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Alexandra Smith

Date 5/7/2016

This Honors paper by Alexandra Smith has been read and approved for Honors in Neuroscience.

Dr. David Kabelik
Project Advisor

Dr. Kim Gerecke
Second Reader

Dr. Sarah Boyle
Extra-Departmental Reader

Dr. Kim Gerecke
Department Chair

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Peripheral Injections of Dopamine (D1) and Dopamine 2 (D2) Agonists and Antagonists Do Not Affect Sexual and Aggressive Behaviors in Male Green Anoles

Alexandra Smith
Neuroscience Program, Rhodes College

Social behaviors are modulated by neurotransmitters, including dopamine. Dopamine is a conserved neurotransmitter among vertebrates. Dopaminergic receptors of the D1 and D2 subtype are also conserved among taxa, and are involved in many different kinds of social behaviors, such as sexual and aggressive behaviors in mammals and birds. However, the functions of the receptors vary across taxa. In reptiles there have only been two studies examining the relationship between the receptors and behaviors. This study examined the effects of D1 and D2 agonists and antagonists on sexual and aggressive behaviors in the male green anole lizard (*Anolis carolinensis)*. For the D1 agonist, the following doses were tested: 0.005 μg/kg, 0.05 μg/kg, 0.001 mg/kg, 0.01 mg/kg, 0.1 mg/kg, 1.0 mg/kg, and 10.0 mg/kg. For the D2 agonist only the 1.0 and 10.0 mg/kg doses were tested. Both the D1 and D2 antagonists were tested at 0.1 and 1 mg/kg. Neither the agonists nor antagonists affected social behaviors. These findings differ from previous research, which demonstrated an effect of D1 and D2 agonists and antagonists on social behaviors in mammals and birds. One potential reason for the lack of significance is that the drug may be binding to receptors in various regions in the brain that could be causing counteracting effects. Future studies should look at individually administering the drugs directly into brain regions known to regulate sexual and aggressive behaviors.

Keywords: dopamine, lizards, social behaviors

Introduction

Social behaviors are the interactions of individuals from the same species (Scott, 1972). In animals, social behaviors are regulated by various brain regions that are interconnected to form a social behavior network (Goodson and Kabelik, 2009). The brain regions in this network differentially affect certain social behaviors, and neurochemicals are released to inhibit and facilitate behaviors (Goodson and Kabelik, 2009). A category of neurochemicals involved in modulating social behaviors are catecholamines, a class of monoamines in the central nervous system (CNS) that includes epinephrine, norepinephrine, and dopamine (Smeets and Gonzalez, 2000). Dopamine in particular is known to be involved in motivation and reward-seeking behaviors (reviewed in Abraham et al., 2014), locomotion (Clemens et al., 2012), and social behaviors, including sexual (see Guiliano and Allard for review) and aggressive behaviors (see Miczek et al., 2002 for review). These dopamine-mediated social behaviors include sexual behavior in a variety of species, including birds (Kleitz-Nelson et al., 2010a; Kleitz-Nelson et al., 2010b; Kleitz-Nelson et al., 2010c), mammals (Dominguez and Hull, 2005), and reptiles (Woolley et al., 2001; see Woolley et al., 2004 for review). These social behaviors also include aggressive behaviors in mammals (Ferrari et al., 2003) and birds (Dennis and Cheng, 2011; Kabelik et al., 2010).

Dopamine's involvement in sexual and aggressive behaviors in birds and mammals is varied, and this variation may be due to which dopamine receptor is activated. Dopamine has five receptor groups to which it binds: dopamine 1 (D1)

through dopamine 5 (D5) (see Zeng et al., 2008 for review). However, the D1 and D2 receptors are predominantly the only receptors addressed in studies regarding social behaviors (Table 1).

In the periphery, the activation of D1 receptors by agonists, drugs that stimulate receptors, increases both sexual and aggressive behaviors in quail (*Coturnix japonica*) (Balthazart et al., 1997), rats (*Rattus norvegicus*) (Beck et al., 2002; D'Aquila et al., 2003), and hens (*Gallus gallus*) (Dennis and Cheng, 2011) (Table 1). However in regards to aggressive behaviors in lizards, there has not been any research on the effects of D1 agonists and antagonists (Table 2), the latter of which are drugs that inactivate receptors. The little that is known about sexual behavior in lizards is limited to the effects of D1 agonists and antagonists in only three lizard species, whiptail lizards (*Cnemidophorus inoratus* and *C. uniparens*) and leopard geckos (*Eublepharis macularius)* (see Woolley et al., 2004 for review). In addition, only consummatory sexual behaviors (i.e. mounting and copulation), and not appetitive sexual behaviors (i.e. anticipatory behaviors) have been documented in relation to dopamine receptor manipulation in lizards (see Woolley et al., 2004 for review). Courtship behaviors were studied in leopard geckos, but since these are unpublished data, it is unknown whether both appetitive and consummatory behaviors were examined (see Woolley et al., 2004 for review). Furthermore, in the one study on the effects of a D1 agonist on sexual behavior in lizards, the D1 agonist doses that caused a significant change in behavior were extremely small at 0.005 and 0.05 μg/mg (Woolley et al., 2001), and comparably small doses were not used in any other studies (Table 1). Thus while the effects of D1 receptor activation

increases sexual and aggressive behaviors in mammals and birds, the specific effects in reptiles are still unclear.

Unlike the effects of D1 receptor activation, it is difficult to conclude how D2 receptor activation affects either sexual or aggressive behaviors, due to the varied results in the literature in regards to whether activation of D2 receptors increases or decreases social behaviors in mammals and birds (Balthazart et al., 1997; Bitran et al., 1989; Kabelik et al., 2010; Dennis and Cheng, 2011). In lizards, the effects of D2 receptor activation are still unclear, due to a lack of research. In fact, to the best of our knowledge, no study has examined the effects of D2 agonists and antagonists on aggression or sexual behaviors in reptiles.

Reptiles are a taxonomic group that has been largely overlooked in studies on dopamine's involvement in sexual and aggressive behaviors; however, they are an important model for examining the effects of dopamine on social behaviors, since they are the closest relatives to birds and mammals (Smeets, 1994; Benton and Donoghue, 2007). Previous research suggests that the structure of the dopaminergic system is a conserved pattern among reptiles (Smeets, 1994), as well as across amniotic vertebrates (Smeets and Reiner, 1994). However, it is unknown whether the functions of the dopaminergic system are similar across taxa.

Therefore, to further understand the conservation of the structure and function of the dopaminergic system, dopamine receptors and behaviors associated with dopamine must also be studied in lizards. In whiptail lizards, systemic injection of a D1 agonist increased mounting behavior (Woolley et al., 2001), and in leopard geckos systemic injection of a D1 antagonist inhibited courtship behavior (see

Woolley et al., 2004 for review). These two studies are the only ones that examine the effects of dopamine receptor activation on social behaviors in lizards, and these studies are limited to just sexual behaviors and D1 receptor activation or inactivation.

Although there is only minimal evidence for dopaminergic regulation of sexual behaviors, and no evidence of regulation of aggressive behaviors in lizards, there is evidence that neural dopamine is involved in aggressive encounters. In the brown anole (*Anolis sagrei)*, different brain regions express varying levels of colocalization of tyrosine hydroxylase, an enzyme involved in dopamine synthesis, and Fos (Kabelik et al., 2014), a protein used as an indicator for neural activation (Kovacs, 2008). The percentage of colocalization, which indicates the percentage of tyrosine hydroxylase neurons that were activated during the behavioral encounter, was correlated with the frequency of courtship and aggressive behaviors (Kabelik et al., 2014). In the related green anole (*A. carolinensis*), extracellular dopamine levels increased in the nucleus accumbens (NAC) and amygdala (AMY) in animals after exposure to a mirror, which the animals perceive as a social challenge from another invading male (Watt et al., 2007). Even catecholamines in the peripheral nervous system (PNS) are involved in social behaviors in lizards. The postorbital patch of skin behind the eye, known as an eye spot, darkens as a display of and response to aggression as a result of an increase in plasma catecholamine levels (Korzan et al., 2000). Hence, dopamine release in both the CNS and PNS is involved in sexual and aggressive behaviors in reptiles.

I hypothesize that the activation of D1 and D2 receptors plays a role in sexual and aggressive behaviors in male green anoles because of their role on such behaviors in other species, as reported in previous research (Table 2). I predict that the activation of D1 receptors will have similar effects as seen in other species, specifically, that a D1 agonist will increase sexual and aggressive behaviors in male green anoles. However, while D2 receptor activation has been shown to be involved in sexual and aggressive behaviors in other species, the effects do not alter behavior in a consistent manner (Table 2.). Therefore, I predict that the D2 agonist will have an affect, but I do not make a prediction on the directionality of this effect because of the inconsistency of previous research, as well as a lack of research on reptiles.

Methods

Subjects

The subjects used in this experiment were male green anoles (*Anolis carolinensis*). They were purchased from Sullivan Amphibians in Nashville, TN, and housed on a 14:10 hour light-dark schedule, and a temperature range of 76-88 °F, with additional heat provided by a 60-watt light bulb suspended above half of each terrarium (30.5 cm Hx26 cm Wx51 cm L). The focal males were housed separately, while the stimulus males were each housed with two stimulus females. All the focal males were kept in visual isolation from each other with an opaque divider between terraria. All animals were fed live crickets three times a week.

Testing ran from June 2014 to April 2016, allowing for several months off from September 2014 to January 2015 between the second and third experiments, as well as several months off from May 2015 to August 2015 between the third and fourth experiments, and, additionally, from September 2015 to January 2016 between the fourth and fifth experiments. During these off months, which imitated non-breeding conditions, the lizards were on a 10:14 hour light dark schedule, and supplementary heat was provided by 40-watt bulbs. In addition, they were only fed twice a week, and housed at approximately 65 F. Prior to resuming experiments, lizards were given several weeks of long days and warm temperatures to restore them to breeding condition.

Each focal male was sized-matched with a stimulus male based on snout-vent length, with the stimulus male being no more than 0.2 cm longer or shorter than the focal. All procedures were conducted according to IACUC standards.

Behavioral Testing

The aggressive and sexual display behaviors (Table 3) examined in this study were the same as those observed in other studies investigating courtship and aggression in lizards (Woolley et al., 2001; Kabelik et al., 2013; Kabelik et al., 2014). For the courtship trials, 2 females were placed in the focal male's terrarium and the behaviors of both stimulus females and the focal male were recorded for 10 minutes. Once the females were removed after the 10 minutes, a mirror aggression trial was run by placing a mirror on the outside of the focal male's terrarium opposite of the heat lamp and opposite the top of the perch, and behaviors were recorded for 10 minutes. The mirror trial was included to test for the initiation of aggressive behavior. The animals do not recognize their reflection, so the focal males view the image as an intruding male. The mirror was then removed and a stimulus male was placed in the focal male's terrarium for the aggression trial, and both focal and stimulus males' behaviors were recorded for 10 minutes. The same methods for the behavioral trials were used in all experiments.

Drugs

Because of the wide range of doses used in previous experiments (Table 1), five sets of experiments were conducted. The D1 agonist used was SKF 38393 (Sigma-Aldrich, St. Louis, MO, USA; catalog item D047), and the D2 agonist used was quinpirole (Sigma-Q111). The D1 antagonist used was SCH-23390 (Sigma-Aldrich, St. Louis, MO, USA; catalog item D054), and the D2 antagonist used was raclopride (Sigma-R121). All agonists and antagonists have been successfully used in previous studies to examine the effects of D1 or D2 agonists or antagonists on sexual and

aggressive behaviors across a variety of species (Table 1). All drugs were dissolved in 0.9% NaCl, and administered intraperitoneally at a volume of 0.05 mL

The D1 and D2 agonists were systemically injected at a 1.0 mg/kg dose, a standard dose that has achieved effects in other species (Balthazart et al., 1997; Beck et al., 2002; Kabelik et al., 2010). Because the 1.0 mg/kg dose produced non-significant trends, the dose for each agonist was increased by an order of magnitude to 10.0 mg/kg. Smaller doses of the D1 agonist were injected at 0.001, 0.01, 0.1 mg/kg doses to test the effects of small doses like those used by Balthazart et al. (1997). A final D1 agonist experiment tested the very small doses used by Woolley et al. (2001) at 0.005 and 0.05 μg/kg. After the final conclusion of the D1 agonist experiments, a D1 and D2 antagonist study was conducted at doses of 0.1 and 1.0 mg/kg to test for a ceiling effect, a phenomenon in which adding the dopamine agonists to a system would not cause any effect if the system is already saturated with endogenous dopamine.

Experiment 1: D1 Agonist at 1.0 and 10.0 mg/kg Doses

The D1 agonist SKF 38393 was used at a 1.0 mg/kg dose in Experiment 1A, and at a 10.0 mg/kg dose in Experiment 1B. Both doses were compared with a saline control in each experiment. Behavioral trials were repeated with the previously untested drug, SKF 38393 or saline, on the same individuals two weeks apart to eliminate any potential for carry-over effects. For Experiment 1, 20 focal males were tested once per week. The injections were given to the focal males 30 minutes before the behavioral trials began in order to allow the drug time to take effect. Repeated testing of same subjects was used because no short-term or long-

term behavioral effects of the same D1 and D2 agonists were seen in Japanese quail (Balthazart et al., 1997). The two-week gap also controlled for the possible effect of recognition between the male anoles. Male green anoles learn information about their challengers during an initial aggressive interaction, and this recognition can last for seven to ten days (Forster et al., 2005). The treatment order was counterbalanced, and the focal males were always paired with the same stimulus animals to minimize behavioral variation.

A small follow-up study to Experiment 1A with 19 focal males was conducted to test the D1 agonist at 1.0 mg/kg dose on courtship behavior at an increased latency of 60 minutes between the drug injection and the start of the courtship trial. Only the courtship trial was included because the scoring of both aggressive trials in the original experiments already occurred at a latency when the drug had maximal effectiveness. The latency was increased because it became clear by cessation of motor ability that the drugs were not maximally binding until after the conclusion of the courtship trials, which was approximately 50 minutes post-injection. The 1.0 mg/kg dose in this follow-up study was compared with a saline control.

Experiment 2: D2 Agonist at 1.0 and 10.0 mg/kg Doses

A second round of experiments with the same 20 focal males, paired with the same stimulus males as in Experiment 1, was conducted. The D2 agonist quinpirole at a 1.0 mg/kg dose in Experiment 2A and at a 10.0 mg/kg dose in Experiment 2B were each compared with saline. The only change was an increase in time between the drug injection and the start of the behavioral trials from 30 minutes in

Experiment 2A to 55 minutes in Experiment 2B because of the reasons described above in Experiment 1.

Experiment 3: D1 Agonist at 0.001, 0.01, 0.1 mg/kg Doses

The same anole lizards used in the previous experiments were again used approximately five months later, beginning in February of 2015. Because of changes in body size due to growth in the intervening period, new focal-stimulus pairings were established. The number of subjects was also increased (focal males N=22). The doses for the drugs were 0.001, 0.01, and 0.1 mg/kg. The drugs were administered to the focal males 65 minutes before the behavioral trials began. The timing was changed from 55 minutes to 65 minutes due to procedural timing constraints.

A small follow-up study immediately following the conclusion of the previous experiment was conducted to test the repeatability of significance obtained for the 0.001 mg/kg D1 agonist dose in the mirror aggression trials (focal males N=22). The 0.001 mg/kg dose in this follow-up study was compared with a saline control. The mirror trials were run as described in previous experiments.

Experiment 4: D1 Agonist at 0.005 and 0.05 μg/kg Doses

A fourth experiment was conducted beginning in August 2015 using D1 doses that replicated the significantly low doses of 0.005 and 0.05 μg/kg used by Woolley et al. (2001). The doses used were 0.05 and 0.005 μg/kg. Again, because of changes in body size, new focal-stimulus males were established. The number of subjects was also decreased (focal males N=10) due to time constraints. Had any trends been apparent, a second group of 10 animals would have been tested. The

latency to the start of behavioral trials after drug injection was increased from 50 to 55 minutes because of procedural constraints. The manner in which the behavioral trials were conducted is the same as described in previous experiments.

Experiment 5: D1 and D2 Antagonists at 0.1 and 1.0 mg/kg Doses

A fifth experiment was conducted beginning in February 2016 using the D1 antagonist SCH-23390 and the D2 antagonist raclopride at 0.1 and 1.0 mg/kg doses. The number of subjects tested was 10 focal males different from the 10 previously tested in Experiment 4. The behavioral trials were the same as described in previous experiments.

Data Analysis

All analyses utilized non-parametric tests because the data did not meet the assumptions of parametric tests. For Experiments 1 and 2, the frequencies and latencies of social behaviors with the drug or with saline were analyzed using a Wilcoxon Signed Ranks test, with significance set at $p<0.05$. For Experiments 3, 4, and 5, the frequencies and latencies of social behaviors with the various drug doses and with saline were analyzed using a Friedman, a non-parametric repeated measures ANOVA, with significance set at $p<0.05$. Post-hoc analyses were conducted using Wilcoxon Signed Rank tests.

Results

Experiments 1: D1 Agonist at 1.0 and 10.0 mg/kg Doses

Results from Experiment 1A found that there were no significant differences in the D1 agonist at the 1.0 mg/kg dose treatment (Tables 4). Separate analyses of the frequencies and latencies of the dorsal crest and eye spot did not offer any additional insight into the role of D1 and D2 receptors on social behaviors, so those data are not reported for any experiment.

For Experiment 1B, there were significant differences found in the 10.0 mg/kg treatments. Both frequencies of and latencies to behaviors were affected in the mirror and intermale aggression trials (Figures 1 and 2). Additionally, the latency of behaviors in the courtship trial was also significantly higher with the 10.0 mg/kg dose of the D1 agonist. A transient cessation of all locomotor activity at approximately 50 minutes followed the 10.0 mg/kg D1 agonist treatment (Table 5). Lizards remained in a state of very low motor activity for a period of minutes to hours after the cessation of behavioral trials, but regained normal motor functioning when the drugs wore off.

After data from Experiment 1 were obtained, it was evident by the lack of motor ability that the drug bound maximally after the conclusion of the courtship trials. The small follow-up experiment to Experiment 1A, using the D1 agonist at 1.0 mg/kg in just the courtship trial, revealed no significant difference in the behaviors observed (Table 6).

Experiment 2: D2 Agonists at 1.0 and 10.0 mg/kg Doses

The results from Experiment 2 revealed that there were no significant differences between the 1.0 mg/kg and saline treatments (Table 7).

However, the lizards treated with 10.0 mg/kg treatment displayed significantly fewer aggressive behaviors than lizards treated with saline (Figure 3 and Table 8). The latency to display courtship behaviors, as well as aggressive behaviors in both the mirror and intermale aggression trials were greater when given the 10 mg/kg dose (Figure 4). While there was no noticeable motor impairment as with the D1 agonist at 10.0 mg/kg, there was a nonspecific decrease in all behaviors.

Experiment 3: D1 Agonists at 0.001, 0.01, 0.1 mg/kg Doses

For Experiment 3, the frequencies and latencies for courtship and intermale aggression were not significantly different for any doses (Table 9). However, the frequency of aggressive behaviors in the mirror trial was significantly different between groups. Post-hoc analysis indicated that the saline significantly differed from the 0.001 mg/kg dose. However, the 0.1 mg/kg or 0.01 mg/kg doses did not significantly differ from the control.

The small follow-up experiment to Experiment 3, conducted to ensure the repeatability of significance obtained by the 0.001 mg/kg dose in the mirror aggression trial, revealed no significant differences in the behaviors observed. This suggests the initial significance was obtained by chance (Table 10).

Experiment 4: D1 Agonists at 0.005 and 0.05 μg*/kg*

Following treatment with 0.005 and 0.05 μg/kg doses of the D1 agonist, there were no significant differences or trends found in Experiment 4 between the frequencies and latencies of behaviors in the males treated with saline and either drug dose (Tables 11).

Experiment 5: D1 and D2 Antagonists at 0.1 and 1.0 mg/kg

Following treatment with 0.1 and 1.0 mg/kg doses of both the D1 and D2 antagonists, there were no significant differences or trends found between the behavior frequencies (Figure 5) and latencies (Figure 6) of the males treated with saline and either drug dose (Table 12).

Discussion

Despite the evidence of for the effects of D1 and D2 receptor activation on sexual and aggressive behaviors in other species, neither the agonists nor antagonists significantly affected those social behaviors in the male green anoles.

The lack of specific effects on social behaviors was not due to the inability of the agonists to have a biological effect. The D1 agonist had an effect, evident by the motor inhibition after the 10.0 mg/kg D1 agonist dose. The D2 agonist also clearly had an effect, since the 10.0 mg/kg dose significantly increased the latencies in all three behavioral trials (Table 8). Both D1 and D2 receptors are involved in motor skills (Clemens et al., 2012), so a high dose affecting motor ability is not surprising. Though treatment with the D2 agonist at the 10.0 mg/kg dose did not result in a transient cessation of all motor activity like the D1 agonist at 10.0 mg/kg, the nonspecific decrease in all social behaviors could not be trusted to be specific to social behavior circuitry. Therefore, neither of the D1 nor D2 agonists directly affected social behaviors.

There was a trend at the 1.0 mg/kg D1 agonist dose for courtship behavior (Table 4), though the drug was only given 30 minutes to take effect. It was later discovered that this time did not allow the drug sufficient time to be maximally effective. The D1 agonist treatment at the 1.0 mg/kg treatment in the replicated experiment on courtship behavior at a time when the drug would be more effective did not significantly differ from the control treatment, suggesting that the initial trend of a decrease in courtship behavior with the 1.0 mg/kg drug treatment was due to chance, and not a result of the effects of the drug on sexual behavior.

For the follow-up study to Experiment 3, replicating just the mirror aggression trial at 0.001 mg/kg dose of the D1 agonist, no significant difference between treatment groups was found. This indicates that the significance obtained in Experiment 3 for the dose 0.001 mg/kg in the mirror aggression trial was due to chance of multiple comparisons.

The lack of significance for the very low D1 agonist doses that were found to be significant in whiptail lizards could be due to the different type of agonists used between the studies (Woolley et al., 2001). The drug, SKF 81297, used in Woolley et al. (2001) is a full D1 agonist with an efficacy rate comparable to dopamine itself (Andersen and Jansen, 1990). However, SKF 81297 is not one that is commonly utilized in such experiments on social behaviors, so its effects from peripheral injections in other animals are unclear (Table 1).

Following the lack of significant results obtained from all of the D1 agonist doses studied, the effects of D1 and D2 antagonists were examined to determine if the lack of significant results could be due to a ceiling effect. Because antagonists inactivate receptors, we thought that testing the effects of D1 and D2 antagonists could clarify the role of D1 and D2 receptors in sexual and aggressive behaviors in male green anoles. However, no significant results were found for either the D1 or D2 antagonist, suggesting that there is not a ceiling effect with the agonists, and the lack of significant results is due to another cause.

There is little known about dopamine's role in aggression in lizards, but previous research on mammals and birds suggested that peripheral injections of dopamine agonists and antagonists should have had an effect. For instance, a D1

agonist increased aggressive behaviors, while a D1 antagonist decreased these same behaviors in hens (Dennis and Cheng, 2011), and rats (Couppis and Kennedy, 2008). The role of D2 receptors in aggression is slightly more variable; a D2 agonist decreased aggression in zebra finches (*Taeniopygia guttata*) (Kabelik et al., 2010), while it increased aggression in hens (Dennis and Cheng, 2011). However, effects of D2 antagonists are slightly more consistent. A D2 antagonist decreased aggression in both hens (Dennis and Cheng, 2011) and rats (Couppis and Kennedy, 2008). Thus the functionality of D2 receptors appears to be involved in aggression, but the manner in which it is involved in still unknown.

A more direct approach of drug delivery to brain regions involved in aggression might yield more telling results. For example, the NAC, AMY, prefrontal cortex (PFC), and parts of the hypothalamus (HYP) are regions known to be involved in regulating aggression (Puglisi-Allegra and Cabib, 2000). A direct method of delivering dopaminergic drugs into these regions individually might prevent the drug from binding to various brains regions that are involved in aggression, but do not affect aggression in the same direction, and thus may counteract one another (Puglisi-Allegra and Cabib, 2000). Dopamine specifically is involved in aggression in these regions in mammals, evident by an increase in dopamine in the NAC in mice (Haney et al., 1990), and both the NAC and PFC in rats after an aggressive encounter (Van Erp and Miczek, 2000). In addition, there are changes in dopaminergic tone in the NAC, PFC, and AMY following an aggressive encounter in mice (Puglisi-Allegra and Cabib, 1990). Specifically, in regards to the effects of D1 and D2 receptors, aggressive behaviors are decreased after D1 and D2

antagonists are separately administered directly into the NAC (Couppis and Kennedy, 2008). When a D2 agonist is injected into a sub-region of the hypothalamus in cats, aggressive behavior is facilitated, while aggression was inhibited by a D2 antagonist (Sweidan et al., 1991). Therefore, specifically targeting regions known to be involved in aggression would more clearly elucidate the functions of D1 and D2 receptors in lizards.

The effect of dopamine on sexual behaviors has been characterized to a greater extent than its effects on aggression. Because all of the previous research in other species did show an effect of D1 and D2 agonists and antagonists on sexual behaviors, it was surprising that effects were not seen in male green anoles.

In previous studies, D1 agonists and antagonists had consistent results on sexual behavior in various species. Intraperitoneal injections of D1 agonists have been found to promote appetitive and consummatory sexual behaviors in quail (Balthazart et al., 1997) and rats (Beck et al., 2002; D'Aquila et al., 2003). In addition, systemic injection of a D1 antagonist decreases appetitive and copulatory behaviors in rats (Pfaus and Phillips, 1991; Ahlenius and Larsson, 1990) and quail (Balthazart et al., 1997). Therefore, the activation of D1 receptors via an agonist is largely consistent in that it facilitates sexual behaviors, and a D1 antagonist produces the opposite results.

The activation of D2 receptors has a less consistent effect on sexual behaviors. Appetitive and consummatory sexual behaviors are inhibited by a D2 agonist in quail (Balthazart et al., 1997) and rats (Bitran et al., 1989); these same behaviors are also inhibited by a D2 antagonist in rats (Pfaus and Phillips, 1991;

Ahlenius and Larsson, 1990). It is odd that an antagonist would produce the same results as an agonist, so the functionality of D2 receptors in regards to sexual behavior is still unclear.

The non-significant results in this study could be due to the peripheral administration of the drugs, which might be a too general means of drug delivery. As mentioned above in relation to aggression, a more effective way of administering the drugs would be to directly inject the dopaminergic drugs into those brain regions of the male green anole that are known for controlling male sexual behavior, such as the medial preoptic area (mPOA) (McHenry et al., 2012). The mPOA is a brain region that is involved with male sexual behavior (McHenry et al., 2012). When apomorphine (APO), a dopamine agonist that binds to both D1 and D2 receptors, is microinfused into the mPOA, there is an increase in consummatory behaviors in male rats (Hull et al., 1986). More specifically, in male Japanese quail, D1 and D2 antagonists separately administered directly into the mPOA inhibited appetitive and consummatory sexual behaviors (McHenry et al., 2012; Kleitz-Nelson et al., 2010c).

However, these specific effects on sexual behavior only occur if the drug is delivered directly into the mPOA. When APO is injected into the ventricles of male rats, consummatory behaviors are decreased, as opposed to increased, when delivered directly into the mPOA (Hull et al., 1986). Similarly in male quail, when a D2 antagonist was injected into the ventricles it facilitated consummatory and appetitive sexual behavior, which was opposite of what was seen when the drug was administered directly into the mPOA (Kleitz-Nelson et al., 2010c). Therefore, non-

specific injections could be acting on different regions of the brain that might be opposing each other (Hull et al., 1986). This idea is further supported by the fact that when APO is peripherally injected into male quail, there is a decrease in appetitive and consummatory sexual behaviors (Castagna et al., 1997), while the opposite effect occurs in rats (Hull et al., 1986). In quail, the APO seems to be binding primarily to D2 receptors, apparent by stereotypic pecking that occurs when D2 receptors are activated, whereas APO appears to be binding primarily to D1 receptors in rats (Castagna et al., 1997). The difference in binding could be due to birds having a higher general density of D2 receptors than D1 throughout the brain, whereas rats have more D1 receptors than D2 (Kleitz et al., 2009). The peripheral injections used in the present study could thus be acting in different brain regions that are involved in social behaviors, but produce counteracting effects.

The lack of significant results for both sexual and aggressive behaviors in the present study could also be attributed to potential differences in receptor densities. While distributions for D1 and D2 receptors are conserved among different taxa, the densities of these receptors are not conserved (Richfield et al., 1987). Turtles and pigeons were found to have more D2 receptors than D1 compared throughout the brain with mammals, such as rats, cats, and monkeys (Richfield et al., 1987), and rats had more D1 receptors than D2, while quail had more D2 receptors than D1 (Kleitz et al., 2009). However, it does not appear that the reptilian brain has a radically different brain than birds or even mammals. In green anoles, D1 receptor distribution and density was similar to those found in mammals, though the density

of D2 receptors is higher in some brain regions compared with the densities of D2 receptors in mammals and birds (Clark et al., 2000). However, this relative conservation of densities is an exception to the pattern observed in previous studies, which have found density differences between taxa (Richfield et al., 1987; Kleitz et al., 2009). Because of this ambiguity in receptor densities, a more specific approach to administering the dopaminergic drugs could elucidate the functions of D1 and D2 receptors in sexual and aggressive behaviors in the male green anole, especially since the ratio of D1/D2 receptors in reptiles is still not entirely clear.

Conclusion

Peripheral injections of D1 and D2 receptor agonists and antagonists at various doses did not affect sexual or aggressive behaviors in male green anoles, despite evidence from previous studies that did find an effect in other species. Future studies should examine the effects of D1 and D2 receptor agonists and antagonists on social behaviors in lizards by directly administering the drugs into brain regions known to be involved in social behaviors. Overall, the role of D1 and D2 receptors in sexual and aggressive behaviors in lizards is still largely unknown.

Table 1. Previous studies on sexual and aggressive behaviors and the various drugs and doses used.

References	Animal	Drug	Dose 1	Dose 2	Dose 3	Dose 4
D1 Agonists						
Woolley et al., 2001	Lizards	SKF81297	0.005 µg/kg[A]	0.05 µg/kg[B]	0.5 µg/kg	
Beck et al., 2002	Rats	SKF38393	1.0 mg/kg[C]	2.5 mg/kg[C]	5.0 mg/kg[C]	
Balthazart et al., 1997	Quail	SKF38393	0.1 mg/kg[C]	1.0 mg/kg[C]		
Dennis and Cheng, 2011	Hens	SKF38393	0.5 mg/kg[E]			
Kabelik et al., 2010	Finches	SKF38393	1.0 mg/ kg			
D2 Agonists						
Balthazart et al., 1997	Quail	Quinpirole	0.1 mg/kg	1.0 mg/kg[F]		
Dennis and Cheng, 2011	Hens	Quinpirole	0.5 mg/kg[E]			
Kabelik et al., 2010	Finches	Quinpirole	100.0 ug/kg[E]	1.0 mg/kg[E]		
D1 Antagonists						
Pfaus and Phillips, 1991	Rats	SCH23390	0.01 mg/kg	0.05 mg/kg	0.1 mg/kg	0.5 mg/kg[C]
Ahlenius and Larsson, 1990	Rats	SCH23390	25.0 µg/kg[C]	50.0 µg/kg[C]	100.0 µg/kg[C]	
Balthazart et al., 1997	Quail	SCH23390	0.1 mg/kg	1.0 mg/kg[C]		
Dennis and Cheng, 2011	Hens	SCH23390	0.5 mg/kg[E]			
D2 Antagonists						
Pfaus and Phillips, 1991	Rats	Sulpiride	0.5 mg/kg	1.0 mg/kg	5.0 mg/kg	10.0 mg/kg[F]
Ahlenius and Larsson, 1990	Rats	Raclopride	0.1 mg/kg[C]	0.6 mg/kg[C]	1.6 mg/kg[C]	
Balthazart et al., 1997	Quail	Spiperone	2.0 mg/kg[C]	10.0 mg/kg[C]		
Dennis and Cheng, 2011	Hens	Raclopride	0.5 mg/kg[E]			

[A] Significant in the *C. uniparens* individuals for consummatory sexual behavior
[B] Significant in male *C. inornatus* for consummatory sexual behavior
[C] Significant for consummatory sexual behavior
[E] Significant for aggressive behaviors
[F] Significant for appetitive and consummatory sexual behavior

Table 2. The results of D1 and D2 receptor agonists (AG) and antagonist (ANT) on sexual and aggressive behaviors among taxa, as well as predictions for the effects in male green anoles. A ↑ indicates an increase in behaviors, while a ↓ indicates a decrease. A **?** indicates that there are no known studies on the effects of D1 or D2 receptor agonists or antagonists.

Previous Findings: Sexual Behaviors					Predictions for Male Green Anoles			
	D1 AG	D1 ANT	D2 AG	D2 ANT	D1 AG	D1 ANT	D2 AG	D2 ANT
Mammals	↑[A]	↓[D]	↓[F]	↓[D]				
Birds	↑[B]	↓[B]	↓[B]	?				
Reptiles	↑[C]	↓[E]	?	?	↑	↓	?	?

Previous Findings: Aggressive Behaviors					Predictions for Male Green Anoles			
	D1 AG	D1 ANT	D2 AG	D2 ANT	D1 AG	D1 ANT	D2 AG	D2 ANT
Mammals	↑[G]	↓[G]	?	↓[G]				
Birds	↑[H]	↓[H]	↓[I] ↑[H]	↓[H]				
Reptiles	?	?	?	?	↑	↓	?	?

[A] Beck et al., 2002; D'Aquila et al 2003
[B] Balthazart et al., 1997
[C] Woolley et al., 2001
[D] Pfaus and Phillips, 1991; Ahlenius and Larsson, 1990
[E] see Woolley et al., 2004 for review
[F] Bitran et al., 1989
[G] Couppis and Kennedy, 2008
[H] Dennis and Cheng, 2011
[I] Kabelik et al., 2010

Table 3. An ethogram of behaviors recorded for the focal males and stimulus animals during behavioral trials.

Behaviors	Description
Head Bob	Nodding up and down of the head, while the rest of the body remains immobile, with each differentiated by a slight pause
Push Up	Lifting up and down of the entire body, with each differentiated by a slight pause
Dewlap Extension	A full extension of the dewlap (throat fan)
Dewlap/Push Up	Combined dewlap extension and push up, with each differentiated by a slight pause
Chase	Rapid pursuit of the conspecific
Copulate	Copulation with the conspecific (only occurred in male-female trials
Dorsal Crest	Elevation of the dorsal crest
Eye Spot	Darkening of the postorbital skin

Table 4. The frequencies and latencies of scored behaviors for all three behavioral trials (male-female, male-mirror, male-male), using the D1 agonist 1 mg/kg drug treatment (N=20).

	Saline		D1 1 mg/kg		Wilcoxon Signed Ranks	
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Z-score	P-value
Courtship Frequency	17.8	2.2791	14.4	2.0109	-1.625	0.104
Courtship Latency	1.4	0.2209	1.8	0.4679	-0.947	0.344
Mirror Aggression Frequency	21.7	3.5947	15.6	4.1596	-1.592	0.111
Mirror Aggression Latency	3.3	0.5567	4.1	0.8223	-0.634	0.526
Intermale Aggression Frequency	24.3	4.5497	22.5	4.0740	-0.093	0.926
Intermale Aggression Latency	2.5	0.6135	3.0	0.7398	-0.226	0.821

Table 5. The frequencies and latencies of scored behaviors for all three behavioral trials (male-female, male-mirror, male-male), using the D1 10 mg/kg drug treatment (N=20). P values <0.05 denoted in bold.

	Saline		D1 10 mg/kg		Wilcoxon Signed Ranks	
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Z-score	P-value
Courtship Frequency	18.1	2.2395	15.1	2.1636	-1.065	0.287
Courtship Latency	1.2	0.2000	2.6	0.7236	-2.414	**0.016**
Mirror Aggression Frequency	14.7	2.2725	0.6	0.2938	-3.624	**0.000**
Mirror Aggression Latency	4.2	0.7800	8.0	0.7980	-3.008	**0.003**
Intermale Aggression Frequency	21.4	2.8520	2.0	0.8159	-3.921	**0.000**
Intermale Aggression Latency	1.5	0.1846	7.8	0.8754	-3.57	**0.000**

Table 6. The frequencies and latencies of scored behaviors for the repeated courtship behavioral trials using the D1 agonist 1 mg/kg drug treatment at a longer latency before behavioral scoring (N=19).

	Saline		D1 1 mg/kg		Wilcoxon Signed Ranks	
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Z-score	P-value
Courtship	27.4	2.7459	28.6	2.9601	-0.282	0.778
Courtship Latency	1.1	0.1053	0.5	0.0000	-1.000	0.317

Table 7. The frequencies and latencies of scored behaviors for all three behavioral trials, using the D2 agonist 1 mg/kg drug treatment (N=20).

	Saline		D2 1 mg/kg		Wilcoxon Signed Ranks	
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Z-score	P-value
Courtship Frequency	19.4	2.3444	19.3	3.3442	-0.403	0.687
Courtship Latency	1.5	0.1846	1.6	0.4500	-0.368	0.713
Mirror Aggression Frequency	18.5	4.8534	15.0	4.0565	-0.741	0.459
Mirror Aggression Latency	3.9	0.8642	4.3	0.7605	-0.283	0.777
Intermale Aggression Frequency	25.5	4.1381	28.8	4.9032	-0.479	0.632
Intermale Aggression Latency	1.7	0.4818	2.3	0.6203	-1.156	0.248

Table 8. The frequencies and latencies of scored behaviors for all three behavioral trials, using the D2 10 mg/kg drug treatment (N=20). P values <0.05 denoted in bold.

	Saline		D2 10 mg/kg		Wilcoxon Signed Ranks	
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Z-score	P-value
Courtship Frequency	21.5	2.9491	15.5	2.4479	-1.999	0.460
Courtship Latency	1.3	0.2036	2.6	0.5305	-2.572	**0.010**
Mirror Aggression Frequency	10.0	3.4690	7.9	2.4782	-0.699	0.484
Mirror Aggression Latency	4.8	0.8363	6.4	0.8379	-2.349	**0.019**
Intermale Aggression Frequency	28.2	4.2106	16.5	4.2491	-2.287	**0.022**
Intermale Aggression Latency	2.1	0.6152	5.4	0.9825	-2.737	**0.006**

Table 9. The frequencies and latencies of scored behaviors for all three behavioral trials, using the D1 agonist 0.001, 0.01, 0.1 mg/kg drug treatments (N=22). P values <0.05 denoted in bold.

	Saline		0.001 mg/kg		0.01 mg/kg		0.1 mg/kg		Friedman		
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	$\chi 2$	df	P value
Courtship Frequency	22.4	2.7942	21.8	3.0221	21.6	3.1963	24.9	2.3953	1.26	3	0.738
Courtship Latency	1.7	0.4595	2.0	0.5915	2.2	0.6001	1.5	0.4288	3.00	3	0.392
Mirror Aggression Frequency	14.2	3.2187	7.4	1.9886	8.9	2.4889	10.7	2.7169	11.89	3	**0.008**
Mirror Aggression Latency	3.6	0.5454	4.9	0.8403	4.8	0.7765	3.7	0.7082	1.81	3	0.612
Intermale Aggression Frequency	25.0	3.7432	28.3	3.6691	25.6	2.9052	30.3	4.1256	7.33	3	0.062
Intermale Aggression Latency	2.5	0.6348	1.9	0.4624	2.0	0.4602	3.7	0.5031	4.14	3	0.246

Table 10. The frequencies and latencies of scored behaviors for the repeated mirror behavioral trials using the D1 agonist 0.001 mg/kg drug treatment (N=22). Though significance was obtained in a previous experiment (Table 9), significance was not replicated in the follow-up experiment.

	Saline		D1 0.001 mg/kg		Wilcoxon Signed Ranks	
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Z-score	P-value
Mirror Aggression Frequency	6.2	2.1153	4.1	1.9321	-1.335	0.182
Mirror Aggression Latency	6.9	0.8294	6.9	0.7551	-0.891	0.373

Table 11. The frequencies and latencies of scored behaviors for the behavioral trials using the D1 agonists at the low doses (N=10).

	Saline		0.005 μg/kg		0.05 μg/kg		Friedman		
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	$\chi 2$	df	P value
Courtship Frequency	19.7	3.4060	17.4	4.0694	19.7	5.1339	4.974	2	0.083
Courtship Latency	2.0	0.8944	1.9	0.9000	1.9	0.6904	0.667	2	0.717
Mirror Aggression Frequency	5.6	2.5219	11.9	3.9085	15.4	6.0004	2.87	2	0.239
Mirror Aggression Latency	6.0	1.3416	5.3	1.0858	5.3	1.3254	0.595	2	0.595
Intermale Aggression Frequency	11.7	3.6999	19.1	6.2707	23.4	7.3997	0.649	2	0.723
Intermale Aggression Latency	3.3	1.1358	4.4	1.3013	3.5	1.2758	0.707	2	0.707

Table 12. The frequencies and latencies of scored behaviors for the behavioral trials using the D1 and D2 antagonists (N=10).

Antagonists	Saline		D1 0.1 mg/kg		D1 1 mg/kg		D2 0.1 mg/kg		D2 1 mg/kg		Friedman		
Behaviors	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	$\chi 2$	df	P value
Courtship Frequency	6.8	2.1385	7.8	2.0966	8.1	1.8345	12	2.6791	10.8	3.0470	4.14	4	0.388
Courtship Latency	3.8	1.1719	4.3	1.3598	3.1	1.1590	2.8	1.0520	3.1	1.1590	8.32	4	0.080
Mirror Aggression Frequency	8.4	4.0337	3	2.0440	3.6	1.7651	7.4	2.9143	6.2	3.5113	1.85	4	0.764
Mirror Aggression Latency	6.1	1.3454	7.2	1.0934	5.3	1.2387	6.1	1.3119	6.3	1.1260	1.96	4	0.743
Intermale Aggression Frequency	17.8	6.3365	25.9	6.3183	17.3	6.1102	31.1	6.5174	21.9	6.5548	3.39	4	0.495
Intermale Aggression Latency	3.3	1.0225	3	1.1738	5.2	1.3888	2.4	0.9333	3.4	1.1470	3.13	4	0.536

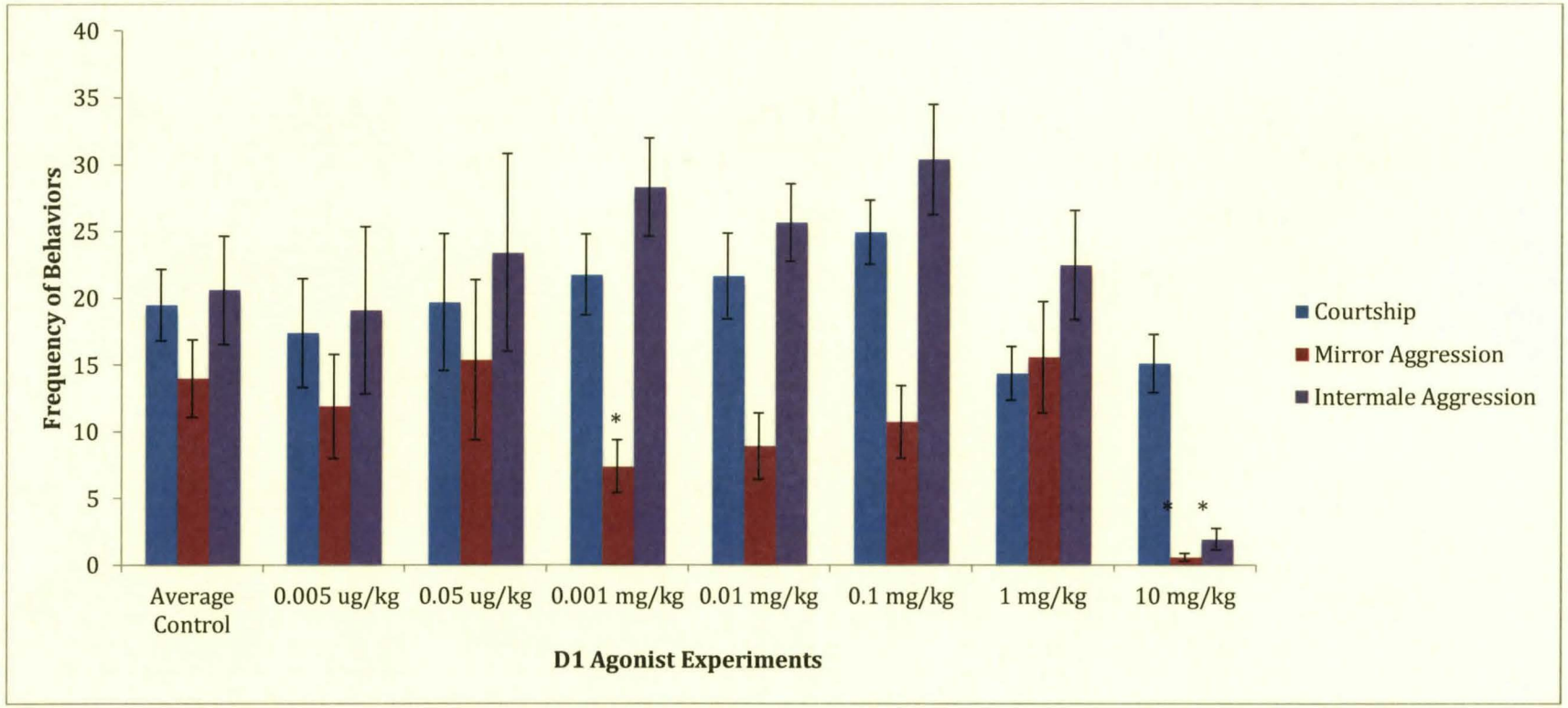

Figure 1. A dose response curve for the frequencies of sexual and aggressive behaviors in experiments using D1 agonists. The control values were averaged from all experiments using D1 agonists. The error bars represent ± S.E.M. The * represents significance of $p<0.05$ compared to control.

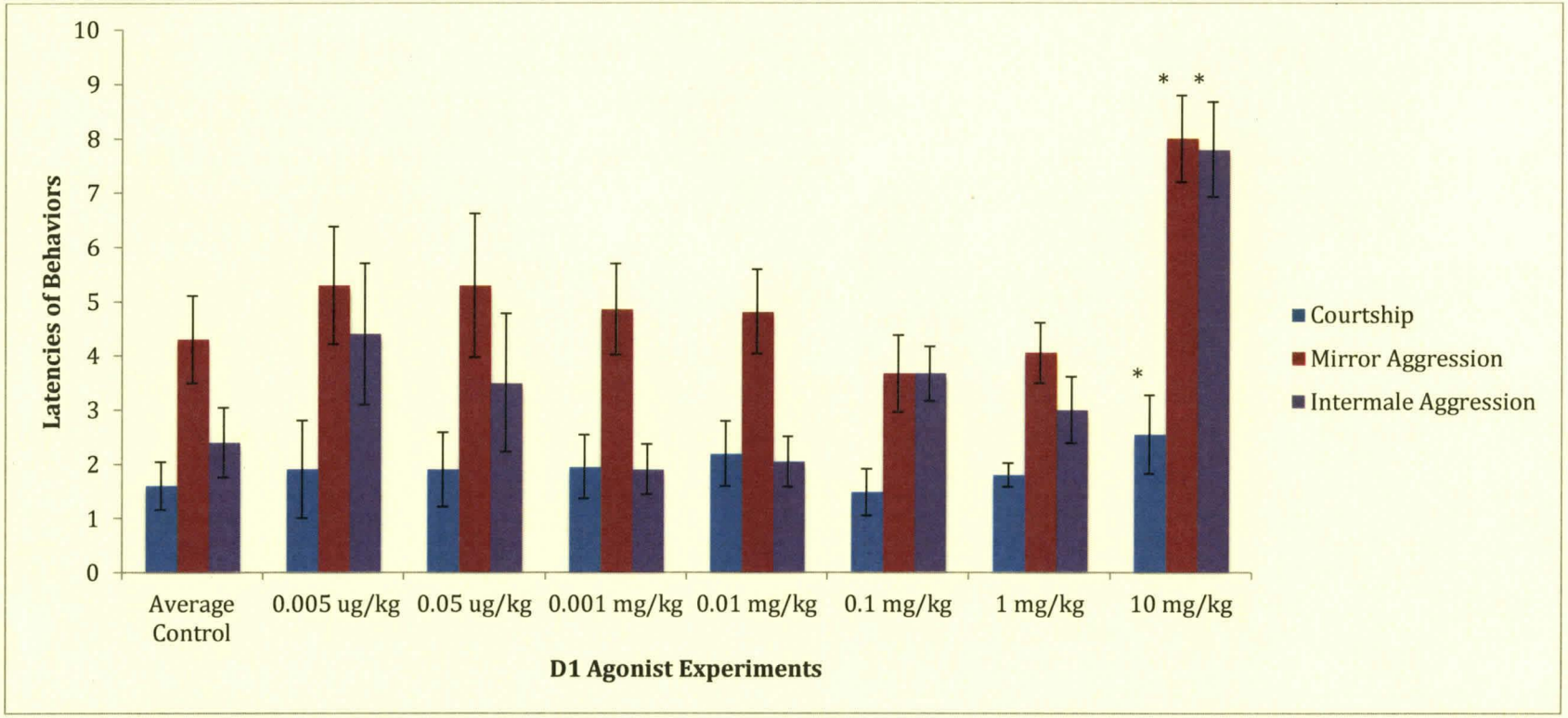

Figure 2. A dose response curve for the latencies of sexual and aggressive behaviors in experiments using D1 agonists. The control values were averaged from all experiments using D1 agonists. The error bars represent ± S.E.M. The * represents significance of $p<0.05$ compared to control.

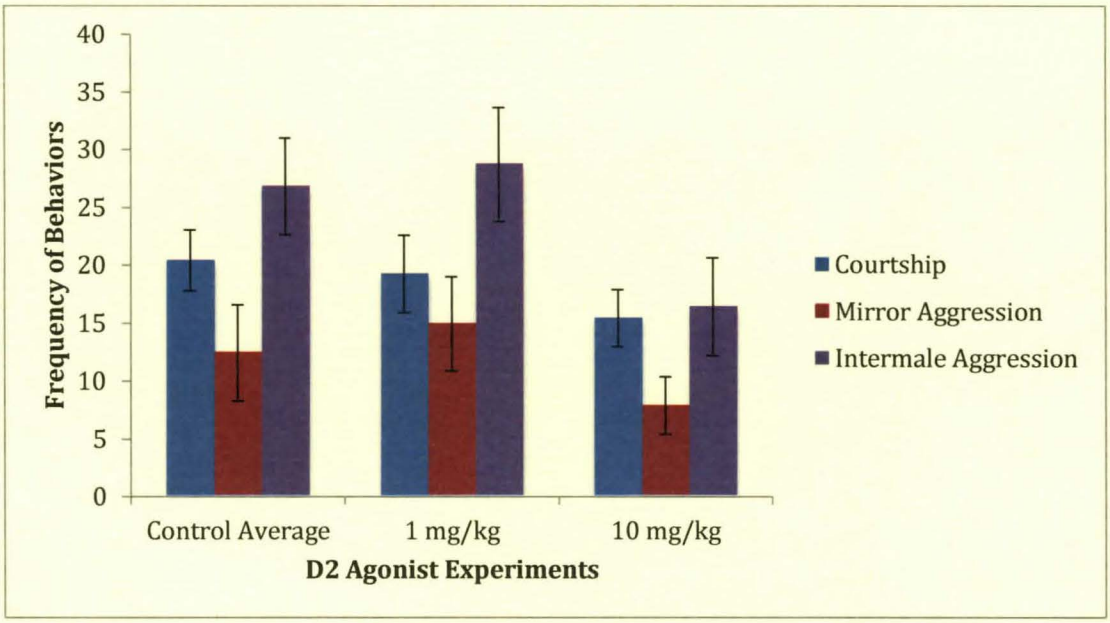

Figure 3. The frequencies of sexual and aggressive behaviors in the social trials were not significantly different after treatment. The error bars represent ± S.E.M. The * represents significance of $p<0.05$ compared to control.

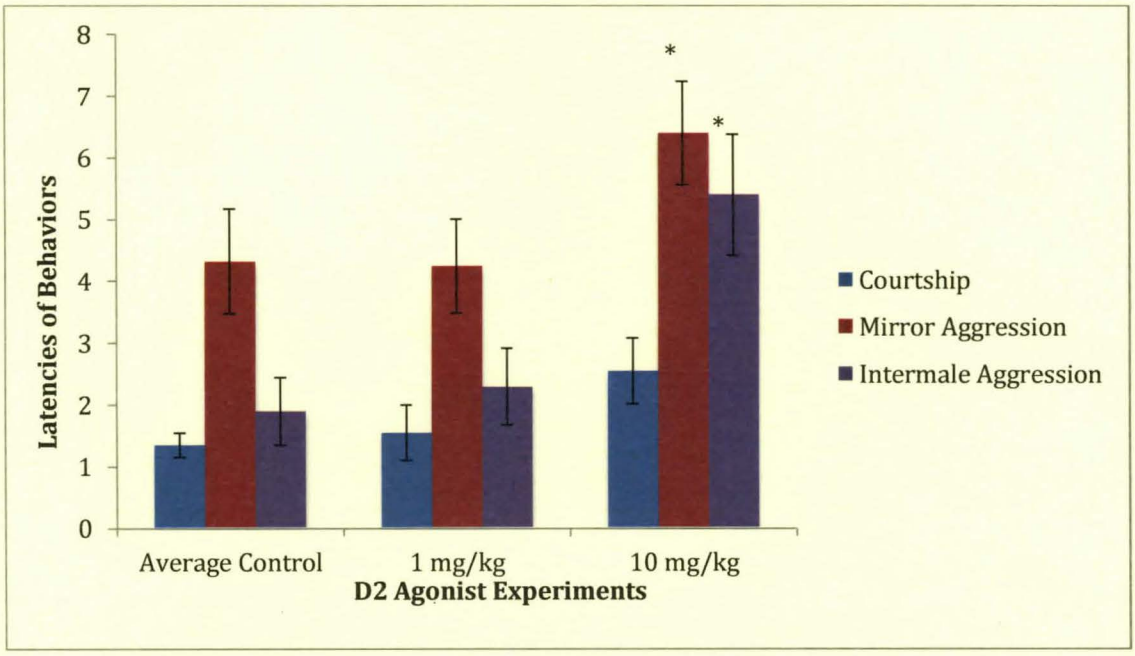

Figure 4. The latencies of sexual and aggressive behaviors in the social trials were not significantly different after treatment. The error bars represent ± S.E.M. The * represents significance of $p<0.05$ compared to control.

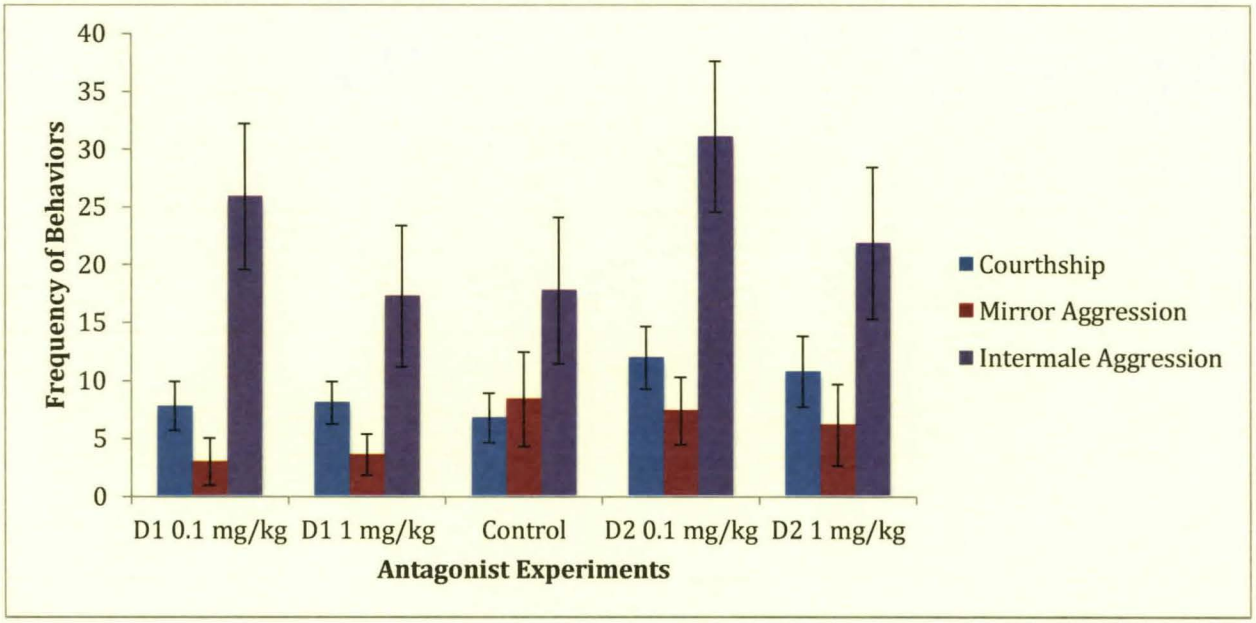

Figure 5. The frequencies of sexual and aggressive behaviors in the social trials were not significantly different after treatment with the antagonists. The error bars represent ± S.E.M.

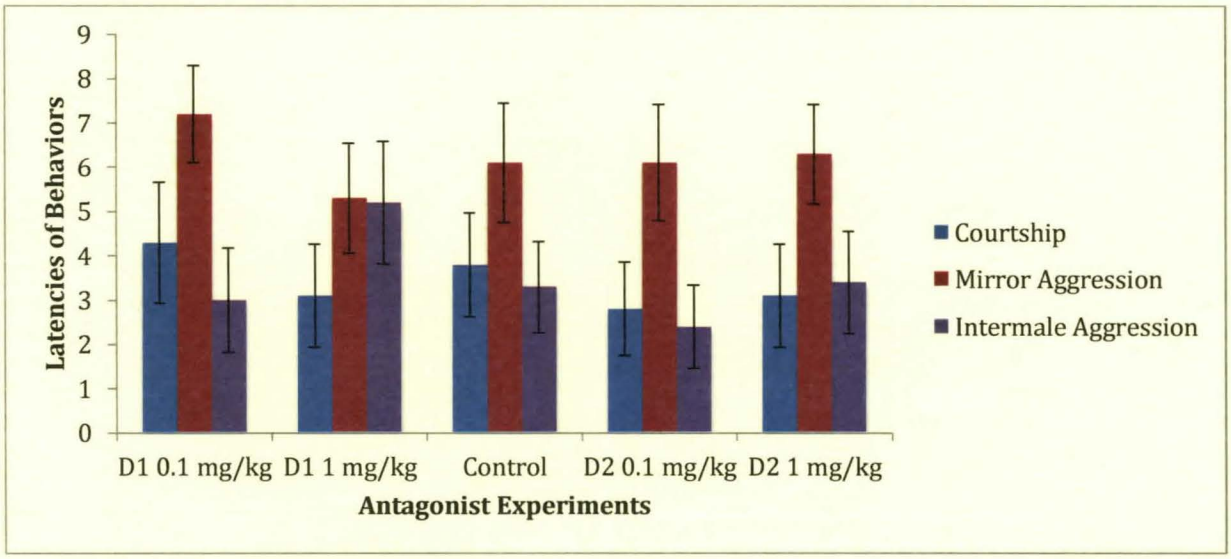

Figure 6. The latencies of sexual and aggressive behaviors in the social trials were not significantly different after treatment with the antagonists. The error bars represent ± S.E.M.

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