

EFFECT OF ATOMOXETINE ON ATTENTIONAL LAPSES: AN ANIMAL MODEL

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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College

Oxford  
May 2015

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## ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Sabol, for allowing me to work in her lab for the past year. I could not have completed this project without her guidance and support. I have learned much more about my capabilities and myself as a researcher through this entire experience.

Furthermore, I would also like to express gratitude to the Sally McDonnell Barksdale Honors College for the multitude of opportunities it has provided me as an undergraduate student. My college experience would not have been the same without the academic and moral support extended by the honors college.

## ABSTRACT

Atomoxetine (ATX) is a non-stimulant drug that has been used to treat symptoms of ADHD, including lapses of attention. These attentional lapses are depicted as a longer, positive skew in a reaction time distribution comprised of a normal and an exponential curve. The central tendency is thought to represent sensory-motor processing; while the positive skew is thought to be caused by attentional lapses in animal models. This positive skew is larger in individuals with ADHD and is thought to be a result of an increased number of attentional lapses. In the animal model used in this experiment reaction time is divided into initiation time and movement time, and further divided into the mode and deviation from the mode (DevMode) of the initiation time distribution. The DevMode represents the positive skew of the initiation time distribution. The aim of this experiment was to study the effect of atomoxetine on each of these measures. Rats were trained using a 2-choice reaction time task. The effects of ATX (vehicle, 0.1, 0.5, and 1.0 mg/kg) on the mode and DevMode were observed. Increasing the dose of ATX did not have a significant effect on the measure of mode. However, a significant decrease of DevMode occurred at 1.0 mg/kg dose of ATX. This study provides further evidence that the mode and skew of initiation time distribution are associated with different behavioral processes. The results support the idea that this task can be used as an animal model for attentional lapses as observed in ADHD.

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## LIST OF ABBREVIATIONS

ADHD	Attention-Deficit/ Hyperactivity Disorder
ATX	Atomoxetine
DA	Dopamine
DAT	Dopamine Transporter
NET	Norepinephrine Transporter
NE	Norepinephrine
PFC	Prefrontal Cortex
5CSRTT	5-choice Serial Reaction Time Task

## INTRODUCTION

### Symptoms of ADHD

Attention deficit- hyperactivity disorder (ADHD) is becoming an increasing problem among individuals. The main symptoms of ADHD include inattention and hyperactivity. In adolescents, this disorder causes individuals great difficulty in completing tasks that require long periods of inactivity, such as sitting still and paying attention in the classroom (Turner et al., 2013) Quantitatively, the children with ADHD demonstrate increased reaction time variability during timed tasks (Tamm, 2012). The response time distributions of the ADHD children are distinguished from those of control children by the presence of a greater number of slow responses, but not an overall pattern of slow responses.

### Introduction to Ex-Gaussian Analysis

The ex-Gaussian distribution curve is characterized as a combination of a Gaussian and exponential curve. The three components of an ex-Gaussian distribution include  $\mu$ ,  $\sigma$ , and  $\tau$ .  $\mu$  and  $\sigma$  are the mean and standard deviation of the normal component, respectively.  $\tau$  is the mean of the exponential distributional elements, and the measure of reaction time variability (Leth-Steenson et al., 2000).

A longer tail is a depiction of an increased number of slower responses and is believed to represent occasional lapses in attention among subjects. On an ex-Gaussian distributional model, the tail of the reaction time distribution is longer for individuals with ADHD than for the control subjects. An attentional lapse is defined as a temporary and often brief shift of conscious attention away from some primary task to unrelated internal information processing (Cheyne et al., 2010). Specifically, the attentional lapses are unique to each individual and are referred to as inconsistencies, and are defined as within-person variability in performance on a single task measured on multiple occasions, either across

testing sessions or across separate trials within the same testing session (Hultsch et al., 2002)

### Selectivity of Atomoxetine

Atomoxetine, a non-stimulant drug commonly used to alleviate symptoms of ADHD, acts as a selective norepinephrine transporter (NET) inhibitor. Activation of the noradrenergic pathway has been known to increase attention, especially in the arousal and maintenance of attention on a single object/task (Biederman & Spencer, 1999) In 2002, Bymaster and colleagues obtained results showing increased levels of norepinephrine (NE) and dopamine (DA) in the prefrontal cortex. NE levels were raised to a peak value of  $290 \pm 33\%$  of basal concentrations after administration of 3.0 mg/kg dose of ATX, and DA levels were raised to  $323 \pm 17\%$  of basal levels (Bymaster et al., 2002). The increase in DA at the 0.3-mg/kg dose is approximately 160 % of baseline, whereas NE is increased to 290 % of the baseline. At doses lower than 0.3 mg/kg ATX one might expect an even smaller effect on DA accumulation. It is suggested that NETs nonselectively uptake DA in the prefrontal cortex, as well as NE. Unlike stimulants, atomoxetine does not increase dopamine levels in other structures such as the nucleus accumbens or striatum (Bymaster et al., 2002). Both, the stimulant and non-stimulant medications have similar effects on the cortex, but different effects on the subcortical structures (Robinson et al., 2008).

### Human Studies Using Atomoxetine

Research assessing the role of atomoxetine on attentional lapses in humans has been limited. In a study conducted in 2011, Kratz concluded that children with ADHD had increased reaction time medians and variability during an attention network test (ANT). Kratz and colleagues utilized a version of the flanker paradigm (a version of the stroop task) combined with cue processing. The subjects of

this study included two groups of children – one experimental group consisting of individuals with ADHD, and another with typically developing children. Each of these groups was presented with a congruent and an incongruent version of the stimuli. The dependent measures of attention included: number of hits, median reaction time (RT), and variability of RT, among a few others. The ADHD group showed higher variability of reaction times while completing this task when compared to the typically developing control group (Kratz et al., 2011). Kratz replicated this study in 2012, but administered either atomoxetine or methylphenidate to the novel experimental group with ADHD. The dependent measures of attention remained the same.

Overall, atomoxetine had a significant effect on each of these measures when compared to baseline. It increased the total number of hits, while decreasing both, the median reaction time and variability of the reaction times (Kratz et al., 2011,2012). A decrease in the variability of reaction times can be interpreted as a decrease in inconsistency as defined by Hultsch and colleagues (Hultsch et al., 2004). On an ex-Gaussian reaction time distribution, this decrease in inconsistency would be seen as a decrease in tau, or the size of the tail, as suggested by Leth-Steensen (Leth –Steensen et al., 2000).

Kratz conducted research based on the Attention Network Theory, proposed by Posner and Peterson, which suggests three different networks of attention, each associated with a different neurotransmitter. The alerting network (consisting of the locus coeruleus, parietal and right frontal cortex) is modulated by noradrenaline. The orienting network (frontal eye fields, superior colliculus, temporal parietal junction and superior parietal cortex) is modulated by acetylcholine, and the conflict network (basal ganglia, anterior cingulate and lateral ventral prefrontal cortex) is modulated by dopamine. Atomoxetine was shown to have an effect on both the orienting network and the conflict network. This provides support for the idea that atomoxetine may act in a nonselective manner on these dopaminergic receptors at certain doses, along with noradrenergic receptors (Kratz et al., 2012). This

suggests that both norepinephrine and dopamine are important neurotransmitters in relation to attention.

### Animal Models of Attention

Studies of atomoxetine's effects on attention have been conducted on animal models of attention, mainly rats. The typical task that has been used in order to measure attention has been the 5-choice serial reaction time task (5CSRTT) (Navarra et al., 2008; Robinson et al., 2008, 2012; Koffarnus et al., 2011; Sun et al., 2012; & Wilson et al., 2012). Some researchers have used other tasks as well – lateralized reaction time task (Jentsch, 2009) and a two-choice visual reaction time task (Sabol et al., 2003).

The 5-choice serial reaction time task is conducted in an operant chamber in which there are 5 nose-poke apertures located horizontally across one wall, and a magazine at the opposite wall. The magazine is the location at which a food reward is dispensed. Each of these apertures has a light located above it, which acts as the visual stimuli throughout the task. The trial typically starts with a food pellet being dispensed into the magazine. After a set time (controlled by the experimenter), a light above one of the apertures is illuminated. Upon illumination, the rat is required to make a nose-poke in the corresponding aperture. Another trial begins as another food pellet is dispensed in the magazine on the opposite wall (Navarra et al., 2008; Robinson et al., 2008, 2012; Koffarnus et al., 2011; Sun et al., 2012; & Wilson et al., 2012).

The 5CSRTT tests the focused attention of animals by measuring factors such as: percent correct responses, premature responses, and omissions. 'Correct responses', reported as a percentage of the total responses made, is simply the number of times the rat makes a nose-poke in the correct aperture. Premature responses include the subject making a nose poke into an aperture before a

stimulus has been presented. An omission is recorded when the subject fails to make a nose poke throughout the entire trial (Navarra et al., 2008; Robinson et al., 2008, 2012; Koffarnus et al., 2011; Sun et al., 2012; & Wilson et al., 2012).

Another task previously used in atomoxetine research on animals is the lateralized reaction time task. In this task, the rat waits for a target stimulus light to be presented to either their right or left while placing its nose in the center nosepoke hole for a predetermined, variable amount of time. The variability of the fixation time and the uncertainty of where the target will be presented requires the rat to divide its attention between the two areas (Jentsch et al., 2009).

Both tasks involve the subject making a nose poke in the aperture over which a target stimulus light is illuminated. In the 5CSRTT, the target is presented after the rat retrieves a food pellet on the opposite side of the operant chamber. This increases the odds of the rat not facing the appropriate direction. This differs from the two-choice reaction-time task in which the subject is already facing the wall where the nose-poke apertures are located. The orientation of the rat allows it to divide its attention between two locations where the target stimulus may appear. Another self-evident difference is the number of choices presented to the rats. As inferred from the name, the 5CSRTT includes five areas that the subject must focus on, whereas the two-choice reaction-time task only offers two. Overall, it can be concluded that the 5CSRTT demands more control over attention on the part of the subject, as compared to the two-choice reaction-time task, but introduces more variability within the reaction times.

A 2-choice visual reaction time task is also used by Sabol and colleagues, but differs from Jentsch's in several ways (Sabol et al., 2003). These differences require different data analysis procedures (discussed below) to be implemented. Another key difference between the two tasks is the length of time that the target stimulus is presented. In Jentsch's lateralized reaction time task, the target

stimulus presentation varied between 0.2 and 2 seconds. In comparison, the target stimulus in Sabol's choice reaction time task was presented until the subject made a choice by inserting its nose in either the right or left nose poke hole. This continuous presentation eliminates the possibility of the subject failing to see the target. It also decreases the memory load on the subject as it is making the choice. In the lateralized reaction time task, the subject must maintain information about the direction in which the target was presented while it is making the choice. Sabol's two-choice task, therefore, allows more focus to be placed on analyzing the reaction time (Jentsch et al., 2009; Sabol et al., 2003).

#### Atomoxetine's Effect on Accuracy in Animal Models

One of the direct measurements of the effect of atomoxetine in the 5CSRTT and lateralized reaction time task was that of accuracy. This measurement was typically made as a total number of correct responses made by the subjects while completing a task. The results of atomoxetine on accuracy were dependent on the drug dose administered to the subjects and by the behavioral task employed by the researcher. The range of atomoxetine doses includes: 0.1 mg/kg, 0.3 mg/kg, 0.5 mg/kg, 1.0 mg/kg, and 3.0 mg/ kg.

Although a 0.1 mg/kg dose of atomoxetine was commonly administered to subjects completing a 5CSRTT (Navarra et al., 2008; Robinson, 2012; Sun et al., 2012), it was not until a 0.3 mg/ kg dose was administered that any significant increases in accuracy were observed. Robinson only observed increased accuracy in poor-performing subjects while they completed a 5CSRTT with variable inter-trial intervals of 5, 6, and 7 seconds. The parameters of performance groups were set based on their accuracy scores under vehicle treatment. The low baseline accuracy group was considered to be poor-performing (Robinson, 2012). Still, other researchers did not see a significant effect on accuracy while using this same atomoxetine dose (0.3 mg/kg) (Koffarnus et al., 2011; Sun et al., 2012).

Navarra and colleagues administered a 0.5mg/kg dose of atomoxetine to subjects participating in a 5CSRTT with variable inter-trial intervals. The significant increase in accuracy was found to be dependent on the inter-trial interval length. The rats only showed an increase in accuracy with a 10-second inter-trial interval during the task (Navarra et al., 2008).

More significant findings were obtained when the atomoxetine dose was increased to 1.0 mg/kg. Subjects that were required to complete the 5CSRTT with a 5-second inter-trial interval showed an increase in accuracy when compared the control group (Navarra et al., 2009) The inter-trial interval was also an important variable in the lateralized reaction time task when assessing accuracy. In 2009, Jentsch found a significant inter-trial interval x drug interaction. During a short inter-trial interval of 0.2 seconds, 1.0 mg/kg dose of atomoxetine caused significant impairments in response accuracy when compared to the saline group. On the other hand, when the inter-trial interval was lengthened to 1.0 second, the same dosage of atomoxetine caused the response accuracy to improve within the same group (Jentsch et al., 2009). Still, some researchers (Koffarnus et al., 2011; Sun et al., 2012) did not find any significant results, even at the 1.0 mg/kg dose of atomoxetine.

The 3.0 mg/kg dose was only administered during a 5CSRTT and did not have significant effects on accuracy as reported by Koffarnus in 2011.

In summary, some researchers found that the administration of atomoxetine increased the number of correct responses made by the rat at certain doses. A dose of 0.1 mg/kg did not yield any significant results (Navarra et al., 2008; Robinson et al., 2008; Sun et al., 2012). Although there was a significant increase in accuracy in one study by Robinson using a 0.3 mg/kg dose of ATX (Robinson, 2012), other researchers did not have success with this dose (Koffarnus et al., 2011; Sun et al., 2012).

Higher doses (0.5 and 1.0 mg/kg) were shown to improve accuracy. Navarra and colleagues, in 2008, observed a significant increase in percent correct responses at these doses. Jentsch found that at a

1.0 s interval, a 1.0-mg/kg dose of atomoxetine caused a significant increase in accuracy (Navarra et al., 2008; Jentsch et al., 2009).

It has been suggested that as the dose of ATX increases, NET blockade by ATX causes extracellular increases in both NE and DA, in the PFC (Bymaster et al., 2002). The results from these studies suggest that both norepinephrine and dopamine are necessary to observe any significant changes in accuracy.

### Atomoxetine Effect on Omissions in Animal Models

Another measure of attention used by researchers is the occurrence of omissions. An omission is recorded when the subject fails to respond to the target stimulus during a trial. These omissions can be due to the subject missing the target presentation or simply not moving at a quick enough rate to the target. In a 5CSRTT, the odds of the subject missing the target presentation increase because the position and body orientation of the rat are unknown and tend to be more variable. These omissions are less likely to be seen in a lateralized reaction time task due to the position of the rat being oriented in the same direction as the targets.

When given any dose of atomoxetine (0.1 – 0.3 mg/ kg), very few significant results were observed among researchers. Sun observed an increase in omissions at a 0.1-mg/kg dose of atomoxetine while rats completed a 5CSRTT (Sun et al., 2012).

Both Sun and Jentsch observed significant increases in omissions at 1.0 mg/kg dose of atomoxetine while their subjects completed the 5CSRTT and lateralized reaction time task, respectively (Sun et al., 2012; Jentsch et al., 2009).

The only other dose that resulted in an increase in omissions was 10 mg/kg of atomoxetine. It was used in a study conducted by Koffarnus in 2011, which employed the 5CSRTT. A unique, fixed-

ratio reward system was implemented by the researcher. The fixed- ratio was set to be 1, 3, or 10, but the focus was on the FR-1 study. It is unusual that this increase in omissions only occurred when the dosage was 10 mg/kg, and did not occur at the lower doses.

In summary, omissions have been found with a range of ATX doses including 0.1 mg/kg, 1.0 mg/kg and 10.0 mg/kg (Koffarnus et al., 2011; Sun et al., 2012; Jentsch et al., 2009). Two studies did not find any increase in omissions, even with increasing demands on attention and doses (0.1, 0.3, 0.3, 0.6, 1.0 and 3.0 mg/kg) of atomoxetine (Robinson et al., 2008, 2012). According to the literature, not enough evidence has been found to state whether or not dopamine is required, in conjunction with norepinephrine, to affect omissions.

#### Atomoxetine's Effect on Reaction Time in Animal Models

The response time is defined as the time taken for the subject to react correctly to a presented stimulus, such as a light. For a response to take place, the subject must process sensory information (in this case the visual presentation of a target stimulus light), and execute proper motor output, including moving to the target location. This is considered a measure of attention because it determines how quickly the rat can orient its attention to the target. This orientation towards the target is more difficult for those completing the 5CSRTT, due to their body position relative to the targets being unknown.

Using the 5CSRTT, Robinson et al. (2012) noticed an increase in correct latency (the time taken for the subject to make a correct response) when the group was administered a 0.3 mg/kg dose of atomoxetine and using a short stimulus duration of 0.125 seconds (Robinson, 2012). Other researchers did not obtain any significant changes in reaction time when using the same dose and task (Koffarnus et al., 2011).

In 2009, Jentsch, using the lateralized reaction time task, observed an increase in reaction times

among subjects treated with 1.0 mg.kg dose of atomoxetine, with preparatory interval times of both of 0.2 s and 1.0 s, when compared to the control group administered saline solution (Jentsch et al., 2009)

The reaction times recorded in these cases only included the time taken to make the correct response (correct latency or correct response time). Only a couple of the researchers succeeded in finding significant changes in reaction times, and these increases were seen at different doses of atomoxetine. Robinson observed an increase in correct latency at 0.3 mg/kg, whereas Jentsch recorded a significantly increased correct response time at 1.0 mg/ kg dose of atomoxetine. (Robinson, 2012; Jentsch et al., 2009)

The effects of atomoxetine on reaction times were seen at both 0.3 and 1.0-mg/kg doses (Jentsch et al., 2012; Robinson, 2012). However, with only two researchers obtaining any significant findings, no conclusions can be reached regarding the roles of dopamine and norepinephrine in atomoxetine's effect on reaction time. It can be speculated, however, that both dopamine and norepinephrine are required for an effect on reaction time during a reaction time task due to the doses at which any effect was observed, but the contribution of DA would be less than that of NE.

### Use of Mode and DevMode in Data Analysis

In the experiment reported here, using a 2-choice task, we focused on the reaction time to measure attention. The reaction time measure was analyzed in two ways. It was first subdivided into initiation time and movement time. The initiation time was defined as the time it takes for the subject to remove its nose from the center hole after presentation of the stimulus light above either the left or right water dispenser. The movement time was the time between the subject removing its nose from the center hole and inserting its nose into the correct water dispenser. Furthermore, these reaction time distributions were divided into distribution mode and distribution skew.

Studying the distribution mode and distribution skew stems from the ex-Gaussian approach, discussed above, to distribution analysis. The data obtained from this study was analyzed using a method similar to the ex-Gaussian approach. The ex-Gaussian approach is based on the idea that a reaction time distribution is comprised of two components - the exponential and the Gaussian components. The Gaussian, or normal component is believed to be representative of sensory motor processing, while the positive skew, or exponential component, represents lapses in attention (Leth-Steensen et al., 2000). The analysis used in our experiment still includes the central tendency and the rightward, positive skew, but includes the idea of the mean being greater than the mode due to the positive skew. The difference between the modal (the most frequent value in a set of data) and mean values is known as the DevMode. An increase in the positive skew results in an increase in the DevMode value.

#### Applying Results of 5CSRTT to 2-choice reaction time task

It is difficult to apply results from previous work done using the 5CSRTT to the present 2-choice reaction time task for several reasons. A difference between the 5CSRTT and the 2-choice reaction time task is the length of time for which the target stimulus is presented. The 5CSRTT target stimulus is only shown for a predetermined time period before being turned off. In the 2-choice reaction time task, this target light remains lit until the subject has made a choice for that trial. This reduces the memory load of the subject and allows the researcher to focus on measurements of attention.

A second difference is present in the procedural requirements of the two tasks. Because the 5CSRTT requires the subject to move away from the target apertures in order to retrieve a food pellet at the end of each trial, it is difficult to determine the position of the subject within the chamber when

the succeeding trial begins. For example, the subject may be facing in such a way that it is not able to see the target. The trial is thus counted as an omission, or slower reaction time (Robinson, 2012). This increases the variability of the reaction times that are recorded. The 2-choice reaction time task, alternatively, requires the subject to make a nose-poke in an aperture located between the two target apertures for a set time before presentation of the target. This forces the subject to be in close proximity to the apertures above which the target lights are located.

Accuracy has been used as the main measure of attention by researchers using the 5CSRTT. This differs from the present paradigm, which uses distributions of reaction time as the main measure of attention.

#### Difference in Data Analysis Between Lateralized Reaction Time Task and Present 2-choice reaction time task

The lateralized reaction time task is a second behavioral task that has been used, by Jentsch and colleagues in 2009, to measure the effects of atomoxetine on attention. This task shares more similarities to the present 2-choice reaction task compared to the 5CSRTT. The uncertainty about the position of the rat is eliminated in Jentsch's lateralized reaction time task as the subject is placed between the two apertures. This is identical to the two-choice visual reaction time task paradigm. Although the variability of reaction times is reduced, the ways in which these values are analyzed and interpreted differ from the present study.

The first difference lies in the measurements of reaction times by Jentsch compared to our group. Jentsch defines the reaction time as time taken for the subject to remove its nose from the center nose-poke aperture and insert it into the proper target aperture. In the present paradigm, the reaction

time is split into initiation and movement time, with the focus of the study being the initiation time – the time taken for the subject to remove its nose from the center nose-poke aperture after the onset of the target stimulus. There is a greater emphasis placed on initiation time because it is the time period during which significant effects on attention are likely to be detected. Nevertheless, Jentsch’s reaction time measures can be applied to our paradigm more directly than other measures of attention, including the reaction time measured during a 5CSRTT and accuracy.

The second difference between the lateralized reaction time task and the present 2-choice reaction time task lies in the dependent variable that is used as the main marker of attention. Because the measures of mode and DevMode of initiation time are attributed to different processes, effects on either of these measures can be interpreted differently. The mode is thought to be representative of sensory-motor processing, whereas the DevMode is representative of attentional lapses (Sabol et al., 2003). The separation of the initiation from movement times increases the specificity with which one can study the exact effects this drug has on attention. A decrease in DevMode is indicative of a decrease in lapses of attention. Therefore, the analysis of the initiation times is a more direct study of the lapses of attention seen in ADHD.

## Hypotheses

After assessing previous research testing the effects of atomoxetine, two conflicting hypotheses present themselves.

The first hypothesis is drawn from the results obtained by Kratz and colleagues in 2012. They found that atomoxetine treatment resulted in an overall decrease in reaction times and reaction time variability in children completing an attention networking task (Kratz et al., 2012). This reaction time variability is analogous to the measure of DevMode in the present study. Based on the Kratz 2012

finding, we hypothesize that the DevMode will decrease, indicating a decrease in lapses of attention. On an ex-Gaussian reaction time distribution, this decrease in attentional lapses will be depicted as a decrease in the longer distributional tail as seen in those of individuals with ADHD (Leth-Steensen et al., 2000).

In contrast to the hypothesis suggested by human studies, animal studies present a hypothesis in which the reaction time is predicted to increase. Jentsch found a significant increase in reaction time during the lateralized reaction time task at 1.0 mg/kg of atomoxetine. This finding suggests an increase in our present paradigm, also. Because of differences in data analysis procedures, however, it is difficult to assess whether this increase in reaction time will be seen in the initiation time DevMode – our main measure of attentional lapses.

It is unclear which hypothesis will be supported because each of the two hypotheses has similarities to the present paradigm. Although the study conducted by Kratz used humans as subjects, the analysis of reaction time variability closely resembles the current study's measure of DevMode. The paradigm employed by Jentsch and colleagues in 2000 is most similar to the present two-choice reaction task, and they used rats. However, Jentsch did not measure distribution skew as part of the lateralized reaction time task. Both hypotheses are considered probable.

## METHODS

### Subjects

Sixteen male Sprague Dawley (Harlan) rats, weighing between 250-275 grams were used during this experiment. They were housed in pairs in clear plastic cages lined with bedding. The subjects had unlimited access to food. Water access was restricted to 20 minutes / day with the exception of 24-hour access on the weekends (Friday – Saturday). The experimental group's weights were monitored to make sure they did not fall below 80% of the control group's weights.

### Drugs

Atomoxetine hydrochloride (Sigma Aldrich) doses were dissolved in saline solution, and were administered by intraperitoneal injection 30 minutes prior to testing. Four doses of atomoxetine were administered including: vehicle, 0.1, 0.5, and 1.0 mg/kg.

### Apparatus

The apparatus included four experimental chambers (22.5 x 22.5 x 20 cm) enclosed in sound-attenuating boxes measuring. They had stainless steel grid floor, aluminum front and back walls, Plexiglass sides, and a Plexiglass top. One side of the chamber had a house light. The test panel, located on the opposite side, had two water dispensers on either side of a nose poke hole. Stimulus lights were mounted above each water dispenser at eye level with the rat as it interrupted the infrared photo beam in the center nose poke hole at the beginning of each trial. Infrared beams also monitored nose pokes into the water dispensers. Each nose-poke was programmed to deliver a 50- $\mu$ L drop of water to the rat.

### Training Sequence

The training sequence was split into three phases before the final parameters were implemented. The first phase focused on the rat learning the task. The house light was not lit for the duration of this phase of training. The rat was required to poke its nose into the center aperture for a maximum time period of 10 cs, or 0.1 s (the foreperiod) before the light above either the right or left water dispenser was illuminated. The rat could, then, choose a water dispenser by making a nosepoke in the appropriate (either right or left) hole and received water reinforcement. Before moving onto the next phase of training, the rat had to complete 100 trials within 30 minutes with 70% accuracy. During the second training phase the maximum foreperiod was increased by one second each day until the 6-s parameter was reached, although the required foreperiod for each trial was variable. A reaction time requirement was introduced during the last phase of training in which the rats had to respond within some criterion value in order to receive water. The criterion was made easier if the rat failed to make a response within the criterion and made more stringent if the rat made the correct response within the criterion time. The house light in the operant chamber was turned on either five days later or when they met another criterion: completing one hundred trials within thirty minutes with  $\geq 70\%$  accuracy. The final phase continued until the rats met the criteria for stable performance. The average initiation times for 5 consecutive blocks of 100 trials did not differ by more than 4%.

### Summary of Final Parameters

The house light was turned on as part of the final parameters. Each trial began with the subject inserting its nose into the center aperture. The required length of time for the subject to make center nose poke (foreperiod) varied for each trial, but the maximum length was 6 seconds. The foreperiod was cumulative, so if the subject removed its nose prematurely, it was able to complete the total

foreperiod in several increments. After the foreperiod had lapsed, a target stimulus was presented over either the left or right water dispenser. This target light remained lit until the subject made a choice by placing its nose in the correct water dispenser within a certain time period. The subject was reinforced with a 50- $\mu$ L drop of water.

### Procedure

The rats, upon arrival, were placed in quarantine for 1 week. They were then housed in pairs and were put on water restriction 1 week prior to the start of training. Once the rats' performance was stable, all rats were administered four doses of atomoxetine (vehicle, 0.1, 0.5, and 1.0 mg/kg). The sequence of doses received by the subjects was determined by a 4 x 4 latin square design. These doses of atomoxetine or the vehicle were administered i. p. 30 minutes prior to the behavioral testing on Tuesdays and Fridays during the two weeks that testing took place.

### Dependent Variables

The dependent measures in this study include *initiation time*, *movement time*, *omissions*, *premature initiations*, and *premature responses*. The reaction time is the time taken for the subject to make a nosepoke in either the left or right water dispenser after the onset of the target stimulus presentation. The reaction time is split into *initiation time* – the time between the onset of the target stimulus presentation and the removal of the subject's nose from the center hole and the *movement time*- the time between the removal of the nose from the center hole and the insertion of the subject's nose into either the right or left water dispenser. This report will not include analysis of movement time measures.

For the initiation times, the mode and DevMode were calculated and analyzed using the running

average technique. The DevMode is a measure of the skew of the reaction times recorded. The DevMode is the difference between the mode and mean of the initiation time distribution.

An *omission* was counted if the subject failed to respond within the reaction time criterion of 2 seconds. A *premature initiation* was counted if the subject removed its nose from the center hole before the complete foreperiod lapsed, but did not place its nose into either of the water dispensers. A *premature response* was counted when the subject removed its nose from the center hole and made a response before the target stimulus light was presented. Both, measures of premature initiations and premature responses, were reported as rates of responses per second.

#### Data Analysis

All the data collected for this experiment for each drug dose was averaged. Each dependent variable (mode, DevMode, correct omissions, premature initiations/s, premature responses/s) was analyzed using a one – way within-subjects analysis of variance (ANOVA), using SPSS version 22. A value of  $P \leq 0.05$  was considered significant. The one-way factor was drug dose (vehicle, 0.1, 0.5, or 1.0 mg/kg ATX). Contrast analysis between conditions was also conducted when significant findings were found within the ANOVA.

## RESULTS

The effect of ATX treatment (vehicle, 0.1, 0.5, and 1.0 mg/kg) on the two-choice visual reaction time task was measured.

### Mode

There was no significant effect of ATX treatment on modal initiation time [ $F_{(3,33)}=5.535$ ;  $p=.661$ ]. See Figure 1.

### Deviation from the Mode (DevMode)

ATX dose had a significant effect on the DevMode measurement [ $F_{(3,33)}=5.034$ ;  $p < .01$ ]. A contrast analysis revealed a significant decrease in DevMode at the 1.0 mg/kg dose of atomoxetine compared to the vehicle ( $p < .05$ ). A non-significant borderline effect was observed when rats were administered 0.5 mg/kg dose of atomoxetine ( $p = .067$ ). See Figure 2.

### Omissions

There was no effect of ATX on omissions [ $F_{(3,33)}= .538$ ;  $p = .660$ ]. See Figure 3.

### Rate of Premature Initiations

No significant effect was found for ATX on the rate of premature initiations made per second [ $F_{(3,33)} = 1.872$ ; ( $p= 0.154$ )]. See Figure 4.

### Rate of Premature Responses

A significant effect of ATX was observed on the rate of premature responses made per second [ $F_{(3,33)} = 2.890$ ;  $p = 0.050$ ]. In a contrast analysis, a significant effect was seen at the 0.1 mg/kg dose [ $F_{(3,33)} = (p < 0.05)$ ]. Non-significant, but notable decreases occurred at the 0.5 mg/kg dose ( $p = 0.092$ ) and the 1.0 mg /kg dose ( $p = 0.059$ ). See Figure 5.

## DISCUSSION

The results of this experiment provide evidence that ATX is efficacious when administered to rats completing a two-choice, visual, reaction time task. ATX significantly decreased the deviation from the mode (DevMode) of the measured initiation times when compared to vehicle. This significant decrease occurred at a 1.0 mg/kg dose. There was also a non-significant borderline effect seen at the 0.5 mg/kg dose ( $p= 0.067$ ). In addition, there was a significant decrease in the rate of premature responses at the 0.1 mg/kg dose of ATX. There were nonsignificant, borderline decreases seen at both the 0.5 mg/kg dose ( $p=0.092$ ) and at the 1.0 mg/kg dose of atomoxetine ( $p=0.059$ ). There were no significant effects seen on modal initiation time, omissions, and rate of premature initiations.

### Effect of ATX on DevMode

The findings of our experiment are in congruence with our hypothesis suggested by human studies- a decrease of DevMode. Namely, our experiment was in accord with the study conducted by Kratz in 2012 that found a significant decrease in reaction time variability with the administration of ATX during an attention network task. Reaction time variability is seen in individuals with ADHD and is attributed to increased attentional lapses while completing a task (Leth-Steensen et al., 2000). Our interpretation of the decrease in DevMode is a decrease in attentional lapses.

### Effect of ATX on Mode and DevMode

Atomoxetine did not have a significant effect on the modal initiation time, but did significantly decrease DevMode. The fact that the drug acted independently on each measure further supports the idea that each of the two components of the initiation time distribution- mode and DevMode-

represents a separate behavioral process. The central tendency, or mode, is indicative of sensory-motor processing, while the DevMode is indicative of attentional lapses (Sabol et al., 2003). A decrease in our measure of DevMode is representative of a decrease in attentional lapses. The lack of effect on mode helps to negate any idea that the increased reaction times observed in subjects with ADHD are due to motor impairments.

#### Implication of Atomoxetine Selectivity on DevMode Measure

It is known that the noradrenergic pathway is involved with ADHD (Biederman & Spencer, 1999). There is great modulation of the noradrenergic and dopaminergic pathways that must occur in the prefrontal cortex to properly arouse and maintain attention. ATX works as a noradrenaline transporter (NAT) inhibitor, thus increasing extracellular levels of NE. In a study conducted by Bymaster in 2002, it was seen that ATX affects NE levels and also DA levels. Bymaster and colleagues found that NE and DA levels increased significantly when compared to baseline levels after administering various doses of ATX (0.3, 1.0, and 3.0 mg/kg). While the levels of NE were elevated to about 300% of baseline at all three doses, the levels of DA increased to about 290% of baseline at 1.0 mg/kg, but only to 160% at 0.3 mg/kg. It can, therefore, be speculated there would be an even greater selectivity for NE at 0.1 mg/kg dose of ATX. We observed significant findings at the 1.0-mg/kg dose, without an effect at the 0.1 mg/kg dose, which implies that DA was required to observe a significant effect on our measure of DevMode.

#### Implication of effect of ATX on premature responses of present experiment

The overall effect of ATX on the rate of premature responses was found to be significant

( $p=0.05$ ). The rate of premature responses was significantly decreased at the 0.1 mg/kg dose of ATX ( $p<0.05$ ), and a non-significant, but notable decreases were observed at the 0.5 mg/kg dose ( $p=0.092$ ) and at the 1.0 mg/kg dose ( $p=0.059$ ). Based on the Bymaster (2002) study, it is thought that NE is the only neurotransmitter needed to significantly decrease the rate of premature responses. It can be speculated that the introduction of DA at the 0.5 mg/kg and 1.0-mg/kg doses causes a slight reversal in the rate of premature responses. However, this is considered unlikely due to the proximity of the mean rates of premature responses. These values are much closer in value to each other than when compared to vehicle. The means for the rate of premature responses are: vehicle = 0.2 ( $\pm 0.03$ ) responses/second (resp/s) ; 0.1 mg/kg = 0.16 ( $\pm 0.023$ ) resp/s; 0.5 mg/kg = 0.17 ( $\pm 0.026$ ) resp/s; 1.0 mg/kg = 0.17 ( $\pm 0.022$ ) resp/s. ATX administration likely has a significant effect on the impulsive activity as depicted by a decrease in the rate of premature responding compared to vehicle. This significant decrease occurred primarily at the 0.1-mg/kg dose of ATX.

### Comparison to Animal Studies

The difference in analysis procedure of the data obtained from this 2-choice reaction time task and previous studies that used a 5CSRTT makes it difficult to compare the effects on reaction time. Many researchers define reaction time in a different manner than the present study (Navarra et al., 2008; Robinson et al., 2008, 2012; Sun et al., 2012, Koffarnus et al., 2011). While completing a 5CSRTT, the reaction time typically is the time between a stimulus light being presented above any of the five apertures and the rat making a choice by placing its nose in the correct aperture. There is much more variability introduced in the reaction times recorded by those using the 5CSRTT due to the demands of the task, and the position of the rat within the chamber.

A lateralized reaction time task is more directly comparable to the present 2-choice reaction time task due to the requirements of the tasks. The measure of reaction time in the lateralized reaction time task was most similar to the present study's reaction time, although the present study focused on initiation time. The reaction time of the lateralized reaction time task was defined as the time between the rat removing its nose from the center aperture and inserting it into the aperture over which the target stimulus was presented. The initiation time in our task was specified as the time elapsed between target stimulus presentation and the rat's removal of its nose from the center aperture. The present study, therefore, had a smaller movement component than the lateralized reaction time task, when focusing on initiation time. Our study was not congruent with the predictions based on the lateralized reaction time task, which suggested that there would be an increase in the reaction time at the 1.0 mg/kg dose of ATX. In our experiment, initiation time mode stayed the same and the DevMode decreased at 1.0 mg/kg dose of ATX.

The difference in results obtained by the lateralized reaction time task (Jentsch et al., 2009) and the current experiment may be related to the differences in data analysis. Because we eliminated movement times from our analysis, and looked at both mode and DevMode, we were able to focus on the time more concerned with inattention: initiation time.

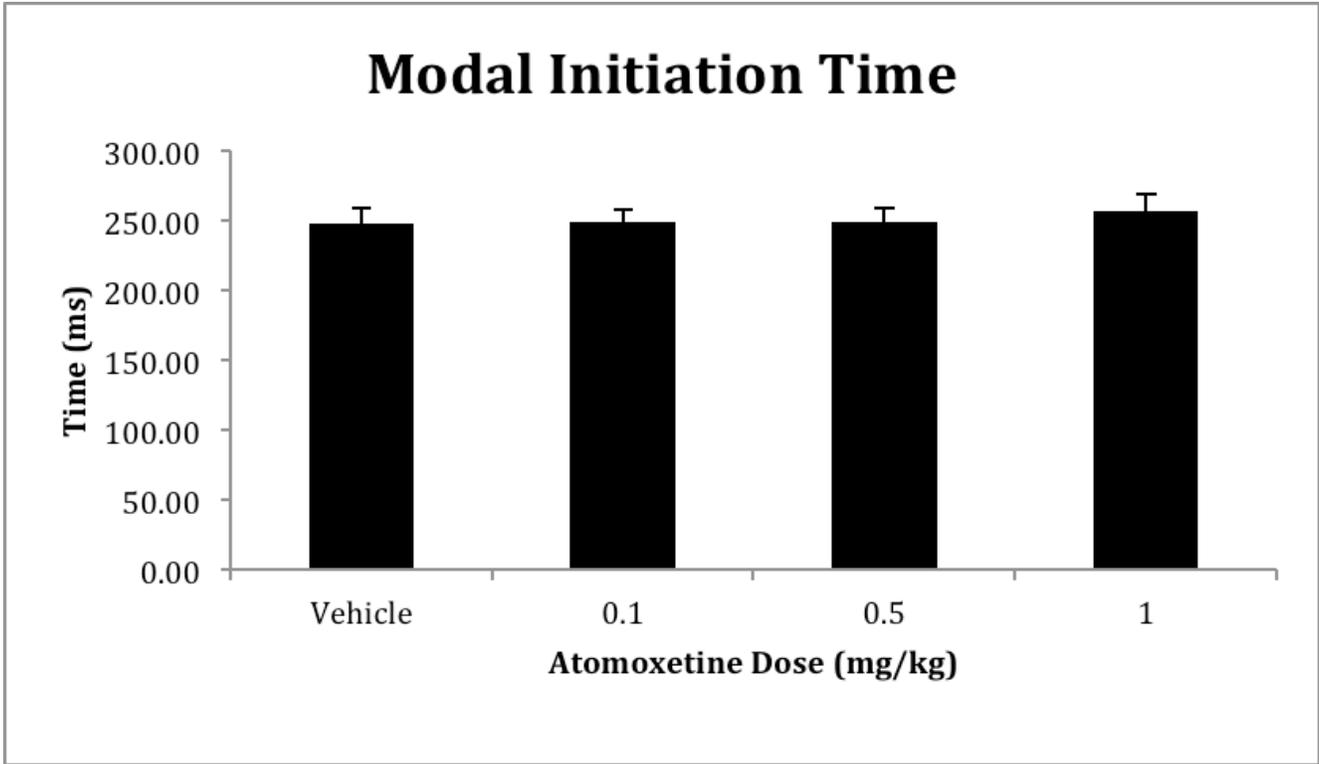
#### Further improvements to the paradigm

Significant results were obtained with the 2-choice reaction time task. Further studies should develop this paradigm to document the effect of ATX on attention. One change that may result in differing outcomes is the switching off of the house light. The light could have served as a distraction to the rats, and the removal of this element may help to further focus the rat's attention to the reaction time task.

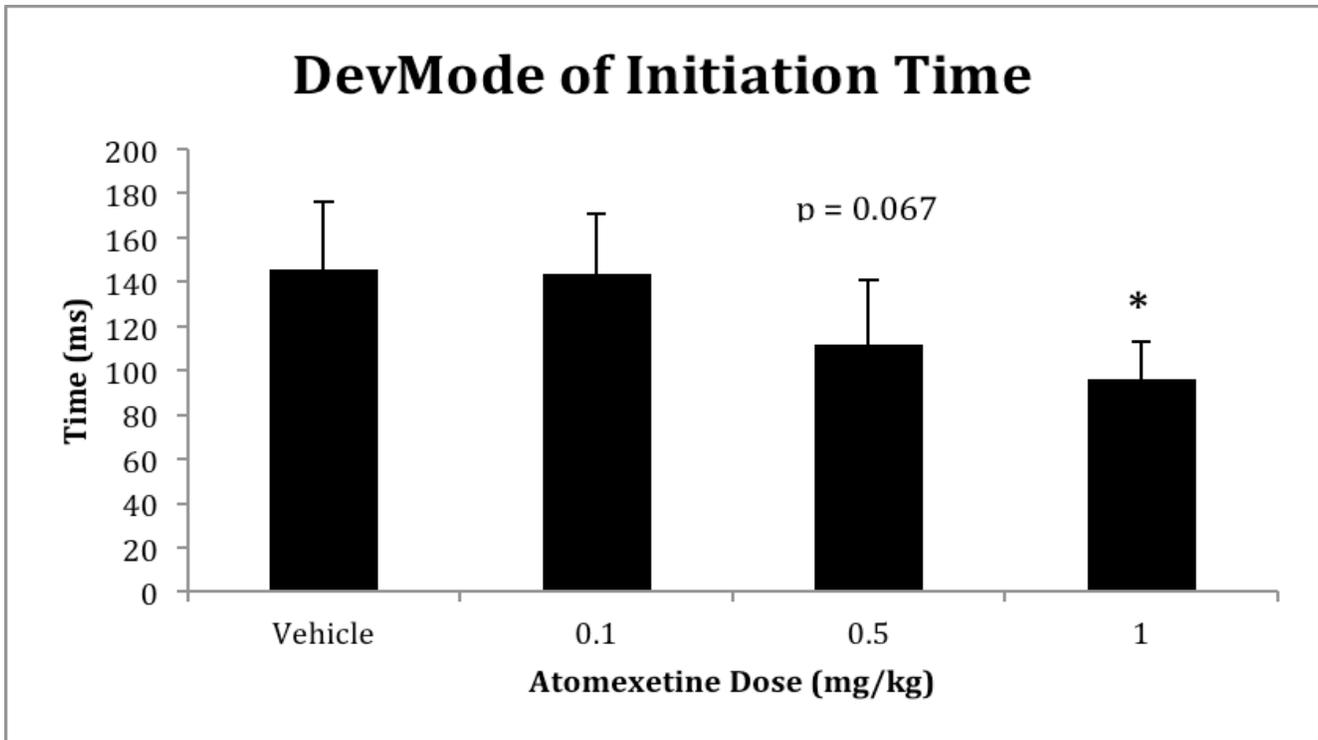
Another element that may have an effect on the results is the addition of sound during the length of the task. This addition of an auditory element will increase the attentional load of the rat while it is completing the task. Researchers will be better able to determine the parameters of ATX efficacy by adding elements that require increased attention.

## CONCLUSIONS

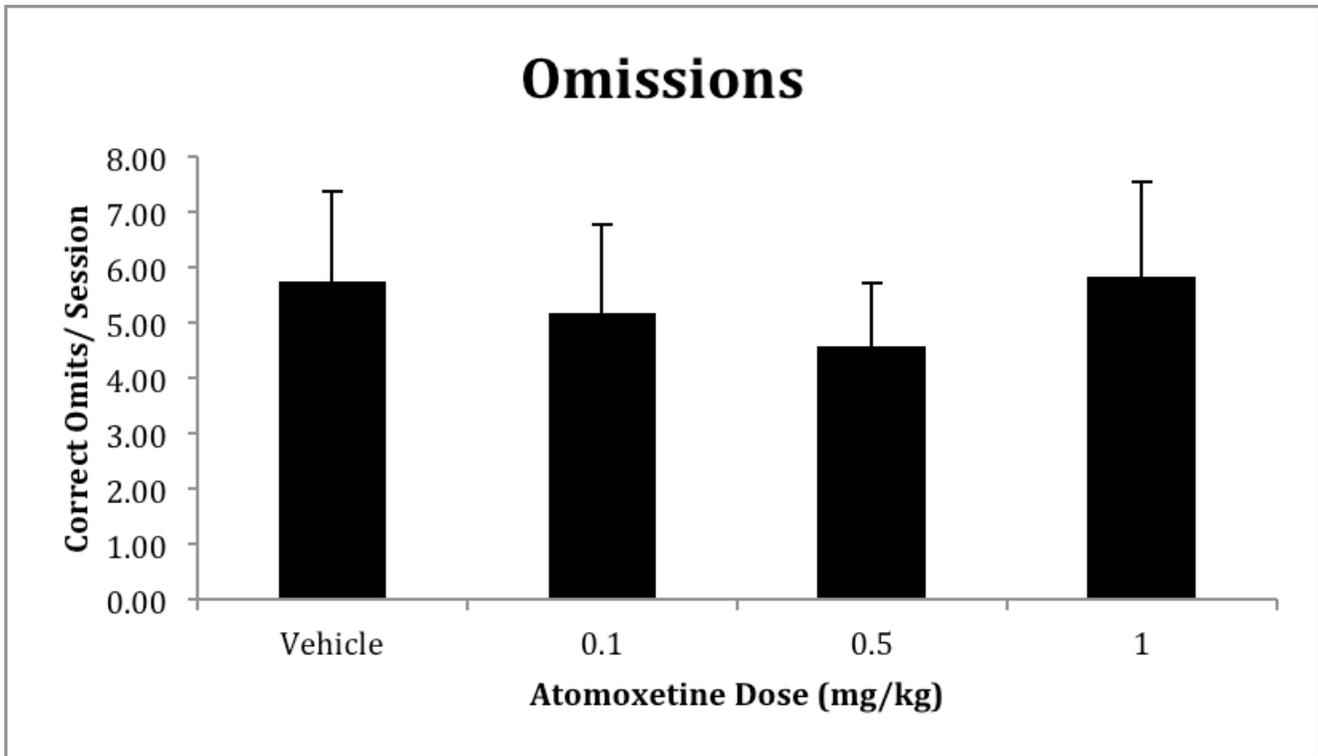
Overall, this study provided further evidence for the hypothesis that the Mode and DevMode are representative of separate behavioral processes, and can be manipulated independently of each other. ATX was shown to successfully decrease attentional lapses. These findings, therefore, support the use of the two-choice visual reaction time task and the analysis method, as an animal model of the ADHD symptom attentional lapses.



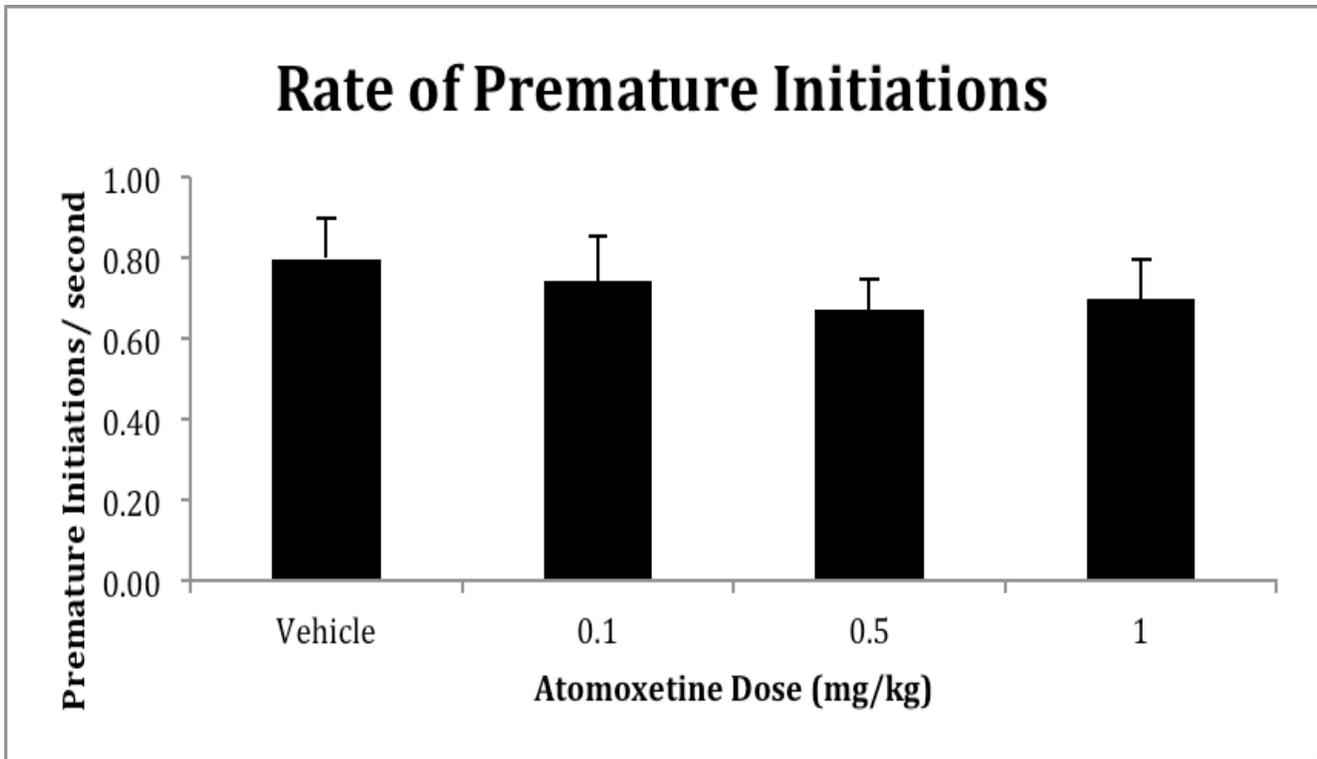
**Fig 1:** The mean modal values, in milliseconds, [ $\pm$  standard error of the mean (SEM)] at various doses of atomoxetine include: vehicle treatment = 247.5 ( $\pm$  11.18); 0.1 mg/kg ATX = 249.17( $\pm$  9.20); 0.5 mg/kg ATX = 248.75 ( $\pm$  10.53); 1.0 mg/kg ATX = 256.25 ( $\pm$  12.32).



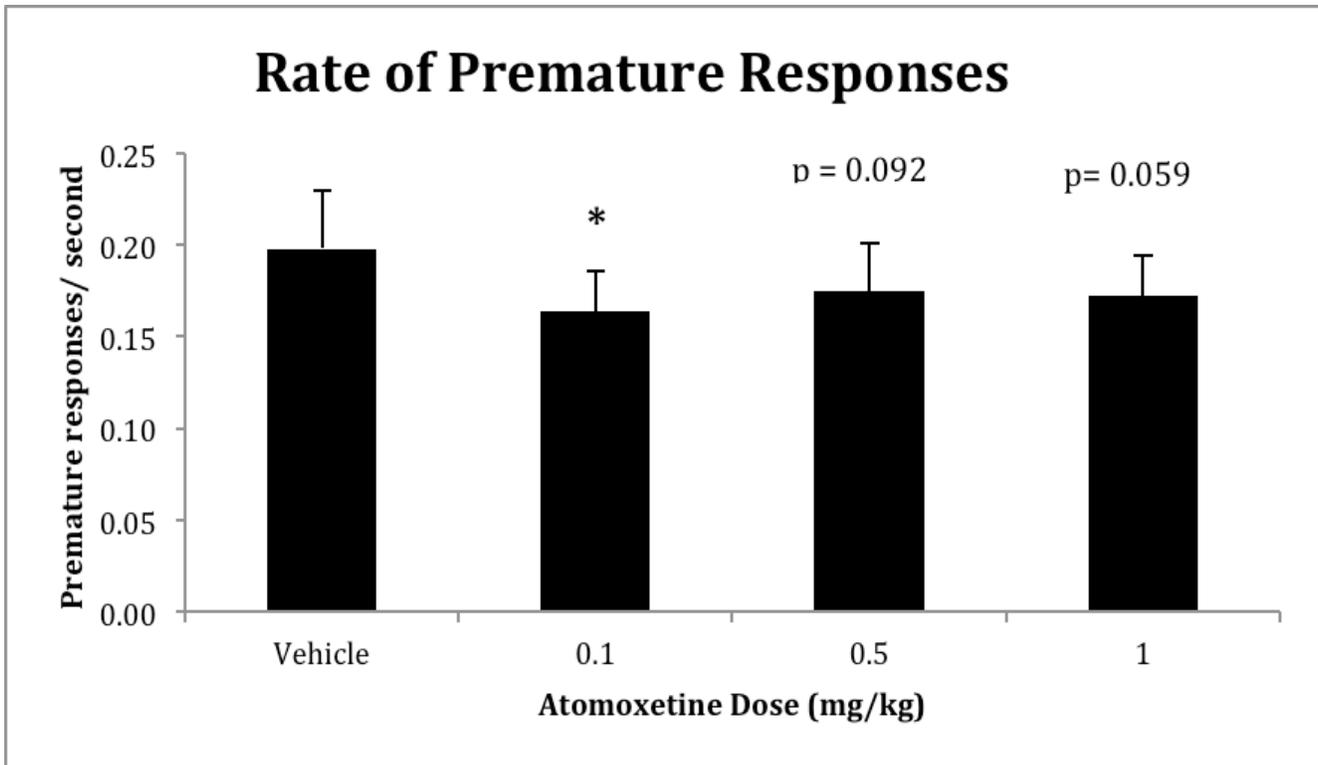
**Fig 2:** The DevMode value, in milliseconds, [ $\pm$  standard error of the mean (SEM)] at various doses of atomoxetine include: vehicle treatment = 145.96 ( $\pm$  30.45); 0.1 mg/kg ATX = 143.78( $\pm$  27.05); 0.5 mg/kg ATX = 112.03 ( $\pm$  29.13); 1.0 mg/kg ATX = 95.74 ( $\pm$  17.22). \* = Significant effect of ATX compared to vehicle.



**Fig 3:** The total number of correct omissions [ $\pm$  standard error of the mean (SEM)] at various doses of atomoxetine include: vehicle treatment = 5.75 ( $\pm$  1.63); 0.1 mg/kg ATX = 5.17 ( $\pm$  1.62); 0.5 mg/kg ATX = 4.58 ( $\pm$  1.14); 1.0 mg/kg ATX = 5.83 ( $\pm$  1.71).



**Fig 4:** The rate of premature initiations (per second), [ $\pm$  standard error of the mean (SEM)] at various doses of atomoxetine include: vehicle treatment = .80 ( $\pm$  0.10); 0.1 mg/kg ATX = 0.74( $\pm$  0.11); 0.5 mg/kg ATX = 0.67 ( $\pm$  0.08); 1.0 mg/kg ATX = 0.70 ( $\pm$  0.10).



**Fig 5:** The rate of premature responses (per second), [ $\pm$  standard error of the mean (SEM)] at various doses of atomoxetine include: vehicle treatment = .20 ( $\pm$  0.03); 0.1 mg/kg ATX = 0.16( $\pm$  0.02); 0.5 mg/kg ATX = 0.17 ( $\pm$  0.03); 1.0 mg/kg ATX = 0.17 ( $\pm$  0.02). \* = Significant effect of ATX compared to vehicle.

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