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Effect of reward type on object discrimination learning in socially monogamous coppery titi monkeys (*Callicebus cupreus*)

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Abstract

Highly valued food items are often used as rewards to reinforce an animal's behavior. For social species, social interaction is rewarding and can drive an individual's behavior as well. In the currently study, we wanted to compare the efficacy of a food reward and a social reward on object discrimination learning in socially monogamous titi monkeys. We hypothesized that titi monkeys would perform more accurately for a social reward (their pair mate) than for a food reward (a highly desired food item). Eleven adult titi monkeys were tested with a two-object visual discrimination task for both types of reward. The colors and shapes of the objects in the two-object discrimination task were counterbalanced across subjects. During each trial, subjects were shown two objects, and the trial ended when the subject touched the reinforced shape (S+) or after 5 min. A correct trial was defined as one when the subject touched S+ first. We found that 45.5% of subjects were able to learn the task with a social reward, and 83.3% were able to learn the task with a food reward. We found that subjects balked more often and had fewer correct trials for the social reward. Finally, subjects took longer to approach the shapes for a social reward, possibly indicating lower motivation to engage in the task when a social reward is used compared to a food reward. Although significantly fewer subjects met criteria of success with the social reward than with the food reward, our results show that titi monkeys can learn a visual discrimination task with either type of reward.

Keywords

monogamy; nonhuman primate; positive reinforcement; social reward; visual discrimination

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

1 | INTRODUCTION

Operant, or instrumental, conditioning is a type of animal learning where the strength or frequency of a certain behavior is altered by positive reinforcement (reward) or negative reinforcement (punishment). In operant conditioning, also referred to as associative learning, an animal also learns to associate certain signals with the positive or negative consequences of their behavior. Operant conditioning has been studied for over a century (Skinner, 2012; Thorndike, 1898), and numerous studies have now contributed to a rich understanding of the environmental and biological factors that underlie animal learning across many species, including invertebrates (Hawkins & Byrne, 2015), birds (Park, Okanoya, & Dooling, 1985), fish (Lucon-Xiccato & Bisazza, 2016; Thompson & Sturm, 1965), and mammals (Grant, 1964), including monkeys (Alvarado, Malkova, & Bachevalier, 2016).

Many behavioral tests that were designed to assess animal learning rely on an animal's ability to discriminate between two stimuli, such as sounds or objects, which are paired with reinforcing or neutral outcomes. The simplest version of this concept is classical conditioning, in which a single, neutral stimulus, such as a bell (conditioned stimulus), gains the ability to trigger a conditioned response after repeated pairings with the unconditioned stimulus, such as a food reward (Pavlov, 1960). It is possible to expand the number of stimuli to include two objects or sounds, and only pair one of them with a reinforcer, in order to test a subject's ability to distinguish between the two and selectively respond to the conditioned stimulus.

This approach is referred to discrimination learning, and much of the early work to understand this type of learning in nonhuman primates was done by Harlow and colleagues (Harlow, 1945; Moss & Harlow, 1947), who also developed the Wisconsin General Test Apparatus (WGTA) to test the learning abilities of rhesus macaques (Harlow & Bromer, 1938). One way that the WGTA can be used is in a two-object visual discrimination task, in which two objects that differ in shape, size, and/or color are presented side-by-side to a test subject. For positive reinforcement learning, after repeated trials in which one of the objects is paired with a reward, a subject will learn the difference between the stimuli and will consistently respond only to the stimulus that is associated with a reward. The studies employing the WGTA have provided a framework upon which our understanding of nonhuman primate learning have been built, including marmosets (Ridley, Bowes, Baker, & Crow, 1984), capuchins (Tavares & Tomaz, 2002), and baboons (Deruelle, Barbet, Dépy, & Fagot, 2000), and it has also been modified for use in rats (Rollin, Thaller, & Shepp, 1963).

Frequently, in studies of animal learning, food is used as the rewarding stimulus for positive reinforcement. Comparatively, relatively few studies of animal learning have used social rewards, such as gaining access to one or more conspecifics, to assess the animal learning. However, a variety of studies have provided evidence that different categories of social interactions are rewarding for animals (Trezza, Campolongo, & Vanderschuren, 2011). For example, it has been demonstrated in mice that social play among juveniles is rewarding (Panksepp & Lahvis, 2007). In addition, mother rats will learn complex mazes to be with their litters, and they will perform more crossings of an electrified grid in order to gain access to their litters than they would to gain access to water or food after water or food

deprivation (Wilsoncroft, 1968). In addition, early postpartum, lactating dams find interactions with pups more rewarding than cocaine (Mattson, Williams, Rosenblatt, & Morrell, 2003, 2001), even when the dose of cocaine is increased (Seip & Morrell, 2007). Thus, a social reward, in this case the offspring, can act as an efficient motivator and a reinforcer for performance on a task. Rats will also work to free a trapped, familiar cage mate from a restraining enclosure, even if they are presented with a concurrent, alternative choice to open a container containing chocolate chips (Bartal, Decety, & Mason, 2011). In socially monogamous prairie voles, cohabitation with mating, which begins the process of pair-bond formation, produces a conditioned place preference in males, which indicates that this interaction is rewarding (Ulloa et al., 2017). Nonhuman primates have also been shown to find social stimuli rewarding, and gaining visual access to a conspecific or a photograph or video of a conspecific have been successfully used as reinforcers in studies of primate learning (Anderson, 1998; Butler, 1954; Deaner, Khera, & Platt, 2005; Gray, Pearce, Thiele, & Rowe, 2017).

If social rewards can reinforce an individual's learning, then this effect should be particularly strong in species that form strong social bonds, such as monogamous species, in which adult male-female pairs form a lasting attachment relationship after mating. Thus, we hypothesized that for a pair-bonded individual of a monogamous species, that individual's pair mate should act as an efficient positive reinforcer in learning tasks. The current study used coppery titi monkeys (*Callicebus cupreus*), which are monogamous, New World primates (Bales et al., 2017). Adults in this species form a stable, bidirectional social bond between opposite sexed individuals after mating (Mason, 1974). Once bonded, titi monkeys prefer to spend extended periods of time in close physical proximity to their pair mate (Anzenberger, 1988; Mendoza & Mason, 1997). Previous studies in titi monkeys have used the WGTA to assess their ability to learn to associate an object with a food reward outcome (Fragaszy, 1981), but it has not been determined whether a social reward would also act as a potent reinforcer in an object discrimination learning task for this species. Based on their ability to learn cognitive tasks and their strong monogamous attachments, titi monkeys are an excellent animal model for the study of social reward efficiency.

In the current study, we developed a two-object visual discrimination task that was modeled roughly after the features of the WGTA and was adapted for testing titi monkeys in their home cage environment. The two reinforcing stimuli for the task were either gaining access to a highly desired food item (food reward) or gaining access to their pair mate (social reward). We sought to determine if the subject's pair mate would serve as an effective positive reinforcer in the discrimination task. We also wanted to directly compare the efficacy of a food reward and a social reward on titi monkey learning outcomes. Compared to the food reward, we predicted that individuals who were being tested with the social reward should (a) need fewer trials to reach the criterion for successfully learning to discriminate two objects, (b) balk less, and (c) make fewer incorrect choices. This study expands previous investigations of learning in titi monkeys and expands our knowledge of the value of social bonding in this species.

2 | MATERIALS AND METHODS

2.1 | Animals

Subjects were 28 adult laboratory-born titi monkeys who had been living as heterosexual pairs in stable family units at the California National Primate Research Center in Davis, CA. All testing took place between November 2013 and January 2014. Their age ranged from 2.2 to 24.0 years old (mean $\pm$ SD = 7.9 ± 5.5 years). Animals were housed in living cages ($1.2 \times 1.2 \times 2.1$ m) that were situated such that each family group was visually isolated from others, but auditory and olfactory interactions were possible. All cages were equipped with four perches. Animals were on a 12:12 light: dark cycle with lights on at 0600 and lights off at 1800. Temperature was maintained at 21 °C. Animals were fed a diet of monkey chow, rice cereal, apple, and carrot at 0800 and at 1300. Water was available ad libitum. Subjects were living with their pair mate only or with their pair mate and offspring. None of the subjects had ever participated in a discrimination-learning experiment before. This research complied with protocols that were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California Davis, adhered to the legal requirements of the United States of America, and adhered to the American Society of Primatologists' Principles for the Ethical Treatment of Primates.

2.2 | Test apparatus

In order to minimize negative effects of novel surroundings (Cubicciotti & Mason, 1975; Mendoza & Mason, 1986), all testing was carried out in the animal's home cage (Figure 1). During testing, a pair of shapes was fastened to the outside the cage, in front of a perch and in reach of the subject. The "trial zone" was defined as the $\sim 14 \text{ cm}^3$ zone immediately surrounding this perch, where titi monkeys regularly sit in order to interact with lab members or care providers. The visual discrimination task used wooden shapes: triangles, squares, and circles (base $\times$ height = 11×11 cm, side $\times$ side = 12×12 cm, radius = 6.5 cm). Each shape was painted with acrylic paint (Craft smart®, water based) and covered with semigloss polyurethane sealant (Varathane®, water based) for easy cleaning. All males and half of the females of this species are dichromatic, so we painted the shapes black, white, and gray rather than using color (Bunce et al., 2011).

2.3 | Training

Before testing, subjects went through a training period in order to shape their behavior and habituate them to both the testing procedure and the brief separation from their pair mate (and offspring, if relevant). This training also helped to avoid any issues related to object novelty, which could be an issue with this highly neophobic species (Mayeaux & Mason, 1998). Due to the prevalence of neophobia in this species, we expected a considerable portion of subjects to fail this training phase, simply because of their unwillingness to approach novel objects. This training phase also served the purpose of determining which individuals exhibited enough novelty seeking behavior to be ideal subjects for further testing.

Before training began, the subject's pair mate and any offspring were caught and removed from the home cage for the duration of the training session (one male subject was

occasionally carrying his infant offspring). The transport cage containing the subject's pair mate (and offspring, if applicable) was moved to a distant part of the room, out of visual range but still within auditory range of the subject; the transport box was covered with a towel, to calm the pair mate and reduce the likelihood of vocalization. Subjects were first trained to approach the trial zone and sit on the perch to get a peanut as a reward. Then, subjects were trained to reach out and touch any shape that was presented in the front of their cage. The shapes that were used in the training phase included any of the nine options that were used for testing (white, gray, or black circles, triangles, or squares) and were chosen at random throughout training so that the subjects would not habituate to or form preferences for any specific shape/color. The training was composed of 3–6 trials per day, with each training trial lasting at most 2 min. The training lasted until the monkey reached the criterion of stability: the subject approaches and grabs the shape without any peanut cue for 5 successive days. Time of training alternated between mornings (10:30–12:30) and afternoon (1:30–4:30). From the 28 animals that began training, 12 animals (7 males, 5 females) met criteria to continue to the testing phase of the experiment. The age of these 12 animals ranged from 2.2 to 24.0 years old (mean $\pm$ SD = 7.9 ± 5.5 years). The length that each animal had been paired with their pair mate ranged from 2 months to 5.9 years and was taken into account in the analysis (mean $\pm$ SD = 21.1 ± 24.7 months).

2.4 | Two-object discrimination task

After successfully meeting success criteria for training, animals were assigned to one of two groups based on the type of reinforcer that would be used for their first round of discrimination testing: food reward first or social reward first. The purpose of this task was to test whether animals can learn to distinguish two different shapes of different colors. For all tests, the subject's pair mate (and offspring, if applicable) were removed from the home cage as described above, and placed out of view of the test subject but within reach of one experimenter. Subjects were shown a pair of two different shapes of different colors, which were quickly clamped in place in the front of their cage so that a second experimenter could step out of view immediately after the start of a trial. One shape was predesignated as the "correct" or reinforced shape (S+) and the other as the non-reinforced shape (S-). Colors, shapes, and assignment as S+ or S- were counterbalanced across individuals. A correct response was defined as touching S+, and an incorrect response was defined as touching S-. If a correct response was given, the subject received the reinforcer immediately. In the food condition, a piece of a high value food (banana) was presented to the test subject in the palm of the experimenter who was located out of view of the monkey, but close enough to the trial zone to present the food reward within reach of the test subject. After a correct response in the social condition, the test subject's pair mate was released into the home cage from the release door (Figure 1), a process that was initiated immediately by the experimenter who had been standing within reach of the transport cage but out of view of the test subject. After a correct response was given, the trial was over.

If an incorrect response was given (touching S-), nothing happened, and the subject could try again until a correct response was given or until the end of 5 min. The shapes were not repositioned if an incorrect response was made; the shapes remained in place until the subject touched S+ or until the end of 5 min. Balks were defined as trials in which no

response was made before the end of 5 min. Incorrect trials were defined as trials in which only an incorrect response was made and no correct response was made before the end of 5 min. Presentation sides (left vs. right) of S+ were counterbalanced across trials. Subjects received two trials per day. After the shapes were presented, the latency to enter the trial zone and the latency to touch both S+ and S- were also measured.

When a trial was over, the stimulus shapes were removed from the front of the cage, and the pair mate was returned after at least a 2-min delay, in order to prevent the pair mate's return from being contingent on the subject's behavior during testing. Thus, there was no punishment for not performing the correct behavior. After a subject performed 10 correct trials in 12 successive sessions, the subject was considered to have reached criteria as successfully learning to choose S+. After this criterion was met, the animal began testing on the other type of reinforcer. In round 2 of testing, a different shape of a different color from the two that were previously used in round 1 was designated as the new reinforced shape (S +') for the second reinforcer. The S- remained the same. All individuals completed testing for both types of reward. If an animal balked in five out of six successive trials, their testing for that reinforcer was ended, and they were considered to have failed to meet criteria for successful learning of the discrimination task. Individuals with more than 30 trials without meeting the success criterion were also considered to have failed to meet criteria for successful learning of the discrimination task.

2.5 | Analysis

Data was analyzed using a general linear mixed model to determine the effects of the following variables on our outcome measures (described in Table 1): reward type (food vs. social), the order of testing (food first vs. social first), the length of the pair bond (number of days since pairing), sex, and age, as well as potential interactions. Animal ID was included as a random factor in order to account for individual differences. Twelve subjects were tested, but we dropped one subject because she balked in five out six trials in a row, so all analyses were done on the remaining 11 subjects. Residuals for all tests were normally distributed. *p* values of less than 0.05 were considered statistically significant.

3 | RESULTS

The aim of the current study was to compare the effectiveness of the food and the social reward on monkeys' performance in a two-object visual discrimination task. There were no significant effects of the testing order on any of the outcome measures listed in Table 1, so we eliminated order as a variable and collapsed the data for further analyses. We found no significant sex differences, and there were no effects of pair bond length on any of our outcomes in the social reward condition.

We found a significant effect of reward type on the latency to enter the trial zone ($F[1,21] = 7.84$; $p = 0.023$; $\eta^2 = 0.398$), such that the animals had a shorter latency to engage in the task when they were tested with a food reward than when they are tested with a social reward (Figure 2). However, there was no effect of reward type on the latency to make either a correct or incorrect response once the subject had entered the trial zone (Figure 2). In other

words, once the subjects decided to enter the trial zone, they did not take additional time to decide which shape to choose.

We found several significant effects of the type of reward on performance and accuracy. First, we found a significant effect of reward on the percentage of correct trials ($F[1,21] = 8.41$; $p = 0.020$; $\eta^2 = 0.292$) such that animals tested with a food reward had more correct trials than when they were tested with a social reward (Figure 3a). We found a significant effect of the reward on the number of trials needed to reach the criterion of success ($F[1,21] = 5.77$; $p = 0.043$; $\eta^2 = 0.223$); animals working for a food reward succeeded after fewer trials than when they were working for a social reward (Figure 3b). We found the social group had a significantly higher percent of trials that ended with an incorrect response only ($F[1,21] = 6.48$; $p = 0.034$; $\eta^2 = 0.401$). Only subjects of the social group had trials that ended with choosing S−; every subject in every trial with the food reward eventually touched S+ (Figure 3c). Finally, there was a significant effect of the type of reward on the percent of trials where the animal balked ($F[1,21] = 9.14$; $p = 0.017$; $\eta^2 = 0.457$); the social group had a significantly higher percentage of trials with balks than the food group (Figure 3d). Although a higher proportion of animals succeeded in learning a two-object visual discrimination task when tested for a food reward compared to a social reward (Figure 4), nearly half of the animals tested with a social reward (45.5%) succeeded in learning the task.

4 | DISCUSSION

This study is the second to our knowledge to assess learning capacities in the coppery titi monkey. In the previous study, it was found that titi monkeys can learn to associate an object with a food reward (Fragaszy, 1981). Our current study extended these previous findings by examining whether a social reward can act as a reinforcer for this species and by directly comparing food reward and social reward in the same task. Our results confirmed previous findings that titi monkeys are capable of learning by positive reinforcement. Our findings also indicated that they could be trained to distinguish two objects with either a food reward or a social reward. However, it is important to note that these two reward types were examined only in this one experimental situation—a visual object discrimination task—so future studies are warranted to evaluate their relative effectiveness across a wider range of testing conditions.

However, we found that a social reward is a less effective reinforcer of titi monkey learning in a discrimination task than a food reward. Our results supported this idea in several ways. When a social reward was used, titi monkeys had a higher proportion of incorrect trials, had trials when no correct response is ever given, and balked more often. For the 45.5% of animals who successfully learned the discrimination task with a social reward, they required more trials in order to reach criteria of success than the animals tested with a food reward.

In addition, we found that monkeys who are tested with the social reward take longer to enter the trial zone than monkeys who are tested with a food reward. This result could be interpreted in two different ways. The subjects could have been less motivated to participate in the two-object discrimination task when they are working for the return of their pair mate compared to a food reward. Alternatively, they may have taken longer to decide which was

the correct shape, and this decision making process was occurring before they entered the trial zone. Our results indicated that the subjects did not take additional time to decide which shape to choose once they arrived in the trial zone; they made a choice as soon as they landed in the trial zone, and there were no differences in the latency to choose the correct or incorrect shape. Thus, it is possible that the subjects were deciding what shape to choose during the latency period between the time the shapes were presented and when they jumped to the perch in the trial zone. If this was the case, then animals tested for the social reward were taking more time to make the decision of which shape is S+, which would support the rest of our findings that social reward was a less effective reinforcer of their behavior.

Taken together, these results indicate that food was a more effective reinforcer of titi monkey behavior than a social reward, which is not what we hypothesized for this highly social, monogamous species. Titi monkeys form lasting pair bonds with their mate and show behavioral and endocrine markers of stress (agitation and increased plasma cortisol) when they are involuntarily separated from their pair mate (Fernandez-Duque, Mason, & Mendoza, 1997; Mendoza & Mason, 1986). Thus, we hypothesized that the return of their pair mate (a rewarding social stimulus) would be a potent reinforcer of their behavior and would be more efficient than a food reward. However, our results did not support this idea. It is possible that the subjects were experiencing some mild separation stress during our testing sessions (because the pair mate was removed prior to all tests), which could have impacted their behavior during testing. However, because the removal of the pair mate was consistent across both the food and social reward conditions, it is unlikely that stress from social separation impacted their behavior only in the social reward tests. In fact, if there were a differential effect of social separation on their behavior in these tests, we would assume that it would act as a motivator specifically in the social reward condition to increase their effort to end the separation by choosing S+, but this was not the case. Furthermore, the pair mate was removed during all training sessions prior to the testing phase of the experiment, which should minimize any effect of social separation on cognitive outcomes. We also did not note any behavioral signs of distress/arousal, such as distress calling, self scratching, or pacing, during any of our testing sessions. Thus, the increased latency to enter the trial zone and decreased accuracy of responses indicates to us that the pair mate's return was a less effective reinforcer than food in this monogamous species.

There are some potential caveats in our test design that are worth discussing in the context of our results. First, the stimulus value between food and social reward may not be equivalent for a titi monkey. In order to directly compare the influence of two classes of reward, the subjective value of those rewards should be equivalent. A previous study of titi monkey food preferences showed that fruit was their most preferred class of food (Fragaszy & Mason, 1983). In rhesus macaques, food preference tests revealed that bananas are preferable to apples, and apples are preferable to carrots (Belzung & Anderson, 1986). Thus, in the current study, it is possible that the food reward we chose—banana—is a particularly palatable and preferred food item for titi monkeys and would then operate as a strong reinforcer. It would be interesting and useful in future investigations to determine food preferences prior to testing and then use the appropriate type of food based on this established scale of reward. In future studies, we could also reduce the value of the food

reward by using alternative food options in order to make the value equivalent to that of the social reward.

Similarly, the subjective reward value of the return of the pair mate in our study could be low, relative to the value of the food reward. Our test subjects were habituated to short social separations during the training phase of our study, and in our colony, a subject's pair mate and/or family members are somewhat frequently removed from the home cage for short periods (minutes) in order to accommodate the specifics of research-related testing or veterinary needs, like medical treatments. After these brief separations, the animals are always promptly returned to their home cage. Thus, it is possible that the monkeys have learned over time that their pair mate will be returned to them. If this idea is true, then our subjects may perceive the temporary social separation during testing as routine and as a result, would not be motivated to engage in the task in order to work for their pair mate's return or would not find the return of the pair mate rewarding enough to consolidate their learning of the distinction between S+ and S-. In future tests, in order to increase the stimulus value in the social reward condition to match the value of a highly desirable food reward, we could increase the amount of time that the test subject is alone prior to testing.

There are a few explanations worth considering for why the food reward was more effective in our discrimination task than a social reward. One possibility is that the subjects were trained to approach and touch the shapes using food. Although banana was used during testing and peanuts were used during training, these items both belong to the same class of reward, which could have biased the behavior of our subjects in favor of the food reward during testing. Another explanation involves the concept of "preparedness" of existing behavioral systems in the titi monkey. The idea of behavioral preparedness proposes that learning modifies innate sensorimotor and motivational systems (Timberlake, 1993), such that an organism should be more likely to learn new associations if they activate existing propensities, such as, in the current study, those systems that are involved in food acquisition and feeding. Thus, it is possible that our subjects had an increased propensity to learn our task in the food reward condition because titi monkeys use the same reaching and grasping motions that were required to select S+ to engage in feeding-related behaviors. However, titis also use reaching and grasping movements to engage in partner-directed social interactions such as grooming and mate-guarding, but Timberlake's behavioral systems approach is still worth considering in the interpretation of our results. This concept could also explain why the subjects that did reach criterion in the social reward task required more test sessions in order to successfully form the association; learning the new association is possible, but slower, because it does not activate existing behavioral systems.

Finally, it is possible, but unlikely, that differences in temporal or spatial contingencies across the two reward types could have impacted our results (Gallistel, Craig, & Shahan, 2014). Temporal contingency between the action of the individual and the outcome of its behavior is critical in reinforced learning paradigms (Flaherty, 1985). Increasing the time between the subject's correct action and the delivery of the reward has been used in studies of learning and memory to understand the maximum duration of time before a subject no longer associates their specific action with reward (for a review of delayed reinforcement on learning outcomes, see reference Lattal (2010)). Furthermore, if the delay is longer, other

behavioral responses may be expressed and associated to the reinforcer (Bouton, 2007). We made every effort to immediately reveal/release/deliver the reinforcer (presenting the food reward for the subject to grab and eat, or opening the release door so that the pair mate could run into the home cage) after the subject touched S+, but we cannot control the monkeys' behavior after the reinforcer is delivered. For example, the subjects would occasionally wait to grab the banana after they touched S+, or they would wait to eat the banana after grabbing it. Also, although the pair mate always ran immediately into the home cage from the release door, we of course cannot control where in the cage the pair mate would run upon entering. On some occasions, the pair mate would run immediately into close proximity or contact with the test subject, but sometimes the pair mate would arrive in the cage at a location more distant to the test subject. However, because the return of a family member through the release door is a regular occurrence for animals in their home cage, the visual and auditory stimuli associated with the process should be an equivalent indicator of the return of their pair mate, which was initiated immediately after a correct response was given by the test subject. In all cases, the test subject immediately looked toward the reward, either to the piece of banana that appeared within reach or to the release door where their pair mate was being released. The fact that the subjects immediately attended to the reward deliveries in all tests and did not exhibit other behaviors which could be associated with the reinforcer, should resolve any potential issues of temporal contingency in the animal's understanding that their action triggered the reinforcer.

Similarly, differences in the spatial contiguity between the location of a reward can cause differences in learning (McClearn & Harlow, 1954; Murphy & Miller, 1955). In the current study, the location of the delivery of the food reward (within reach of where the subject was sitting in the trial zone) was different from the location of the delivery of the social reward (the release door at the bottom corner of the home cage). We recognize that this difference could have potentially contributed to our results. However, prior to beginning this experiment, we piloted a variety of different experimental designs to directly address the spatial difference between the reward delivery locations. Although we ultimately had to compromise on spatial contiguity, we chose the option that caused the fewest additional issues that would impair associative learning and that was the most ethologically appropriate for this species. For example, if we were to deliver the food reward at the release door, then the test subjects would have to travel toward the bottom of the cage in order to receive their reward, which introduces issues with temporal contingency as described above and also demands that the subjects approach the floor—a rare behavior for these arboreal animals, which are rarely observed on the ground (Gron, 2007). These pilot experiments directly informed our final design to ensure that the onset of all reward-related cues would be perceived by the subjects identically across conditions and immediately after a correct response was given.

Overall, this study indicates that titi monkeys can learn a two-object discrimination task, which provides a new behavioral outcome measure in our assessments of titi monkey sociality and cognition. It may be possible in future studies to combine this test of animal learning with pharmacological treatments in order to investigate the neural basis of reward learning and motivation in this species. A recent study in mice showed that social reward requires both the oxytocin and serotonin systems in the brain, acting specifically in the

nucleus accumbens, which is an important region in the reward circuitry (Dölen, Darvishzadeh, Huang, & Malenka, 2013). Although the social reward was less effective in shaping titi monkey behavior, nearly half of our subjects successfully learned the discrimination task with a social reinforcer. Thus, titi monkeys can learn to associate an object with a food reward or with a social reward. It is possible that treatment with oxytocin, which is a neuropeptide that modulates several aspects of social cognition (Caldwell & Albers, 2016), could improve the behavior of titi monkeys in the social condition of this task and further contribute to our understanding of the neurobiology of social cognition.

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REFERENCES

- Alvarado MC, Malkova L, & Bachevalier J (2016). Development of relational memory processes in monkeys. *Developmental Cognitive Neuroscience*, 22, 27–35. 10.1016/j.dcn.2016.10.007. [PubMed: 27833046]
- Anderson JR (1998). Social stimuli and social rewards in primate learning and cognition. *Behavioural Processes*, 42(2–3), 159–175. [PubMed: 24897460]
- Anzenberger G (1988). The pairbond in the titi monkey (*Callicebus moloch*): intrinsic versus extrinsic contributions of the pairmates. *Folia Primatologica; International Journal of Primatology*, 50(3–4), 188–203. [PubMed: 3240912]
- Bales KL, Arias Del Razo R, Conklin QA, Hartman S, Mayer HS, Rogers FD, ... Wright EC (2017). Titi monkeys as a novel non-human primate model for the neurobiology of pair bonding. *The Yale Journal of Biology and Medicine*, 90(3), 373–387. [PubMed: 28955178]
- Bartal IB-A, Decety J, & Mason P (2011). Empathy and pro-social behavior in rats. *Science*, 334(6061), 1427–1430. 10.1126/science.1210789. [PubMed: 22158823]
- Belzung C, & Anderson JR (1986). Social rank and responses to feeding competition in rhesus monkeys. *Behavioural Processes*, 12(4), 307–316. 10.1016/0376-6357(86)90001-X. [PubMed: 24924690]
- Bouton ME (2007). *Learning and behavior: A contemporary synthesis* Sunderland, MA: Sinauer Associates.
- Bunce JA, Isbell LA, Neitz M, Bonci D, Surridge AK, Jacobs GH, & Smith DG (2011). Characterization of opsin gene alleles affecting color vision in a wild population of titi monkeys (*Callicebus brunneus*). *American Journal of Primatology*, 73(2), 189–196. 10.1002/ajp.20890. [PubMed: 20938927]
- Butler RA (1954). Incentive conditions which influence visual exploration. *Journal of Experimental Psychology*, 48(1), 19–23. [PubMed: 13174728]
- Caldwell HK, & Albers HE (2016). Oxytocin, vasopressin, and the motivational forces that drive social behaviors. *Current Topics in Behavioral Neurosciences*, 27, 51–103. 10.1007/7854_2015_390. [PubMed: 26472550]
- Cubiccioni D, & Mason WA (1975). Comparative studies of social behavior in *Callicebus* and *Saimiri*: Male-female emotional attachments. *Behavioral Biology*, 16(2), 185–197.
- Deaner RO, Khera AV, & Platt ML (2005). Monkeys pay per view: Adaptive valuation of social images by rhesus macaques. *Current Biology*, 15(6), 543–548. 10.1016/j.cub.2005.01.044. [PubMed: 15797023]

- Deruelle C, Barbet I, Dépy D, & Fagot J (2000). Perception of partly occluded figures by baboons (*Papio papio*). *Perception*, 29(12), 1483–1497. 10.1068/p3071. [PubMed: 11257971]
- Dölen G, Darvishzadeh A, Huang KW, & Malenka RC (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature*, 501(7466), 179–184. 10.1038/nature12518. [PubMed: 24025838]
- Fernandez-Duque E, Mason WA, & Mendoza SP (1997). Effects of duration of separation on responses to mates and strangers in the monogamous titi monkey (*Callicebus moloch*). *American Journal of Primatology*, 43(3), 225–237. [http://doi.org/10.1002/\(SICI\)1098-2345\(1997\)43:3<225::AID-AJP3>3.0.CO;2-Z](http://doi.org/10.1002/(SICI)1098-2345(1997)43:3<225::AID-AJP3>3.0.CO;2-Z). [PubMed: 9359966]
- Flaherty CF (1985). *Animal learning and cognition* New York City, NY: Random House.
- Fragaszy DM (1981). Comparative performance in discrimination learning tasks in two New World primates (*Saimiri sciureus* and *Callicebus moloch*). *Animal Learning and Behavior*, 9(1), 127–134.
- Fragaszy DM, & Mason WA (1983). Comparisons of feeding behavior in captive squirrel and titi monkeys (*Saimiri sciureus* and *Callicebus moloch*). *Journal of Comparative Psychology*, 97(4), 310–326.
- Gallistel CR, Craig AR, & Shahan TA (2014). Temporal contingency. *Behavioural Processes*, 101, 89–96. <http://doi.org/10.1016/j.beproc.2013.08.012>. [PubMed: 23994260]
- Grant DA (1964). Classical and operant conditioning. In Melton AW (Ed.), *Categories of Human Learning* (pp. 1–31). New York: Academic Press.
- Gray H, Pearce B, Thiele A, & Rowe C (2017). The use of preferred social stimuli as rewards for rhesus macaques in behavioural neuroscience. *PLoS ONE*, 12(5), e0178048 10.1371/journal.pone.0178048. [PubMed: 28542356]
- Gron KJ (2007, December 19). Primate Factsheets: Dusky titi (*Callicebus moloch*) Taxonomy, Morphology, & Ecology. Retrieved March 19, 2018, available online at: http://pin.primate.wisc.edu/factsheets/entry/dusky_titi.
- Harlow HF (1945). Studies in discrimination learning by monkeys; discriminations between stimuli differing in both color and form, and only in color, and only in form. *The Journal of General Psychology*, 33, 225–235. [PubMed: 21006713]
- Harlow HF, & Bromer JA (1938). A test apparatus for monkeys. *The Psychological Record*, 2, 434–436.
- Hawkins RD, & Byrne JH (2015). Associative learning in invertebrates. *Cold Spring Harbor Perspectives in Biology*, 7(5), 10.1101/cshperspect.a021709.
- Lattal KA (2010). Delayed reinforcement of operant behavior. *Journal of the Experimental Analysis of Behavior*, 93(1), 129–139. 10.1901/jeab.2010.93-129. [PubMed: 20676272]
- Lucon-Xiccato T, & Bisazza A (2016). Male and female guppies differ in speed but not in accuracy in visual discrimination learning. *Animal Cognition*, 19(4), 733–744. 10.1007/s10071-016-0969-2. [PubMed: 26920920]
- Mason WA (1974). Comparative studies of social behavior in *Callicebus* and *Saimiri*: Behavior of male-female pairs. *Folia Primatologica*, 22, 1–8.
- Mattson BJ, Williams SE, Rosenblatt JS, & Morrell JJ (2003). Preferences for cocaine- or pup-associated chambers differentiates otherwise behaviorally identical postpartum maternal rats. *Psychopharmacology*, 167(1), 1–8. 10.1007/s00213-002-1351-4.
- Mattson BJ, Williams S, Rosenblatt JS, & Morrell JJ (2001). Comparison of two positive reinforcing stimuli: Pups and cocaine throughout the postpartum period. *Behavioral Neuroscience*, 115(3), 683–694. [PubMed: 11439457]
- Mayeaux DJ, & Mason WA (1998). Development of responsiveness to novel objects in the titi monkey, *Callicebus moloch*. *Primates*, 39(4), 419–431.
- McClearn GE, & Harlow HF (1954). The effect of spatial contiguity on discrimination learning by rhesus monkeys. *Journal of Comparative and Physiological Psychology*, 47(5), 391–394. [PubMed: 13221689]
- Mendoza SP, & Mason WA (1986). Contrasting responses to intruders and to involuntary separation by monogamous and polygynous New World monkeys. *Physiology & Behavior*, 38(6), 795–801. [PubMed: 3823197]

- Mendoza SP, & Mason WA (1997). Attachment relationships in new world primates. *Annals of the New York Academy of Sciences*, 807, 203–209. [PubMed: 9071352]
- Moss EM, & Harlow HF (1947). The role of reward in discrimination learning in monkeys. *Journal of Comparative and Physiological Psychology*, 40(5), 333–342. [PubMed: 20267825]
- Murphy JV, & Miller RE (1955). The effect of spatial contiguity of cue and reward in the object-quality learning of rhesus monkeys. *Journal of Comparative and Physiological Psychology*, 48(3), 221–224. [PubMed: 13242695]
- Panksepp JB, & Lahvis GP (2007). Social reward among juvenile mice. *Genes, Brain and Behavior*, 6(7), 661–671. 10.1111/j.1601-183X.2006.00295.x.
- Park T, Okanoya K, & Dooling R (1985). Operant conditioning of small birds for acoustic discrimination. *Journal of Ethology*, 3(1), 5–9.
- Pavlov IP, (1960). In Anrep GV (Ed.), *Conditioned reflex: An investigation of the physiological activity of the cerebral cortex* New York: Dover Publications, Inc.
- Ridley RM, Bowes PM, Baker HF, & Crow TJ (1984). An involvement of acetylcholine in object discrimination learning and memory in the marmoset. *Neuropsychologia*, 22(3), 253–263. [PubMed: 6431311]
- Rollin AR, Thaller KE, & Shepp BE (1963). A modified wisconsin general test apparatus for rat. *American Journal of Psychology*, 76(3), 500–&.
- Seip KM, & Morrell JI (2007). Increasing the incentive salience of cocaine challenges preference for pup- over cocaine-associated stimuli during early postpartum: Place preference and locomotor analyses in the lactating female rat. *Psychopharmacology*, 194(3), 309–319. 10.1007/s00213-007-0841-9. [PubMed: 17589831]
- Skinner BF (2012). How to teach animals. *Scientific American Reprints*, No. 423
- Tavares MCH, & Tomaz C (2002). Working memory in capuchin monkeys (*Cebus apella*). *Behavioural Brain Research*, 131(1–2), 131–137. [PubMed: 11844580]
- Thompson T, & Sturm T (1965). Visual-reinforcer color, and operant behavior in Siamese fighting fish. *Journal of the Experimental Analysis of Behavior*, 8(5), 341–344. [PubMed: 14342375]
- Thorndike EL (1898). Animal intelligence: An experimental study of the associative processes in animals. *Psychological Monographs: General and Applied*, 2(4), i–109.
- Timberlake W (1993). Behavior systems and reinforcement – an integrative approach. *Journal of the Experimental Analysis of Behavior*, 60(1), 105–128. 10.1901/jeab.1993.60–105. [PubMed: 8354963]
- Trezza V, Campolongo P, & Vanderschuren LJMJ (2011). Evaluating the rewarding nature of social interactions in laboratory animals. *Developmental Cognitive Neuroscience*, 1(4), 444–458. 10.1016/j.dcn.2011.05.007. [PubMed: 22436566]
- Ulloa M, Portillo W, Díaz NF, Young LJ, Camacho FJ, Rodríguez VM, & Paredes RG (2017). Mating and social exposure induces an opioid-dependent conditioned place preference in male but not in female prairie voles (*Microtus ochrogaster*). *Hormones and Behavior*, 97, 47–55. 10.1016/j.yhbeh.2017.10.015. [PubMed: 29111331]
- Wilsoncroft WE (1968). Babies by bar-press: Maternal behavior in the rat. *Behavior Research Methods and Instruments*, 1(6), 229–230. 10.3758/BF03208105.

FIGURE 1.
Schematic of the testing set-up in the subject's home cage

FIGURE 2.

Average behavioral latencies during the two-object discrimination task between the two types of reward

(a) Trials with a correct response **(c) Trials with no correct response** **(b) Trials to reach criteria for success** **(d) Trials with balks** **FIGURE 3.**

Learning outcomes in a two-object visual discrimination task. (a) Percent of trials ending with a correct response. (b) Number of trials required to reach success criteria. (c) Percent of trials ending with an incorrect response only and no correct response ever given. (d) Percent of trials when the subject balked

FIGURE 4.
Percent of animals who succeeded in learning the two-object discrimination task

TABLE 1

Outcome measures

Average latency (sec) to enter the trial zone
Average latency (sec) to make a correct choice after entering the trial zone
Average latency (sec) to make an incorrect choice after entering the trial zone
Percent of correct trials
Percent of trials where no correct response was given
The percent of trials where the subject balked
The number of trials to reach criteria

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