2014

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THE EFFECT OF FLUOXETINE ON SELF-CONTROL IN *BETTA SPLENDENS*

by

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Dissertation

presented in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy
in Experimental Psychology

The University of Montana
Missoula, MT

June 2014

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Lamp, Kathryn, M.A. Summer 2014 *Psychology*

The Effect of Fluoxetine on Self-Control in *Betta splendens*

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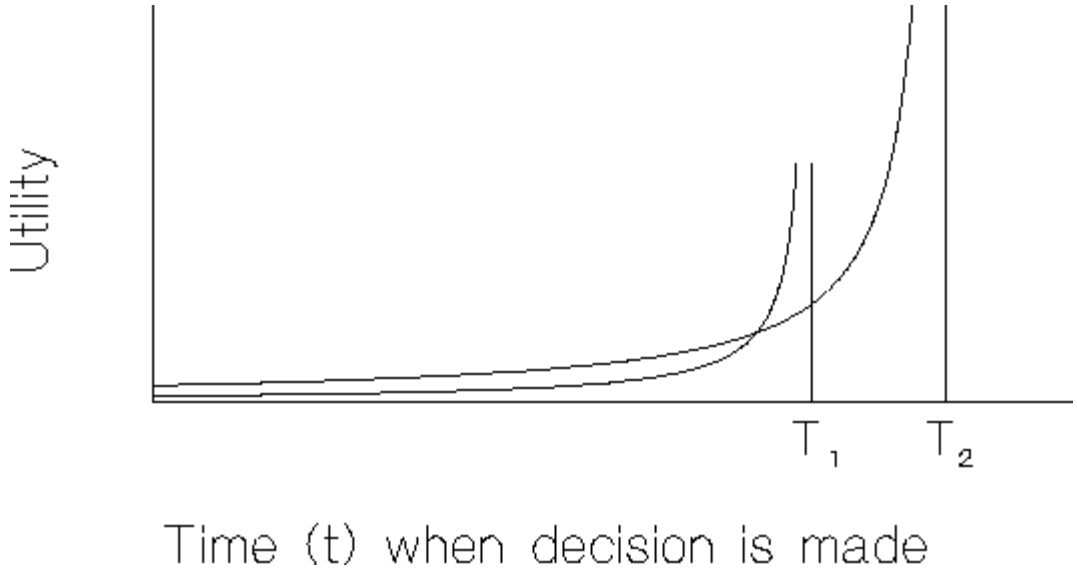
The present study examined the effect of fluoxetine on self-control in Siamese fighting fish (*Betta splendens*). The subjects included 17 male *Betta splendens* that were exposed to varying levels of fluoxetine, a selective serotonin reuptake inhibitor that increases levels of serotonin, and instrumental choice trials were run. A subject began each trial in the start box and, when a guillotine divider door was lifted, entered one side of a divided goal box. The checkerboard side of the choice door represented either the smaller-sooner choice (SS) or the larger-later choice (LL). When the subject had entered one side of the goal box, the guillotine divider door was lowered and the subject was given food pellets, 1 pellet immediately or 3 pellets after 18 seconds, depending on which side of the choice door the subject entered. Prior to these trials, subjects experienced various levels of fluoxetine exposure (0 μMol, 7.5 μMol, or 12.5 μMol). Fish exposed to higher levels of fluoxetine were expected to show a greater preference for self-control than subjects exposed to lower levels of fluoxetine. Contrary to the hypothesis, subjects in all groups did not demonstrate a significant preference for either the smaller-sooner choice or the larger-later choice, nor did the groups differ significantly from one another in their choice preference. Subjects exposed to fluoxetine did demonstrate higher response latencies than subjects not exposed to fluoxetine, and though these differences were not significant, they suggest that fluoxetine may have impacted learning or motivation.

The Effect of Serotonin on Self-Control in *Betta splendens*

Self-control and impulsivity are ever-present in the lives of both humans and non-human animals; thus, it is important to understand these constructs and the factors that impact them. Self-control has been defined as the preference for a larger but delayed reinforcer over a smaller, immediate reinforcer, and impulsiveness as the opposite, when the organism's preference switches to a smaller, more immediate reinforcer over a larger but delayed reinforcer (Ainslie, 1974, 1975). An oft-cited example of a self-control choice paradigm is that found in a discussion by Mischel and his colleagues (1989) on the delay of gratification in children. Children were given a choice between either one marshmallow available immediately or two marshmallows available after a delay period. Children choosing the smaller reward immediately, one marshmallow, were said to have demonstrated impulsivity, while children who choosing the larger but delayed reward, two marshmallows, were said to have demonstrated self-control.

The construct of self-control has been examined with the purpose of understanding why an individual would demonstrate impulsivity when self-control is overall the more beneficial choice (a greater, or larger, reward is more often the better choice than a smaller reward). Reviewing how self-control plays a role in areas of human activity such as the economy, behavioral psychology and psychopathology, Ainslie (1975) argued that as delay to reward increases, the perceived value of the reward decreases, a phenomenon known as hyperbolic discounting. Below is a visual depiction of such a function:



As this graph shows, at time 1, the second option (or option B) is preferred because of its higher perceived value. At time 2, there is a preference reversal because the first option (or option A) now has a higher perceived value.

Prelec and Herrnstein (1997) offer further explanation as to how this delay to reward can impact the cost-benefit analysis that individuals engage in when making choices between alternatives. Known as the temporal mismatch, an instance in which some time interval separates the cost(s) and benefit(s) of a choice may lead to an individual choosing to act impulsively if the benefit is immediately present while any cost will not immediately occur. An individual is also more likely to act impulsively if the benefit to exercising self-control is not immediately present. For example, a person can choose to either act impulsively or exhibit self-control when he or she has a craving to eat fast food. Choosing to eat fast food will satisfy the craving immediately. Even though the costs associated with eating fast food (obesity, diabetes, heart disease, etc.) are high, the person may ignore these costs because they do not occur at the same time as the benefit of craving and/or hunger satisfaction (i.e. immediately). Choosing to exhibit self-control and not eat fast food is the optimal choice for the person's long-term health, but the long-term health

benefits do not typically occur immediately while the costs of an increasingly strong craving and increasing feelings of hunger will be immediately felt by the person.

In addition to research that has demonstrated how perceived value impacts self-control, other research has demonstrated that self-control can be manipulated by various factors such as age and reward type. Tobin and Logue (1994) reviewed studies examining self-control across age groups, including 3- and 5-year olds as well as adults. A typical choice paradigm was used wherein subjects were given a choice between a smaller but immediate reward and a larger but delayed reward. Children were given a food reward while adults were given either a food reward or points that could be redeemed for money. Researchers found that 5-year old children showed more self-control than 3-year old children, demonstrating the importance of age as a factor. Researchers also found that adults showed more self-control when the reward was points redeemable for money rather than food, demonstrating that reward type is another important factor in determining self-control.

The study of self-control in non-human animals has taken a different approach due to the differences inherent in the behavioral observations often utilized in research with non-human animal subjects. For example, key pecking is a commonly used behavior in self-control research using pigeons. Ainslie (1974) found that these subjects could be trained not only to differentiate between two available choice options (a smaller more immediate reward and a larger but delayed reward, as in the previously discussed choice paradigm), but also to acquire self-control through training. Other researchers examining self-control in pigeons have also used key pecking as a behavior measure and have found that self-control in these subjects can be manipulated by increasing the delay to reward (Chelonis, et al, 1994; Jackson & Hackenburg, 1996), using food-deprivation, and altering frequency of reinforcement (Logue, et al, 1988).

Another behavior utilized in self-control research using non-human animals is lever pressing, a commonly used measure in research with rats (Tobin, et al, 1993; Eisenberger, et al, 1982; Chelonis, et al, 1998). Such research has demonstrated that, similar to pigeons, rats are capable of not only differentiating between available choice options but will also show preference for self-control in certain conditions (e.g. when force required to operate levers is increased to a certain point (Chelonis, et al, 1998)).

Still other research on self-control in non-human animals has employed the use of mazes. For example, research on self-control in domestic hens (Abeyesinghe, et al, 2004) utilized a two-choice return maze in order to test self-control in this species. These researchers demonstrated that when the temporal difference between the availability of a smaller but immediate reward and a larger but delayed reward was increased, subjects showed a significant preference for the larger but delayed option.

Research on self-control has been conducted across several species encompassing humans and non-human animals alike. The establishment of one of the most commonly used paradigms in self-control research (Ainslie, 1974, 1975), as well as research investigating the factors influencing self-control in both humans and non-human animals (Mischel, 1989; Ainslie, 1975; Prelec & Herrnstein, 1997; Tobin & Logue, 1994; Chelonis, et al, 1994; Jackson & Hackenburg, 1996; Logue, et al, 1988), have provided a strong foundation for understanding this construct. Examining the neurological substrates of behavior is a more recent but still critical development in this research.

One such neurotransmitter implicated in behavior across species is serotonin. More specifically, the serotonergic system in the human brain appears to play a role in impulse control and related behaviors such as aggression. Ciccocioppo (1999) investigated the involvement of

serotonin (5-HT) in craving related to addiction in humans. He discussed how the 5-HT system affects cognitive and learning processes; more specifically, this system appears to significantly impact motivation and the effectiveness that reinforcers will have on behavior. A deficit or decrease in serotonin, he argued, may therefore lead to an increase in impulsive behavior.

Other research has also examined the potential link between serotonin and behavior related to low self-control. In an investigation on self-control as a predictor of antisocial behavior, Beaver and his colleagues (2009) focused in part on the role of the serotonin transporter gene in the development of low self-control. This gene is responsible for the function and levels of serotonin present in an individual's system (Heils, et al, 1996; Hu, et al, 2006; Lesch, et al, 1996; Reist, Mazzanti, Vu, Tran & Goldman, 2001). Lower levels of serotonin are associated with increases in impulsivity, aggression, and violence (Raine, 1993); therefore, the serotonin transporter gene may be linked to lower levels of self-control.

There is strong evidence to suggest a link between serotonin and behavior related to self-control including impulsivity and aggression in humans (Ciccocioppo, 1999; Beaver, et al, 2009). There is also research that has been conducted examining this link in several non-human animal species. Some such research examining this relationship in rats (Olivier, et al, 1995) focused specifically on territorial aggression in males and maternal aggression in females. Researchers found that certain 5-HT_{1A} agonists (buspirone, ipsapirone, and 8-OH-DPAT) and nonselective 5-HT1 receptor agonists (like RU24969, eltoprazine, and TFMPP) decreased both territorial and maternal aggression in both males and females.

While some research has investigated the relationship between serotonin and aggression in rats (Olivier, et al, 1995), still other research has examined this relationship in Syrian golden hamsters. Ferris and his colleagues (1997) focused on the role of the 5-HT_{1B} receptor, particularly

in the anterior hypothalamic region of the basolateral hypothalamus, in offensive aggression behaviors (e.g. instigating attacks, biting, etc.). When placed in a resident/intruder paradigm and faced with an intruder (conspecific), subjects treated with fluoxetine (Prozac, a specific serotonin reuptake inhibitor or SSRI) demonstrated significantly longer response latencies in exhibiting offensive aggression behaviors. Researchers concluded that an increase in serotonin decreased aggression by way of the 5-HT_{1B} receptor in the basolateral hypothalamus.

Still other research has investigated the relationship between serotonin and aggression in the context of a natural environment so as to take into consideration the organic social and physical influences found there. Sperry and his colleagues (2005) observed the effect of fluoxetine on aggression during the breeding season for male American tree sparrows (*Spizella arborea*). Subjects were treated and observed during this season due to the fact that these animals demonstrate their highest levels of aggression during this time. Over the course of 15 days, subjects were injected with either fluoxetine or saline and observed for aggressive territorial behaviors. Not only did researchers find that fluoxetine significantly decreased aggression in this species, but they also noted that subjects treated with fluoxetine were significantly less aggressive in the time period ranging from days 11 to 15 as compared to days 1 to 5 and 6 to 10, demonstrating that aggression also decreased further over time when serotonin was increased.

Much of the research on the serotonergic system and the behaviors that it appears to impact has focused on mammalian and avian species (Ciccocioppo, 1999; Beaver, et al, 2009; Olivier, et al, 1995; Ferris, et al, 1997; Sperry, et al, 2005). An important distinction must be made regarding teleost species as fish are considered the most diverse groups of vertebrates and have unique characteristics, particularly in regards to their neuroanatomy. Like other vertebrate species, serotonergic neurons have been found in the diencephalon, hindbrain and/or spinal cord

(with few exceptions) of several fish species including zebrafish, goldfish and stickleback (Lillesaar, 2011). Zebrafish in particular have served as an excellent model for understanding the serotonergic system in fish species due to their evolutionarily conserved features, one being their 5-HT system (Lillesaar, 2011).

While zebrafish do share similarities in their 5-HT system with other vertebrate species, as noted above, it is important to understand the locations of serotonergic populations (and their apparent functions) specific to the system in this species. Serotonergic populations in the diencephalon of zebrafish are located in the pineal and retinal glands, and similar to what has been found in other vertebrates, 5-HT serves as a precursor to melatonin, which regulates circadian rhythms.

Cells containing 5-HT have also been found in the boundary region between the thalamus and pretectum in several species of fish, but not in amphibians, reptiles, birds or mammals, suggesting that this is a feature specific to fish alone (Lillesaar, 2011). This area appears to be responsible for the regulation of visuomotor behaviors as well as the integration of visual input in fish including zebrafish. 5-HT populations have also been located in the posterior tuberculum and hypothalamus of zebrafish, but their function is not yet known; however, it has been suggested based on past research in other species that these populations could be related to hypothalamic functions like aggression, appetite, reproduction and circadian rhythms.

Serotonergic populations found in the raphe nuclei (both the superior and inferior raphe) of zebrafish are the most easily studied populations due to their similarity to those found in the raphe nuclei in mammals. Based on these similarities, it has also been postulated that they may serve functions similar to those seen in mammalian brains; however, further investigation into the division of populations of 5-HT neurons in the raphe nuclei and their apparent projections in

zebrafish reveals that the system is not nearly as complex or specialized as the one found in many mammals. Regardless, the similarities in the presence of 5-HT populations in this structure in both fish species and other vertebrates supports the idea that this system is one that has been evolutionarily conserved.

Finally, 5-HT cells have been identified in the hindbrain of zebrafish, namely in the medulla oblongata and into the spinal cord. While it is not yet known what the apparent function of these cells is in the medulla oblongata (although again, a similarity to such populations found in other vertebrates suggests there could be similarities in function worth exploring), there is evidence to suggest that the 5-HT populations found along the spinal cord influence motor behavior.

As with existing research examining analogous systems across species, there are comparative implications for understanding the variety of behaviors that appear to be impacted by the 5-HT system. Research on the 5-HT system in this species has revealed several serotonergic functions including locomotion, aggression and fear/anxiety responses. Motor behavior in zebrafish is impacted differently by the 5-HT system depending on the developmental stage of the animal: during early stages, when spontaneous swimming first appears, activation of the 5-HT system appears to increase the frequency of swimming behavior (Brustein, et al, 2003), whereas activation of the system appears to decrease locomotion in adult zebrafish (Gabriel, et al, 2009).

The 5-HT system has also been implicated in the manipulation of aggressive behavior in a number of fish species including stickleback (Bell et al, 2007), rainbow trout (Winberg & Lepage, 1998), arctic charr (Winberg, et al, 1992), Bluehead wrasse (Perreault, et al, 2003),

Siamese fighting fish (Lynn et al, 2007) and zebrafish (Filby et al, 2010). In general, increased activation of the 5-HT system appears to decrease aggressive behavior.

Finally, the 5-HT system has also been implicated in fear and anxiety responses in several fish models including zebrafish (Cachat, et al, 2010; Egan, et al, 2009; Gerlai, 2010; Levin & Cerutti, 2009). This has been assessed through several behaviors including a "tank-behavior" (where the fish swims to the bottom of the tank when placed in a new environment), freezing, and/or erratic swimming patterns. All of these behaviors have been successfully manipulated with the introduction of drugs acting on the serotonin system, including buspirone (Bencan, et al, 2011) and fluoxetine (Maximino, et al, 2011), providing further evidence that the 5-HT system plays a role in fear and anxiety responses.

Research using zebrafish has provided a robust model for understanding the serotonergic system in teleost species. A similar species, Siamese fighting fish, has also recently been used to further investigate the link between the 5-HT system and aggressive behavior. Kania and his colleagues (2012) examined the effect of serotonin on aggressive behavior in these animals. Subjects were exposed to varying levels of fluoxetine over the course of 28 days. Fluoxetine was added to aquarium water in which the subjects were housed, and between 14 and 28 days of exposure, researchers found that fluoxetine increased levels of 5-HT at synaptic levels in these subjects. This increase in 5-HT resulted in decreased levels of conspecific aggression, demonstrating the effect of serotonin on aggression in this species. Similar research was conducted by Lynn and her colleagues (2007) in which researchers found that the effect of fluoxetine on aggressive behavior in this species could be seen in an even shorter amount of time; specifically, after three hours of exposure to the chemical, subjects demonstrated a significant decrease in their aggressive behavior.

Research conducted examining the role serotonin plays in various processes and behaviors across species including humans (Ciccocioppo, 1999; Beaver, et al, 2009), rats (Olivier, et al, 1995), hamsters (Ferris, et al, 1997), sparrows (Sperry, et al, 2005), Siamese fighting fish (Kania, et al, 2012; Lynn, et al, 2007), and goldfish (Beulig & Fowler, 2005) strongly suggests that serotonin plays an important role in behavior in several species, particularly those behaviors related to self-control.

Additionally, research on serotonin and aggressive behavior in several species including rats, (Olivier, et al, 1995), hamsters (Ferris, et al, 1997), sparrows (Sperry, et al, 2005) and Siamese fighting fish (Kania, et al, 2012; Lynn, et al, 1997) demonstrates the existence of a link between these two variables. It is important to note, however, that this research has often adopted a definition of aggression that relies on "intent to harm" (Berkowitz, 1993). Other recent definitions with a more psychological focus have incorporated fear-, anger-, and pleasure-driven motives underlying aggressive behavior (Scarpa & Raine, 1997; Blanchard, et al, 2001; Ingle, 2002). This shift in focus can be attributed, in part, to researchers that have found that aggression occurring in the absence of any clear variable of consequence (a resident intruder, for example) appears to have a reinforcing component resulting from pleasure (Potegal, 1979). It could then be argued that the neurological underpinnings of impulsive behavior (namely, serotonin) could also be related to those responsible for aggressive behavior not relying entirely on harmful intent. Examples of this can be seen in research that found that a deficiency in serotonin resulted in an increase in impulsive aggression; that is, aggression without regard to consequences (Coccaro, 1989; Virkkunen & Linnola, 1993; Mehlman, et al, 1994).

While aggression is a trait that has been studied in relation to serotonin in Siamese fighting fish (Kania, et al, 2012; Lynn, et al, 2007), there has been little if any research

examining the effect of serotonin on self-control in this species. Thus, the purpose of this study was to investigate the effect of serotonin manipulation on self-control choice behavior in *Betta splendens*. Based on existing research, it was hypothesized that an increase in serotonin would result in an increase in the proportion of delayed but larger reward choices compared to immediate but smaller reward choices.

Methods

Subjects

Subjects included 17 male Siamese fighting fish (*Betta splendens*) purchased from Live Aquaria. Subjects were housed in tanks containing dechlorinated water kept at a constant temperature of 25 degrees Celsius (72.5 degrees Fahrenheit), under a constant 12:12 h light-dark cycle. Subjects were fed a daily diet consisting of nine Betta Baby pellets (Hikari, Himeji, Japan). During choice trial days, subjects were given an adequate amount of food for completing the choice task (a minimum of 9 pellets per day). All subjects were treated in accordance with the ethical principles regarding animal treatment set forth by the American Psychological Association (APA, 2002).

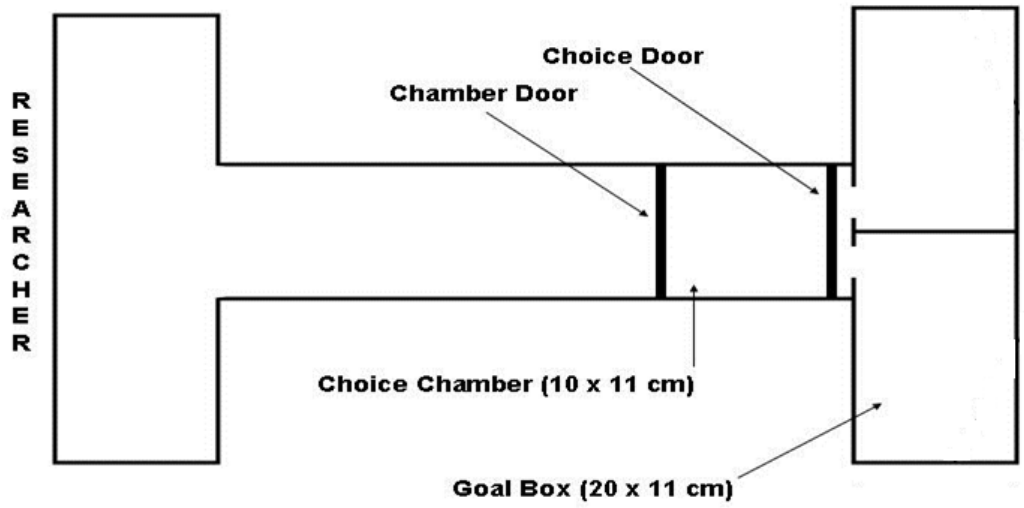
Materials

Each fish was housed singly in a 67.3 x 40.6 x 16.8 cm tank filled with approximately 28 L of dechlorinated water. Each tank was equipped with a modified T-maze (see Figure 1 for diagram of the apparatus), a gravel base, a tank heater and a temperature gauge. The discriminative stimulus consisted of contact paper printed with a black and white checkerboard pattern attached to the inside of one goal arm and the corresponding choice door. The other arm and corresponding choice door were a solid black color.

Fluoxetine, a selective-serotonin reuptake inhibitor, was used to increase serotonin levels at synapse sites. A pilot study conducted on the effect of fluoxetine on aggression in *Betta splendens* demonstrated that peak effects of fluoxetine on aggressive behavior were seen approximately 3 hours after exposure; thus, subjects were given an acclimation period of three hours after exposure prior to beginning trials.

Figure 1

Choice apparatus



Procedure

Subjects were housed in the T-mazes of their tank and completed 6 trials each day following an early morning drug administration and an acclimation period during which the subject was reintroduced to its tank. Subjects were randomly assigned to one of three fluoxetine groups, a high-level group (12.5 μMol), a mid-level group (7.5 μMol), or a control group that was not exposed to fluoxetine, such that all groups had equal numbers of subjects. Daily drug administration began at approximately 8:00 AM each day with a 5 minute interval between each subject, and involved placing the subject via a dip net into a separate container (11 cm x 7.5 cm x 7.5 cm) which contained fluoxetine dissolved into 200 mL of the subject's own tank water. The subject was exposed to the drug for 30 minutes during which the container was floated inside the subject's home tank in order to reduce stress. The control group was treated similarly to the drug

groups but no fluoxetine was added to the water. Following the 30-minute drug exposure, each subject was placed back into their home tank.

Choice trials consisting of two forced choice trials and four free choice trials began approximately 2 hours and 30 minutes after termination of the drug administration. Two forced choice trials began approximately 2 hours and 30 minutes after drug administration was completed, where the subject was only allowed access to one goal arm of the maze: during one trial, the available choice was the checkerboard side of the goal arm, and during a second trial, the available choice was the solid black side of the goal arm. The order of this availability varied depending on the day, as the discriminative stimulus was pseudo-randomly assigned each day to one side of the goal arm such that it was not assigned to the same side of the goal arm for more than two consecutive days. Free choice trials were conducted beginning approximately 3 hours and 30 minutes after the completion of drug administration. Free choice trials were run in two-trial blocks, such that two were conducted approximately 3 hours and 30 minutes after the completion of drug administration, and two were conducted approximately 4 hours and 30 minutes after the completion of drug administration. During free choice trials, each subject was given access to freely choose either side of the goal arm. The discriminative stimulus was counterbalanced across subjects such that the checkerboard pattern was associated with the larger reward, longer delay (LL) choice for half of the subjects in each group and the smaller reward, shorter delay (SS) for the other half of the subjects in each group.

To begin each trial, the subject was guided into the choice box, an area separated from the subject's living area by a divider door. A guillotine door was then raised, beginning the trial and the choice box latency (response latency) measure. Once the subject swam through a choice door opening into one of the goal arms, the choice latency period ended and the guillotine door

to the goal arm was lowered. The subject was then given either 1 pellet immediately after making a choice or 3 pellets after an 18 s delay, depending on the side of the goal arm the subject entered. The subject remained in the goal arm afterwards to be given adequate time (a maximum of 5 minutes) to consume all food pellets. The subject was then returned to the choice box and remained there until the beginning of the next trial. Choice and choice latency were recorded for each trial. Subjects remained in the choice box for the duration of trials as this reduced the stress to the animal. When a subject had completed all daily trials, all doors were removed and the subject was allowed to swim freely throughout the T-maze.

Results

The number of choices for the larger-later reward (e.g. self-control) was averaged across the last four days of the experiment for each subject. The effect of drug exposure on the proportion of larger-later choices was analyzed using a one-way analysis of variance (ANOVA). There were no significant differences in larger-later choice preference among the three groups, $F(2,16) = 1.63$, $p = 0.23$, partial $\eta^2=0.19$. A one sample *t*-test was conducted for each group compared to chance or indifferent choice (p=0.50) to determine the presence of a choice bias. All groups failed to show significant deviation from indifference; Control group ($t(5)=1.190$, p=0.29, d=0.49), 7.5 μMol group ($t(4)=0.492$, p=0.65, d=0.22), and the 12.5 μMol group ($t(5)= -1.25$, p=0.28, d=0.50).

TABLE 1

DESCRIPTIVE STATISTICS FOR PROPORTION OF LL CHOICES FOR EACH GROUP

	Control Group (0.0 µMol)	Mid-level Group (7.5 µMol)	High-level Group (12.5 µMol)
M	0.57	0.53	0.44
SD	0.15	0.11	0.13
n	6	5	6

The proportion of larger-later choices for each counterbalance group was also analyzed using a one-way analysis of variance. There were no significant differences in self-control choice preference among the three groups for either counterbalance group; counterbalance group 1 (S^D = LL), F (2, 7) = 2.43, p = 0.18, partial η^2=0.49, and counterbalance group 2 (S^D = SS), F (2,8) = 0.04, p = 0.97, partial η^2=0.01. It should be noted, however, that counterbalance group 1 demonstrated greater range of mean group differences compared to counterbalance group 2 (see table 2).

Additionally, a one sample *t*-test was conducted for each group compared to chance performance (p=0.50) to determine the presence of a choice bias in relation to the counterbalance group assignment (see Table 3). All groups failed to show significant deviation from indifference; counterbalance group 1 (S^D=LL): control group (t(2)=1.26, p=0.34, d=0.73), 7.5 µMol group (t(1)=1.00, p=0.50, d=0.71), and the 12.5 µMol group (t(2)= -1.73, p=0.23, d=1.00), counterbalance group 2 (S^D=SS): control group (a *t*-test could not be completed as the standard deviation for the group was zero), 7.5 µMol group (t(2)=0.23, p=0.84, d=0.18), and the 12.5 µMol group (t(2)=0.00, p=1.00, d=0.00).

TABLE 2

DESCRIPTIVE STATISTICS FOR PROPORTION OF LL CHOICES FOR EACH COUNTERBALANCE GROUP

Counterbalance Group 1 (S^D = Larger-Later option)

	Control Group (0.0 µMol)	Mid-level Group (7.5 µMol)	High-level Group (12.5 µMol)
M	0.65	0.53	0.37
SD	0.20	0.04	0.13
n	6	5	6

Counterbalance Group 2 (S^D = Smaller-Sooner option)

	Control Group (0.0 µMol)	Mid-level Group (7.5 µMol)	High-level Group (12.5 µMol)
M	0.50	0.52	0.50
SD	0.00	0.16	0.11
n	6	5	6

Response latency was averaged across the last four days of the experiment for each subject. The effect of drug exposure on response latency was analyzed using a one-way analysis of variance (ANOVA). There were no significant differences in response latency among the three groups, F (2,16) = 2.46, p = 0.12, partial η^2=0.26.

TABLE 3

DESCRIPTIVE STATISTICS FOR RESPONSE LATENCY FOR EACH GROUP

	Control Group (0.0 µMol)	Mid-level Group (7.5 µMol)	High-level Group (12.5 µMol)
M	58.54	124.68	107.79
SD	33.80	59.23	60.54
N	6	5	6

The average response latency for each counterbalance group was also analyzed using a one way analysis of variance. There were no significant differences in response latency among the three groups for either counterbalance group; counterbalance group 1 (S^D = LL), F (2, 7) = 0.84, p = 0.48, partial η^2=0.25, and counterbalance group 2 (S^D = SS), F (2,8) = 1.92, p = 0.23, partial η^2=0.39

TABLE 4

DESCRIPTIVE STATISTICS FOR RESPONSE LATENCY FOR EACH COUNTERBALANCE GROUP

Counterbalance Group 1 (S^D = Larger-Later option)

	Control Group (0.0 µMol)	Mid-level Group (7.5 µMol)	High-level Group (12.5 µMol)
M	51.12	113.95	73.21
SD	40.85	74.66	51.01
N	6	5	6

Counterbalance Group 2 (S^D = Smaller-Sooner option)

	Control Group (0.0 µMol)	Mid-level Group (7.5 µMol)	High-level Group (12.5 µMol)
M	65.96	131.83	142.37
SD	31.97	63.54	54.54
n	6	5	6

Discussion

In the present study, subjects were expected to have differing levels of self-control based on their exposure to varying levels of fluoxetine. One group was exposed to no fluoxetine, one group was exposed to a 7.5 μMol solution of fluoxetine, and one group was exposed to a 12.5 μMol solution of fluoxetine, in order to examine whether levels of self-control were impacted by changes in serotonin levels as a result of exposure to fluoxetine. Subjects exposed to higher levels of fluoxetine were expected to make more self-control (larger-later) choices than subjects exposed to lower levels of fluoxetine.

Overall, none of the groups demonstrated a significant preference for either choice option (larger-later or smaller-sooner), and subjects in the control group did not differ significantly in their preferences from subjects in either treatment group. The results of this study are inconsistent with existing literature on the possible relationship between the serotonergic system and behavior, and the role of fluoxetine in impacting choice behavior. One possible explanation is the impact fluoxetine may have on fear and anxiety through the 5-HT system in aquatic species. Acute exposure to fluoxetine in zebrafish resulted in more time spent exploring novel environments, less time engaging in "tank behavior" (swimming to the bottom of a tank when exposed to a new environment) (Maximino, et al, 2011), and a significant reduction in erratic movements (Egan, et al, 2009). These findings suggest that fluoxetine could impact fear and/or anxiety, specifically a reduction in stress as measured through cortisol levels (Mennigen, et al, 2011). In the present study, significant changes in anxiety, fear, or stress level may have interfered with choice preference. This explanation is only a partial one, however, due to the fact that neither the treatment groups nor the control group demonstrated a significant preference for either choice option.

Another possibility is that fluoxetine may impact learning and discrimination, and this phenomenon has previously been seen in some other aquatic species (Beulig & Fowler, 2008; Mennigen, et al, 2011). When goldfish were exposed to fluoxetine, their performance on a two-way avoidance learning task was lower than control subjects or subjects exposed to a 5-HT $_{1A}$ agonist (Beulig and Fowler, 2008) and when fathead minnows were exposed to fluoxetine, their predator avoidance behavior decreased, indicating a possible decrease in learned association (Mennigen, et al, 2011). The ability to discriminate between two choice options and to form associations between discriminative stimuli and rewards may have been impacted by fluoxetine exposure; however, it is important to note that since none of the groups in the present study demonstrated a preference for either choice option, it is possible that subjects did not fully learn the task. While fluoxetine exposure could provide explanation for why the treatment groups did not demonstrate a preference for either choice option, it is less clear why subjects in the control group did not demonstrate a preference for either choice option, but learning and association could still be factors worth considering.

While the overall results from the present study do not indicate that the subjects in any of the groups demonstrated a preference for either choice option, it is worth noting that when the results were analyzed by counterbalance groups, a trend was observed. Subjects in counterbalance group 1 (S^D=Larger-Later option) demonstrated a trend across treatment groups that suggests that when the discriminative stimulus indicated the larger-later reward option, fluoxetine may have had more of an impact on choice preference across groups (see Table 2). Subjects in counterbalance group 2 (S^D=Smaller-Sooner option) did not demonstrate a similar trend; rather, subjects across all groups in this counterbalance group demonstrated indifference in

their choice preferences (see Table 2), suggesting that the meaning of the discriminative stimulus may have influenced choice preference.

Response latency across groups is also worth discussing in further detail. While response latency did not differ significantly between the control group and the treatment groups (likely due to variation within groups, see Table 3), there was an apparent difference in groups that could be attributed to fluoxetine exposure, as the control group had a lower average response latency than either of the treatment groups (see Table 3). Fluoxetine has been found to have both a motoric effect (fluoxetine exposure decreases locomotion in adult zebrafish, Airhart, et al, 2007; Egan, et al, 2009; Gabriel, et al, 2009) and a negative effect on eating behavior in both fathead minnows (Weinberger & Klaper, 2014) and goldfish (De Pedro, et al, 1998), and both of these factors could play a role in the latency differences seen in the control group and the treatment groups in the present study. Higher average latency seen in the two treatment groups could be the result of the impact of fluoxetine on swimming behavior in these subjects, as response latency indicated the time it took a subject to swim towards then through a choice door. It could also be the result of fluoxetine's impact on food intake and thus motivation to complete the choice task in which food was the reinforcer.

There are several limitations that may also address why the hypothesis was not supported and why the results of the present study are not consistent with existing literature. First, while a two-choice task like the one used in the present study has been used frequently in past research on self-control and choice behavior (Ainslie, 1974, 1975; Mischel, 1989; Prelec & Herrnstein, 1997; Tobin & Logue, 1994; Chelonis, et al, 1994; Jackson & Hackenburg, 1996; Logue, et al, 1988), less research has been conducted using this task with *Betta splendens*. Based on past research, it was anticipated that choice behavior in *Betta splendens* could be assessed using this

task, but the results of the present study suggest that subjects did not learn the task as it was presented. It is possible that the nature of the task did not access behavior based on choice preference in the subjects in the present study.

Secondly, an aquatic model of the 5-HT system has been further developed and investigated in earnest in recent years, but some of the species most commonly used (zebrafish (*Danio rerio*), goldfish (*Carassius auratus* auratus) and fathead minnows (*Pimephales promelas*)) may differ from *Betta splendens,* particularly in regards to this system. Zebrafish, goldfish, and fathead minnows belong to the order of fish known as cypriniformes, while *Betta splendens* belong to the order known as perciformes. In addition to the physiological differences found in these two orders (Helfman, Collette & Facey, 1997), there are neurological differences between the two orders that may be of interest. Research on motor neuron organization in *Betta splendens* has demonstrated that motor neuron distribution differs between fish found in the perciformes and cypriniformes orders (Gorlick, 1989). The projections from the trigeminal motor nucleus to dilator opercula muscles and the facial motor nucleus in teleost fishes such as *Betta splendens* are responsible for respiratory and feeding movements, so differences in this particular neural organization between cypriniformes and perciformes could result in differences in motor and feeding behavior.

The role of the telencephalon in learning in teleost fishes is also important to consider, as this structure may play a different role in behavior across different aquatic species. Telencephalic ablation studies have demonstrated that this structure appears to play a role in both short-term memory and instrumental learning in teleost fish such as *Betta splendens* (Flood, 1976; Shapiro, et al, 1974). Conversely, other research using goldfish, which are cypriniformes, found that

telencephalic ablation did not impair instrumental learning (Savage, 1969), suggesting that the telencephalon may play a different role in learning in *Betta splendens* and goldfish.

Differences found in the nervous systems of perciformes and cypriniformes, as well as apparent differences in the role of certain structures in both learning and behavior across several aquatic species suggest that the 5-HT system in *Betta splendens* may be different from that of existing models. Comparisons should be made cautiously at this point, however, due to the difference in volume of research on the 5-HT system between existing models such as zebrafish and less complete models such as *Betta splendens*. Zebrafish been studied much more extensively and the organization of their serotonergic nervous system is more clearly understood than that of *Betta splendens* (Lillesaar, et al, 2011). Continued investigation into the organization of the nervous system in *Betta splendens* would be necessary to make more accurate comparisons between the two species and for further comparative applications to other fish species regarding the 5-HT system.

The findings of the present study point to the idea that *Betta splendens* may provide a unique model for studying the serotonergic system in aquatic species. While zebrafish and goldfish have served as informative models for such research thus far (Lillesaar, 2011; Beulig and Fowler, 2008), the present study suggests that the serotonergic system in Siamese fighting fish may not be as similar to that of other aquatic species as was hypothesized. Additionally, the choice task used in the present study may have presented unanticipated challenges in measuring learning and preference in this species. It is possible that such a traditional learning preparation may not produce results indicating choice preference in Siamese fighting fish; thus, this information may help inform future research on choice behavior in this species.

Future research on the neurological underpinnings of behavior, specifically the role of the serotonergic system in behavior in aquatic species, could take one of several paths. One possibility would be to investigate whether a stimulus other than food could serve as an effective reinforcer for a two-choice preparation with this species. Previous research with *Betta splendens* has demonstrated that this species finds mirror exposure highly reinforcing given the opportunity to engage in aggressive behavior towards a dummy-predator. Using mirror exposure instead of food as reinforcement in the two-choice task could produce results more in line with existing research on the impact of fluoxetine on behavior in this species (Lyn, et al, 2007).

Another possible direction for future research would be to manipulate the two-choice model such that the checkerboard pattern serves as the S^+ for reinforcement while the other side serves as the S^- for no reinforcement. In the present study, counterbalance group 1 (S^D=Larger-later option) demonstrated a greater ability to discriminate between the two choice options than counterbalance group 2 (S^D=Smaller-sooner option), suggesting that the checkerboard pattern may have impacted this learning (or lack thereof) in some way. While it is not clear why this was the case, further studies using the checkerboard pattern as an S^D for reinforcement might provide further evidence that this species has the ability to discriminate between patterns. It would also be of interest to experiment with variations of the discriminative stimulus to determine whether this species can discriminate between different patterns similar to the checkerboard pattern used in the present study.

The construct of self-control in both humans and non-human animals is one that has been examined substantially and the literature provides strong evidence for the argument that this phenomenon not only exists, but that choice behavior can be manipulated. When given the choice between a smaller, more immediate reward and a larger but delayed reward, an individual

should choose the more valuable reward even when a delay is present. However, organisms frequently display impulsive behavior when given this choice, and Ainslie (1975) presented the hyperbolic discounting theory to explain this counter-intuitive response. His theory provides the explanation that delay of a reward devalues the option and organisms act impulsively as a result. Another explanation for impulsive choice behavior is a biological one; namely, that the neurotransmitter serotonin plays a role in impulsive behavior in humans and some non-human animals species. Because serotonin appears to have a strong effect on impulsivity (and conversely, self-control), the present study aimed to find evidence supporting a relationship between fluoxetine, an SSRI found to increase levels of serotonin, and self-control. While the results did not indicate that fluoxetine exposure significantly impacted self-control, the present study was attempting to expand on previous research findings in a different species, *Betta splendens*; therefore, further research could provide more information as to the potentially unique nature of the serotonergic system in this species.

References

Abeyesinghe, S.M., Nicol, C.J., & Wathes, C.M. (2005). Can domestic fowl, *Gallus gallus domesticus,* show self-control? *Animal Behaviour, 70,* 1-11.

Ainslie, G.W. (1974). Impulse control in pigeons. *Journal of the Experimental Analysis of Behavior, 21,* 485-489.

Ainslie, G.W. (1975). Specious reward: A behavioral theory of impulsiveness and impulse control. *Psychological Bulletin, 82,* 463-496.

Airhart, M.J., Lee, D.H., Wilson, T.D., Miller, B.E., Miller, M.N., Skalko, R.G. (2007). Movement disorders and neurochemical changes in zebrafish larvae after bath exposure to fluoxetine (PROZAC). *Neurotoxicology and Teratology, 29,* 652-664.

American Psychological Association. (1992). Ethical principles of psychologists and code of conduct. *American Psychologist*, *47*, 1597-1611.

Beaver, K.M., Rachford, M., & Ferguson, C.J. (2009). Evidence of genetic and environmental effects on the development of low self-control. *Criminal Justice and Behavior, 36,* 1158-1172.

Berkowitz, L. (1993) *Aggression: Its causes, consequences and control*. New York: McGraw-Hill.

Beulig, A. & Fowler, J. (2008). Fish on Prozac: Effect of serotonin reuptake inhibitors on cognition in goldfish. *Behavioral Neuroscience, 122,* 426-432.

Blanchard, D.C., Griebel, G., Blanchard, R.J. (2001) Mouse defensive behaviors: Pharmacological and behavioral assays for anxiety and panic. *Neuroscience & Biobehavioral Reviews, 25,* 205-218.

Chelonis, J.J., King, G., Logue, A.W., & Tobin, H. (1994). The effect of variable delays on self-control. *Journal of the Experimental Analysis of Behavior, 62,* 33-43.

Ciccocioppo, R. (1999). The role of serotonin in craving: From basic research to human studies. *Alcohol & Alcoholism, 34,* 244-253.

Coccaro, E.F. (1989). Central serotonin and impulsive aggression. *The British Journal of Psychiatry, 155,* 52-62.

De Pedro, N., Pinillus, M.L., Valencio, A.I., Alonso-Bedate, M., & Delgado, M.J. (1998). Inhibitory effect of serotonin on feeding behavior in goldfish: Involvement of CRF. *Peptides, 19,* 505-511.

Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kalueff, A.V. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research, 205,* 38-44.

Ferris, C.F., Melloni, R.H., Koppel, G., Perry, K.W., & Fuller, R.W. (1997). Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. *The Journal of Neuroscience, 17,* 4331-4340.

Flood, N.C. (1976). Teleost telencephalon and learning: An interpretive review of data and hypotheses. *Physiology & Behavior, 16,* 783-798.

Gabriel, J.P., Mahmood, R., Kyriakatos, A., Soll, I., Hauptmann, G., Calabrese, R.L., El Manira, A. (2009). Serotonergic modulation of locomotion in zebrafish: endogenous release and synaptic mechanisms. *The Journal of Neuroscience, 29,* 10387-10395.

Gorlick, D.L. (1989). Motor innervation of respiratory muscles and an opercular display muscle in Siamese fighting fish. *Journal of Comparative Neurology, 290,* 412-422.

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., et al. (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry*, *66*, 2621-2624.

Helfman, G., Collette, B., & Facey, D. (1997). *The Diversity of Fishes.* Retrieved from http://www.fishbase.org.

Herrnstein, R.J. (1961). Relative and absolute strength of response as a function of frequency of reinforcement. *Journal of the Experimental Analysis of Behavior, 4,* 267-272.

Hu, X. Z., Lipsky, R. H., Zhu, G., Akhtar, L. A., Taubman, J., Greenberg, B. D., et al. (2006). Serotonin transporter promoter gain-of-function genotypes are linked to obsessive compulsive disorder. *American Journal of Human Genetics*, *78*, 815-826.

Ingle, D. (2002). Recreational aggression: Cultural and biological dimensions. *Aggressive Behavior*.

Jackson, K. & Hackenberg, T.D. (1996). Token reinforcement, choice, and self-control in pigeons. *Journal of the Experimental Analysis of Behavior, 66,* 26-49.

Kania, B.F., Gralak, M.A., & Wielgosz, M. (2012). Fluoxetine (SSRI) exposure diminishes aggressive behavior of male Siamese fighting fish (*Betta splendens*). *Journal of Behavioral and Brain Science, 2,* 185-190.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, *274*, 1527-1531.

Lillesaar, C. (2011). The serotonergic system in fish. *Journal of Chemical Neuroanatomy, 41,* 294-308.

Logue, A.W. (1986). *The Psychology of Eating and Drinking.* New York: W.H. Freeman and Company. *104-106.*

Logue, A.W., Chavarro, A., Rachlin, H., & Reeder, R.W. (1988). Impulsiveness in pigeons living in the experimental chamber. *Animal Learning & Behavior, 16,* 31-39.

Logue, A.W., Tobin, H., Chelonis, J.J., Wang, R.Y, Geary, N., & Schachter, S. (1992). Cocaine decreases self-control in rats. *Psychopharmocology, 109,* 245-247.

Lynn, S.E., Egar, J.M., Walker, B.G., Sperry, T.S. & Ramenofsky, M. (2007). Fish on Prozac: A simple, non-invasive physiology laboratory investigating the mechanisms of aggressive behavior in *Betta splendens*. *Advances in Physiology Education, 31,* 358-363.

Maximino, C., da Silva, A.W., Gouveia Jr, A., Herculano, A.M. (2011). Pharmacological analysis of zebrafish (*Danio rerio*) scototaxis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 25,* 624-631.

Mehlman, P.T., Higley, J.D., Faucher, I., Lilly, A.A., Taub, D.M., Vickers, J., Suomi, S.J., Linnoila, M. (1994). Low CSF 5-HIAA concentrations and severe aggression and impaired impulse control in nonhuman primates. *American Journal of Psychiatry, 151,* 1485-1491.

Mennigen, J.A., Stroud, P., Zamora, J.M., Moon, T.W., & Trudeau, V.L. (2011). Pharmaceuticals as neuroendocrine disruptors: lessons learned from Fish on Prozac. *Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 14: 5-7,* 387-412.

Miczek, K.A., Fish, E.W., de Bold, J.F., de Almeida, R.M.M. (2002). Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine, and γ-aminobutyric acid systems. *Psychopharmacology, 163,* 434-458.

Mischel, W., Shoda, Y., & Rodriguez, M.L. (1989). Delay of gratification in children. *Science, 244,* 933-938.

Olivier, B., Mos, J., van Oorschot, R. & Hen, R. (1995). Serotonin receptors and animal models of aggressive behavior. *Pharmacopsychiatry, 28,* 80-90.

Potegal M (1979) The reinforcing value of several types of aggressive behavior: a review. *Aggressive Behavior, 5,* 353–373.

Prelec, D. & Herrnstein, R.J. (1997). Preferences or principles: Alternative guidelines for choice. *The Matching Law: Papers in Psychology and Economics.* Cambridge, MA: Harvard University Press. *293-312.*

Raine, A. (1993). *The psychopathology of crime: Criminal behavior as a clinical disorder.* San Diego, CA: Academic Press.

Reist, C., Mazzanti, C., Vu, R., Tran, D., & Goldman, D. (2001). Serotonin transporter promoter polymorphism is associated with attenuated prolactin response to fenfluramine. *American Journal of Medical Genetics*, *105*, 363-368.

Sauvage, G.E. (1969). Some preliminary observations on the role of the telencephalon in food-reinforced behavior in the goldfish (*Carassus auratus*). *Animal Behavior, 17,* 760-772.

Scarpa, A. & Raine, A. (1997). Psychophysiology of anger and violent behavior. *Psychiatric Clinics of North America, 20,* 375-394.

Shapiro, S., Shuckman, H., Sussman, S., & Tucker, A.M. (1974). Effects of telencephalic lesions on the gill cover response of Siamese fighting fish. *Physiology & Behavior, 13,* 749-755.

Sperry, T.S., Moore, I.T., Meddle, S.L., Benowitz-Fredericks, Z.M., Wingfield, J.C. (2005). Increased sensitivity of the serotonergic system during breeding season in free-living American tree sparrows. *Behavioural Brain Research, 157,* 119-126.

Tobin, H. & Logue, A.W. (1994). Self-control across species. *Journal of Comparative Psychology, 108,* 126-133.

Tobin, H., Logue, A.W., Chelonis, J.J., & Ackerman, K.T. (1996). Self-control in the monkey *Macaca facicularis*. *Animal Learning & Behavior, 24,* 168-174.

Virkkunen, M. & Linnoila, M. (1993). Brain serotonin, type II alcoholism, and impulsive violence. *Journal of Studies on Alcohol and Drugs, 11,* 163-169.

Weinberger, J. & Klaper, R. (2014). Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in fish (*Pimephales promelas*) (fathead minnows). *Aquatic Toxicology, 15,* 77-83.