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The Effects of Antidepressant Drugs on the Schedule-Induced Polydipsia

By

Sean Mooney-Leber

THESIS

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In partial fulfillment of the requirements  
For the degree of

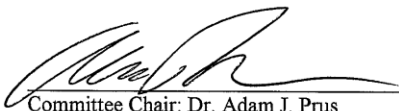
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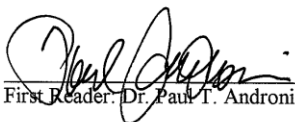
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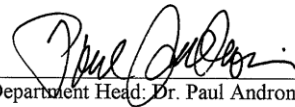
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## ABSTRACT

### THE EFFECTS OF ANTIDEPRESSANT DRUGS ON SCHEDULE-INDUCED POLYDIPSIA

By

Sean Mooney-Leber

Anxiety disorders are characterized by excessive fear about future uncertainties and can interfere with functioning. Among current medications, selective serotonin reuptake inhibitors (SSRI) and serotonin norepinephrine reuptake inhibitors (SNRI) have shown high levels of efficacy in the treatment of various anxiety disorders. Although these treatments appear to be effective in ameliorating symptoms associated with anxiety, the therapeutic onset of action with these drugs are delayed and thus presents a significant problem with producing immediate effects. To address this issue valid animal models are necessary. However, there is no current animal model that provides a measurement of the onset of these compounds. One putative model that has been suggested to measure the delay in these compounds is the schedule-induced polydipsia animal paradigm. The present study has sought to further characterize the effects of antidepressant drugs on schedule-induced polydipsia through the use of fluoxetine (SSRI) and duloxetine (SNRI). Fluoxetine and duloxetine both produced a robust decrease in water consumption in a time sensitive manner. Furthermore, the present findings add to previous literature suggesting that schedule-induced polydipsia is a valid animal model for measuring the onset of antidepressant drugs.

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Sean Mooney-Leber

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## LIST OF ABBREVIATIONS

5-HT: Serotonin

## **Introduction**

### *Anxiety*

Due to the rising prevalence rates of certain disorders, mental health has become a major concern in the United States. Currently, the lifetime prevalence rate for any mental disorder is approximately 46%. When looking at the lifetime prevalence by disorder, approximately 28% have an anxiety disorder (Kessler et al., 2005). Similar results are found when looking at a 12-month period of time as opposed to a lifetime (Kessler et al., 2012). Due to the large amount of people afflicted by these disorders extensive research has been conducted to develop effective pharmacotherapeutic approaches.

The Diagnostic and Statistical Manual of Mental Disorders 4<sup>th</sup> Edition Text Revision, provides classifications for anxiety and other mood disorders. The current disorders listed under anxiety disorders are as follows: panic attack, agoraphobia, panic disorder without agoraphobia, agoraphobia without history of panic disorder, specific phobia, social phobia, obsessive-compulsive disorder, posttraumatic stress disorder, acute stress disorder, and generalized anxiety disorder (DSM-IV-TR). The symptoms that accompany anxiety disorders tend to be marked by heightened levels of anxiety due to certain stimuli and environments. This level of anxiety can persist over an extended period of time.

As mentioned above, these disorders affect a high number of individuals in the United States. Unlike other disorders there is not a specific population of

individuals who are affected by anxiety disorders. The typical onset of anxiety disorders is 11 years of age but can be seen in a wide range of ages (Kessler et al., 2005). However, there is a significantly higher rate of anxiety disorders present in female populations. These results tend to remain consistent in younger populations (Kessler et al., 2012).

Anxiety disorders can be debilitating and negatively impact one's life. A study conducted by Wittchen, Carter, Montgomery, and Kessler (2000) found that individuals with generalized anxiety disorder tend to have a higher number of days impaired within a month, higher levels of reduced activity, and higher self reports of bodily pain.

### *Neurobiology*

The exact pathology of anxiety disorders has yet to be discovered. However, certain structures of the limbic system appear to be affected due to this illness. The use of neuroimaging has given researchers the ability to investigate neuroanatomical differences in certain individuals. Bellis et al. (2000) found that children with generalized anxiety disorder had a significantly larger amygdala when compared to control groups. However, they did not find any alterations in the volume of the hippocampus. In individuals experiencing post-traumatic stress disorder, magnetic resonance imaging results have found an overall decrease in hippocampus volume when compared to controls (Letizia et al., 2008). Finally, through the use of functional magnetic resonance imaging techniques, research has shown that individuals with social phobia display heightened levels of activity in the amygdala

bilaterally when presented with pictures of neutral faces (Birbaumer et al., 1998).

Despite the inconsistent results from previous neuroimaging studies, recent research suggests there is an inverse correlation between hippocampal and amygdalar size in the presence of an anxiety disorder.

One possible explanation for the differences in neurological anatomy between the hippocampus and amygdala in individuals suffering from anxiety disorders is the monoamine hypothesis. This premise explains anxiety disorders as a diminishment of monoamine neurotransmitters, particularly norepinephrine and serotonin, in the central nervous system. The monoamine hypothesis was derived from the discovery of early antidepressant drugs that enhanced levels of monoamines, and is still supported by the actions of modern antidepressant drugs (for review see Schildkraut, 1995)

### *Treatments*

Individuals suffering from anxiety have experienced relief from their symptoms through taking pharmacological treatments. The first treatments produced to ameliorate symptoms of anxiety disorders were barbiturates. These compounds produced their therapeutic effect by modulating GABA receptor binding in the brain. Although they do not bind to the GABA receptor site, barbiturates facilitate and potentiate the binding of GABA to its receptor through an allosteric site (for review see Olsen, 1981). This produces a sedative-like feeling that can help alleviate symptoms of anxiety. Although these compounds display efficacy, they produced cognitive impairments and the body over time builds a tolerance which would require

higher doses. Additionally, due to the mechanism by which these compounds exert their effects, there is a concern for their abuse potential (Connell, 1976).

With pharmacological advancements, a new agent emerged as a potential treatment for anxiety disorders. The compounds were called “benzodiazepines.” Similar to barbiturates, benzodiazepines produced their effect by facilitating GABA binding at receptors (for review see Olsen, 1981). This increase in GABA binding leads to sedative effects which produces anxiolytic effects. Although these compounds tend to be much safer than barbiturates, they still display drowsiness, mental confusion, motor inhibition, and cognitive impairments (for review see Lader, 2008).

Due to the adverse effects produced by both barbiturates and benzodiazepines there was a need for newer anxiolytic compounds. One of the first of these compounds was buspirone. Buspirone, a 5-HT<sub>1A</sub> receptor antagonist produced its effect through a completely different mechanism than its predecessors. This change in mechanisms did not produce the side effects associated with GABA receptor enhancement, but it still produced alleviation of symptoms that accompany anxiety disorders (Taylor, 1988)

With the efficacy of buspirone new compounds were produced that were centered around the augmentation of serotonin. These modern antidepressants fall under the classes of selective serotonin reuptake inhibitors and serotonin norepinephrine reuptake inhibitors. These compounds block the reuptake of serotonin or both serotonin and norepinephrine, respectively. Today, most antidepressant drug prescriptions for treating anxiety tend to be either selective serotonin reuptake

inhibitors or serotonin norepinephrine reuptake inhibitors. The prototype for selective serotonin reuptake inhibitors is fluoxetine (Prozac) and the prototype for serotonin norepinephrine reuptake inhibitors is duloxetine (Cymbalta). Fluoxetine was developed by Eli Lilly and Company in 1974 and was not available until 1987 when it was approved for distribution by the U.S. Food and Drug Administration. Due to its high levels of efficacy in clinical trials fluoxetine is one of the most prescribed antidepressants to date. Similarly, duloxetine was also developed by Eli Lilly and Company, but at a much later date in 1986. Duloxetine was approved for distribution in 2001 by the U.S. Food and Drug Administration.

## **Comparisons Between Fluoxetine and Duloxetine**

### *Physiological Changes*

Fluoxetine displays a robust effect by inhibiting serotonin reuptake into the presynaptic cell, while displaying no effect on the reuptake of norepinephrine or dopamine (Fuller, 1994, Harms, 1983; Wong et al., 1974). Wong et al. (1985) found that fluoxetine did not display any binding affinity for  $\alpha_1$ ,  $\alpha_2$ , or  $\beta$  adrenoceptors or any binding affinity for histamine  $H_1$  or opioid receptors. Bymaster et al. (2001) found that duloxetine inhibited the reuptake of both radiolabeled serotonin and norepinephrine, although greater inhibition occurred for serotonin. Furthermore duloxetine has been shown to have virtually no affinity for cholinergic receptors or found to inhibit monoamine oxidase (Bymaster et al., 2001; Koch et al. 2003; Wong

et al., 1993). Gould et al. (2007) reported that duloxetine significantly inhibited serotonin reuptake but failed to find inhibition of norepinephrine reuptake.

Beyond the effects of these drugs on serotonin and norepinephrine transporters, microdialysis techniques have been used to assess synaptic overflow of serotonin or dopamine. Using these techniques in rodents, acute administration of 10 or 20 mg/kg fluoxetine resulted in significant increases of extracellular serotonin in the frontal cortex, ventral hippocampus, and raphe nuclei (Malagie et al., 1995). These results are not found after administration of 1.0 mg/kg fluoxetine. Conversely, Beyer et al. (2002) indicated no increase in extracellular serotonin in the rat frontal cortex following acute administration of 30.0 mg/kg of fluoxetine. They also reported no change in extracellular levels of norepinephrine following the same dose.

Muneoka et al. (2009) investigated the effects of duloxetine on tissue from the medial prefrontal cortex, dorsal later frontal cortex, hippocampus, nucleus accumbens, caudate putamen, substantia nigra, ventral tegmental area, hypothalamus, midbrain, pons-medulla, and cerebellum of Sprague-Dawley rats. They found that duloxetine, at a 20.0 mg/kg dose, significantly increased extracellular serotonin concentrations in the dorsal lateral prefrontal cortex, hippocampus, nucleus accumbens, hypothalamus, and midbrain when compared to vehicle. Duloxetine only increased norepinephrine in the nucleus accumbens. Duloxetine, 15.0 mg/kg, has also been reported to increase serotonin and norepinephrine concentrations in the hypothalamus and prefrontal cortex (Englman et al., 1995; Koch et al., 2003).

Finally, electrophysiological methods have been used to investigate the effects of fluoxetine and duloxetine on cell firing. Dorsal raphe nucleus neurons have been



shown to be completely suppressed after administration of 10.0 mg/kg fluoxetine and partially suppressed after administration of 5.0 mg/kg of fluoxetine. These results were not seen in CA1 and CA3 pyramidal neurons in the hippocampus (Smith & Lakoski, 1997). Furthermore, chronic administration of a 10.0 mg/kg fluoxetine dose has been shown to suppress prefrontal cortical cell firing. This suppression was not seen after acute fluoxetine administration (Gronier & Rasmussen, 2003). In the dorsal raphe nucleus, duloxetine has been shown to suppress cell firing at a lower dose (1.4 mg/kg) than needed for fluoxetine (Smith & Lakoski, 1997). Moreover, chronic administration of duloxetine, at a 20 mg/kg dose, has failed to suppress cell firing in the dorsal raphe nucleus (Rueter, Montigny, and Blier, 1998).

### *Clinical Trials*

The efficacy of fluoxetine and duloxetine has been tested extensively in clinical trials, with both compounds showing efficacy for anxiety disorders. In a study conducted by Jenike et al. (1997) fluoxetine was found to significantly reduce the symptoms of obsessive-compulsive disorder. Similar results have been documented in children with obsessive-compulsive disorder (Riddle et al., 1992). Conversely, a positive result was found with the use of fluoxetine in the treatment of obsessive-compulsive disorder (Jenike, Baer, Minichiello, Rauch, and Buttolph, 1997). Additionally, fluoxetine has also ameliorated symptoms of panic disorder and post-traumatic stress disorder (Amore et al., 1999; Connor, Sutherland, Tupler, Malik, & Davidson, 1999; Martenyi, Brown, Zhang, Prakash, & Stephanine, 2002). In children and adolescents with mixed anxiety disorders, fluoxetine displayed efficacy in

separation anxiety disorder, social phobia, specific phobia, and panic disorder, but not in generalized anxiety disorder (Fairbanks et al., 1997).

With the efficacy displayed by fluoxetine for treatment of both mood and anxiety disorders, researchers have investigated the potential use of fluoxetine in individuals suffering from depression with comorbid anxiety. For these patients, fluoxetine provides significant improvements in both depressive and anxiety derived symptoms. The findings from these clinical trials indicated that fluoxetine is an effective treatment for mood and anxiety disorders (Sonawalla et al., 2002).

Clinical trials also provide documentation of various adverse effects produced through the use of fluoxetine. The most commonly reported adverse effects in these studies were as follows: nausea, dry mouth, fatigue, sexual side effects, excessive sweating, sedation, insomnia, anorexia, drowsiness, and nervousness (Amore et al., 1999; Bremner, 1984; Cohn, & Wilcox, 1985; Feighner, 1985; Fairbanks, 1997; Riddle et al., 1992; Wernicke et al., 1987). Although fluoxetine provides a risk for the symptoms, fluoxetine is generally considered well tolerated.

Duloxetine has shown similar results to fluoxetine in clinical trials for anxiety disorders. In patients with generalized anxiety disorder, duloxetine administered at 20, 60, and 120 mg per day displayed clinically significant reductions in anxiety after 1-2 weeks (Hartford et al., 2007; Kopene et al., 2007; Nicolni et al., 2008; Rynn et al., 2008). Additionally, a study conducted by Davidson et al. (2008) found reductions in anxiety occurring 1-2 weeks with 60 or 120 mg per day of duloxetine and that these reductions continued during long term administration, which was continued over 50 weeks. Reductions in anxiety symptoms occurred after 1-2 weeks of administration

and these effects remained throughout the 50 week study. Due to the comorbid nature of mood and anxiety disorders, duloxetine has also been investigated for alleviating anxiety symptoms that accompany major depression. Dunner et al. (2003) found that daily administration of 60 mg of duloxetine per day reduced anxiety symptoms in patients with major depressive disorder and comorbid anxiety.

The most commonly reported adverse effects for duloxetine during clinical testing were nausea, drowsiness, headache, dry mouth, diarrhea, dizziness, constipation, fatigue, decreased libido, insomnia, hyperhidrosis, and anorexia (Berk, du Plessis, Birkett, & Richardt, 1997; Dunner et al., 2003; Nicolini et al., 2008; Koponen et al., 2007; Perahia et al., 2006; Rynn et al., 2008). Like fluoxetine, duloxetine is considered to be well tolerated by patients.

Although current medications have been shown to be effective in the treatment of anxiety disorders there is still an issue concerning onset. Both fluoxetine and duloxetine display a lag in their onset of therapeutic effect. That is these compounds typically require 2-4 weeks of continuous administration to display efficacy in the alleviation of anxiety disorder symptoms. During this lag individuals may stop taking their medication. In the progression of antidepressant medications this lag is an issue that must be addressed.

### **Preclinical Models**

There are a number of preclinical animal models used for screening experimental antidepressant drugs. One of the most widely used models is the forced

swim test. In these model animals, usually rats or mice, are placed in a container of water and are measured for the length of time they swim versus assuming a passive behavior where the animals emit only minimal movements necessary to keep their heads above water. In this paradigm antidepressants will increase the duration of time an animal spends actively swimming. A similar paradigm to the forced swimming paradigm is the tail suspension test. In this model, an animal is suspended by its tail and the time spent immobile is measured. Administration of antidepressant drugs will decrease the amount of time spent immobile in this test (Nestler et al., 2002).

Another model used to produce anxiety-like behavior is the chronic stress model. In this paradigm animals are exposed to lengthy periods of time with various stressors such as isolated housing, tilted home cages, or disrupted light-dark cycles, in addition to brief periods of time of food or water deprivation. Although this model produces high levels of stress, it is very hard to measure the long term efficacy of antidepressants while using it because they only provide the acute effects (Nestler et al., 2002).

Aside from the models listed above, there are several paradigms that use the animals' exploratory behavior as an indicator of stress or anxiety. These models include the elevated plus maze, zero maze, and the dark/light box. In these paradigms animals are placed in apparatuses that have areas that would be considered safe (closed areas) and areas that produce anxiety, such as the elevated open arms in the elevated plus maze. When antidepressants are introduced, animals tend to spend more time exploring the open areas (Deussing, 2006).

The preclinical animal models described above are responsive to the acute effects of antidepressant drugs. However, these models may not be ideal for assessing the delayed onset effects of antidepressant drugs for treating anxiety. One model that has been proposed to measure and provide an index for the onset of antidepressants is the schedule-induced polydipsia model. This model has been shown to be time sensitive to current antidepressant drugs such as fluoxetine by measuring the decrease in water consumption of animals participating in the task (Hogg & Dalvi, 2004).

### **Schedule-Induced Polydipsia**

Schedule-induced polydipsia is a phenomenon in which a non-water deprived organism consumes excessive amounts of water as a result of a contingency for an unrelated behavioral process. Typically, training for the production of this behavior consists of food restricting an animal to 80% -90% of their free-feeding bodyweight and then presenting a behaviorally contingent or non-contingent schedule of food delivery in an apparatus that is equipped with a water bottle. Over repeated training sessions, animals will exhibit excessive water consumption during times in between food pellet access or delivery.

This form of "adjunctive" behavior was first reported by John Falk in 1961 (Falk, 1961). In his initial experiment Falk employed a variable interval 60 second reinforcement schedule, in which the rats received a food pellet after pressing a lever after a non-signaled average of 60 seconds. The session time was 3.17 hours. Falk

found that the animals' average water intake was 3.43 times higher than their 24 hour pre-experimental amount of water intake in their home cages. Also the rats displayed their peak amount of drinking immediately after consuming a pellet. This behavior has been documented in various species such as monkeys, mice, and humans (Mittle, Van Brunt, & Matthews, 2003; Grant, Leng, Green, Szeliga, Rogers, & Gonzales, 2008; Porter, Brown, & Goldsmith, 1982).

After the discovery of schedule-induced polydipsia, researchers started examining variations in these methodological procedures. In a study conducted by Falk (1966), rats were trained on a variable interval schedule for pellet delivery but were also required to respond through a fixed ratio schedule for access to water. He found that rats still developed schedule-induced polydipsia even when water was not freely available. In this particular study only two rats were used, but both maintained polydipsic-like behaviors up to a fixed ratio of 20 for access to water. One rat maintained high levels of water consumption until reaching a fixed ratio of 50.

Falk also assessed whether the type of reinforcer would have an impact on the amount of water consumed in schedule-induced polydipsia. Liquid reinforcers used in this study were SKF (liquid standard monkey diet), metrecal, and 30% sucrose. Pellet reinforcers used were lab rat pellets, dextrose pellets, and sucrose pellets. Results showed that all pellets used produced polydipsia and only SKF from the liquids produced polydipsia. Additionally, the smaller amount of SKF used as a reinforcer was correlated with higher rates of water consumption (Falk, 1967).

As mentioned above, rats trained on schedule-induced polydipsia tend to drink the most immediately after consumption of their pellet with water intake decreasing

throughout the rest of the inter-pellet interval. This pattern of licking was manipulated in a study by only granting access to water during certain times of the inter-pellet delivery. Rats were placed into separate groups and were habituated to the testing apparatus. Rats in the first group were only allowed access to water during the first 15 seconds of the 30 second fixed time interval. The second group was only allowed access to water during the second 15 seconds of the 30 second fixed time interval. The third group was allowed access throughout the whole 30 seconds and the fourth group was only tested on a 15 second time interval with free access to water throughout the interval. Results showed that rats allowed access to water during the full 30 seconds consumed the highest amount of water. The first group displayed the second highest intake of water followed by the third group and then the fourth group. However the only significant finding between the groups was observed when comparing the full 30 second group to the rest of the groups. These results were similar when using this methodology in a fixed time interval of 90 seconds (Lopez-Crespo, Rodriguez, Pellon, and Flores, 2004).

The duration of a fixed-time pellet delivery can also have an impact on water consumption and licks made. In a study conducted by Patterson & Boakes (2012), rats were presented with a 30, 60, 120, or 240 second fixed time interval. They found that rats trained on the shortest fixed-time interval displayed the highest amount of water consumption and rats trained on the longest time interval displayed the lowest amount of water consumption. Moreover, when these rats were all placed on a fixed-time interval of 120 seconds, rats previously trained on the 30 second fixed-time interval displayed the highest amount of water consumed.

### *Neurobiological*

The formation and maintenance of adjunctive behavior has been studied from a neurobiological standpoint. The two biological mechanisms implicated in research on schedule-induced polydipsia are the pituitary-adrenal axis and the limbic system. Mittleman, Blaha, and Philips (1992) investigated the influence of the pituitary-adrenal axis in adrenalectomized rats or in rats administered the cortisol inhibitor metyrapone. Animals who underwent an adrenalectomy or received metyrapone 25 or 50 mg/kg i.p. consumed significantly less water during training. Moreover, rats who received corticosterone during schedule-induced polydipsia training also displayed a decrease in water consumption. When corticosterone was abstracted through adrenalectomy in rats that had developed schedule-induced polydipsia, there was a decrease in water consumption compared to their pre-adrenalectomy levels. Finally, rats given corticosterone who were trained on schedule-induced polydipsia displayed an increase in water consumption. Furthermore, Brett and Levine (1979) investigated the effects of schedule-induced polydipsia on endogenous corticosterone in rats. Their results found that corticosterone levels were significantly lower following a training session in schedule-induced polydipsia in comparison to home cage and pre-session drinking levels.

Although it seems that the pituitary-adrenal axis plays a large role in the acquisition and modulation of schedule-induced polydipsia, similar findings have been documented in studies investigating structures of the limbic system. In the acquisition of polydipsia, it has been shown that lesions to the hippocampus results in



an increase in water consumption. This rapid acquisition, however, does not lead to overall higher levels of water consumption compared to baseline (Davenport, 1978). Conversely, a full hippocampectomy has been shown to lead to decreased water consumption, licks, and efficiency in licks. Similar results are seen in rats with bilateral lesions to the nucleus accumbens. Bilateral lesions to the caudate putamen result in an increase in licking but no change in the amount of water consumed, which indicated a lower level of licking efficacy (Mittleman et al., 1990). Although the locus coeruleus is not part of the limbic system, it projects to many areas within the limbic system and its influence on schedule-induced has been investigated. Rats with a lesioned locus coeruleus have decreased water consumption and licks when compared to control rats. This decrease is larger when subsequent lesions are made in the ventral tegmental area (Lu et al., 1992).

#### *Pharmacological Aspects of Schedule-Induced Polydipsia*

The maintenance of schedule-induced polydipsia is effected by compounds used to treat anxiety disorders. The common effect of these compounds is a gradual decrease in water consumption (Woods et al., 1993; Loullis, 1979; Roehner, 1995). Additionally, previous research has noted saline administration alone has no effect on water consumption (Porter, Young, & Moeschl, 1978). This effect makes the use of schedule-induced polydipsia a putative model for screening experimental pharmacotherapies for mood and anxiety disorders.

Mittleman, Jones, and Robbins (1988) investigated the effects of the benzodiazepine diazepam on schedule-induced polydipsia. They found a decrease in

water consumption, panel pressing for food, and locomotor activity in animals administered diazepam. Conversely, an increase in water consumption was found with the use of the benzodiazepine clordiazepoxide (Barret & Weinberg, 1975). In a study conducted by Hogg and Dalvi (2004) the use of the selective serotonin reuptake inhibitor fluoxetine in combination with either a 5-HT<sub>1A</sub> or 5-HT<sub>1B</sub> receptor antagonist was employed to investigate the number of sessions necessary for reductions in water consumption to occur. Fluoxetine, at a 27 mg/kg dose given p.o., significantly decreased water consumption during the sixth daily session. Fluoxetine at the same dose in combination with the 5-HT<sub>1A</sub> antagonist WAY-100635, at a 0.52 mg/kg dose given s.c., produced a significant decrease in water consumption during the first session. These results were similar to fluoxetine in combination with the 5-HT<sub>1B</sub> receptor antagonist GR-127935 at a 4.5 mg/kg dose given s.c. Neither WAY-100635 nor GR-127935 alone had any effect on water consumption. Furthermore, WAY-211612, a selective serotonin reuptake inhibitor and 5-HT<sub>1A</sub> antagonist, have been studied in this model. WAY-211612, intraperitoneally administered at a 56 mg/kg dose, produced a significant decrease in water consumption when compared to baseline on the first session. Additionally, a trend in decreased water consumption was found after administration of WAY-211612, at a 30 mg/kg dose given i.p. (Beyer et al., 2009). These results are comparable to the fluoxetine in combination with WAY-100635 reported earlier.

Aside from the 5-HT<sub>1</sub> receptor sub family a role for 5-HT<sub>2</sub> receptors in the regulation of schedule-induced polydipsia has been suggested. Rosenzweig-Lipson et al. (2007) found that modulation of the 5-HT<sub>2C</sub> receptor has an impact on schedule-

induced polydipsia behavior. Administration of WAY-163409, a 5-HT<sub>2C</sub> receptor agonist, significantly decreased water consumption after administration of a 3.0 or 5.6 mg/kg dose. This decrease was negated by the administration of either the 5-HT<sub>2C</sub> antagonist SB-206553 or SB-242084. Finally, no effects on water consumption were produced by WAY-163409 when SB-215505, a 5-HT<sub>2B</sub> antagonist, was co-administered. These results are similar to other studies measuring the effects of 5-HT<sub>2C</sub> agonists and antagonists (Martin, Ballard, & Higgins, 2002; Martin et al., 1998).

The effects that antidepressants have on reducing schedule-induced polydipsia is consistent and robust. As mentioned above the decrease in water consumption produced by antidepressant drugs happens over a number of test sessions. The lag in onset of therapeutic effects is one of the major issues with antidepressant treatment. The research described above suggests that this onset can be accelerated and delayed with alterations to various serotonergic receptors. Furthermore, schedule-induced polydipsia has been proposed as a model to test the onset of novel antidepressant compounds.

Although various classes of antidepressant compounds have been investigated in the schedule-induced polydipsia procedure, not all have been tested. Further research is needed to validate this model as a tool to measure the onset of effect for antidepressant drugs. One class of antidepressants that has not been investigated using the schedule-induced polydipsia animal model is the serotonin norepinephrine reuptake inhibitors. When looking at past research with antidepressants and schedule-induced polydipsia one would expect a serotonin norepinephrine reuptake inhibitor to

respond in the same time sensitive manner by producing a reduction in schedule-induced polydipsia.

## RATIONALE

The schedule-induced animal model appears to be a putative model for investigating novel antidepressant drugs, based on previous literature. Schedule-induced polydipsia produces a robust increase in the amount of water consumed during a session, which is sensitive to various antidepressant drugs and modulation of receptors involved in the effects of novel antidepressant drugs. The selective serotonin reuptake inhibitor fluoxetine has been shown to decrease water consumption in this model in a time-sensitive manner (Hogg & Dalvi, 2004). Moreover, the lag in decreased water consumption is decreased by 5-HT<sub>1</sub> antagonist administration (Beyer et al., 2009).

No previous studies have investigated the effects of an serotonin norepinephrine reuptake inhibitor on the schedule-induced polydipsia animal model. In order to evaluate the effects of an serotonin norepinephrine reuptake inhibitor, duloxetine, was tested in rats acclimated to the schedule-induced polydipsia animal model. The goal of this study was to assess the ability of duloxetine to decrease water consumption and replicate previous findings that the selective serotonin reuptake inhibitor fluoxetine decreases water consumption in this paradigm.

## METHODS

### *Subjects*

The subjects were male Sprague Dawley rats which were obtained from Charles River Laboratories (Portage, MI, USA) and housed 3 per cage (type of cage) for at least 1 month before schedule-induced polydipsia training. All animals were housed in a temperature- and humidity-controlled environment, which was kept on a 12 hour light/dark cycle. Food administration was limited to once a day to maintain 85% of free-feed bodyweights. Animals had free access to water in their home cages at all times. All procedures were consistent with the Guide for the Care and Use of Laboratory Animals (2011) and were approved by the Institutional Animal Care and Use Committee at Northern Michigan University.

### *Apparatus*

This study used eight rat operant chambers enclosed in sound-attenuating cabinets equipped with fans for ventilation and masking noise (Med-Associates, St. Albans, VT, USA). Each chamber had a lickometer, which was connected to the metal spout water bottle containing 100ml of tap water (Company - Location). A stainless steel ball located at the end of the spout prevented excess water spillage. A food trough for the food pellet dispenser was located to the left of the water bottle and delivered 45 mg dustless food pellets (BioServ, Frenchtown, NJ, USA). This wall also contained a house light, located near the ceiling. All experimental events and

data collection were performed using Med-PC Version IV software (Med-Associates).

### *Drugs*

Doses, pretreatment times, and routes of administration of fluoxetine were chosen on the basis of previous literature (Hogg and Dalvi, 2004). Both fluoxetine hydrochloride and duloxetine hydrochloride were dissolved in physiological saline (0.9%) and were prepared fresh every day. Fluoxetine and duloxetine were administered via oral gavage 60 minutes in a 6 ml/kg volume. The salt form of the drugs was used.

### *Procedure*

A fixed-time 60 second schedule for food pellet delivery was used for each 50 minute session. Training sessions were conducted daily (5-7 days per week). Sessions were terminated once the animal had developed a stabilized level of water intake, as determined when a rat exhibited less than ten percent variation in water consumption over three consecutive sessions. Once this criterion was met, rats were assigned to one of three treatment groups (fluoxetine, duloxetine, and saline) in a counterbalanced order according to polydipsic drinking levels.

Test sessions were conducted during the course of 14 daily, consecutive sessions. During the first 7 days, rats were given an administration of drug or saline, depending on the group a rat belonged to. During the final 7 days, all rats were given daily administrations of saline. After completing these sessions, a mass feeding

session was administered in which animals received all 50 pellets upon start of the sessions, but received no pellets during the session.

### *Data Analysis*

The dependent variables measured were total water consumption, total amount of licks, and number of licks made in five 12-second bins in between food pellet deliveries. Total water consumption and total number of licks were reported as means (+/- the standard error of the mean [SEM]). During the minute that preceded each pellet delivery, the number of licks occurring were collected in five 12-second bins and then the mean for each bin for each animal and session was calculated.

Using these means for each bin, an index of curvature was calculated to determine the curvature for the frequency of licks occurring between each food pellet. The index of curvature was calculated by

$$IC = \frac{4(Bin1 + Bin2 + Bin3 + Bin4 + Bin5) - 2(4 \cdot Bin1 + 3 \cdot Bin2 + 2 \cdot Bin3 + Bin4)}{5(Bin1 + Bin2 + Bin3 + Bin4 + Bin5)}$$

(Fry, Kelleher, & Cook, 1960). Index of curvature values are expressed as means (+/- SEM). Pretreatment baseline values for water consumption, total licks, and index of curvatures were calculated as a mean of these values from the three training session's immediately preceding drug or saline administration. A one-way repeated measures analysis of variance (ANOVA) was conducted to assess water consumption, total licks during the days of drug (or saline) treatment and baseline. Another repeated measures ANOVA was conducted to assess water consumption or total licks during the wash-out days and baseline. Statistically significant differences were further analyzed using a Dunnett's post hoc test to compare treatment days to baseline. A



paired samples t test was used to assess differences in mass feeding session water consumption, total licks, and index of curvature from baseline. All analyses were conducted using GraphPad Prism for Windows version 6 (GraphPad Software, La Jolla, CA, USA).

## RESULTS

Acquisition of schedule induced polydipsia was found in all rats ( $n=24$ ) after 12.4 ( $\pm 0.9$ ) training sessions. Training continued until level of water consumption was stable.

### *Repeated saline administration*

Figure 1 presents the percentage of water consumed, compared to baseline, during each session over seven consecutive sessions. Saline administration did not significantly affect water consumption compared to baseline drinking amounts,  $F(7, 49) = 1.16, p > 0.05$ .

Figure 2 presents the percentage of water consumed, compared to baseline, during the washout period over seven consecutive sessions. Washout administration did not significantly affect water consumption compared to baseline water consumption,  $F(7, 35) = .789, p > 0.05$ .

Figure 3 presents the percentage of licks made, compared to baseline, during each session over seven consecutive sessions. Saline administration did not significantly affect the amount of licks made compared to baseline licking amounts,  $F(7, 49) = 2.112, p > 0.05$ .

Figure 4 presents the percentage of licks made, compared to baseline, during the washout period over seven consecutive sessions. Washout administration did not

significantly affect licks made compared to baseline amount of licks made,  $F(7, 35)=0.446$ ,  $p > 0.05$ .

Figure 5 presents index of curvature during each session over seven consecutive sessions. Saline administration did significantly decrease in index of curvature compared to baseline index of curvature,  $F(7, 49)= 2.88$ ,  $p < 0.025$ . Post hoc test revealed a significant decrease in the index of curvature, on day 6.

Figure 6 presents the index of curvature during the washout period over seven consecutive sessions. Washout administration did not significantly affect licks made compared to baseline amount of licks made,  $F(7, 35)= 1.407$ ,  $p > 0.05$ .

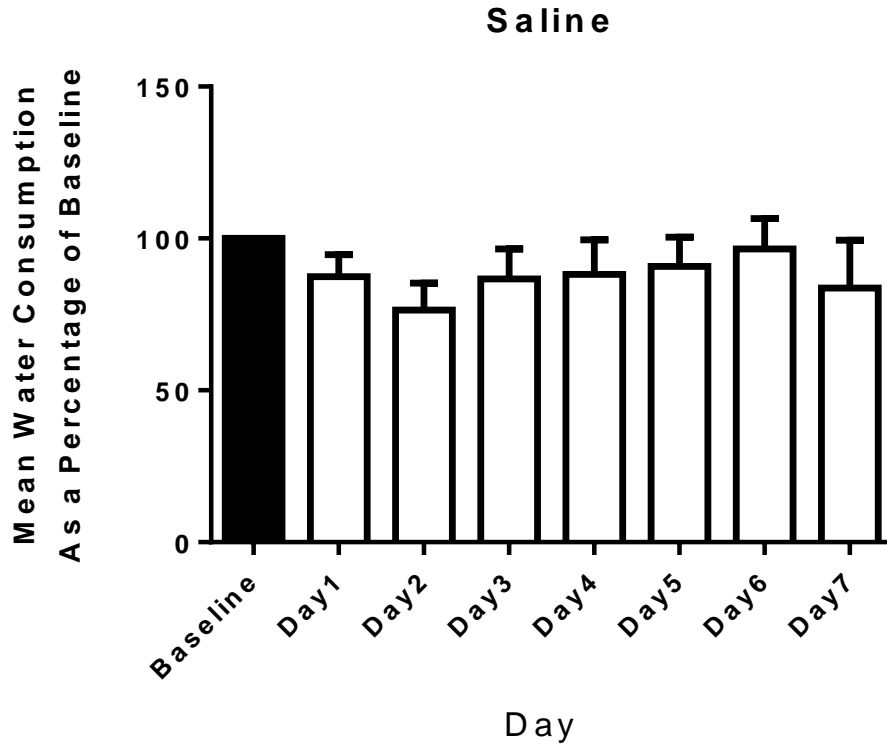


Figure 1 shows the amount of water consumed (in the form of a percentage of baseline) as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. Baseline data is the mean of the three prior sessions to drug testing.

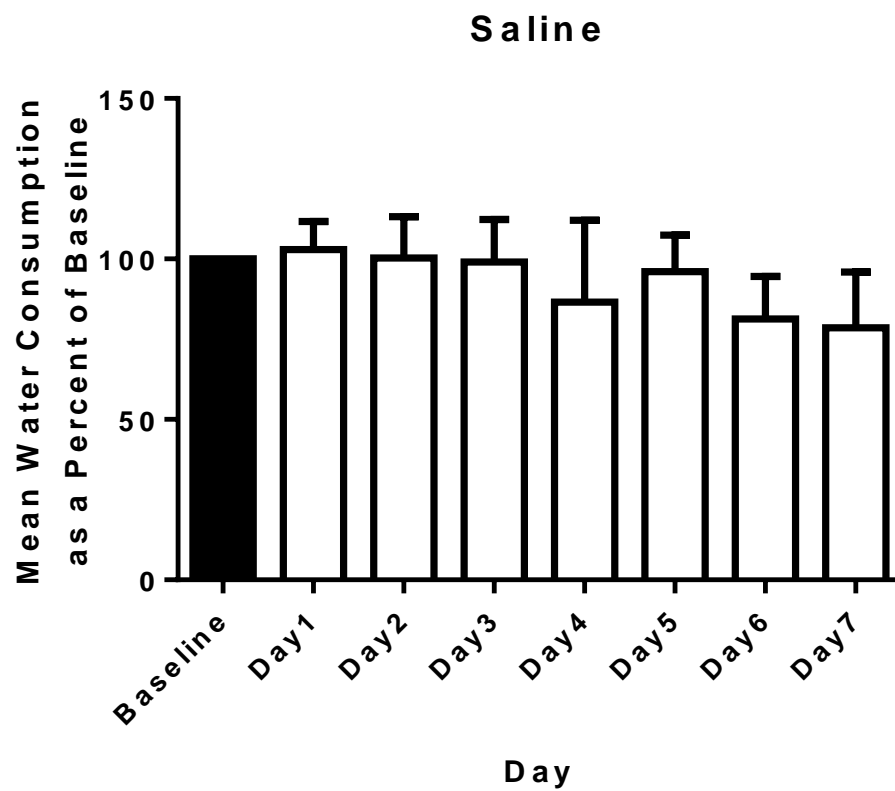


Figure 2 shows the amount of water consumed during washout (in the form of a percentage of baseline) as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

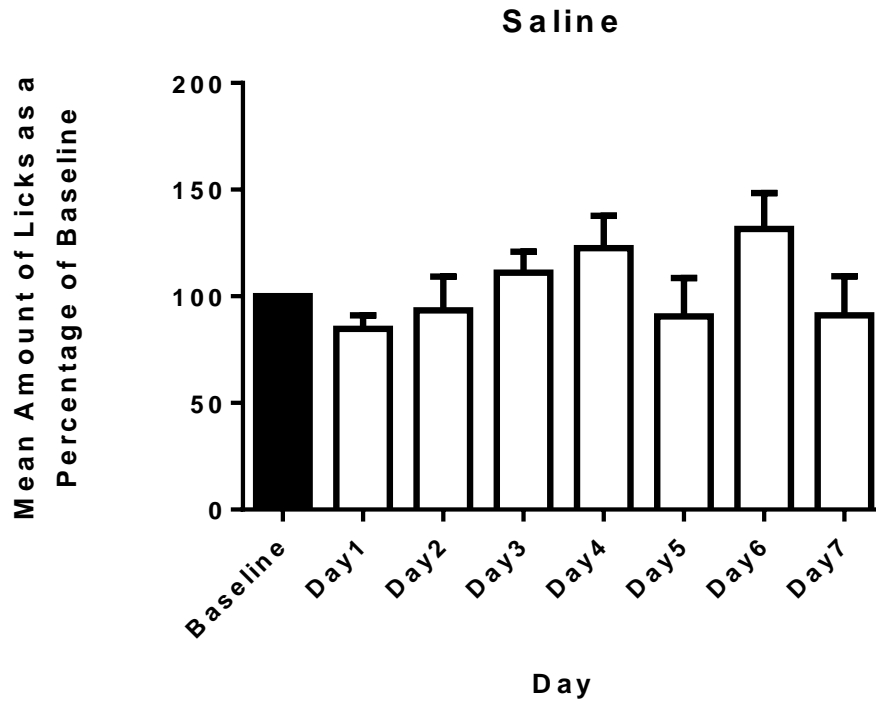


Figure 3 shows the amount of licks made (in the form of a percentage of baseline) as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

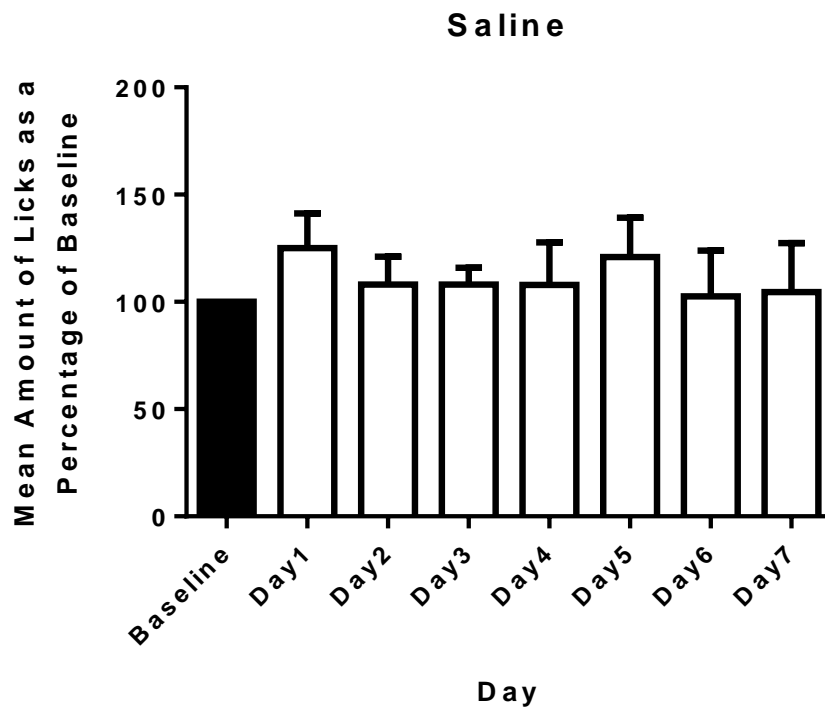


Figure 4 shows the amount of licks made during washout (in the form of a percentage of baseline) as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

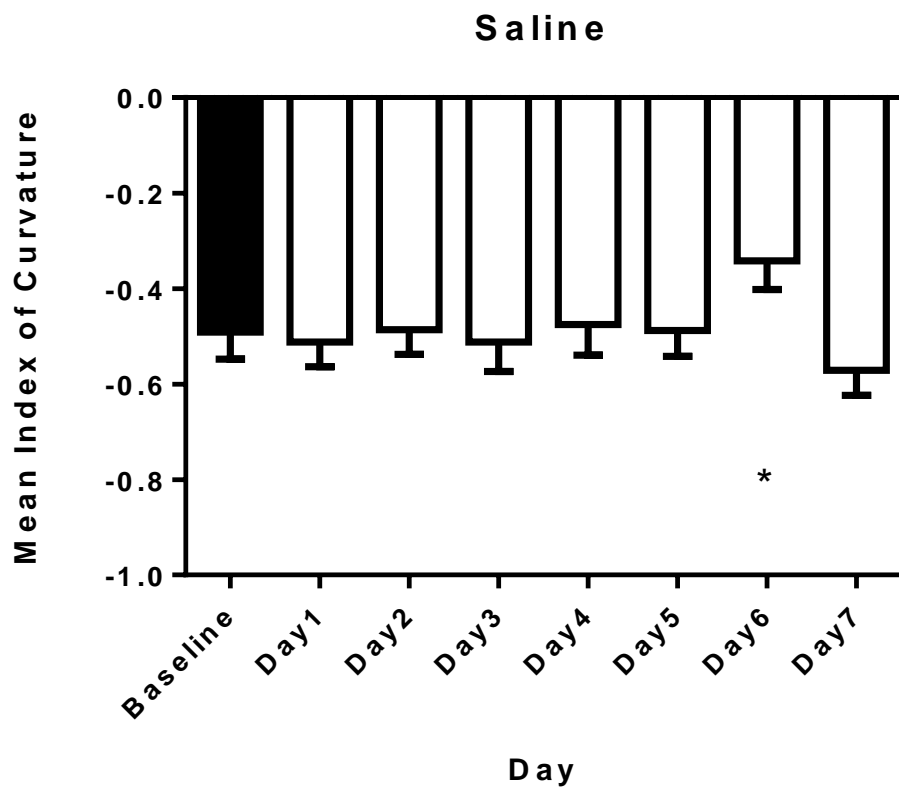


Figure 5 shows index of curvature as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.



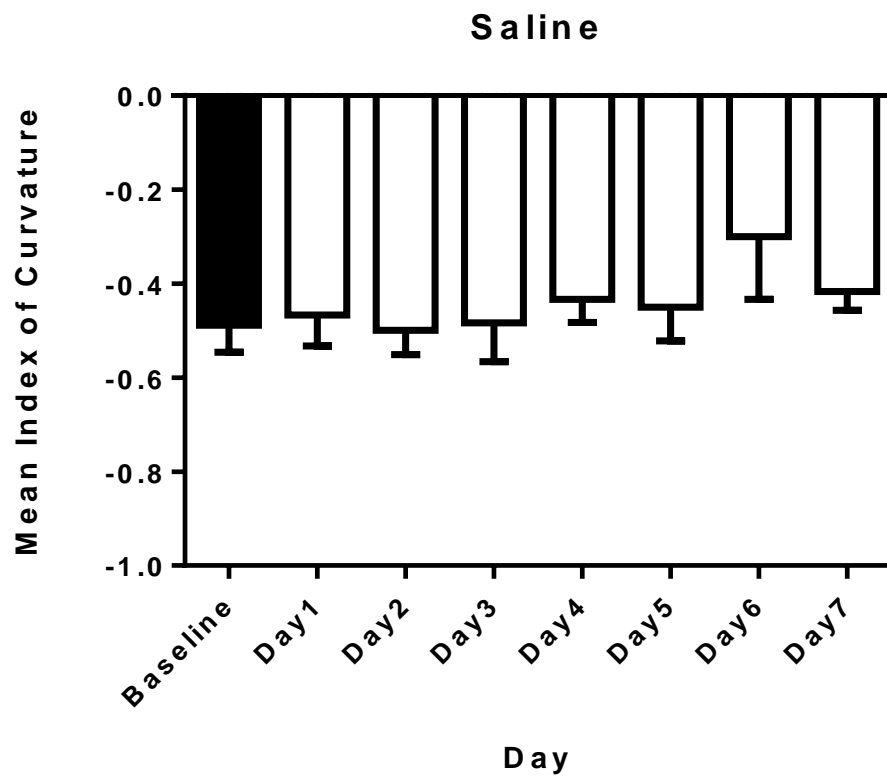


Figure 6 shows index of curvature during washout as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

### *Repeated fluoxetine administration*

Figure 7 presents the mean percentage of water consumed, compared to baseline, during each session over seven consecutive sessions. Fluoxetine administration at 10 mg/kg significantly decreased water consumption compared to baseline drinking amounts,  $F(7, 49) = 2.33$ ,  $p < 0.05$ . Post hoc tests found a significant decrease in water consumption on days 6 and 7. Fluoxetine administration at 30 mg/kg significantly decreased water consumption compared to baseline drinking amounts,  $F(7, 49) = 3.872$ ,  $p < 0.001$ . Post hoc tests revealed a significant decrease in water consumption on days 1 through 7, as compared to baseline.

Figure 8 presents percentage of water consumed, compared to baseline, during the washout period over seven consecutive sessions. Saline washout administration following fluoxetine 10 mg/kg administration significantly affected water consumption compared to baseline,  $F(7, 49) = 5.786$ ,  $p < 0.001$ . However, post hoc tests did not reveal significant differences on any of the seven days as compared to baseline. Saline washout administration following fluoxetine 30 mg/kg administration significantly decreased water consumption compared to baseline,  $F(7, 35) = 16.16$ ,  $p < 0.001$ . Post hoc tests found a significant difference on days 1, 2, 3, 4, 5, and 6.

Figure 9 presents the percentage of licks made, compared to baseline, during each session over seven consecutive sessions. Fluoxetine administration at 10 mg/kg did not significantly affect licks made compared to baseline amount of licks made,  $F(7, 42) = 0.3896$ ,  $p > 0.05$ . Fluoxetine administration at 30 mg/kg significantly decreased the amount of licks made compared to baseline amount of licks made,  $F(7,$

49)= 4.78,  $p < 0.001$ . Post hoc tests found a significant decrease in licks made on days 4, 5, 6, and 7.

Figure 10 presents percentage of licks made, compared to baseline, during the washout period over seven consecutive sessions. Saline washout administration following fluoxetine 10 mg/kg significantly increased licks made compared to baseline amount of licks made,  $F(7, 42) = 3.43$ ,  $p < 0.025$ . Post hoc tests found a significant increase in licks made on days 3, 4, 6, and 7 compared to baseline. Washout administration, post fluoxetine 30 mg/kg, did significantly affect licks made compared to baseline amount of licks made,  $F(7, 28) = 6.23$ ,  $p < 0.001$ . Post hoc tests found a significant decrease of licks made on days 1, 2, and 3.

Figure 11 presents index of curvature during each session over seven consecutive sessions. Fluoxetine administration at 10 mg/kg did not significantly affect index of curvature compared to baseline index of curvature,  $F(7, 42) = 0.479$ ,  $p > 0.05$ . Fluoxetine administration at 30 mg/kg significantly decreased index of curvature compared to baseline index of curvature,  $F(7, 49) = 2.56$ ,  $p \leq 0.025$ . Post hoc tests found a significant decrease in the index of curvature on day 6.

Figure 12 presents index of curvature during the washout period over seven consecutive sessions. Washout administration, post fluoxetine 10 mg/kg, did not significantly affect index of curvature compared to baseline index of curvature,  $F(7, 42) = 0.727$ ,  $p > 0.05$ . Washout administration, post fluoxetine 30 mg/kg, did not significantly affect index of curvature compared to baseline index of curvature,  $F(7, 35) = 1.53$ ,  $p > 0.05$ .

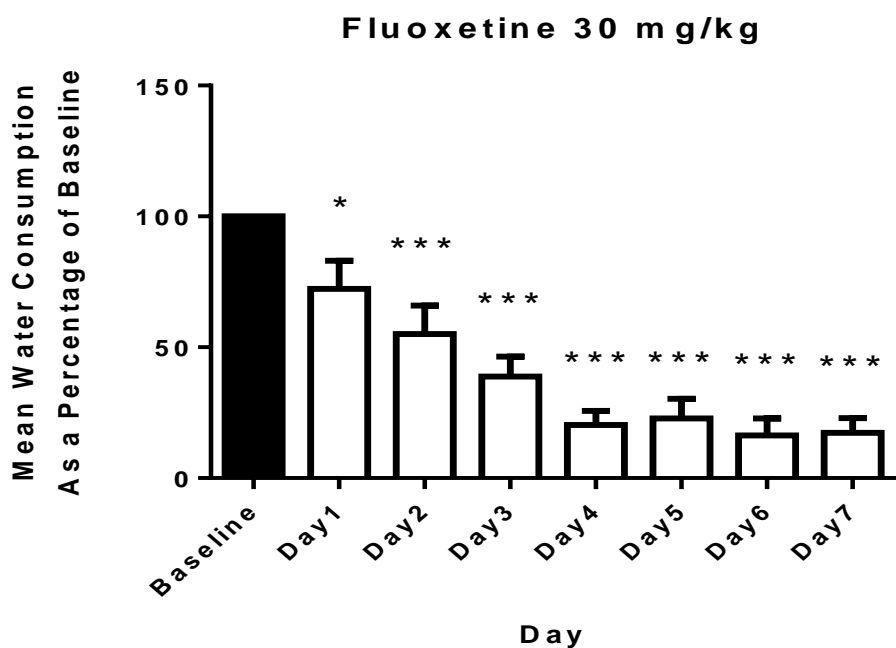
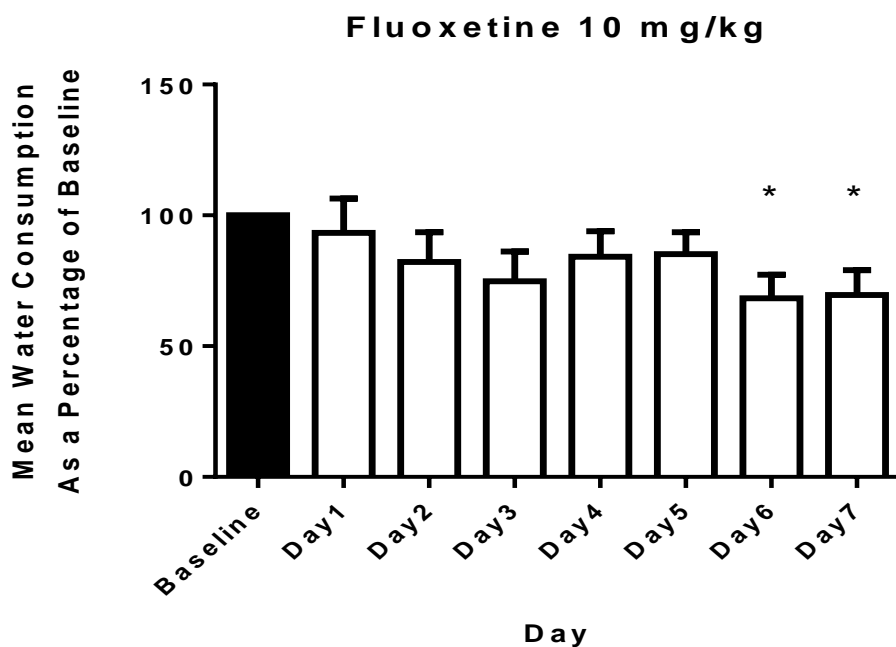


Figure 7 shows the amount of water consumed (in the form of a percentage of baseline) during Fluoxetine 10 mg/kg and 30 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

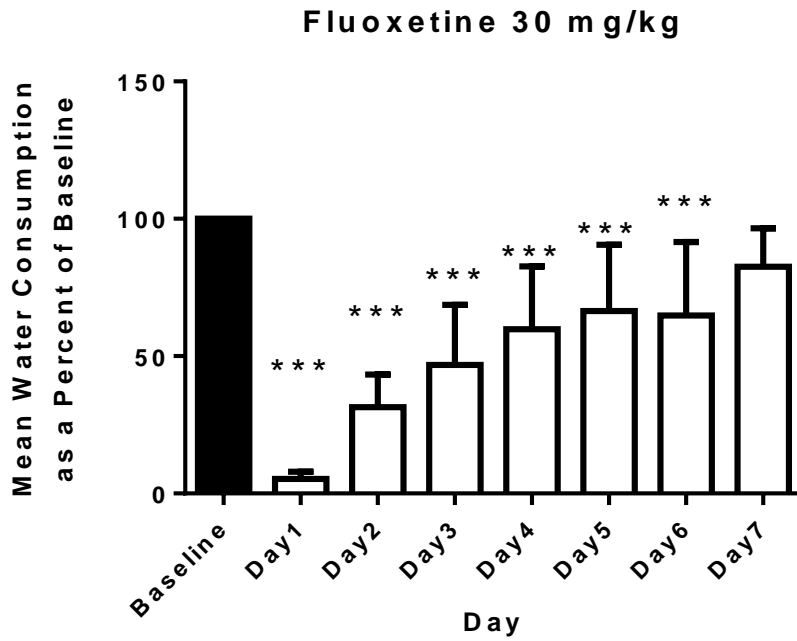
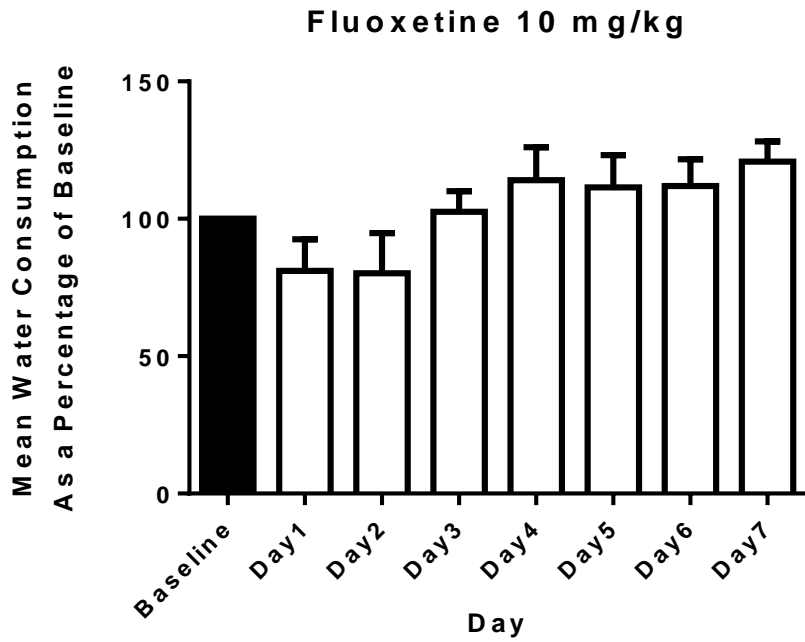


Figure 8 shows the amount of water consumed (in the form of a percentage of baseline) during washout following Fluoxetine 10 mg/kg and 30 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

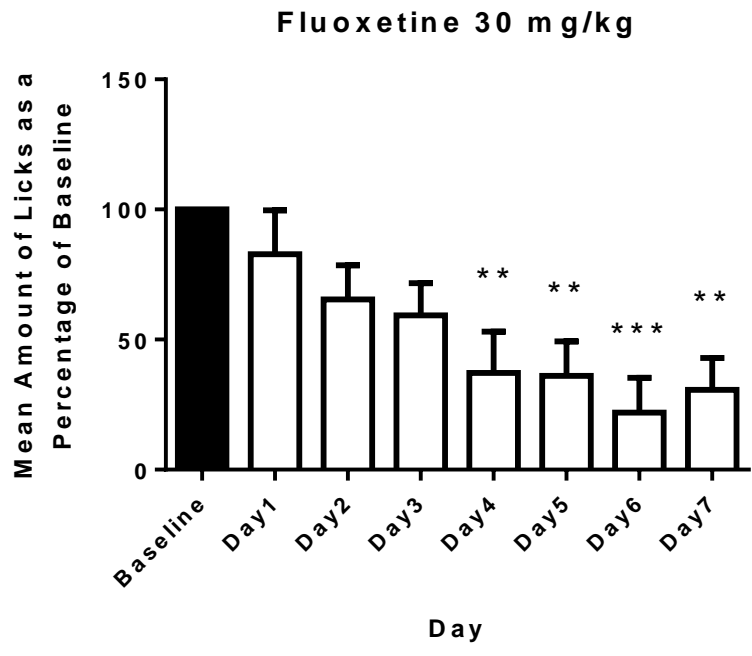
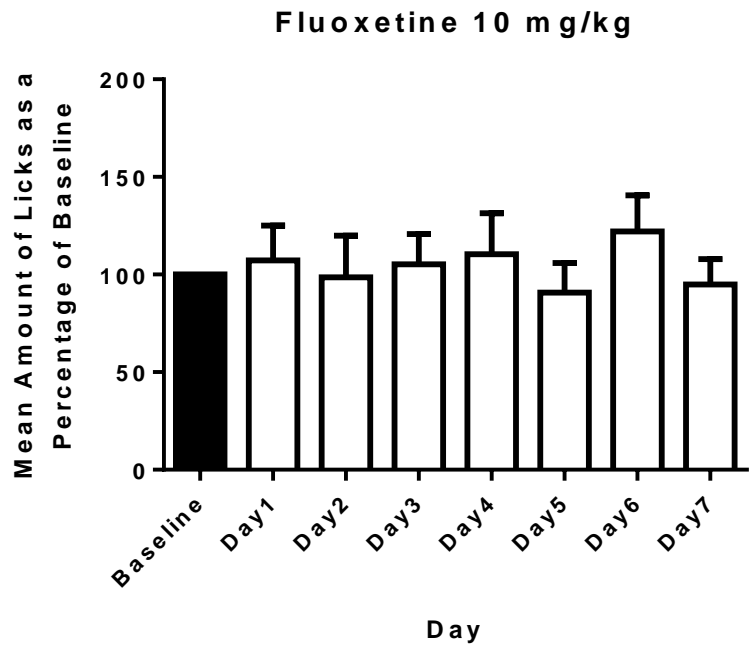


Figure 9 shows the amount of licks made (in the form of a percentage of baseline) during Fluoxetine 10 mg/ kg and 30 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

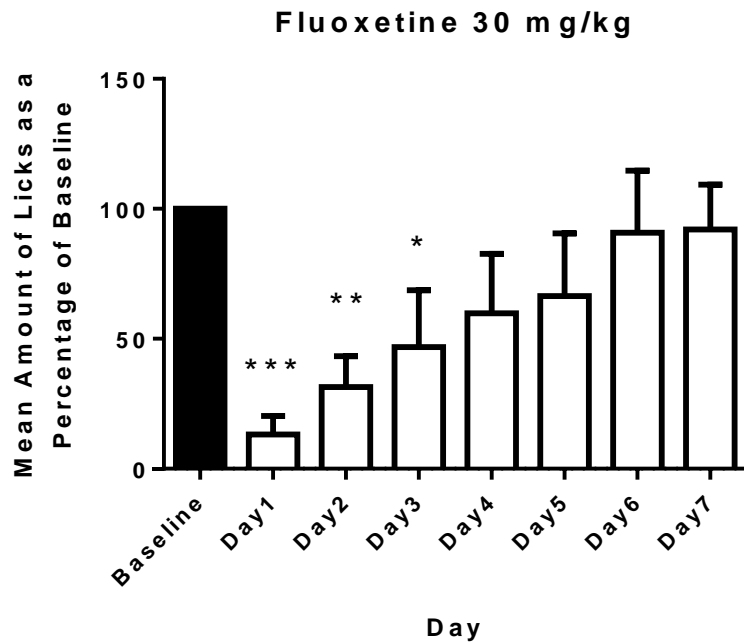
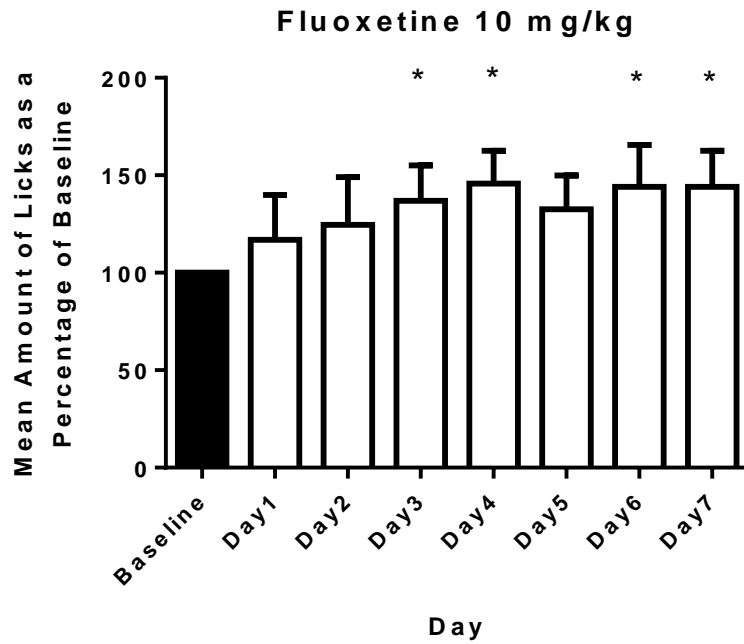


Figure 10 shows the amount of licks made (in the form of a percentage of baseline) during washout following Fluoxetine 10 mg/kg and 30 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

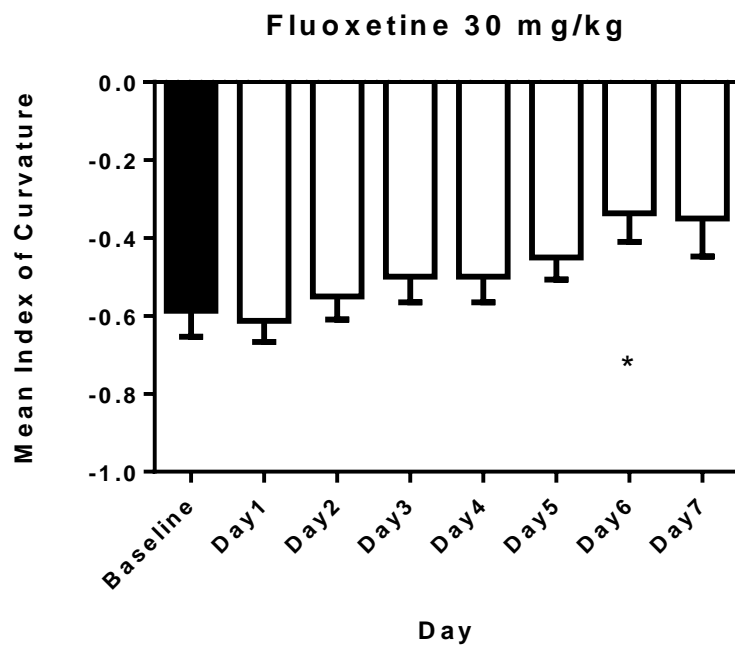
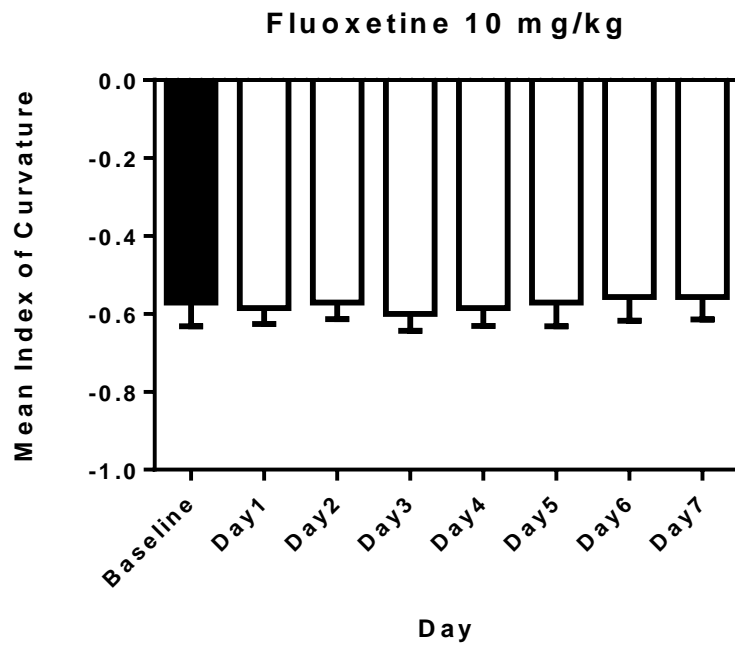


Figure 11 shows index of curvature during Fluoxetine 10 mg/ kg and 30 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.



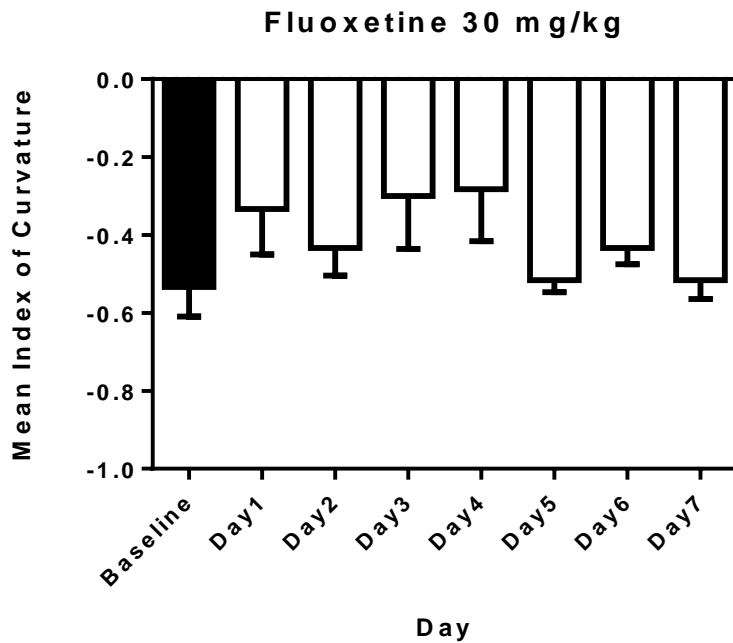
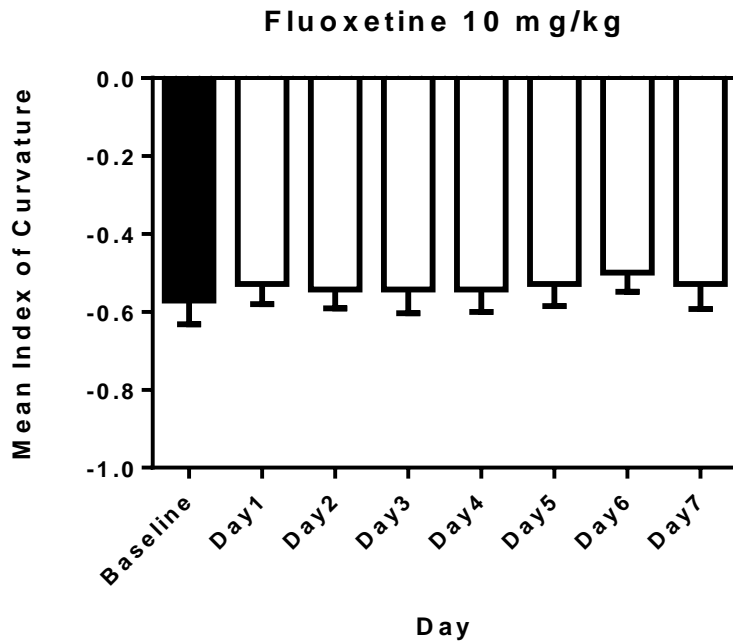


Figure 12 shows index of curvature during washout following Fluoxetine 10 mg/ kg and 30 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

### *Repeated duloxetine administration*

Figure 13 presents the percentage of baseline water consumed during each session over seven consecutive sessions of daily duloxetine treatment. Duloxetine administered at 30 mg/kg significantly decreased water consumption compared to baseline water consumption,  $F(7, 49) = 4.860$ ,  $p < 0.001$ . Post hoc tests revealed a significant decrease in water consumption on days 3, 4, 5, and 7 compared to baseline. Duloxetine administered at 100 mg/kg significantly decreased water consumption compared to baseline water consumption,  $F(7, 35) = 13.37$ ,  $p < 0.001$ . Post hoc tests revealed a significant decrease in water consumption on all seven days.

Figure 14 presents percentage of water consumption, compared to baseline, during the washout period over seven consecutive sessions. Washout administration, post duloxetine 30 mg/kg, did not significantly affect water consumption compared to baseline water consumption,  $F(7, 21) = 1.64$ ,  $p > 0.05$ . Washout administration, post duloxetine 100 mg/kg, did not significantly affect water consumption compared to baseline water consumption,  $F(7, 35) = 1.88$ ,  $p > 0.05$ .

Figure 15 presents the total licks, expressed as a percentage of baseline, during each session over seven consecutive sessions. Duloxetine administered at a 30 mg/kg dose did not significantly affect the amount of licks made,  $F(7, 49) = 0.337$ ,  $p > 0.05$ . Duloxetine administered at 100 mg/kg also did not significantly affect the amount of licks made,  $F(7, 35) = 1.77$ ,  $p > 0.05$ .

Figure 16 presents percentage of total licks, compared to baseline, during washout period over seven consecutive sessions. Washout administration, post duloxetine 30 mg/kg, did not significantly affect total licks compared to baseline

amount of total licks,  $F(7, 21) = 0.719$ ,  $p > 0.05$ . Washout administration, post duloxetine 100 mg/kg, did not significantly affect total licks compared to baseline amount of licks made,  $F(7, 35) = 0.244$ ,  $p > 0.05$ .

Figure 17 presents the index of curvature during each session over seven consecutive sessions. Duloxetine administered at 30 mg/kg did not significantly affect the index of curvature,  $F(7, 49) = 2.03$ ,  $p > 0.05$ . Duloxetine administered at 100 mg/kg did not significantly affect the index of curvature,  $F(7, 35) = 2.03$ ,  $p > 0.05$ .

Figure 18 presents index of curvature during the washout period over seven consecutive sessions. Washout administration, post duloxetine 30 mg/kg, did not significantly affect index of curvature compared to baseline index of curvature,  $F(7, 21) = 1.52$ ,  $p > 0.05$ . Washout administration, post duloxetine 100 mg/kg, did significantly decrease index of curvature compared to baseline index of curvature,  $F(7, 35) = 2.61$ ,  $p < 0.05$ . Post hoc tests revealed a significant decrease in the index of curvature on day 1.

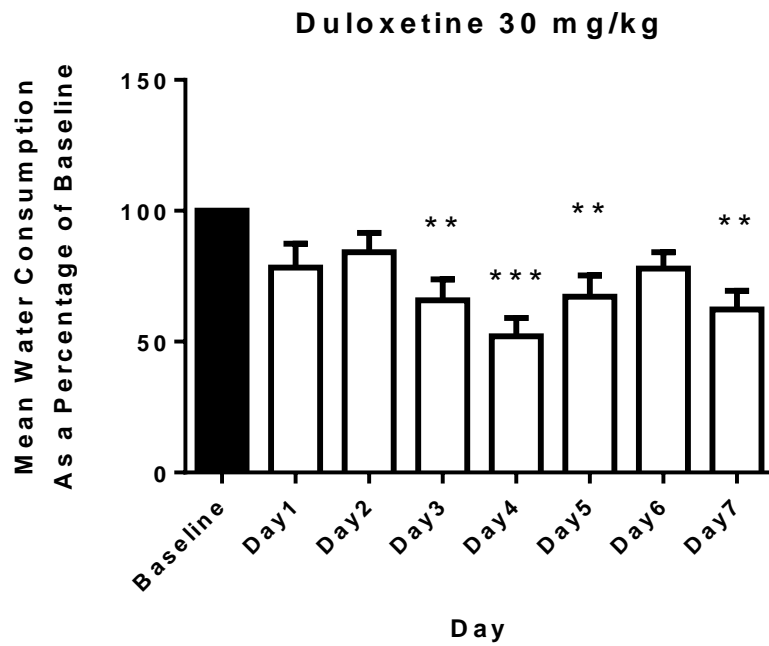


Figure 13 shows water consumption (in the form of a percentage of baseline) during Duloxetine 30 mg/ kg and 100 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

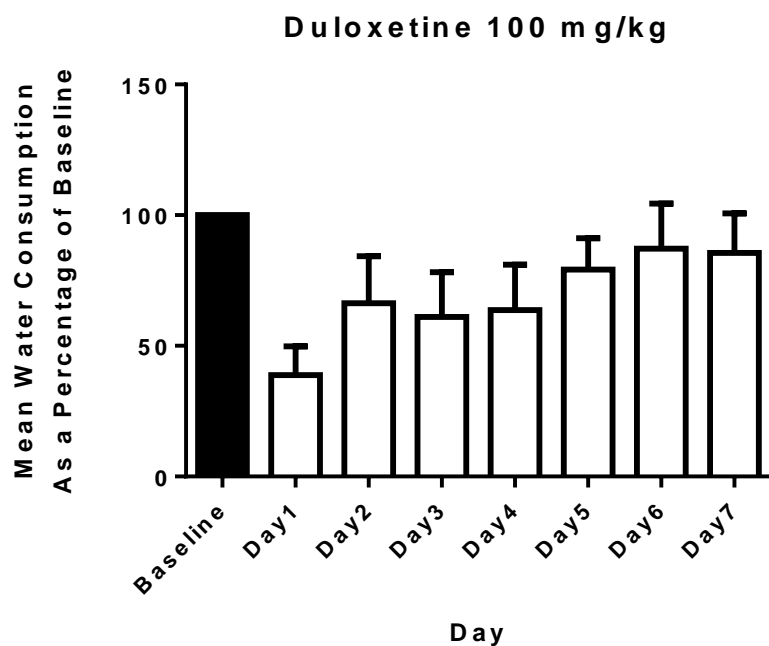
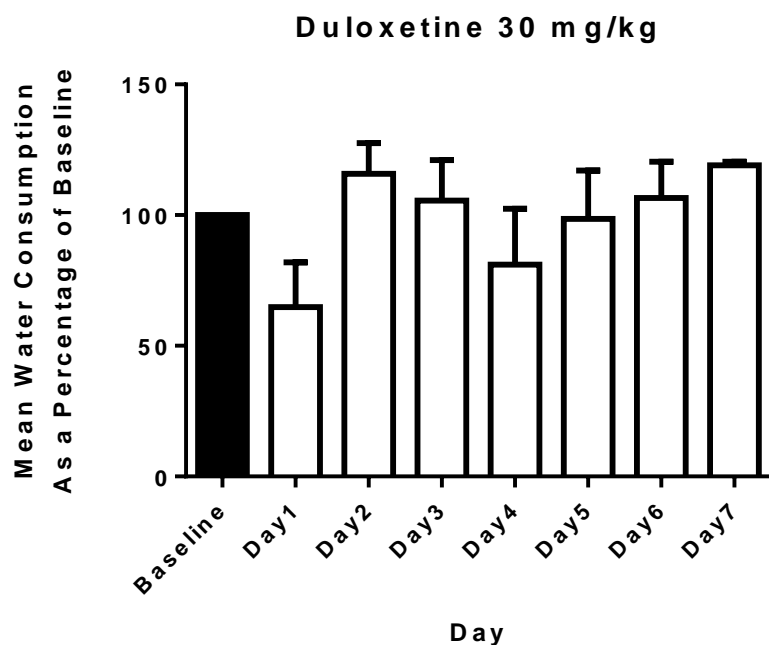


Figure 14 shows water consumption (in the form of a percentage of baseline) during washout following duloxetine 30 mg/kg and 100 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

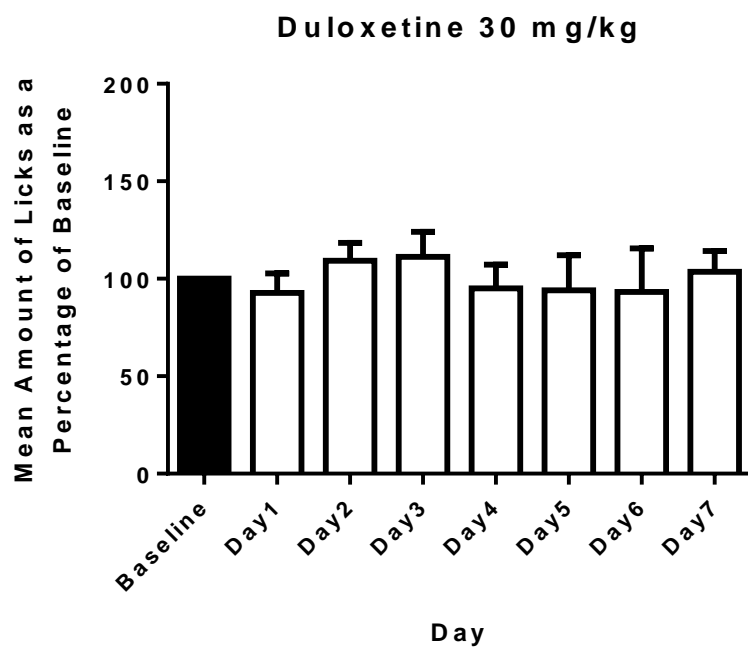


Figure 15 shows the amount of licks made (in the form of a percentage of baseline) during duloxetine 30 mg/ kg and 100 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

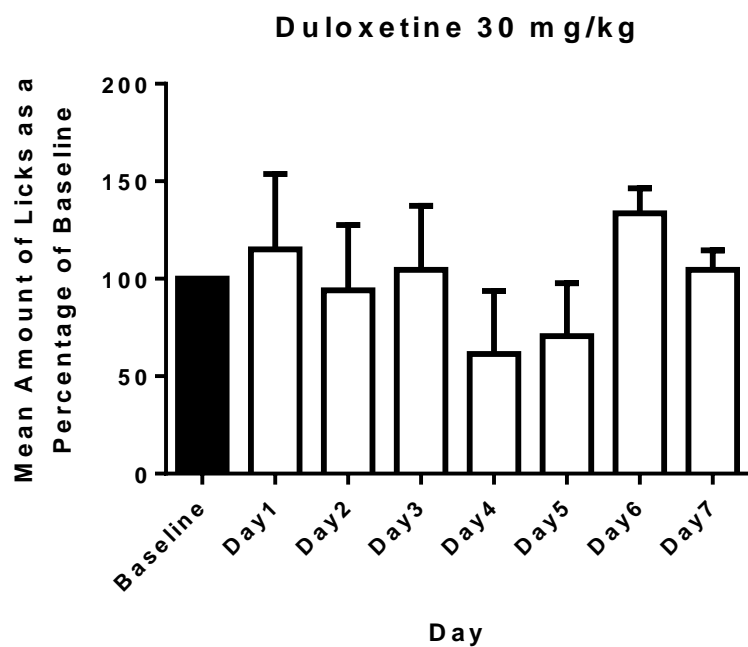


Figure 16 shows the amount of licks made (in the form of a percentage of baseline) during washout following duloxetine 30 mg/kg and 100 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

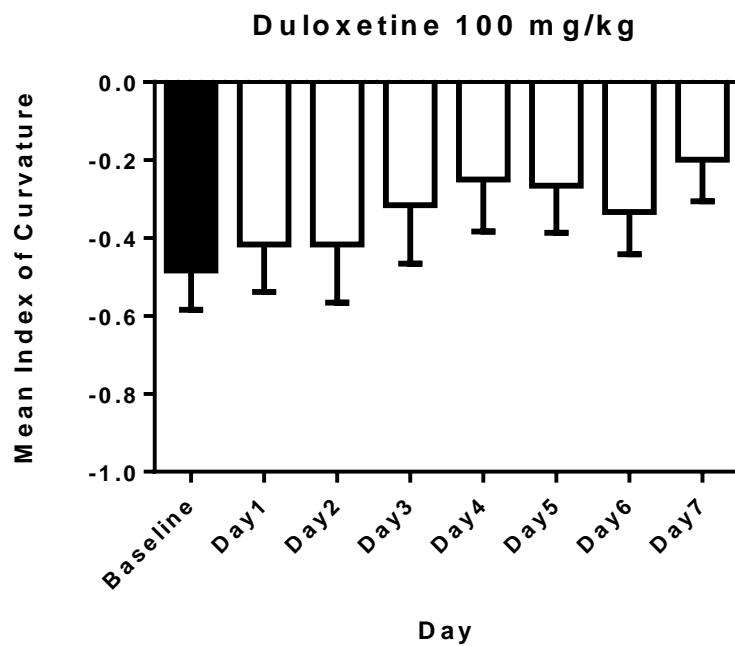
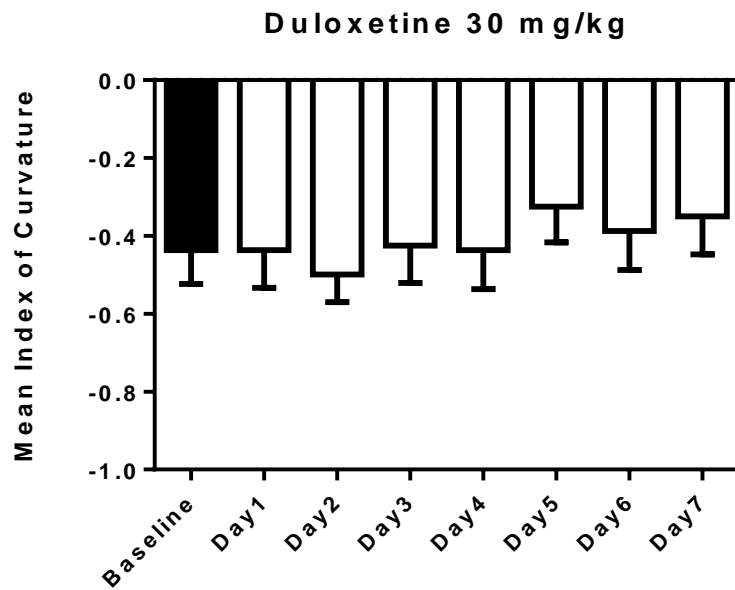


Figure 17 shows index of curvature (in the form of a percentage of baseline) during duloxetine 30 mg/kg and 100 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.



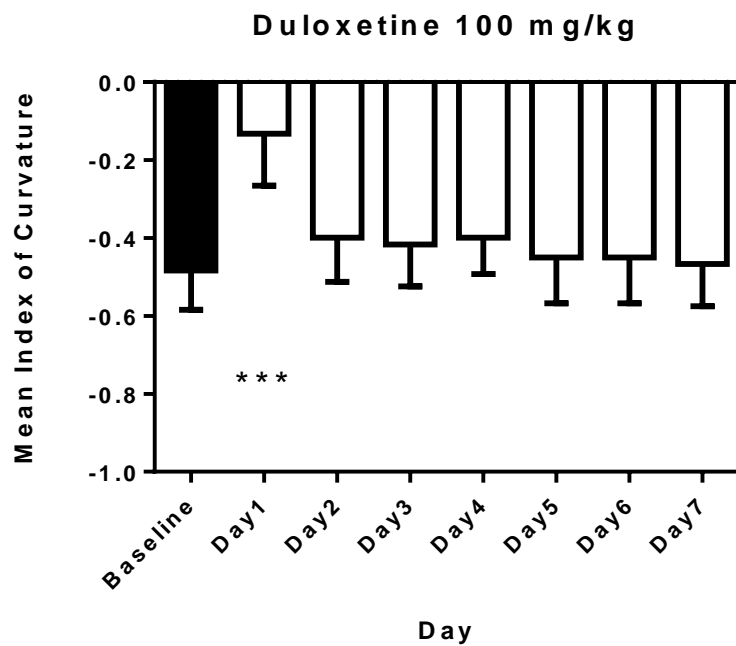
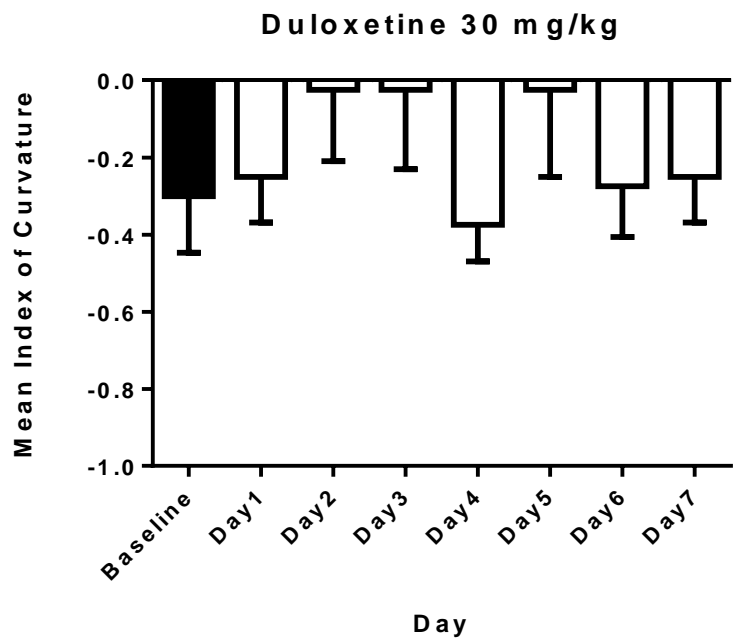


Figure 18 shows index of curvature (in the form of a percentage of baseline) during washout following duloxetine 30 mg/ kg and 100 mg/kg administration as a function of repeated sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

*Mass feeding session*

Figure 19 presents the percentage of water consumption, percentage of total licks, and index of curvature compared to baseline. Mass feeding significantly decreased water consumption compared to baseline,  $t(21) = 19.73$ ,  $p < 0.001$ . Mass feeding significantly decreased total licks compared to baseline,  $t(19) = 17.96$ ,  $p < 0.001$ . Mass feeding significantly decreased index of curvature compared to baseline,  $t(19) = 6.77$ ,  $p < 0.001$ .

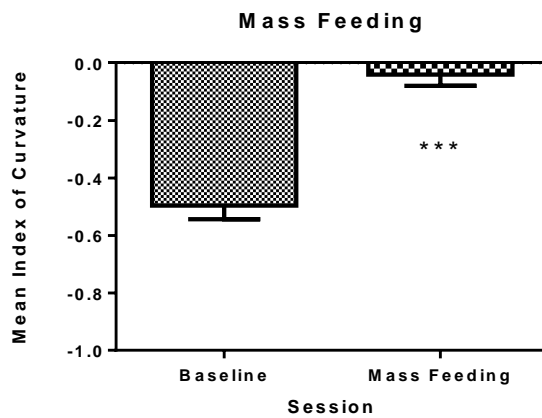
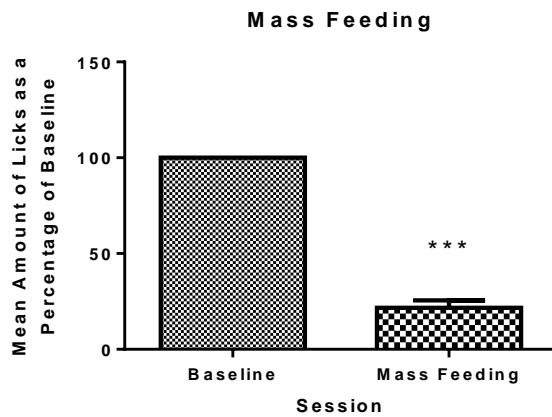
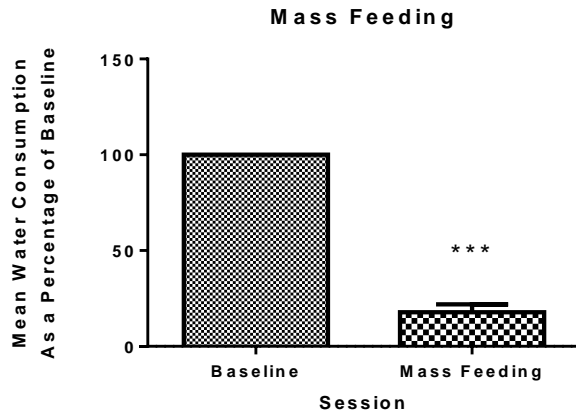


Figure 19 shows water consumption, amount of licks made (in the form of a percentage of baseline), and index of curvature during mass feeding administration as a function of sessions in Sprague Dawley rats in the schedule-induced polydipsia animal model. For more detail see figure 1.

## DISCUSSION

The present study replicates the findings by Hogg and Dalvi (2004) that fluoxetine decreases water consumption in a time-sensitive and dose-dependent manner. Fluoxetine at 10 mg/kg produced a significant decrease in water consumption only on days 6 and 7. However, this dose failed to affect total licks and index of curvature. Fluoxetine at 30 mg/kg produced a significant decrease in water consumption, total licks, and index of curvature. Decrease in water consumption was observed starting on day 1 and remained significantly lower throughout drug administration. For total licks, fluoxetine produced a significantly lower amount of licks on days 4, 5, 6 and 7. Index of curvature was significantly lower only on day 6.

In washout sessions, water consumption, total licks, and index of curvature measures returned to baseline fairly rapidly. Saline washout, following fluoxetine (10 mg/kg), produced a significant increase in total water consumption. However, post hoc tests failed to find a significant difference between days. For total licks made, saline washout produced a significant increase in licks made on days 3, 4, 6, and 7. Conversely, saline washout did not produce an affect on index of curvature following low dose of fluoxetine. Furthermore, saline washout following the high dose of fluoxetine (30 mg/kg) found no difference from baseline only on day 7 when the behavior returned to baseline levels. Similarly, licking behavior returned to baseline starting on day 4 and remained at baseline level throughout the remainder of saline administration.

Previous studies investigating fluoxetine using various animal models of anxiety such as the forced swim test, tail suspension test, and the elevated plus maze have shown similar results (Ciulla et al., 2007; Drapier et al., 2007; Kamei et al., 2008; Perrault, Morel, Zivkovic, & Sanger, 1992; Rogoz & Skuza, 2011). However, these studies do not indicate the onset of fluoxetine's effects. The results from the present study provide a measurement of time for the onset of fluoxetine. Furthermore, these results suggest that fluoxetine may display a faster onset in a dose dependent manner. Similarly, studies using a modified version of the forced swim task have reported that fluoxetine at 2.5 mg/kg, 5.0 mg/kg, and 15.0 mg/kg doses had no effect on immobility, swimming, and climbing when administered continuously for 3 days. However, after administration for 14 days there was a significant increase in all measurements except for climbing (Cryan, Page, & Lucki, 2005; Detve, Johnson, & Lucki, 1997). Finally, the results from the present study replicate previous findings of a decrease in schedule-induced water consumption produced by fluoxetine (Hogg & Dalvi, 2004; Beyer et al., 2009).

In the present study duloxetine only affected total water consumption. Both doses decreased water consumption over a number of sessions. The low dose of duloxetine (30 mg/kg) decreased water consumption on days 3, 4, 5 and 7. The high dose of duloxetine (100 mg/kg) decreased water consumption on all seven days. Although there was a robust decrease in water consumption there was no affect observed on the amount of licks made. During the saline washout period following duloxetine administration only the index of curvature was affected on day 1 for the high dose of duloxetine and water intake quickly returned to baseline levels.

Similar to fluoxetine, duloxetine has been investigated in many behavioral paradigms used to measure the efficacy of antidepressants. Previous studies have found that duloxetine's effects in these paradigms are typical of antidepressant drugs (Bardin et al., 2010; Berrocoso et al., 2013; Menezes et al., 2008; Reneric & Lucki, 1998; & Xue et al., in press). The studies mentioned above, failed to provide a measurement of the onset of duloxetine's effect. In the present study duloxetine at both dosages decreased water consumption without having an effect on the amount of licks made. This may be due to the high variability and individual differences expressed in licking behaviors. Furthermore, in the low dose there was a time sensitive lag to achieve a significant decrease in water consumption. Similar results have been documented in studies using the zero maze, which is a modified version of the elevated plus maze. Troelsen, Nielsen, and Mirza (2005) found no effect produced by acute administration of duloxetine in the zero maze. However, after 21 days of administration, duloxetine displayed a significant increase in the amount of entries and time spent in the open areas. Although this study indicates that there is a lag in the onset of effects, it does not provide an exact time at which the compound displays its effects. The present study is the first to use duloxetine in the schedule induced-polydipsia animal model and provides a quantitative measurement for the onset of its effects, which is expressed as a decrease in water consumption.

During the mass feeding session all measurements were significantly affected. The decrease in water consumption and total licks suggests that the schedule was both effective and necessary for establishing polydipsia. The decrease in index of

curvature demonstrates that the temporal distribution of licks is a product of the fixed-time schedule of reinforcement.

The lag in onset of the therapeutic effects produced by fluoxetine and duloxetine may be due to similar mechanisms. It has been proposed that the systems modulating serotonin and norepinephrine are two distinct systems. However, it has been shown that serotonergic heteroreceptors are localized on norepinephrine cell bodies in the locus coeruleus, which implies that these systems inhibit as well as enhance each other (Leger & Descarries, 1977; Leger, Wiklund, Descarries, & Persson, 1979). Microdialysis studies have found that the serotonin agonist 5-carboxamindotryptamine administered to rat hippocampal slices increased radioactive labeled norepinephrine levels (Feverslein & Hertting, 1986). Additionally, administration of the selective norepinephrine reuptake inhibitor reboxetine resulted in an increase of dorsal raphe nuclei firing and an increase in serotonin output in the medial prefrontal cortex (Linner, Wiker, Arborelius, Schalline, & Svensson). Behaviorally serotonin-mediated actions were exacerbated through the use of a  $\beta$ -androgen receptor antagonist, which would imply an increase in serotonin (Cowen, Grahame-Smith, Green, & Heal, 1982).

One explanation for the cause of the lag in the onset of therapeutic effects is the activation of the inhibitory 5HT<sub>-1</sub> receptor family. As a putative model for measuring the onset of antidepressants, schedule-induced polydipsia provides an assay for investigating the effects of the 5-HT<sub>1</sub> receptor family on this delay. As mentioned above, Hogg and Dalvi (2004) found a rapid decrease in water consumption when fluoxetine was used in combination with either a 5-HT<sub>1A</sub> or 5-

HT<sub>1B</sub> receptor antagonist. The decrease produced by the combination of these compounds was significantly faster as compared to fluoxetine alone.

When comparing the results of fluoxetine and duloxetine at similar dosages (30 mg/kg), fluoxetine displayed a decrease in water consumption faster than duloxetine. This difference in onset may be due to inhibitory effect produced by the  $\alpha$ -2 noradrenergic receptor. This inhibitory heteroreceptor may produce a decrease in serotonin firing resulting in an immediate decrease in serotonin. However, the difference in onset may also be due to differences in potencies and the compound's effect on inhibiting reuptake. It has been shown that duloxetine is a more potent inhibitor of serotonin reuptake than fluoxetine (Bymaster, Katner, Nelson, Hemerick-Luecke, Threlkeld, 2002; Bymaster, Dreshfield-Ahmas, Threlkeld, Shaw, Thompson, 2001). Furthermore, the differences found during washout period may be due to the half-life time of each compound. Studies have shown that the half-life of fluoxetine is longer than duloxetine which would result in a longer effect produced by fluoxetine (Bergstrom, Chappell, Knadler, & Lobo, 2011; Gram, 1994).

Along with the results found in the present study, schedule-induced polydipsia has been shown to respond to various types of antidepressants in a manner that allows for the measurement of the onset of their effects. Due to the responsiveness of a serotonin norepinephrine reuptake inhibitor in this model and the interaction between norepinephrine and serotonin systems, schedule-induced polydipsia may be used to measure the influence that  $\beta$ 1&2 and  $\alpha$ 1&2 receptors have on antidepressant efficacy.

Possible applications for the use of schedule-induced polydipsia could be in measuring the onset changes with various drug combinations. One such combination



could be the  $\alpha_2$  receptor antagonist yohimbine with a selective serotonin reuptake inhibitor. Furthermore, the administration of an selective norepinephrine reuptake inhibitor, such as reboxetine, may be used to further characterize the effects of monoamine altering compounds on schedule-induced polydipsia.

In conclusion, both the selective serotonin reuptake inhibitor fluoxetine and the serotonin norepinephrine reuptake inhibitor duloxetine produced a significant decrease in water consumption in the schedule-induced polydipsia animal model. Compared to duloxetine, fluoxetine displayed a faster onset in the decrease in water consumption and produced a decrease in total licks made. These results suggest that the schedule-induced polydipsia animal model is a viable paradigm that can be used to measure the onset of antidepressant drugs.

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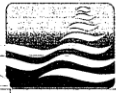
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APPENDIX A



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**MEMORANDUM**

September 24, 2012

**TO:** Dr. Adam Prus  
Psychology Department

**FROM:** Brian Cherry, Ph.D. *BC*  
Assistant Provost/IACUC Administrator

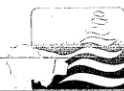
**RE:** **Application to use Vertebrate Animals**  
**Amendment: Protocol IACUC 196**  
**Approval Period: 09/24/2012-01/24/2014**

The Institutional Animal Care and Use Committee has approved your amendment to change the drug administration of fluoxetine/duloxetine from subcutaneous to oral by administrative review in research for "Antidepressant Drug Effects on Schedule-Induced Polydipsia".

If you have any questions, please contact me.

ljc

APPENDIX B



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**MEMORANDUM**

February 2, 2012

**TO:** Dr. Adam Prus  
Psychology Department

**FROM:** Brian Cherry, Ph.D. *BC*  
Dean of Graduate Studies & Research

**RE:** **Application to use Vertebrate Animals**  
**Application # IACUC 196**  
**Approval Period: 01/24/2012-1/24/2014**

The Institutional Animal Care and Use Committee, has approved your application by designated member review to use vertebrate animals in research for "Antidepressant drug effects on schedule-induced polydipsia".

If you have any questions, please contact me.

kjm