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Dopamine receptor manipulation does not alter patterns of partner preference in long-term marmoset pairs

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ABSTRACT

The relationship between socially monogamous mates is dynamic and regulated by neurobiological influences. Research in rodent models has indicated a key role for the neurotransmitter dopamine (DA) and its receptors (DAR) in mediating the formation and maintenance of monogamous bonds. DAR activation was pharmacologically manipulated in marmosets housed in long-term pairs. Marmosets exposed to DAR manipulation were tested in a partner preference test under two social conditions: one in which their mate could visually observe their interactions with an opposite-sex individual, and one in which their pair mate could not visually observe these interactions. Marmosets displayed a spatial preference for the mate compared to an unfamiliar conspecific, however, they displayed a sexual preference for an unfamiliar conspecific over their mate. D1R manipulation had no impact on marmoset partner preference. However, activation of D2Rs reduced the time marmosets spent in contact with either stimulus animal, indicating a decrease in social interest, but did not reduce time spent in proximity to the stimulus animals nor number of sexual solicitations. Additionally, social context (visibility of the mate) did not influence marmoset behavior. These findings suggest that D2Rs may be involved in regulating generalized, but not partner-specific, social interest in marmoset monkeys.

1. Introduction

Interactions between socially monogamous mates are qualitatively unique from other social interactions, and can be characterized by high levels of affiliation, aggression toward same- or opposite-sex conspecifics, biparental care, and/or selective social and sexual preferences for the mate compared to other potential sexual partners [1–3]. The expression of these behavioral components is dynamic and changes over time [4,5]. Pair bonds, or the attachments between socially monogamous mates, are operationalized in rodent models as the formation of a selective social preference for the mate over a stranger of the opposite-sex [6], and behavioral changes associated with pair bond formation appear to be mediated by shifts in underlying neurobiology [6–8].

Notably, the monoamine neurotransmitter dopamine (DA), commonly recognized for its involvement in reward, plays a prominent role in regulating behavior between mates in socially monogamous rodents [9,10]. DA is involved in normative reward processes such as those associated with eating, drinking and mating, as well as in co-opted reward processes such as drug addiction [11]. DA's role in reward can be partially attributed to the facilitation of memory for events with motivational importance [12]. In fact, DA's action in aiding associative learning in contexts such as these may explain its role in social bonding [13]. For instance, DA is released in response to mating, which is a key behavior in the formation of bonds between monogamous rodents [13]. Thus, DA release associated primarily with a single mate in socially monogamous species may provide a mechanism through which selective (and rewarding) social attachments are formed.

The DA system contains two subtypes of receptors: D1-like (D1R, D5R; henceforth D1R) and D2-like (D2R, D3R, D4R; henceforth D2R). In the socially monogamous prairie vole (*Microtus orchrogaster*), D2R activation facilitates the formation of bonds between mating partners, while D1R activation plays a role in the maintenance of already established bonds. D1Rs are upregulated in the nucleus accumbens post-pairing in male prairie voles as pairs transition from the formation phase into the maintenance phase [14]. Likewise in the titi monkey (*Callicebus cupreus*), a monogamous nonhuman primate, recently paired males show increased D1R binding in key regions of the brain associated with social reward [15], indicating that D1R upregulation may also occur during social bonding in primates as in rodents. In non-monogamous Japanese macaques, treatment with either a D1R or D2R antagonist reduces interest in social images [16], indicating that the D1R and D2R systems may function together in processing of some social situations. DA also differentially influences the behavior of

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newly-paired and long-term pairs of marmosets (*Callithrix jacchus*) upon reunion with their partners after an hour-long physical separation during behavioral testing. When female marmosets from short term pairs receive manipulation (agonist or antagonist) of the D2R, the pair display reduced proximity behavior during reunion compared to when males receive the same treatment. This pattern is not observed when females in short term pairs receive treatment manipulating the D1R. However, when female marmosets from long term pairs receive manipulation (agonist or antagonist) of the D1R, the pair displays reduced proximity behavior during reunion compared to when males receive the same treatment, while proximity behavior patterns during reunion are unaffected by manipulation of the D2R [17]. Together, this set of findings suggests that the distinct roles of the D1Rs in mediating maintenance of attachment, and D2Rs in regulating formation of attachments in socially monogamous primate behavior may be conserved from rodents to primates.

While neurobiology clearly plays an essential role in regulating social behavior, the context in which an interaction takes place also has the potential to alter behavior. For example, several species of "monogamous" New World monkeys show increased social interest in, and sexual solicitation of, an opposite sex conspecific when their mate is not present [18–20]. These alterations in behavior can be extreme: male subjects tested with a female stranger engage in sexual solicitation behavior when their pair mate is not present, but behave aggressively toward female strangers when their pair mate is present [20]. Social context also appears to alter the behavior of animals in preference tests. Monogamous titi monkeys display altered patterns of proximity with their mate in the presence of a stranger [21]. Further, watching their mate interact with a potential sexual rival induces specific patterns of neural activity in male titi monkeys that are not seen when males watch a stranger female interact with a stranger male [22]. The differences observed across social contexts may be driven by changes in behavior from both members of the pair. For example, a female may respond aggressively to seeing her mate interact with a rival, shifting her mate's response toward the rival from affiliative to aggressive. Differences in social context may help to explain variability in social interest and associated behavioral output. Manipulating the social environment presents an opportunity to examine the interaction between neurobiological and contextual social factors in the expression of affiliative and aggressive behavior.

In the current study, we aimed to examine the role of the DA system in maintaining long-term bonds between marmoset mates, and to compare these effects to the known role of the DA system in other species (e.g., prairie voles and non-monogamous primates). In order to tease apart the effects of the D1 and D2 systems separately in regulating behavior, we administered D1R- and D2R-specific agonists and antagonists to marmosets and utilized a standardized partner preference test to assess how manipulation of DA receptors (DARs) altered behavior directed toward both a marmoset's long-term partner and an unfamiliar conspecific. Furthermore, we manipulated the social context of the preference test with the use of a removable barrier, such that the subject's mate either could or could not observe the subject's interactions with the unfamiliar conspecific. The environment in which the mate can observe interactions allows for a more complex social environment in which the mate, the unfamiliar stimulus animal, and the subject are potentially all able to communicate more readily with one another.

We hypothesized that the effect of DAR manipulation in long-term pairs of marmoset monkeys would follow a similar pattern as observed in pair bonded monogamous rodents. While in prairie voles, blocking D1Rs reduces species-typical aggression toward an opposite-sex stranger (a potential mate) [14], marmosets typically show social interest in opposite-sex conspecifics rather than aggression toward them [23,24]. Thus, rather than anticipating enhanced aggression toward a novel marmoset, we hypothesized that marmosets treated with a D1R antagonist would spend less time with, and express less affiliative behavior toward, their mate, and would increase these metrics for the unfamiliar stimulus animal (**H1**). We also hypothesized that activation of the pair bond "maintenance" system with a D1R agonist would result in the opposite effect. We predicted that D1R-agonsit treated marmosets would show increased affiliation and proximity with their mate and decreases in these measures toward the unfamiliar stimulus animal (**H2**). Given that the mates in this study had been paired for an extended period of time, we hypothesized that treatment with a D2R agonist (which in voles selectively alters behavior associated with relationship formation) would not alter social behavior (**H3**). However, we expected that marmosets treated with a D2R antagonist, which has been associated with decreased interest in social images in non-monogamous primates [16], would show reduced social interest overall, in either their mate or unfamiliar stimulus animal (**H4**). Finally, we anticipated that the direction of the behavioral patterns as a result of DAR manipulation would be consistent across social context, given that the role of the DA system in regulating behavior toward a mate likely evolved across multiple social contexts. However, we predicted that the visual access allowed condition would result in a magnification of the pattern induced by DAR manipulation (**H5**).

2. Materials and methods

2.1. Dose evaluation of D1R/D2R compounds

Prior to the start of the experiment six marmoset monkeys (*Callithrix jacchus*) were used to evaluate dose selection. Given DA's known role in motor control it was important to evaluate the effects of treatments on both gross and fine motor control to avoid selecting treatment doses at which behavioral effects could have been a side effect of altered motoric function. Each treatment (D1R agonist – SKF 38393, D1R antagonist – SCH 23390, D2R agonist – quinpirole, and D2R antagonist – raclopride) was administered at three doses selected from previous literature examining the effects of these treatments on primate behavior. Each marmoset was treated with a selective agonist or antagonist for the D1R (agonist: SKF38393, antagonist: SCH23390) and D2R (agonist: quinpirole, antagonist: raclopride) and observed for gross motor immobility (time spent immobile in the home cage during a 15-min observation) and performance on a fine motor task. In the fine motor task, a clear plastic box with one open side (opening: 12 × 12 cm) was presented in three different orientations (opening to the front, opening to the left, opening to the right) and marmosets were given 30 s to retrieve a preferred food reward from the box. Subjects completed nine consecutive trials of the fine motor task with order of presentation semi-randomized with each direction of opening occurring three times per testing day. The number of attempts to grab the treat was recorded as well as "time to grasp," a derived measure of the difference (in sec) from the first attempt to grasp the food item to successful grasp of the food reward. Doses of each drug for administration in social behavior testing were generally selected based on the following criteria: the highest tested dose at which no adverse side effects (i.e., gastrointestinal upset), gross, or fine motor differences were observed, relative to saline-treated control conditions. There was one treatment (D2R agonist) for which the dose selected for the social behavior study was lower than this threshold because of nausea induced by the higher dose treatment in two animals. Additional information on methods, results, and dose selection can be found in Supplemental Materials. Results from the dose selection study are presented in Supplemental Fig. 1.

2.2. Subjects

Ten common marmosets in established heterosexual pairs were subjects in the study (five pairs, $M_{\text{pairing length}} = 3.07$ years, $SD = 1.25$ years). None of the subjects had previous parental experience. Marmosets were housed at the Callitrichid Research Center at the

University of Nebraska at Omaha in enclosures with dimensions of 1.0 × 2.5 × 2.0 m. Marmosets received a daily diet of a prepared commercial marmoset food (Zupreem) supplemented by fresh fruits, vegetables, yogurt, apple sauce, eggs and mealworms. The production of offspring was prevented by surgical vasectomy of the male. All procedures were approved by the University of Nebraska Medical Center/University of Nebraska at Omaha Institutional Animal Care and Use Committee (protocol # 15-033-05-FC) and adhere to guidelines of the National Institutes of Health guide for the care and use of laboratory animals.

2.3. Drug treatments

Subjects were administered an intramuscular (IM) injection of one of five treatments at doses determined by the results of the dose evaluation study: D1R agonist (SKF 38393, 0.05 mg/kg), D1R antagonist (SCH 23390, 0.01 mg/kg), D2R agonist (quinpirole, 0.05 mg/kg), D2R antagonist (raclopride, 0.03 mg/kg) or a saline vehicle. There is evidence for each of the described compounds to penetrate the blood brain barrier (Raclopride: [25]; SCH 23390: [15]; Quinpirole: [26]; SKF 38393: [27,28]). Treatment order was counterbalanced and there was a minimum of three days between treatments for any individual. Injections were given IM in a volume of 0.5 mL/kg. All compounds were purchased from Sigma Aldrich, and prepared in the same manner as in the preliminary dose selection study (Supplemental Materials).

2.4. Behavioral testing

Subjects were removed from their home enclosure, administered a treatment, and housed alone in a transport cage (30 × 30 × 30 cm) for 30 min during the drug uptake period. At the completion of drug uptake, subjects were transferred to the T-shaped partner preference test apparatus for 45 min [23,29]. While in the partner preference test, subjects had simultaneous auditory, olfactory, and limited tactile access to their untreated mate and to an untreated unfamiliar opposite-sex marmoset in stimulus cages at each end of the long arm of the T (Fig. 1). Each monkey was tested twice with each DAR treatment, once in each of two social contexts. In one social context we placed a visual barrier in the center of the long arm of the T maze, blocking the ability of the stimulus animals to view one another, such that the subject could interact with one stimulus animal without the other seeing the interactions. In the second social context we removed this barrier and all animals had visual access to one another. The same unfamiliar stimulus animal was utilized for a single subject across all tests, with the exception of a single stimulus animal that was replaced partway through the study. Stimulus animals were net caught 15 min prior to the beginning of testing and placed in transport cages (30 × 30 × 30 cm) at the ends of the preference test apparatus to habituate prior to the start of the observation. Regardless of the experimental condition, the visual barrier remained in place during habituation until testing began. Prior to the beginning of the study, the test subject had two habituation sessions (30 min each) to the apparatus with both their pair mate and the stranger stimulus animal. Testing order was counterbalanced for both DAR manipulation and social context. Test animals had full access to the entire T-shaped apparatus, while stimulus animals (mate and unfamiliar conspecific) were each confined to transport cages at the end of one arm of the apparatus. The location of the stimulus animals (left or right arm) was counterbalanced across tests. Partner preference testing lasted for 45 min after which all animals were returned to their home environments.

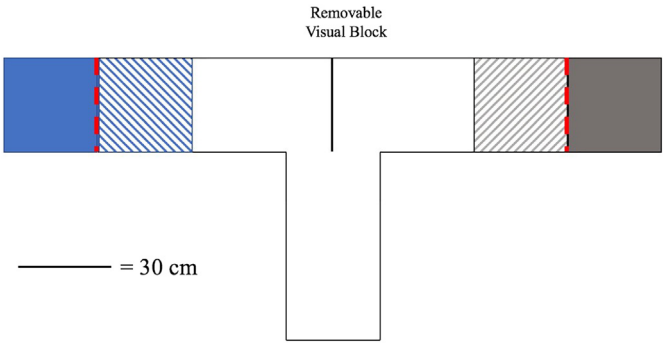

Fig. 1. Preference Test Apparatus. The hashed zones indicate the proximity zones of 30 cm closest to the stimulus cages. The solid boxes denote the stimulus cages (30 × 30 × 30 cm) and are attached at opposite ends of each arm of the T. The measure of contact with stimulus cage occurs whenever the subject is touching the grate (dashed red line) between the T apparatus and stimulus cage. The removable block either allows visual access between the two stimulus animals, or blocks visual access. All zones in white are considered neutral locations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We recorded the duration of proximity to each stimulus animal (the 30 cm closest to the stimulus cage; see Fig. 1), time spent in contact with each stimulus cage, and sexual solicitations (open mouth displays consisting of tongue flicks and lip smacks) directed toward each stimulus animal. Proximity and contact were measured for latency, duration and number of occurrences. Sexual solicitations were measured only for latency and number of occurrences. Behavior was live scored by trained observers who had attained a minimum of 90% inter-rater reliability scores.

2.5. Data analysis

The 45-min preference test was divided into three 15-min segments to assess change over time. Behavioral expressions of partner preference (time spent in proximity to each stimulus animal, time spent in contact with each animal, and sexual solicitations toward each stimulus animal) were analyzed using a repeated measures ANOVA analysis with drug treatment (D1R Agonist, D1R Antagonist, D2R Agonist, D2R Antagonist, Saline), time bin (first, second or third 15-min segment), social context (visual vs no visual access), and stimulus animal identity (pair mate vs stranger) as within subjects factors in the model, and sex (male, female) as a between subjects factor. Only significant main effects and interactions were probed via post hoc comparisons. In order to limit the number of post hoc tests for drug treatment, only comparisons with saline were made. Main effects, interactions, and pairwise post hoc comparisons with $p < .05$ were considered significant. Trends were considered when $p < .06$ and were in a direction that made sense based on other data. All data are available upon request.

3. Results

3.1. Social context: marmoset behavior was not altered by social context

The social context in which the preference test took place (i.e., whether the subject's mate could view the subject's interactions with the unfamiliar stimulus animal or not) did not alter marmoset behavior. No measures of social behavior were altered by changing social context including time spent in proximity to either social stimulus [$F(1, 8) = 3.26$, $p = .108$, $\eta^2 = 0.290$, 95% CI (−8.28, 68.25 s), $M_{\text{visual access}} = 277.39$, $SE = 15.78$, $M_{no\ \text{visual access}} = 247.41$, $SE = 23.90$], time spent in contact with either social stimulus [$F(1, 8) = 0.307$, $p = .595$, $\eta^2 = 0.037$, 95% CI (−32.78, 53.50 s), $M_{\text{visual access}} = 160.94$, $SE = 21.85$, $M_{no\ \text{visual access}} = 150.89$, $SE = 23.61$], and degree of sexual interest expressed in a social partner [$F(1, 7) = 0.40$, $p = .548$, $\eta^2 = 0.054$, 95% CI (−1.49, 0.86 count), $M_{\text{visual access}} = 1.27$, $SE = 0.47$, $M_{no\ \text{visual access}} = 1.59$, $SE = 0.70$]. There was no sex difference in how marmosets responded to the two social contexts on any measure of affiliation (proximity, contact, or sexual interest) (all $p > .05$).

3.2. Duration of testing: marmosets show largely stable behavior across the preference test

Marmosets spent equal amounts of time in proximity to [$F(2, 16) = 0.41$, $p = .670$, $\eta^2 = 0.049$], or in contact with [$F(2, 16) = 0.07$, $p = .936$, $\eta^2 = 0.008$], a social partner during the first, second, and third 15 min of the preference test. Marmosets did alter the rate at which they engaged in sexual solicitations overall toward either stimulus animal during the preference test [$F(2, 14) = 5.17$, $p = .021$, $\eta^2 = 0.425$], however, post hoc comparisons indicated that while the rates of sexual solicitations trended to be higher during the first 15 min of testing, it was not a statistically significant difference [first 15 vs. second 15 $p = .055$, 95% CI (−0.03, 2.09 count); first 15 vs. third 15 $p = .057$, 95% CI (−0.41, 2.25 count); second 15 vs. third 15 $p = .409$, 95% CI (−0.27, 0.13 count)]. However, marmosets did alter the rate at which they engaged in sexual solicitations based on stimulus animal identity across time bins [$F(2,14) = 4.10$, $p = .040$, $\eta^2 = 0.370$], indicating that the pattern of sexual solicitations directed toward the mate or unfamiliar stimulus animals changed differently over time. Post hoc comparisons show that during the first and third 15 min of the preference test marmosets sexually solicited the unfamiliar stimulus animal significantly more than their mate [first 15: $p = .036$, 95% CI (0.36, 7.74 count); third 15: $p = .048$, 95% CI (0.03, 4.03 count)]. While the same direction (more sexual solicitations directed toward the unfamiliar stimulus animal than toward the mate) is observed in the second time bin, it does not reach statistical significance [$p = .055$, 95% CI (−0.07, 4.46 count)]. Males and females did not differ in any measure of behavior throughout the preference test (all $p > .05$).

3.3. Partner preference: marmosets have a sociospatial, but not sociosexual, partner preference

Marmosets spent a greater amount of time both in proximity to [$F(1, 8) = 12.92$, $p = .007$, $\eta^2 = 0.618$, 95% CI (86.68, 397.06 s); Fig. 2], and in contact with [$F(1, 8) = 9.16$, $p = .016$, $\eta^2 = 0.534$, 95% CI (37.84, 279.79 s); Fig. 5], their mates during the preference test, compared to time spent with the unfamiliar stimulus animal. In contrast, marmosets displayed a greater number of sexual solicitations directed toward the unfamiliar stimulus animal than toward their mate [$F(1, 7) = 6.59$, $p = .037$, $\eta^2 = 0.485$, 95% CI (0.22, 5.30 count); Fig. 3]. Males and females did not differ in any measure of partner preference (all $p > .05$).

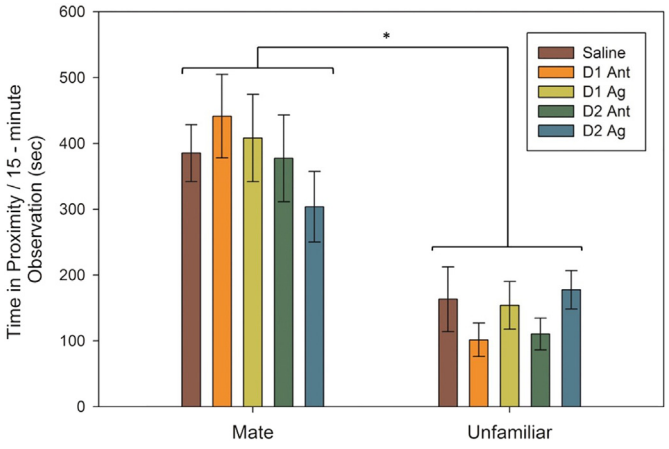

Fig. 2. Proximity Preference for the Partner During Preference Test is Unaffected by DAR Manipulation. Marmosets did not alter time spent in proximity (*M* ± *SEM*) to either their mate or an unfamiliar stimulus animal as a result of DAR manipulation. No post hoc comparisons were run for DAR manipulation as the omnibus test of the interaction was not significant ($p > .05$). Overall, marmosets spent more time in proximity to their mate than to the unfamiliar stimulus animal. *Denotes $p < .05$.

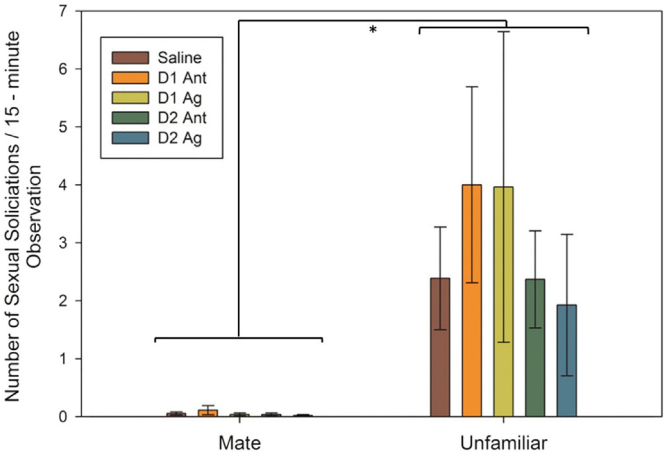

Fig. 3. Sexual Solicitation Preference for the Unfamiliar Stimulus Animal During Preference Test is Unaffected by DAR Manipulation. Marmosets did not alter their pattern of sexual solicitations (*M* ± *SEM*) toward either their mate or an unfamiliar stimulus animal as a result of DAR manipulation. No post hoc comparisons were run for DA treatment as the omnibus test of the interaction was not significant ($p > .05$). Overall, more sexual solicitations were displayed toward unfamiliar stimulus animals than toward mates. *Denotes $p < .05$.

3.4. DAR manipulation: DAR manipulation did not alter proximity or sexual solicitation behavior, but did impact time spent in contact with a social partner

DAR manipulation did not significantly alter proximity behavior patterns [$F(4, 32) = 2.01$, $p = .117$, $\eta^2 = 0.201$]. The interaction between DAR manipulation and stimulus identity was not significant, such that marmosets did not differentially alter patterns of proximity behavior toward their mate or stranger depending on DA treatment [$F(4, 32) = 1.45$, $p = .242$, $\eta^2 = 0.153$; Fig. 2].

Similarly, DAR manipulation did not significantly alter patterns of sexual solicitation [$F(4, 28) = 0.45$, $p = .774$, $\eta^2 = 0.060$], and marmosets did not differentially alter patterns of sexual solicitation by DAR manipulation based on stimulus identity [$F(4, 28) = 0.410$, $p = .800$, $\eta^2 = 0.055$; Fig. 3].

The three-way interaction between DAR manipulation, social context and stimulus identity was not significant for duration of time in proximity to [$F(4, 32) = 0.72$, $p = .585$, $\eta^2 = 0.083$], or in contact with [$F(4, 32) = 0.81$, $p = .531$, $\eta^2 = 0.091$], or sexual solicitation directed toward [$F(4, 28) = 0.08$, $p = .987$, $\eta^2 = 0.012$], a stimulus animal, meaning that marmosets behaved consistently toward both their mate and the unfamiliar stimulus animal regardless of social context or DAR manipulation.

DAR manipulation did significantly alter the amount of time that marmosets spent in contact with the stimulus animals [$F(4, 32) = 2.91$, $p = .037$, $\eta^2 = 0.266$; Fig. 4], and marmosets treated with a D2R agonist spent significantly less time in contact with either stimulus cage overall compared to when marmosets were treated with saline [$p = .015$, 95% CI (16.03, 113.25 s)]. No other DAR manipulations altered contact behavior compared to saline (p's > 0.05). This reduction in time spent in contact was not dependent on animal identity; DAR treatment did not differentially alter marmoset behavior toward the two stimulus options [$F(4, 32) = 1.75$, $p = .165$, $\eta^2 = 0.179$; Fig. 5]. There were no sex differences in response to DAR manipulation (all $p > .05$).

4. Discussion

In this study, we sought to determine the effect of the DA system in the expression of behavior critical to marmoset social monogamy, as well as the impact that social context would have on DA treatment

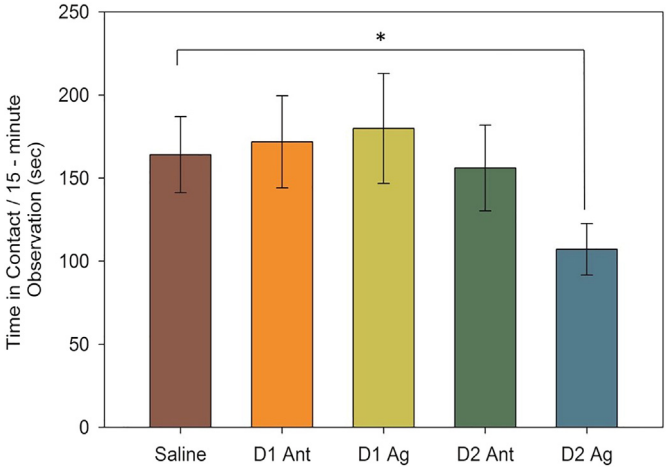

Fig. 4. Marmosets Treated with a D2R Agonist Spent Reduced Time in Contact with a Social Partner. Marmosets treated with a D2 agonist spent less time in contact with a stimulus cage ($M \pm SEM$) compared to when they were treated with saline. *Denotes $p < .05$.

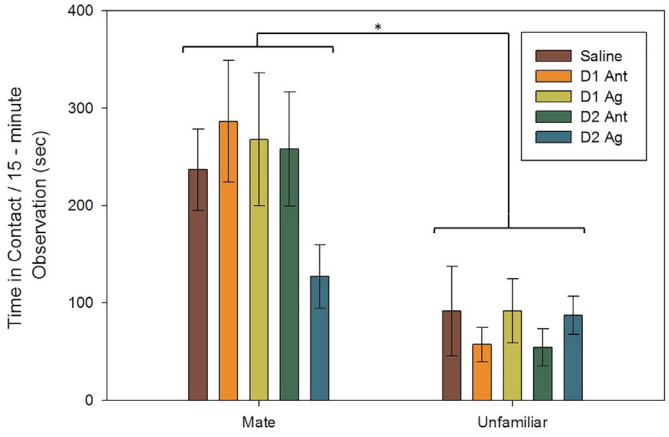

Fig. 5. Contact Preference for the Partner During Preference Test is Unaffected by DAR Manipulation. Marmosets did not alter time spent in contact ($M \pm SEM$) with either their mate or an unfamiliar stimulus animal as a result of DAR manipulation. No post hoc comparisons were run for DA treatment as the omnibus test of the interaction was not significant ($p > .05$). Overall marmosets spent more time in contact with their mate than with the unfamiliar stimulus animal. *Denotes $p < .05$.

effects. Marmoset social behavior in the partner preference test was relatively stable across the preference test, with the exception of sexual solicitation patterns directed toward mates compared to unfamiliar opposite sex conspecifics. Marmoset behavior was, however, largely uninfluenced by experimental manipulations such as social context (i.e. whether the pair mate could see the subject's interactions with the stranger) or pharmacological manipulation of DARs. Only D2R agonism affected social behavior by reducing overall close social proximity-seeking with either stimulus animal compared to saline treatment. No DAR manipulation affected the expression of social behavior directed toward a specific stimulus partner, familiar or unfamiliar.

We predicted that the DA system would regulate behavior in marmosets similarly to how it has been documented to in monogamous rodent models. In general, manipulation of the DA system did not affect behavior in long-term pairs of marmosets as it does in monogamous rodent models, highlighting variability in the neurobiology regulating social interactions across species. We hypothesized that manipulation of the D1R system would alter behavior in long-term pairs such that antagonist treatment would reduce partner preference (**H1**) and agonist treatment would enhance a partner preference (**H2**). These hypotheses were not supported, as D1R system manipulation did not affect time spent in proximity to or in contact with the mate or the unfamiliar stimulus animal, nor did it affect sexual solicitations. The D2R system plays a role in regulating behavior between newly introduced prairie vole mates (i.e., pair bond formation) [14,30], thus, we expected in the present study that long-term marmoset pairs would be unaffected by D2R agonism with regard to specific social preferences (**H3**). However, in non-monogamous primates blocking D2Rs reduces the time spent looking at a social stimulus [16], and as such we hypothesized that marmosets treated with a D2R antagonist would show reduced interest in either their mate or unfamiliar stimulus animal indicative of reduced social engagement (**H4**). This hypothesis was not supported. In fact, we found that it was not marmosets treated with a D2R antagonist that showed reduced social interest, but rather marmosets treated with a D2R agonist. Marmosets treated with a D2R agonist spent reduced amounts of time in contact with either stimulus cage compared to when marmosets were treated with saline. This broad decrease (e.g., not specific to either the mate or the unfamiliar stimulus animal) may indicate a reduced social interest in close contact following activation of the D2Rs. The fact that contact behavior, but not general proximity, was affected by this treatment highlights the potential that the DA system regulates specific aspects of social behavior in marmosets. The pairs presently studied would be considered within the maintenance phase of their relationship – a bond has already formed between mates and now the mates are maintaining that bond [5]. Thus, it is possible that for established bonds in marmosets, activation of the D2Rs dampens some measures of general social interest rather than increases interest in a stranger. If an unfamiliar opposite-sex stimulus animal represents a potential new partner for a pair bond, then activation of the D2R may protect the existing pair bond by reducing overall interest, and thereby reducing the likelihood of establishing a new pair bond. This phenomenon by which the existing pair bond is maintained by reducing interest in new partners has already been shown to be affected by oxytocin, another potent regulator of social bonds [23,24]. This potential mechanism of pair bond maintenance in which interest is reduced under pharmacological manipulation highlights a distinction between the marmoset and rodent models of sociality in which marmoset bonds may be more heavily maintained by reduced affiliation rather than increased aggression.

The limited effects of DAR manipulation are surprising given the body of literature in both rodents and nonhuman primates indicating the importance of the DA system in regulating social behavior in some capacity. While DA has a role in social behavior, it is not the only neurobiological regulator of social interactions. Specifically, the neuropeptide oxytocin (OT) has well-documented roles in facilitating social interactions, including pair bonding [10,31]. In marmosets, OT treatment increases affiliative behavior in newly formed pairs [29], and decreases interest in and prosociality toward opposite-sex conspecifics [23,24], while blocking the OT system results in reduced levels of food sharing with a mate, a prosocial and affiliative behavior [29]. Together, these studies provide evidence of the way in which the OT system may play a role in maintaining pair bonds by increasing interactions between mates and reducing the potential for affiliative interactions with other conspecifics. While the interaction between the DA and OT system in mediating interactions between mates has not been evaluated in a nonhuman primate model, there is evidence from rodent models that these two systems work together to facilitate pair bond formation. Specifically, prairie voles treated with an antagonist for either OT or D2R fail to form a partner preference [32], suggesting that both the DA and OT systems are necessary for the establishment of a social bond. Anatomically, OTRs are present in the nucleus accumbens, a DA-rich region of the brain [33], providing a physical link for how these two systems may interact, and administration of either DA or OT increases central release of the other neurochemical in rats [10]. It may therefore be that by examining manipulation of DARs alone we were not able to capture the complex interactions between DA and other neurobiological

systems that together facilitate the maintenance of social relationships.

Other potential reasons for the small effects observed with DAR manipulation may be specific to our study design. For instance, our study had a small sample size which may have resulted in underpowered analyses. Finally, though a dose selection study was conducted (see Supplemental Materials) it is possible that dose selection was too conservative. In an effort to ensure that doses did not have any motoric effects, they may have been too low to elicit effects on social behavior. A potential reason that the effect of DA manipulation in marmosets did not mirror that in prairie voles may be methodological differences between rodent and nonhuman primate studies. While prairie voles were administered DAR manipulations directly to specific brain regions using guide cannula injections [13,14,32], treatments were administered via intramuscular (IM) injection to marmosets in this study. IM treatments can produce peripheral effects in addition to central effects, and the central effects that are produced with IM administration can be widespread in the brain, as opposed to the region-specific effects that can be induced by canula injections. These concerns are partially mediated by the fact that all agents are known to cross the blood brain barrier (Raclopride: [25]; SCH 23390: [15]; Quinpirole: [26]; SKF 38393: [27,28]), and the preliminary screen of doses (see Supplemental Materials) was designed to minimize effects that may alter behavior because of peripheral effects and/or effects in areas other than social (e.g., movement). However, it is possible that with region-specific treatment administration effects of DAR manipulation in marmosets may be more similar to that observed in prairie voles.

Marmosets spent significantly more time both in proximity to, and in contact with, the stimulus cages of their mates relative to those of unfamiliar opposite-sex marmosets. These findings are consistent with displaying a partner preference for measures of proximity and contact, one of the hallmark behaviors associated with social monogamy [3,34]. Previous examinations of marmoset behavior have not revealed a proximity preference, and in fact, in some cases have shown a preference for spending time in proximity to an unfamiliar conspecific rather than the mate [23,29]. These differences may be due to several factors. No proximity preference for the mate was observed in marmoset pairs housed together for 24 h [29], three weeks [29] or eight weeks [23]; however, marmosets did display such a preference in the present study (all paired > 24 weeks, and average 3.01 years). Thus, it is likely that the dynamics of pair interactions continue to change across time in ways that induce a proximity preference throughout development of the pair bond. Despite the demonstration of a sociospatial preference to spend time near the mate, subjects did display more sexual solicitations toward the unfamiliar stimulus animal than they did to their mate. This discrepancy between spatial data (where they spent time) and content data (what are they doing) highlights the complexity of the marmoset as a model of social behavior. These two classes of behavior (sociospatial and sociosexual) may be reflective of multiple neural systems that differentially regulate distinct components of a relationship, namely sexual interest vs. attraction vs. attachment [35]. Similarly, research on human engagement in serial socially monogamous mating systems indicates a discrepancy between the evolutionary pressures that should benefit from multiple sexual partners (and thereby increased offspring) and the tendency for human couples to maintain a relationship for at least several years post birth [36]. The observed pattern in marmosets of a distinction between sexual and spatial interest may be reflective of these different pressures between sexual activity and social security. These differential patterns are not observed in model species such as prairie voles, which show consistency in both sociospatial and sociosexual preference for their mates [6]. This conditional behavioral flexibility, or dissonance in different behavioral measures within the same social context, highlights the potential social complexity of the marmoset and value of marmosets as a translational model for behavioral pharmacology.

Previous literature has identified that contextual features may be important in regulating social interactions in socially monogamous marmosets and tamarins [19,20], however, this effect was not observed in this study. As such, our hypothesis regarding enhanced differences in social behavior in conditions in which the subject's pair mate could observe interactions was not supported (**H5**). Several factors may have contributed to this difference. For instance, in the present study the subject's mate was always present in the preference test, while in Evans et al., the mate of the subject was either present in the testing room or absent [20]. Thus, marmosets may only alter their behavior toward an opposite-sex stranger when their mate is not present at all rather than when their mate is present and only visual communication is blocked. Finally, though changes in social context have been documented to alter behavior in marmosets there is potential that the ecological characteristics of marmosets may limit the ability to respond to a cognitively demanding task. For instance, in nature marmosets live in relatively small groups resulting in a reduced need for the enhanced social cognitive capacity required to maintain social cohesion in primate species with larger social groupings [37–39]. The cognitive demand of the social context manipulation, either alone or in combination with the increased stress associated with treatment administration, may have resulted in reduced sensitivity to social environment. Alternatively, as with sociospatial partner preferences, it is possible that pairing length also affects context-specific expression of extrapair behavior.

5. Conclusions

This study provides several novel additions to the field of translational neuropharmacology. First, to the best of our knowledge this study is the first demonstration of a spatial partner preference in marmoset monkeys. This is likely due to the extended length of cohabitation in the current study compared to previous studies in marmosets. Therefore, this study, along with previous studies in marmoset monkeys involving partner preference testing [23,29] provides a full spectrum of the development of a partner preference in marmosets. Second, this study provides an important stepping stone between the well-documented role of the DA system in rodent models of social monogamy and the less understood role of DA in regulating nonhuman and human primate social behavior. While previous research has found similarities in the way in which the DA system regulates social interest and behavior between primates and rodents [16,17], the current study highlights the potential for the DA system to function differently in primates than in prairie voles in regulating social interactions. This lack of specialization in the DA system may underlie the behavioral flexibility that is characteristic of both marmoset and human social relationships.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.physbeh.2019.03.004.

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