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# Sex Differences In The Dopaminergic Regulation Of Courtship, But Not Pairing Behaviors In Zebra Finches

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**SEX DIFFERENCES IN THE DOPAMINERGIC REGULATION OF COURTSHIP, BUT NOT PAIRING BEHAVIORS IN ZEBRA FINCHES**

**by**

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## DEDICATION

This work is dedicated to my wonderful parents, Pat and Cheryl Lowrey, for instilling in me the values of education, motivation, and hard work. Thank you for your unconditional love, belief, and support. To my brother, Patrick Lowrey, for his love, support, and constant cheerleading. To Dr. Tina Lowrey, Dr. L.J. Shrum, and Dr. Ronald Hess for setting the bar so high. Your love, support, and advice are greatly appreciated and you continue to inspire me. I have been blessed to have so many wonderful friends and loved ones in my life and for this, I shall always be grateful. To Aki, Bailey, and Mimi for their patience, unconditional love, and for always being excited when I get home, no matter how late.

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# CHAPTER 1
# INTRODUCTION

Relationships are strongly linked to physical and mental well being in social species. Dopamine (DA) is one of the key ingredients in the glue that cements social bonds in vertebrates (Aragona & Wang, 2009; Bales, Mason, Catana, Cherry, & Mendoza, 2007; Broad, Curley, & Keverne, 2006). Dopamine receptors are widely distributed throughout the limbic system, a network of brain regions that has been strongly implicated in reward (Missale, Nash, Robinson, Jaber, & Caron, 1998). Dopamine plays a role in several appetitive and rewarding behaviors, especially those associated with sexual activity (Balthazart & Absil, 1997; Castagna, Ball, & Balthazart, 1997; Hull & Dominguez, 2007; Woolley, Sakata, Gupta, & Crews, 2001). Research has shown that dopamine is also implicated in pair relationships in several monogamous species, including humans (Aragona & Wang, 2009; Fisher, Aron, & Brown, 2006; Goodson, Kabelik, Kelly, Rinaldi, & Klatt, 2009).

Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain and is implicated in a variety of behaviors including locomotor activity, emotion, positive reinforcement, cognition, learning and memory, arousal, and food intake (Durstewitz, Kroner, & Gunturkun, 1999; Missale, et al., 1998). DA receptors are classified in two groups, D1-like receptors (D1 and D5) and D2-like receptors (D2, D3, and D4) (Neve, Seamans, & Trantham-Davidson, 2004). D1 and D2 receptors are segregated on separate neurons, with only a small number exhibiting co-expression (Missale, et al., 1998), indicating that these two groups of neurons have different neuronal effects. In mammals, D2 receptor expression is largest in the striatum (or caudate putamen), the olfactory tubercule, and the core of the nucleus accumbens (Missale, et al., 1998). D2 receptor mRNA is located in the prefrontal, cingulate, temporal, and

entorhinal cortices, the amygdala, the hippocampus, the substantia nigra pars compacta (SNC), and the ventral tegmental area (VTA) (Durstewitz, et al., 1999; Missale, et al., 1998). D2 receptor expression, distribution, and segregation all indicate that it has differential functions from D1 receptors in the mammalian brain.

Decades of dopaminergic research have shown that there is similar structural/anatomical organization of the dopaminergic system in birds and mammals, as well as similar functional organization (Durstewitz, et al., 1999). Though the organization of avian telencephalic areas is quite different from that of mammals (Durstewitz, et al., 1999), there are many homologous brain areas between mammalian and avian species. For instance, the striatum, or caudate putamen, receives the densest amount of dopaminergic fiber input, and contains the largest amount of D2 receptors in rats, primates, and birds (Ding & Perkel, 2002; Durstewitz, et al., 1999; Levey et al., 1993). Comparable to mammals, the dopaminergic innervations of the avian telencephalon begin from cell populations located in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) (Durstewitz, et al., 1999). From these two areas, D2 receptor fibers innervate the basal ganglia, dorsal hippocampus, and medial area parahippocampalis (APH) (Durstewitz, et al., 1999). Evidence from the avian telencephalon is congruent with evidence from the mammalian telencephalon, thus, the differential expression and distribution of D2 and D1 receptors indicate differential neuronal effects.

Researchers have used several techniques to measure or approximate dopamine receptor expression in the avian and mammalian brain. One technique is tyrosine hydroxalayse (TH) immunoreactivity; TH is the rate-limiting enzyme in the synthesis of all catecholamines and must be present in dopaminergic neurons, with a few exceptions (Durstewitz, et al., 1999; Missale, et al., 1998; Neve, et al., 2004). In general, the distribution of TH fibers closely follows

the distribution of DA fibers, except in the APH and the Nucleus taeniae of the amygdala (TnA), which are high in TH fibers, but low in DA fibers (Durstewitz, et al., 1999). It is likely that norepinephrine is more prevalent in these two regions. Others have used Fos-immunoreactivity; FOS is the protein product of the immediate-early gene c-fos and is considered to be a general marker of neuronal activity. Fos has been shown to be one of the final targets in the signaling cascade of DA receptors and appears to be required for long-lasting modifications of gene expression in response to acute stimuli (Missale, et al., 1998). TH and Fos expression are the two primary methods used to measure dopaminergic activity and expression by the researchers in the field, but neither technique is specific to a particular receptor class, or even to dopamine in general. Perhaps the best technique for locating specific dopamine receptors is *in situ* hybridization, which uses species-specific probes to measure mRNA expression (Kubikova et al, 2010).

Dopamine is implicated in a variety of appetitive and reward behaviors, such as sexual activity and drug use (Balthazart & Absil, 1997; Castagna, et al., 1997; Hull & Dominguez, 2007; Pfaus, 2009; Woolley, et al., 2001). Dopamine and reward pathways in the brain have been implicated in drug use and abuse behaviors, as well as addiction (Berridge, 2004). Specifically, in an animal model of cocaine-seeking behavior, the D2 receptor has been shown to mediate the incentive to seek further cocaine reinforcement (Missale, et al., 1998). Sexual activity, a highly rewarding behavior, has also been strongly associated with the release of DA in reward areas of the brain, similar to the areas activated by drug use (Besson et al., 2010; Dalley & Everitt, 2009) in both mammals and birds. This research provides evidence for dopamine as an important chemical messenger in the rewarding aspects of sexual activity.

However, do social relationships, such as those involving members of a monogamous pair, also involve the dopaminergic system beyond DA release during sexual behavior? Dopamine has even been indirectly linked to the feeling of "love" in humans (Fisher, et al., 2006). Researchers have used fMRI imaging to link feelings of love to activation of particular brain regions implicated in dopaminergic reward and motivation pathways, including the right VTA and right caudate-putamen (Fisher, et al., 2006), two brain areas shown to be dense in D2 receptor expression in both mammalian and avian species. This indicates that dopamine, specifically the D2 receptor, may be implicated in monogamous pair bonding behavior in humans.

Dopamine is one of the primary regulators of monogamous pair bonding behavior in a popular animal model, the prairie vole. Research in this small rodent has shown that the D1 and D2 dopamine receptors have differential effects on pairing. D2 antagonists block the formation of a partner preference in mating female voles, while D2 agonists facilitate formation of a partner preference in the absence of mating (Gingrich, Liu, Cascio, Wang, & Insel, 2000). Additionally, mating-induced DA release selectively activates D2 receptors to promote pair bond formation in male prairie voles (Aragona & Wang, 2009). These studies indicate that the D2 receptor is implicated in the motivation to form a pair bond in both male and female prairie voles.

The D1 receptor is not activated during initial pair bonding, but is important for the maintenance of the pair bond once it has been formed. Activation of D1-like receptors within the shell of the nucleus accumbens fails to induce partner preferences and can override the activation of D2-like receptors, which induces partner preferences when D1 and D2 receptors are simultaneously activated in female prairie voles (Aragona & Wang, 2009). Female prairie voles in an established pair bond have enhanced D1-like signaling system in the nucleus accumbens,

which may serve to maintain the pair bond (Aragona & Wang, 2009). The density of D1-like receptors present in the nucleus accumbens is significantly greater in pair bonded males than sexually naïve males and sibling-paired controls (Aragona & Wang, 2009). A separate control group of males who had mated, but had not established a pair bond did not demonstrate an increase D1-like receptor density, indicating that mating alone is not sufficient to induce this change (Aragona & Wang, 2009). Pair bonded males show preferences for their partners and will ignore or even attack a novel female. This demonstration of selective aggression and the increase in D1-like receptor density indicate that D1-like dopamine receptors may be responsible for maintaining pair bonds in male prairie voles, while previous studies implicate D2-like receptors in the establishment of the initial pair bond in both male and female prairie voles (Aragona & Wang, 2009). The prairie vole literature indicates a role for dopamine in monogamous pair bonding that extends beyond sexual reward, but this role is dependent on the dopamine receptor type.

Although research across a wide array of species has demonstrated the role of dopamine in courtship and sexual behavior, at present, the role of dopamine in pairing behavior has been largely restricted to a single study species, the prairie vole. While the prairie vole has provided important insight into the neural processes of monogamous social relationships in mammals, this species relies heavily on chemosensory cues to establish a pair bond (Aragona & Wang, 2009). Thus, direct links between results from prairie vole research and less olfactory species, such as humans, primates, or birds, need to be made with caution. Perhaps a more appropriate model of monogamous social relationships is the zebra finch, a species that relies heavily on visual and vocal cues to establish pair bonds and recognize partners. Zebra finches live in complex social colonies, establish lifelong pair bonds even after weeks of acoustic and visual separation, and are

bi-parental, making them behaviorally similar to humans (Zann, 1996). In terms of neuroanatomy, areas of the mammalian brain containing large numbers of dopaminergic neurons are similar in structure and function to those in the avian brain (Gale & Perkel, 2005).

Additionally, a prairie vole's average lifespan is 40-65 days in the wild and they typically form pair bonds at 30-40 days of age (Curtis, 2010), leaving little time to study the pair bond. Zebra finches typically live for 5 years in the wild, as long as 12 years in captivity, and they form pair bonds relatively early in life, beginning at age 60 days post-hatching (Zann, 1996). This indicates that monogamous pair bonds are a vital part of lifetime social development in the zebra finch. Taken together, these facts support the use of the zebra finch as a good animal model of social monogamy.

There are several studies indicating that monogamous behavior activates reward pathways in the brain in the zebra finch. The VTA is an area of the midbrain containing a great number of dopaminergic neurons and is highly involved in reward and motivation in humans, mammals, and birds. Comparisons of various estrildids (finch species) that differ in their degree of monogamy and sociality have shown that the number of TH-ir neurons in the caudal VTA positively correlates with the degree of sociality (Goodson, et al., 2009). In male zebra finches, a highly social, monogamous species, immediate early gene (FOS) activity in TH-imunoreactivity (tyrosine hydroxylase, the rate limiting enzyme in catecholamine production) neurons of the caudal VTA is significantly correlated with the amount of courtship singing (Goodson, et al., 2009), such that the number of TH-ir neurons in the caudal VTA is greater in courting male zebra finches than in non-courting males. Huang and Hessler (2008) have also shown that VTA dopaminergic neurons are more strongly activated during courtship singing than non-courtship singing. In the male European starling, a social songbird, Heimovics and Riters (2008) also

found a significant relationship between measures of tyrosine-hydroxylase immunoreactive (TH-ir) neurons in the VTA in response to female-directed songs. Male starlings treated with GBR-12909, a DA reuptake inhibitor, increased their female-directed singing behaviors and showed increased levels of TH-ir in the VTA, as well as in the preoptic area (POM), an area involved in mating behaviors, and Area X, an area involved in song learning. These data indicate that dopaminergic receptors in the VTA are implicated in the socially monogamous behavior of male zebra finches.

Areas of the brain with dopaminergic innervations from the VTA have also been implicated in courtship and pair bonding behaviors in the male zebra finch. An area associated with dopaminergic activity and the expression of courtship behaviors is the midbrain central gray (CG), homologous to the mammalian periaqueductal gray, which is implicated in sexual activity. The number of TH-ir neurons in this area correlates significantly with levels of courtship motivation and that TH-FOS co-localization in this area was strongly correlated with the number of female-directed songs produced by males in the final trial (Goodson, et al., 2009). The expression of the immediate early gene *egr-1* is higher during undirected than during directed singing in the song nuclei Area X, the lateral magnocellular nucleus of the anterior nidopallium (LMAN) and the robust nucleus of the arcopallium (RA) in the male zebra finch (Hara, Kubikova, Hessler, & Jarvis, 2007). These song areas are also innervated by the midbrain ventral tegmental area–substantia nigra pars compacta (VTA–SNc) complex, which modulates motivation via dopaminergic input (Hara, et al., 2007). It has been demonstrated that DA neurons in the male zebra finch had a significantly heightened FOS response to sexual interactions and to the performance of courtship singing (Bharati and Goodson, 2006). Singing has been associated with increased dopamine levels in Area X. Dopamine levels in Area X were

significantly higher with directed relative to undirected singing (Sasaki, Sotnikova, Gainetdinov, & Jarvis, 2006). These studies provide evidence for the link between courtship behaviors in male zebra finches, such as directed signing, and dopamine activation in reward areas of the brain.

More direct manipulations of courtship behaviors via dopamine receptor agonists or antagonists have also supported the link between dopamine and pair bond formation in the male zebra finch. Males implanted with an osmotic pump containing 0.5 μg/g/day of the D1/D2 receptor antagonist *cis*-flupenthixol demonstrate decreases in female-directed singing, high-intensity courtship displays, copulation, and grooming behaviors than males receiving saline (Harding, 2004). In a similar study, Rauceo and colleagues (2008) compared the above doses to a high dose of the D1/D2 antagonist (saline, 0.5, or 5 μg/g/day). Males treated with a high dose (5 μg/g/day) sang to females significantly less than males in the other two groups. Low-dose (0.5 μg/g/day) males demonstrated significantly fewer high-intensity courtship displays. These studies demonstrate a role for dopamine in male courtship behaviors, but the role of specific dopamine receptor types was not addressed. Further study is needed to determine if the D2 receptor is responsible for pair bond formation zebra finches as in prairie voles (Aragona & Wang, 2009).

Despite the preponderance of studies linking dopamine and male courtship behavior, there have been few studies on the role of dopamine in female courtship behaviors in avian species. However, some researchers are beginning to acknowledge the importance of the female's role in pair bonding. A region in the social behavior network (nucleus taeniae of the amygdala, the avian homologue of the mammalian amygdala) has been found to have higher levels of ZENK (an immediate early gene similar to FOS) expression when females were

reunited with their male partners than with a novel male (Svec, Licht, & Wade, 2009). Pawlisch and Riters (2010) have studied female preference for male song in female European starlings treated with GBR-12909, a DA reuptake inhibitor. Untreated females showed greater preference for high incentive songs (long songs from male starlings) than for lower incentive songs (short songs from male starlings or heterospecific songs from male purple martins), while treated females responded to all songs. These treated females also displayed increased FOS immunolabeling in the ventromedial nucleus of the hypothalamus (VMH). Taken together, these studies support the role of dopamine in female courtship and pair bonding behaviors and the necessity of further study in this area.

Prairie vole studies have implicated the dopamine D2 receptor in the establishment of the pair bond (Aragona & Wang, 2009), but the role of this receptor in pair bonding in the zebra finch is unknown. Studies of the zebra finch have established the relationship between DA expression in reward areas of the avian brain and courtship behaviors (Bharati & Goodson, 2006; Hara, et al., 2007; Harding, 2004; Rauceo, et al., 2008; Svec, Lookingland, & Wade, 2009). Female mate choice and pair bonding behaviors also deserve further study, as females are highly involved in the courtship process, and may have the majority of control over the establishment of the pair bond (Tomaszycki & Adkins-Regan, 2005; Zann, 1996). Our first hypothesis is that the administration of raclopride, a specific D2 receptor antagonist, will decrease the demonstration of courtship and pairing behaviors in both male and female zebra finches. Raclopride has been shown to significantly alter D2 activity in the brain (Svec, Lookingland, et al., 2009), and a D1/D2 receptor antagonist decreases courtship behaviors in male zebra finches (Harding, 2004; Rauceo, et al., 2008) Our second hypothesis is that the administration of vanoxerine (GBR-12909), a DA reuptake inhibitor, would have the opposite effect, increasing such behaviors in

both males and females, as vanoxerine has been used to study female starlings responses to male song (Pawlisch & Riters, 2010) and increases singing behavior in the male European starling (Schroeder & Riters, 2006).

## Chapter 2

## Methods

### *2.1 Subjects*

Thirty two adult zebra finches (*Taenopygia guttata*) were subjects in this study (16 males and 16 females). One female had to be removed from the study due to an adverse reaction to the DA reuptake inhibitor. The birds were housed in stainless steel flight cages in a temperature (70°F) and humidity (50%) controlled room with a regulated light cycle (12:12). A vitamin-supplemented commercial finch seed mix (Simple System Breeder Crumb 5-Day Product, The Bird Care Company, Hemet, CA), water, grit, and cuttlebone was available *ad libitum*. This diet was supplemented with hard-boiled eggs, wheat bread, and calcium supplement. All animals were housed in isosexual aviaries prior to the experiment. During the testing phase, the test subjects (four males and four females) were housed in smaller cages (91.4 ×76.2 × 76.2 cm), and separated by sex in between tests.

### *2.2 Experimental Design*

For each test, eight birds of each sex (4 cohorts total) were placed into the following testing paradigm, using a repeated measures design (see Figure 1). These birds were chosen at random from the male and female aviaries. When these birds were returned to the aviaries after the study, they had a second leg band added so that they would not be chosen for later studies. Four birds of one sex were injected 15 minutes prior to introducing 4 birds of the opposite sex. To control for possible cohort effects, two of these birds received saline (control) and two received the drug. The birds were then observed for one hour, and all courtship and pairing behaviors were noted. After one hour, the sexes were separated overnight. Twenty-four hours later, subjects were again placed in the same conditions with the same animals without receiving

any injection, and their behavior was observed for one hour. The purpose of this testing day was to determine if the administration of dopamine agonists/antagonists from the previous day increased or decreased, respectively the probability of pair bond formation. One week separated the tests, to allow birds to become re-motivated to engage in courtship and pairing. Each bird in each group received one of two trial sets: Raclopride during one session and saline during one session or Vanoxerine during one session and DMSO during one session.

*2.3 Drug administration*

Animals were injected with raclopride (Raclopride; Sigma [cat # R-121]), 1mg/kg in 0.05ml of 0.9% sterile saline, a D2 receptor antagonist, or the same volume of saline. In the second phase of the experiment, animals were injected with vanoxerine/GBR-12909 (GBR; Sigma [cat # D-052]), 10 mg/kg in 0.05ml of DMSO, a DA reuptake inhibitor, or the same volume of DMSO. Once the drugs were prepared, observers were blind to treatment groups.

*2.4 Behavioral Tests*

After the sexes were placed in the cage together (4 males and 4 females), their behavior was recorded on data sheets over the course of an hour, which was divided into 15 minute intervals. Two observers observed two birds each per 15 minute interval, so the four injected birds were observed for the entire hour session. These observers were trained until there was a high degree of inter-observer reliability. The behaviors recorded are all associated with courtship and mating, as demonstrated through naturalistic observations and previous research (Tomaszycki & Adkins-Regan, 2005). These behaviors are female-directed singing (only males sing, and the song that is measured is directed toward a female); allopreening, clumping, tail-quiver, nest building or entering a nest together, and copulation (see Table 1). Singing and

clumping are measured in seconds using a timer and tail-quivers, nest building, allopreening, and copulation are counted using tally marks (one mark for each bout of the behavior).

Many researchers seek to normalize group behavior at the outset of an experiment. When using zebra finches, researchers will test males with several different females and record their behavior. The researchers will then choose only the males with the highest level of courtship behaviors to participate in the study. Additionally, to ensure the continuation of this behavior, researchers will administer exogenous testosterone (Bharati & Goodson, 2006; Rauceo, et al., 2008). In our quest to use a more naturalistic paradigm, we have not pre-tested birds for courtship behaviors. We chose both male and female birds at random from our population and these birds may have had differing baseline levels of courtship behavior. The repeated measures experimental design, however, controls for any baseline behavioral differences among the subjects because each bird serves as its' own baseline and experimental condition. Any difference in baseline behaviors between subjects should be controlled for by repeated measures. The birds were also allowed a choice between four opposite sex birds per session, which allows for a more natural choice condition.

*2.5 Statistical Analysis*

All behavioral observation data (see Table 1) were analyzed using repeated measures ANOVAs. Post-hoc analyses were conducted using paired sample t-tests. We also assessed preference using an association index. The association index was determined by finding the opposite-sex partner that each subject spent the greatest amount of time with (clumping for females, singing and clumping for males) on Day 1. For example, for males, the number of seconds each male spent singing and clumping with his "preferred female" each day was divided by the total number of seconds engaged with all females in these behaviors for that day to create

a percentage. An arcsin transformation was performed on these percentages. A repeated measures ANOVA was then conducted to compare the effects of treatment and day on the association index. Paired t-tests were used to analyze any significant interactions in the ANOVA. Furthermore, we examined the percentage of opposite-sex individuals that our subjects interacted with on each day (see Table 2). Again, an arcsin transformation was performed to normalize the data and a repeated measures ANOVA was conducted to compare the effects of treatment and day. Paired t-tests were used to analyze any significant interactions in the ANOVA.

# Chapter 3

# Results

## *3.1 Raclopride: Males*

### *Behavioral Observations*

Raclopride treatments did not alter pairing behavior (clumping, allopreening and nest building) female receptivity (tail quivers) or sexual behavior (copulations) on either testing day. Within treatment groups, there was no effect of day on these behaviors (see Table 4 for means and standard errors).

We predicted that raclopride, a dopamine antagonist, would decrease courtship behavior. There was a significant main effect of treatment on directed singing, $F(1,7) = 15.64$, $p = 0.01$, such that males treated with raclopride spent less time courting females than they did in the saline condition (see Table 2). There was also a treatment by day interaction, $F(1,7) = 5.63$, $p = 0.05$, such that this difference occurred on the injection day (day 1) ($t(7) = -3.19$, $p < .05$; see Figure 2), and not on testing day 2 ($t(7) = -0.02$, $p = .986$; see Table 2 and Figure 2). Finally, there were no significant effect of day on directed singing, $F(1,7) < 1$ (see Table 2).

### *Association Index*

There was a significant main effect of day, $F(1,7) = 19.18$, $p < 0.001$, such that males spent more time singing and clumping with their "preferred female" on day 1 ($M = .82$, $SE = .13$) than on day 2 ($M = .20$, $SE = .06$; see Table 2 and Figure 3). There was no significant effect of treatment, $F(1,7) = 1.53$, $p = 0.26$ or an interaction between treatment and day, $F(1,7) = 3.98$, $p = 0.09$ on the likelihood of forming a partner preference (see Table 2 and Figure 3).

*Percentage of Females Courted*

When treated with raclopride, males (M = 0.41, SE = 0.09) courted significantly fewer females than when treated with saline (M = 0.92, SE = 0.08; see Table 2 and Figure 4). There was a significant main effect of treatment, $F(1,7) = 18.85$, $p < 0.001$, such that males courted a lower percentage of females when treated with raclopride (M = 0.41, SE = 0.09) than when treated with saline (M = 0.92, SE = 0.08; see Table 2 and Figure 4). There was also a significant effect of treatment by day, $F(1,7) = 37.9$, $p < 0.001$ (see Table 2). On Day 1, males treated with raclopride courted a smaller percentage of females (M = 0.19, SE = 0.07) than when treated with saline (M = 1.21, SE = 0.14; see Table 2 and Figure 4). On Day 2, this effect was no longer apparent (raclopride: M = 0.64, SE = 0.17; saline: M = 0.62, SE = 0.11; see Table 2 and Figure 4).

*3.2 Raclopride: Females*

*Behavioral Observations*

As with males, no significant effects of treatment, day, or treatment by day were found for directed singing, clumping, allopreening, nest building, tail quivers, or copulation behaviors (see Table 5 for means and standard errors).

*Association Index*

The best marker of female choice is clumping behavior (Zann, 1996). However, there were no significant main effects for day, $F(1,7) = 0.42$, $p = 0.54$, treatment, $F(1,7) = 0.01$, $p = 0.94$, or treatment by day, $F(1,7) = 0.01$, $p = 0.95$; see Table 3 and Figure 3) on the association index.

*Interactions with Different Males*

There were no significant main effects for day, $F(1,7) = 2.49$, $p = 0.16$, treatment, $F(1,7) = 0.01$, $p = 0.92$, or treatment by day, $F(1,7) = 0.12$, $p = 0.74$, on the percentage of males with whom females clumped (see Table 3 and Figure 4)

*3.3 Vanoxerine: Males*

Vanoxerine treatments did not alter pairing behavior (clumping, allopreening and nest building) female receptivity (tail quivers) or sexual behavior (copulations) on either testing day. Within treatment groups, there was no effect of day on these behaviors (see Table 4 for means and standard errors).

*Directed Singing Behavior*

We predicted that vanoxerine would have the opposite effect on courtship behavior from raclopride, such that it would increase courtship behavior relative to the control condition. There was a significant main effect of treatment, $F(1,7) = 8.63$, $p = 0.02$ and treatment by day, $F(1,7) = 16.86$, $p = 0.01$, but not for day, $F(1,7) = 0.70$, $p = 0.43$ (see Table 2). Surprisingly, vanoxerine had a similar effect on behavior to raclopride in that males spent significantly less time singing to females during the vanoxerine condition than the DMSO condition ($t(7) = -6.43$, $p < 0.001$; see Table 2 and Figure 5). The effects from day 1 were not apparent on day 2. Males spent equal amounts of time singing to a female regardless of treatment ($t(7) = 0.84$, $p = 0.43$; see Table 2 and Figure 5). The decrease in male behavior during the DMSO condition on day 2 was evident in day 1 vs. day 2 comparisons. Males, when treated with DMSO, spent significantly more time singing to females on day1 in comparison to day 2 ($t(7) = 2.61$, $p < 0.05$; see Table 2 and Figure 5).

*Association Index*

There was no significant main effect of treatment, $F(1,7) = 0.28$, $p = 0.61$, day, $F(1,7) = 1.57$, $p = 0.25$, or treatment by day, $F(1,7) < 1$ (see Table 2 and Figure 6).

*Percentage of Females Courted*

There was a significant main effect of treatment, $F(1,7) = 5.40$, $p = 0.05$, such that males courted a significantly lower percentage of females when being treated with vanoxerine than when treated with DMSO ($t(7) = -2.27$, $p = 0.04$), indicating that vanoxerine decreased courtship behaviors in males compared to DMSO treatment (see Table 2). There was no significant effect for day, $F(1, 7) = 1.18$, $p = 0.31$ or treatment by day, $F(1, 7) = 3.50$, $p = 0.10$ (see Table 2 and Figure 7).

*3.4 Vanoxerine: Females*

*Behavioral Observations*

As with males, no significant effects of treatment, day, or treatment by day were found for directed singing, clumping, allopreening, nest building, tail quivers, or copulation behaviors (see Table 5 for means and standard errors).

*Association Index*

There were no significant main effects for day, $F(1,7) = 0.65$, $p = 0.45$, treatment, $F(1,7) = 3.61$, $p = 0.10$, or treatment by day, $F(1,7) = 0.01$, $p = 0.91$ (see Table 3 and Figure 6) on association with a preferred male.

*Interactions with Different Males*

A one way ANOVA revealed that there was a significant main effect of day, $F(1,7) = 7.31$, $p = 0.03$, such that females interacted with a lower percentage of males on day 1 (see Table

3). There were no significant main effects for treatment, $F(1,7) = 0.08$, $p = 0.79$, or treatment by day, $F(1,7) = 3.11$, $p = 0.12$ (see Table 3 and Figure 7).

# Chapter 4

# Discussion

## *4.1 Raclopride*

We hypothesized that raclopride, a selective D2 receptor antagonist, treatments would decrease courtship and pairing in male and female zebra finches. In male zebra finches, raclopride treatment did decrease courtship behavior, as measured by directed singing. This finding replicates previous findings which used a D1/D2 antagonist (Rauceo, et al., 2008). Our results suggest that the effect on singing in those studies might be attributable to DA acting at D2 receptors. However, studies using a selective D1 antagonist should be done before firm conclusions can be made.

Despite differences in courtship behavior, raclopride treatments did not decrease behaviors related to pairing, nor did it alter the likelihood that a male would form a pair relationship. Research has shown that male song output and song quality is highly important for female mate choice (Tomaszycki & Adkins-Regan, 2005) and that females make very sophisticated judgments on the basis of song (Zann, 1996). If females are deciding between males based on the amount of song, then raclopride treatments would have decreased pairing behaviors. However, our data suggests that females may be more sensitive to song quality (which was presumably unaltered by raclopride since song, once learned, is largely stereotyped) than to total amount of singing. Studies over longer time courses may be more likely to reveal significant effects of this decreased singing on female mate choice.

This was the first study to examine the effects of a D2 antagonist on male pairing behavior in zebra finches. These results suggest that, while dopamine acting at D2 receptors may be linked to courtship motivation, it may not ultimately affect the outcome of that behavior—

pairing. These findings differ from those in prairie voles, which clearly show that DA acting at D2 receptors regulates pairing in males (Aragona & Wang, 2009). Our research suggests that the mechanisms of pairing in male zebra finches might be different from the mechanisms of pairing in male prairie voles.

Despite the effects on courtship in males, we have no evidence that raclopride affected courtship or pairing behavior in females. Very little research has focused on courtship in female zebra finches. Courtship behaviors in females tend to be more receptive in nature, usually measured by tail quivers in response to male directed singing. One study which investigated the effects of blocking sex steroids (androgens and estrogens) found no effects on courtship behavior in females (Tomaszycki, Banerjee, & Adkins-Regan, 2006). Future studies should elucidate the mechanisms regulating this infrequent and subtle behavioral response.

Raclopride treatments did not interfere with pairing in females. No study, to date, has found a neurohormonal mechanism of pairing in female zebra finches. Studies have primarily focused on neurochemical mechanisms of female song preferences. These studies have shown that norepinephrine (another catecholamine) antagonists or the administration of DSP-4, a neurotoxin specific to norepinephrine, can interfere with a female's ability to discriminate between songs (Lynch, Diekamp, & Ball, 2008). It will be interesting to study whether or not raclopride would similarly interfere with a female's ability to distinguish between songs, or if norepinephrine is actually important for pairing in females, not dopamine.

Taken together, our raclopride results show that dopamine acting at D2 receptors does not alter pairing behavior in either sex. These findings are very different from those in prairie voles, which find clear effects of D2 antagonists on inhibiting pair formation. There are three possible reasons for this difference. 1) Our raclopride treatments were not sufficient to block D2 receptors

in the brain. 2) Our testing paradigm was not sufficiently long enough to significantly alter pairing. 3) Mechanisms of pairing differ according to species.

It is unlikely that the raclopride treatments were insufficient. Previous work in zebra finches has used a similar systemic dosage and found significant effects on dopaminergic activity in the brain (Pawlisch & Riters, 2010; Schroeder & Riters, 2006; Svec, Lookingland, et al., 2009). Other studies have found a significant reduction in courtship behaviors, including directed singing, in males zebra finches, using a D1/D2 receptor antagonist, *cis*-flupenthixol (Harding, 2004; Rauceo, et al., 2008). Since we obtained a similar effect, it suggests that the dosage was sufficient to alter male behavior. However, we cannot rule out the possibility that higher or lower doses would alter pairing behavior.

It is possible that our testing paradigm was not sufficiently long enough to alter pairing. Animals were only allowed contact with the same group of opposite-sex partners for two hours over a two day period. These time periods were designed so that the animals were only in contact when the drugs were active (day 1) and then allowed to display their preferences (day 2). This may not actually be enough time to form a partner preference or pair relationship, although this is the first study to examine pairing behavior, and similar studies in prairie voles only use testing periods of a couple of hours in duration.

At present, it appears that DA acting at D2 receptors is not necessary for pairing to occur. This suggests that results from one species (prairie voles) may not generalize to other species (zebra finches). Further research should consider whether D2 antagonists inhibit pairing in other monogamous species.

*4.2 Vanoxerine*

We hypothesized that vanoxerine, a DA reuptake inhibitor, treatments would increase courtship and pairing behaviors in male and female zebra finches. However, the results of the vanoxerine treatment were similar to the results of the raclopride treatment, in that we saw a decrease in courtship behaviors. In males, vanoxerine treatment resulted in a decrease in directed singing behavior and a decrease in the percentage of females courted. In females, vanoxerine treatment did not result in any significant behavioral changes. In both males and females, pairing behaviors were not observed frequently enough to be analyzed statistically. These results conflict with previous studies, which found that vanoxerine increased singing behavior in male starlings, a seasonally breeding songbird, and increased receptivity to male starling song in treated female starlings (Heimovics & Riters, 2008; Schroeder & Riters, 2006) These results support the involvement of dopamine in courtship, but the mechanism may be contrary to what we had hypothesized.

Vanoxerine has been shown to affect courtship behaviors in the male European starling, a territorial songbird. Treatment with vanoxerine in male starlings resulted in increased female-directed singing behaviors and showed increased levels of TH-ir in the VTA and the POM, areas involved in mating behaviors, and Area X, an area involved in song learning (Heimovics and Riters, (2008). Female starlings treated with vanoxerine showed an increased preference for male songs from both starlings (high-incentive songs) and purple martins (low-incentive songs), while untreated females only showed a preference for the high incentive male starling songs (Pawlisch & Riters, 2010). These treated females also displayed increased FOS immunolabeling in the ventromedial nucleus of the hypothalamus (VMH). Taken together, the starling studies and the results of the current study indicate that vanoxerine has an effect on directed singing in

males, an important courtship behavior, the perception of that song in females (an important aspect of mate choice), and IEG expression in reward areas of the brain.

Vanoxerine or GBR 12909, (1-(2-(bis(4-fluorphenyl)-methoxy)-ethyl)-4-(3-phenyl-propyl)piperazine), is a highly selective inhibitor of the presynaptic dopamine uptake complex both in vivo and in vitro (Andersen, 1989; Kelley & Lang, 1989). Vanoxerine inhibits the uptake of dopamine by binding to the DA binding site on the DAT carrier protein itself, blocking the carrier process and allowing DA to stay in the synapse (Andersen, 1989). The high potency and selectivity of vanoxerine for the DAT is similar to that of cocaine and other drugs of abuse. Vanoxerine has been shown to reliably maintain drug self-administration in the squirrel monkey (Andersen, 1989) and the rhesus monkey (Lindsey et al., 2004). These studies support the selectivity for vanoxerine at the DAT complex and the importance of that action in the reward pathway of the brain.

Vanoxerine has been shown to have a steady response curve, with lower doses being less effective and higher doses mimicking the effects of amphetamine (Young et al., 2010) in mice. Vanoxerine has been shown to increase courtship behaviors in both male and female starlings, a social songbird, at a dose of 10 mg/kg and 20 mg/kg (Pawlisch & Riters, 2010; Schroeder & Riters, 2006). This is the first study to use vanoxerine to study the effects of DA on courtship and pairing behaviors in the zebra finch. The dose used in the present study, 10 mg/kg, is the lower dose and was used as a starting point. In fact, this dose had adverse effects on motor ability in one female, suggesting that the 20mg/kg dose would have been too high. It is possible, in light of the few studies available, that our dose was too low and therefore did not have the desired effect. In the future, it is possible that a variety of doses may be used to determine the balance between changes in courtship behaviors and amphetamine-like activity.

Despite the fact that vanoxerine is a highly potent and specific DA reuptake inhibitor, it is an equally potent norepinehprine transport inhibitor (Lindsey, et al., 2004). It is possible that this property could have interfered with the courtship behaviors we were interested in studying, as norepinephrine is ten times as prevalent as dopamine in the zebra finch brain (Lynch, et al., 2008). Norepinephrine receptors are present in many of the same reward areas of the brain as dopamine and have been implicated in the motivational and attentional components of courtship behaviors (Lynch, et al., 2008). In the future, norepinephrine should be studied in conjunction with dopamine to determine the specific functions of these two catecholamines on courtship and pairing behaviors in the zebra finch. It is also possible that vanoxerine may have a different effect on zebra finches than the other species it has been previously tested in, such as rodents, primates, and other birds. This is unlikely, given the similar functional organization between the mammalian and avian telencephalon (Durstewitz, et al., 1999). Given the evidence, it is unlikely that the results of this study are due to any neurochemical differences in vanoxerine or species differences in test subjects.

Raclopride treatments decreased the courtship behavior of directed singing similar to previous studies (Harding, 2004; Rauceo, et al., 2008; Svec, Lookingland, et al., 2009), indicating that activation of the D2 receptor is important during this behavior. Raclopride did not have any effect on other courtship behaviors or pairing behaviors. Vanoxerine treatments, however, did not result in an increase in courtship or pairing behaviors, and actually resulted in a decrease in the courtship behavior of directed singing. Vanoxerine did not have any significant effect on other courtship and pairing behaviors. The results of this study do support the role of dopamine, specifically the D2 receptor, in the courtship behavior, directed singing, in the male zebra finch, but do not support the role of the D2 receptor in pair bonding behaviors. This is the

first study to show evidence for the role of the D2 receptor specifically in the male zebra finch courtship behavior. Further research is needed to understand the role of the D2 receptor in female courtship behaviors and for pair bonding in both sexes

| **Behavior** | **Description** |
|---|---|
| Directed Singing | Duration of song directed at a female |
| Clumping | Duration of time spent in direct physical contact with another animal |
| Nesting | The number of times two animals share a nest box |
| Allopreening | The number of times one animal grooms another |
| Tail quivers | The number of times a female rapidly shakes her tail while near a male |
| Copulation | Mounting of a female by a male |

*Table 1*: Description of the courtship and pairing behaviors of the zebra finch observed during the experiments.

*The Effect of Treatment and Day on Time Spent Singing to Females*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 3.75 | 1.50 | 87.63 | 26.97 | Day 1 | 28.00 | 12.33 | 100.50 | 25.23 |
| Day 2 | 46.13 | 13.47 | 45.50 | 13.60 | Day 2 | 123.38 | 20.69 | 0.34 | 0.10 |
| Total | 24.94 | 7.23 | 66.56 | 16.67 | Total | 75.69 | 15.34 | 81.63 | 20.81 |

*The Effect of Treatment and Day on Percentage of Time Spent with Preferred Female*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.63 | 0.24 | 0.51 | 0.04 | Day 1 | 0.63 | 0.23 | 0.57 | 0.06 |
| Day 2 | 0.55 | 0.24 | 0.31 | 0.11 | Day 2 | 0.42 | 0.10 | 0.34 | 0.10 |
| Total | 0.59 | 0.16 | 0.41 | 0.07 | Total | 0.53 | 0.10 | 0.46 | 0.05 |

*The Effect of Treatment and Day on Association Index*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.19 | 0.07 | 1.21 | 0.14 | Day 1 | 0.51 | 0.20 | 1.21 | 0.14 |
| Day 2 | 0.64 | 0.17 | 0.62 | 0.11 | Day 2 | 1.02 | 0.22 | 1.14 | 0.18 |
| Total | 0.41 | 0.09 | 0.92 | 0.08 | Total | 0.76 | 0.16 | 1.17 | 0.10 |

*Table 2*: Repeated Measures ANOVA means and standard errors for treatment and day effects for male zebra finches only.

*The Effect of Treatment and Day on Time Spent Singing and Clumping with Males*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 89.38 | 16.57 | 99.00 | 28.84 | Day 1 | 107.00 | 19.59 | 167.50 | 19.01 |
| Day 2 | 120.38 | 30.60 | 81.13 | 10.52 | Day 2 | 113.25 | 21.69 | 80.50 | 11.48 |
| Total | 104.88 | 17.81 | 90.06 | 17.49 | Total | 110.13 | 16.70 | 124.00 | 11.12 |

*The Effect of Treatment and Day on Percentage of Time Spent with Preferred Male*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.54 | 0.09 | 1.14 | 0.18 | Day 1 | 0.87 | 0.18 | 0.57 | 0.09 |
| Day 2 | 0.27 | 0.06 | 0.91 | 0.22 | Day 2 | 0.41 | 0.13 | 0.69 | 0.16 |
| Total | 0.41 | 0.06 | 1.03 | 0.19 | Total | 0.64 | 0.12 | 0.63 | 0.10 |

*The Effect of Treatment and Day on Association Index*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 1.13 | 0.17 | 1.05 | 0.17 | Day 1 | 1.19 | 0.20 | 1.39 | 0.12 |
| Day 2 | 0.81 | 0.04 | 0.86 | 0.22 | Day 2 | 1.30 | 0.13 | 0.96 | 0.19 |
| Total | 0.97 | 0.08 | 0.95 | 0.13 | Total | 1.24 | 0.14 | 1.18 | 0.14 |

*Table 3*: Repeated Measures ANOVA means and standard errors for treatment and day effects for female zebra finches only.

*The Effect of Treatment and Day on Time Spent Clumping with Females*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 1.63 | 1.10 | 0.00 | 0.00 | Day 1 | 0.00 | 0.00 | 0.00 | 0.00 |
| Day 2 | 0.00 | 0.00 | 0.00 | 0.00 | Day 2 | 5.25 | 5.11 | 0.00 | 0.00 |
| Total | 0.81 | 0.55 | 0.00 | 0.00 | Total | 2.63 | 2.55 | 0.00 | 0.00 |

*The Effect of Treatment and Day on Time Spent Nesting*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.00 | 0.00 | 3.75 | 3.75 | Day 1 | 0.25 | 0.25 | 0.00 | 0.00 |
| Day 2 | 0.88 | 0.88 | 3.63 | 3.22 | Day 2 | 0.00 | 0.00 | 1.25 | 0.73 |
| Total | 0.44 | 0.44 | 3.69 | 3.48 | Total | 0.13 | 0.13 | 0.63 | 0.36 |

*The Effect of Treatment and Day on Time Spent Allopreening with Females*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.13 | 0.13 | 0.00 | 0.00 | Day 1 | 0.00 | 0.00 | 0.00 | 0.00 |
| Day 2 | 0.00 | 0.00 | 0.00 | 0.00 | Day 2 | 0.13 | 0.13 | 0.00 | 0.00 |
| Total | 0.06 | 0.06 | 0.00 | 0.00 | Total | 0.06 | 0.06 | 0.00 | 0.00 |

*The Effect of Treatment and Day on the Number of Tail Quivers towards Males*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.00 | 0.00 | 0.25 | 0.25 | Day 1 | 0.50 | 0.50 | 0.38 | 0.38 |
| Day 2 | 0.00 | 0.00 | 0.00 | 0.00 | Day 2 | 0.00 | 0.00 | 0.00 | 0.00 |
| Total | 0.00 | 0.00 | 0.13 | 0.13 | Total | 0.25 | 0.25 | 0.19 | 0.19 |

*The Effect of Treatment and Day on the Number of Copulations with Females*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.50 | 0.27 | 2.38 | 1.69 | Day 1 | 0.38 | 0.26 | 1.13 | 0.52 |
| Day 2 | 0.13 | 0.13 | 0.38 | 0.38 | Day 2 | 0.00 | 0.00 | 0.13 | 0.13 |
| Total | 0.31 | 0.19 | 1.38 | 1.03 | Total | 0.19 | 0.13 | 0.63 | 0.25 |

*Table 4*: Repeated Measures ANOVA means and standard errors for treatment and day effects for other courtship and pairing behaviors in male zebra finches only.

*The Effect of Treatment and Day on Time Spent Clumping with Males*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 2.38 | 1.87 | 0.00 | 0.00 | Day 1 | 1.00 | 0.76 | 3.00 | 2.27 |
| Day 2 | 5.25 | 4.97 | 8.50 | 4.85 | Day 2 | 1.13 | 1.13 | 0.50 | 0.33 |
| Total | 3.81 | 2.48 | 4.25 | 2.43 | Total | 1.06 | 0.93 | 1.75 | 1.10 |

*The Effect of Treatment and Day on Time Spent Nesting*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 2.38 | 2.38 | 1.14 | 0.18 | Day 1 | 0.00 | 0.00 | 0.00 | 0.00 |
| Day 2 | 2.75 | 2.03 | 0.88 | 0.58 | Day 2 | 0.00 | 0.00 | 0.00 | 0.00 |
| Total | 2.56 | 1.73 | 1.00 | 0.66 | Total | 0.00 | 0.00 | 0.00 | 0.00 |

*The Effect of Treatment and Day on Time Spent Allopreening with Males*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.88 | 0.58 | 0.00 | 0.00 | Day 1 | 0.88 | 0.74 | 0.00 | 0.00 |
| Day 2 | 0.00 | 0.00 | 6.50 | 4.82 | Day 2 | 1.13 | 1.13 | 0.13 | 0.13 |
| Total | 0.44 | 0.29 | 3.25 | 2.41 | Total | 1.00 | 0.93 | 0.06 | 0.06 |

*The Effect of Treatment and Day on the Number of Tail Quivers towards Males*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 0.00 | 0.00 | 0.38 | 0.38 | Day 1 | 0.00 | 0.00 | 1.00 | 0.76 |
| Day 2 | 0.00 | 0.00 | 0.00 | 0.00 | Day 2 | 0.00 | 0.00 | 0.00 | 0.00 |
| Total | 0.00 | 0.00 | 0.19 | 0.19 | Total | 0.00 | 0.00 | 0.50 | 0.38 |

*The Effect of Treatment and Day on the Number of Copulations with Males*

| | Raclopride | | Saline | | | Vanoxerine | | DMSO | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | Std Error | Mean | Std Error | | Mean | Std Error | Mean | Std Error |
| Day 1 | 4.75 | 1.93 | 1.63 | 1.07 | Day 1 | 1.75 | 0.77 | 1.38 | 0.73 |
| Day 2 | 0.25 | 0.16 | 0.00 | 0.00 | Day 2 | 0.63 | 0.50 | 0.13 | 0.13 |
| Total | 2.50 | 0.97 | 0.81 | 0.53 | Total | 1.19 | 0.37 | 0.75 | 0.38 |

*Table 5*: Repeated Measures ANOVA means and standard errors for treatment and day effects for other courtship and pairing behaviors in female zebra finches only.

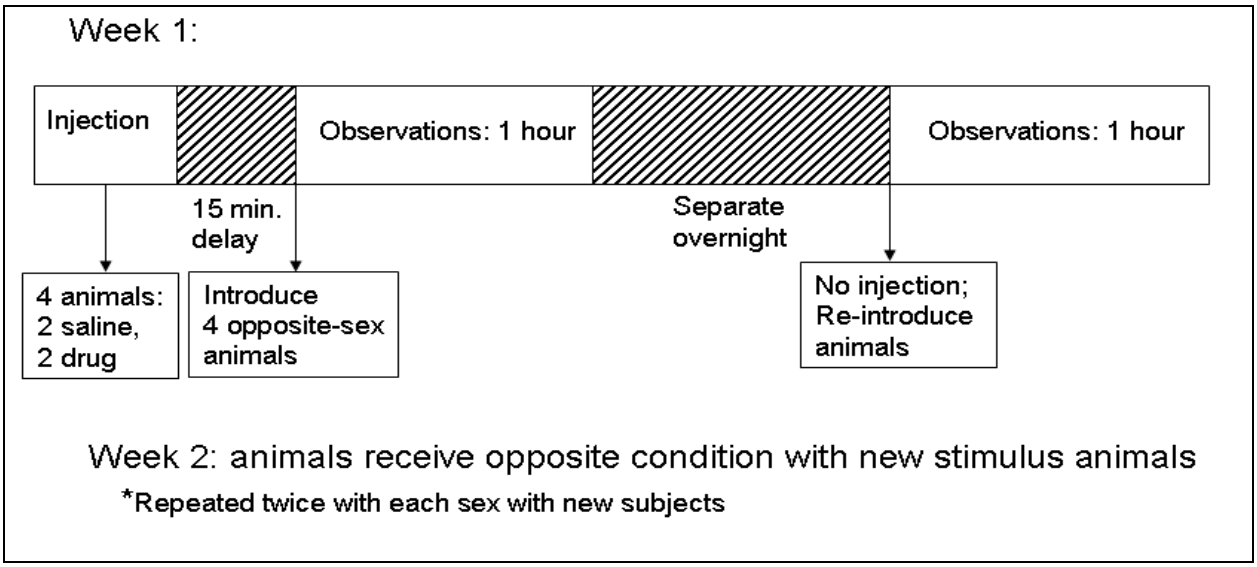


*Fig 1*: Experimental Design. This figure is a graphical representation of the experimental design followed during experiments. The first experiment was conducted with two cohorts of raclopride-treated males and two cohorts of vanoxerine-treated males. The second experiment was conducted with two cohorts of raclopride-treated females and two cohorts of vanoxerine-treated females.

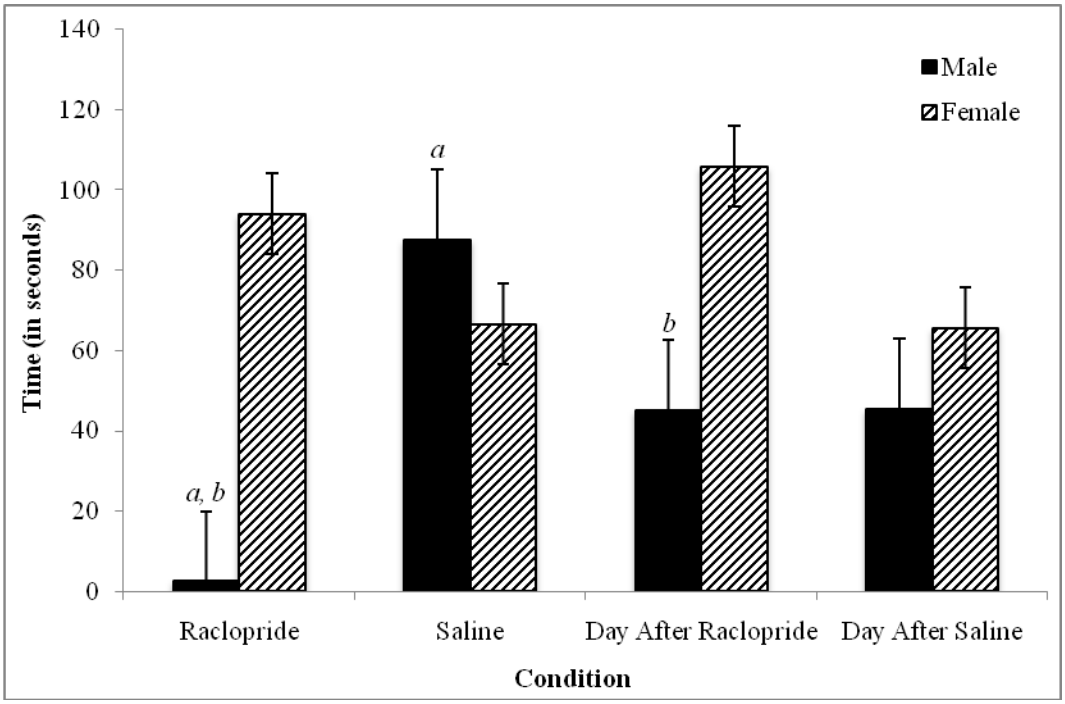


*Figure 2*: Time (in seconds) engaged in directed singing and clumping by male zebra finches and clumping in females when treated with a D2 receptor antagonist or saline. The black bars represent the total time in seconds that males spent singing to females when males were treated with raclopride, saline, and the day after treatments. The black and white stripe bars represent the total time in seconds that females spent clumping with males when treated with raclopride, saline, and the day after treatments. Letters above the bars denote significant differences in behavior.

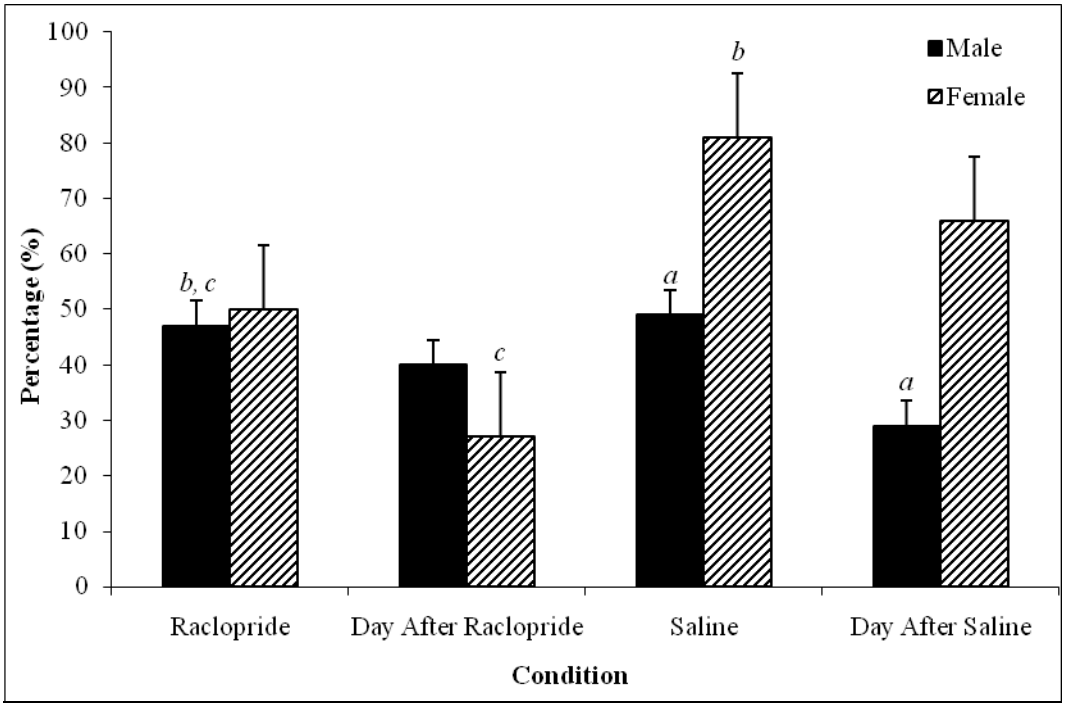


*Figure 3*: Association index in male and female zebra finches treated with a D2 antagonist or saline. The preferred member of the opposite sex was determined by finding the member of the opposite sex the male or female spent the most total time with during the first day they were introduced. The percentage of time was determined by taking the total amount of time in seconds the male or female engaged in courtship/pairing behaviors to the preferred member of the opposite sex and dividing it by the total amount of time spent with all member of the opposite sex and multiplying by 100. The black bars represent the percentage of time the males spent with their preferred females when the males were treated with raclopride, saline, and the day after treatments. The black and white striped bars represent the percentage of time the females spent with their preferred males when the females were treated with raclopride, saline, and the day after treatments. Letters above the bars denote significant differences in behavior.

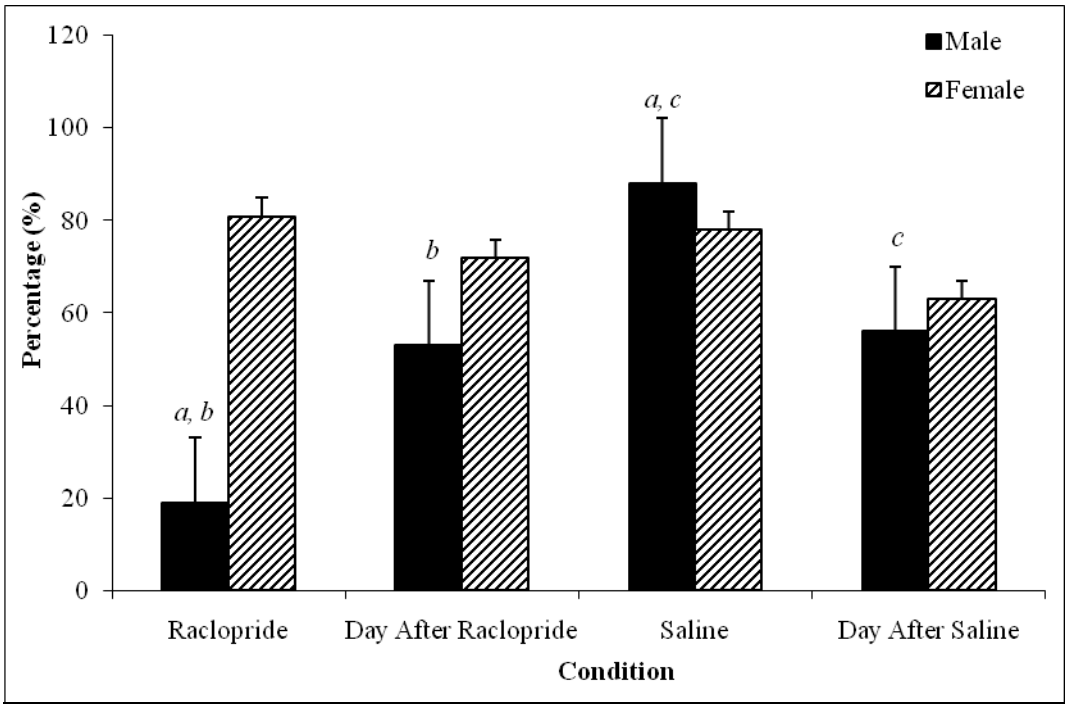


*Figure 4*: Percentage of members of the opposite sex that males and females spent time with during D2 antagonist treatment. The percentage of members of the opposite sex spent time with was determined by counting the number of different members of the opposite sex toward which males or females demonstrated courtship/pairing behaviors and divided this number by the total number of the opposite sex available (four members of the opposite sex per cohort) and multiplied by 100. The black bars denote the percentage of females courted by males treated with raclopride, saline, and the day after treatments. The black and white striped bars denote the percentage of males that females spent time with when females were treated with raclopride, saline, and the day after treatments. Letters above the bars denote significant differences in behavior.

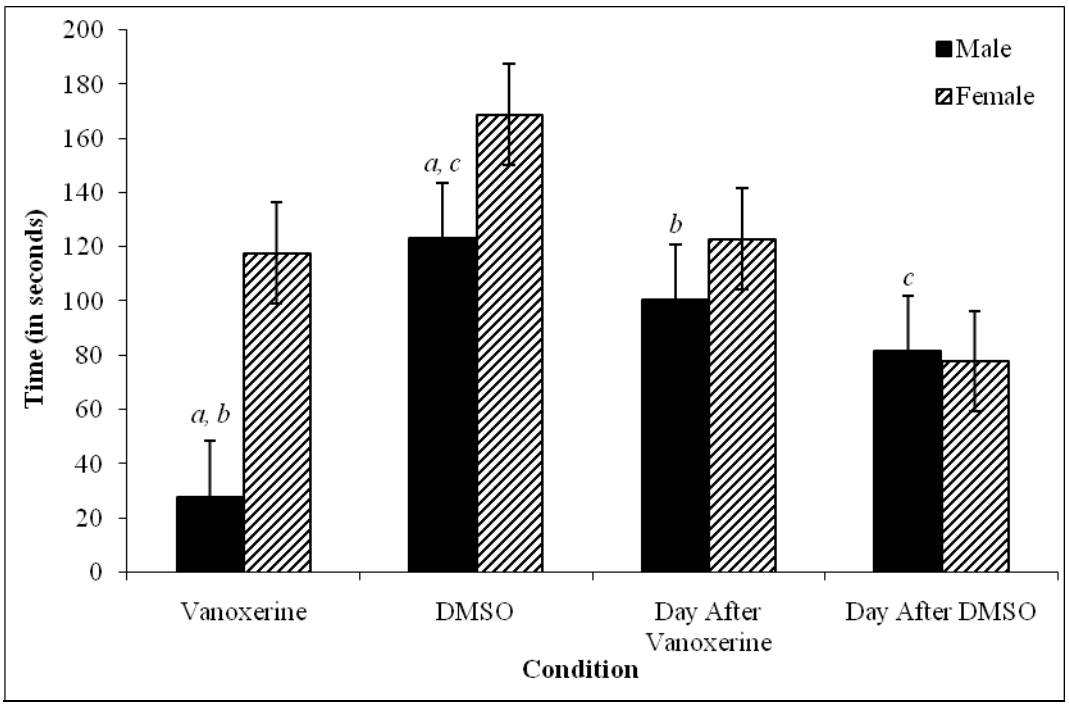


*Figure 5*: Time (in seconds) engaged in directed singing and clumping by male zebra finches and clumping in females when treated with a DA reuptake inhibitor or DMSO. The black bars represent the total time in seconds that males spent singing to females when males were treated with vanoxerine, DMSO, and the day after treatments. The black and white stripe bars represent the total time in seconds that males spent singing to females and females spent clumping with males when females were treated with vanoxerine, DMSO, and the day after treatments. Letters above the bars denote significant differences in behavior.

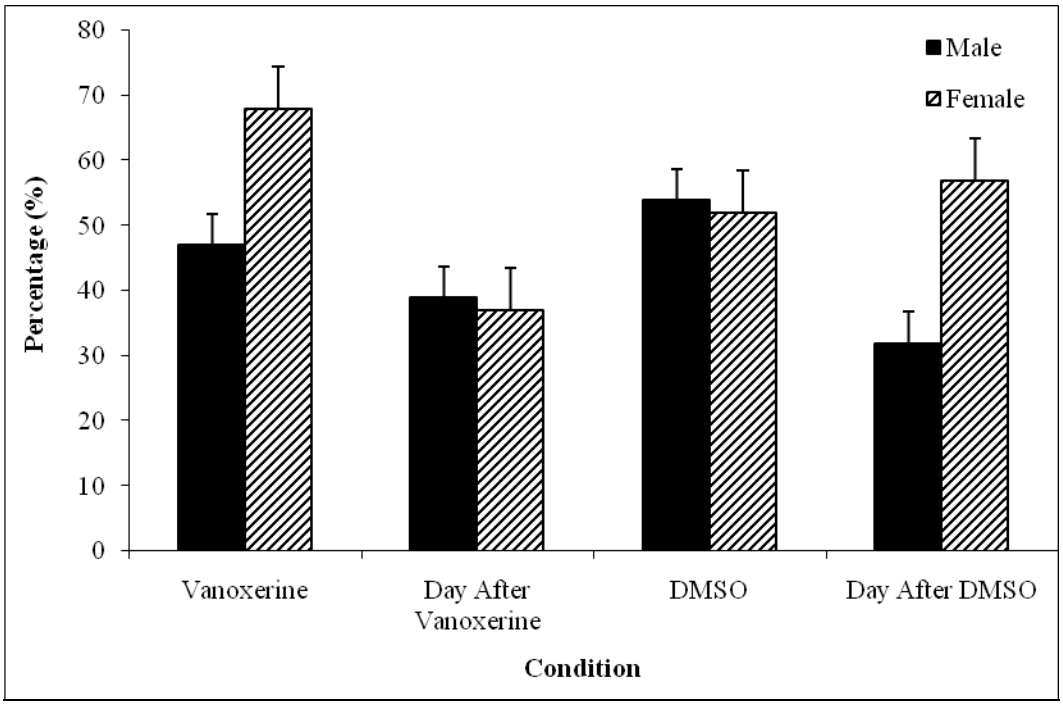


*Figure 6*: Association index in male and female zebra finches treated with a DA reuptake inhibitor or DMSO. The preferred member of the opposite sex was determined by finding the member of the opposite sex the male or female spent the most total time with during the first day they were introduced. The percentage of time was determined by taking the total amount of time in seconds the male or female engaged in courtship/pairing behaviors to the preferred member of the opposite sex and dividing it by the total amount of time spent with all member of the opposite sex and multiplying by 100. The black bars represent the percentage of time the males spent without their preferred females when the males were treated with vanoxerine, DMSO, and the day after treatments. The black and white stripe bars represent the percentage of time the females spent with their preferred males when the females were treated with vanoxerine, DMSO, and the day after treatments.

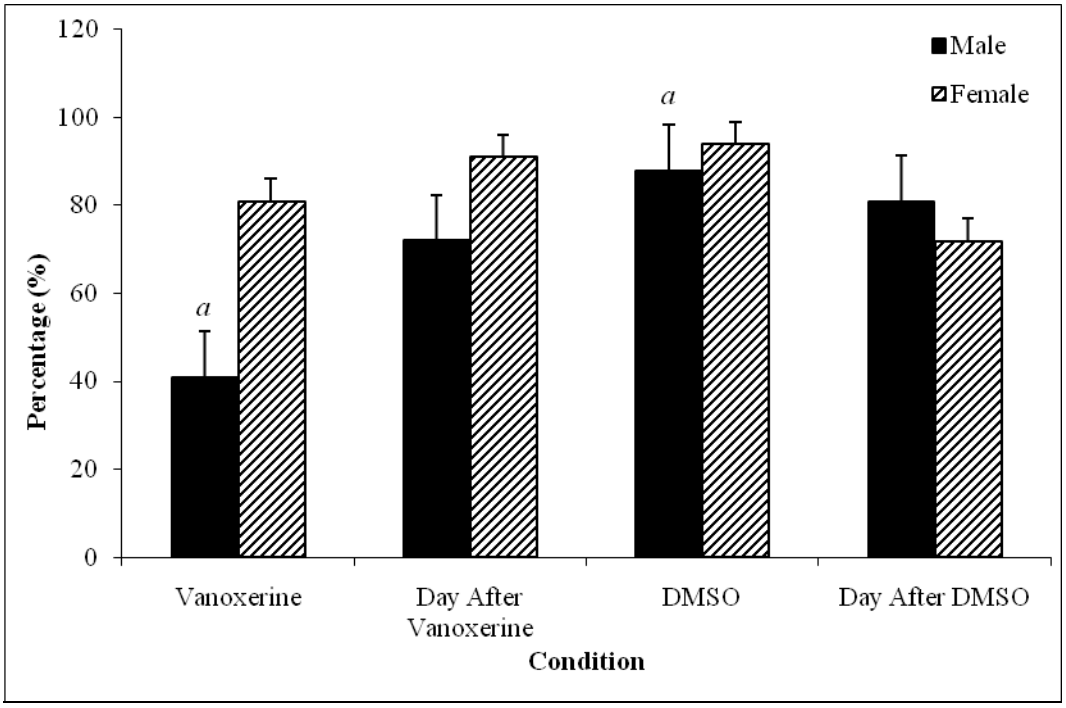


*Figure 7*: Percentage of members of the opposite sex that males and females spent time with during DA reuptake inhibitor treatment. The percentage of members of the opposite sex spent time with was determined by counting the number of different members of the opposite sex toward which males or females demonstrated courtship/ or pairing behaviors and divided this number by the total number of the opposite sex available (four members of the opposite sex per cohort) and multiplied by 100. The black bars denote the percentage of females courted by males treated with vanoxerine, DMSO, and the day after treatments. The black and white striped bars denote the percentage of males that females spent time with when females were treated with vanoxerine, DMSO, and the day after treatments. Letters above the bars denote significant differences in behavior.

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# ABSTRACT

## SEX DIFFERENCES IN THE DOPAMINERGIC REGULATION OF COURTSHIP, BUT NOT PAIRING BEHAVIORS IN ZEBRA FINCHES

**by**

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**August 2012**

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**Degree:** Master's of Arts

Dopamine is one of the key ingredients in the glue that cements social bonds in vertebrates. The D2 dopamine receptor has been implicated in the regulation of monogamous pair bonding in the prairie vole. While dopamine affects courtship behaviors in the male zebra finch, the behavioral role of dopamine acting at D2 receptors in both males and females deserves further attention. We hypothesized that the D2 receptor would regulate courtship and pairing behaviors in the male and female zebra finch. Sixteen males and females were tested using a repeated measures design. On day 1, the zebra finches were injected with 0.5 μg/ml of raclopride, a D2 receptor antagonist, GBR-12909, a dopamine reuptake inhibitor, or the vehicle. Then, birds of the opposite sex were introduced and behavior was recorded. On day 2, the same males and females were placed together without injections and their behavior was recorded. Data were analyzed using repeated measures ANOVAs and followed by paired samples t-tests. Surprisingly, neither treatment affected female behavior. However, directed singing was decreased when males were injected with

raclopride compared to the day they were injected with saline. There was also a significant increase in directed singing the day after the males were injected with raclopride. Contrary to our hypothesis, GBR-12909 also caused significant decreases in courtship behavior. These results indicate that dopamine acting at the D2 dopamine receptor plays a role in the courtship of the male zebra finch, but does not appear to alter the behavior of females.

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