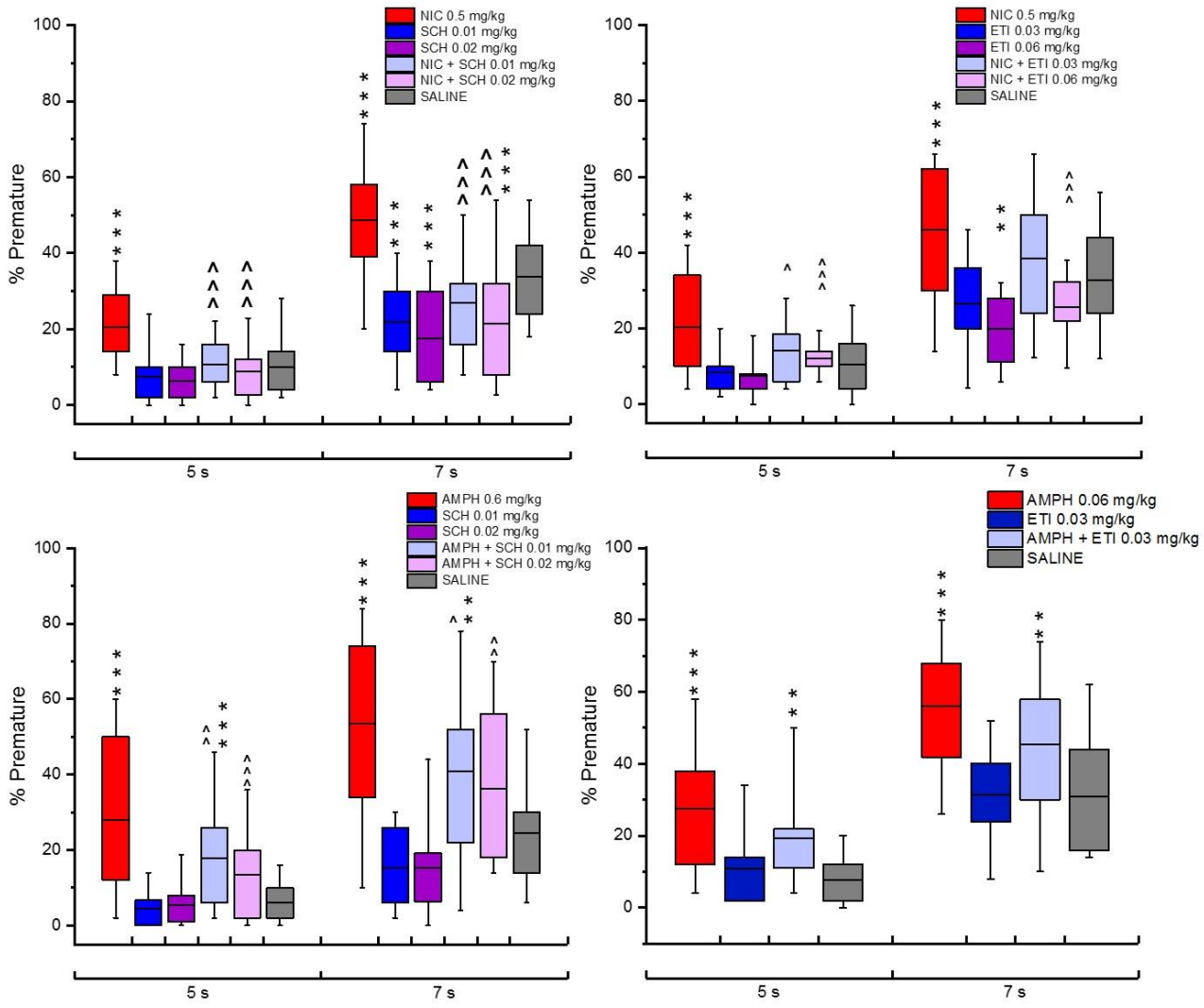

Figure 5.4. Effects of nicotine or amphetamine in combination with dopamine antagonists on premature responding. Both nicotine and amphetamine increased premature responses, when compared to saline at both cue delays. SCH 23390 significantly attenuated the effects of nicotine and amphetamine. Eticlopride significantly attenuated the effects of nicotine. *Compared to saline. ^Compared to nicotine or amphetamine. * ^$p \leq 0.05$, ** ^^$p \leq 0.01$, *** ^^^$p \leq 0.001$.

Drugs	Dose (mg/kg)	Time before testing (min)	% Omissions			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
			Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
			3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s
Nicotine	**0.5**	20	0.6 ± 0.2	0.8 ± 0.0	0.9 ± 0.3	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.07 9 ± 0.04	0.78 ± 0.05	0.85 ± 0.7	**4.02 ± 0.29 *****	3.06 ± 0.26	2.5 ± 0.25	1.44 ± 0.09	1.47 ± 0.1	1.36 ± 0.05
SCH 23390	**0.01**	30	4.3 ± 0.8	3.3 ± 0.9	2.3 ± 0.4	0.05 2 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	**1.3 ± 0.08 ^^**	**0.95 ± 0.06 ^^**	0.92 ± 0.07	**6.27 ± 0.33 ^^^**	**4.78 ± 0.26 ^^**	3.03 ± 0.35	1.53 ± 0.08	1.51 ± 0.06	1.56 ± 0.09
	0.02		**8.5 ± 0.8** ** ^^^**	**6.6 ± 1.4 * ^^**	**6.0 ± 1.2** * ^^**	0.07 ± 0.03	0.06 ± 0.01	0.07 ± 0.03	**1.23 ± 0.08 ^^**	1.08 ± 0.08	0.96 ± 0.06	**6.58 ± 0.37 ^^^**	**5.38 ± 0.35 ^^^ ****	**3.82 ± 0.29 ^**	1.72 ± 0.18	1.99 ± 0.32	1.78 ± 0.15
Nicotine + SCH 23390	**0.5 + 0.01**		2.6 ± 0.8	2.6 ± 0.9	1.8 ± 0.7	0.03 8 ± 0.01	0.03 2 ± 0.08	0.04 ± 0.01	**1.19 ± 0.09 ^**	0.87 ± 0.04	0.94 ± 0.06	5.22 ± 0.35	**4.6 ± 0.37 ****	2.89 ± 0.27	1.48 ± 0.05	1.48 ± 0.05	2.14 ± 0.62
	0.5 + 0.02		**10.9 ± 2.5** ** ^^^**	**7.9 ± 2.1** ** ^^^**	**7.4 ± 1.9** ** ^^^**	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	**1.32 ± 0.18 ^^^**	1 ± 0.07	0.88 ± 0.05	**6.86 ± 0.14 ** ^^^**	**5.23 ± 0.48 *** ^^^**	**3.94 ± 0.38 ^^**	1.78 ± 0.15	1.8 ± 0.13	1.68 ± 0.09
Vehicle	**Saline**	20	1.2 ±0.2	1.8 ± 0.5	1.5 ± 0.3	0.04 ± 0.01	0.04 ± 0.01	0.08 ± 0.03	1.08 ± 0.06	0.88 ± 0.05	0.81 ± 0.07	5.40 ± 0.31	3.46 ± 0.31	3.73 ± 0.40	1.36 ± 0.05	1.35 ± 0.05	1.45 ± 0.08

Table 5.9. Effects of the combination of nicotine and SCH 23390 on other dependent measures of 5-CSRTT performance from Experiment 2. *Compared to saline. ^Compared to nicotine. * ^$p \leq 0.05$, ** ^^$p \leq 0.01$, *** ^^^$p \leq 0.001$.

Drugs	Dose (mg/kg)	Time before testing (min)	% Omissions			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
			Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
			3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s
Nicotine	0.5	20	1.4 ± 0.5	1.7 ± 0.6	1.2 ± 0.5	0.03 ± 0.01	0.04 ± 0.01	0.07 ± 0.03	0.98 ± 0.07	0.81 ± 0.06	0.88 ± 0.05	4.25 ± 0.3^	3.52 ± 0.35	2.65 ± 0.26	1.63 ± 0.15	1.63 ± 0.17	1.88 ± 0.23
Eticlopride	0.03	20	6.5 ± 1.9	5.7 ± 1.5	4.9 ± 1.7	0.04 ± 0.01	0.04 ± 0	0.04 ± 0.01	1.35 ± 0.09	**1.17 ± 0.08 ^**	0.97 ± 0.08	**5.47 ± 0.29 ^^**	**4.85 ± 0.33 ***	3.43 ± 0.37	1.4 ± 0.05	1.4 ± 0.04	1.43 ± 0.07
	0.06		**8.3 ± 2.2 ** ^^**	**7.2 ± 2.0 ****	**7.5 ± 1.9 ^ ****	0.09 ± 0.03	0.07 ± 0.01	0.05 ± 0.01	**1.68 ± 0.19 *** ^^^**	**1.14 ± 0.11 ^**	1.11 ± 0.11	**6.43 ± 0.3 ^^^**	**5.71 ± 0.43 ^^^ ****	**4.19 ± 0.28 ^**	1.49 ± 0.05	1.48 ± 0.05	1.53 ± 0.06
Nicotine + Eticlopride	0.5 + 0.03		5.0 ± 1.8	3.1 ± 1.4	4.1 ± 1.8	0.07 ± 0.03	0.06 ± 0.02	0.07 ± 0.02	1.05 ± 0.07	0.95 ± 0.11	0.92 ± 0.12	4.81 ± 0.28	3.69 ± 0.38	2.7 ± 0.38	1.58 ± 0.08	1.64 ± 0.1	1.56 ± 0.07
	0.5 + 0.06		**13.5 ± 2.9* ** ^^^**	**11 ± 2.4* ** ^^^**	**10.7 ± 2.8* ** ^^^**	0.06 ± 0.03	0.04 ± 0.01	0.05 ± 0.03	1.33 ± 0.15	**1.33 ± 0.15 ^^^ ****	0.9 ± 0.1	**5.62 ± 0.46 ^^**	4.87 ± 0.43	**4.03 ± 0.49 ^**	1.61 ± 0.12	1.51 ± 0.1	1.57 ± 0.11
Vehicle	Saline	20	1.3 ± 0.3	0.8 ± 0.2	0.5 ± 0.2	0.08 ± 0.02	0.06 ± 0.02	0.09 ± 0.03	1.04 ± 0.06	0.86 ± 0.05	0.8 ± 0.06	5.44 ± 0.31	3.79 ± 0.28	2.85 ± 0.3	1.82 ± 0.19	1.72 ± 0.13	1.87 ± 0.23

Table 5.10. Effects of combination of nicotine and eticlopride on other dependent measures on 5-CSRTT from Experiment 2. *Compared to saline. ^Compared to nicotine. * ^p ≤ 0.05, ** ^^p ≤ 0.01, *** ^^^p ≤ 0.001.

Drugs	Dose (mg/kg)	Time before testing (min)	% Omissions			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
			Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
			3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s
Amphetamine	**0.6**	10	2.9 ± 1.0	2.2 ± 0.7	3.8 ± 1.5	0.07 ± 0.01	0.11 ± 0.02	0.07 ± 0.02	0.87 ± 0.07	0.82 ± 0.07	0.96 ± 0.12	**3.79 ± 0.37 ****	3.19 ± 0.31	2.69 ± 0.35	1.55 ± 0.11	1.82 ± 0.27	1.53 ± 0.15
SCH 23390	**0.01**	30	9.3 ± 2.4	**8.8 ± 2.4 *^**	5.8 ± 1.6	0.16 ± 0.1	0.14 ± 0.07	0.13 ± 0.06	**1.39 ± 0.13 ^^**	1.08 ± 0.07	0.98 ± 0.06	**6.87 ± 0.44 ^^^**	**6.03 ± 0.41 ^^^ ***	**4.22 ± 0.46 ^**	2.43 ± 0.9	1.75 ± 0.2	4.93 ± 3.32
	0.02		**15.8 ± 3.4 ** ^^**	**16.3 ± 3.5 *** ^^^**	**13.5 ± 3.2 ** ^**	0.04 ± 0.01	0.01 ± 0.01	0.07 ± 0.03	**1.66 ± 0.24 ^^^ ****	**1.35 ± 0.25 ^^ ***	1.03 ± 0.09	**7.35 ± 0.45 ^^^**	**5.33 ± 0.31 ^^**	**5.01 ± 0.57 ^^^**	1.82 ± 0.14	2.16 ± 0.56	3 ± 1.27
Amphetamine + SCH 23390	**0.6 + 0.01**		3.6 ± 1.4	4.0 ± 1.2	4.8 ± 1.3	0.11 ± 0.04	0.14 ± 0.06	0.09 ± 0.02	0.89 ± 0.05	0.86 ± 0.05	0.96 ± 0.11	4.7 ± 0.43	4.05 ± 0.56	2.57 ± 0.34	1.57 ± 0.08	1.72 ± 0.12	1.65 ± 0.11
	0.6 + 0.02		5.9 ± 2.56	4.9 ± 2.4	5.6 ± 2.5	0.1 ± 0.03	0.1 ± 0.04	0.13 ± 0.05	1 ± 0.06	0.82 ± 0.06	0.86 ± 0.07	4.58 ± 0.47	3.59 ± 0.44	**3.3 ± 0.43 ^^**	1.6 ± 0.15	1.66 ± 0.13	1.65 ± 0.16
Vehicle	**Saline**	20	3.1 ± 0.8	2.2 ± 0.6	2.0 ± 0.5	0.05 ± 0.01	0.06 ± 0.02	0.13 ± 0.07	1.17 ± 0.08	0.88 ± 0.05	0.79 ± 0.04	5.82 ± 0.36	4.32 ± 0.32	2.93 ± 0.26	1.51 ± 0.07	1.46 ± 0.05	1.56 ± 0.08

Table 5.11. Effects of combination of amphetamine and SCH 23390 on other dependent measures on 5-CSRTT from Experiment 2. *Compared to saline. ^Compared to nicotine or amphetamine. * ^$p \leq 0.05$, ** ^^$p \leq 0.01$, *** ^^^$p \leq 0.001$.

Drugs	Dose (mg/kg)	Time before testing (min)	% Omissions			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
			Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
			3 s	5 s	7 s	3s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s
Amphetamine	**0.6**	10	2.7 ± 0.7	2.5 ± 1.0	2.5 ± 0.8	0.08 ± 0.02	0.07 ± 0.01	0.1 ± 0.02	0.8 ± 0.04	0.89 ± 0.11	0.87 ± 0.08	**3.94 ± 0.32 ^^**	2.37 ± 0.33	2.59 ± 0.29	1.46 ± 0.06	1.63 ± 0.13	1.54 ± 0.09
Eticlopride	**0.03**	20	**6.4 ± 2.2 ***	**5.6 ± 2.0 ***	**4.8 ± 1.8 ***	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	**1.36 ± 0.13 ^^^ ***	1.18 ± 0.17	1.12 ± 0.2	**5.25 ± 0.26 ^**	**4.26 ± 0.39 ^**	2.47 ± 0.32	1.48 ± 0.05	1.48 ± 0.05	1.48 ± 0.05
Amphetamine + Eticlopride	**0.6 + 0.03**		5.5 ± 2.2	5.4 ± 2.1	5.2 ± 1.8	0.09 ± 0.03	0.11 ± 0.05	0.1 ± 0.03	1.05 ± 0.12	0.88 ± 0.08	1 ± 0.13	**4.64 ± 0.43 ***	3.45 ± 0.44	3.18 ± 0.57	1.48 ± 0.07	1.51 ± 0.07	1.59 ± 0.11
Vehicle	**Saline**	20	1.6 ± 0.3	0.9 ± 0.3	0.5 ± 0.2	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	1.03 ± 0.05	0.9 ± 0.06	0.86 ± 0.08	5.34 ± 0.3	3.44 ± 0.28	2.5 ± 0.3	1.62 ± 0.15	1.46 ± 0.06	1.45 ± 0.06

Table 5.12. Effects of combination of amphetamine and eticlopride on other dependent measures on 5-CSRTT from Experiment 2. *Compared to saline. ^Compared to nicotine or amphetamine. * ^$p \leq 0.05$, ** ^^$p \leq 0.01$, *** ^^^$p \leq 0.001$. The higher eticlopride dose of 0.06 mg/kg was not included in the AMPH-eticlopride analysis due to omissions > 50%.

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Chapter 6 Interaction between Cholinergic and Dopaminergic Neurotransmitters that Influence Attention and Response Inhibition under Conditions of Circadian Disruption

6.1 Abstract

Circadian rhythms are endogenous rhythms governing behavior and physiology. Circadian disruption is an environmental factor that impacts cognition and increases the risk of neurodegenerative disease by altering the circadian clock at a molecular level. Conventional sources of circadian disruption in human populations include working beyond the regular hours of '9 to 5' (shift work) and untimely exposure to light (light-at-night, LAN). Our study investigated the effect of 2 models of circadian disruption on response inhibition, which has previously been unaddressed, and attention using a 5-choice serial reaction time task (5-CSRTT). Adult Long-Evans rats of both sexes were maintained on a 12h:12h light:dark cycle and were tested under one of 3 light conditions: light at night, light phase, and control (no light). Our hypothesis that rats tested under both models of circadian disruption would have reduced response inhibition and attention versus controls was confirmed. Because acetylcholine (ACh) governs circadian rhythms and attention, and dopamine (DA) modulates response inhibition, we performed drug challenges to examine for an interaction between the 2 neurotransmitter systems in our models. We combined an ACh agonist (nicotine) and a DA receptor 1 (DR1) antagonist (SCH 23390) under the 3 circadian conditions to identify differential drug responses between treatment groups. We also repeated the same process with a DA receptor 2 (DR2) antagonist (eticlopride). SCH 23390 and eticlopride ameliorated the effect of nicotine in both models. The 2 circadian disruption models showed increased sensitivity to nicotine and the combination of nicotine and 0.01 mg/kg SCH 23390 compared to control. This response to the combination of drugs

confirms an interaction between cholinergic and dopaminergic neurotransmitters and identifies novel effects of circadian disruption on response to nicotine.

6.2 Introduction

Circadian rhythms are 24-hour endogenous rhythms that govern both behavior and physiology. The suprachiasmatic nucleus (SCN), the master clock, located in the hypothalamic region of the brain governs these rhythms. Factors like light and demanding cognitive tasks have a direct effect on these internal rhythms (Gritton et al., 2013; Silver and Kriegsfeld, 2014). These rhythms can be disturbed when there is a conflict between the external cues and intrinsic rhythms (Arble et al., 2010; Karatsoreos et al., 2011; Potter and Newhouse, 2008). We continue to use the two models, shift work (Wright et al., 2013) and light at night (LAN) (Dominoni et al., 2016; Fonken and Nelson, 2014) that we established in Chapter 4. Both the models we used in Chapter 4 showed a significant deficit in attention and response inhibition compared to the control group which was tested in their active phase under red light.

The deficits in attention due to circadian disruption match with previously established literature (Gritton et al., 2009) as attention and circadian rhythmicity are both modulated by acetylcholine (ACh) signaling (Gritton et al., 2012; Landgraf et al., 2014; Wright et al., 2012). We also reported the effects of circadian disruption on impulsive behavior for the first time. Previous research and our results from Chapter 5 establish that impulsive behavior is predominantly governed by dopamine (DA) via binding to dopamine-1 or -2 receptors (DR1s or DR2s) (Dalley and Roiser, 2012). In the prefrontal cortex (PFC), which is crucial for both attention and impulsive behavior, nucleus accumbens (NAc), striatum and the ventral tegmental area (VTA), cholinergic signaling interacts with

dopaminergic neurotransmission. ACh stimulates the release of DA from presynaptic terminals through the nicotinic ACh receptors (nAChRs) in these brain regions (van Gaalen et al., 2006a; Livingstone and Wonnacott, 2009; Picciotto et al., 2012). These effects were established in Chapter 5 under the LAN condition where we observed that impulsive behavior was affected by cholinergic drugs.

Studies using clock gene mutations have established that regulation of various aspects of dopaminergic signaling are under circadian control. *Clock* expression in VTA and NAc is implicated in reward processing and mood (McClung et al., 2005; Roybal et al., 2007). *Clock*Δ19 mutant mice show abnormal mesolimbic dopaminergic signaling and exhibit increased sensitivity to rewarding substances compared to wild-type mice (McClung et al., 2005; Ozburn et al., 2012). REV-ERBα, a circadian nuclear receptor, represses the expression of tyrosine hydroxylase (*Th*) in the midbrain implicating the circadian proteins in the transcriptional control of DA-related proteins (Chung et al., 2014). Both DR1 and DR2 receptors also possess canonical E-box sites where the CLOCK/BMAL1 heterodimer binds to regulate the rhythmic expression of these receptors (Akhisaroglu et al., 2005).

Interestingly, DA receptors (DR1 and DR2) also affect the expression of clock genes (Imbesi et al., 2009). Weaver et al. established that D1 receptors are expressed in the SCN and that DA signaling could influence the entrainment to cues (Weaver et al., 1992, 1995). This observation was further substantiated by a study where timed D1 agonists administered to SCN-lesioned pregnant hamsters caused fetal entrainment (Viswanathan and Davis, 1997). This study helped establish that in the absence of input from the SCN, stimulation of DR1 aided in the alignment of external cues. This SCN

DR1 expression is known to persist into adulthood (Rivkees and Lachowicz, 1997; Weaver et al., 1992). Luo et al. identified previously unknown populations of neurons in the VTA. A large portion of these novel neurons in the VTA were recorded in the dark phase, the active phase for rats (Luo et al., 2008). They also identified a novel projection pathway from the SCN to VTA via the medial preoptic nucleus (Luo and Aston-Jones, 2009). These studies establish that, like cholinergic signaling (Gritton et al., 2009, 2012, 2013), there is a possibility of a bidirectional relationship between dopaminergic signaling and circadian rhythms. The disruption of one system could have detrimental effects on the other.

The purpose of this experiment was to allow us to understand the effect of circadian disruptions on the interaction between cholinergic and dopaminergic neurotransmission. We accomplished this by studying the effects of cholinergic and dopaminergic drugs on impulsive behavior under different conditions of circadian disruption which have been previously unexplored. We examined the effects of combinations of the cholinergic agonist, nicotine, and dopaminergic antagonists for both DR1 (SCH 23390) and DR2 (eticlopride) on accuracy and premature responding on 5-CSRTT, where the behavioral measures correspond to attention and impulsive behavior (Robbins, 2002). Chapter 5 and other studies have established that nAChRs have a critical role in modulating attention (Havekes et al., 2011; Wallace and Bertrand, 2013), and an interaction between nicotinic signaling and the DA pathways modulates impulsive behavior (Livingstone and Wonnacott, 2009). Like in Chapter 5, we administered these drugs individually and then combined nicotine with DA receptor antagonists.

6.3 Materials and Methods

Subjects

Two cohorts of Long-Evans rats, with 30 rats (15 of each sex) in the first cohort and 42 (21 of each sex) in the second cohort, approximately 70 days of age were purchased from Envigo (Indianapolis, IN.) Rats were single-housed in polycarbonate shoebox cages with wood-chip bedding (Beta Chip, Northeastern Products Corp., Warrensburg, NY) in a temperature- and humidity-controlled room (targeted 22^{o} C, 40-55% humidity). 2020X Teklad Rodent Diet (Envigo) was fed to the rats. Food restriction was initiated after a one-week acclimation period to reduce rats' body weights over 2 weeks to target weights of 85% of their free-feed body weights. After that, target weights were incrementally increased by 5-10 g every 2 weeks, with a maximum of 250 ± 10 g for female rats and 350 ± 10 g for male rats, to allow for growth. Food restriction was intended to maintain motivation for performing operant-based behavioral tasks. Tap water was provided *ad libitum*. TestDiet sucrose pellets (AIN-76A 5TUL, 45 mg pellets, St. Louis, MO) were used for food-based reinforcement during behavior testing. After 1 week of acclimation the rats were randomly assigned to 3 different light conditions and were housed in chambers where the light cycle was regulated and maintained in the same light condition until the end of the study. Ultimately, 12 males and 12 females were maintained under each light condition.

The two conditions of circadian disruption included light at night (LAN) group and light phase (shift work) group. The rats in LAN were tested 4 h after the lights were switched off, during their active period. They were subjected to ambient light during testing and transportation to the testing room. The light phase group of rats was tested 4 h after the lights were switched on which was during their physiological resting phase.

The control group of rats was tested 4 h after the lights were switched off during their active phase, with no exposure to ambient light. Control rats were handled under red lights exclusively. In the testing chambers, control rats were exposed to a red LED stimulus cue light as well as low-intensity, yellow LEDs from the 5 nose-poke holes.

During the daily testing sessions for both circadian disruption groups, rats were exposed to overhead fluorescent white. While in the testing chambers, rats were exposed to the house light (2.8 watt bulb) in each chamber, a 2.8-watt stimulus cue light, and a low-intensity, yellow LED from the 5 nose-poke holes during both behavior tasks. The light intensity in the home cages ranged from 220 to 360 lux (average 290 lux). Rats were behavior tested at either zeitgeber time (ZT) 4 or 16 where the environmental cues are called zeitgebers (timegiver). Timers were staggered to allow light-dark transitions for a maximum of 12 rats at a time, counterbalanced across sexes and treatment groups, to permit adequate time for daily testing of all rats. This schedule was maintained across cohorts. Figure 1 shows the schematic of the experimental plan.

Apparatus and the 5-Choice Serial Reaction Time Task (5-CSRTT)

Behavioral testing began 3 weeks after the rats arrived when rats were approximately 90 days of age. Training and testing sessions, 1 per day, were performed 6 days each week in 12 5-choice operant behavior-conditioning chambers housed in sound-insulated and ventilated cubicles (Med Associates Inc., St. Albans, VT). Each chamber consisted of 5 evenly-spaced nose-poke cue holes (2.5 x 2.5 x 2.5 cm and 2 cm above the floor) on one wall. Each aperture had a yellow LED light centered in the back and an infrared photocell to detect head entries. The opposite wall had a pellet trough with a head-entry

detector in the center panel, a stimulus cue light directly above it, and then a house light mounted 6 cm above the cue light. Experimental contingencies were programmed using MedState Notation programming language, and data acquisition was performed using MED-PC IV software (version 4.38, Med Associates). Behavioral-testing programs were modified from those used by Beaudin et al. (Beaudin et al., 2017).

During the 7 initial training phases of the 5-CSRTT, rats learned to associate nose pokes in the pellet trough and the 5 nose-poke holes with food reinforcement. The house light remained on during all these phases. The criterion to advance from one phase to the next was 99 or 100 successful nose pokes. Each session during the 7 initial phases lasted until 100 pellets were earned or 60 min elapsed. Rats took an average of 8 days, ranging from 7 to 15 days, to complete initial training. The next phase of training was Visual Discrimination 1, during which cue lights were introduced. Cue lights were illuminated for 15 s during each trial. Nose pokes in the illuminated cue hole resulted in reinforcer delivery. Poking in any other cue hole, or not poking during the 15 s period, resulted in a time-out (see next section). Rats were tested on this phase until criteria of making 75% or more correct responses for 2 out of three consecutive days were met within 15 sessions. All but one rat in this study met the criteria, taking an average of 8.4 days, ranging from 5 to 12 days to progress to next phase. In the next phase, Visual Discrimination 2, the duration of cue light illumination was shortened to 1 s, requiring rats to be more attentive to optimize performance. Rats still had a 15 s limited-hold period to nose poke. Rats were tested on this phase for 5 days.

Sustained Attention Task

Following the training sessions, rats were tested on the Sustained Attention phase for 21 days. During daily sessions in each phase, the yellow LED cue lights in the 5 nose-pokes holes would randomly illuminate in one hole per trial, in a counterbalanced manner, so that each hole was illuminated during an equal number of trials per session. The house light was illuminated except during time-out periods. Each trial began with a nose-poke in the pellet trough. If it was the first trial, or if the previous trial resulted in sucrose pellet delivery, the rat was given 3 s to consume the pellet (reinforcer duration). After the 3 s reinforcer duration, the rat was given a 3 s turn-around time to allow the rat time to orient toward the wall with the 5 nose-poke holes. If the previous trial had resulted in a time-out, there was no reinforcer duration after a nose poke in the trough. In this phase of the test, there were variable delays of 3, 5 or 7 s until a cue light would illuminate in a nose-poke hole. These delays consisted of the 3 s turn-around time plus an additional delay of 0, 2 or 4 s. The cue light in the nose-poke holes would illuminate for a maximum of 1 s and, concurrently, a 15 s limited-hold period would commence during which a nose poke could be registered.

One of 4 trial outcomes could result. A **correct** trial was when the rat poked in the hole in which the cue light was illuminated during the 15 s limited-hold period. An **incorrect** trial was when the rat poked in one of the other 4 holes. A **premature** trial was when the rat poked during the delay before the cue light illuminated. Premature nose-pokes were not recorded during the 3 s turn-around time, so premature trials only occurred when the cue delay was 5 or 7 s. An **omission** occurred when the rat did not poke during the limited-hold period. Correct trials resulted in a food pellet being

dispensed, the cue light over the feeder illuminating, and the beginning of a new trial when the rat retrieved the reinforcer. If the rat continued to make nose pokes in any of the 5 cue holes after making a correct response, those nose pokes were recorded as **perseverative** responses but had no consequence. Perseverative responses were recorded only when there was a correct trial. Incorrect, premature, and omission responses triggered an immediate 5 s time-out, during which all cue lights and the house light extinguished. Poking in any of the 5 nose-poke holes during a time-out reset the time-out timer. When 5 s elapsed without any nose pokes, the cue light above the pellet trough illuminated until the rat poked in the trough, thus beginning the next trial. Each daily session lasted until 150 trials or 60 min elapsed. For the control group of rats, the house light was turned off, and the cue light over the feeder was replaced with a red LED light.

Drug Challenges

After rats completed 21 sessions of Sustained Attention, they continued testing on that phase until all rats in the experiment completed 21 sessions. Subsequently, rats tested for an average of 5.9 extra days (range 0 to 12), prior to drug challenges commencing. Between the Sustained Attention phase and drug testing, rats were tested with only one cue delay of 5 s to maintain what was termed a baseline level of performance. Drug testing occurred daily Monday through Friday. Mondays and Wednesdays were baseline performance days with only the 5 s cue delay, Tuesdays and Fridays were drug administration days, and all rats received a saline injection on Thursdays. During

drug and saline days, all 3 cue delays (3, 5 and 7 s) were present during testing sessions.

Rats in this study received single and then paired drug combinations (see Table 6.1). Drugs were administered in the order listed in the table. For each drug, all doses were administered using a Latin square design, with a minimum of 3 days between each dose, before moving to the next drug. All of the drugs were dissolved in sterile 0.9% normal saline (Baxter Healthcare Corp., Deerfield, IL) and drugs were administered intraperitoneally. Concentrations of each dosing solution were adjusted so that 1 µL/g body weight was always administered. The drugs used and times of administration before beginning testing sessions were (-)-nicotine ditartrate (NIC) 20 min (Tocris Bioscience, Minneapolis, MN), SCH 23390 hydrochloride (SCH) 30 min (Tocris), S-(-)-eticlopride hydrochloride (ETI) 20 min (Sigma-Aldrich, St. Louis, MO), and saline vehicle 20 min, based on available pharmacokinetic information. Results from Chapter 5 were used to select and modify the doses for this study. Like the study in Chapter 5, all rats were initially administered 0.5 mg/kg NIC for 3 consecutive days to acclimate rats to the undesirable physiologic effects of NIC that might impair performance (Bizarro and Stolerman, 2003). One female did not make the 75% correct criteria within 15 days in Visual Discrimination 1 and was omitted from the study, and another female died during the NIC and ETI administration phase and thus was only included in analyses of drugs through that point. Behavior testing lasted 17 weeks.

Data Analysis

R program for statistical computing and graphics (R Core Team, 2016) was used to calculate **percent correct** (number of correct responses/total trials *100), **percent incorrect** (number of incorrect responses/total trials *100), **percent accuracy** (percent correct/(percent correct + percent incorrect)), **percent premature** (number of premature responses/total trials *100), **percent omissions** (number of omitted responses/total trials *100), and **average perseverative responses** (sum of perseverative responses in all 5 nose-poke holes/number of correct responses). **Latency to correct responses** (sum of latencies to all correct responses/number of correct responses), **latency to incorrect responses** (sum of latencies to all incorrect responses/number of incorrect responses), and **latency to collect reinforcers** (sum of latencies to collect reinforcers/number of correct responses) were also calculated. Percent accuracy indicates the ability of the subject to sustain attention. Percent premature responses indicate deficits in response inhibition. Percent omissions is a measure of inattentiveness, a lack of motivation, or both (Robbins, 2002). Data are reported as mean ± SEM.

All statistical analyses were conducted using SPSS for Windows (version 24, SPSS Inc., Chicago, IL). Mixed model ANOVAs were used to analyze all data, with statistical significance set at $p \leq 0.05$. The data from the 21 days of Sustained Attention phase were evaluated to characterize learning of the 5-CSRTT. Experimental factors of block (7 3-day blocks) and cue delay (3 cue delays, except for 2 for percent premature) were within-subjects factors, and treatment (3 light conditions) and sex were between-subjects factors. Post-hoc testing (Bonferroni) was performed when appropriate using

SPSS. The data from the last 3 days of the Sustained Attention phase (block 7), when asymptotic performance had been reached, was evaluated with cue delay as a within-subjects factor and treatment (3 light conditions) and sex as between-subjects factors. For data from drug trials, dose (2-6 doses, including controls) was a within-subjects factor and treatment (3 light conditions) and sex were between-subjects factor. Two sets of comparisons were made in this study for drugs: 1) individual and combination doses were compared with control, and 2) combination doses were compared with NIC alone to gauge the effectiveness in attenuating the effects caused by NIC. For drug trials, post-hoc testing (Sidak) was performed when appropriate using SPSS.

6.4 Results

For all data on initial learning and drug challenges, significant sex-related differences were infrequent and thus are only included, when present, for the 2 primary measures of interest, accuracy and premature responding. Data on other measures including perseverative responding, which was rarely affected, omissions, and latencies during drug trials are presented in tabular form in the Tables section to assist with interpretation of drug effects. Due to substantial significant differences in premature responding and latency to incorrect responses between cue delays, data for the drug trials were separately analyzed for each cue delay (3, 5, 7 s for accuracy; 5, 7 s for premature responses). Full F test results are reported for accuracy and premature responding, but not for other measures in the interest of space.

Baseline 5-CSRTT Performance

Across the 7 blocks of testing on the Sustained Attention phase, accuracy increased, and premature responding decreased until asymptotic performance was reached by block 6 for both measures (not shown). In block 7, (See Table 6.2 and Figures 6.2-6.3) when the rats were said to have reached baseline performance levels, there was not a significant difference in accuracy between cue delays, but there was an effect of light condition across all 3 cue delays ($F_{2, 65} = 43.9$, $p < 0.001$), where LAN ($p < 0.001$) and shift work ($p < 0.001$) groups both showed significantly lower accuracy (Figure 6.2). For premature responding, the main effects of cue delay ($F_{1, 65} = 283.5$, $p < 0.001$) and light conditions ($F_{2, 65} = 35.1$, $p < 0.001$) were both significant. There was a significant cue delay by light condition interaction ($F_{2, 65} = 27.8$, $p < 0.001$). Post hoc analysis revealed that the rats under all 3 light conditions made more premature responses at 7 than at 5 s ($p_{con} = 0.008$, p_{LAN} and $p_{lightphase} < 0.001$). Control rats made fewer premature responses compared to rats in both models of circadian disruption of LAN ($p < 0.001$ at both cue delays) and light phase ($p_{5s} = 0.009$ and $p_{7s} < 0.001$) (Figure 6.3).

Omissions were not affected by light condition but were affected by cue delay ($F_{2, 130} = 7.9$, $p = 0.001$). There was a decrease in omissions at longer cue delays compared to 3 s ($p_{5s} = 0.001$ and $p_{7s} = 0.003$). There was a decrease in average perseverative responses in block 7 under both conditions of circadian disruption compared to control ($F_{2, 65} = 3.4$, $p = 0.04$; $p_{LAN} = 0.024$, $p_{lightphase} = 0.002$). Additionally, the main factors of light condition ($F_{2, 65} = 9.1$ $p < 0.001$) and cue delay ($F_{2, 130} = 8.4$, $p < 0.001$) were significant for latency to correct response but the interaction was not significant. Post hoc analysis revealed that both LAN ($p = 0.006$) and light phase ($p < 0.001$) groups took

longer to make a correct response. Rats in all three light conditions group took less time to make a correct response at both the longer cue delays of 5 s ($p = 0.001$) and 7 s ($p = 0.003$). There was an effect of light condition on latency to make incorrect responses ($F_{2, 65} = 3.4$, $p = 0.041$), where post hoc analysis revealed that the light phase ($p = 0.044$) group took longer to make incorrect responses compared to control. There was also a significant effect of cue delay on this measure ($F_{2, 130} = 172.7$, $p < 0.001$). Post hoc analysis showed that rats took less time to make incorrect responses at longer cue delays of 5 s and 7s ($p < 0.001$ for both) compared to 3 s. The rats also took less time to make an incorrect response at 7s ($p < 0.001$) compared to 5s. Reinforcement latency did not differ between light conditions or by cue delay.

Nicotine and Dopamine 1 Receptor Antagonist (SCH 23390) Trials

Percent accuracy graphs for each cue delay are in presented in Figure 6.4, 6.5 and 6.6. There was an effect of light condition on accuracy at the cue delays of 3 s ($F_{2, 71} = 30.9$, $p < 0.001$), 5 s ($F_{2, 70} = 28.6$, $p < 0.001$) and 7 s ($F_{2, 70} = 26.5$, $p < 0.001$) with both conditions of circadian disruption having lower accuracy ($p < 0.001$ at all three cue delays for both LAN and light phase versus control). There were dose effects at 3 s ($F_{5, 316} = 8.6$, $p < 0.001$), 5 s ($F_{5, 316} = 4.2$, $p = 0.001$), and 7 s ($F_{5, 315} = 3.9$, $p = 0.002$). Post hoc analysis revealed that at the shortest cue delay of 3 s only, NIC increased accuracy compared to saline ($p = 0.013$) (See Figure 6.4). At 5 s, SCH at the lower dose of 0.01 mg/kg lowered accuracy compared to saline ($p = 0.023$). At the longest cue delay of 7s the combination of NIC-SCH (0.02 mg/kg) differed from NIC ($p = 0.004$) and saline ($p =$

0.012). There was no interaction observed between drug and light condition with respect to accuracy.

For premature responding, at both cue delays there was a significant effect of light condition (5 s - $F_{2, 67} = 20.7$, $p < 0.001$ and 7 s - $F_{2, 70} = 32.5$, $p < 0.001$), where both LAN and light phase group showed greater premature responding (both $p < 0.001$) than controls as shown in Figures 6.7 and 6.8. Doses of NIC and SCH also influenced premature responding at both cue delays of 5 s ($F_{5, 315} = 57.4$, $p < 0.001$) and 7s ($F_{5, 318} = 56.9$, $p < 0.001$). There was an interaction between light condition and drug at both cue delays of 5 s ($F_{10, 315} = 13.6$, $p < 0.001$) and 7 s ($F_{10, 318} = 8.0$, $p < 0.001$).

At the cue delay of 5 s, post hoc analysis revealed that NIC increased impulsive behavior compared to saline in LAN and light phase groups (both $p < 0.001$) but not in the circadian control group. NIC + 0.01 mg/kg SCH increased premature responding compared to saline in the light phase ($p = 0.005$). In LAN group, there was a significant decrease in premature responding with NIC + 0.01 mg/kg SCH ($p < 0.001$) and NIC + 0.02 mg/kg SCH ($p < 0.001$) compared to NIC. The light phase group also showed a significant decrease in premature responding in NIC + 0.01 mg/kg SCH ($p = 0.002$) and NIC + 0.02 mg/kg SCH ($p < 0.001$) compared to nicotine.

At the cue delay of 7 s, NIC increased premature responding compared to saline in both LAN and light phase groups (both $p < 0.001$). In the LAN group, 0.01 mg/kg SCH decreased premature responding compared to saline ($p < 0.001$) while the higher SCH dose did not alter behavior. NIC + 0.02 mg/kg SCH also decreased premature responding compared to saline in LAN group ($p = 0.01$). There were decreases in

premature responding associated with NIC + 0.01 mg/kg SCH and NIC + 0.02 mg/kg SCH compared to NIC alone in both LAN and light phase groups (all $p < 0.001$) .

Table 6.4 shows the effects of drugs and light conditions on the other dependent measures on 5-CSRTT. There was an effect of dose on omissions at 3 s ($F_{5, 323} = 37.8$, $p < 0.001$), 5 s ($F_{5, 323} = 41.9$, $p < 0.001$) and 7 s ($F_{5, 325} = 31.7$, $p < 0.001$). Light conditions altered omissions only at the longest cue delay of 7 s ($F_{2, 74} = 5.4$, $p = 0.006$). There was an interaction between dose and light condition at all 3 cue delays of 3 s ($F_{10, 323} = 1.9$, $p = 0.042$), 5 s ($F_{10, 324} = 3.1$, $p = 0.021$) and 7 s ($F_{10, 324} = 2.2$, $p = 0.013$). Post hoc analysis for this interaction revealed that at the shortest cue delay of 3 s, 0.02 mg/kg SCH increased omissions compared to saline ($p = 0.015$) in the control group. In the LAN group, both doses of SCH increased omissions compared to saline ($p < 0.001$) and NIC + 0.02 mg/kg SCH increased omissions compared to saline and NIC alone ($p < 0.001$). In the light phase group the higher dose of 0.02 mg/kg SCH increased omissions compared to saline ($p < 0.001$) and NIC + 0.02 mg/kg SCH increased omissions compared to both saline ($p = 0.019$) and NIC ($p < 0.001$).

At the cue delay of 5s, post hoc analysis revealed that in the control group, the higher dose of 0.02 mg/kg SCH also increased omissions compared to saline ($p = 0.003$). In the LAN group, both doses of SCH increased omissions compared to saline ($p < 0.001$), and NIC + 0.02 mg/kg SCH significantly increased omissions compared to both saline and NIC ($p < 0.001$). In the light phase group NIC + 0.02 mg/kg SCH significantly increased omissions compared to saline ($p = 0.019$) and NIC ($p = 0.003$).

At the 7s cue delay, there was no effect of drug dose in the circadian control group. Both doses of SCH (0.01 mg/kg SCH, $p = 0.006$ and 0.02 mg/kg SCH, $p < 0.001$) and

NIC + 0.02 mg/kg SCH ($p < 0.001$) increased omissions compared to saline in the LAN group. Omissions caused by NIC + 0.02 mg/kg SCH also increased compared to NIC in LAN group ($p < 0.001$). In light phase group the higher dose of 0.02 mg/kg SCH increased omissions compared to saline ($p < 0.001$).

There was an effect of light condition on the latency to correct responses at all three cue delays of 3 s ($F_{2, 71} = 3.6$, $p = 0.032$), 5 s ($F_{2, 73} = 9.1$, $p < 0.001$) and 7 s ($F_{2, 75} = 4.4$, $p = 0.015$). Post hoc analysis revealed that only the light phase group took longer than control group to make correct responses ($p_{3s} = 0.028$, $p_{5s} < 0.001$, $p_{7s} = 0.014$). There was a light condition and dose interaction observed only at the longest cue delay of 7 s ($F_{10, 323} = 2.1$, $p < 0.021$). Post hoc analysis revealed that NIC + 0.01 mg/kg SCH made the rats take longer to make a correct response in light phase group compared to saline ($p = 0.009$). Latency to incorrect responses showed an effect of light condition at cue delays of 3 s ($F_{2, 65} = 3.4$, $p = 0.032$), 5 s ($F_{2, 60} = 8$, $p = 0.001$) and 7 s ($F_{2, 68} = 4.5$, $p = 0.014$) where post hoc analysis revealed that circadian disruption caused the rats to take longer to make an incorrect response ($p_{lightphase3s} = 0.044$; $p_{LAN5s} = 0.003$ and $p_{lightphase5s} = 0.002$; $p_{LAN7s} = 0.015$). Additional drug related effects are listed in Table 6.4. There were limited effects of dose and light condition on average perseverative responses and reinforcement latency.

Nicotine and Dopamine 2 Receptor Antagonist (Eticlopride) Trials

The effect of drugs and light condition on percent accuracy and percent premature are in presented in Table 6.5, and the other 5-CSRTT dependent measures are detailed in Table 6.6. The dose of 0.06 mg/kg ETI was excluded from analysis as it caused more

than 50% omissions in more than half the animals. Percent accuracy graphs for each cue delay are in presented in Figures 6.9, 6.10 and 6.11.

The effect of light condition on percent accuracy was consistent with previous results at all 3 cue delays: 3 s ($F_{2, 68} = 34.5$, $p < 0.001$), 5 s ($F_{2, 68} = 23.7$, $p < 0.001$) and 7 s ($F_{2, 69} = 38.7$, $p < 0.001$). Post hoc analysis revealed that the control group had significantly greater percent accuracy compared to both conditions of disruption (all $p < 0.001$) at all three cue delays. While the main effect of dose was significant at the 3 s ($F_{3, 182} = 7.3$, $p < 0.001$), 5 s ($F_{3, 183} = 5.6$, $p = 0.001$) and 7 s ($F_{3, 184} = 3.9$, $p = 0.01$) cue delays, findings on post hoc analysis were limited. The dose of 0.03 mg/kg of ETI and NIC + 0.03 mg/kg ETI decreased accuracy compared to NIC alone ($p = 0.001$) at 3 s. At 5 s cue delay, NIC + 0.03 mg/kg ETI decreased accuracy compared to both NIC ($p < 0.001$) and saline ($p = 0.035$). At the longest cue delay of 7 s, NIC + 0.03 mg/kg ETI decreased accuracy compared to saline ($p = 0.022$).

Figures 6.12 and 6.13 shows the effects of light condition, NIC and ETI on premature responding. Light condition altered premature responding at both 5 s ($F_{2, 68} = 8.8$, $p \leq 0.001$) and 7 s ($F_{2, 70} = 22.5$, $p \leq 0.001$) cue delay. Post hoc analysis showed that the control group made fewer premature responses compared to both LAN ($p < 0.001$ at both cue delays) and light phase groups ($p_{5s} = 0.029$ and $p_{7s} < 0.001$). There was a dose effect at both 5 s ($F_{3, 183} = 20.7$, $p < 0.001$) and 7 s ($F_{3, 188} = 19.5$, $p \leq 0.001$). Post hoc analysis revealed that at 5 s, NIC ($p < 0.001$) and NIC + 0.03 mg/kg ETI ($p = 0.002$) increased premature responding compared to the saline. At the 7 s cue delay, the post hoc analysis showed that NIC increased premature responding compared to saline ($p < 0.001$), while 0.03 mg/kg ETI and NIC + 0.03 mg/kg ETI

decreased premature responding ($p < 0.001$) compared to NIC. Effects of NIC and ETI on other parameters are presented in Table 6.6 but are not discussed.

6.5 Discussion

The present study examined the effects of combinations of cholinergic and dopaminergic drugs on accuracy and premature responding on 5-CSRTT, where the behavioral measures correspond to attention and impulsive behavior (Robbins, 2002). The motivation for the study in this chapter was based on the effects we saw on attention and impulsive behavior in both models of circadian disruption (LAN and light phase) in Chapter 4. We successfully reproduced the effects of circadian disruption detailed in Chapter 4 during the 21 days of the Sustained Attention phase before starting the drug trials. The overall results of the study indicate minimal effects of drugs (single and combination) on percent accuracy, which is a proxy for attention. The effects were more significant for percent premature which is an indicator of impulsive behavior. This study is the first of its kind to examine the effects of circadian disruption on the interaction between cholinergic and dopaminergic neurotransmitters in their role in governing impulsive behavior.

The combination of NIC (0.05 mg/kg) and SCH (0.01 and 0.02 mg/kg) had minimal effect on attention. The effect of light condition was consistent with our previously observed results, with the control group being more attentive. The combination of NIC and SCH had prominent effects on impulsive behavior. We hypothesize that this was due to NIC increasing DA release from presynaptic terminals, increasing impulsive behavior. Meanwhile SCH countered the effects of NIC, reducing premature responding by itself and in combination with NIC, consistent with previous results and literature (van

Gaalen et al., 2006b; Hahn et al., 2002). We saw these effects of drugs in light phase and LAN groups but not in the control group of rats at both cue delays when the combination of NIC and SCH was administered. The cumulative effects of drug treatment and light condition were more pronounced at the longer cue delay of 7 s. There was an increase in impulsive behavior separate from drug effects in both LAN and light phase models. Nicotine increased impulsive behavior in both models of circadian disruption compared to control group. Even though SCH ameliorated the effects of NIC within the LAN and light phase groups, within these 2 groups there still was a significant increase in impulsive behavior as compared to controls, reflecting the underlying effect of circadian disruption. Although speculative at this time, it is possible that SCH 23390 bound DR1 in the SCN and altered the already disrupted rhythms in LAN and light phase groups, thus increasing deficits in cognition (Rivkees and Lachowicz, 1997; Weaver et al., 1992). Studies by Rivkees and Weaver established the presence of DR1 in the SCN, but this is the first study to explore the effects they could have on circadian disruption and cognition. Further studies are needed to validate this relationship.

Nicotine (0.05 mg/kg) and ETI (0.03 mg/kg), individually and in combination, had limited effects on attention, compared to the effects on impulsivity. The main effect of light condition was consistent with previously observed results. Nicotine and ETI more strongly altered impulsive behavior in this study. Nicotine (0.05 mg/kg) increased impulsive behavior and the combination of nicotine and 0.03 mg/kg ETI ameliorated the effect of nicotine. The control group of rats had better response inhibition compared to both groups of circadian disruption. We hypothesized that administration of NIC

increased the baseline levels of DA, thus increasing impulsive behavior, but the antagonistic nature of SCH 23390 and eticlopride limited these effects, which are identical to the effects we saw in Chapter 5. Our results in the previous chapter established the relationship between cholinergic and dopaminergic signaling in influencing impulsive behavior on 5-CSRTT under one light condition which was the LAN model. Now we have been able to demonstrate that differential response to drugs is more prominent in 2 different models of circadian disruption as compared to the control group.

It has been shown that in vivo administration of NIC increases the release of glutamate, a neurotransmitter in the retinal hypothalamic tract. Glutamate signals photic cues to the SCN and can increase the firing of SCN neurons to produce phase shifts (Miller et al., 1987). The concentration of the endogenous agonist, ACh, can be altered by untimely exposure to light (Murakami et al., 1984). Additionally, a pulse of light can stimulate the synthesis of choline acetyltransferase, an enzyme catalyzing the synthesis of ACh, which is present in the SCN (Brownstein et al., 1975). Considering the above, our findings suggest that there is an interaction between nicotine administration and circadian disruption (LAN and light phase) which alters premature responding. Part of this effect may be due to nicotine stimulating the nAChRs present on the presynaptic terminals of DA neurons (Livingstone and Wonnacott, 2009), but other types of interactions between nicotine and circadian disruption may also be important.

It is possible that circadian disruption alters the endogenous levels of DA as various aspects of DA neurotransmission are under direct clock control. For example, monoamine oxidase A (*Maoa*) is directly regulated by the CLOCK/BMAL1 heterodimer

(Hampp et al., 2008). Tyrosine hydroxylase (*Th*), the rate limiting enzyme in the synthesis for DA, also exhibits diurnal variation under normal conditions (Sleipness et al., 2007; Webb et al., 2009). Imbesi et al. showed that DA receptors differentially altered the expression of clock genes in the striatum. Their study established that the dopamine receptors mediate psychostimulant-induced changes in clock gene expression (Imbesi et al., 2009).

DA receptor expression has also been shown to be rhythmic, and canonical E-box sites are present in dopamine receptor 1 (*Drd1*) and dopamine 2 receptor (*Drd2*) genes, which are differentially expressed, suggesting that these genes are also directly controlled by clock genes (Parekh et al., 2015). Mohawk et al. established that the DR1 antagonist SCH 23390, disrupted phase synchrony in peripheral tissues like liver and lungs and had similar effects even when combined with methamphetamine injections (Mohawk et al., 2013). It is a possibility that we are seeing similar effects on circadian disruption by the DR1 antagonist in our system as well. Hampp et al. showed that *Per2* mutant mice (another clock-component gene) had lower expression of DR1 (Hampp et al., 2008). We speculate that circadian disruption caused altered expression of clock genes, which in turn affected the expression of D1 and D2 receptors. The differential expression of the D1 and D2 receptors due to circadian disruption, in turn, could have modulated their behavioral responses observed in our study. Hood et al. showed a direct relationship between extracellular DA and *Per2* in the dorsal striatum where DA concentrations peak 6 h ahead of *Per2*. Alterations in dopaminergic neurotransmission had direct effects on the expression of *Per2* (Hood et al., 2010).

We hypothesize that the increased sensitivity to drugs that we observed in the models of circadian disruption is due to altered expression of molecular components under circadian control. The bidirectional relationship between dopaminergic and clock-component genes is potentially the main cause for the increase in impulsive behavior we observed. Additionally, our results suggest a more significant role for modulation by DR1s. These receptors are present in a greater majority than DR2s in the SCN and assist in entraining the organism to photic cues and help modulate response inhibition. Our model of circadian disruption appears to consistently yield deficits in attention and response inhibition. Despite both D1 and D2 receptors playing a role in modulating response inhibition, the combined effects of circadian disruption and drugs seem to be predominantly modulated by DR1. We need to further explore the effects of D1 receptors on responses to drugs and circadian disruption to better understand the underlying mechanism by which circadian disruption alters behavior and causes differential responses to nicotine.

6.6 Figures and Tables

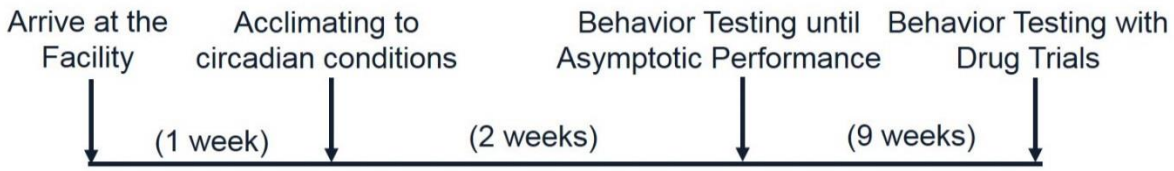

Figure 6.1. Schematic of the experiment plan that was followed for both the cohorts of rats.

Drugs	Mechanism	Doses (mg/kg)
Nicotine	nAChR agonist	0.5 x 3 sessions
Nicotine/SCH 23390	nAChR agonist/DR1 antagonist	0.5/0.01, 0.02
Nicotine/Eticlopride	nAChR agonist/DR2 antagonist	0.5/0.03, 0.06

Table 6.1. Drug combinations and doses administered. DR1/DR1, dopamine receptors 1 and 2; nAChR, nicotinic acetylcholine receptor

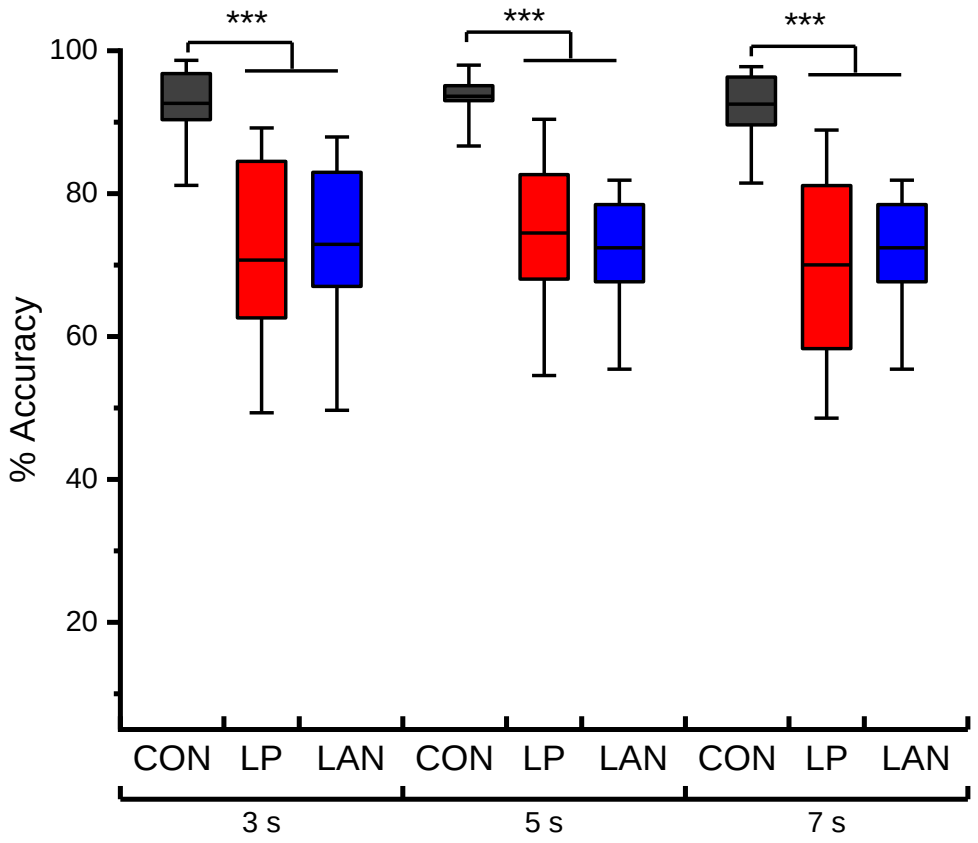

Figure 6.2. In block 7, % accuracy, a measure of attention was siginificantly diminished in rats in Light at Night (LAN) and Light Phase (LP) groups compared to control group (CON). ***p < 0.001 compared to control.

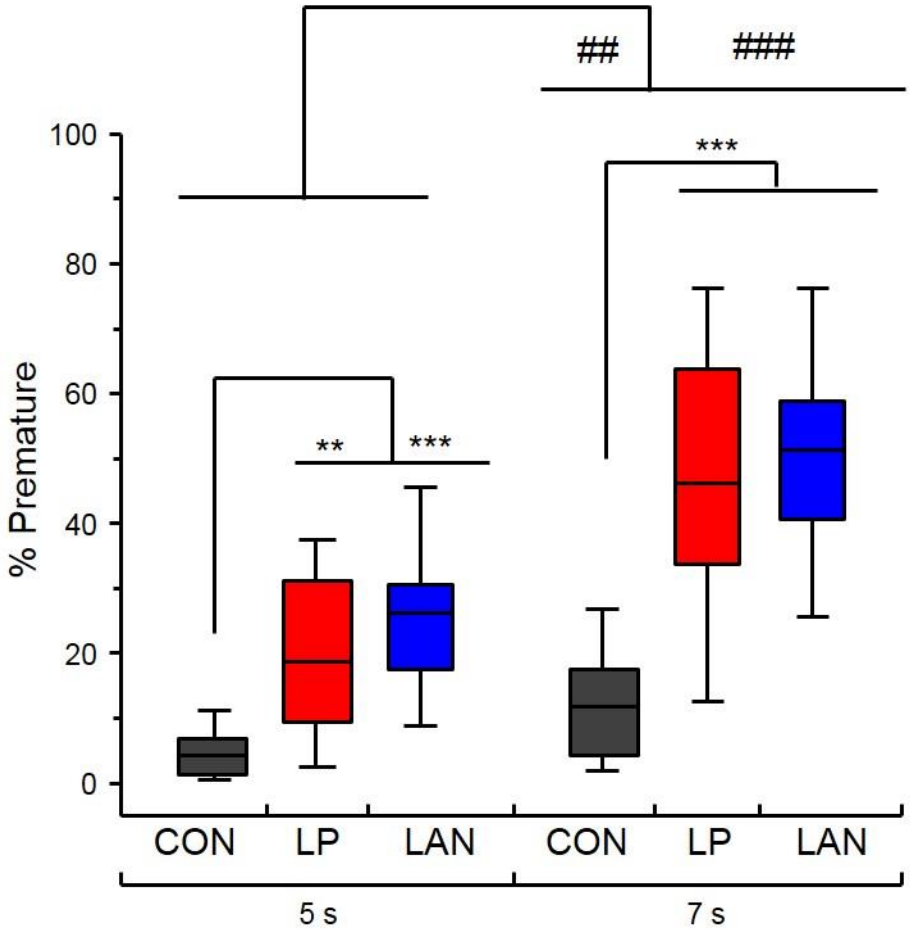

Figure 6.3. Percent premature, a measure of impulsive behavior, in block 7, for the three light conditions. Control (CON), Light at Night (LAN) and Light Phase (LP) shows increased impulsivity compared to control (*). Rats at the longer cue delay of 7 s also had greater % premature responding compared 5 s (# compared to 5 s). ***/### $p < 0.001$, **/## $p < 0.01$.

	Treatment Group	% Accuracy			% Premature		% Omission			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
		Cue Delay			Cue Delay		Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
		3 s	5 s	7 s	5 s	7 s	3 s	5 s	7 s	3 s	5 s	7 s	3s	5s	7s	3s	5s	7s	3s	5s	7s
Block 7	**Light at Night**	**72.9 ± 2.4** ###	**72.4 ± 1.8** ###	**71.7 ± 2.3** ###	**26.2 ± 2.9** ###	**51.2 ± 3.5** ### †††	2.5 ± 0.6	**1.8 ± 0.7** ***	**1.8 ± 0.7** ^^	**0.07 ± 0.01** *	**0.06 ± 0.01** *	**0.07 ± 0.01** *	**1.13 ± 0.07** ##	**1.01 ± 0.05** ## ***	**0.99 ± 0.07** ## ^^	4.07 ± 0.25	**3.66 ± 0.2** ***	**0.99 ± 0.07** ^^^ †††	1.29 ± 0.05	1.28 ± 0.04	1.32 ± 0.06
	Light Phase	**70.6 ± 2.7** ###	**74.4 ± 2.3** ###	**70 ± 2.7** ###	**19 ± 2.3** ##	**46.2 ± 4.2** ### †††	2.7 ± 0.7	**1.6 ± 0.4** ***	**1.7 ± 0.6** ^^	**0.06 ± 0** **	**0.07 ± 0.01** **	**0.03 ± 0** **	**1.24 ± 0.07** ###	**1 ± 0.05** ### ***	**1.07 ± 0.09** ### ^^	**4.67 ± 0.26** #	**3.46 ± 0.24** # ***	**1.07 ± 0.09** # ^^^ †††	1.47 ± 0.1	1.49 ± 0.08	1.42 ± 0.05
	Control	92.6 ± 1	93.6 ± 0.6	92.5 ± 1	4.4 ± 0.7	**11.9 ± 1.9** ††	4.4 ± 1.1	**2.7 ± 0.8** ***	**2.9 ± 0.8** ^^	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.84 ± 0.02	**0.81 ± 0.02** ***	**0.78 ± 0.02** ^^	3.92 ± 0.5	**2.58 ± 0.29** ***	**0.78 ± 0.02** ^^^ †††	1.34 ± 0.05	1.34 ± 0.05	1.37 ± 0.06

Table 6.2. Dependent measures from Block 7 of the Sustained Attention phase with cue delay 3 s, 5 s and 7s, for all three light conditions. Significant differences in accuracy and premature responding were seen in the light at night and light phase groups compared to the control group. Premature responding was significantly greater at the longest cue delay of 7s. #Compared to control group. *Compares cue delay 3 s to 5 s. ^Compares 3 s to 7 s. †Compares 5 s to 7 s. * ^ †$p \leq 0.05$, ** ^^ ††$p \leq 0.01$, *** ^^^ †††$p \leq 0.001$.

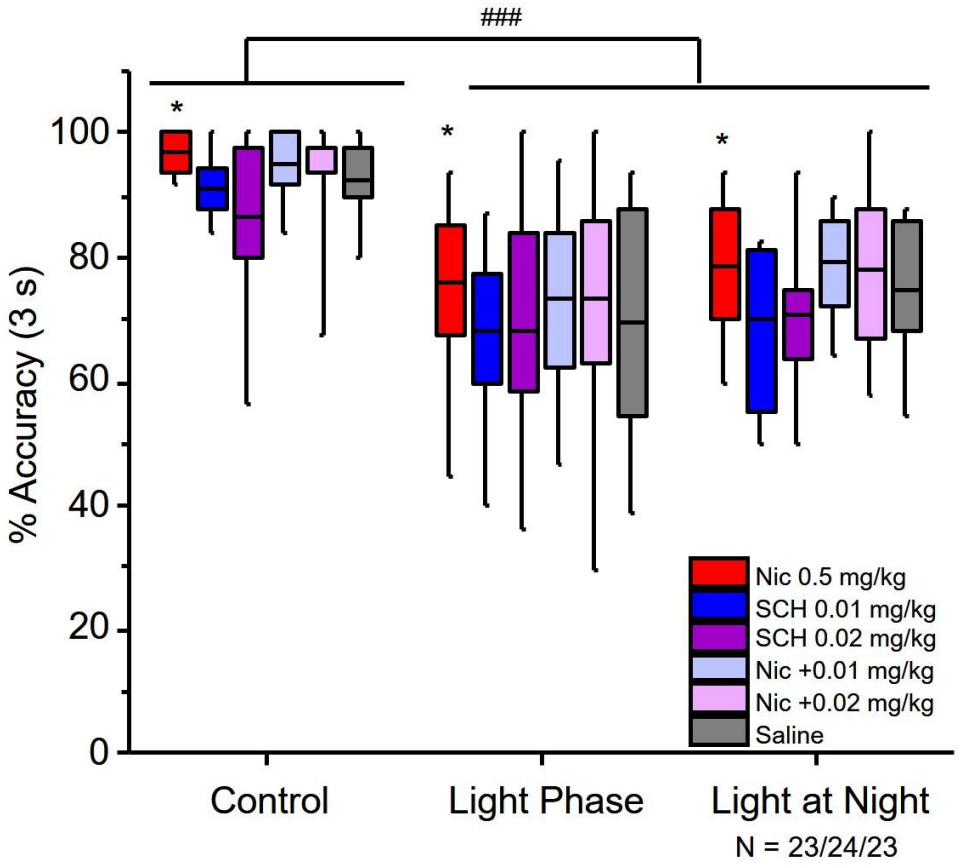

Figure 6.4. Effects of light condition on attention when NIC (0.05 mg/kg) and SCH 23390 (0.01mg/kg and 0.02 mg/kg) were administered individually and in combination at cue delay of 3 s. Both LAN and light phase groups showed a reduction in accuracy compared to control. Nicotine reduced accuracy in all groups only at this cue delay. $^{\#}$Compares control group to LAN and light phase.*Compares dose to saline. $^{\#\#\#}p < 0.001$. $^{*}p < 0.05$.

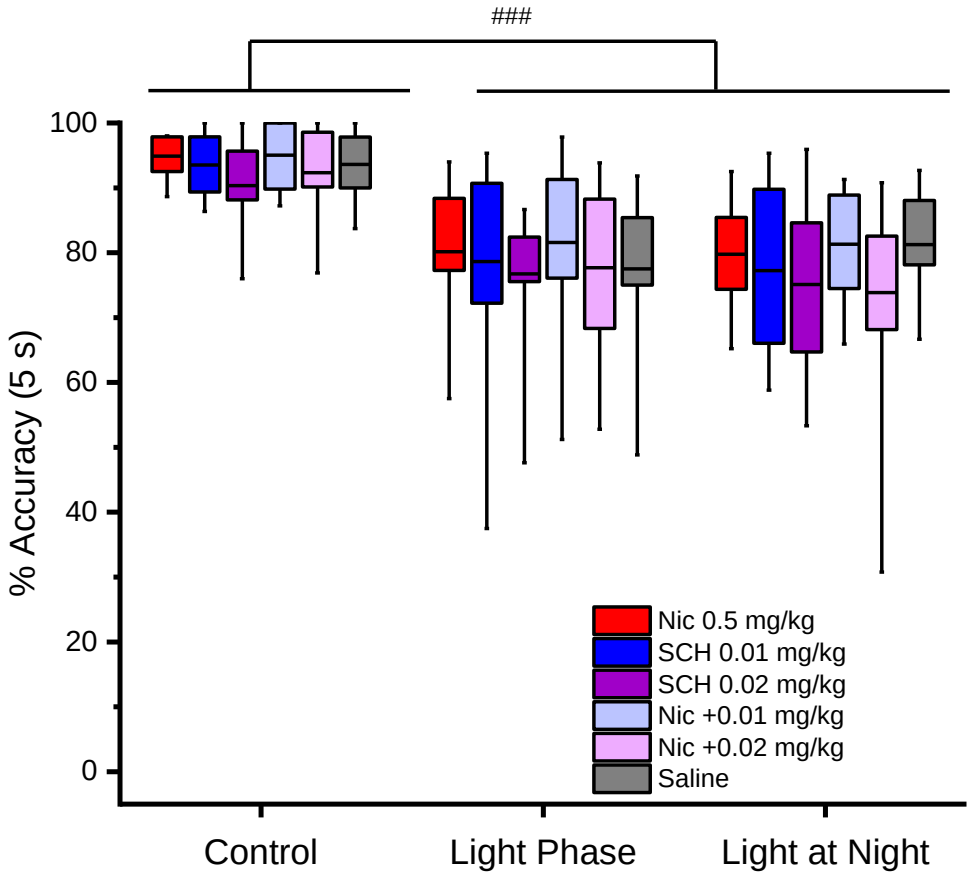

Figure 6.5. Effects of light condition on attention when NIC (0.05 mg/kg) and SCH 23390 (0.01 mg/kg and 0.02 mg/kg) were administered individually and in combination at cue delay of 5 s. Both LAN and light phase groups showed a reduction in accuracy compared to control. Limited effects of drugs were observed. #Compares control group to LAN and light phase. ### $p < 0.001$.

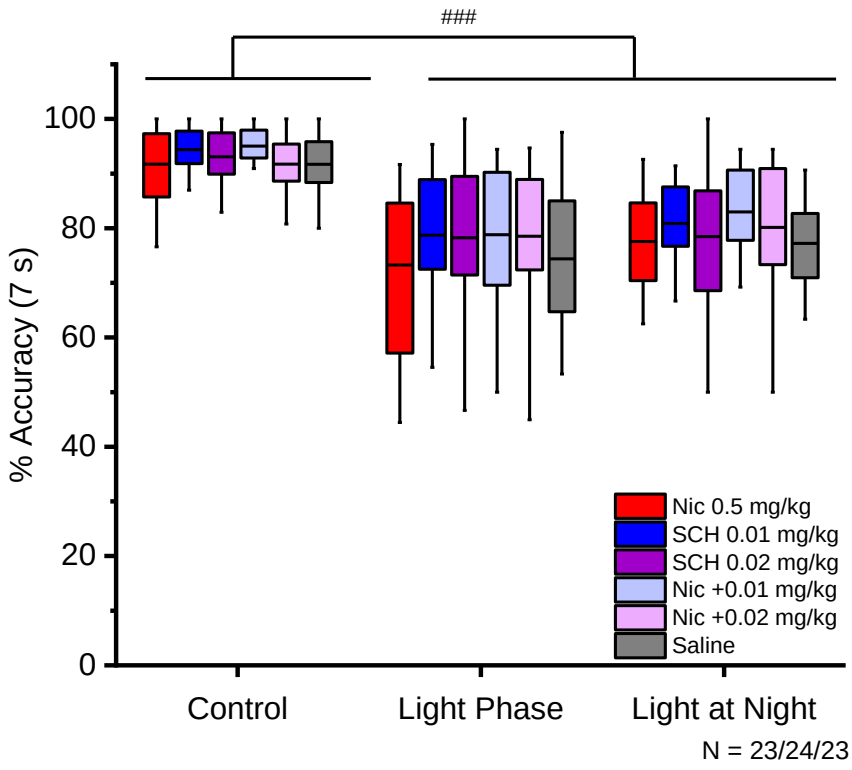

Figure 6.6. Effects of light condition on attention when NIC (0.05 mg/kg) and SCH 23390 (0.01 mg/kg and 0.02 mg/kg) were administered individually and in combination at cue delay of 7s. Both LAN and light phase groups showed a reduction in accuracy compared to control. Limited effects of drugs were observed. #Compares control group to LAN and light phase. ### $p < 0.001$.

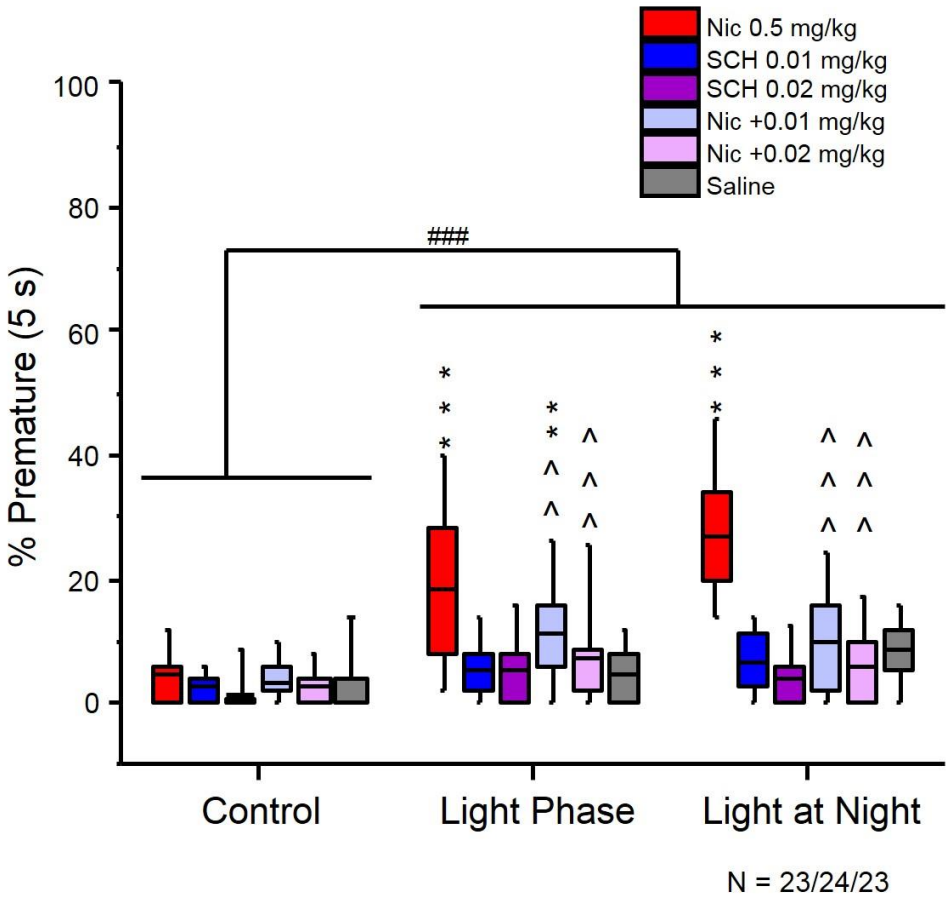

Figure 6.7. Effects of light conditions and the cholinergic agonist, nicotine, and the DR1 antagonist, SCH 23390, on impulsive behavior at 5 s cue delay. Both LAN and light phase groups had significantly more premature responding compared to control. Significant dose effects of NIC and SCH were seen in both conditions of circadian disruption but not in the control group. #Compares Control group to Light at Night and Light phase. *Compared to Saline within each light condition group. ^Compared to nicotine within each light condition group. ** ^^ p <0.01, ### *** ^^^ p < 0.001

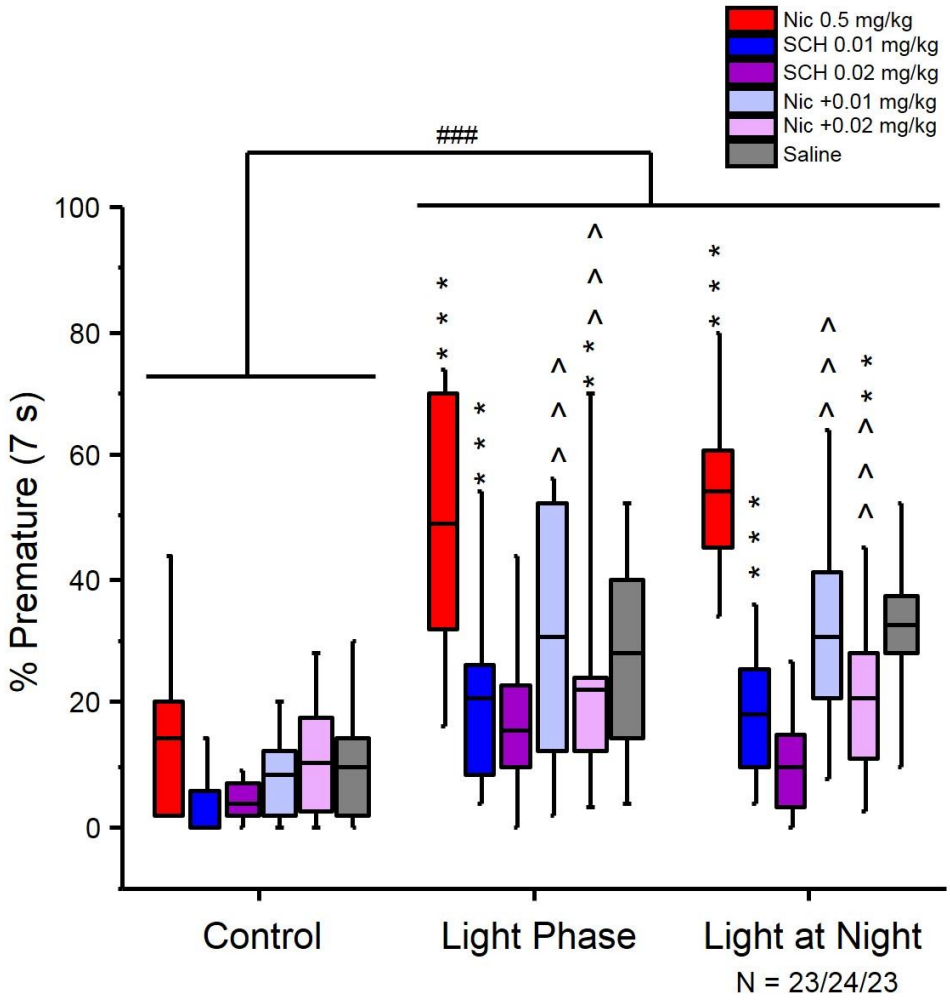

Figure 6.8. Effects of light conditions and the cholinergic agonist, nicotine, and the DR1 antagonist, SCH 23390, on impulsive behavior at 7 s cue delay. Both LAN and light phase groups had significantly more premature responding compared to control. Significant dose effects of NIC and SCH were seen in both conditions of circadian disruption but not in the control group. #Compares Control group to Light at Night and Light phase. *Compared to Saline within each light condition group. ^Compared to nicotine within each light condition group. ** ^^ p <0.01, ### *** ^^^ p < 0.001

Drugs	Dose (mg/kg)	Time before testing	Treatment Group	%Accuracy			%Premature	
				Cue Delay			Cue Delay	
				3s	5s	7s	5s	7s
Nicotine	**0.5**	20	**Light at Night**	**78.6 ± 2.2** ### *	**79.7 ± 1.6** ###	**77.5 ± 2** ###	**26.7 ± 2.2** ### ***	**54.1 ± 2.9** ### ***
SCH 23390	**0.01**	30		**70.1 ± 2.5** ###	**77.2 ± 2.6** ### *	**80.8 ± 1.5** ###	6.7 ± 1.1	**18.3 ± 2.3** ***
	0.02	30		**70.9 ± 2.8** ###	**75.1 ± 3** ###	**78.4 ± 3.1** ###	3.9 ± 0.8	10 ± 1.8
Nicotine + SCH 23390	**0.5 + 0.01**			**79 ± 1.9** ###	**81.3 ± 1.8** ###	**82.9 ± 1.7** ###	**9.8 ± 1.6** ^^^	**30.5 ± 3.4** ### ^^^
	0.5 + 0.02			**78.1 ± 2.6** ###	**73.8 ± 4.3** ###	**80.1 ± 2.7** ### * ^^	**5.9 ± 1.3** ^^^	**20.8 ± 2.7** ^^^ **
Vehicle	**0**	20		**74.9 ± 2.6** ###	**81.2 ± 1.7**	**77.2 ± 1.7** ###	8.5 ± 1	**32.9 ± 2.5** ###
Nicotine	**0.5**	20	**Light Phase**	**75.9 ± 3.3** ### *	**80.1 ± 2.7** ###	**73.2 ± 3.2** ###	**18.6 ± 2.6** ### †† ***	**48.8 ± 4.4** ### ***
SCH 23390	**0.01**	30		**68.3 ± 3.1** ###	**78.6 ± 3.7** ### *	**78.7 ± 2.9** ###	5.2 ± 0.9	20.8 ± 3.4
	0.02	30		**68.4 ± 4.3** ###	**73.5 ± 3.8** ###	**78.2 ± 3.4** ###	5 ± 1.2	15.4 ± 2.9
Nicotine + SCH 23390	**0.5 + 0.01**			**73.3 ± 3.9** ###	**81.5 ± 2.8** ###	**78.8 ± 3.4** ### * ^^	**11.4 ± 1.8** ## ^^ **	**30.7 ± 4.1** ### ^^^
	0.5 + 0.02			**73.5 ± 4.7** ###	**77.6 ± 2.8** ###	**78.5 ± 3.2** ### * ^^	**6.9 ± 1.7** ^^^	**22 ± 4.1** ^^^
Vehicle	**0**	20		**69.4 ± 3.8** ###	**77.5 ± 2.9** ###	**74.3 ± 3.6** ###	4.7 ± 0.8	**27.9 ± 3.3** ###
Nicotine	**0.5**	20	**Control**	**96.9 ± 0.6** *	94.9 ± 0.7	91.7 ± 1.5	4.5 ± 1	14 ± 2.6
SCH 23390	**0.01**	30		91.2 ± 1.2	**93.5 ± 1** *	94.3 ± 0.8	2.5 ± 0.9	5.6 ± 2
	0.02	30		86.6 ± 3.8	90.3 ± 2.1	93 ± 1.4	1 ± 0.7	4 ± 0.9
Nicotine + SCH 23390	**0.5 + 0.01**			94.9 ± 1.1	95 ± 1.1	95 ± 1	3.3 ± 0.9	8.2 ± 1.5
	0.5 + 0.02			93.5 ± 2.6	92.3 ± 1.6	**91.7 ± 1.2** ### * ^^	2.6 ± 0.5	10.1 ± 1.9
Vehicle	**0**	20		92.4 ± 1.5	93.5 ± 1.2	91.7 ± 1.2	3.7 ± 1	10 ± 2.2

Table 6.3 Combined effect of light condition and cholinergic agonist, nicotine, and dopaminergic receptor 1 (DR1) antagonist on attention and impulsive behavior. #Compares Light at Night and Light Phase to Control group. † Compared Light at night to Light Phase *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. * ^ $p \leq 0.05$, ** ^^ $p \leq 0.01$, *** ^^^ $p \leq 0.001$.

Drugs	Dose (mg/kg)	Time before testing	Treatment Group	%Omission			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
				Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
				3s	5s	7s	3s	5s	7s	3s	5s	7s	3s	5s	7s	3s	5s	7s
Nicotine	**0.5**	20	**Light at Night**	0.7 ± 0.4	0.6 ± 0.3	0.6 ± 0.3	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.85 ± 0.05	**0.74 ± 0.04 ***	0.63 ± 0.04	**3.41 ± 0.35 *** **	**2.52 ± 0.34 ## **	**2.1 ± 0.33 # **	1.21 ± 0.04	1.22 ± 0.04	1.26 ± 0.08
SCH 23390	**0.01**	30		**15.1 ± 2.1 *** **	**13.9 ± 2.4 *** **	**10.3 ± 1.9 ** **	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	**6.62 ± 0.42 *** **	1.16 ± 0.09	1.02 ± 0.07	**6.62 ± 0.42 * **	**5.74 ± 0.42 ## * **	**4.03 ± 0.38 # * **	1.61 ± 0.18	1.42 ± 0.06	1.63 ± 0.19
	0.02	30		**24.4 ± 3 *** **	**25.5 ± 3.1 *** **	**20.7 ± 2.8 *** **	0.06 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	1.96 ± 0.28	**1.2 ± 0.13 ** **	1.16 ± 0.13	**7.72 ± 0.4 * **	**6.01 ± 0.52 ## *** **	**4.88 ± 0.68 # *** **	1.57 ± 0.12	1.74 ± 0.23	1.48 ± 0.06
Nicotine+ SCH 23390	**0.5 + 0.01**			7.1 ± 2.3	3.9 ± 1.3	5.2 ± 1.7	0.07 ± 0.02	0.1 ± 0.06	0.06 ± 0.01	1.14 ± 0.07	0.96 ± 0.04	**1.14 ± 0.07 ^^^**	**5.46 ± 0.42 ^**	**4.53 ± 0.46 ##**	**3.08 ± 0.41 #**	1.42 ± 0.05	3.54 ± 2.06	1.58 ± 0.12
	0.5 + 0.02			**22.3 ± 5.1 # ^^^ *** **	**17.4 ± 4.2 ^^^ *** **	**18.2 ± 5 ### ^^^ *** **	0.05 ± 0.01	0.07 ± 0.02	0.04 ± 0.01	1.18 ± 0.09	1.13 ± 0.1	0.77 ± 0.04	**6.99 ± 0.63 ^^^**	**6.18 ± 0.58 ## ^^^**	**5.51 ± 0.76 ^^^ # *** **	1.46 ± 0.05	1.47 ± 0.05	1.54 ± 0.07
Vehicle	**0**	20		1.5 ± 0.5	0.4 ± 0.1	0.5 ± 0.2	0.07 ± 0.01	0.04 ± 0.01	0.04 ± 0	1.32 ± 0.13	0.88 ± 0.07	0.88 ± 0.07	4.98 ± 0.38	**3.54 ± 0.4 ##**	**2.37 ± 0.32 #**	1.34 ± 0.06	1.31 ± 0.06	1.32 ± 0.05

Table 6.4. Combined effect of light condition and cholinergic agonist, nicotine, and dopaminergic receptor 1 (DR1) antagonist on other dependent measures of 5-CSRTT performance. # Compares Light at Night and Light phase group to Control group. *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. * ^ # $p \leq 0.05$, ** ^^ ## $p \leq 0.01$, *** ^^^ ### $p \leq 0.001$.

Nicotine	**0.5**	20	**Light Phase**	1.3 ± 0.5	0.5 ± 0.2	0.6 ± 0.4	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.03	**1.04 ± 0.08 # *****	**0.87 ± 0.08 # ***	**0.66 ± 0.03 # ****	**4.34 ± 0.38 # *****	**3.75 ± 0.64 ##**	2.25 ± 0.34	1.34 ± 0.06	1.37 ± 0.07	1.36 ± 0.06
SCH 23390	**0.01**	30		10.3 ± 2.8	8.8 ± 2.3	6 ± 1.5	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	**6.68 ± 0.53 #**	**1.21 ± 0.1 #**	**0.98 ± 0.1 #**	**6.68 ± 0.53 # ***	**5.24 ± 0.42 ## ***	**4.28 ± 0.52 ***	1.44 ± 0.06	1.57 ± 0.11	1.52 ± 0.07
	0.02	30		**18.3 ± 3 *****	**19.7 ± 3.2 *****	**14.8 ± 3 *****	0.05 ± 0.01	0.14 ± 0.1	0.25 ± 0.19	**1.97 ± 0.24 #**	**1.37 ± 0.14 # ****	**0.99 ± 0.1 #**	**7.04 ± 0.45 # ***	**6.89 ± 0.73 ## *****	**4.26 ± 0.6 *****	1.57 ± 0.09	1.77 ± 0.23	12.73 ± 11.13
Nicotine+ SCH 23390	**0.5 + 0.01**			3.5 ± 1.1	2.7 ± 0.7	2 ± 0.6	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	**1.28 ± 0.11 #**	**1.01 ± 0.09 #**	**1.28 ± 0.11 # ^^^**	**5.72 ± 0.52 # ^^**	**4.04 ± 0.62 ##**	2.67 ± 0.33	1.68 ± 0.25	1.67 ± 0.15	3.35 ± 1.84
	0.5 + 0.02			**14.1 ± 3 ^^^ ***	**12.1 ± 2.9 ^^ ***	9 ± 2.2	0.09 ± 0.03	0.13 ± 0.04	0.07 ± 0.02	**1.75 ± 0.24 #**	**1.21 ± 0.16 # ^^^**	**1.09 ± 0.13 #^^^**	**6.23 ± 0.57 # ^^^**	**4.88 ± 0.47 ## ^^^**	4.55 ± 0.61 *** ^^^	1.87 ± 0.27	4.1 ± 2.18	2.26 ± 0.61
Vehicle	**0**	20		2.6 ± 0.7	1.8 ± 0.4	1.9 ± 0.4	0.07 ± 0.01	0.08 ± 0.02	0.04 ± 0.01	**1.55 ± 0.16 #**	**1.07 ± 0.07 #**	**0.91 ± 0.06 #**	**5.86 ± 0.34 #**	**4.15 ± 0.41 ##**	2.52 ± 0.28	1.58 ± 0.11	1.59 ± 0.12	1.54 ± 0.1

Table 6.4 (Continued). Combined effect of light condition and cholinergic agonist, nicotine, and dopaminergic receptor 1 (DR1) antagonist on other dependent measures of 5-CSRTT performance. # Compares Light at Night and Light phase group to Control group. *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. * ^ # $p \leq 0.05$, ** ^^ ## $p \leq 0.01$, *** ^^^ ### $p \leq 0.001$.

Nicotine	**0.5**	20	**Control**	1.3 ± 0.6	1.5 ± 0.6	1.2 ± 0.5	0.14 ± 0.03	0.12 ± 0.04	0.12 ± 0.04	0.71 ± 0.02	**0.68 ± 0.02 ***	0.67 ± 0.02	**3.71 ± 0.84 *****	2.53 ± 0.48	1.25 ± 0.29	1.31 ± 0.07	1.33 ± 0.06	1.51 ± 0.2
SCH 23390	**0.01**	30		10.2 ± 2.8	8.4 ± 2.4	6.8 ± 1.9	0.12 ± 0.03	0.12 ± 0.02	0.1 ± 0.03	**6.32 ± 0.89 *****	0.87 ± 0.03	0.87 ± 0.03	**6.32 ± 0.89 ***	**3.35 ± 0.64 ***	**2.11 ± 0.46 ***	3.23 ± 1.27	1.51 ± 0.1	2.45 ± 0.94
	0.02			**17.5 ± 4.5 ***	**15.4 ± 3.4 ** ^**	**11.9 ± 2.1 ^**	0.09 ± 0.03	0.11 ± 0.03	0.08 ± 0.01	1.28 ± 0.24	0.94 ± 0.07 **	0.9 ± 0.04	**5.62 ± 0.87 ***	**4.83 ± 1.13 *****	**3.98 ± 1.05 *****	1.56 ± 0.08	1.58 ± 0.08	1.74 ± 0.15
Nicotine+ SCH 23390	**0.5 + 0.01**			6.2 ± 2	6.1 ± 1.9	4.4 ± 1.3	0.09 ± 0.01	0.09 ± 0.02	0.1 ± 0.03	0.89 ± 0.05	0.8 ± 0.02	0.89 ± 0.05	**4.27 ± 0.88 ***	2.72 ± 0.54	2.79 ± 0.69	1.57 ± 0.15	1.47 ± 0.07	1.72 ± 0.21
	0.5 + 0.02			10.1 ± 2	8.5 ± 1.8	7.9 ± 2	0.05 ± 0.01	0.09 ± 0.02	0.09 ± 0.02	0.97 ± 0.08	**0.83 ± 0.02 ^^^**	0.77 ± 0.01	**3.98 ± 0.68 *****	**2.75 ± 0.57 ^^^**	**3.09 ± 0.57 ^^^ ***	1.46 ± 0.06	1.45 ± 0.05	1.48 ± 0.06
Vehicle	**0**	20		4.4 ± 1.3	2.3 ± 0.7	2.4 ± 0.9	0.12 ± 0.02	0.11 ± 0.02	0.1 ± 0.02	0.96 ± 0.04	0.87 ± 0.03	0.79 ± 0.02	5.31 ± 0.73	3.2 ± 0.77	2.19 ± 0.56	1.37 ± 0.06	1.38 ± 0.06	1.39 ± 0.06

Table 6.4 (Continued). Combined effect of light condition and cholinergic agonist, nicotine, and dopaminergic receptor 1 (DR1) antagonist on other dependent measures of 5-CSRTT performance. # Compares Light at Night and Light phase group to Control group. *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. * ^ # $p \leq 0.05$, ** ^^ ## $p \leq 0.01$, *** ^^^ ### $p \leq 0.001$.

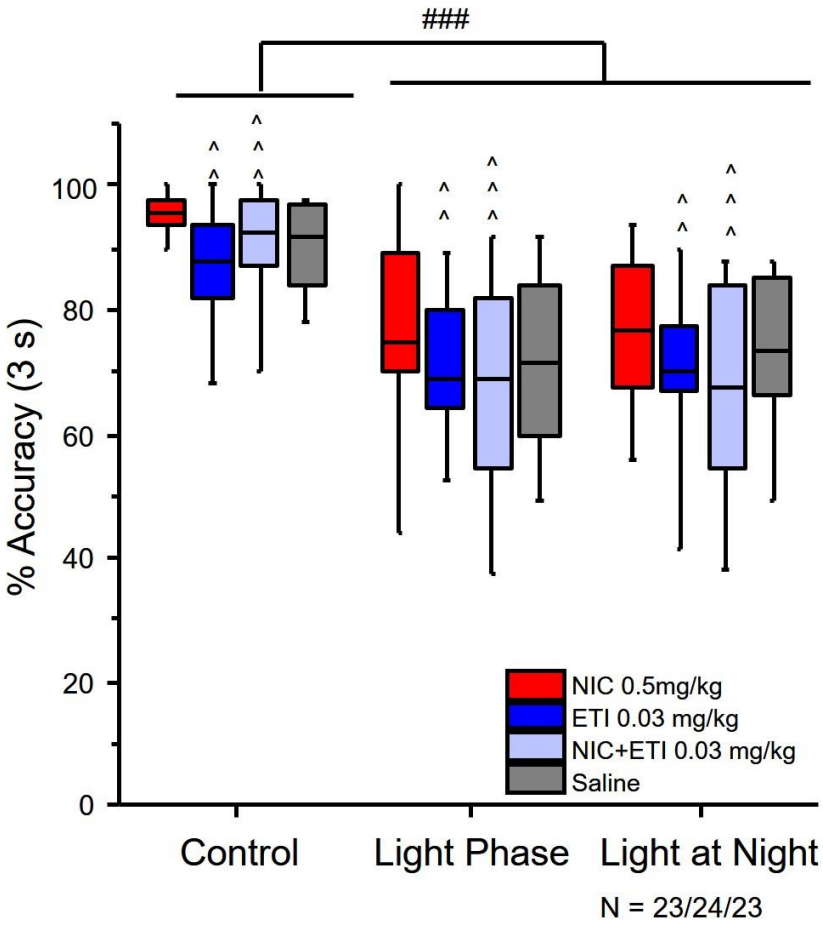

Figure 6.9. Effects of light condition on attention when NIC (0.05 mg/kg) and eticlopride (ETI) (0.03 mg/kg) were administered individually and in combination at 3 s. Both LAN and light phase groups showed deficits in accuracy compared to control. Limited effects of drugs were observed. #Compares Control group to Light at Night and Light phase groups. ^Compared to NIC ^^^ ### $p \leq 0.001$, ^^$p < 0.01$.

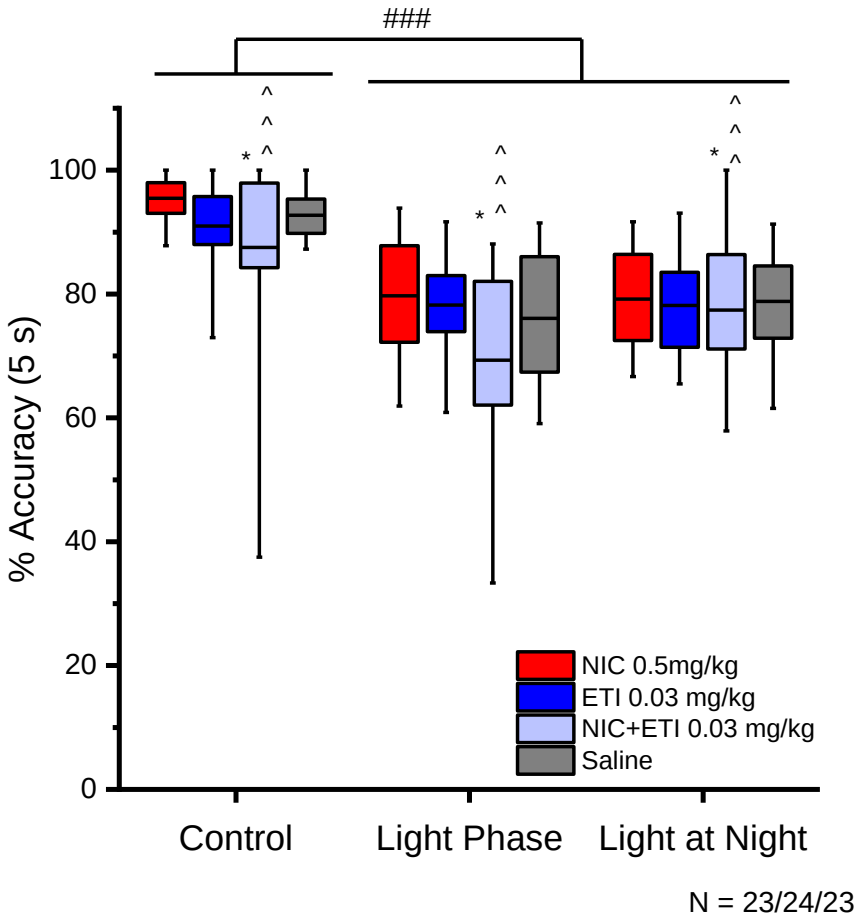

Figure 6.10. Effects of light condition on attention when NIC (0.05 mg/kg) and eticlopride (ETI) (0.03 mg/kg) were administered individually and in combination at 5 s. Both LAN and light phase groups showed deficits in accuracy compared to control. Limited effects of drugs were observed. #Compares Control group to Light at Night and Light phase groups. ^Compared to NIC. *Compares doses to saline. ###/^^^ $p < 0.001$ and * $p < 0.05$.

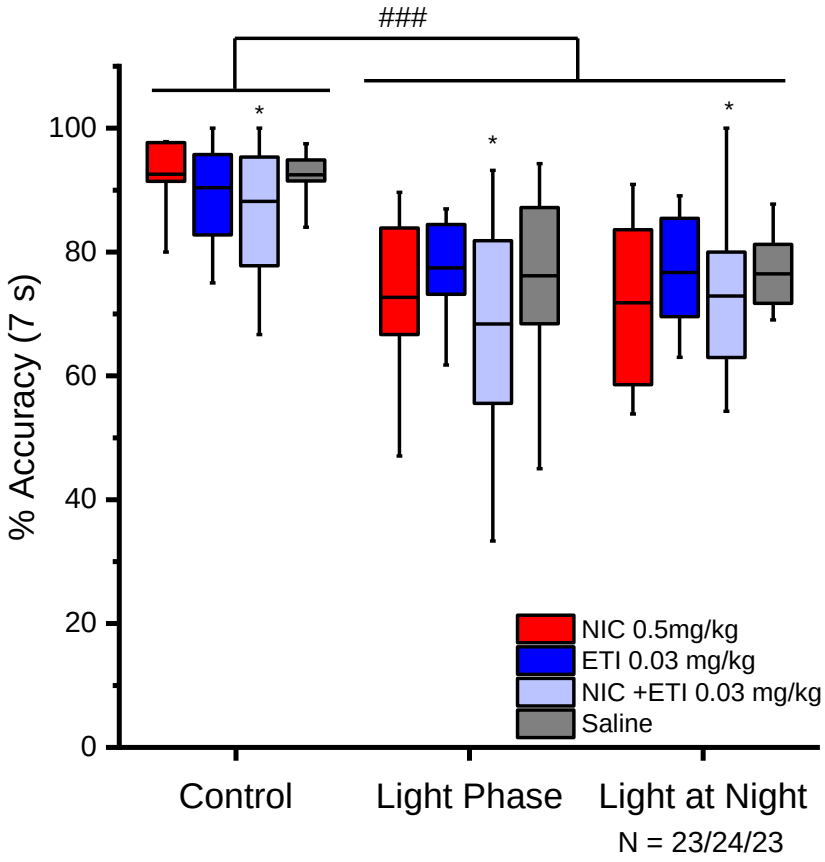

Figure 6.11. Effects of light condition on attention when NIC (0.05 mg/kg) and eticlopride (ETI) (0.03 mg/kg) were administered individually and in combination at 7 s. Both LAN and light phase groups showed deficits in accuracy compared to control. Limited effects of drugs were observed. $^{\#}$Compares Control group to Light at Night and Light phase groups. *Compared to saline. $^{\#\#\#}$ $p < 0.001$ and * $p < 0.05$.

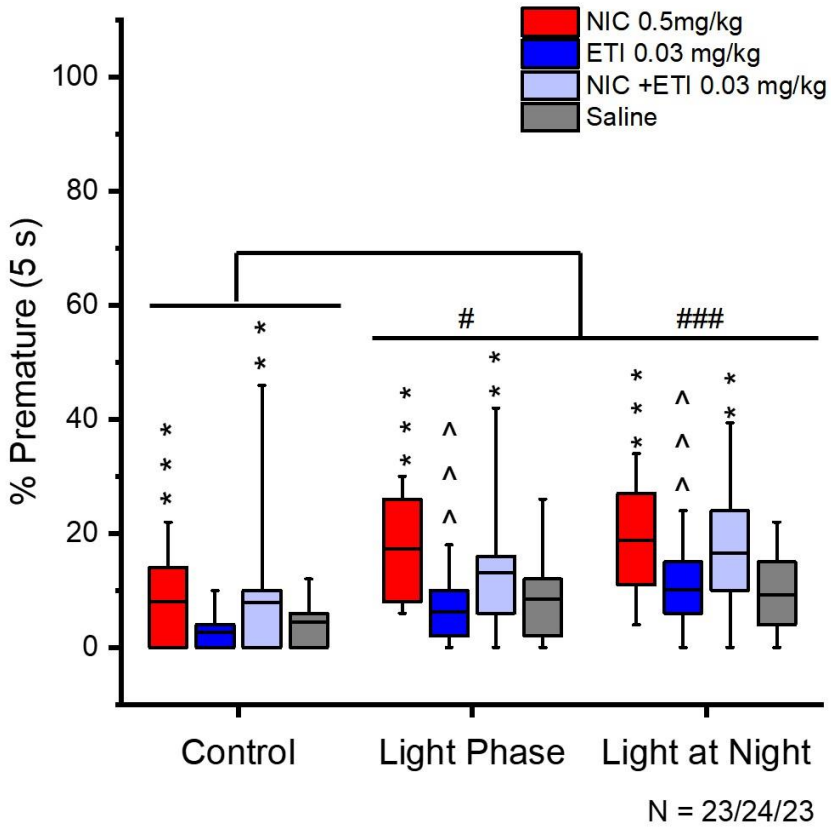

Figure 6.12. Effects of light conditions and the cholinergic agonist, nicotine, and the DR2 antagonist, eticlopride, on impulsive behavior at 5 s cue delay. In LAN and light phase NIC increased impulsive behavior compared to saline, and nicotine + 0.03 mg/kg ETI reduced the effects of NIC alone. There was no interaction between drug and light condition. #Compares Control group to Light at Night and Light phase. *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. ## ** ^^ p <0.01, ### *** ^^^ p < 0.001

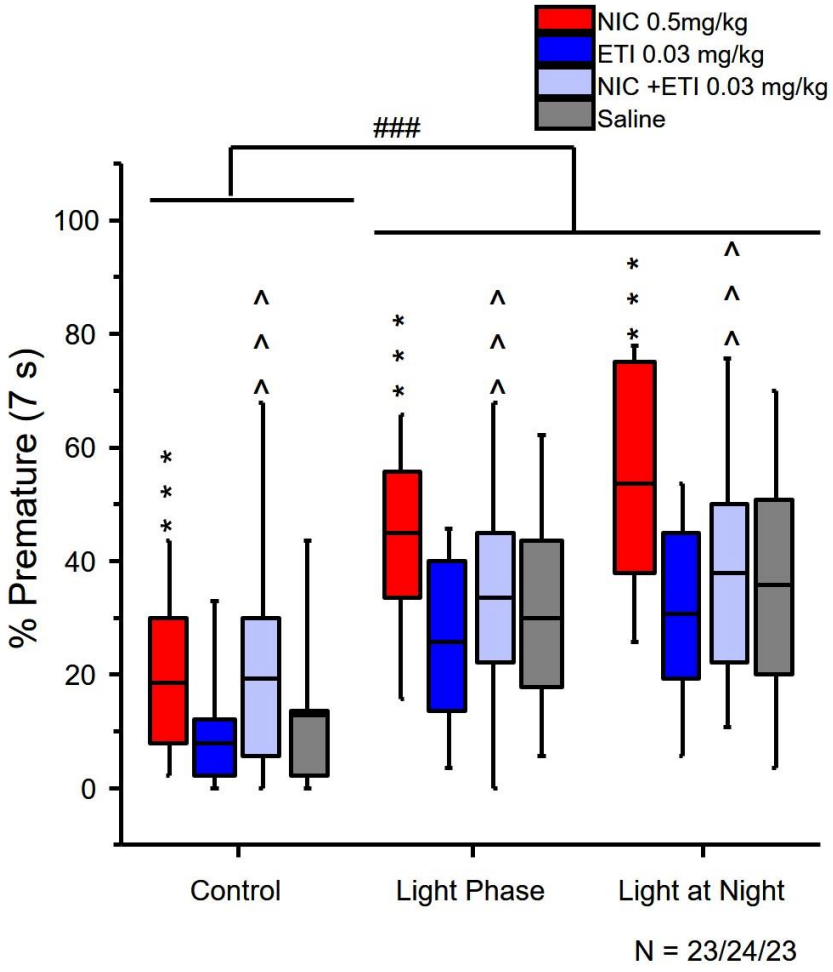

Figure 6.13. Effects of light conditions and the cholinergic agonist, nicotine, and the DR2 antagonist, eticlopride, on impulsive behavior at 7 s cue delay. In LAN and light phase, NIC increased impulsive behavior compared to saline, and nicotine + 0.03 mg/kg ETI reduced the effects of NIC alone. There was no interaction between drug and light condition. #Compares Control group to Light at Night and Light Phase. *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. ## ** ^^ p <0.01, ### *** ^^^ p < 0.001

Drugs	Dose (mg/kg)	Time before testing	Treatment Group	% Accuracy			% Premature	
				Cue Delay			Cue Delay	
				3s	5s	7s	5s	7s
Nicotine	**0.5**	20	**Light at Night**	**76.3 ± 2.5** ###	**79.7 ± 1.9** ###	**70.5 ± 2.7** ###	**18 ± 2.1** ### ***	**52.9 ± 3.9** ### ***
Eticlopride	**0.03**	20		**70.2 ± 3** ### ^^^	**77.4 ± 1.7** ###	**76 ± 2** ###	**9.6 ± 1.7** ### ^^^	**30.2 ± 3.7** ### ^^^
Nicotine + Eticlopride	**0.5 + 0.03**			**67.5 ± 4.1** ### ^^^	**77.3 ± 3.1** ### ^^^ *	**73.3 ± 3.2** ### *	**16.4 ± 2.9** ### **	**38.2 ± 4.5** ### ^^^
Vehicle	**0**	20		**73.7 ± 2.7** ###	**78.3 ± 1.8** ###	**75.8 ± 1.1** ###	**8.7 ± 1.4** ###	**34.8 ± 4.2** ###
Nicotine	**0.5**	20	**Light Phase**	**75 ± 3.9** ###	**79.7 ± 2.7** ###	**72.6 ± 3.7** ###	**17.3 ± 2.3** # ***	**45 ± 4** ### ***
Eticlopride	**0.03**	20		**69 ± 3.3** ### ^^^	**78.2 ± 2.3** ###	**77.4 ± 1.8** ###	**6.2 ± 1.3** # ^^^	**26 ± 3.3** ### ^^^
Nicotine + Eticlopride	**0.5 + 0.03**			**68.7 ± 3.8** ### ^^^	**69.3 ± 4.2** ### ^^^ *	**68.3 ± 3.9** ### *	**13.1 ± 2.4** # **	**33.4 ± 4.4** ### ^^^
Vehicle	**0**	20		**71.7 ± 3.4** ###	**76 ± 3** ###	**76.1 ± 3.2** ###	**8.4 ± 1.6** #	**30 ± 3.9** ###
Nicotine	**0.5**	20	**Control**	95.7 ± 0.6	95.4 ± 0.8	92.5 ± 1.2	**8 ± 1.9** ***	18.3 ± 3.5
Eticlopride	**0.03**	20		**87.9 ± 1.9** ^^^	90.9 ± 1.5	90.3 ± 1.7	2.6 ± 0.7	8.1 ± 1.9
Nicotine + Eticlopride	**0.5 + 0.03**			**92.5 ± 1.9** ^^^	**87.5 ± 4.9** ^^^ *	**88.1 ± 2.2** *	**7.9 ± 2.9** **	19 ± 4.5
Vehicle	**0**	20		91.4 ± 1.5	92.7 ± 0.7	92.4 ± 0.9	4.4 ± 1.4	12.9 ± 3.3

Table 5.5 Combined effect of light condition and cholinergic agonist, nicotine, and dopaminergic receptor 2 (DR2) antagonist, eticlopride on attention and impulsive behavior. #Compares Control group to Light at Night and Light Phase. *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. * ^ $p \leq 0.05$, ** ^^ $p \leq 0.01$, *** ^^^ $p \leq 0.001$.

Drugs	Dose (mg/kg)	Time before testing	Treat-ment Group	% Omission			Perseverative Responses			Correct Response Latency			Incorrect Response Latency			Reinforcement Latency		
				Cue Delay			Cue Delay			Cue Delay			Cue Delay			Cue Delay		
				3s	5s	7s	3s	5s	7s	3s	5s	7s	3s	5s	7s	3s	5s	7s
Nicotine	**0.5**	20	**Light at Night**	0.7 ± 0.2	0.8 ± 0.3	0.6 ± 0.4	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	**1.05 ± 0.07** # *	**0.83 ± 0.07** ***	0.83 ± 0.07	4.18 ± 0.31	**2.79 ± 0.3** ###	2.02 ± 0.37	1.27 ± 0.06	1.28 ± 0.05	1.32 ± 0.06
Eticlopride	**0.03**	20		**16.1 ± 5.4** ^^^	**15.3 ± 5.1** ^^ **	**15.9 ± 5** ^^^ ***	0.07 ± 0.02	0.06 ± 0.02	0.04 ± 0.01	**1.45 ± 0.18** # ^^^	**1.13 ± 0.12** ^^^	**1.01 ± 0.11** ^^	5.79 ± 0.39	**4.29 ± 0.37** ### ^^	3.45 ± 0.84	**1.61 ± 0.21** ^^	**1.53 ± 0.16** ^^	**1.48 ± 0.12** ^^ *
Nicotine + Eticlopride	**0.5 + 0.03**			**27.4 ± 6.8** ^^^	**25.1 ± 6.1** ^^^ ***	**21.9 ± 6** ^^^ ***	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	**1.32 ± 0.15** #	1.02 ± 0.11	0.8 ± 0.08	**5.08 ± 0.43** #	**4.07 ± 0.54** ### ^^^	**3.82 ± 1.01** ^^	1.35 ± 0.07	**1.5 ± 0.12** ^	**1.54 ± 0.11** ^^^ **
Vehicle	**0**	20		1.7 ± 0.7	1.8 ± 0.8	1 ± 0.3	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	**1.5 ± 0.17** #	0.97 ± 0.08	0.77 ± 0.06	4.95 ± 0.37	**3.75 ± 0.34** ###	2.59 ± 0.37	1.44 ± 0.1	1.48 ± 0.14	1.36 ± 0.06

Table 5.6. Combined effect of light condition and cholinergic agonist, nicotine, and dopaminergic receptor 2 (DR2) antagonist, eticlopride, on other dependent measures of 5-CSRTT performance. # Compares Control group to Light at Night and Light Phase. *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. * ^ $p \leq 0.05$, ** ^^ $p \leq 0.01$, *** ^^^$p \leq 0.001$.

Nicotine	**0.5**	20	**Light Phase**	8.8 ± 5.2	8.5 ± 5	6.1 ± 3.9	0.06 ± 0.01	**0.04 ± 0** #	0.03 ± 0	**1.2 ± 0.13** ### *	**0.87 ± 0.09** # ***	0.71 ± 0.05	4.54 ± 0.44	**2.61 ± 0.33** #	1.87 ± 0.31	1.37 ± 0.07	1.39 ± 0.07	1.36 ± 0.06
Eticlopride	**0.03**	20		**12.2 ± 4.7** ^^^	**7.7 ± 2.9** ^^ **	**9.1 ± 3.6** ^^^ ***	0.04 ± 0	**0.06 ± 0.01** #	0.05 ± 0.01	**1.97 ± 0.36** ### ^^^	**1.12 ± 0.1** # ^^^	**0.85 ± 0.06** ^^	5.87 ± 0.4	**4.39 ± 0.46** # ^^	2.94 ± 0.42	**1.6 ± 0.11** ^^	**1.53 ± 0.07** ^^	**1.69 ± 0.14** ^^ *
Nicotine + Eticlopride	**0.5 + 0.03**			**20.1 ± 5.3** ^^^	**18.2 ± 5.6** ^^^ ***	**15.5 ± 4.7** ^^^ ***	0.04 ± 0.01	**0.04 ± 0.01** #	0.04 ± 0.02	**1.43 ± 0.27** ###	**1.58 ± 0.32** #	0.89 ± 0.09	**5.81 ± 0.61** ###	**3.61 ± 0.41** # ^^^	**3.36 ± 0.66** ^^	1.52 ± 0.1	**1.5 ± 0.09** ^	**1.57 ± 0.1** ^^^ **
Vehicle	**0**	20		4.9 ± 1.8	2.8 ± 1.5	2.9 ± 1.4	0.05 ± 0.01	**0.05 ± 0.01** #	0.08 ± 0.02	**1.52 ± 0.13** ###	**1.06 ± 0.12** #	0.8 ± 0.05	5.2 ± 0.3	**3.49 ± 0.34** #	3.04 ± 0.59	1.53 ± 0.12	1.51 ± 0.11	1.48 ± 0.09
Nicotine	**0.5**	20	**Control**	2 ± 0.9	2 ± 1	1.7 ± 0.8	0.12 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	**0.75 ± 0.03** *	**0.72 ± 0.03** ***	0.73 ± 0.02	3.86 ± 0.8	2.03 ± 0.71	1.67 ± 0.35	1.35 ± 0.06	1.41 ± 0.09	1.46 ± 0.11
Eticlopride	**0.03**	20		**21 ± 5.7** ^^^	**21.3 ± 6.3** ^^ **	**20.3 ± 6.3** ^^^ ***	0.13 ± 0.02	0.09 ± 0.02	0.11 ± 0.03	**1.02 ± 0.04** ^^^	**1 ± 0.07** ^^^	**0.87 ± 0.03** ^^	5.09 ± 0.78	**2.63 ± 0.51** ^^	2.21 ± 0.43	**1.6 ± 0.16** ^^	**1.53 ± 0.12** ^^	**1.53 ± 0.12** ^^ *
Nicotine + Eticlopride	**0.5 + 0.03**			**16.2 ± 5.8** ^^^	**17.1 ± 5.3** ^^^ ***	**16.6 ± 5.5** ^^^ ***	0.12 ± 0.05	0.11 ± 0.04	0.14 ± 0.07	0.82 ± 0.05	0.8 ± 0.04	0.79 ± 0.04	2 ± 0.44	**3.49 ± 0.95** ^^^	**2.45 ± 0.51** ^^	1.59 ± 0.18	**1.5 ± 0.09** ^	**1.65 ± 0.15** ^^^ **
Vehicle	**0**	20		5 ± 2	4.6 ± 1.6	3.5 ± 1.1	0.13 ± 0.03	0.11 ± 0.02	0.13 ± 0.03	1.02 ± 0.07	0.85 ± 0.03	0.8 ± 0.03	4.32 ± 0.78	2.38 ± 0.39	1.74 ± 0.3	1.39 ± 0.08	1.4 ± 0.08	1.42 ± 0.07

Table 5.6(Continued). Combined effect of light condition and cholinergic agonist, nicotine, and dopaminergic receptor 2 (DR2) antagonist, eticlopride, on other dependent measures of 5-CSRTT performance. # Compares Control group to Light at Night and Light Phase. *Compared to Saline within each treatment group. ^Compared to nicotine within each treatment group. * ^ $p \leq 0.05$, ** ^^ $p \leq 0.01$, *** ^^^$p \leq 0.001$.

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Chapter 7 Conclusions

7.1 Discussion

The overall aim of my doctoral dissertation was to examine the effects of circadian disruption on cognition, specifically attention and response inhibition in adult Long-Evans rats of both sexes. Circadian rhythms are endogenous 24-hour rhythms in all organisms that govern both behavior and physiology. Environmental factors like light alter these endogenous rhythms which modulate behavior and physiology (Silver and Kriegsfeld, 2014). I modeled two types of circadian disruption to study the effects they have on attention and impulsive behavior:

1. Shift work is where the work-time schedules fall beyond the regular working hours of “9 am – 5 pm” and there is a conflict between the external cues that the body perceives (Wright et al., 2013);
2. Light at night (LAN) is untimely exposure to light at night (Dominoni et al., 2016; Fonken and Nelson, 2014; Russart and Nelson, 2017).

Specific Aim 1 detailed in Chapter 4 discusses the effects that these two models have on behavior. We established that both light phase and LAN model caused deficits in attention and response inhibition. Attentional deficits in shift work model have been previously shown by Gritton et al. (Gritton et al., 2009, 2012, 2013) but our study is the first to identify deficits associated with response inhibition in a light phase model. Nelson et al. have previously examined the physiological alterations associated with LAN model of circadian disruption (Dominoni et al., 2016; Fonken and Nelson, 2014; Russart and Nelson, 2017). This study uses the LAN model for the first time to establish deficits in both attention and response inhibition. Chapter 4 also identified changes at a molecular level caused by circadian disruption. This study identified deficits in the expression of

Per2 in the light phase work model of circadian disruption, but not in the LAN model. The altered expression of *Per2* in the light phase group could be due to alterations in dopaminergic neurotransmission. We base this on studies by Hood et al. where circadian clock-controlled genes in the dorsomedial striatum govern the expression of dopaminergic neurotransmission (Hood et al., 2010; Verwey et al., 2016). Although we did not investigate the expression of monoamine oxidase (*Maoa)* gene, previous studies have established that alteration in the expression of *Per2* directly affects *Maoa,* thus altering levels of endogenous dopamine (DA) (Hampp et al., 2008). We speculate that our shift work model of circadian disruption results in similar alterations. The absence of a difference in the expression of *Per2* in the LAN model from the controls indicates a possibility of an alternative mechanistic pathway. Our observation of a higher expression of choline acetyltransferase (ChAT) associated with the active phase establishes that there are rhythmic alterations of neurotransmitter-related genes in our rats, which is consistent with the literature (Hut and Van der Zee, 2011). Both models of disruption also showed higher expression of dopamine 1 receptor (DR1) which when coupled with binding to the elevated DA levels, could initiate post-synaptic signaling cascades at a higher rate, leading to higher expression of impulsive behavior.

These results identify deficits caused by both models of circadian disruption in attention and response inhibition. ACh governs both attention, and circadian rhythms (Gritton et al., 2012; Landgraf et al., 2014; Wright et al., 2012) and DA governs response inhibition (Dalley and Roiser, 2012; Dalley et al., 2008). The effects of both models of disruption on attention could be potentially due to alteration in the endogenous ACh levels. We speculate that the effects of the two models of disruption

on response inhibition is due to alterations in DA signaling. In brain regions like the prefrontal cortex (PFC), a brain region critical for both attention and impulsivity, and nucleus accumbens and striatum, cholinergic functioning interacts with dopaminergic signaling. In these regions, ACh release stimulates DA neurons to release DA via binding of nicotinic subtypes of ACh receptors (nAChRs) by ACh (van Gaalen et al., 2006; Livingstone and Wonnacott, 2009a). We speculate that in our models of circadian disruption, effects on behavior were mediated through this signaling pathway.

Specific Aim 2 focused on understanding the effects of circadian disruption on dopaminergic neurotransmission and impulsive behavior which have been previously unexplored. By applying pharmacological challenges in both models of circadian disruption, we identified that circadian rhythms which are governed by ACh were altering impulsive behavior. Combinations of the cholinergic agonist, nicotine, and dopaminergic antagonists for both DR1 (SCH 23390) and DR2 (eticlopride) did not influence accuracy but significantly altered premature responding on 5-CSRTT in both models of disruption, where the behavioral measures correspond to attention and impulsive behavior (Robbins, 2002). Chapter 5 as well as studies by others have established that nicotinic modulation of ACh has a critical role in modulating attention (Havekes et al., 2011; Wallace and Bertrand, 2013) and nicotinic acetylcholine receptors (nAChRs) on the dopaminergic presynaptic terminals play a role in modulating impulsive behavior (Livingstone and Wonnacott, 2009b). The effects of our drug challenges on impulsive behavior in Chapter 6 are novel and help us understand how circadian disruption increases impulsive behavior and sensitivity to drugs. Our findings are potentially due to altered expression of molecular components, especially

those components of dopaminergic neurotransmission that are under circadian control (Hampp et al., 2008; Hood et al., 2010; Verwey et al., 2016).

7.2 Future Studies

These studies are only the first step in learning how circadian disruption affects behavior. We need to further explore the effects of circadian disruption on the receptors and enzymes that are involved in modulating optimal cholinergic and dopaminergic neurotransmission. Future studies will parse out further mechanisms underlying these two models of circadian disruption by quantifying mRNA levels for TH, DAT and the DRs in the ventral tegmentum and substantia nigra, regions containing the dopaminergic neuronal bodies that project to the prefrontal cortex, nucleus accumbens and the striatum. Additionally, quantifying the protein levels corresponding to the genes tested in this study in both infralimbic cortex and dorsomedial striatum could provide us more information on how regulation of protein translation may alter the underlying neurochemistry in both our models of circadian disruption.

One final point is that we repeatedly reproduced our effects of both models of circadian disruption on attention and response inhibition. These established models could further serve as the base to investigate the effects of how other environmental factors, including chemical sources of endocrine disruption, may affect neurochemical signaling and behavior.

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