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Urinary oxytocin in capuchin monkeys: Validation and the influence of social behavior

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In highly social species, like primates, oxytocin plays an important role in cooperation, and in the formation and maintenance of social relationships. Despite recent interest in the relationship between oxytocin and social behavior in nonhuman primates, relatively little is known about endogenous oxytocin in social New World Monkeys. In this paper, we investigate the relationship between oxytocin and affiliative behaviors in socially-housed captive capuchin monkeys (*Sapajus* [*Cebus*] *apella*) by first validating methods of analysis of urinary oxytocin in this species and, second, examining the effects of grooming and fur-rubbing behavior on oxytocin concentrations and further affiliative behavior. In the validation, we found that intranasal exogenous oxytocin significantly increased urinary oxytocin 15–60 min post-administration. Oxytocin was also implicated in both grooming and fur-rubbing behaviors. We found that oxytocin concentrations increased after subjects engaged in grooming or fur-rubbing. In addition, we found that fur-rubbing influenced affiliative behaviors, both during and after a social fur-rubbing bout. While individuals spent more time in contact and proximity while fur-rubbing, immediately following the fur-rubbing event (15–30 min afterwards) all affiliative behaviors decreased. This supports previous research that oxytocin may, in fact, initially be related to increased social distance in this species. Yet, an increase in all affiliative behaviors 30–45 min after the onset of fur-rubbing suggests that fur-rubbing, like grooming, may ultimately function to strengthen social relationships. Overall, these results support a critical role for oxytocin in affiliative behaviors that maintain and strengthen social relationships in capuchin monkeys, and highlight the complexity of the interactions among oxytocin, affiliative behaviors, and social bonding.

KEYWORDS
affiliation, capuchin, hormone, oxytocin, social bonding

1 | INTRODUCTION

The neuropeptide hormone oxytocin (OT) plays an important role in the formation and maintenance of mammalian social bonds. It is considered an integral catalyst in mother-infant bond formation early in life (Finkenwirth, van Schaik, Ziegler, & Burkart, 2015; Ross & Young, 2009), and an important mechanism in facilitating sexual (Carter, 1992; Cavanaugh, Mustoe, Taylor, & French, 2014; Smith, Agmo, Birnie, & French, 2010) and social bonds (Ross & Young, 2009) later in life. In highly social species, oxytocin promotes affiliative behaviors important

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in maintaining cooperation and group cohesion (De Dreu, 2012). Oxytocin has been linked to increased food sharing behavior and grooming in vampire bats (Carter & Wilkinson, 2015), guarding and pup-feeding in meerkats (Madden & Clutton-Brock, 2011), social play in domestic dogs (Romero, Nagasawa, Mogi, Hasegawa, & Kikusui, 2014, 2015), prosocial choices in rhesus macaques (Chang, Barter, Ebitz, Watson, & Platt, 2012), and reciprocity (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008; Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005) and generosity (Zak, Stanton, & Ahmadi, 2007) in economic games in humans. In sum, higher oxytocin levels are typically associated with stronger social bonds and increases in cooperative or prosocial behaviors.

There has been a growing interest in the evolutionary role of oxytocin in bond formation and affiliative behaviors, with an increase in studies on non-human primates. In chimpanzees (*Pan troglodytes*), for example, elevated oxytocin levels are linked with food sharing behavior (Wittig et al., 2014) and greater within-group cohesion during intergroup encounters (Samuni et al., 2017). Similarly, in macaques (*Macaca spp.*), administering oxytocin amplified social behavior in infants (Simpson et al., 2014), and adults (Chang et al., 2012). Despite the growing interest in the evolutionary role of oxytocin in influencing affiliative behaviors in non-human primates, an important question remains: does oxytocin enhance social behaviors in general, or can oxytocin influence behaviors in a species-specific way? To address this question requires a thorough understanding of the relationship between oxytocin and social behavior across the primate taxa. To date, however, research on oxytocin has been largely confined to a handful of species (*Pan troglodytes*; Chang et al., 2012; Crockford et al., 2013; *Macaca spp.*; Ebitz, Watson, & Platt, 2013; Finkenwirth et al., 2015; Finkenwirth, Martins, Deschner, & Burkart, 2016; *Callitrix spp.*; e.g., example,; Freeman et al., 2014; Mustoe, Harnisch, Hochfelder, Cavanaugh, & French, 2016 Saito & Nakamura, 2011; Samuni et al., 2017; Simpson et al., 2014; Smith et al., 2010; *Callicebus spp.*; Snowdon et al., 2010; Winslow & Insel, 1992; *Saimuri scitureus*; Wittig et al., 2014) with the vast majority of studies conducted on pair-bonding behavior in callitrichids (e.g., Cavanaugh et al., 2014; Finkenwirth et al., 2016, 2015; Mustoe et al., 2016; Saito & Nakamura, 2011; Snowdon et al., 2010). While these studies highlight the importance of oxytocin in parental (e.g., Saito & Nakamura, 2011) and sexual relationships (e.g., Cavanaugh et al., 2014), we know considerably less about the role of oxytocin in maintaining social bonds and facilitating cooperative behaviors in group-living New World monkeys. In this study, we investigate the relationship between oxytocin and affiliative social behaviors in socially-housed captive capuchin monkeys (*Sapajus [Cebus] apella*).

Capuchin monkeys are an excellent species to examine the relationship between oxytocin and social behavior. Like humans and chimpanzees, capuchins are highly gregarious and exhibit strong social bonds with both kin and non-kin (See Fragaszy, Visalberghi, & Fedigan, 2004); however, in contrast to the other New World monkey species in which oxytocin has been studied, capuchin monkeys do not form monogamous pair-bonds, and rather live in large multi-male, multi-female social groups (Fragaszy et al., 2004). In addition, capuchins are highly cooperative (Brosnan, Salwiczek, & Bshary, 2010). Capuchins routinely form alliances and coalitions (Crofoot, Rubenstein, Maiya, & Berger-Wolf, 2011; Scarry et al., 2013), engage in food sharing (de Waal, 2000), and are one of the few non-human primates to show consistent experimental evidence of prosocial behavior (De Waal, Leimgruber, & Greenberg, 2008; Jaeggi, Burkart, & Van Schaik, 2010; Lakshminarayanan & Santos, 2008; Leimgruber et al., 2014). If oxytocin plays an important role in maintaining social bonds across primates, we expect a positive relationship between affiliative behaviors and oxytocin in capuchins that is similar to those that have been found in other primate species (e.g., Crockford et al., 2013; Saito & Nakamura, 2011; Simpson et al., 2014; Smith et al., 2010; Wittig et al., 2014).

However, recent research suggests oxytocin may function somewhat differently in capuchins (Brosnan et al., 2015). Oxytocin in capuchin monkeys, along with several other New World monkey species, possesses a position-8 amino acid substitution, resulting in a novel form of oxytocin, Pro^8 oxytocin, that is, different from that of other mammalian species (Lee et al., 2011; Mustoe et al., 2018; Parreiras-E-Silva et al., 2017). This substitution could alter the regulation of oxytocin and subsequently impact reproductive and social behavior. To date, the only study on oxytocin and capuchins suggests that this might be the case (Brosnan et al., 2015), however, this study was run prior to the recognition of this novel form of oxytocin (although it was published subsequently) and so used the mammalian consensus oxytocin, complicating interpretation. When administered intranasally, oxytocin increased distance from social partners, and subsequently decreased food sharing behavior between them (Brosnan et al., 2015). These results are intriguing considering oxytocin increases prosocial food choices in macaques (Chang et al., 2012) and has been linked to greater food sharing behavior in chimpanzees (Wittig et al., 2014). One possibility is that oxytocin in capuchins functions slightly differently, perhaps having a predominant anxiolytic effect, and thus reducing desire for social contact (Brosnan et al., 2015). If this is the case, this suggests that oxytocin may have more of a species-specific effect on cooperative behaviors.

A necessary next step is to examine the relationship between naturally-occurring endogenous peripheral oxytocin levels and social behavior in capuchin monkeys. Exogenously administering oxytocin is useful in determining what oxytocin does, whereas endogenous oxytocin levels highlight the factors that influence oxytocin release, providing useful insight into the adaptive function of the peptide (Crockford, Deschner, Ziegler, & Wittig, 2014; Ebitz & Platt, 2013). While there is still debate about whether peripheral oxytocin levels are indicative of central oxytocin level (e.g., Crockford et al., 2014; Lefevre et al., 2017; Leng & Ludwig, 2016; Valstad et al., 2017; Veening & Olivier, 2013), there are growing number of studies that have linked peripheral oxytocin in blood and urine to behavioral functions (Crockford et al., 2014). For example, peripheral oxytocin increases after social contact in humans (Morhenn, Beavin, & Zak, 2012), grooming among close social partner in chimpanzees (Crockford et al., 2013), and is positively associated with rates of affiliative behaviors in pair-bonded tamarins (Snowdon et al., 2010). If oxytocin functions the same in capuchins as it does in other primate taxa, we expect to find a similar relationship between an increase in endogenous oxytocin levels and increase in affiliative behaviors in this species.

Because there have been no studies on endogenous measures of oxytocin in capuchins, we first validated a commercially available enzyme immunoassay kit for use in this species. To do this, we examine the effect of intranasal (Pro^8) oxytocin on urinary oxytocin levels in capuchins. This functions as both a validation of our methods of analyzing endogenous urinary oxytocin in capuchins and to determine a time window of urinary clearance of oxytocin in this species. Second, we examined the effect of two affiliative social behaviors, grooming, and fur-rubbing, on endogenous oxytocin levels. We chose grooming and fur-rubbing specifically because of their role in affiliation in capuchins. Grooming plays an important role in the formation and maintenance of social bonds across the primate taxa (e.g., Mitani, 2009; Schülke, Bhagavatula, Vigilant, & Ostner, 2010; Silk et al., 2009, 2010). Moreover, recent research on chimpanzees found that urinary oxytocin levels increased after grooming with preferred social partners, suggesting that oxytocin is involved in the maintenance of strong social bonds (Crockford et al., 2013). Capuchins, like chimpanzees, form strong social bonds with social partners, which are often characterized by increased proximity, contact, and grooming behavior (Fragaszy et al., 2004). We predicted that oxytocin levels should be higher after an individual is groomed when compared to baseline oxytocin levels, taken after a period with no affiliative social interactions.

We expected a similar relationship between oxytocin and fur-rubbing, a less widespread behavior than grooming, but one that is believed to also function in maintaining social relationships in capuchins. In both the wild and in captivity, capuchin monkeys engage in a frantic fur-rubbing behavior with plants (e.g., Piper, Citrus) or other pungent materials (e.g., onions, vinegar) (Baker, 1996, 1997). When fur-rubbing, capuchins rub the object vigorously over their entire body, and often congregate to fur-rub in contact with other social partners (Ludes & Anderson, 1995). While there is some evidence of a medicinal function for fur-rubbing (Baker, 1996), the social nature of this behavior has led to an alternative hypothesis that fur-rubbing may function similarly to grooming, by increasing social bonding, and group cohesion (Leca, Gunst, & Petit, 2007; Meunier & Petit, 2008; but see, Paukner & Suomi, 2008, 2012). To test this hypothesis, we examined behavioral and hormonal changes during and following fur-rubbing bouts. If fur-rubbing has a social function, we predict the following: 1) oxytocin levels should increase following social fur-rubbing bouts; 2) rates of affiliative behaviors should increase during and following social fur-rubbing bout; and 3) individuals will be more likely to fur-rub in contact with closely bonded social partners than more distant ones.

Because oxytocin does seem to increase social behaviors in other social mammals, we suspect that its *adaptive function*, to form and maintain social bonds, is more generally conserved. However, the previous research on capuchins does suggest that there might be some species specific behavioral *responses* to oxytocin. This paper aims to examine this further by looking at endogenous measures which focuses on the circumstances in which oxytocin is released, providing more insight into the function of oxytocin in capuchins. More specifically, we examined the relationship between affiliative social behavior and endogenous oxytocin levels in capuchin monkeys. To do this, we first validated a method for urinary oxytocin analysis, by comparing baseline urinary oxytocin levels with oxytocin concentration after a subject received intranasal oxytocin, and second, examined the effects of grooming and fur-rubbing behavior on oxytocin levels. We predicted that oxytocin should increase in all three manipulations. In addition to oxytocin, we examined differences in affiliative behavior during and after fur-rubbing to assess the social function of this behavior.

2 | METHODS

2.1 | Subjects and study site

Our subjects were 18 capuchin monkeys (five males and 13 females) living in four multi-male, multi—female social groups at the Language Research Center of Georgia State University. Subjects lived in large indoor-outdoor enclosures. During the day, subjects were given the opportunity to voluntarily enter individual testing chambers affixed to the indoor section of the group enclosure. Water was available ad libitum, including in the testing chambers. Food, including primate chow and fresh fruits and vegetables, was provided multiple times per day, regardless of the testing schedule. All urine and data collection was non-invasive and conducted between May and December 2017 in the indoor chambers. All protocols were approved by the Georgia State University IACUC (A16031) and adhered to all applicable legal requirements of the United States; in addition, all research was conducted in adherence with the ASP Principles for the Ethical Treatment of Primates.

2.2 | Urine collection and analysis

We collected urine samples opportunistically from all subjects between 8:30 am to 11:00 am while they were separated in their testing chambers. All urine samples were collected on sterile trays when subjects were separated into individual testing boxes. For samples associated with behaviors of interest (e.g., grooming), subjects were placed into testing boxes immediately following observation, and samples were collected within 1 hr of the observed behavior. Urine was transferred with a disposable pipette into 5 ml vials within 5 min of urination. Upon collections, urine samples were immediately placed in a frozen freezer cooler (FlexiFreeze freezable cooler) and kept cold until samples were transferred to the Language Research Center Hormone Laboratory within 2 hr of collection. Following urine collection, samples were centrifuged at 2,700 rpm for 3 min and aliquoted into 1.0 ml increments into microcentrifuge tubes containing 100 μl of 0.5 N phosphoric acid. Samples were stored at −20 °C and transferred in IsoPacks to the Georgia State University Core Assay Facility for analysis.

At the Core Assay Facility, Sep-Pak Light C18 cartridges (55–150 mm 50/box; WAT023501) were conditioned with 1 ml of 100% MeOH, then 1 ml distilled water. This method of extraction using

MeOH aligns with several previously published studies (e.g., Crockford et al., 2013; Finkenwirth et al., 2015) and is more "field friendly" than TFA extractions. Urine (1 ml) was transferred to a syringe and loaded onto the cartridge by slowly pushing the syringe so that the flow rate was approximately 1 ml per min. The cartridge was then washed with 1 ml of 10% acetonitrile (ACN) containing 0.1%t TFA. Samples were then eluted with 1 ml of 80% ACN. Eluted samples were dried down in a lyophilizer at −50° for 16–24 hr until dried and stored at −20 °C until assayed. Dried samples were reconstituted in 250 µl of assay buffer and analyzed using a commercially available enzyme immunoassay kit (Assay Designs; catalog no. 901–153) previously used for measuring urinary oxytocin in nonhuman primates (Crockford et al., 2013; Finkenwirth et al., 2015; Samuni et al., 2017; Seltzer & Ziegler 2007; Wittig et al., 2014). The assay standard curve ranged from 7.8 to 1,000 pg/ml. Mean intra-assay coefficients of variation were 11.8% low and 3.95% high. Mean inter-assay coefficients of variation were 2.48%, respectively. Linearity validation (Mean% Recover = 102, t(8) = 0.68, $n = 5$, $p = 0.52$) and extraction efficiency (Mean% Recovery = 105, t(6) = 0.36, $n = 4$, $p = 0.77$) were satisfactory (see Supplementary Materials).

In addition to oxytocin, all samples were analyzed for creatinine levels to control for variable urine concentration (Seltzer & Ziegler 2007). Samples were diluted with distilled water (1:2 dilution) and run according to manufacturer's instructions (Assay Designs; catalog no. 907–030). The creatinine detection assay standard curve ran from 0.31–20 mg/dl and assay sensitivity was 0.042 mg/dl.

As previously established for other primate species, for all samples we assumed a time window of urinary clearance of oxytocin of 15–60 min (Amico, Ulbrecht, & Robinson 1987; Crockford et al., 2013). Note, however, that we tested this time window for our subjects in the exogenous validation.

2.3 | Exogenous oxytocin

To validate our methods of measuring endogenous oxytocin, we administered ~15 IU of crystallized New World monkey oxytocin (Pro^8-OT from Anaspec; http://www.anaspec.com/) reconstituted in saline via an inhalation method commonly used in non-invasive oxytocin manipulations (Brosnan et al., 2015; Van & Bakermans-Kranenburg, 2012). We aerosolized the reconstituted oxytocin using a NebPak handheld baby ultrasonic nebulizer (MABIS; catalog no. 40-366-000). Seven of our subjects, three males and four females, were previously trained to hold onto a target attached to the cage and present their nose to the mesh for oxytocin inhalation (Brosnan et al., 2015). Subjects were given ~15 IU over the course of six 10 s bouts for a total of 60 s of nebulization (a 1 mg vial of crystalized new world monkey OT dissolved in 33.5 ml saline administered at a rate of 1 ml per minute for 60 s). Subjects were rewarded at the end of each bout with a small apple cube. We visually monitored subject inhalation throughout administration to ensure that the subjects were breathing the aerosolized vapor in during each bout; as subjects breathed in and out, a distinct pulling and "puffing" of the vapor flow indicated subject inhalation. All subjects completed administration of their full dose within 3 min of the onset of oxytocin vaporization.

We administered oxytocin five times on five different days a minimum of a week apart. We collected urine samples prior to and up to 1 hr following oxytocin administration. When possible, we collected multiple samples from the same individual within that 1 hr window of collection.

2.4 | Behavioral data collection

We collected data on two different affiliative behaviors, grooming, and fur-rubbing, that we predicted should influence oxytocin levels. Both behaviors have been hypothesized to function in maintaining and strengthening social bonds, and as oxytocin is hypothesized to play an important role in mediating social relationships, we predicted that both grooming and fur-rubbing would increase urinary oxytocin in capuchins monkeys.

2.5 | Grooming

We collected grooming samples after a subject received grooming from a conspecific. As part of the standard behavioral data collection protocol for our lab, we conducted routine observational group scans at minimum once per week on all capuchin groups. During these scans, all subjects in the group were observed for 30 min and state behaviors (e.g., locomote, grooming, feeding behaviors, and inactivity) were recorded at 3 min increments. Every 3 min we also recorded whether individuals were grooming, or were in contact (i.e., touching another individual) or in proximity (i.e., within arm's reach of another individual) to others in their social group (behaviors are mutually exclusive and coded in the listed order of importance). All scans were conducted on Kindle Fires using an application designed in-house specifically for our data collection protocol (WhatsOb; Watzek & Brosnan, 2016). In addition to our routine data collection, for the scans used in this study, all instances of grooming behavior were recorded *ad libitum* during group scans. This included: grooming received, grooming given, time grooming began, time grooming ended, and the individuals involved in each grooming bout. Urine samples were considered a "grooming sample" if the sample was collected in the individual test boxes within 15–60 min after an individual received grooming.

2.6 | Fur-rubbing

Second, we examined the effects of fur-rubbing on urinary oxytocin levels and group dynamics in two capuchin social groups. Fur-rubbing is a common behavior in capuchins and is often done in direct contact with one or more individuals within the social group (DeJoseph, Taylor, Baker, & Aregullin, 2002). To initiate fur-rubbing, subjects were each given ⅓ of an onion while in their main social enclosure and allowed to fur-rub until they lost interest or for up to 10 min, whichever came first. During fur-rubbing bouts, we recorded duration of fur-rubbing, whether individuals were in contact with conspecifics (social fur-rubbing) or alone (solo fur-rubbing), and the identity of fur-rubbing partner. After 10 min, all subjects either entered individual testing boxes for urine collection or remained in the social group for behavioral observation. Urine samples were collected opportunistically from all subjects within 1 hr after the end of a fur-rubbing bout.

On separate days from urine collection, we conducted modified group scans post-fur-rubbing that consisted of 1 hr of data collection from the onset of the fur-rubbing bout; these modified scans allowed us to examine how fur-rubbing might change subsequent behaviors. These scans followed the same protocol as the routine group scans but were longer in duration to capture changes in social behavior during and after fur-rubbing bouts.

2.7 | Data analysis

We collected 242 urine samples from 18 adult capuchin monkeys between May and December 2017. Of those samples, only 120 from 11 individuals (three males and 11 females) had enough urine for both creatinine and oxytocin analysis (>1.5 ml; see Supplementary Material). All oxytocin measures were corrected for creatinine levels to control for variable urine concentration and log-transformed (log pg OT/mg Cr) to reach normal distribution prior to statistical analysis. Prior to our analysis, we identified and removed one outlier greater than 2 s.d. from the mean (a post fur-rubbing sample). Nonetheless, all models were run both with and without the outlier, which produced similar results (see Supplementary Materials).

Grooming, fur-rubbing, and exogenous OT samples were compared to baseline urine samples. A urine sample was considered a baseline sample if the subject did not engage in any grooming interaction during their observation period and did not receive any experimental manipulation (i.e., OT administration or onion) in the hour prior to collection. In total, our analysis consisted of 49 baseline samples ($N = 10$), 16 post grooming samples ($N = 4$), 19 post fur-rubbing samples ($N = 6$), and 36 post exogenous OT samples ($N = 7$).

First, to test the effect of exogenous oxytocin on endogenous levels ($n = 75$ urine endogenous and baseline samples, $N = 7$), we fit a Linear Mixed Model (LMM) comparing urinary oxytocin measures taken at four 15 min time intervals after administration to baseline oxytocin samples for each individual. Because our main goal is to examine changes from baseline samples, which is the referent in the model, each time interval is compared only to the baseline and not to each other (Table 1). In our model, we used urinary oxytocin concentration (log pg OT/mg CR) as the dependent variable, and time interval (baseline, 0–15 min, 15–30 min, 30–45 min, 45–60 min) as a fixed categorical effect. Since our dataset contained incomplete values (not all subjects had samples in each time interval), we included identity of subject as a random effect.

Second, to investigate the impact of social behavior on endogenous oxytocin, we fit a second LMM examining the effect of grooming and fur-rubbing behavior on urinary oxytocin concentrations. In this model, we included sample type as a fixed effect (baseline, post-grooming, post-fur-rubbing, and post-exogenous OT) and identity of subject as a random effect. We controlled for two demographic factors that may influence oxytocin levels, age (continuous fixed effect), and sex (categorical fixed effect).

Third, to assess whether fur-rubbing further influenced social behavior, we constructed an additional generalized linear mixed model (GLMM) using a Poisson distribution. In this model, we examined the effects of fur-rubbing on affiliative behaviors. To do this, we compared rates of affiliative behavior during the routine morning group scans (Control) to rates of behaviors during fur-rubbing (Fur-Rubbing) and at three 15 min time intervals after the onset of a fur-rubbing bout. Because individuals were allowed to fur-rub for 15 min, the first time interval (Time 1) reflects a period 15–30 min after the onset of fur-rubbing, the second time interval (Time 2) is 30–45 min after the onset of fur-rubbing, and the third time interval (Time 3) is 45–60 min after the onset of fur-rubbing. For affiliative behaviors, we combined all counts of proximity, contact, and grooming for each routine group scan and at each time interval for the post fur-rubbing behaviors. In the affiliative model, the dependent variable was count of affiliative behaviors per scan offset by time (minutes) to compare differences in the rate of occurrence of these behaviors. We included condition as a fixed effect (Control, Fur-Rubbing, Time 1, Time 2, Time 3) and identity of subject as a random effect.

Lastly, we examined whether subjects were more likely to engage in social fur-rubbing with closely affiliated social partners than with more peripheral members of their social circle. We examined relationship strength between partners by examining how often dyadic pairs spent in proximity, contact, and grooming with each other. For each dyadic pair within a social group, we compared the rates of these affiliative behaviors during normal group scans (Control) to rates of these behaviors during a fur-rubbing bout (Fur-Rubbing). To do this, we constructed a GLMM, with a Poisson distribution. In our model, the dependent variable was counts of affiliative behaviors between dyadic pairs offset by total count of affiliative behaviors. We included scan type, control if during routine group scans or fur-rubbing if during fur-rubbing bout, as a fixed effect and controlled for subject difference by including both individuals in the dyad as random effects.

For each model, we determined the statistical significance of the full model by comparing its fit using a likelihood test with that of a null model including only the intercept and the random effect. We conducted all model analyses in R v.3.2.0 (R Core Team, 2015) using the “lmer” function in the lme4 packages v.1.1–11 (Bates et al., 2015).

TABLE 1 Effects of exogenous oxytocin administration on endogenous urinary oxytocin levels

Predictors	*B*	SE	*t*-value	*p*-value
(Intercept)*	1.81	0.06	28.70	2.53e–09
0-15 min	0.32	0.21	1.56	0.123
15-30 min	0.52	0.12	4.26	6.39 e–05***
30-45 min	0.76	0.15	5.22	1.75 e–06***
45-60 min	0.69	0.15	2.56	2.13 e–05***

Urinary oxytocin levels were compared to baseline sample at four time intervals after intranasal oxytocin administration. Results show an increase in endogenous oxytocin from baseline as early as 15–30 min and remain elevated (compared to baseline) up to 60 min after administration.
Full versus null-model $\chi 2$ (df = 4) = 37.72, $p < 0.001$.
Significance at *0.05, **0.01, ***0.001.

We visually inspected each model using a Q-Q plot, histogram of residuals, and scatterplot of fitted versus residual values. Residual values for all models were normally distributed.

3 | RESULTS

3.1 | Exogenous oxytocin

Urinary oxytocin levels increased significantly from baseline 15–30 min after exogenous oxytocin administration (LMM; $\beta = 0.52$, SE = 0.12, $t = 4.26$, $p < 0.001$; Figure 1) and remained elevated 30–45 min ($\beta = 0.76$, SE = 0.15, $t = 5.22$ $p < 0.001$; Figure 1) and 45–60 min ($\beta = 0.69$, SE = 0.15, $t = 2.56$, $p < 0.001$; Figure 1) after administration. We found no difference in oxytocin levels between baseline samples and urine samples collected 0–15 min ($\beta = 0.32$ SE = 0.21, $t = 0.56$, $p = 0.12$) after administration (Table 1).

3.2 | Effect of grooming and fur-rubbing on oxytocin

On average, grooming bouts were 1 min 35 s (+/−12 s) and ranged from 30 s −10 min in duration. Fur-rubbing bouts average 2 min 3 s and ranged from 8 s −9 min 57 s in duration. Capuchins spent more time fur-rubbing in contact with other individuals (social fur-rub: M = 3 min 8 s, SE = 31 s) than fur-rubbing alone (solo fur-rub: M = 45 s, SE = 12 s). All subjects were observed fur-rubbing in contact with at least one other individual in their social group. Social fur-rubbing bouts ranged from two to five individuals in contact.

We found that both grooming (LMM; $\beta = 0.28$, SE = 0.13, $t = 2.10$, $p = 0.038$; Figure 2) and fur-rubbing behaviors ($\beta = 0.27$, SE = 0.13, $t = 2.07$, $p = 0.041$; Figure 2) were associated with significantly increased urinary oxytocin concentrations in capuchins. We found no overall effect of age ($\beta = 0.01$, SE = 0.01, $t = 0.88$, $p = 0.448$) or sex ($\beta = -0.10$, SE = 0.11, $t = -0.95$, $p = 0.421$) on oxytocin concentrations. Again, we found that samples collected after exogenous oxytocin administration were also significantly higher than baseline levels ($\beta = 0.54$, SE = 0.11, $t = 5.06$, $p < 0.001$; Table 2).

3.3 | Effect of fur-rubbing on social behavior

In the 15 min during which individuals were fur-rubbing, we found a significant increase in the rate of affiliative behaviors as compared to the control observation condition (GLMM; Fur-Rubbing, $\beta = 0.69$, SE = 0.12, $z = 5.75$, $p < 0.001$; Figure 3). However, in 15–30 min after the onset of fur-rubbing (Time 1), rates of all affiliative behaviors decreased to below control levels (Time 1, $\beta = -0.85$, SE = 0.24, $z = -3.62$, $p < 0.001$; Figure 3). 30–45 min after the onset of fur-rubbing affiliative behaviors increased again to above control levels (Time 2, $\beta = 0.31$, SE = 0.14, $z = 2.23$, $p = 0.026$; Figure 3). We found no difference in affiliative behaviors during normal observations and 45–60 min after fur-rubbing (Time 3, $\beta = 0.17$, SE = 0.15, $z = 1.17$, $p = 0.244$; Table 3).

In addition, we found that individuals were more likely to fur-rub in contact with individuals with whom they had strong affiliative relationships (GLMM; $\beta = 1.20$, SE = 1.05, $z = 2.09$, $p = 0.036$), as measured by contact, proximity, and grooming behavior.

4 | DISCUSSION

We found that exogenous oxytocin administration, grooming, and fur-rubbing were all associated with increased urinary oxytocin concentrations, and that the increase was effective from 15 to at least 60 min subsequent to the event. This study is, to our knowledge, the first to validate and characterize endogenous oxytocin levels in capuchin monkeys. We found that exogenous oxytocin was associated with increased peripheral oxytocin levels, as measured by our urinary assays. Specifically, urinary oxytocin levels increased 15–30 min window, and were still significantly elevated in the 45–60 min window (although a visual inspection of the data indicates that they may have been declining by this point; we did not collect further data so we do not know when they returned to baseline levels). In humans, the window for clearance of oxytocin in urine is 30–60 min (Amico et al., 1987). In chimpanzee urine samples collected 15–60 min after prosocial behaviors exhibited increased oxytocin levels (Crockford et al., 2013; Samuni et al., 2017; Wittig et al., 2014), however, that window of clearance was never tested. Our results indicate 15–60 min

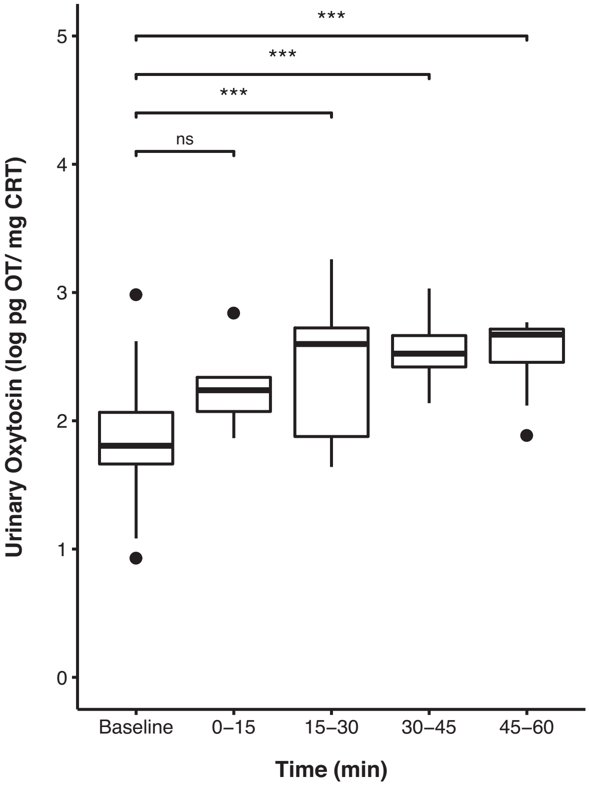

FIGURE 1 Changes in urinary oxytocin concentrations from baseline samples at four 15 min time intervals after intranasal oxytocin administration. Box plots show median and quartiles, whiskers show the 95%CI, and circles indicate values 95%CI. Significant differences across time denoted as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

between administration and testing is an appropriate window of oxytocin clearance in experimental studies in capuchin monkeys.

Considering each of these findings in more detail, we think that our most interesting result is that fur-rubbing was associated with both increased endogenous oxytocin concentration and fluctuations in subsequent affiliative behaviors. As predicted, we found a rise in urinary oxytocin levels following a fur-rubbing bout. In addition, while fur-rubbing, capuchins spent more time in proximity, and in contact with others in their social group. However, contrary to our predictions, 15–30 min after the onset of fur-rubbing we found a decrease in affiliative behaviors. During this period, capuchins spent less time in proximity and contact, and groomed at lower rates, than during the baseline observation condition. Previous studies on captive tufted capuchin have shown similar results; individuals are highly social during fur-rubbing bouts, but afterwards social distance appeared to increase (Paukner & Suomi, 2008, 2012). When taken together with the increase in oxytocin following fur-rubbing, these results appear to support the previous finding in capuchins that an increase in oxytocin may be associated with an initial decrease in social distance and a reduction in affiliative behavior (Brosnan et al., 2015).

Results from this study support the view that oxytocin does not universally enhance prosocial behaviors (Beery, 2015; Brosnan et al., 2015; Crockford et al., 2013; Ebitz & Platt, 2013; Mustoe, Cavanaugh, Harnisch, Thompson, & French, 2015) but may do so in context and in species-specific ways. In both capuchins (Brosnan et al., 2015) and marmosets (Mustoe et al., 2015), individuals treated with oxytocin exhibited a decrease in prosocial behaviors whereas in macaques oxytocin administration increased prosocial tendencies (Chang et al., 2012). While these studies suggest differences in how oxytocin influences affiliative behaviors, there are limitations in exogenous studies, such as isolation to administer oxytocin, stress related to administration, and discrepancies in dosage that may be responsible for these contradictory results (Cardoso, Ellenbogen, Orlando, Bacon, & Joober, 2013; Crockford et al., 2014). This study contributes to these findings by showing that naturally-occurring increases in oxytocin as a result of fur-rubbing behavior had an initial effect of decreasing affiliative behaviors in capuchins, suggesting that results from previous studies are not simply a byproduct of exogenous administration.

TABLE 2 Effects of social behavior on urinary oxytocin (OT) levels

Predictors	*B*	SE	*t*-value	*p*-value
(Intercept)	1.75	0.18	9.49	0.002
Grooming	0.28	0.13	2.10	0.038*
Fur-rubbing	0.27	0.13	2.07	0.041*
Exogenous OT	0.54	0.11	5.06	1.65e−06***
Age	0.01	0.01	0.88	0.448
Sex	−0.10	0.11	-0.95	0.421

Urinary oxytocin was significantly higher than baseline samples after individuals engaged in both affiliative behaviors, grooming and fur-rubbing, and after exogenous oxytocin administration.
Full versus null-model $\chi 2$ (df = 5) = 30.87, $p < 0.001$
Significance at *0.05, **0.01, ***0.001.

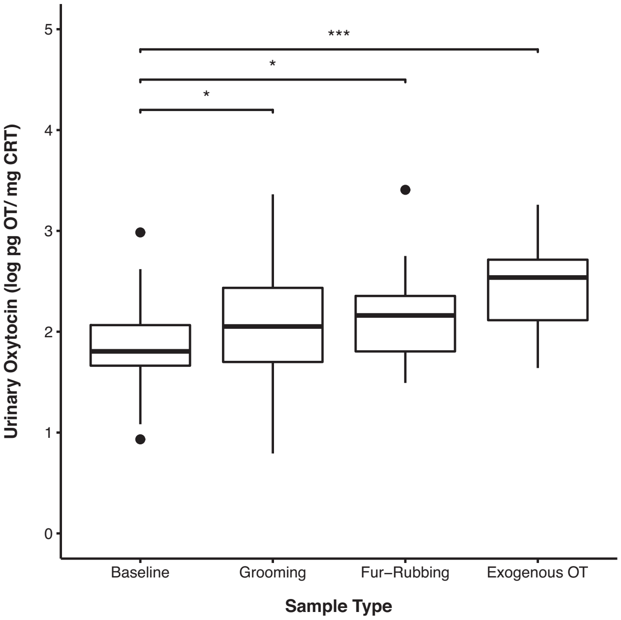

FIGURE 2 Effects of two affiliative behaviors, grooming and fur-rubbing, and exogenous oxytocin administration on urinary oxytocin (OT) concentrations (log pg OT/ mg CRT). Urinary oxytocin levels were collected within an hour following a grooming bout, a fur-rubbing bout, intranasal oxytocin administration (Endogenous), or resting or feeding (Baseline). Box plots show median and quartiles, whiskers show the 95%CI, and circles indicate values 95%CI. Significant differences across conditions denoted as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

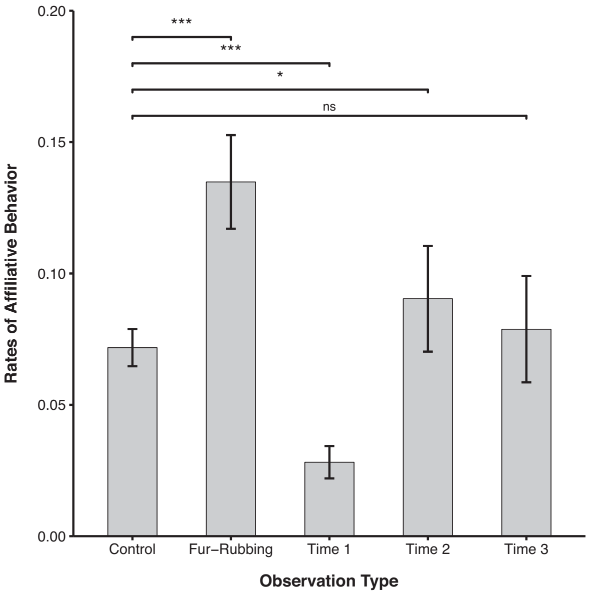

FIGURE 3 Mean (+/−SE) rates of affiliative during standard group scans (Control), during a 15 min fur-rubbing bout (Fur-Rubbing), and at three 15 min time periods after fur-rubbing: Time 1 (15–30 min), Time 2 (30–45 min), Time 3 (45–60 min). Rates were calculated as counts per minute of proximity, contact, and grooming. Significant differences across observation type denoted as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

TABLE 3 Effect of fur-rubbing on affiliative behavior

Predictors	B	SE	z-value	p-value
(Intercept)	−2.88	0.23	−12.25	2.00e−16
Fur-rubbing	0.69	0.12	5.75	8.73e−09***
Time 1	−0.85	0.24	-3.62	0.000***
Time 2	0.31	0.14	2.23	0.025*
Time 3	0.17	0.15	1.17	0.244

Changes in rates of affiliative behaviors (proximity, contact, and grooming) measured as counts per minute during fur-rubbing and at three time periods (Time 1, 15–30 min; Time 2, 30–45 min; Time 3, 45–60 min) after the onset of a fur-rubbing bouts to rates of affiliative behaviors during normal group scans.
full versus null-model $\chi 2$ (df = 4) = 56.24, $p < 0.001$.
Significance at *0.05, **0.01, ***0.001.

Despite the initial decrease in proximity and contact, we found a significant increase above baseline in the rate of affiliative behaviors between conspecifics 30–45 min after a fur-rubbing bout. Specifically, individuals spent more time in contact during this period of time than during routine morning scans when no fur-rubbing occurred (Table 4). Results from our exogenous administration show that the window of clearance for oxytocin in urine is 15–60 min in capuchins, such that in both the 15–30 min and the 30–45 min time period, endogenous oxytocin levels were elevated. This suggests that increased oxytocin, either directly or indirectly, may be implicated in both initially decreasing social distance and eventually increasing social contact.

While the exact mechanism for this fluctuation in affiliative behaviors is beyond the scope of this paper, one prominent hypothesis is that oxytocin is interacting with the hypothalamic-pituitary-axis response, and that together, these two neuroendocrine systems modulate prosocial behavior. Through down-regulation of the amygdala, oxytocin can inhibit the stress-induced activity of the hypothalamic-pituitary-axis response, suppressing the release of stress hormones (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Neumann, Wigger, & Torner, 2000; Uvnäs-Moberg, Ahlenius, Hillegaart, & Alster, 1994; Windle, Shanks, Lightman, & Ingram, 1997). In humans, for example, the combination of oxytocin and social support had the biggest reduction in cortisol concentration after a psychosocial stressor, as well as resulting in an increase in calmness and decrease in anxiety during stress (Heinrichs et al., 2003). Fur-rubbing in capuchins may function similarly, as individuals are experiencing both an increase in oxytocin and an increased rate of contact with preferred social partner during fur-rubbing bouts. In chimpanzees, grooming with preferred social partners both increased urinary oxytocin (Crockford et al., 2013), and decreased cortisol levels (Wittig et al., 2016). Therefore, an increase in oxytocin after fur-rubbing bouts may decrease stress levels, resulting in lowered motivation to seek social closeness (Brosnan et al., 2015). Future studies would benefit from measuring cortisol levels after fur-rubbing behavior to test this hypothesis.

We propose two hypotheses to explain the increase in social contact 30–45 min after a fur-rubbing bout. First, the prosocial effect of oxytocin may linger while the anxiolytic effects of oxytocin wears off. However, this seems unlikely given that previous studies on exogenous oxytocin in capuchins found an overall decrease in affiliative behaviors 30–60 min after oxytocin administration (Brosnan et al., 2015). Instead, it might be that the increase in oxytocin resulted in a decrease in cortisol levels, which eventually promoted social contact. In marmosets, individuals with low cortisol levels engaged more in prosocial behavior with strangers than those with high cortisol levels (Mustoe et al., 2015). Changes in cortisol levels, which may have resulted in an initial decrease in proximity, could have eventually led to an increase in affiliative behaviors. Future work that carefully explores this dynamic is needed to understand the relationships between cortisol and oxytocin on the one hand, and stress and affiliation on the other.

While oxytocin may be context-specific in how it influences social behavior, our results align with oxytocin's adaptive function of strengthening social bonds in primates. Similar to what has been found in chimpanzees (Crockford et al., 2013), we found that urinary oxytocin concentrations increased after grooming, an important behavior implicated in the formation and maintenance of social bonds. In chimpanzees, social relationship strength mediated oxytocin release (Crockford et al., 2013), however, we found an overall effect of grooming despite not taking into account the strength of social bonds between individuals. One limitation of the current study is that our sample size was too small to compare oxytocin levels after individuals groomed with bonded versus non-bonded individuals, as was done with chimpanzees (Crockford et al., 2013). Additionally, in our socially-housed capuchin colony, group sizes are relatively small, ranging from four to 10 individuals. Given this small group size, it is likely that all individuals within the social group had affiliative social bonds, at least to some degree. Finally, it may be that our samples only reflected socially bonded individuals, as these dyadic pairs would have groomed more often.

Fur-rubbing, like grooming, has been hypothesized to function to strengthen social bonds. However, there has been some debate as to whether this is the case in tufted capuchin monkeys. First, one study found that while white-faced capuchins fur-rubbed mainly in subgroups, tufted capuchins fur-rubbed mainly alone, and showed no particular motivation to seek out other group members (Leca et al.,

TABLE 4 Mean and standard error of rates of proximity, contact, and grooming behaviors

	Proximity		Contact		Grooming	
Condition	Mean	SE	Mean	SE	Mean	SE
Control	0.020	0.003	0.021	0.004	0.029	0.004
Fur-rubbing	0.050	0.012	0.096	0.013	0.000	0.000
Time 1	0.013	0.005	0.006	0.003	0.011	0.004
Time 2	0.024	0.008	0.034	0.011	0.033	0.009
Time 3	0.007	0.003	0.051	0.099	0.026	0.009

Counts per minute of the three affiliative behaviors measured before, during, and at three time points (Time 1, 15–30 min; Time 2, 30–45 min; Time 3, 45–60 min) after fur-rubbing.

2007). This is very different from our population of tufted capuchins, in which all individuals fur-rubbed with at least one other social partner and spent more time fur-rubbing in contact with other individuals (mean duration social fur-rubbing = 3 min 8 s) than fur-rubbing alone (mean duration solo fur-rubbing = 45 s). Furthermore, individuals were more likely to fur-rub in contact with their grooming partners, suggesting that, like white-faced capuchins, tufted capuchins choose specific fur-rubbing partners.

Second, recent research on this species found that after fur-rubbing bouts, tufted capuchins, but not white-faced capuchins, both increased social distance and decreased affiliative behaviors, suggesting that fur-rubbing behavior may have an anti-social effect on this species (Paukner & Suomi, 2008, 2012). We found similar results in behavior immediately following a fur-rubbing bout and in the same time frame as these previous studies. However, we argue that the increase in oxytocin following fur-rubbing, and the subsequent increase in affiliative behaviors that occurred 30–45 min after fur-rubbing bout, nonetheless suggest a social bonding function of fur-rubbing, similar to grooming. If oxytocin is associated with the differences in affiliative behaviors after fur-rubbing, further research should examine differences in oxytocin and social proximity between white-faced and tufted capuchins, given that they exhibit different social behaviors after fur-rubbing.

An interesting avenue of future research might be to explore the specific cause of the oxytocin increase; that is, future work might seek to investigate whether it is the act of fur-rubbing itself, the increased contact with other individuals during fur-rubbing, or some combination of both that raises oxytocin levels. We could not separate these potential causes in our study as all individuals from which we collected post fur-rubbing urine samples engaged in both solo and social fur-rubbing in every fur-rubbing session. It is possible that mere physical contact may be inducing the release of oxytocin in capuchins, as some evidence exists in rodents that touch increases plasma oxytocin (e.g., Stock & Uvnäs-Moberg, 1988); however, a growing body of evidence suggests that touch alone is not sufficient to raise oxytocin level (Beery & Zucker, 2010; Crockford et al., 2013; Dantzer, Bluthe, Koob, & Le Moal, 1987; Kosfeld et al., 2005).

An important practical implication of this study is that fur-rubbing may be used as a non-invasive experimental manipulation to increase oxytocin in capuchins. Capuchins are a model species for studies on social cognition, cooperation, and prosociality (Fragaszy et al., 2004). Surprisingly, relatively little is known about the effects of oxytocin on capuchin social behavior and cognition. This may be due to the fact that endogenous measures are lacking, and exogenous measures of administering intranasal oxytocin are expensive, relatively invasive, and often require extensive training and separating individuals from their social group. Unlike exogenous administration, social fur-rubbing, which can be experimentally induced by providing subjects with pungent materials like onions, allows for a natural rise in oxytocin levels within a social context, providing a biologically relevant oxytocin manipulation in this species. This provides researchers interested in the relationship between oxytocin and social cognition, an alternative method to exogenous methods for experimentally inducing endogenous oxytocin release.

Results from this study highlight the effect of exogenous oxytocin on peripheral oxytocin levels in capuchins and support the role of oxytocin in mediating affiliative behaviors and strengthening social bonds. Specifically, we found that urinary oxytocin concentrations increased 15–60 min after individuals engaged in two affiliative behaviors, grooming and fur-rubbing. In general, our results support the hypothesis that the adaptive function of oxytocin is to strengthen social bonds in primates. However, changes in affiliative behavior after an increase in oxytocin associated with fur-rubbing suggest that oxytocin may have a species and context-specific effect on affiliative behaviors that should be explored to more fully understand its function.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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