Pharmacological and Behavioral Modulation of Impulsivity in the Touchscreen-based Mouse 5-choice Serial Reaction Time Test

by

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Abstract

This work characterized the performance of mice on a new touchscreen version of the 5-choice serial reaction time test (5CSRTT). We investigated whether task-parameter and pharmacological manipulations that influence impulsivity in the older rat 5CSRTT exerted similar influences in the mouse 5CSRTT. Using premature responding as a measure of impulsive actions, we found that increasing the inter-trial interval from 5 to 9s reliably increased impulsivity. The psychostimulant cocaine increased premature responding but had a weaker effect in mice than in rats. The α2-adrenoceptor antagonist yohimbine, which heightens impulsivity in rats, reduced impulsivity in mice. The serotonin 5-HT2C receptor agonist lorcaserin, noradrenaline re-uptake inhibitor atomoxetine, and selective serotonin re-uptake inhibitor citalopram decreased premature responding. These results indicate that the touchscreen version of the 5CSRTT can reliably measure impulsivity, and that there is some correspondence between the effects of pharmacological and behavioral manipulations on the 5CSRTT in rats and mice.

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Chapter 1
Introduction

1 Impulsivity as a multi-faceted construct

Impulsivity is a multi-dimensional behavioral trait that can be both beneficial or detrimental to an individual's daily life. For example, impulsively applying for a new job may afford a person a valuable opportunity to grow their career, or the opportunity to make an ill-advised career change that begets financial instability. Trait impulsivity is generally thought to function as a normal aspect of a person's behavior, but at high levels, may become pathological and contribute to the presence of substance abuse and psychiatric disorders (Dalley & Rosier, 2012). Impulsivity is not a unitary construct and different aspects of impulsivity are thought to contribute to different aspects of behavior and decision making.

In general, impulsivity is defined as a lack of self-control, which is characterized by making decisions before all relevant information has been acquired (Dalley et al., 2011). In recent years however, there has been a growing consensus that impulsivity is a multi-faceted construct that reflects separate underlying processes (de Wit, 2008). Impulsivity can be decomposed into at least two different processes, impulsive choice and impulsive action (Winstanley et al., 2004a). In humans, impulsive choice is measured on tasks of delay-discounting and is operationalized as preferring an immediate small reward over a delayed bigger reward (Cardinal et al., 2001). In this way, a person is "discounting" the more rewarding delayed-option relative to the less-rewarding immediate-option. As a person becomes more impulsive, they discount the delayed-choice to a larger degree.

Impulsive action, on the other hand is the inability of an individual to stop responses that they are primed to make. This is referred to as a prepotent response. Impulse control is the inhibitory mechanism by which prepotent responses towards primary reinforcers, such as food, money, and sex are inhibited (Nigg, 2000). Impulsive action, or the failure of impulse control, therefore arises when rapidly prepotent responses are not inhibited (Winstanley et al., 2006). Impulsive action is commonly assessed using the Go/No-Go Task (Eagle et al., 2009). In each trial, participants are conditioned to make a particular response following presentation of a "Go" cue. However, during certain trials, a "No Go" cue is presented, either simultaneously, or preceding

the “Go” cue. Behavioral inhibition is measured by calculating the amount of time needed to inhibit an already-initiated response (de Wit, 2008).

2 Impulsivity using animal models

Impulsivity also occurs in animals, and both impulsive choice and impulsive action can be measured using well-established tasks (De wit, 2008). Impulsive choice is typically tested using an adapted rodent delay-discounting task. This task works in the same manner as the human version – rodents, typically rats, choose between an immediate but small food reward, or larger reward that is presented following an ever-increasing delay. Accordingly, impulsive choice making is measured by how much the animal discounts the larger delayed reward.

A common measure of impulsive action in rodents is premature responding in the five choice serial reaction time task (5CSRTT), a well-validated test of sustained visual attention (Bari et al., 2008). In this task, animals are trained to make a response in one of five response areas when presented with a light cue for food reward. Each trial begins with a five second inter-trial interval (ITI), during which animals must refrain from responding. If the animals respond during this ITI, the response is counted as a premature response. These premature responses therefore act as a measure of motoric impulsivity, or impulsive action (Bari et al., 2008). This task is particularly useful as it measures various aspects of an animal’s performance, including motivation, accuracy, attention, motor activity, and impulsivity; this allows for the parsing of the animal’s behavior into specific constituent factors of interest (Robbins, 2002).

3 Neural substrates of impulsivity

Impulsive choice and action have overlapping, but separate neurobiological substrates in the medial temporal and frontal cortices. Impulsive choice in humans is mediated by the orbitofrontal cortex, and the ventrolateral prefrontal cortex (Fineberg et al., 2010). In rodents, impulsive choice has been linked to the activity of the nucleus accumbens (NAc), subthalamic nucleus (STN), and the medial striatum (MS). For example, lesioning the STN of rats increases the degree which they discount delayed rewards on the delay-discounting task (Uslaner & Robinson, 2006).

Frontal-striatal circuitry are thought to be the primary contributors to impulsive actions (Naaijen et al., 2015). In humans, response inhibition has been correlated with the activity of the right

inferior frontal cortex, caudate putamen, and STN. For example, on the Go/No-Go task, deep-brain stimulation of the STN in Parkinson's patients is correlated with enhanced inhibitory control on no/go tasks (van den Wildenberg et al., 2006). Likewise, damage to the right inferior frontal gyrus leads to delayed ability to internally suppress a motoric response (Aron et al., 2003). Furthermore, impaired Go/No-Go performance has been seen in persons with lesions to the basal ganglia and caudate nucleus (Rieger et al., 2003).

Complementary results have been found using animal models, with the infralimbic cortex, anterior cingulate cortex, orbitofrontal cortex, NAc, MS (analogous to the human caudate nucleus), and STN of rodents (Fineberg et al., 2010) shown to mediate response inhibition. For example, lesioning the MS and STN of rats increases premature responding on the 5CSRTT (Baunez & Robbin, 1997), and leads to a marked inability to inhibit responses on the Go/No-Go task (Eagle & Robbins, 2003). This effect is similar to the one seen in Rieger et al's (2003) human study. Similarly, excitotoxic lesioning of the anterior cingulate cortex leads to an increase in premature responding on the 5CSRTT (Muir et al., 1996). These results indicate that while there is some overlap between the substrates of impulsive choice and impulsive action, the two forms of impulsivity nonetheless depend on distinct neural circuits in the brain.

4 Pharmacological mechanisms of impulsivity

A wealth of studies highlight the role of the neurotransmitters norepinephrine (NE) and dopamine (DA) in impulsivity. Dopaminergic compounds, such as amphetamine, cocaine, and methylphenidate decrease impulsive choices in discounting paradigms (Richards et al., 1999; Wade et al., 2000; Krebs and Anderson, 2012; Cottone et al., 2013). In the opposite manner, amphetamine and cocaine greatly increase impulsive actions in the 5CSRTT (van Gaalen et al., 2006b; Blondeau and Dellu-Hagedorn, 2007). These divergent effects are thought to arise due to a combination of brain region-specific and receptor sub-type specific activation by dopaminergic agents. For example, van Gaalen et al (2006) have demonstrated that activation of dopamine D2 receptors in the NAc shell and NAc core increase and reduce premature responding in the 5CSRTT. Likewise, Winstanley et al (2010) have shown that injection of dopamine D2 and D3 agonists but not D1 agonists into the orbitofrontal cortex attenuate premature responding on the 5CSRTT.

Norepinephrine also affects the expression of impulsivity. Systematic administration of NA re-uptake inhibitors (e.g. atomoxetine) reduces premature responding on the 5CSRTT and the SSRTT, and impulsive choices in delay discounting paradigms (Robinson et al., 2008). More specific studies have elucidated the contribution of different NA sub-receptors to these effects. NA α1-adrenoceptor antagonists (e.g. Prazosin), and α2-adrenoceptor agonists (e.g. Guanfacine) generally reduce premature responding in the 5CSRTT (Liu et al., 2009; Fernando et al., 2012). In opposition to this, α2-adrenoceptor antagonists (e.g. yohimbine) increase impulsive actions (Sun et al., 2010). β-adrenoceptors have also been implicated in impulsive actions but results so far have been mixed; while some studies show that administration of selective β2-adrenoceptor agonists reduces premature responding on the 5CSRTT (Pattij et al., 2012), a similar result is seen with non-specific β-adrenoceptor antagonism (Milsten et al., 2010). As such, while NA plays a role in impulsive behaviors, more work is needed to delineate the exact mechanisms underlying these effects.

5 The role of serotonin in impulsivity

Serotonin (5-hydroxytrtyptamine, 5-HT) is an important neurotransmitter that has been linked to impulsivity. 5-HT neurons project to the mid- and forebrain via two main ascending projections originating in the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN). These brainstem raphe 5-HT systems are among the most widely distributed neurotransmitter systems in the brain and project diffusely to various areas (Lesch & Merschdorf, 2000). The terminal projections from the MRN and DRN innervate partially overlapping but distinct regions of the frontal cortices and medial temporal lobes. MRN neurons project via the ventromedial forebrain bundle and provide serotonergic input to the medial septum, cingulate cortex and the hippocampus. On the other hand, 5-HT neurons of the DRN travel via the ventrolateral medial forebrain bundle and innervate the amygdala, substantia nigra, nucleus accumbens, caudate-putamen, and globus pallidus (Harrison et al., 1997). At the synaptic level, 5-HT supplied by the MRN and DRN helps to modulate and regulate the activity of other neurotransmitters, including dopamine neurons from the ventral tegmental area and the substantia nigra. These modulatory effects arise from 5-HT interactions with different receptor-subtypes, with 5-HT1A, 5-HT1B, 5-HT2A, and 5-HT2C receptor subtypes thought to be important in the expression of impulsivity (Carli & Samanin, 2000; Higgins et al. 2003; Winstanley et al. 2004b; Bouwknecht et al. 2001)

There is a large body of evidence that demonstrates that 5-HT activity modulates impulsivity. In humans, evidence for the role of 5-HT comes primarily from 5-HT depletion and genetic studies investigating aggression and substance abuse. Early work implicated low levels of 5-HT and its metabolites in impulsive behavior. For example, lower levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), a marker of overall 5-HT levels in the brain, are correlated with impulsive aggression, and impulsive alcohol-drinking (Linnoila et al., 1983; Nielsen et al., 1998; Kreek et al., 2004). Furthermore, Coccaro et al., (1997) showed that low 5-HT metabolism, as assessed by prolactin release after fenfluramine challenge, is associated with increased behavioral impulsivity and higher risk of having impulsive personality traits in first-degree relatives. Additionally, studies looking at variations in genes coding for tryptophan hydroxylase 1 and 2 (enzymes important for the synthesis of 5-HT) have demonstrated that low 5-HT producing alleles are associated with increased impulsivity and impulsive aggression (Nielsen et al., 1994). A more commonly used line of research used to investigate the association between 5-HT and impulsivity is 5-HT depletion. Studies using this technique ask participants to ingest an amino acid mixture that is selectively-lacking tryptophan, the precursor to 5-HT. This leads to a marked reduction in 5-HT synthesis and cerebrospinal fluid 5-HT levels. Using this technique, Crean et al. (2002) have shown that acute reduction of 5-HT functioning via a tryptophan-depleting diet leads to impairments in behavioral inhibition as assessed by the Stop Task. This result has been corroborated by numerous studies demonstrating increased impulsivity as assessed by impaired conditioned suppression (Robinson et al., 2012), increased premature responding (Walderhaug et al., 2007, Booij et al., 2006) and increased delayed reward discounting (Schweighofer et al., 2008). These results combined with the previously mentioned studies suggest that 5-HT exerts an important influence on impulsivity.

The role of 5HT on impulsivity has also been studied extensively in animals. Early studies have demonstrated that central 5-HT depletion via 5,7-dihydroxytryptamine (5,7-DHT) lesioning leads to increased premature responding on the 5CSRTT (Harrison et al., 1997), and decreased behavioral inhibition on the Go/No-Go task (Harrison et al., 1999). Likewise, other studies have shown that pharmacological depletion of 5-HT (via parachloroamphetamine) also increases premature responding (Masaki et al., 2006). Further converging evidence for the link between 5-HT and impulsivity has come from studies using more selective 5-HT manipulations: administration of 5-HT2A/2C agonist 2,5-Dimethoxy-4-iodoamphetamine (DOI) increases

impulsive action in the 5CSRTT and impulsive decision-making on the delay discounting tasks (Evenden and Ryan, 1999; Blokland et al., 2005). Likewise, Fletcher et al., (2007) found that selective blockade of the 5-HT2C receptor by the selective antagonist SB242084 increases premature responding in the 5-CSRTT. Related studies have further established that selective antagonism of 5-HT2A receptors by the drug M100907 reduces behavioral impulsivity on the 5-CSRTT in a dose-dependent fashion (Fletcher et al., 2007; Winstanley et al., 2004). A similar decrease in impulsive action has been seen following administration of 5-HT1A agonist 8-OH-DPAT. Overall, these studies suggest that 5-HT plays an important role in the expression of impulsivity; with reductions or enhancements of impulsive behavior depending on the sub-type of 5-HT receptor that is involved.

6 Move towards mouse models and automated touchscreen chambers

While the animal approaches used in the studies above have provided a wealth of knowledge about the role of different brain regions and neurotransmitters in the expression of impulsivity, their findings have often not been translated into viable treatment options for persons with impulsivity disorders. This problem has often been decomposed into two separate challenges. First, there are few dose-sensitive pre-clinical behavioral assays that produce reliable results across different contexts, and pharmacological and genetic manipulations (Insel, 2010). Often, pre-clinical assays of cognition are run by hand, in non-automated testing chambers that vary in form and parts. For example, the lights used in the response areas of 5CSRTT boxes may drastically differ in brightness across different labs and time points. This may result in considerable variation across different testing boxes and lab contexts (Hvoslef-Eide et al., 2015). Second, it is difficult to find behavioral tests that demonstrate high construct validity and whose results can readily be translated from pre-clinical to clinical contexts (Moore et al., 2013). For example, few tests demonstrate back-translational effects, wherein drug treatments used in clinical settings demonstrate similar affects in animal tested on comparable tasks.

In order to address these issues, scientists have begun to adopt the use of touchscreen operation chambers and validated rodent tests of cognition that provide pre-clinical measures which closely resemble those seen in clinical contexts. Under the guide of the NEWMEDS (Novel Methods leading to New Medications in Depression and Schizophrenia) consortium, a new rodent

touchscreen-based cognitive test battery has been created which aims to ameliorate some of the aforementioned challenges (Hvoslef-Eide et al., 2015). As part of this new battery, the rodent 5CSRTT has been extended to touch screen boxes. This touchscreen task is slightly changed from the classic test. In this newer version of task, rodents make a response directly on the touch screen following the presentation of a white square located in one of five locations, as opposed to making nose pokes in response to light flashes. These changes are designed to allow for greater species cross-validation; the test now very closely resembles the human touchscreen 4CSRTT (Voon et al. 2014), in terms of both the presented stimulus and the form of responding. In support of this, early work using the touchscreen versions of the 5CSRTT and the 4CSRTT demonstrated that serotonin depletion in humans (using diet-induced tryptophan depletion) and rats results in a very similar pattern of increased premature responses (Worbe et al. 2014). Furthermore, as the touchscreen 5CSRTT is administered in a similar apparatus as other NEWMEDS test, there is improved comparability with results from other tests of memory, learning, and attention in both humans and rodents (Bussey et al., 2012).

The new touchscreen version of the 5CSRTT has increasingly begun to be used with transgenic mice models of various disorders. For example, Bartko et al. (2011) used the touchscreen 5CSRTT in conjunction with muscarinic M1 acetylcholine knock-out mice. They showed that M1 knock-out mice displayed heighted levels of perseverative and impulsive responding. Likewise, Kolisnyk et al. (2013) have shown that overexpressing the vesicular acetylcholine transporter in the prefrontal cortex of leads to increased premature responding at short stimulus durations. Interestingly, they also found that decreasing the levels of the same transporter led to a more subtle change in accuracy and omissions but did not lead to any changes in impulsive responding. Taken together, these results would suggest that the touchscreen 5CSRTT may offer a new, more efficient and reliable method of testing impulsivity in pre-clinical settings; it offers improved ecological validity, an ability to detect both impairments and improvements in task performance, and sensitivity to subtle effects.

7 Framework

The 5CSRTT has proven to be a reliable method of testing impulsive actions. It measures not only the level of impulsive actions, but also other aspects of an animal's performance, including attention, and motivation. As such, it allows for a detailed look at the impulsive actions arising from experimental manipulations. While the 5CSRTT has been used quite extensively in rat models, relatively less attention has been placed on the study of mice in the paradigm. This is a noteworthy gap in the literature as the use of mice allows not only for standard pharmacological and task parameter manipulations of impulsive action, but also for the incorporation of behavioral genetic techniques. For example, transgenic mice can be used to directly examine the role of specific neurotransmitter subtypes and sub-regions of the brain in mediating impulsive actions.

Likewise, due to the relatively recent arrival of the touchscreen version of the 5CSRTT, there has been limited work examining whether task parameter and pharmacological manipulations of impulsivity used in the classic version of the task reliably affect behavior in the same way in the touchscreen version. Therefore, it is important to extensively characterize the expression of impulsive behavior in the mice-adapted touchscreen version of the 5CSRTT. Studying the responses of mice to both behavioral and pharmacological challenges will set the foundation for more advanced research into the antecedents of impulsive behaviors using transgenic and optogenetic approaches, and more generally, allow for a more thorough understanding of the behavioural construct of impulsivity.

7.1 Objectives

1) The first goal of these experiments was to optimize a mouse-version of the touchscreen 5CSRTT that produced consistent and reliable expression of impulsive action. Mice were trained on a baseline version of the 5CSRTT with a 5s ITI. They were then challenged by three task parameter manipulations which are known to increase premature responding in rats (Robbins, 2002). These include a longer 9s ITI, a variable 5-9-15s ITI, and a combination of 9s ITI and reduced brightness. The inclusion of brightness manipulations, which are known to tax attention (Muir et al., 1996), allowed us to examine whether our ITI manipulations were taxing response inhibition only, or whether our task was also acting as an attentional challenge. Overall, these

tests allowed us to determine whether elevated levels of impulsive action could be reliably induced in mice in the same manner as rats.

2) The second goal of these experiments was to evaluate whether the optimized 5CSRTT is sensitive to well-characterized pharmacological manipulations of impulsivity. To this end, mice were tested in two conditions. First, they were tested on the baseline 5s of the task following administration of the DA re-uptake inhibitor cocaine, and the NE α2 receptor antagonist yohimbine. These drugs produce reliable and significant increases in premature responding in rats (Jupp & Dalley, 2014). Second, mice were tested on the 9s ITI 5CSRTT following injections of yohimbine, the systemic 5-HT reuptake inhibitor citalopram, 5-HT2C receptor agonist lorcaserin, and norepinephrine reuptake-inhibitor atomoxetine. These drugs have been shown to decrease impulsive actions in the 5CSRTT (Jupp & Dalley, 2014, Funk et al., 2019). Together, these tests allowed us compare how systemic and more specific manipulations of neurotransmitter systems influence impulsivity in rats and mice. Demonstrating successful bidirectional manipulation of impulsivity using our test is an important first step towards the use of more advanced behavioral genetic techniques.

7.2 Hypotheses

1) All three task parameter changes will lead to elevated levels of premature responding. Mice challenged with the variable ITI will show higher overall levels of premature responding when compared to the 9s ITI challenges. This is due the fact that variable ITI manipulation is thought to not allow for the formation of response strategies as a result of task experience. However, due to this increase in difficulty, the mice will likely complete fewer overall trials. Lastly, the combination of decreased brightness and 9s ITI will lead to more premature responses and generally worse performance when compared to the standard 9s ITI manipulation. This is due to the test taxing both response inhibition and attentional resources.

2) Cocaine, and yohimbine will increase premature responding seen during the 5s ITI tests. However, the drugs' overall effect on impulsivity may be more subtle in mice than rats. This is due to the fact that mice have a lower baseline level of impulsivity. Citalopram, lorcaserin, and atomoxetine will all decrease premature responding on the 9s ITI. Citalopram is a systemic 5-HT reuptake inhibitor and will affect the activity of multiple 5-HT receptor subtypes. As these

subtypes can both reduce or enhance impulsive behaviors, citalopram's overall effect on impulsivity may be more subtle than the more selective drugs lorcaserin and atomoxetine.

Chapter 2
Methods

1 Subjects and housing

Adult male C57BL/6J mice (n =24) were obtained from Jackson Laboratories (Bar Harbor, Maine, United States). Mice were group-housed, and water and food were available ad libitum in home cages under a 12-hour light-dark cycle (lights off at 7pm). The study began with mice aged approximately two-and-a-half months and weighing between 20–30g. Two weeks prior to the start of training, mice were food restricted to 85-90% of their free-feeding body weight using a 3h/day limited-access schedule beginning 1 h after completion of behavioral procedures. Once mice acclimated to limited food access, the access duration was reduced and held at 1.5h/day during training and testing days of the experiment. During non-training or testing days, mice had unlimited access to food. Training and testing occurred during the light period. Experimental procedures conformed to the guidelines set by the Canadian Council on Animal Care, and the CAMH Animal Care Committee. All behavioral testing was conducted using the same group of mice.

2 Apparatus

Mice were trained and tested on the 5-CSRT in eight touch-screen operant chambers (Lafayette Instrument Co., Lafayette, IN) contained within sound and light-attenuating boxes. Chambers were trapezoid-shaped (20 cm high × 18 cm long × 24 cm or 6 cm wide), and consisted of two black Perspex walls, a see-through Perspex lid, and a perforated steel floor. A 3-W house light was located at the top of the chamber. An infrared touchscreen monitor (24.5 × 18.5 cm) was located at the front of the chamber. This monitor was covered by a black Perspex mask containing five equal-sized (4 x 4 cm) response windows wherein mice could make a touch response (e.g., nose poke). An automated liquid pump was located opposite to the touchscreen monitor. This pump delivered strawberry milkshake (Nestle, CH) into a reward-tray equipped with an LED light and infrared-sensors to detect entries. The operant chambers were controlled using ABET II touchscreen software (Lafayette Instrument Co., Lafayette, IN).

3 Touchscreen training

Mice were first habituated to the touchscreen chambers in two sessions. In each session, the reward tray was illuminated and primed with 150µl of strawberry milkshake reward. When mice made a tray entry, the reward tray light was turned off for 10 s, and 7µl of milkshake was delivered. Mice repeated this procedure approximately sixty times during the session. Mice then progressed to initial-touch training. In this phase, a white square stimulus was displayed randomly in one of the five response windows. After a 30 s delay, the stimulus was removed, and milkshake was delivered. If mice correctly nose-poked the response window where a stimulus was displayed, they received three times the milkshake reward. Collection of the reward initiated a new trial. Mice who reached criterion (30 trials in 30 minutes) moved to "must-touch" training. In this phase, a white square stimulus was randomly displayed in one of five response windows. Mice were required to touch the response window displaying the stimulus to receive a reward. A new trial was initiated 5 s after reward collection. Touching the other blank response windows had no programmed consequences. Mice who reached criterion (20 trials in 30 min on two consecutive days) progressed to 5-choice serial reaction time testing.

4 5-choice serial reaction time test training

Each session began with the illumination of the reward tray and the delivery of strawberry milkshake. A nose-poke into the reward tray initiated the trial. Following a fixed inter-trial interval (ITI) of 5s, a white square stimulus was presented randomly in one of five response windows. A correct nose poke on the response window containing the stimulus, or in the brief period following stimulus display (limited hold period), illuminated the reward tray and led to the delivery of milkshake reward. Collection of the reward initiated the ITI for the next trial. Incorrect nose-pokes on blank reward windows or non-responses (omissions) were not reinforced and led to a 5s time-out period where the house light was illuminated. Following the time-out period, mice had to make a nose-poke into the reward tray to begin a new trial. Nose-pokes into any response window made during the ITI (premature response) also resulted in a time-out. Sessions lasted for 30 minutes or for 100 trials.

Performance on the task was determined by measuring accuracy of responding expressed as percentage of correct responses (correct responses/(correct + incorrect responses) x 100); percent omissions (number of omission trials/total trials completed x100); and percent premature

responses (premature responses/ total trials initiated x 100). Perseverative responses (additional nose-pokes made prior to collection of reward), and latencies for correct responses and reward collection were also recorded.

Training started with a stimulus duration (SD) of 30s. Based on mice's performance (reaching a criterion of >80% accuracy and <30% omissions over two consecutive training sessions), SD was systematically reduced to 1s. Time-out periods and limited holds were always 5 s. ITI was 5 s except in tests in which ITI was a manipulation variable.

Training sessions took place five days a week between Monday and Friday. Testing sessions always took place on Tuesdays and Fridays. Mice were run on the standard 5-CSRTT task in between testing sessions.

5 Behavioral manipulations

5.1 Experiment 1: performance with 9s inter-trial interval

In order to assess whether premature responding rates could be increased above baseline, mice were tested systematically on four separate test days with the ITI increased to 9s. These 9s ITI probe tests were separated by three days, during which mice were tested on the standard 5 s ITI version of the task.

5.2 Experiment 2: performance with a 9s inter-trial interval and reduced stimulus brightness

This experiment was conducted to investigate whether the addition of reduced stimulus brightness would induce higher levels of impulsive actions compared to the standard 9s ITI version of task. Mice were tested on a modified version of the task with stimulus brightness reduced to thirty percent. Four separate tests were conducted, with each 9s test separated by three days of testing on the baseline 5s ITI version of the task.

5.3 Experiment 3: performance with a variable inter-trial interval

This experiment assessed whether a variable ITI version of the 5CSRTT would induce a higher level of premature responding when compared to the standard 9s ITI version of the task. This task used ITI values of 5, 9, and 15 s. Each testing session consisted of sets of 15 trials. In each set, each ITI was presented five times in a random order. This ensured that each ITI was not presented more than 3 times in a row. Mice were tested on four separate days, with each probe test separated by three days of testing on the baseline 5s ITI version of the task.

6 Pharmacological manipulations

In the following experiments, animals were tested with drugs that have previously been shown to increase or decrease impulsive actions. When testing with drugs expected to increase premature responding, a 5s ITI version of the task was used. Mice typically show low premature on this version of the task, making it ideal for the detection of potentially elevated impulsive responding. When testing with drugs expected to decrease premature responding, a 9s ITI version of the task was used. This version of the task induces high levels of premature responding and can therefore be used to better detect reductions in impulsive responding.

6.1 Experiment 4: effects of cocaine

In this experiment, mice were tested on the baseline 5s ITI version of the task following injections of vehicle, 7.5 or 15 mg/kg cocaine. Injections were administered IP 10 min before the start of tests.

6.2 Experiment 5: effects of yohimbine using 5s inter-trial interval

In this experiment, mice were tested on the baseline 5s ITI version of the task following injections of vehicle, 0.313 and 0.625 mg/kg yohimbine. Injections were administered IP 30 min before the start of tests.

6.3 Experiment 6: effects of citalopram

In this experiment, mice were tested on the 9s ITI version of the task following injections of vehicle, 5 and 10 mg/kg citalopram. Drugs were injected interperitoneally 20 minutes before testing.

6.4 Experiment 7: effects of lorcaserin

In this experiment, mice were tested on the 9s ITI version of the task following injections of vehicle, 0.05, 0.1 and 0.2 mg/kg lorcaserin. Vehicle and lorcaserin were injected subcutaneously 30 min before the start of the tests.

6.5 Experiment 8: effects of atomoxetine

In this experiment, mice were tested on the 9s ITI version of the task following injections of saline, 0.5 and 1 mg/kg atomoxetine. Injections were administered IP 40 min before the start of tests

6.6 Experiment 9: effects of yohimbine using 9s inter-trial interval

Following the results of experiment 4, we wished to assess whether yohimbine reduces impulsivity in mice. Accordingly, mice were tested on the 9s ITI version of the task following injections of vehicle, 0.313 and 0.625 mg/kg yohimbine. Injections were administered IP 30 min before the start of tests.

6.7 Drugs and injections

Cocaine hydrochloride (Medisca, St-Laurent, Canada) was dissolved in 0.9 % saline solution and injected intraperitoneally (IP) 10 minutes before testing. Atomoxetine (Toronto Research Chemicals, Toronto, Canada) was prepared in 0.9 % saline and administered 40 min before the start of the session. Yohimbine (Sigma Aldrich, Oakville, Canada) was dissolved in distilled water and injected IP 30 minutes prior to testing. Lorcaserin (NPS Pharmaceuticals, Toronto, Canada) was prepared in 0.9% saline solution and administered subcutaneously 30 minutes before testing. Citalopram HBr (Toronto Research Chemicals, Toronto, Canada) was dissolved in 0.9% saline and administered IP 20 minutes before the start of testing. Drug solutions were prepared fresh each day. In all experiments, drug treatment followed a within-subject, Latin-

square design. Drug treatment sessions were always separated by a 72h washout period. Drug doses for cocaine, atomoxetine, and citalopram were based on prior work (Fletcher et al., 2011; Robinson et al., 2007; Humby et al., 2013; Tomlinson et al., 2014; Browne & Fletcher, 2016). Drug doses for lorcaserin, and yohimbine were chosen based on pilot data.

6.8 Statistical analyses

Repeated-measures one-way ANOVAs or paired *t* tests were used to assess the significance of effects related to behavioral and pharmacological manipulations. A two-way repeated measures ANOVA was used to assess the stability of mice's performance on the 9s ITI task at six different time points. In all cases, assumptions of the ANOVA procedure or *t* tests, including sphericity and equality of variances, were assessed with Mauchly's test or Leven's test. Post hoc analyses were performed with Dunnett's test, Tukey's multiple comparison test, and Sidak's post hoc test.

Chapter 3
Results

1 Experiment 1: performance with 9s inter-trial interval

Figure 1 shows performance of mice on the 9s ITI task. Increasing the ITI from 5s to 9s led to an increase in premature responses (t (23) = 15.275, p = < 0.001, d = 3.12) and rate of omissions (t (23) = 4.17, p = < 0.001, d = 0.85), and a decrease in accuracy (t (23) = 4.204, p = < 0.001, d = 0.86) and number of trials completed (t (23) = 11.673, p = < 0.001, d = 2.38).

In order to better characterize mice's performance, we analyzed the stability of responding across multiple testing days. Furthermore, we also investigated as to whether low and high impulsive mice performed differently over time. Figure 2 shows results of these analyses. In the case of premature responding, there was a significant effect of testing day ($F_{3.816,53.42}$ = 3.453, p = .015), a significant effect of impulsivity group ($F_{1,14}$ = 5.076, p = .0408) and a significant interaction between the two ($F_{5,70}$ = 3.126, p = .0132). Post hoc tests determined that mice were more impulsive on the first testing day than on subsequent days. This effect was driven primarily by high-impulsive mice. There were no differences between the two groups on later testing days. Analysis of omission rates revealed a main effect of testing day ($F_{3.302, 46.22}$ = 5.736, p = .002), with mice making fewer omission during the last two testing days when compared to the first day. Analysis of trial completions revealed a significant main effect of testing day ($F_{2.972,41.60}$ = 11.37, p = <0.001). Post-hoc analysis showed that animals completed more trials on the last two testing days.

2 Experiment 2: performance with 9s inter-trial interval and reduced stimulus brightness

Figure 3 shows the performance of mice on the standard and reduced brightness versions of the 9s ITI task. Reducing the brightness led to a decrease in accuracy (t (95) = 7.146, p = < 0.001). It had no effect on premature responding (t (95) = 0.299, p = .765), rate of omissions (t (95) = 1.239, p = .218), or the number of trials completed (t (95) = 0.624, p = .534).

3 Experiment 3: performance with a variable inter-trial interval

Figure 4 shows the performance of mice on the variable ITI task. There was no difference in the number of trials completed ($F_{2,94} = 1.045$, $p = .354$) or latency to collect rewards ($F_{2,94} = 0.444$, $p = .621$) when comparing across the three ITIs. Varying the ITI significantly affected accuracy ($F_{2,94} = 10.499$, $p = <0.001$). Post hoc tests revealed that mice were less accurate during 9s ITI and 15s ITI trials. There was no difference between accuracies during 9s and 15s ITI trials. Varying the ITI also significantly affected the rate of omissions ($F_{2,94} = 11.728$, $p = <0.001$). Post hoc tests revealed that mice omitted significantly more 15s ITI trials compared to 5s and 9s ITI trials. Furthermore, there were significant changes in latency to correct responses across the three ITIs ($F_{2,94} = 13.509$, $p = <0.001$). Post hoc tests revealed that animals were slower to make a correct response during 15s ITI trials when compared to 5s and 9s ITI trials. Lastly varying the ITI had a significant effect on the percentage of premature responses ($F_{2,94} = 384.948$, $p = <0.001$). Post hoc tests reveals that increasing the ITI from 5s to 9s, and from 9s to 15s significantly increased the percentage of premature responses.

4 Experiment 4: effects of cocaine

Figure 5 shows the effects of cocaine on performance in the 5s ITI task. Cocaine increased premature responding ($F_{2,42} = 3.61$, $p = .036$), omissions ($F_{2,42} = 8.83$, $p = .003$), and reward collection latency ($F_{2,42} = 15.13$, $p = .002$), and decreased the number of trials completed ($F_{2,42} = 10.14$, $p = .002$), and accuracy ($F_{2,42} = 6.73$, $p = .008$). Cocaine had no effect on correct latency ($F_{2,42} = 10.14$, $p = .224$). Post-hoc tests revealed that the effects of cocaine on premature responding and accuracy were significantly different from vehicle at both 7.5 and 15 mg/kg doses. Significant effects of cocaine on omissions and trials completed were only seen at the 15 mg/kg dose while reward latency was decreased at the 7.5 mg/kg dose.

5 Experiment 5: effects of yohimbine using 5s inter-trial interval

As seen in Figure 6, yohimbine increased omissions ($F_{2,44} = 14.78$, $p = < .001$), reward ($F_{2,44} = 21.07$, $p = < .001$) and correct ($F_{2,44} = 5.80$, $p = .018$) latencies. These effects were seen at the 0.625 mg/kg dose. The trend towards reduced trials completed and increased premature responding were not significant. Yohimbine did not alter accuracy.

6 Experiment 6: effects of citalopram

Figure 7 shows the effects of citalopram. Citalopram decreased premature responding ($F_{2,22} = 5.35$, $p = .013$) and the number of trials that mice completed ($F_{2,22} = 4.38$, $p = .036$), and increased omissions ($F_{2,22} = 8.71$, $p = .002$). Post-hoc tests indicated that the premature responding was significantly lower than vehicle at both 5 and 10 mg/kg. Omissions and number of trials completed were only altered at the 10 mg/kg dose. The trends toward increase reward collection latency and latency to correct responses were not significant.

7 Experiment 7: effects of lorcaserin

As seen in figure 8, lorcaserin reduced premature responding ($F_{3,30} = 15.52$, $p = <.001$) and the number of trials completed ($F_{3,30} = 4.49$, $p = .01$), and increased omissions ($F_{3,30} = 4.578$, $p = .009$), and reward correct latencies ($F_{3,30} = 7.011$, $p = .001$). Accuracy was marginally higher ($F_{3,30} = 2.67$, $p = .065$). Reward latencies were not altered by lorcaserin. The majority of these effects were seen at 0.2 m/kg with the exception of premature responding, which was reduced at 0.1 and 0.2 mg/kg. The marginal increase in accuracy was seen at 0.1 mg/kg.

8 Experiment 8: effects of atomoxetine

As seen in figure 9, atomoxetine reduced omissions ($F_{2,22} = 5.12$, $p = .015$) and significantly decreased premature responding ($F_{2,22} = 36.7$, $p = <.001$). Post-hoc tests revealed that omissions were lower at the 0.5 mg/kg dose, and that premature responding was attenuated at both 0.5 and 1 mg/kg doses. The trend towards increased completed trials and accuracy were not significant. Latencies for reward collection and correct responses were not affected by atomoxetine.

9 Experiment 9: effects of yohimbine using 9s inter-trial interval

Figure 10 shows the effects of yohimbine. Yohimbine significantly reduced premature responding at 0.625 mg/kg ($F_{2,22} = 6.518$, $p = .006$). Omissions ($F_{2,22} = 14.00$, $p = .002$) and latency for correct responses ($F_{2,22} = 13.181$, $p = <.001$) were likewise significantly higher at the 0.625 mg/kg dose. The number of trials completed, overall accuracy, and reward collection latency were all unaltered by yohimbine.

Chapter 4
Discussion

The experiments presented here explored the performance of mice on the touchscreen five choice serial reaction time test (5CSRTT). Using premature responding as an index of impulsive actions, these studies assessed whether task parameter changes and pharmacological challenges could be used to manipulate mice's impulsivity. There were two sets of main findings. The first series of experiments showed that elevated levels of impulsive responding can be reliably induced by elongating the inter-trial-interval (ITI) to 9s, and by using a variable 5-9-15s ITI. Second, by using the baseline 5s ITI and 9s ITI versions of the 5CSRTT, we were able to show that impulsive responding could be bidirectionally manipulated using well characterized dopaminergic, serotonergic, and norepinephrinergic pharmacological agents. More specifically, we showed that cocaine increases premature responses, while yohimbine, citalopram, lorcaserin, and atomoxetine decrease premature responses. Collectively, the results of these experiments show that performance on the touchscreen version of the 5CSRTT is a reliable method for analyzing impulsive action in mice.

1 Behavioral manipulations of premature responding

1.1 Characterization of impulsive responding on the 9s ITI 5CSRTT

The first goal of these experiments was to optimize a version of the touchscreen 5CSRTT that produced consistent and reliable expression of impulsive action in mice. Mice were first trained using the 5 s ITI task. They readily acquired the task, and maintained a high level of performance, shown by high accuracies (above eighty percent) and low levels of premature responding (around five percent of trials). Mice were then challenged with a version of the task where the 5s ITI was elongated to 9s. This is a common task manipulation in studies using rat 5CSRTT (Fletcher et al., 2007, 2011). Across four different testing days, mice showed a significant four-fold increase in premature responses. This effect is in accordance with previous findings from our lab and others which have shown that elongated ITIs lead to increased impulsive action in both rats and mice. Importantly, the results also clearly demonstrated that this increase in premature responding was stable and reproducible.

Increasing the ITI to 9s also affected other performance measures. Overall, mice completed fewer trials, were slightly less accurate, and omitted more trials. However, these changes were relatively minimal, and mice performed well on the task; they completed more than forty trials, omitted roughly thirty percent of trials, and maintained accuracies above eighty percent. This would suggest that the new touchscreen task can elicit high levels of impulsive actions with minimal impact on other performance measures.

Taken as a whole, the present results indicate that the new 9s ITI version of the mouse 5CSRTT is a great candidate task for investigations of impulsivity. Mice perform the task well, and the overall levels of premature responding seen in the task are line with the results of previous studies using non-touchscreen versions of the mouse 5CSRTT. This would suggest that experimental results arising from the use of this version of the 5CSRTT can be confidently compared with results using the non-touchscreen version of the task.

1.2 9s ITI and attentional resources

As part of the process to characterize impulsive behavior on the 9s ITI task, it was important to assess whether the addition of an attentional challenge would enhance the effect of the 9s ITI challenge on premature responding. To this end, mice were also tested on a version of the 9s ITI version of the 5CSRTT where the brightness of the stimuli was reduced to thirty percent. This manipulation is typically used as an attentional challenge in the 5CRTT. It forces animals to allocate more attentional resources to detect the white stimulus boxes (Robbins, 2002). When comparing the standard 9s ITI task and the reduced-brightness version, the only measure that was significantly altered was accuracy of responding, with thirty percent brightness lowering accuracy by roughly ten percent. This suggests that the addition of an attentional challenge does not increase impulsive responding in mice. Accordingly, this version of the task may not offer increased utility over the standard 9s ITI in specific investigations of impulsive actions. However, it should be noted that the decreased accuracy seen in the reduced-brightness task offers external validity that the 9s task is indeed measuring attention as well. As such, the reduced-brightness version of the task may be well suited to situations where experimental manipulations may possibly affect both attentional and impulse-control resources.

1.3 Comparison between variable ITI and 9s ITI

In recent years, there has been some controversy regarding the stability and level of impulsive responding elicited by fixed ITI challenges in the 5CSRTT. Certain studies have demonstrated that using the same ITI duration over the course of many testing days may lead to either very low levels of premature responses (Sanchez-Roige et al., 2012), or to a gradual decrease in premature responses over time (Sanchez-Roige et al., 2012; Weir, et al., 2014). This is thought to arise from task experience and animals' development of temporal-mediated response strategies (Robbins, 2002). Accordingly, studies have started implementing variable ITI schedules. In these tests, the ITI changes randomly from on trial to another, a change that is thought to disallow the easy formation of response strategies (Caballero-Puntiverio et al, 2017).

As part of the optimization process of the 9s ITI task, mice were also tested on a variable 5-9-15s ITI version of the 5CSRTT. Results showed that overall levels of premature responding at 9s and 15s ITI were significantly higher than at 5s. However, this increase in premature responding was correlated with a drastic decrease in the number of trials completed; on average, mice completed roughly fourteen trials at each ITI. This is one third the number of trials that mice complete on the fixed 9s ITI task. Considering that these tasks are tests of baseline and drug-induced impulsive action, the low number of trials completed in the variable ITI may reduce statistical power, thus negatively affecting the ability of researchers to detect small effects. Additionally, the percentage of premature responses seen on the 9s ITI trials of the variable ITI task and the fixed 9s ITI task were quite similar. This suggests that mice may display the same level of impulsive responding regardless of the potential opportunity to form temporal-mediated response strategies. Further support for this idea comes from Fitzpatrick et al.'s (2019) study which used a variable 5-10-15s ITI version of the 5CSRTT. In their study, mice demonstrated premature responses in roughly 8% of 10s ITI and 20% of 15s ITI. This level of impulsive action is lower than what is seen in our studies using the fixed 9s ITI. Based on these results it appears that mouse touchscreen 9s ITI version of the 5CSRTT may be a more sensitive and effective test of impulsive responding than variable ITI 5CSRTT. This is particularly the case for studies involving repeated testing, as is often done in studies investigating the effects of drugs on impulsivity. The next section describes the results of several experiments involving drug challenges.

2 Pharmacological agents predicted to increase impulsivity

2.1 Cocaine

In the present study the psychomotor stimulant cocaine increased impulsive responding. Both 7.5 and 15 mg/kg doses significantly increased premature responses. These findings are consistent with the results of prior studies demonstrating cocaine-induced increases in behavioral disinhibition and impulsive behaviors in rats and mice (Cole & Robbins, 1987; van Gaalen et al., 2006; Fletcher et al., 2011). This effect may arise in part due to cocaine's inhibition of dopamine reuptake, which leads to accumulation of excess dopamine in both the ventral and dorsal striata (Dalley & Roiser, 2012). This in turn may increase dopamine D2 receptor activity (van Gaalen et al., 2006). Additionally, mice injected with the higher 15mg/kg dose completed fewer trials and made more omissions, while those injected with lower 7.5 mg/kg dose had lower reward latencies. This pattern resembles the results of prior work by van Gaalen et al. (2006) and may reflect the presence of an inverted U–shaped curve of dopamine system activity, with very low and very high dopamine levels leading to a worse performance (Caballero-Puntiviero et al., 2017). It may also be the case that the combination of lower completed trials and higher omissions seen at the 15 mg/kg dose arose from cocaine-induced locomotor stimulation, which would interfere with the ability of the mice to perform the sequence of behaviors required to successfully respond on the 5-CSRTT. Lastly, it should be noted that mice showed less premature responses on the 5CSRTT when compared to rats. Mice made premature responses in roughly twelve percent of trials. In prior rat studies, the combination of elongated ITIs and cocaine induce premature responses in thirty to fifty percent of trials (Fletcher et al., 2011) The relatively lower level of impulsivity seen in the present experiment is corroborated by other studies (Humby et al., 1999; Young et al., 2013; Caballero-Puntiviero et al., 2017), and may arise from mice having lower baseline levels of impulsive behaviors.

2.2 Yohimbine

In this study, the effects of yohimbine were tested both on baseline 5s ITI and elongated 9s ITI versions of the 5CSRTT. During 5s ITI tests, yohimbine did not affect premature responses or accuracy. At 0.625 mg/kg it increased the percentage of omissions, as well as latencies for reward collection and to correct choices. This pattern of results is not in line with prior studies of yohimbine in rats, which have shown that yohimbine typically increases premature responding (Sun et al., 2010, Funk et al, 2019). More interestingly, when mice were tested with yohimbine on the 9s ITI version of the task, yohimbine reduced premature responses. This is an interesting result because it points to the possibility that yohimbine's effects on impulsive action may be dependent on the cognitive load that mice are under. In the baseline version of the task, where inhibitory processes are not taxed, yohimbine did not alter impulsivity. However, when challenged with a longer 9s ITI, wherein mice must inhibit a response for an extended period, yohimbine facilitated motor inhibition. This seemingly backward pattern of behavior is comparable to the results of a recent study by Herman et al. (2019). In their study, healthy patients were administered yohimbine or placebo, and then asked to perform the affective stop signal task. Herman et al. found that patients exposed to yohimbine were less impulsive. They correlated this effect to the presence of heightened physiological arousal, as assessed by increased diastolic blood pressure. In our study, mice tested with yohimbine on the 9s ITI version of the task displayed a larger magnitude increase in reward collection latencies, and the percentage of trials that they omitted compared to mice in the 5s ITI task. This points to the possibility that the increased difficulty of the 9s ITI task combined with the anxiogenic effects of yohimbine may have increased physiological arousal, and in turn decreased behavioral impulsivity.

Overall, the disparity between the results of the current study and previously published work using rats, points to the possibility that yohimbine's effect on impulsivity may be species-specific, and/or more nuanced than previously thought. As such, more work needs to be done to elucidate how these differences arise, and the potential neurochemical basis that may drive them.

3 Pharmacological agents predicted to decrease impulsivity

3.1 Citalopram and lorcaserin

The present data revealed that both systemic and more specific manipulations of serotonergic systems lead to changes in impulsive actions. Both citalopram and lorcaserin significantly decreased the percentage of premature responses. These reductions parallel the findings of previous studies which have investigated these drugs in rats (Humpston et al., 2013, Higgins et al., 2012). Interestingly, while citalopram nearly halved premature responding at both high and low doses, lorcaserin overall had a much stronger effect on premature responding. At the higher 0.2 m/kg dose, premature responding had fallen from twenty-one percent of trials down to four percent. This difference between the two drugs may arise due to their mode of interaction with the serotonergic system. Citalopram acts as a selective serotonin reuptake inhibitor (Hyttel, 1982). It may be the case that the increased serotonin following reuptake inhibition may have affected the activity of various 5HT receptors, some of which have opposing influence on impulsive behaviors. For example, an increase in activity at 5HT2C, which decreases impulsivity, may have been simultaneously offset by increased activity at 5HT2A receptors, which typically increase premature responding (Higgins et al., 2003; Fletcher et al., 2007; Navarra et al., 2008). This would lead to overall weaker effects on impulsivity. Lorcaserin on the other hand, is a selective serotonin 5HT2C receptor agonist, and therefore more likely to produce strong effects on premature responses.

The present study also found that unlike previous studies using rats (Higgins et al., 2012) lorcaserin also increased accuracy and reward collection latencies. The presence of small differences between prior rat studies and the current mouse study highlight the need for more research into potential species differences in impulsivity. In particular, it may be important to pay close attention to the doses of drugs given to animals during the testing. For example, in our pilot studies, lorcaserin doses typically given to rats, such as 0.3 and 0.6 mg/kg, significantly impacted the ability of mice to perform the task. As such while the current study indicates that there is much parity between the results of serotonergic manipulations of impulsivity in the new touchscreen version of the mouse 5CSRTT and older methods, further work is required to better understand the role of serotonin in impulsive behaviors.

3.2 Atomoxetine

Animals injected with atomoxetine were less impulsive than animals in the vehicle group. At the 0.5 mg/kg and 1 mg/kg doses, animals were almost sixty-seven and seventy-four percent less impulsive respectively compared to animals injected with vehicle. This large decrease in impulsive action is in line with prior studies of atomoxetine in rats and mice (Navarra et al., 2008; Robinson et al., 2008; Paterson et al., 2011; Pillidge et al., 2014; Fitzpatrick et al., 2019). Interestingly, atomoxetine induced the most selective effects on performance in the present study. It produced robust decreases in premature responding and percentage of omission trials. This may arise due to atomoxetine's ability to elevate norepinephrine levels in brain regions responsible for impulse control. Support for this idea comes from Economidou et al., (2012) who found that atomoxetine injections in the shell of the nucleus accumbens decreased premature responses – an effect they attributed to norepinephrine transporter blockade and subsequently increased norepinephrine transmission. Regardless, while the exact mechanism underlying atomoxetine's effect is unknown, the current results indicate that the 9s ITI version of the task is sensitive to manipulations of the norepinephrine system and produces results that corroborate the findings of studies using older testing methods. Atomoxetine therefore be a useful tool in future investigations of the interaction between norepinephrine activity and impulse control.

4 Stability of impulsivity over time

Several recent studies have found that repeated testing with the same ITI length may lead to gradual decreases in impulsive action. This reduction in impulsivity is thought to arise from animal's acquisition of temporal-mediated strategies (Sanchez-Roige et al., 2012; Oliver et al., 2009). However, this effect has most often been seen in rats, and is thought to reflect rat's comparatively higher utilization of temporal strategies (Cope et al., 2016). We were interested to determine whether this effect occurred in our optimized test. Accordingly, we assessed the stability of premature responding and other test measures across six different 9s ITI tests spanning seven months. Mice maintained similar levels of accuracy and omissions across all six tests. However, there were between-day differences in the number of trials that mice completed and the number of premature responses. In the last two tests, which took place six months after initial testing, mice completed roughly forty percent more trials. Animal's impulsive actions also changed over the course of testing; there was a reduction in premature responses on the third day

of testing, which then stabilized for the rest of the testing. It is known that low-impulsive and high-impulsive animals react differently to pharmacological (Tomlinson et al., 2014) and task parameter (Fitzpatrick et al., 2019) manipulations. With this in mind, we assessed whether the change in impulsive actions seen across our tests could be driven by subgroup-specific effects. Results showed that indeed, the significant change in premature responding over time was driven by an interaction with impulsivity-subgroups. During the first two testing day, high-impulsive mice demonstrated significantly higher percentage of premature responses than low-impulsive mice. However, starting on the second day of testing, the differences between the two groups began to diminish, with both groups reaching parity during the fifth and sixth testing days.

While on the surface, these results would indicate that impulsivity as assessed by our task may be somewhat unstable, a closer look at the results paints a very different picture. Across all six testing days, the 9s ITI challenge induced an almost three-hundred percent increase in premature responding when compared to baseline testing. Furthermore, any changes that may have arisen from the acquisition of temporal-mediated strategies were only evident in the high-impulsivity subgroup. This would suggest that even though low-performing animals may have acquired temporally mediated strategies as a result of task experience, the task still taxed impulse-control enough to create a large magnitude increase in premature responding that was sustained across all six tests.

Accordingly, it seems that the touchscreen 9s ITI version of the 5CSRTT may be suitable for future investigations of impulsive action in mice. However, care should be taken when interpreting the results. It may be useful to plan tests in such a manner as to avoid potential task-driven changes in performance. Furthermore, it may be important to thoroughly investigate the potential presence of subgroup-effects.

Chapter 5
Conclusions

The current experiments were conducted to characterize the performance of mice on a touchscreen version of the 5CSRTT. While the 5CSRTT has a long history of use with rats and in older operant chambers (Robbins, 2002), less work has been done to investigate the performance of mice in the touchscreen implementation of the task. The present data suggest that this version of the task is well suited to testing behavioral impulsivity; we have shown that mice can reliably learn and perform the task and that impulsive actions can be induced with the 9s ITI challenge in a stable manner. Furthermore, we have shown that we are able to bidirectionally manipulate the level of impulsive actions through pharmacological means; well-characterized serotonergic, dopaminergic, and noradrenergic drugs elevated and attenuated impulsive responding. Interestingly, while testing with these drugs, we have found that mice and rats may have different sensitivity to drugs that affect impulsivity. The psychostimulant cocaine had a much weaker effect in mice than in rats, and the α2-adrenoceptor antagonist yohimbine, which heightens impulsivity in rats, had the opposite effect of reducing impulsivity. Lastly, we have elucidated how prior task experience may affect impulsive actions in mice; in our tests, high and low-impulsive mice expressed converging levels of impulsive responding over time. Overall the present data would suggest that touchscreen version of the mouse 5CSRTT may be a great tool for future investigations of impulsivity.

The primary motivation for conducting these experiments was to investigate the touchscreen version of the mouse 5CSRTT as a potential task which could be combined with transgenic approaches. The touchscreen task offers a variety of benefits over prior implementations of the task, including stability and reliability, greater cross-species validation, and improved compatibility with other tests of memory, learning, and attention in both humans and rodents (Bussey et al., 2012). As such, the combination of touchscreen tasks and transgenic approaches would be a powerful method of investigation. One such combination may be the use of the 5CSRTT in conjunction with optogenetics. Optogenetics allows for the precise control of specific neurotransmitter subtypes and has increasingly been used to identify cause-and-effect relationships between specific neurotransmitters and behaviors (Deisseroth, 2011). In the same manner, it may be possible to use the new touchscreen 5CSRTT in concert with optogenetic approaches to clearly isolate the role of different neurotransmitter systems in impulsive

responding. Through pharmacological means, the current study and others have already identified several candidate neurotransmitter systems, such as serotonergic and noradrenergic systems. The logical next step would be to probe the role of these neurotransmitters with more powerful techniques like optogenetics. As such, future studies may wish to combine the new touch screen task with optogenetic approaches to better explore the underlying mechanisms by which impulsive actions arise.

Chapter 6
References

Aron, A. R., Fletcher, P. C., Bullmore, E. T., Sahakian, B. J., & Robbins, T. W. (2003). Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nature neuroscience*, *6(2)*, 115.

Aron, A. R., Robbins, T. W., & Poldrack, R. A. (2014). Inhibition and the right inferior frontal cortex: one decade on. *Trends in cognitive sciences*, *18(4)*, 177-185.

Bari, A., Dalley, J. W., & Robbins, T. W. (2008). The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nature protocols*, *3*(5), 759.

Bartko, S. J., Romberg, C., White, B., Wess, J., Bussey, T. J., & Saksida, L. M. (2011). Intact attentional processing but abnormal responding in M1 muscarinic receptor-deficient mice using an automated touchscreen method. *Neuropharmacology*, *61(8)*, 1366-1378.

Baunez, C., & Robbins, T. W. (1997). Bilateral lesions of the subthalamic nucleus induce multiple deficits in an attentional task in rats. *European Journal of Neuroscience*, *9*(10), 2086-2099.

Blokland, A., Sik, A., & Lieben, C. (2005). Evaluation of DOI, 8-OH-DPAT, eticlopride and amphetamine on impulsive responding in a reaction time task in rats. *Behavioural pharmacology*, *16*(2), 93-100.

Blondeau, C., & Dellu-Hagedorn, F. (2007). Dimensional analysis of ADHD subtypes in rats. *Biological psychiatry, 61(12*), 1340-1350.

Booij, L., Swenne, C. A., Brosschot, J. F., Haffmans, P. J., Thayer, J. F., & Van der Does, A. W. (2006). Tryptophan depletion affects heart rate variability and impulsivity in remitted depressed patients with a history of suicidal ideation. *Biological psychiatry*, *60*(5), 507-514.

Bouwknecht, J. A., Hijzen, T. H., van der Gugten, J., Maes, R. A., Hen, R., & Olivier, B. (2001). Absence of 5-HT1B receptors is associated with impaired impulse control in male 5-HT1B knockout mice. *Biological psychiatry*, *49*(7), 557-568.

Bromberg-Martin, E. S., Hikosaka, O., & Nakamura, K. (2010). Coding of task reward value in the dorsal raphe nucleus. *Journal of Neuroscience*, *30*(18), 6262-6272.

Browne, C. J., Abela, A. R., Chu, D., Li, Z., Ji, X., Lambe, E. K., & Fletcher, P. J. (2018). Dorsal raphe serotonin neurons inhibit operant responding for reward via inputs to the ventral tegmental area but not the nucleus accumbens: evidence from studies combining optogenetic stimulation and serotonin reuptake inhibition. *Neuropsychopharmacology*, 1.

Bussey, T. J., Holmes, A., Lyon, L., Mar, A. C., McAllister, K. A. L., Nithianantharajah, J., ... & Saksida, L. M. (2012). New translational assays for preclinical modelling of cognition in schizophrenia: the touchscreen testing method for mice and rats. *Neuropharmacology, 62(3)*, 1191-1203.

Caballero-Puntiverio, M., Fitzpatrick, C. M., Woldbye, D. P., & Andreasen, J. T. (2017). Effects of amphetamine and methylphenidate on attentional performance and impulsivity in the

mouse 5-Choice Serial Reaction Time Task. *Journal of Psychopharmacology, 31(2)*, 272-283.

Cardinal, R. N., Pennicott, D. R., Lakmali, C., Robbins, T. W., & Everitt, B. J. (2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science, 292*(5526), 2499-2501.

Carli, M., & Samanin, R. (2000). The 5-HT1A receptor agonist 8-OH-DPAT reduces rats' accuracy of attentional performance and enhances impulsive responding in a five-choice serial reaction time task: role of presynaptic 5-HT1A receptors. *Psychopharmacology, 149*(3), 259-268.

Coccaro, Emil F., Richard J. Kavoussi, and Richard L. Hauger. "Serotonin function and antiaggressive response to fluoxetine: a pilot study." *Biological Psychiatry* 42, no. 7 (1997): 546-552.

Cole, B. J., & Robbins, T. W. (1987). Amphetamine impairs the discriminative performance of rats with dorsal noradrenergic bundle lesions on a 5-choice serial reaction time task: new evidence for central dopaminergic-noradrenergic interactions. *Psychopharmacology, 91(4)*, 458-466.

Cope, Z. A., Halberstadt, A. L., van Enkhuizen, J., Flynn, A. D., Breier, M., Swerdlow, N. R., ... & Young, J. W. (2016). Premature responses in the five-choice serial reaction time task reflect rodents' temporal strategies: evidence from no-light and pharmacological challenges. *Psychopharmacology, 233(19-20)*, 3513-3525.

Cools, R., & D'Esposito, M. (2011). Inverted-U–shaped dopamine actions on human working memory and cognitive control. *Biological psychiatry, 69(12*), e113-e125.

Cottone, P., Iemolo, A., Narayan, A. R., Kwak, J., Momaney, D., & Sabino, V. (2013). The uncompetitive NMDA receptor antagonists ketamine and memantine preferentially increase the choice for a small, immediate reward in low-impulsive rats. *Psychopharmacology*, *226*(1), 127-138.

Crean, J., Richards, J. B., & de Wit, H. (2002). Effect of tryptophan depletion on impulsive behavior in men with or without a family history of alcoholism. *Behavioural brain research*, *136*(2), 349-357.

Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2011). Impulsivity, compulsivity, and top-down cognitive control. *Neuron*, *69*(4), 680-694.

Dalley, J. W., & Roiser, J. P. (2012). Dopamine, serotonin and impulsivity. *Neuroscience*, *215*, 42-58.

Deisseroth, K. (2011). Optogenetics. *Nature methods, 8(1)*, 26.

De Wit, H. (2009). Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addiction biology*, *14*(1), 22-31.

Eagle, D. M., & Robbins, T. W. (2003). Inhibitory control in rats performing a stop-signal reaction-time task: effects of lesions of the medial striatum and d-amphetamine. *Behavioral neuroscience*, *117*(6), 1302.

Eagle, D. M., Lehmann, O., Theobald, D. E., Pena, Y., Zakaria, R., Ghosh, R., ... & Robbins, T. W. (2009). Serotonin depletion impairs waiting but not stop-signal reaction time in rats:

implications for theories of the role of 5-HT in behavioral inhibition. *Neuropsychopharmacology, 34(5),* 1311.

Economidou, D., Theobald, D. E., Robbins, T. W., Everitt, B. J., & Dalley, J. W. (2012). Norepinephrine and dopamine modulate impulsivity on the five-choice serial reaction time task through opponent actions in the shell and core sub-regions of the nucleus accumbens. *Neuropsychopharmacology, 37(9),* 2057.

Evenden, J. L. (1999). Varieties of impulsivity. *Psychopharmacology*, *146*(4), 348-361.

Evenden, J. L., & Ryan, C. N. (1999). The pharmacology of impulsive behaviour in rats VI: the effects of ethanol and selective serotonergic drugs on response choice with varying delays of reinforcement. *Psychopharmacology*, *146*(4), 413-421.

Fenno, L., Yizhar, O., & Deisseroth, K. (2011). The development and application of optogenetics. *Annual review of neuroscience*, *34*.

Fernando, A. B., Economidou, D., Theobald, D. E., Zou, M. F., Newman, A. H., Spoelder, M., ... & Robbins, T. W. (2012). Modulation of high impulsivity and attentional performance in rats by selective direct and indirect dopaminergic and noradrenergic receptor agonists. *Psychopharmacology, 219(2),* 341-352.

Fineberg, N. A., Potenza, M. N., Chamberlain, S. R., Berlin, H. A., Menzies, L., Bechara, A., ... & Hollander, E. (2010). Probing compulsive and impulsive behaviors, from animal models to endophenotypes: a narrative review. *Neuropsychopharmacology*, *35*(3), 591.

Fitzpatrick, C. M., & Andreasen, J. T. (2019). Differential effects of ADHD medications on impulsive action in the mouse 5-choice serial reaction time task. *European journal of pharmacology, 847*, 123-129.

Fletcher, P. J. (1993). A comparison of the effects of dorsal or median raphe injections of 8-OH-DPAT in three operant tasks measuring response inhibition. *Behavioural brain research*, *54*(2), 187-197.

Fletcher, P. J. (1995). Effects of combined or separate 5, 7-dihydroxytryptamine lesions of the dorsal and median raphe nuclei on responding maintained by a DRL 20s schedule of food reinforcement. *Brain research*, *675*(1-2), 45-54.

Fletcher, P. J., Tampakeras, M., Sinyard, J., & Higgins, G. A. (2007). Opposing effects of 5-HT 2A and 5-HT 2C receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. *Psychopharmacology*, *195*(2), 223-234.

Fletcher, P. J., Rizos, Z., Noble, K., & Higgins, G. A. (2011). Impulsive action induced by amphetamine, cocaine and MK801 is reduced by 5-HT2C receptor stimulation and 5-HT2A receptor blockade. *Neuropharmacology, 61(3),* 468-477.

Funk, D., Tamadon, S., Coen, K., Fletcher, P. J., & Lê, A. D. (2019). Kappa opioid receptors mediate yohimbine-induced increases in impulsivity in the 5-choice serial reaction time task. *Behavioural brain research, 359*, 258-265.

Fonseca, M. S., Murakami, M., & Mainen, Z. F. (2015). Activation of dorsal raphe serotonergic neurons promotes waiting but is not reinforcing. *Current Biology*, *25*(3), 306-315.

Grant, J. E., & Chamberlain, S. R. (2014). Impulsive action and impulsive choice across substance and behavioral addictions: cause or consequence?. *Addictive behaviors*, *39*(11), 1632-1639.

Harrison, A. A., Everitt, B. J., & Robbins, T. W. (1997). Doubly dissociable effects of median- and dorsal-raphe lesions on the performance of the five-choice serial reaction time test of attention in rats. *Behavioural Brain Research, 89*(1-2), 135-149.

Harrison, A. A., Everitt, B. J., & Robbins, T. W. (1997). Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. *Psychopharmacology, 133*(4), 329-342.

Harrison, A. A., Everitt, B. J., & Robbins, T. W. (1999). Central serotonin depletion impairs both the acquisition and performance of a symmetrically reinforced go/no-go conditional visual discrimination. *Behavioural brain research, 100*(1-2), 99-112.

Harvey-Lewis, C., Li, Z., Higgins, G. A., & Fletcher, P. J. (2016). The 5-HT2C receptor agonist lorcaserin reduces cocaine self-administration, reinstatement of cocaine-seeking and cocaine induced locomotor activity. *Neuropharmacology, 101*, 237-245.

Herman, A. M., Critchley, H. D., & Duka, T. (2019). The impact of Yohimbine-induced arousal on facets of behavioural impulsivity. *Psychopharmacology, 1-13*.

Higgins, G. A., Enderlin, M., Haman, M., & Fletcher, P. J. (2003). The 5-HT 2A receptor antagonist M100, 907 attenuates motor and impulsive-type behaviours produced by NMDA receptor antagonism. *Psychopharmacology, 170*(3), 309-319.

Higgins, G. A., Silenieks, L. B., Roßmann, A., Rizos, Z., Noble, K., Soko, A. D., & Fletcher, P. J. (2012). The 5-HT 2C receptor agonist lorcaserin reduces nicotine self-administration, discrimination, and reinstatement: relationship to feeding behavior and impulse control. *Neuropsychopharmacology, 37(5),* 1177.

Humby, T., Laird, F. M., Davies, W., & Wilkinson, L. S. (1999). Visuospatial attentional functioning in mice: interactions between cholinergic manipulations and genotype. *European Journal of Neuroscience, 11(8),* 2813-2823.

Humby, T., Eddy, J. B., Good, M. A., Reichelt, A. C., & Wilkinson, L. S. (2013). A novel translational assay of response inhibition and impulsivity: effects of prefrontal cortex lesions, drugs used in ADHD, and serotonin 2C receptor antagonism. *Neuropsychopharmacology, 38(11),* 2150.

Humpston, C. S., Wood, C. M., & Robinson, E. S. (2013). Investigating the roles of different monoamine transmitters and impulse control using the 5-choice serial reaction time task. *Journal of Psychopharmacology, 27(2),* 213-221.

Hvoslef-Eide, M., Mar, A. C., Nilsson, S. R. O., Alsiö, J., Heath, C. J., Saksida, L. M., ... & Bussey, T. J. (2015). The NEWMEDS rodent touchscreen test battery for cognition relevant to schizophrenia. *Psychopharmacology, 232(21-22*), 3853-3872.

Hyttel, J. (1982). Citalopram—pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 6(3),* 277-295.

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., ... & Wang, P. (2010). Research domain criteria (RDoC): toward a new classification framework for research on mental disorders.

Jupp, B., & Dalley, J. W. (2014). Convergent pharmacological mechanisms in impulsivity and addiction: insights from rodent models. *British journal of pharmacology, 171(20),* 4729-4766.

Kolisnyk, B., Al-Onaizi, M. A., Hirata, P. H., Guzman, M. S., Nikolova, S., Barbash, S., ... & Prado, V. F. (2013). Forebrain deletion of the vesicular acetylcholine transporter results in deficits in executive function, metabolic, and RNA splicing abnormalities in the prefrontal cortex. *Journal of Neuroscience, 33(37),* 14908-14920.

Kreek, M. J., Nielsen, D. A., Butelman, E. R., & LaForge, K. S. (2005). Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nature neuroscience, 8*(11), 1450.

Krebs, C. A., & Anderson, K. G. (2012). Preference reversals and effects of D-amphetamine on delay discounting in rats. *Behavioural pharmacology, 23(3),* 228-240.

Lê Dzung, A., Funk, D., Harding, S., Juzytsch, W., Li, Z., & Fletcher, P. J. (2008). Intra-median raphe nucleus (MRN) infusions of muscimol, a GABA-A receptor agonist, reinstate alcohol seeking in rats: role of impulsivity and reward. *Psychopharmacology, 195*(4), 605-615.

Lesch, K. P., & Merschdorf, U. (2000). Impulsivity, aggression, and serotonin: a molecular psychobiological perspective. *Behavioral sciences & the law, 18*(5), 581-604.

Linnoila, M., Virkkunen, M., Scheinin, M., Nuutila, A., Rimon, R., & Goodwin, F. K. (1983). Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior. *Life sciences, 33*(26), 2609-2614.

Liu, Y. P., Lin, Y. L., Chuang, C. H., Kao, Y. C., Chang, S. T., & Tung, C. S. (2009). Alpha adrenergic modulation on effects of norepinephrine transporter inhibitor reboxetine in five-choice serial reaction time task. *Journal of biomedical science, 16(1),* 72.

McDevitt, R. A., Tiran-Cappello, A., Shen, H., Balderas, I., Britt, J. P., Marino, R. A., ... & Bonci, A. (2014). Serotonergic versus nonserotonergic dorsal raphe projection neurons: differential participation in reward circuitry. *Cell reports, 8*(6), 1857-1869.

Masaki, D., Yokoyama, C., Kinoshita, S., Tsuchida, H., Nakatomi, Y., Yoshimoto, K., & Fukui, K. (2006). Relationship between limbic and cortical 5-HT neurotransmission and acquisition and reversal learning in a go/no-go task in rats. *Psychopharmacology, 189*(2), 249-258.

Milstein, J. A., Dalley, J. W., & Robbins, T. W. (2010). Methylphenidate-induced impulsivity: pharmacological antagonism by β-adrenoreceptor blockade. *Journal of Psychopharmacology, 24(3),* 309-321.

Moore, H., Geyer, M. A., Carter, C. S., & Barch, D. M. (2013). Harnessing cognitive neuroscience to develop new treatments for improving cognition in schizophrenia: CNTRICS selected cognitive paradigms for animal models.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1996). The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cerebral cortex, 6(3),* 470-481.

Naaijen, J., Lythgoe, D. J., Amiri, H., Buitelaar, J. K., & Glennon, J. C. (2015). Fronto-striatal glutamatergic compounds in compulsive and impulsive syndromes: a review of magnetic resonance spectroscopy studies. *Neuroscience & Biobehavioral Reviews, 52*, 74-88.

Nakamura, K., Matsumoto, M., & Hikosaka, O. (2008). Reward-dependent modulation of neuronal activity in the primate dorsal raphe nucleus. *Journal of Neuroscience, 28*(20), 5331-5343.

Navarra, R., Graf, R., Huang, Y., Logue, S., Comery, T., Hughes, Z., & Day, M. (2008). Effects of atomoxetine and methylphenidate on attention and impulsivity in the 5-choice serial reaction time test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 32(1),* 34-41.

Nielsen, D. A., Virkkunen, M., Lappalainen, J., Eggert, M., Brown, G. L., Long, J. C., ... & Linnoila, M. (1998). A tryptophan hydroxylase gene marker for suicidality and alcoholism. *Archives of general psychiatry*, *55*(7), 593-602.

Nigg, J. T. (2000). On inhibition/disinhibition in developmental psychopathology: views from cognitive and personality psychology and a working inhibition taxonomy. *Psychological bulletin, 126*(2), 220.

Oliver, Y. P., Ripley, T. L., & Stephens, D. N. (2009). Ethanol effects on impulsivity in two mouse strains: similarities to diazepam and ketamine. *Psychopharmacology, 204(4),* 679-692.

Paterson, N. E., Ricciardi, J., Wetzler, C., & Hanania, T. (2011). Sub-optimal performance in the 5-choice serial reaction time task in rats was sensitive to methylphenidate, atomoxetine and d-amphetamine, but unaffected by the COMT inhibitor tolcapone. *Neuroscience research, 69(1),* 41-50.

Pattij, T., Schetters, D., Schoffelmeer, A. N., & van Gaalen, M. M. (2012). On the improvement of inhibitory response control and visuospatial attention by indirect and direct adrenoceptor agonists. *Psychopharmacology, 219(2),* 327-340.

Pillidge, K., Porter, A. J., Vasili, T., Heal, D. J., & Stanford, S. C. (2014). Atomoxetine reduces hyperactive/impulsive behaviours in neurokinin-1 receptor 'knockout'mice. *Pharmacology Biochemistry and Behavior, 127,* 56-61.

Richards, J. B., Sabol, K. E., & de Wit, H. (1999). Effects of methamphetamine on the adjusting amount procedure, a model of impulsive behavior in rats. *Psychopharmacology, 146(4),* 432-439.

Rieger, M., Gauggel, S., & Burmeister, K. (2003). Inhibition of ongoing responses following frontal, nonfrontal, and basal ganglia lesions. *Neuropsychology*, *17*(2), 272.

Robbins, T. (2002). The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology*, *163*(3-4), 362-380.

Robinson, E. S., Eagle, D. M., Mar, A. C., Bari, A., Banerjee, G., Jiang, X., ... & Robbins, T. W. (2008). Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. *Neuropsychopharmacology, 33(5),* 1028.

Robinson, O. J., Cools, R., & Sahakian, B. J. (2012). Tryptophan depletion disinhibits punishment but not reward prediction: implications for resilience. *Psychopharmacology, 219*(2), 599-605.

Sanchez-Roige, S., Peña-Oliver, Y., & Stephens, D. N. (2012). Measuring impulsivity in mice: the five-choice serial reaction time task. *Psychopharmacology, 219(2*), 253-270.

Schweighofer, N., Bertin, M., Shishida, K., Okamoto, Y., Tanaka, S. C., Yamawaki, S., & Doya, K. (2008). Low-serotonin levels increase delayed reward discounting in humans. *Journal of Neuroscience*, *28*(17), 4528-4532.

Sun, H., Green, T. A., Theobald, D. E., Birnbaum, S. G., Graham, D. L., Zeeb, F. D., ... & Winstanley, C. A. (2010). Yohimbine increases impulsivity through activation of cAMP response element binding in the orbitofrontal cortex. *Biological psychiatry, 67(7),* 649-656.

Tomlinson, A., Grayson, B., Marsh, S., Harte, M. K., Barnes, S. A., Marshall, K. M., & Neill, J. C. (2014). Pay attention to impulsivity: modelling low attentive and high impulsive subtypes of adult ADHD in the 5-choice continuous performance task (5C-CPT) in female rats. *European Neuropsychopharmacology, 24(8),* 1371-1380.

Tye, K. M., & Deisseroth, K. (2012). Optogenetic investigation of neural circuits underlying brain disease in animal models. *Nature Reviews Neuroscience*, *13*(4), 251.

Uslaner, J. M., & Robinson, T. E. (2006). Subthalamic nucleus lesions increase impulsive action and decrease impulsive choice− mediation by enhanced incentive motivation?. *European Journal of Neuroscience, 24*(8), 2345-2354.

van den Wildenberg, W. P., van Boxtel, G. J., van der Molen, M. W., Bosch, D. A., Speelman, J. D., & Brunia, C. H. (2006). Stimulation of the subthalamic region facilitates the selection and inhibition of motor responses in Parkinson's disease. Journal of cognitive neuroscience, 18(4), 626-636.

van Gaalen, M. M., van Koten, R., Schoffelmeer, A. N., & Vanderschuren, L. J. (2006). Critical involvement of dopaminergic neurotransmission in impulsive decision making. *Biological psychiatry*, *60*(1), 66-73.

Voon, V., Irvine, M. A., Derbyshire, K., Worbe, Y., Lange, I., Abbott, S., ... & Wood, J. (2014). Measuring "waiting" impulsivity in substance addictions and binge eating disorder in a novel analogue of rodent serial reaction time task. *Biological psychiatry, 75(2),* 148-155.

Wade, T. R., de Wit, H., & Richards, J. B. (2000). Effects of dopaminergic drugs on delayed reward as a measure of impulsive behavior in rats. *Psychopharmacology, 150(1*), 90-101.

Walderhaug, E., Magnusson, A., Neumeister, A., Lappalainen, J., Lunde, H., Refsum, H., & Landrø, N. I. (2007). Interactive effects of sex and 5-HTTLPR on mood and impulsivity during tryptophan depletion in healthy people. *Biological psychiatry*, *62*(6), 593-599.

Weir, R. K., Dudley, J. A., Yan, T. C., Grabowska, E. M., Peña-Oliver, Y., Ripley, T. L., ... & Hunt, S. P. (2014). The influence of test experience and NK1 receptor antagonists on the performance of NK1R-/-and wild type mice in the 5-Choice Serial Reaction-Time Task. *Journal of Psychopharmacology, 28(3),* 270-281.

Winstanley, C. A., Dalley, J. W., Theobald, D. E., & Robbins, T. W. (2003). Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. *Psychopharmacology, 170*(3), 320-331.

Winstanley, C. A., Theobald, D. E., Dalley, J. W., Glennon, J. C., & Robbins, T. W. (2004). 5-HT 2A and 5-HT 2C receptor antagonists have opposing effects on a measure of

impulsivity: interactions with global 5-HT depletion. *Psychopharmacology*, *176*(3-4), 376-385.

Winstanley, C. A., Dalley, J. W., Theobald, D. E., & Robbins, T. W. (2004). Fractionating impulsivity: contrasting effects of central 5-HT depletion on different measures of impulsive behavior. *Neuropsychopharmacology*, *29*(7), 1331.

Winstanley, C. A., Eagle, D. M., & Robbins, T. W. (2006). Behavioral models of impulsivity in relation to ADHD: translation between clinical and preclinical studies. *Clinical psychology review*, *26*(4), 379-395.

Winstanley, C. A., Zeeb, F. D., Bedard, A., Fu, K., Lai, B., Steele, C., & Wong, A. C. (2010). Dopaminergic modulation of the orbitofrontal cortex affects attention, motivation and impulsive responding in rats performing the five-choice serial reaction time task. *Behavioural brain research, 210(2*), 263-272.

Worbe, Y., Savulich, G., Voon, V., Fernandez-Egea, E., & Robbins, T. W. (2014). Serotonin depletion induces 'waiting impulsivity'on the human four-choice serial reaction time task: cross-species translational significance. *Neuropsychopharmacology*, *39(6)*, 1519.

Young, J. W., Geyer, M. A., Rissling, A. J., Sharp, R. F., Eyler, L. T., Asgaard, G. L., & Light, G. A. (2013). Reverse translation of the rodent 5C-CPT reveals that the impaired attention of people with schizophrenia is similar to scopolamine-induced deficits in mice. *Translational psychiatry*, *3(11*), e324.

Chapter 7
Figure Captions

Figure 1: Effects of increasing the inter-trial interval (ITI) from 5s to 9s on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. The percentage of premature responses and omissions were higher at 9s relative to 5s. Increasing the ITI decreased accuracy and the number of trials completed. Data are presented as means ± SEM. *** indicates p <0.001, paired t-test.

Figure 2: Time-dependent effects of 9s ITI schedule on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. Mice demonstrated higher percentage of premature responding on the first testing day than on subsequent days. This effect was driven primarily by high-impulsive mice. Mice made fewer omission and completed more trials during the last two testing days when compared to the first day. There were no time-dependent effects on accuracy. Data are presented as means ± SEM. *** indicates p <0.001 relative to low-impulsive group, Sidak's test. # indicates p <0.05, ## indicates p <0.01 relative to first testing day, Tukey's test.

Figure 3: Effects of decreasing stimulus brightness from 100% to 30% on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. Decreasing the brightness significantly reduced accuracy, but did not affect number of trials completed, percentage of premature responses, or omissions. Data are presented as means ± SEM. *** indicates p <0.001, paired t-test.

Figure 4: Effects of variable inter-trial interval (ITI) lengths on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. For these tests, the ITIs consisted of 5, 9, and 15s. The percentage of premature responses increased as a function of ITI length. Increasing the ITI decreased accuracy. Increasing the ITI to 15s led to an increase in percentage of omissions relative to 5s. Data are presented as means ± SEM. *** indicates p <0.001.

Figure 5: Effects of 7.5 and 15 mg/kg cocaine or its vehicle on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. During these tests, the inter-trial interval was set to 5s. Administration of 7.5 and 15 mg/kg of cocaine

significantly increased premature responding and decreased accuracy relative to vehicle. Administration of 15 mg/kg cocaine significantly increased the percentage of omissions and lowered the number of completed trials relative to vehicle. White bars represent mean ± SEM for vehicle group and shaded bars represent mean ± SEM scores for drug treatment groups. * indicates $p < 0.05$, ** indicates $p < 0.01$ relative to vehicle, Dunnett's test.

Figure 6: Effects of 0.313 and, 0.625 mg/kg yohimbine or its vehicle on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. During these tests, the inter-trial interval was set to 5s. 0.625 mg/kg yohimbine significantly increased percentage of omissions relative to vehicle. Yohimbine did not significantly affect premature responding, number of trials completed, or accuracy. White bars represent mean ± SEM for vehicle group and shaded bars represent mean ± SEM scores for drug treatment groups. *** indicates $p < 0.001$ relative to vehicle, Dunnett's test.

Figure 7: Effects of 5 and 10 mg/kg citalopram or its vehicle on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. During these tests, the inter-trial interval was set to 9s in order to increase base levels of premature responding. 5 and 10 mg/kg citalopram significantly decreased percentage of premature responding relative to vehicle. 10 mg/kg significantly decreased the number of completed trials, and increased percentage of omissions relative to vehicle. Cocaine did not significantly accuracy. White bars represent mean ± SEM for vehicle group and shaded bars represent mean ± SEM scores for drug treatment groups. * indicates $p < 0.05$, ** indicates $p < 0.01$ relative to vehicle, Dunnett's test.

Figure 8: Effects of 0.05, 0.1, and 0.2 mg/kg lorcaserin or its vehicle on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. During these tests, the inter-trial interval was set to 9s in order to increase base levels of premature responding. 0.1 and 0.2 mg/kg citalopram significantly decreased percentage of premature responding relative to vehicle. 0.2 mg/kg significantly decreased the number of completed trials, and increased percentage of omissions relative to vehicle. 0.1 mg/kg increased accuracy relative to vehicle. White bars represent mean ± SEM for vehicle group and shaded bars represent mean ± SEM scores for drug treatment groups. * indicates $\underline{p} < 0.05$, *** indicates $\underline{p} < 0.001$ relative to vehicle, Dunnett's test.

Figure 9: Effects of 0.5 and 1 mg/kg atomoxetine or its vehicle on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. During these tests, the inter-trial interval was set to 9s in order to increase base levels of premature responding. 0.5 and 1 mg/kg atomoxetine significantly decreased percentage of premature responding relative to vehicle. 0.5 mg/kg significantly decreased the percentage of omissions relative to vehicle. Atomoxetine did not affect the number of trials completed and accuracy. White bars represent mean ± SEM for vehicle group and shaded bars represent mean ± SEM scores for drug treatment groups. * indicates $p < 0.05$, *** indicates $p < 0.001$ relative to vehicle, Dunnett's test.

Figure 10: Effects of 0.313 and, 0.625 mg/kg yohimbine or its vehicle on **a** percentage of premature responses, **b** number of trials, **c** percentage accuracy, and **d** percentage of omissions. During these tests, the inter-trial interval was set to 9s in order to increase base levels of premature responding. 0.625 yohimbine significantly decreased percentage of premature responding, and increased omissions relative to vehicle. Yohimbine did not affected the number of trials completed and accuracy. White bars represent mean ± SEM for vehicle group and shaded bars represent mean ± SEM scores for drug treatment groups. * indicates $p < 0.05$, ** indicates $p < 0.01$ relative to vehicle, Dunnett's test.

Chapter 8
Figures

Figure 1

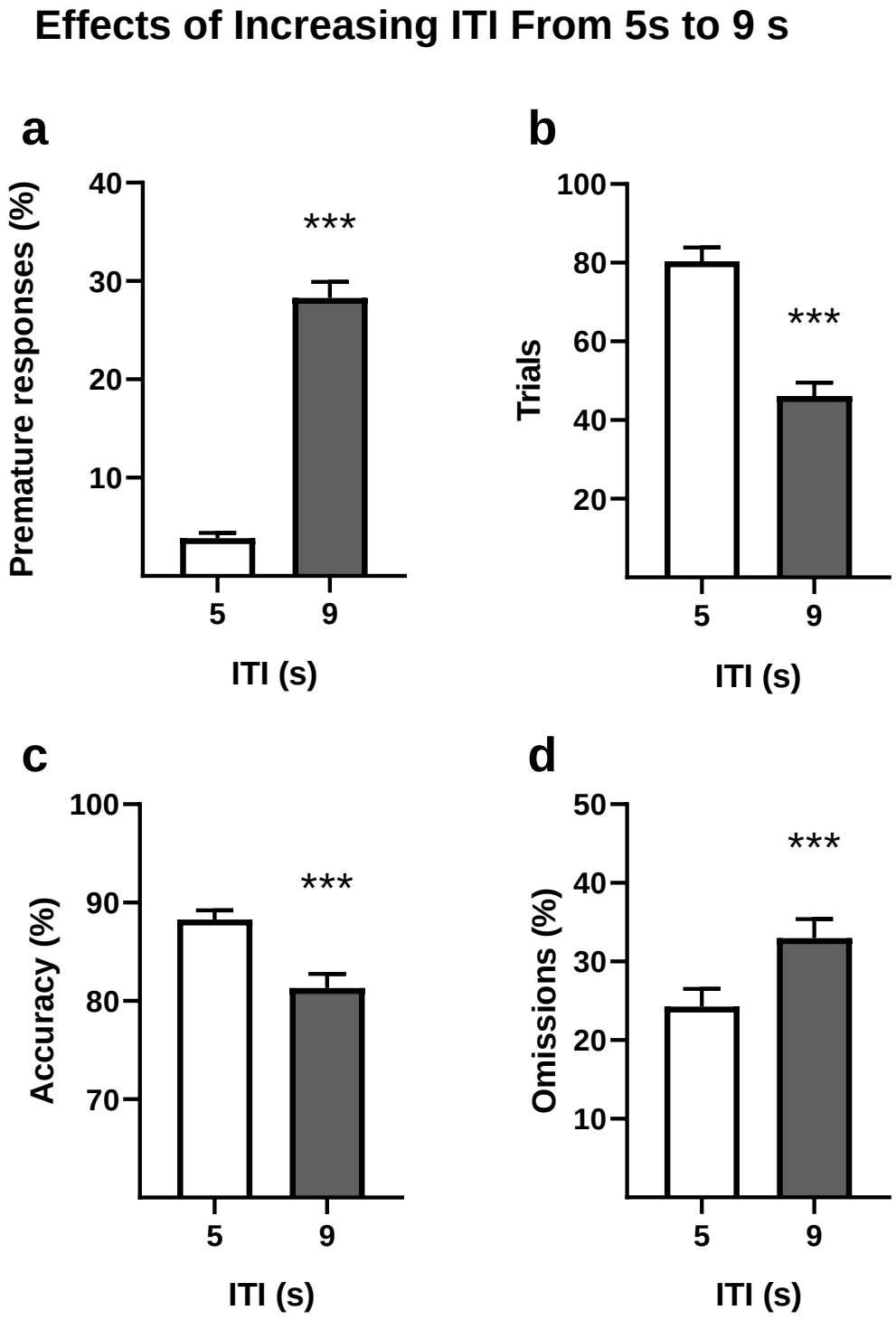


Figure 2

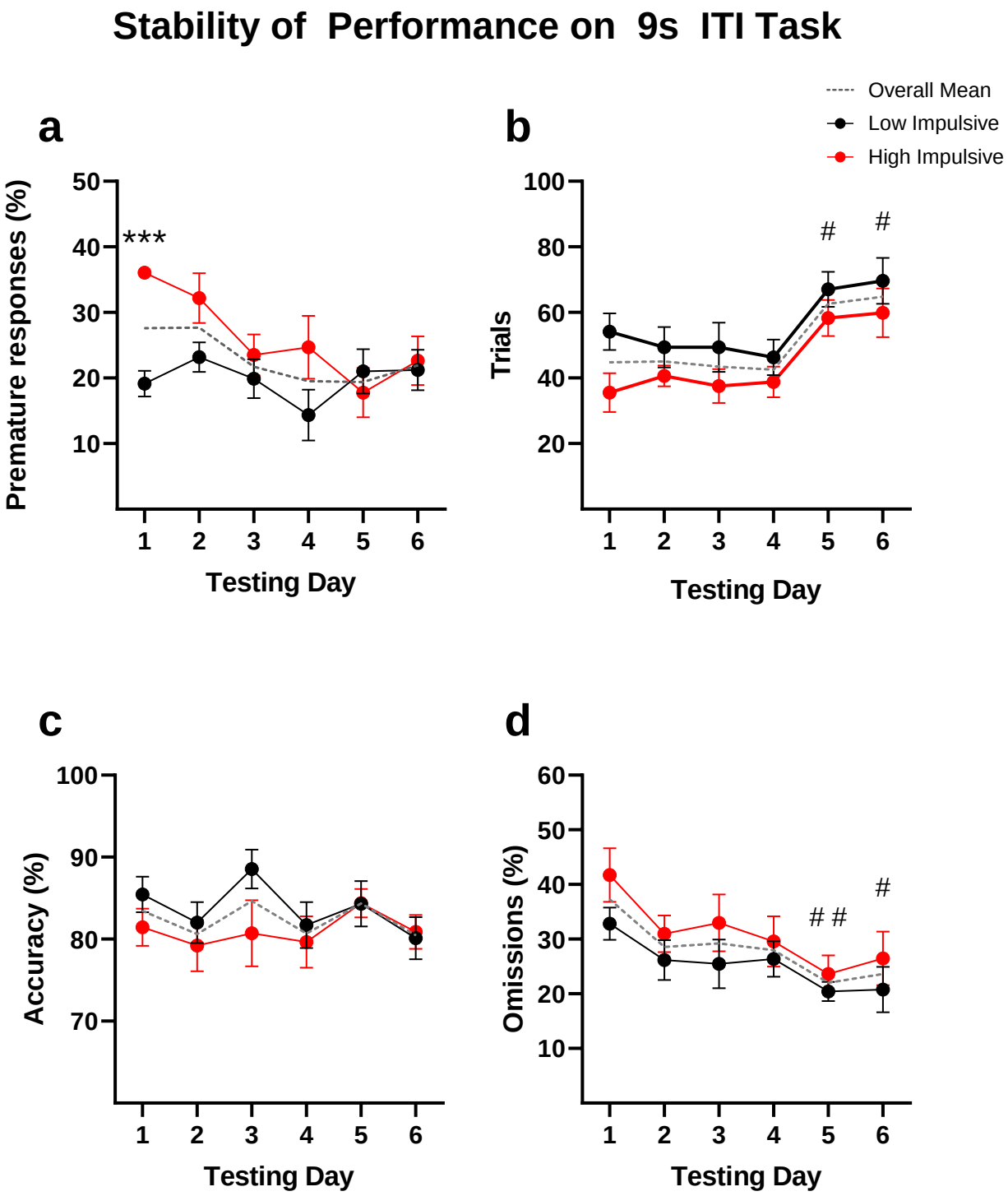

Stability of Performance on 9s ITI Task
Overall Mean
Low Impulsive
High Impulsive
a
Premature responses (%)
***
Testing Day
b
Trials
#
#
Testing Day
c
Accuracy (%)
Testing Day
d
Omissions (%)
# #
#
Testing Day

Figure 3

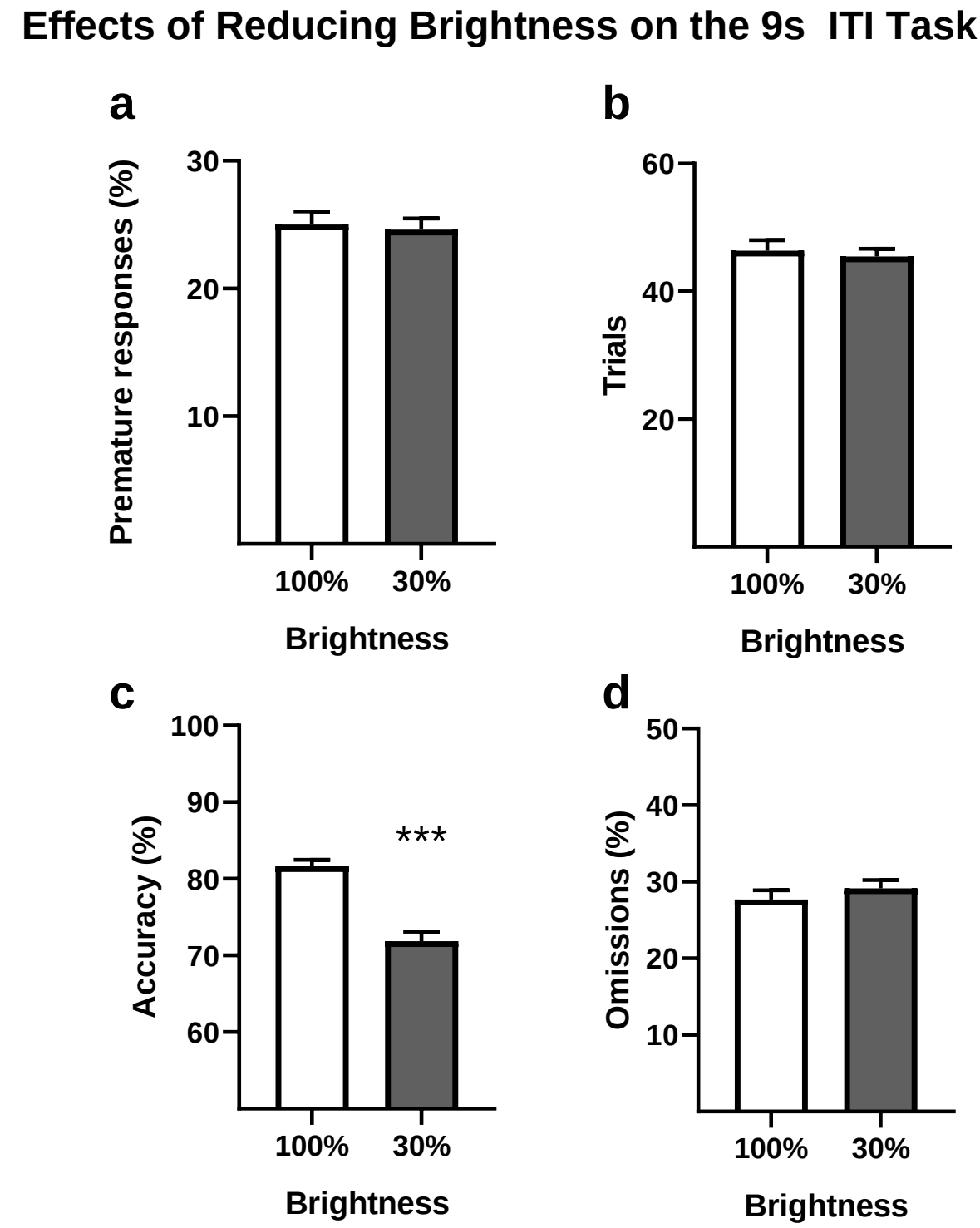

Effects of Reducing Brightness on the 9s ITI Task
a
Premature responses (%)
30
20
10
100%
30%
Brightness
b
Trials
60
40
20
100%
30%
Brightness
c
Accuracy (%)
100
90
80
70
60
***
100%
30%
Brightness
d
Omissions (%)
50
40
30
20
10
100%
30%
Brightness

Figure 4

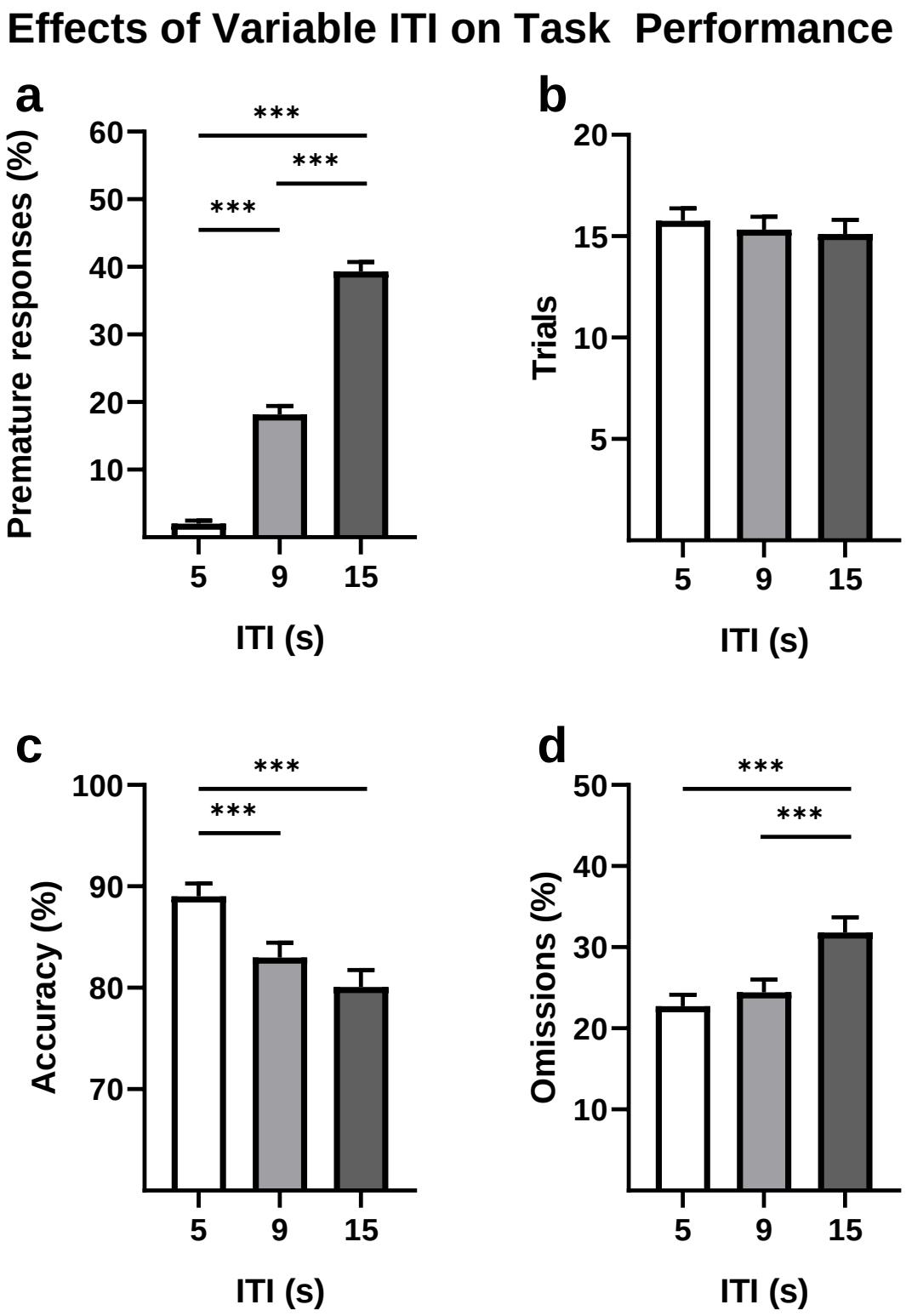

Effects of Variable ITI on Task Performance
a
Premature responses (%)
60
50
40
30
20
10
***
***
***
5
9
15
ITI (s)
b
Trials
20
15
10
5
5
9
15
ITI (s)
c
Accuracy (%)
100
90
80
70
***
***
5
9
15
ITI (s)
d
Omissions (%)
50
40
30
20
10
***
***
5
9
15
ITI (s)

Figure 5

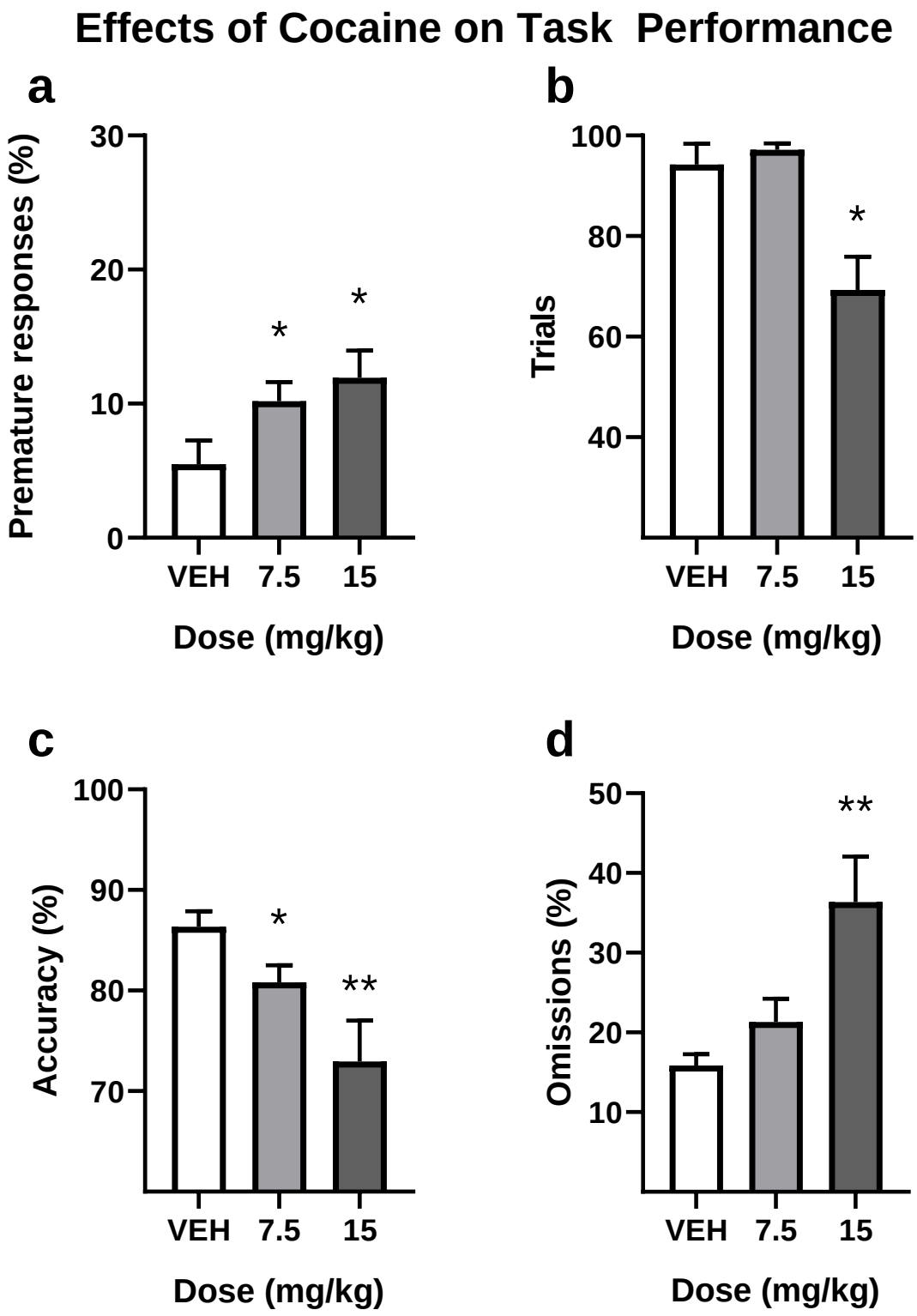

Effects of Cocaine on Task Performance
a
Premature responses (%)
30
20
10
0
*
*
VEH
7.5
15
Dose (mg/kg)
b
Trials
100
80
60
40
*
VEH
7.5
15
Dose (mg/kg)
c
Accuracy (%)
100
90
80
70
*
**
VEH
7.5
15
Dose (mg/kg)
d
Omissions (%)
50
40
30
20
10
**
VEH
7.5
15
Dose (mg/kg)

Figure 6

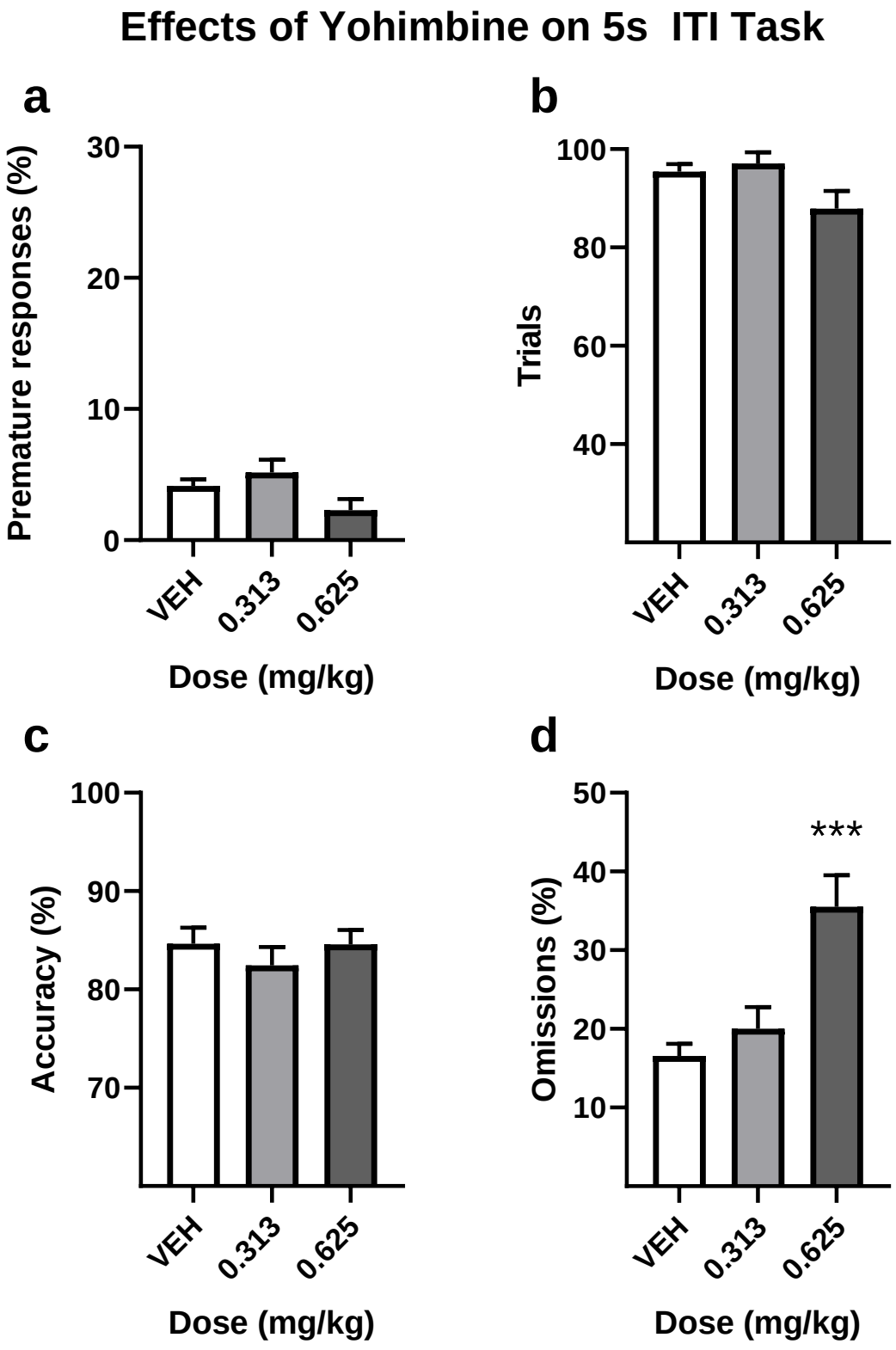

Effects of Yohimbine on 5s ITI Task
a
Premature responses (%)
30
20
10
0
VEH
0.313
0.625
Dose (mg/kg)
b
Trials
100
80
60
40
VEH
0.313
0.625
Dose (mg/kg)
c
Accuracy (%)
100
90
80
70
VEH
0.313
0.625
Dose (mg/kg)
d
Omissions (%)
50
40
30
20
10
***
VEH
0.313
0.625
Dose (mg/kg)

Figure 7

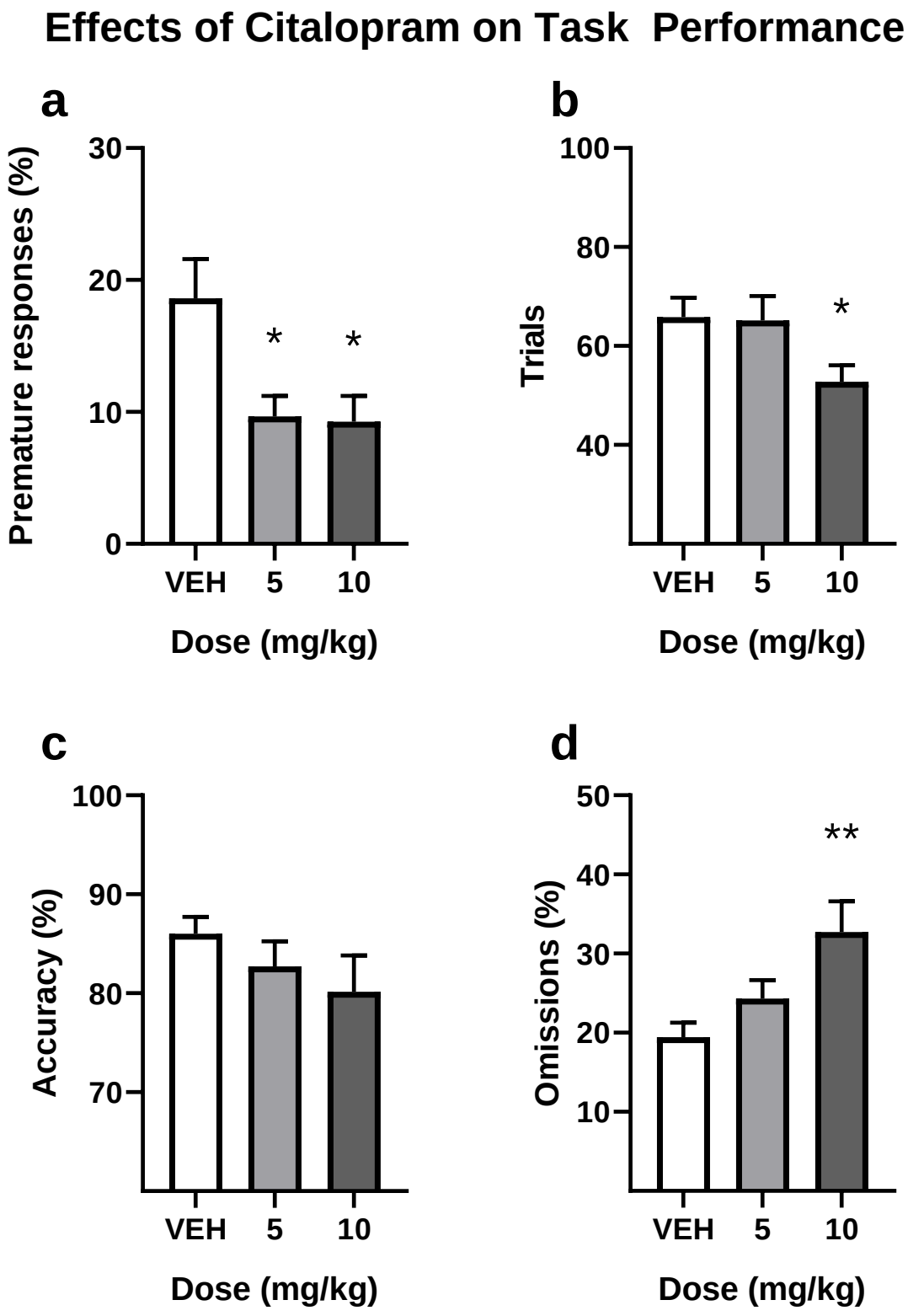

Effects of Citalopram on Task Performance
a
Premature responses (%)
30
20
10
0
*
*
VEH
5
10
Dose (mg/kg)
b
Trials
100
80
60
40
*
VEH
5
10
Dose (mg/kg)
c
Accuracy (%)
100
90
80
70
VEH
5
10
Dose (mg/kg)
d
Omissions (%)
50
40
30
20
10
**
VEH
5
10
Dose (mg/kg)

Figure 8

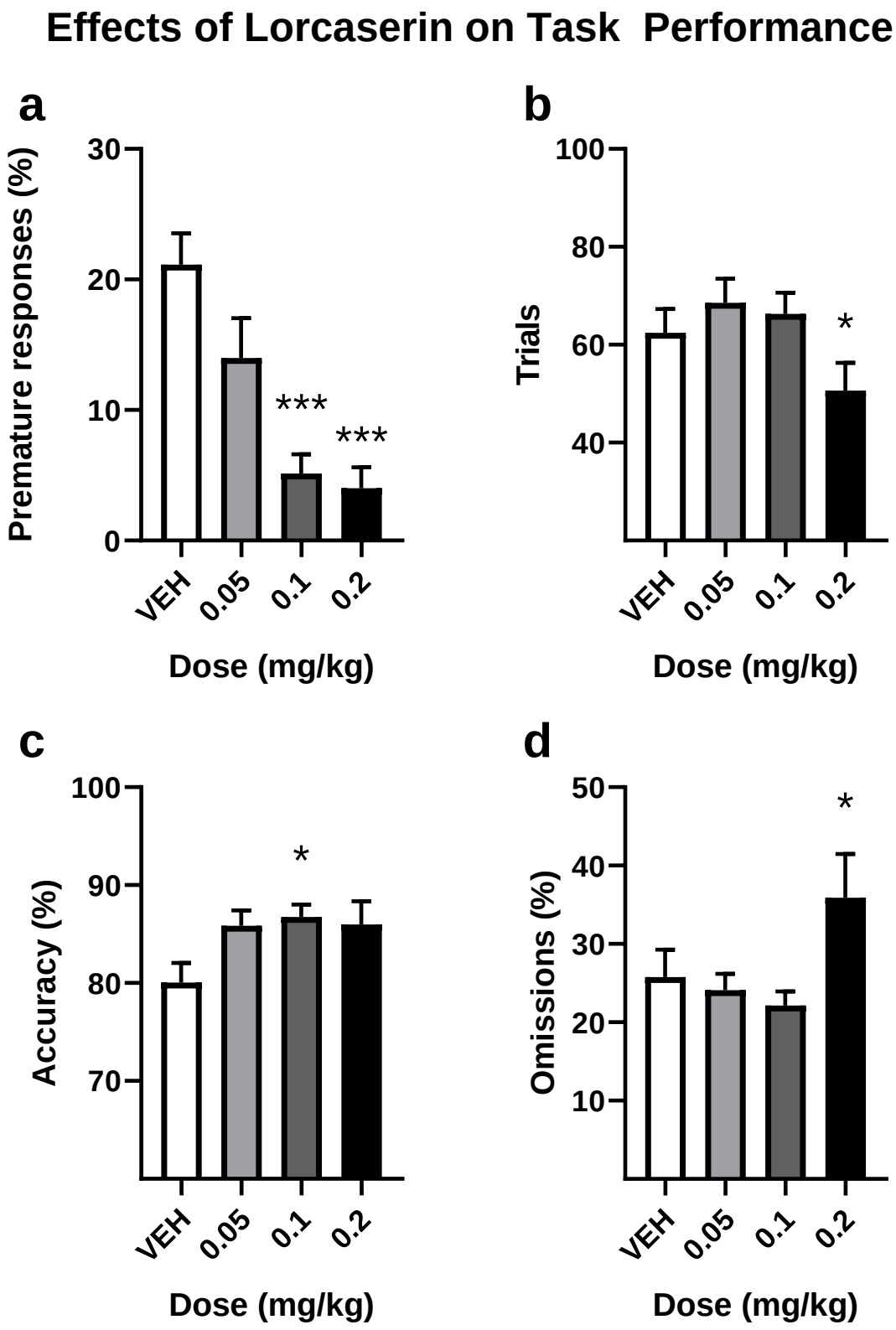

Effects of Lorcaserin on Task Performance
a
Premature responses (%)
30
20
10
0
***
***
VEH
0.05
0.1
0.2
Dose (mg/kg)
b
Trials
100
80
60
40
*
VEH
0.05
0.1
0.2
Dose (mg/kg)
c
Accuracy (%)
100
90
80
70
*
VEH
0.05
0.1
0.2
Dose (mg/kg)
d
Omissions (%)
50
40
30
20
10
*
VEH
0.05
0.1
0.2
Dose (mg/kg)

Figure 9

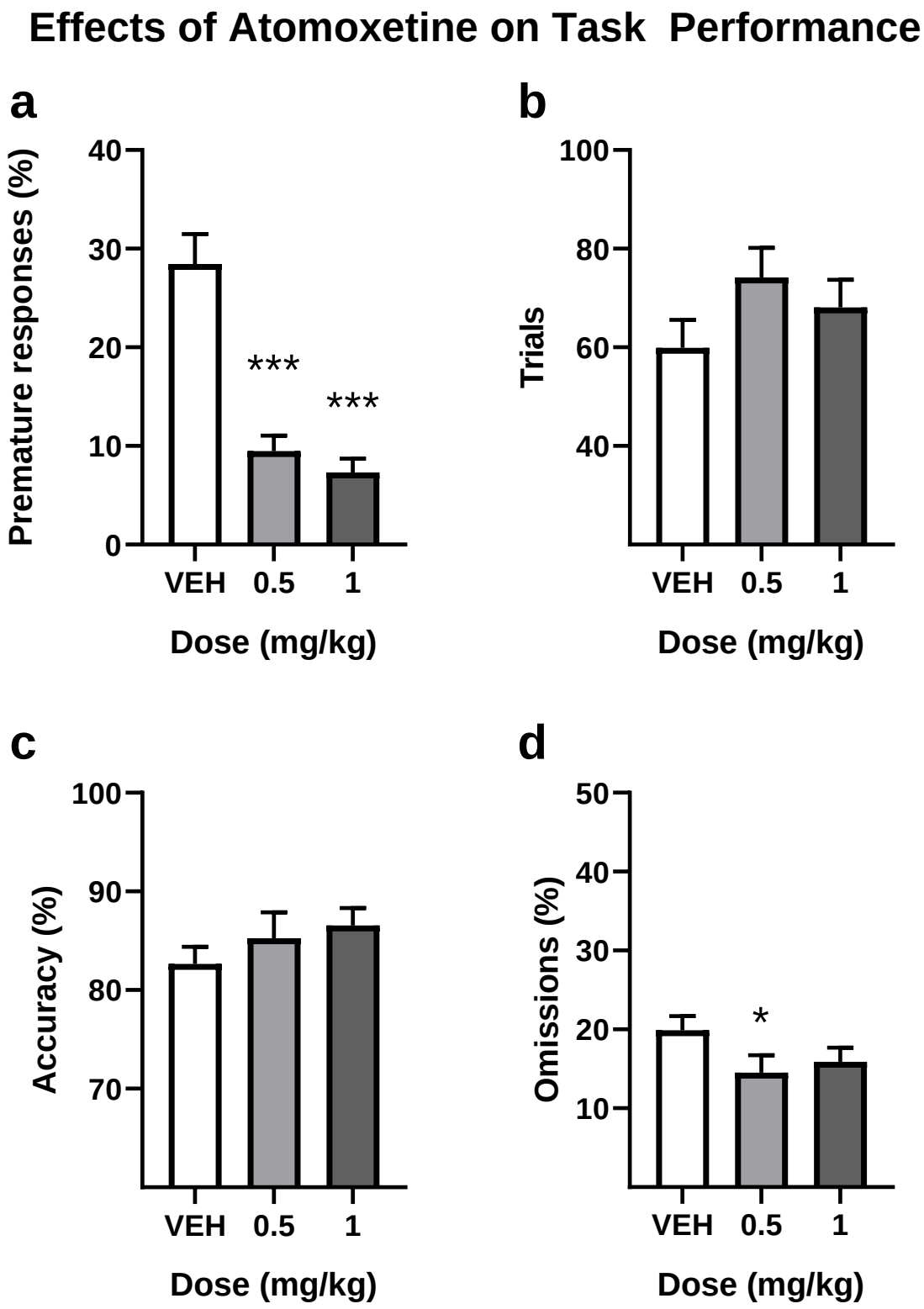
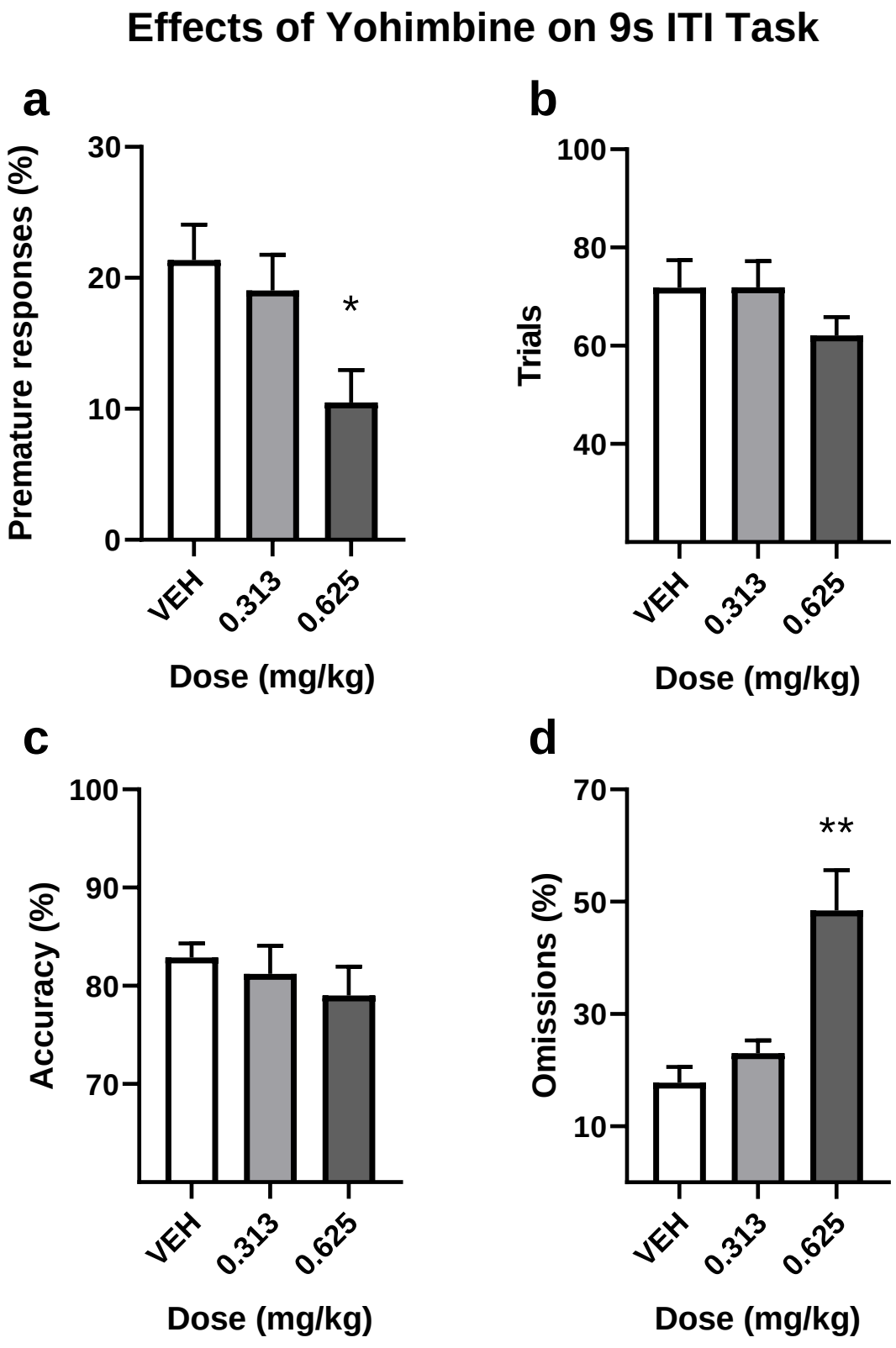

Effects of Atomoxetine on Task Performance
a
Premature responses (%)
40
30
20
10
0
***
***
VEH
0.5
1
Dose (mg/kg)
b
Trials
100
80
60
40
VEH
0.5
1
Dose (mg/kg)
c
Accuracy (%)
100
90
80
70
VEH
0.5
1
Dose (mg/kg)
d
Omissions (%)
50
40
30
20
10
*
VEH
0.5
1
Dose (mg/kg)

Figure 10


Effects of Yohimbine on 9s ITI Task
a
Premature responses (%)
30
20
10
0
*
VEH
0.313
0.625
Dose (mg/kg)
b
Trials
100
80
60
40
VEH
0.313
0.625
Dose (mg/kg)
c
Accuracy (%)
100
90
80
70
VEH
0.313
0.625
Dose (mg/kg)
d
Omissions (%)
70
50
30
10
**
VEH
0.313
0.625
Dose (mg/kg)