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Publication Date

2020-03-01

DOI

10.1016/j.psyneuen.2019.104494

Peer reviewed

HHS Public Access

Author manuscript

Psychoneuroendocrinology. Author manuscript; available in PMC 2021 March 01.

Published in final edited form as:

Psychoneuroendocrinology. 2020 March ; 113: 104494. doi:10.1016/j.psyneuen.2019.104494.

Effects of Chronic Intranasal Oxytocin on Behavior and Cerebral Glucose Uptake in Juvenile Titi Monkeys

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Abstract

Intranasal oxytocin (IN OXT) has been proposed as a treatment for autism spectrum disorder (ASD); however, little is known about the effects of long-term exposure. This is the first study in a non-human primate species to examine how developmental exposure to chronic IN OXT affects juvenile's interactions with family members, social preference for parents versus strangers, anxiety-like behavior, and cerebral glucose metabolism. Titi monkeys are socially monogamous and biparental; their family bonds share important characteristics with human family bonds. Fourteen males and 15 females were treated intranasally with saline (n=14) or 0.8 IU/kg OXT (n=15), daily from 12 to 18 months of age. Compared to SAL-treated animals, OXT-treated animals of both sexes spent significantly more time grooming other family members (F_1=8.97, p=0.006). Overall, OXT-treated subjects were more social (F_1 = 8.35, p = 0.005) during preference tests. OXT-treated females displayed an enhanced preference for their parents (t=2.265, p=0.026). OXT-treated males had a blunted preference for their parents and an increase in the time spent near unfamiliar pairs (F1=10.89, p=0.001). During anxiety tests, OXT-treated males refused to complete the task more often than SAL-treated males and had longer latencies (p< 0.0001). Neuroimaging studies revealed that OXT-treated animals had higher glucose uptake across the social salience network as a whole after one month of treatment ($F_{1,9}$ = 1.07, p = 0.042). Our

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Disclosures

The authors report no conflicts of interest, except for Suma Jacob, who has been an investigator on DOD, NIH, and Roche treatment trials for autism. She has also served on scientific advisory committees for Roche.

results suggest moderate prosocial effects of chronic IN OXT, that did not depend on anxiolytic properties. We also found important sex differences that should be considered in a translational context.

Keywords

Intranasal oxytocin; chronic; social behavior; anxiety; imaging; Autism

1. Introduction

Oxytocin (OXT) is a neurotransmitter involved in the regulation of social and non-social behaviors (Lee et al., 2009). Research efforts for the last several decades have focused on evaluating the effects of intranasal oxytocin (IN OXT) on human behavior and determining its potential as a treatment for psychopathologies characterized by social deficits, such as autism spectrum disorder (ASD) and schizophrenia (Anagnostou et al., 2012; Davis et al., 2013). The potential use of IN OXT as a treatment has become particularly important as there are currently no approved medications to improve social abilities, despite social deficits being considered one of the most debilitating features of these disorders.

Most IN OXT single dose studies in healthy adults have reported beneficial immediate effects, including increased gaze to eye region (Guastella et al., 2008), increased trust towards strangers (Kosfeld et al., 2005), improved empathic accuracy (Bartz et al., 2010), while others have found null results (Tabak et al., 2019) or negative outcomes such as envy (Shamay-Tsoory et al., 2009) and ethnocentrism (Dreu et al., 2011). IN OXT single dose studies in patients with ASD have been shown to temporarily improve social abilities (Andari et al., 2010; Auyeung et al., 2015; Guastella et al., 2010; Hollander et al., 2006). However, other studies using repeated administration of IN OXT have found modest improvements to the core symptoms or only improvements to secondary symptoms of ASD (Anagnostou et al., 2012; Feifel et al., 2012; Tachibana et al., 2013; Watanabe et al., 2015; Yatawara et al., 2016; Yamasue et al., 2018) or no significant improvements (Dadds et al., 2014; Guastella et al., 2015, Munesue and Higashida, 2016).

For many years, no studies of long-term administration of OXT (by any administration route) were available in either human or animal models. More recently, long-term studies in juvenile and young adult rodents and pigs have shown mixed results, including both facilitation and disruption of social behavior (Bales et al., 2014, 2013; Huang et al., 2014; Rault et al., 2013). Therefore, potential use of IN OXT for long-term daily administration (particularly during development) has raised questions about sex-specific and dose dependent effects of IN OXT treatment, as well as about the potential for undesirable side effects on the developing brain.

“Long-term” human studies only typically involve daily treatment for a few weeks to a couple months, rather than the chronic, daily treatment across early life development and adolescence that might be expected in a clinical treatment plan for individuals with ASD. This gap in our knowledge highlights the utility of studying long-term IN OXT treatment in an animal model with more rapid development. Due to the close evolutionary relationship

between primates and humans, we use a non-human primate model in the current study. To our knowledge, this is the first study in a non-human primate species to describe the effects of daily, long-term administration of IN OXT during development on social behavior, brain activity, and reproductive maturation in both sexes.

Our animal model, the coppery titi monkey (*Callicebus cupreus*) is a small, diurnal New World monkey native to the Amazon Basin of South America. Titi monkeys are a socially monogamous species, meaning that adults can form selective long-lasting pair-bonds that share the same characteristics as human attachment (Mason, 1966; Mason and Mendoza, 1998; Mendoza and Mason, 1997). Fathers are the infant's primary care giver and figure of attachment; mothers and siblings also engage in care giving behaviors but for shorter periods of time (Hoffman et al., 1995; Mayeaux et al., 2002). These selective, long-lasting family bonds and the fact that titi monkeys have the same OXT sequence as humans (Lee et al., 2011) make them an ideal non-human primate model to study the effects of IN OXT on social bonding. Like humans, titi monkeys also have OXT receptors (OXTR) and vasopressin 1a receptors (AVPR1a) expressed throughout the "social salience" system; they also have strong expression of OXTR in visual rather than olfactory areas, as rodents do (Freeman et al., 2014; Freeman and Young, 2016). In the current study, we examined regional and global cerebral glucose metabolism using baseline positron emission tomography (PET) scans co-registered with structural magnetic resonance imaging (MRI) after one month of daily IN OXT treatment. We predicted that OXT treated animals will show an increased in [18F]-fluorodeoxyglucose (FDG) uptake in whole brain (based on whole brain glucose increases seen during pair bond formation in this species) (Maninger et al., 2017a), and in brain regions where OXTR and AVPR1a have been found, compared with saline treated (SAL) animals.

We also hypothesized that long-term administration of IN OXT would result in higher levels of sociability by enhancing the innate preference for parents seen in juvenile titi monkeys and accelerating the emergence of responsiveness to strangers (Mayeaux et al., 2002). We followed the reproductive maturation of the juveniles to observe whether or not any changes in social preferences were secondary to pubertal development.

Finally, OXT is involved in the regulation of the HPA axis activity (Neumann, 2002), reduces amygdala activity in humans (Domes et al., 2007; Kirsch et al., 2005; Lancaster et al., 2018) and has been shown to have anxiolytic effects after endogenous release and after exogenous treatment (Carson et al., 2015; De Oliveira et al., 2012). Therefore it is possible that IN OXT may exert its pro-social effect through its anxiolytic and stress-reducing properties (Churchland and Winkielman, 2012; Neumann and Landgraf, 2012). Here, we examined the effects of long-term IN OXT on anxiety-like behavioral responses to a novel non-social stimulus, to help discern to what extent the anxiolytic properties of OXT might influence any changes in social behavior observed after IN OXT administration. In the present study, dosages and age were based on a human clinical trial at the time the study began (Anagnostou et al., 2014) and our previous studies on IN OXT in prairie voles (Bales et al., 2013) and mice (Bales et al., 2014).

2. Materials and methods

2.1 Housing and animal care

All titi monkeys (*Callicebus cupreus*) used in this study were born at the California National Primate Research Center (CNPRC) and housed in indoor home cages measuring 1.2 m × 1.2 m × 2.1 m. Rooms were maintained at 21° Celsius on a 12:12 light dark cycle (lights on at 6:00 am and off at 6:00 pm) for further details of husbandry see Tardif, S. *et al.*(Tardif et al., 2006). Subjects were housed with both parents and any siblings, when applicable. The University of California Davis Institutional Animal Care and Use Committee approved all housing conditions and experimental procedures described in this paper.

2.2 Subjects

All animals born in the colony during the time of the study were included in the sample. Subjects were twenty-nine titi monkeys, OXT= 8 females, 7 males; SAL=7 females, 7 males. Within sex we alternated assignments and treatments, the only exception was when two juveniles were from the same family then they were assigned different treatments. No more than two juveniles were used from the same family. With the purpose of creating additional blinding and avoidance of biased several different color codes were used throughout the study, to represent oxytocin and saline. All dosing, data collection, scoring, and analysis were done while blinded to treatment, and only one person had access to the key.

Sample size was calculated using Monte Carlo simulations based on the proposed analyses for our experiments. Our power analyses (based on the ability to detect an 8% change in glucose uptake) indicated that a sample size of eight subjects per group was sufficient to detect differences between conditions with a power of .82 and a Type I error rate of .05.

2.3 Pharmacological treatment

Subjects received treatments daily between 9:00 and 10:00 am. For the OXT treatment, oxytocin acetate salt (Santa Cruz Biotechnology, Dallas, Texas, USA) was dissolved in saline at a concentration of 0.8 IU/kg of body weight. The treatment was administered using a pipette to drip 25 μl of OXT or SAL in each nostril (50 μl in total). Subjects were habituated and trained using positive reinforcement techniques to all the steps required to receive an intranasal dose before the first day of the treatment. Each animal received either 0.8 IU/kg of IN OXT dissolved in SAL (50 μl), or 50 μl SAL (vehicle control) for six months via intranasal administration starting at 12 months (360 ± 2 days) to 18 months (540 ± 2 days) of age. The age interval encompasses the pubertal period late as well as the late juvenile period (Valeggia et al., 1999). The dosage was roughly equivalent to a weight-adjusted dose used in humans. Specifically, it would be equivalent to a 40 IU dosage given to a 110-lb subject. This dose (0.8 IU/Kg) was used in previous studies in prairie voles (medium dose) and in a mouse autism model (Bales et al., 2014, 2013). Treatments were prepared every month to ensure each monkey received the appropriate dose based on its weight. Aliquots of the treatment solutions were stored in microcentrifuge tubes at −80° Celsius until use.

Subjects received treatments daily between 9:00 and 10:00 am. Subjects were trained to voluntarily enter a transport cage. Doses were kept cold while personnel checked the animal's identification number and wrapped the monkey using a towel. While being held, treatment was administered using a pipette to drip 25 μl of OT or saline in each nostril, alternating nostrils. A thumb was placed individually over each nostril to prevent sneezing out the compound and held for a few seconds until the compound was absorbed into the nasal mucosa. Pieces of banana were given to the monkey as reward. Treatment administration took less than 5 min per animal and took equal amounts of time for SAL and OXT groups.

2.4 Behavior within the family group

After receiving the intranasal treatment (OXT or SAL), subjects were returned to their natal group. Subject's behavior and interactions with family members were video recorded once a month during the period of treatment (12 months to 18 months). They were recorded for 5 min at two time points that were calculated based on the dosing time; the first-time point occurred between 1–15 min (the first-time point occurred within 15 min) after receiving the dose, and the second time point occurred between 45–60 min after dosing. The subjects served as the focal animal, and any social behaviors (contact, tail twine, grooming, being groomed, social play, arousal, approach, leave, carry infant sibling) that were initiated by or directed towards the subject were scored from video recordings, as well as subject's locomotion and arousal behavior (Supplementary table 1).

2.5 Parent preference tests

The parent preference test is a modified version of the partner preference test as standardized for titi monkeys (Carp et al., 2016). The partner preference test measures female-male adult attachment, while the parent preference test measures the attachment that titi monkey offspring have for their fathers. Subjects were tested four times in the parent preference test at 13, 15, 17 and 19 months of age to examine changes in their preference for their natal group and a stranger family during the chronic treatment period (at 13, 15 and 17 months) and after the treatment had ended (month 19). The subject was exposed to two groups of stimulus animals in the test apparatus. The subject's family "Natal group" served as one of the groups and an unfamiliar family—a female-male pair and infant offspring, if present—was used as the "Stranger family group" (Fig. 1).

2.5.1 Test apparatus—The test apparatus consisted of three adjacent metal cages of equal size (2.1m × 1.2m × .8m). The side cages had two parallel wire mesh windows measuring 30 cm × 30 cm. These windows connected the side cages with the center cage. The mesh had square openings of 1.3 cm × 1.3 cm that allowed visual, olfactory, and limited physical contact between the test animal in the center and stimulus animals in the side cages (Fig. 1). The wire mesh windows provided visual access between the stimulus animals when both were located near their respective windows at the same time, also the stimulus animals could observe when the test animal was interacting with stimulus animals from the other cage. Each cage had two perches running the width of the cage, which allowed animals to sit on the perch close to the window. In the center cage the two perches were divided into three equal segments, each 40 cm, and classified as either a preference zone right, left, and neutral

zone (the middle third). All the other areas of the center cage were considered no preference or neutral zones. Further details of the test and testing apparatus are described elsewhere (Carp et al., 2016).

2.5.2 Testing procedure—For this test, stimulus animals were released into one of the side cages and the subject into the center cage. The location of the stimulus groups was counterbalanced with each treatment (OXT and SAL). All test sessions took place in the morning from 10:00 am to 1:15 pm (starting approximately 30–45 min post-dosing during the ages at which the animals were dosed) and animals were provided with food and water throughout.

Each test session consisted of five consecutive 30-min observations with 5-minute breaks between observations, an entire test session lasted approximately 3 hr. Physical location of the subject was live-scored and included time spent in each preference zone (Supplementary table 2). Behavioral data was collected using Behavior Tracker 1.5 (www.behaviortracker.com). Multiple trained observers were validated for live focal animal sampling with greater than 90% observer reliability.

2.5.3 Group of stimulus animals—Different family groups were used as stranger families for each subject. The stranger family groups were chosen based on unfamiliarity to the test animal and its natal group, genetic distance, and similarity to the structure of the subject's natal group. Siblings younger than 6 months joined the parents in the test; siblings older than 6 months stayed in the home cage for the duration of the test, although some exceptions were made for infants that were too distressed by the absence of the parents.

2.6 Novel pattern test

This test determined the level of anxiety-like behavior by examining the reluctance to approach, touch and retrieve a preferred food item when presented in front of a series of novel visual patterns that escalate in complexity. Because titi monkeys are neophobic (Mayeaux and Mason, 1998), we expected them to have longer latencies to retrieve the food item as visual patterns escalate in complexity.

Subjects were tested at three time points, two time points during treatment: 15 and 17 months and one time point after treatment at 19 months of age, with one session per day for four days at each time point. This test examined willingness to retrieve a preferred food item when presented in front of a series of novel visual patterns that escalate in complexity. At 15 and 17 months of age, all test sessions took place between 45 and 60 min after the subject had received the intranasal dose (between 9:00 and 9:45 am). At 19 months of age, when subjects were no longer being dosed, all test sessions took place at an estimated time based on past dosing times (further details in supplementary material). Animals were habituated to the test using a set of blank cards before the first testing session at 15 months of age. Only one habituation session was performed prior to testing at 17 months, and no habituation was needed prior to testing at 19 months.

2.6.1 Testing procedure—Subjects were tested with two different sets of patterns at each time point. Testing was conducted four days in a row, or on two successive days on

consecutive weeks (i.e. Thursday, Friday and Monday, Tuesday). Subjects were tested in their home cages; family members were removed from the home cage for the duration of the test and placed out of view of the subject. A metal basket feeder with the visual patterns (Supplementary table 3) and a small piece of banana (2 cm) was hung at the front of the cage shielded from the subject's view using a white cover card. To start the test, the first card in the basket feeder was uncovered, and two trained observers measured the latency of the subject to approach the basket feeder, to touch the banana or basket feeder, and to retrieve the piece of banana placed next to the pattern card. Then the basket feeder was re-covered, and another piece of banana was placed for the next trial. Each test consisted of six trials, and each trial ended when the subject reached for the piece of banana or when 30 s had elapsed after revealing the basket feeder (time out). A piece of banana was provided for each of the six trials. The first trial was always a blank white card, trials 2–5 were patterns with increased complexity, and the last trial was a blank white card.

2.7 Reproductive hormones

Urine samples were collected from subjects three times per week between 5:45 and 7:00 AM during the entire study to follow reproductive maturation for both treatment groups. Samples were collected from inside the cage using disposable specimen cups, aliquoted and kept frozen at −80°C until hormone analyses were performed. Levels of testosterone were measured in male samples, and estrogen and pregnanediol were measured in female samples.

2.8 Hormone assays

Assays for urinary estrogen conjugate (E1C) and conjugated pregnanediol (PgG) have been established and validated as previously described (Valeggia et al., 1999) using the same primary antisera. Urinary androgen was determined similarly using a polyclonal antisera (R156/7) raised against testosterone. Briefly, urine was diluted in water (E1C, 1:200; PdG, 1:4 for female samples and for urinary androgen in males 1:20). Nunc Maxisorb plates were coated with antiserum (either R522–2 for E1C, R13904 for PdG, both at 1:10,000, and R156/7 for androgen 1:15000 in coating buffer) and washed before addition of standards and sample. The standard curve for E1C ranged from 300–0.6pg/well, for PdG from 10–0.78pg/well and for urinary androgen 1000–1.95 pg/well. Horseradish peroxidase (HRP) conjugated to E1C (1:240,000 dilution), PdG (1:150,000 dilution) and urinary androgen (1:70,000) was added, plates were thoroughly mixed and incubated at 4°C overnight. The following morning, plates were washed 4 times in wash solution, then 100 ul of freshly prepared substrate solution (0.05 M Citrate, pH 4.0, 0.4mM ABTS, 1.6mM H2O2) is added. Plates were read when the average OD of the total binding wells was at an absorbance of 1.0. E1C, PdG and urinary androgen concentrations were expressed after normalization to creatinine concentrations using well-established methods(Taussky, 1954). High medium and low pools were included in all assays and intra- and inter-assay co-efficients of variation are <15% for all assays.

2.9 Imaging study

To characterize brain activity due to OXT intranasal administration during development, subjects underwent a positron emission tomography (PET) scan experiment at 13 months of

age. A structural MRI was performed for each subject within a month of the PET scan, to co-register with the resulting PET images to provide structural information for analysis.

Animals were fasted 10 h prior to the PET scan, with water available throughout the pre-scan period. On the day of the PET scan, the subject was removed from the cage, and manually restrained by trained personal to receive a bolus injection of [18F]-fluorodeoxyglucose (FDG, PETNET Solutions, Sacramento, CA, USA) administered in a volume of <2 ml) into the saphenous vein. Intranasal OXT or SAL was administered immediately after. The subject was returned to their cage with their father for 30 min of conscious uptake. FDG and MRI scans were performed as previously described (Bales et al., 2007; Maninger et al., 2017a, 2017b).

2.9.1 PET and MRI coregistration, quantification of FDG uptake—

The following regions of interest (ROIs) were measured for FDG uptake: anterior cingulate cortex (ACC), lateral septum (LS), caudate nucleus (C), nucleus accumbens (NACC), putamen (PUT); amygdala (AMY), hippocampus (HIPP), paraventricular nucleus of the hypothalamus (PVN), supraoptic nucleus of the hypothalamus (SON). Boundaries for these ROI structures, as well as for the whole brain, were drawn on each subject's MRI image using Siemen's Inveon Research Workplace software (IRW, Siemens Healthcare, USA). For whole brain, the edge of the right and left lobes were outlined through the entire brain, excluding the first and last slices of the MRI where edges were not clearly visible for all subjects. For ROIs anatomical landmarks were established to determine the location (slide) and borders of the ROIs. Static PET images were reconstructed with a 3DRP reconstruction protocol. MRI images were co-registered with PET scan images using the automatic rigid registration algorithm in IRW and checked visually for acceptable registration accuracy. Mean FDG activity for the PET images were determined by applying the ROIs boundaries that were defined on the MRI images to the PET images in IRW.

3. Data analysis

Behavioral data was analyzed by mixed model ANOVA (Littell et al., 1996), with sex, treatment, month, month by treatment and a sex by treatment interaction as fixed variables, and with subject as a random factor in the case of repeated measures on the same animals. For novel pattern data, models included trial, treatment, sex, and a sex by treatment interaction. Glucose uptake was analyzed by multivariate ANOVA, with sex, treatment, and a sex by treatment interaction, followed by two-way ANOVAs for individual regions of interest. Hormonal data was analyzed by ANOVA for females and by growth curve modeling for males (Supplementary figure 1). All tests were two tailed and p-values were considered significant when less than 0.05. Effect size for the significant pairwise comparisons was calculated using Cohen's *d*. When possible, Levene's tests for homogeneity of variance were run for all treatment groups.

4. Results

4.1 Behavior within the family group

Immediately following treatment, interactions with family members in the home cage were observed. These observations occurred once a month during the six months of treatment for each subject, for a total of 10 min divided in 2 time points. The first-time point occurred between 1–15 min after receiving the dose, and the second time point occurred between 45–60 min after dosing. Regardless of treatment, subjects spent more time grooming other family members during the second time point, compared to the first time point (F_1=8.03, p=0.0049, d=0.30; Fig. 2a). Timepoint was not significant for any other behaviors.

There were no sex differences, no sex by treatment interactions, no treatment by time point interactions and no difference in family interactions between months of treatment. When averaging each behavior for the 6 months of treatment, we found that OXT-treated subjects tended to locomote less (F_1 = 3.94, p = 0.058) but spent a similar amount of time in contact with their family members (F_1 = 0.02, p = 0.890; Fig. 2b). OXT-treated subjects approached other animals less often compared to SAL-treated subjects (F_1 = 4.31, p = 0.048, d=0.48; Fig. 2c). However, there was no difference in the frequency with which subjects left contact with other monkeys (F_1 = 2.59, p = 0.120) and no difference in the frequency of approaches by other animals towards OXT- and SAL- treated subjects (F_1 = 1.79, p = 0.193; Fig 2c). While in contact, OXT-treated animals spent significantly more time grooming other family members (F_1 = 8.97, p = 0.006, d=0.22; Fig. 2d). Other animals did not groom OXT-treated subjects more than SAL-treated subjects (F_1 = 1.61, p = 0.216). The number of siblings each subject had in their family group varied between zero and two, however when included into the model, sibling number was not significant for any of the variables.

4.2 Social preference: parents versus strangers

We used the parent preference test to assess total social proximity and preference for the parents over an unfamiliar pair. Subjects were tested multiple times during development (age 13, 15, 17, and 19 months). Considering an average of all tests (ages 13, 15, 17 and 19 months) we found that OXT-treated animals spent more time in total social proximity to stimulus pairs (time in the parent zone and the unfamiliar pair zone combined) compared to saline-treated animals (F_1 = 8.35, p = 0.005, d=0.43; Fig. 3a). Total social proximity changed across months regardless of treatment (F_3 = 10.37, p <0.0001), increasing as animals got older (Fig. 3b).

Time spent in proximity to the parents and to strangers was altered by OXT treatment in a variety of ways and in a sex-specific manner. Across all months, OXT-treated animals spent more time in proximity with their parents than did SAL-treated animals (F_1 = 4.54, p = 0.036). OXT-treated animals also spent significantly more time in proximity to the unfamiliar pair than did SAL-treated animals (F_1 = 4.86, p = 0.030). OXT-treated females spent more time in proximity to their parents, tending to be higher than OXT-treated males (t = 1.770, p = 0.080) and significantly higher than both SAL-treated females (t = 2.265, p = 0.026) and SAL-treated males (t = 2.506, p = 0.014) (Fig. 3c). There was also a significant sex by treatment interaction on proximity to the unfamiliar pairs (F_1 = 10.89, p = 0.001).

OXT-treated males spent significantly more time in the unfamiliar pairs' preference zone than OXT-treated females or SAL-treated animals of either sex (all p's < 0.01) (Fig. 3c).

Considering an average of all tests, parent preference (a summary variable consisting of time in parents' preference zone divided by total time in social proximity) was significantly predicted by sex ($F_1 = 4.56$, $p = 0.036$) and by a sex by treatment interaction ($F_1 = 15.65$, $p = 0.0002$), with a trend for change over month ($F_3 = 2.30$, $p = 0.084$). The parent preference for female OXT-treated animals was significantly higher than female SAL-treated animals ($t = -2.261$, $p = 0.026$), indicating a stronger preference for the parents. The parent preference for OXT-treated males was significantly lower than all other groups (all p's < 0.05), which indicates a weaker preference for the parents. When considering each monthly test separately, we found that the parent preference in both OXT-treated and SAL-treated females did not change significantly between months on treatment (13, 15, 17 months) and the month off treatment (19 month) (Fig. 3d). OXT-treated males at 17 months spent significantly less time in the parent preference zone compared to their previous tests at 13 ($t = -2.28$, $p = 0.026$) and 15 months ($t = -2.17$, $p = 0.033$) where they preferred their parents to a higher degree. However, this effect was lost at 19 months of age (after treatment), with age 17 and 19 months differing significantly ($t = 2.02$, $p = 0.047$; Fig. 3e). SAL-treated males showed a strong preference for their parents over the unfamiliar pair during treatment (13, 15, 17 months), however at their 19 month test, their parent preference was significantly different than in the previous months (all p-values< 0.05) (Fig. 3f).

4.3 Novel pattern testing

Novel pattern testing was used to explore anxiety-like behavior, by examining the subject's reluctance to approach and retrieve a reward (banana) when the reward was placed in front of novel visual patterns of increasing complexity.

4.3.1 Effects of trial number, test day, and age at testing—Trial number (which represents the complexity of the pattern) significantly affected all variables, including latency to arrive at the perch ($F_5 = 11.74$, $p < 0.0001$), the latency to touch the food ($F_5 = 20.63$, $p < 0.0001$), and the latency to take the banana ($F_5 = 23.28$, $p < 0.0001$). Pairwise comparisons showed that in all cases, the two white screen conditions (the trial 1 and trial 6) were not statistically different for any of the variables, (all p's >.05), and trials 4 and 5 were the most complex and had significantly higher latencies compared to the rest of the trials (all p's <.0001) (Fig. 4a). Test day also affected all variables including latency to arrive at the perch (approach) ($F_1 = 8.22$, $p = 0.004$), the latency to touch the food (touch) ($F_1 = 11.79$, $p = 0.0006$), and the latency to retrieve the banana (retrieve) ($F_1 = 13.26$, $p = 0.0003$); these outcome variables were all shorter on the second day of testing. Finally, age at testing affected all variables (perch, $F2 = 35.59$, $p<0.0001$; touch, $F2 = 57.03$, $p<0.0001$; banana, $F_2 = 58.37$, $p<0.0001$); latencies were shorter as animals got older.

4.3.2 Effects of treatment and sex—Balks (time-outs because of refusal to touch the food) were predicted by treatment for males ($\chi^2 = 16.83$, $p <0.0001$) but not for females ($\chi^2 = 2.03$, $p = 0.154$). Males treated with OXT had a higher proportion of balks than SAL-treated males (18.03% of trials vs. 9.12% of trials).

After eliminating trials in which the animals balked, the same pattern of behavior was still evident: OXT-treated animals had longer latencies to arrive at the perch ($F_1 = 10.99$, $p = 0.0009$), touch the food ($F_1 = 26.04$, $p < 0.0001$), and retrieve the banana ($F_1 = 21.72$, $p < 0.0001$). There was no main effect of sex on any variable. However, for each of these variables, there was also a significant sex by treatment interaction: latency to arrive at the perch ($F_1 = 13.33$, $p = 0.0003$), touch the food ($F_1 = 26.75$, $p < 0.0001$), and retrieve the banana ($F_1 = 25.68$, $p < 0.0001$). When directly compared, treatment did not affect female latencies; however, OXT-treated males had higher latencies than SAL-treated males (approach, $t = -4.791$, $p < 0.0001$, $d=0.30$; touch, $t = -7.040$, $p < 0.0001$, $d=0.47$; retrieve, $t = -6.643$, $p < 0.0001$, $d=0.50$) (Fig. 4b and c).

OXT-treated males showed higher latencies compared to SAL-treated males during treatment (15 and 17 months) and after treatment (19 months) for touch and retrieve variables (all p's <.0001; Fig. 4d–f).

4.4 Reproductive hormones

Gonadal hormones did not differ between OXT and SAL treated animals in either sex. Females displayed very low ovarian activity within their natal group, with at most two putative cycles before the age of 2 years. Treatment did not predict the levels of urinary estrogen conjugates (E_1C)($F_1 = 0.38$, $p = 0.546$) or urinary pregnanediol (PdG)($F_1 = 0.05$, $p = 0.831$) or the number of putative cycles ($F_1 = 2.05$, $p = 0.175$) (Supplementary Table 4).

In males, both treatment groups exhibited a non-linear increase in testosterone concentration from age 12 to 24 months (Supplementary Tables 6 and 7 and Supplementary fig. 1). The latent growth curve model where all parameters are constrained to be equal between groups fit best compared to the other five levels of invariance tested (Supplementary table 8). Therefore, we do not have any evidence to suggest that testosterone concentration significantly differed between males treated with OXT and males treated with saline.

4.5 Central glucose uptake

Whole brain glucose uptake did not differ by treatment, sex, or a treatment by sex interaction.

However, OXT-treated animals had higher glucose uptake across the social salience network as a whole, as analyzed by MANOVA (Wilks' lambda = 0.6092, $F_{1,9} = 1.07$, $p = 0.042$), (Fig. 5). Sex and a treatment by sex interaction were not significant.

Because of the significant treatment effect in the multivariate analysis, we followed up with univariate analysis for each region of interest and transformed the data when residuals were non-normal. The supraoptic nucleus (SON) and caudate nucleus (C) displayed non-significant trends with p-values of 0.056 and 0.058, respectively. The treatment effect on glucose uptake in all other individual areas was non-significant with p-values above 0.1.

5. Discussion

The present study addresses an important gap in the current knowledge about IN OXT. Our goals were to provide much needed data on the long-term behavioral and neural effects of chronic IN OXT in different social and non-social contexts in a non-human primate model that has excellent translatability to humans (Bales et al., 2017). Previous studies have shown non-linear dose response curves for intracerebroventricular infusion and IN OXT, divergent acute and chronic effects, and differential long-term effects depending on amount of IN OXT administered (Bales et al., 2012; Guoynes et al., 2018; Huang et al., 2014; Peters et al., 2014). Therefore, one limitation of the results reported here is the use of a single dose; however, we examined acute and chronic effects as well as effects.

Our results suggest that IN OXT increased brain activity across the social salience network after one month of daily treatment as well as social engagement and social behavior during treatment. These effects, although moderate, were not due to a decrease in anxiety or changes in levels of steroid hormones. While these results provide promising evidence for the use of IN OXT as long-term treatment for psychiatric disorders involving social impairments, we found important sex differences and caveats that should be taken into account, such as the increase in anxiety that OXT-treated males displayed during the novel pattern test, which remained consistent during (15 and 17 months) and after treatment (19 months), suggesting that chronic IN OXT treatment effects can persist after treatment ends.

OXT-treated subjects displayed increased grooming behavior towards other family members within one hour of receiving the dose. A study in bats also found an increase in social grooming after repeated administration of IN OXT, with a peak in grooming 30–50 min after OXT exposure (Carter and Wilkinson, 2015). OXT treated subjects also approached other animals less and tended to locomote less. The decrease in approach and locomotion could be secondary to the increase in social grooming, which is a sedentary behavior. However, these behavioral effects would require additional experimentation to untangle. Although these could also be due to differences in energy metabolism, we found no evidence of altered body weight (Supplementary Table 9). OXT- and SAL-treated animals spent similar amounts of time in contact with their family members and broke contact at a similar frequency. IN OXT did not alter the interactions subjects *received* from other family members, such as grooming, approaches, and breaks of contact. Thus, the acute effects of OT treatment on behavior within the natal group appeared to be prosocial but moderate in extent.

When experimentally given the choice between spending time in proximity with their parents or with stranger adults, the typical response of titi monkey offspring is to show a strong preference for their parents. This pattern is seen at 6, 12, 18 and 24 months of age, with responsiveness to strangers slightly increasing at 24 months of age, primarily due to increased antagonism from male offspring towards male strangers (Mayeaux et al., 2002). In the present study, during the parent preference test OXT-treated animals spent more time in the preference zones—a proxy measure of proximity maintenance—of both stimulus pairs (parents and unfamiliar adult female-male pair), compared to SAL-treated animals, which suggests that OXT stimulated overall social behavior. OXT-treated females showed a strong preference for their parents compared to SAL-treated females during treatment. While a

strong preference for parents is expected in juvenile titi monkeys, this result suggests that chronic IN OXT enhanced the innate preference for parents in females. While the time near their parents was not changed by OXT treatment in males, OXT-treated males spent more time near the strangers compared to SAL-treated males, which suggested an accelerated emergence of the interest in strangers.

However, these interactions were primarily friendly, and we saw few signs of antagonism, which is unlike what was reported previously regarding developmental changes in males (Mayeaux et al., 2002). Reduced aggression and increased social exploration towards an unfamiliar animal have also been reported in male rats after repeated administration of IN OXT (Calcagnoli et al., 2015) and in humans, an increased approach toward the stranger, but not towards the friend has been reported after a single dose of IN OXT (24 IU) in adult males (Cohen and Shamay-Tsoory, 2018). It has also been suggested in other contexts that OXT could alter testosterone levels (Gossen et al., 2012; Weisman et al., 2014), however, we found no difference in testosterone levels between OXT- and SAL-treated males. Thus, the interest in an unfamiliar pair seemed to be an increase in positive social approach, rather than an acceleration of sexual interest in the female of the stranger pair.

Another possibility was that OXT-treated animals might be more social because they were less fearful. In other words, IN OXT may exert its pro-social effect through anxiolytic and fear-reducing properties, which would reduce social inhibitions and increase social approach behaviors (Churchland and Winkielman, 2012). The novel pattern test was designed to assess reaction to an anxiety-producing stimulus during and after treatment using a non-social paradigm; this was the first study to use this task in titi monkeys. Titi monkeys are extremely sensitive to novelty (Hennessy et al., 1995) and a similar study was previously done to examining the developmental responsiveness to a novel object (Mayeaux and Mason, 1998), where they found differences in attraction to novel objects were inversely related to age as juveniles approached more objects, more quickly, and spent more time near them than older subjects, who tend to be more conservative, cautious, and restrained. Comparisons across trials supported the claim that the novel patterns were an anxiety-producing stimulus, with trials 4 and 5 (which had the most complex patterns) producing significantly higher approach latencies. Trial 1 (white screen) and Trial 6 (a return to white screen) showed a shorter latency and confirmed that the animals were not satiated by the end of the trial. During the entire study, subjects from both groups were weighed weekly, and no significant differences were found between saline and OXT groups, suggesting that IN OXT treatment had no effect on overall on appetite or weight gain.

OXT-treated animals had longer latencies for each step of the novel pattern task during treatment and one month after treatment: move to the perch, touch the box containing the food and pattern, and take the food reward. A longer latency to approach a novel object (a potential threat) is usually considered a general indicator of anxiety. OXT-treated males in particular refused to complete the task more often than SAL-treated males and had longer latencies for each step of the task, suggesting it made them fearful. These results suggest IN OXT induced an anxiogenic effect in males during treatment and that this effect persisted at least one month after treatment ended. Anxiogenic effects have also been reported in humans after IN OXT exposure (Bartz et al., 2010; Grillon, 2013; MacDonald et al., 2013)

Overall, OXT-treated animals displayed both more pro-social behaviors and more anxiety-like behaviors, suggesting that the prosocial effect of OXT did not depend on anxiolytic properties. It is possible that prolonged exposure to OXT might actually impair its anxiolytic effect. An important aspect of the novel pattern task to consider is that the attachment figure and the rest of the family members except the subject were removed from the home cage to avoid disruptions in the task. Therefore, it is possible that removal of the family was a social stressor that affected the OXT-treated males more profoundly than it did the SAL-treated animals, possibly causing an effect of social context on anxiety. This task has not been pharmacologically validated with anti-anxiety medications, and it is possible that rather than reflecting anxiety-like behavior, it instead reflects another process such as differential attention. Either way, it is a potential cause for concern in that OXT-treated males were either more anxious/vigilant, or altered their attention in a way that could be either deleterious or beneficial.

After one month of daily IN OXT treatment, we examined regional and global cerebral glucose metabolism using baseline positron emission tomography (PET) scans. As predicted, OXT-treated animals had higher [18F]-fluorodeoxyglucose (FDG) uptake across targeted brain areas known to be involved in social behavior (nucleus accumbens, putamen, lateral septum, anterior cingulate cortex, central amygdala, hippocampus, paraventricular nucleus of hypothalamus, supraoptic nucleus). In titi monkeys, OXTR binding has been identified in the lateral septum and hippocampus and AVPR1a in the caudate, putamen, nucleus accumbens, central amygdala and hippocampus (CA4 field) (Freeman et al., 2014). Other studies have also found greater activation in some of these areas (NACC, ACC, right C) after an acute administration of IN OXT in children with ASD a during nonsocial reward anticipation (Greene et al., 2018) and in the NACC during the a social judgment condition (Gordon et al., 2013), suggesting that the NACC may be particularly sensitive to the effects of IN OXT. However, we did not see particularly outstanding increases in any specific area, rather a small increase across the social behavior network. No differences were found in FDG whole brain uptake, which strongly suggests that this change was localized to “social” regions. However, it remains unknown whether this increased uptake persisted during the rest of the treatment or when animals were not actively on treatment.

While these results show moderate promise for the use of IN OXT as treatment for psychiatric disorders that involve social deficits, further research is needed to look at possible long-term effects of chronic exposure. In our prairie vole studies (Bales et al., 2013), negative effects of periadolescent exposure did not manifest until male voles were adults and had been off treatment for some time. In that study, we found that males treated with a similar dose of OXT to that used here, during an analogous developmental period, did not form pair bonds normally as adults. A study in rats showed DNA damage in the hippocampus, a region enriched with oxytocin receptors, after chronic administration of OXT (intraperitoneal) at high and low dosages (Leffa et al., 2017). It will therefore be important to follow up on these results with measures of social behavior, anxiety-like behavior, and brain activity in these same animals as adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We gratefully acknowledge the following for contributions to this project: Rebecca Cotterman, Fang-Shiuan Leung, Charles Smith, Doug Rowland, Rich Larson, Veterinary Staff, Jaleh Janatpour and Kevin Theis, Benjamin Regan, Charlotte McLean, Sarah Carp, and Bales lab volunteers. This research was supported by NICHD grant HD071998, NIH grant OD011107 to the CNPRC, UC Mexus, and the Good Nature Institute.

References

Anagnostou E, Soorya L, Brian J, Dupuis A, Mankad D, Smile S, Jacob S, 2014. Intranasal oxytocin in the treatment of autism spectrum disorders: a review of literature and early safety and efficacy data in youth. Brain Res. 1580, 188–198. [PubMed: 24508578]

Anagnostou E, Soorya L, Chaplin W, Bartz J, Halpern D, Wasserman S, Ting Wang A, Pepa L, Tanel N, Kushki A, Hollander E, 2012. Intranasal oxytocin versus placebo in the treatment of adults with autism spectrum disorders: a randomized controlled trial. Mol. Autism 3, 16. [PubMed: 23216716]

Andari E, Duhamel J-R, Zalla T, Herbrecht E, Leboyer M, Sirigu A, 2010. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proc. Natl. Acad. Sci 107, 4389–4394. [PubMed: 20160081]

Auyeung B, Lombardo MV, Heinrichs M, Chakrabarti B, Sule A, Deakin JB, Bethlehem RAI, Dickens L, Mooney N, Sipple JAN, Thiemann P, Baron-Cohen S, 2015. Oxytocin increases eye contact during a real-time, naturalistic social interaction in males with and without autism. Transl. Psychiatry 5, e507−−6. 10.1038/tp.2014.146 [PubMed: 25668435]

Bales KL, del Razo RA, Conklin QA, Hartman S, Mayer HS, Rogers FD, Simmons TC, Smith LK, Williams A, Williams DR, Witczak LR, Wright EC, 2017. Titi monkeys as a novel non-human primate model for the neurobiology of pair bonding. Yale J. Biol. Med 90.

Bales KL, Mason W. a, Catana C, Cherry SR, Mendoza SP, 2007. Neural correlates of pair-bonding in a monogamous primate. Brain Res. 1184, 245–253. 10.1016/j.brainres.2007.09.087 [PubMed: 17976540]

Bales KL, Perkeybile AM, Conley OG, Lee MH, Guoynes CD, Downing GM, Yun CR, Solomon M, Jacob S, Mendoza SP, 2013. Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles. Biol. Psychiatry 74. 10.1016/j.biopsych.2012.08.025

Bales KL, Perkeybile AM, Conley OG, Lee MH, Guoynes CD, Downing GM, Yun CR, Solomon M, Jacob S, Mendoza SP, 2012. Chronic Intranasal Oxytocin Causes Long-Term Impairments in Partner Preference Formation in Male Prairie Voles. Biol. Psychiatry 1–9. 10.1016/j.biopsych.2012.08.025

Bales KL, Solomon M, Jacob S, Crawley JN, Silverman JL, Larke RH, Sahagun E, Puhger KR, Pride MC, Mendoza SP, 2014. Long-term exposure to intranasal oxytocin in a mouse autism model. Transl. Psychiatry 4. 10.1038/tp.2014.117

Bartz Jennifer A, Zaki J, Bolger N, Hollander E, Ludwig NN, Kolevzon A, Ochsner KN, 2010. Oxytocin Selectively Improves Empathic Accuracy. 10.1177/0956797610383439

Bartz JA, Zaki J, Ochsner KN, Bolger N, Kolevzon A, Ludwig N, Lydon JE, 2010. Effects of oxytocin on recollections of maternal care and closeness. Proc. Natl. Acad. Sci

Calcagnoli F, Kreutzmann JC, de Boer SF, Althaus M, Koolhaas JM, 2015. Acute and repeated intranasal oxytocin administration exerts anti-aggressive and pro-affiliative effects in male rats. Psychoneuroendocrinology 51, 112–121. 10.1016/j.psyneuen.2014.09.019 [PubMed: 25305547]

Carp SB, Rothwell ES, Bourdon A, Freeman SM, Ferrer E, Bales KL, 2016. Development of a partner preference test that differentiates between established pair bonds and other relationships in socially monogamous titi monkeys (Callicebus cupreus). Am. J. Primatol 78. 10.1002/ajp.22450

Carson DS, Berquist SW, Trujillo TH, Garner JP, Hannah SL, Hyde SA, Sumiyoshi RD, Jackson LP, Moss JK, Strehlow MC, Cheshier SH, Partap S, Hardan AY, Parker KJ, 2015. Cerebrospinal fluid

and plasma oxytocin concentrations are positively correlated and negatively predict anxiety in children. Mol. Psychiatry 20, 1085–1090. [PubMed: 25349162]

Carter GG, Wilkinson GS, 2015. Intranasal oxytocin increases social grooming and food sharing in the common vampire bat Desmodus rotundus. Horm. Behav 75, 150–153. 10.1016/j.yhbeh.2015.10.006 [PubMed: 26475061]

Churchland PS, Winkielman P, 2012. Modulating social behavior with oxytocin: how does it work? What does it mean? Horm. Behav 61, 392–399. [PubMed: 22197271]

Cohen D, Shamay-Tsoory SG, 2018. Oxytocin regulates social approach. Soc. Neurosci 13, 680–687. 10.1080/17470919.2017.1418428 [PubMed: 29241395]

Davis MC, Lee J, Horan WP, Clarke AD, McGee MR, Green MF, Marder SR, 2013. Effects of single dose intranasal oxytocin on social cognition in schizophrenia. Schizophr. Res 147, 393–397. 10.1016/j.schres.2013.04.023 [PubMed: 23676253]

De Oliveira DCG, Zuardi AW, Graeff FG, Queiroz RHC, Crippa JAS, 2012. Anxiolytic-like effect of oxytocin in the simulated public speaking test. J. Psychopharmacol 26, 497–505. 10.1177/0269881111400642 [PubMed: 21555332]

Domes G, Heinrichs M, Glascher J, Buchel C, Braus DF, Herpertz SC, 2007. Oxytocin attenuates amygdala responses to emotional faces regardless of valence. Biol.Psychiatry 62, 1187–1190. 10.1016/j.biopsych.2007.03.025 [doi] [PubMed: 17617382]

Dreu C.K.W. De, Greer LL, Kleef G.A. Van, Shalvi S, Handgraaf MJJ, 2011. Oxytocin promotes human ethnocentrism 108. 10.1073/pnas.1015316108

Feifel D, Macdonald K, Cobb P, Minassian A, 2012. Adjunctive intranasal oxytocin improves verbal memory in people with schizophrenia. Schizophr. Res 139, 207–210. [PubMed: 22682705]

Freeman SM, Walum H, Inoue K, Smith AL, Goodman MM, Bales KL, Young LJ, 2014. Neuroanatomical distribution of oxytocin and vasopressin 1a receptors in the socially monogamous coppery titi monkey (Callicebus cupreus). Neuroscience 273. 10.1016/j.neuroscience.2014.04.055

Freeman SM, Young LJ, 2016. Comparative perspectives on oxytocin and vasopressin receptor research in rodents and primates: Translational implications. J. Neuroendocrinol

Gordon I, Vander Wyk BC, Bennett RH, Cordeaux C, Lucas MV, Eilbott JA, Zagoory-Sharon O, Leckman JF, Feldman R, Pelphrey KA, 2013. Oxytocin enhances brain function in children with autism. Proc. Natl. Acad. Sci 110, 20953–20958. 10.1073/pnas.1312857110 [PubMed: 24297883]

Gossen A, Hahn A, Westphal L, Prinz S, Schultz RT, Grunder G, Spreckelmeyer KN, 2012. Oxytocin plasma concentrations after single intranasal oxytocin administration - A study in healthy men. Neuropeptides 46, 211–215. [PubMed: 22884888]

Greene RK, Spanos M, Alderman C, Walsh E, Bizzell J, Mosner MG, Kinard JL, Stuber GD, 2018. The effects of intranasal oxytocin on reward circuitry responses in children with autism spectrum disorder 1–16.

Grillon, 2013. Oxytocin increases anxiety to unpredictable threat (kommentiert Version) 18, 1199–1216. 10.1038/mp.2012.156.Oxytocin

Guastella AJ, Einfeld SL, Gray KM, Rinehart NJ, Tonge BJ, Lambert TJ, Hickie IB, 2010. Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. Biol. Psychiatry 67, 692–694. [PubMed: 19897177]

Guastella AJ, Mitchell PB, Dadds MR, 2008. Oxytocin increases gaze to the eye region of human faces. Biol. Psychiatry 63, 3–5. [PubMed: 17888410]

Guoynes CD, Simmons TC, Downing GM, Jacob S, Solomon M, Bales KL, 2018. Chronic Intranasal Oxytocin has Dose-dependent Effects on Central Oxytocin and Vasopressin Systems in Prairie Voles (Microtus ochrogaster). Neuroscience 369. 10.1016/j.neuroscience.2017.11.037

Hennessy MB, Mendoza SP, Mason WA, Moberg GP, 1995. Endocrine sensitivity to novelty in squirrel monkeys and titi monkeys: species differences in characteristic modes of responding to the environment. Physiol. Behav 57, 331–338. [PubMed: 7716212]

Hoffman KA, Mendoza SP, Hennessy MB, Mason WA, 1995. Responses of infant titi monkeys, Callicebus moloch, to removal of one or both parents: Evidence for paternal attachment. Dev. Psychobiol 28, 399–407. [PubMed: 8557176]

Hollander E, Bartz J, Chaplin W, Phillips A, Sumner J, Soorya L, Anagnostou E, Wasserman S, 2006. Oxytocin increases retention of social cognition in autism. Biol. Psychiatry

Huang H, Michetti C, Busnelli M, Manago F, Sannino S, Scheggia E, Giancardo L, Sona E, Murino V, Chini B, Scattoni ML, Papaleo F, 2014. Chronic and acute intranasal oxytocin produce divergent social effects in mice. Neuropsychopharmacology 39, 1102–1114. [PubMed: 24190025]

Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddanthi S, Gruppe H, Mattay VS, Gallhofer B, Meyer-Lindenberg A, 2005. Oxytocin modulates neural circuitry for social cognition and fear in humans. J. Neurosci 25, 11489–11493. [PubMed: 16339042]

Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E, 2005. Oxytocin increases trust in humans. Nature 435, 673–676. [PubMed: 15931222]

Lancaster K, Goldbeck L, Pournajafi-Nazarloo H, Connelly JJ, Carter CS, Morris JP, 2018. The Role of Endogenous Oxytocin in Anxiolysis: Structural and Functional Correlates. Biol. Psychiatry Cogn. Neurosci. Neuroimaging 3, 618–625. 10.1016/j.bpsc.2017.10.003 [PubMed: 30047477]

Lee AG, Cool DR, Grunwald WCJ, Neal DE, Buckmaster CL, Cheng MY, Hyde SA, Lyons DM, Parker KJ, 2011. A novel form of oxytocin in New World monkeys. Biol. Lett

Lee H-J, Macbeth AH, Pagani JH, Young III WS, 2009. Oxytocin: the great facilitator of life. Prog. Neurobiol 88, 127–151. [PubMed: 19482229]

Leffa DD, Daumann F, Damiani AP, Afonso AC, Santos MA, Pedro TH, Souza RP, Andrade VM, 2017. DNA damage after chronic oxytocin administration in rats: a safety yellow light? Metab. Brain Dis 32, 51–55. 10.1007/s11011-016-9885-z [PubMed: 27488109]

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, 1996. SAS System for Mixed Models. SAS Institute Inc., Cary, NC.

MacDonald K, MacDonald TM, Brüne M, Lamb K, Wilson MP, Golshan S, Feifel D, 2013. Oxytocin and psychotherapy: A pilot study of its physiological, behavioral and subjective effects in males with depression. Psychoneuroendocrinology 38, 2831–2843. 10.1016/j.psyneuen.2013.05.014 [PubMed: 23810433]

Maninger N, Hinde K, Mendoza SP, Mason WA, Larke RH, Ragen BJ, Jarcho MR, Cherry SR, Rowland DJ, Ferrer E, Bales KL, 2017a. Pair bond formation leads to a sustained increase in global cerebral glucose metabolism in monogamous male titi monkeys (Callicebus cupreus). Neuroscience 348. 10.1016/j.neuroscience.2017.02.028

Maninger N, Mendoza SP, Williams DR, Mason WA, Cherry SR, Rowland DJ, Schaefer T, Bales KL, 2017b. Imaging, behavior and endocrine analysis of “jealousy” in a monogamous primate. Front. Ecol. Evol 5. 10.3389/fevo.2017.00119

Mason WA, 1966. Social organization of the South American monkey, Callicebus moloch: a preliminary report. Tulane Stud. Zool 13, 23–28.

Mason WA, Mendoza SP, 1998. Generic aspects of primate attachments: Parents, offspring and mates. Psychoneuroendocrinology 23, 765–778. [PubMed: 9924737]

Mayeaux DJ, Mason WA, 1998. Development of responsiveness to novel objects in the titi monkey, Callicebus moloch. Primates 39, 419–431.

Mayeaux DJ, Mason WA, Mendoza SP, 2002. Developmental changes in responsiveness to parents and unfamiliar adults in a monogamous monkey (Callicebus moloch). Am. J. Primatol 58, 71–89. [PubMed: 12386915]

Mendoza SP, Mason WA, 1997. Attachment relationships in New World primates. Ann. N. Y. Acad. Sci 807, 203–209. [PubMed: 9071352]

Munesue T, Higashida H, 2016. Oxytocin for Male subjects with autism spectrum Disorder and comorbid intellectual Disabilities : a randomized Pilot study 7, 1–11. 10.3389/fpsyt.2016.00002

Neumann ID, 2002. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. Prog. Brain Res 139, 147–162. [PubMed: 12436933]

Neumann ID, Landgraf R, 2012. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci. 35, 649–659. [PubMed: 22974560]

Peters S, Slattery DA, Uschold-schmidt N, Reber SO, Neumann ID, 2014. ScienceDirect Dose-dependent effects of chronic central infusion of oxytocin on anxiety , oxytocin receptor binding and stress-related parameters in mice. Psychoneuroendocrinology 42, 225–236. 10.1016/j.psyneuen.2014.01.021 [PubMed: 24636519]

Rault JL, Carter CS, Garner JP, Marchant-Forde JN, Richert BT, Lay DC, 2013. Repeated intranasal oxytocin administration in early life dysregulates the HPA axis and alters social behavior. Physiol. Behav 112–113, 40–48. 10.1016/j.physbeh.2013.02.007

Shamay-Tsoory SG, Fischer M, Dvash J, Harari H, Perach-Bloom N, Levkovitz Y, 2009. Intranasal administration of oxytocin increases envy and schadenfreude (gloating). Biol. Psychiatry 66, 864–870. [PubMed: 19640508]

Tabak BA, Teed AR, Castle E, Dutcher JM, Meyer ML, Bryan R, Irwin MR, Lieberman MD, Eisenberger NI, 2019. Null results of oxytocin and vasopressin administration across a range of social cognitive and behavioral paradigms: Evidence from a randomized controlled trial. Psychoneuroendocrinology 4530. 10.1016/j.psyneuen.2019.04.019

Tachibana M, Kagitani-Shimono K, Mohri I, Yamamoto T, Sanefuji W, Nakimura A, Oishi M, Kimura T, Onaka T, Ozono K, Taniike M, 2013. Long-term administration of intranasal oxytocin is a safe and promising therapy for early adolescent boys with autism spectrum disorders. J. Child Adolesc. Psychopharmacol 23, 123–127. [PubMed: 23480321]

Tardif S, Bales K, Williams L, Moeller EL, Abbott D, Schultz-Darken N, Mendoza S, Mason W, Bourgeois S, Ruiz J, 2006. Preparing New World monkeys for laboratory research. ILAR J. 47. 10.1093/ilar.47.4.307

Taussky HH, 1954. A microcolorimetric determination of creatine in urine by the Jaffe reaction. J. Biol. Chem 208, 853–861. [PubMed: 13174594]

Valeggia CR, Mendoza SP, Fernandez-Duque E, Mason WA, Lasley BL, 1999. Reproductive biology of female titi monkeys (Callicebus moloch) in captivity. Am. J. Primatol 47, 183–195. [PubMed: 10075433]

Watanabe T, Kuroda M, Kuwabara H, Aoki Y, Iwashiro N, Tatsunobu N, Takao H, Nippashi Y, Kawakubo Y, Kunimatsu A, Kasai K, Yamasue H, 2015. Clinical and neural effects of six-week administration of oxytocin on core symptoms of autism. Brain 138, 3400–3412. 10.1093/brain/awv249 [PubMed: 26336909]

Weisman O, Zagoory-Sharon O, Feldman R, 2014. Oxytocin administration, salivary testosterone, and father-infant social behavior. Prog. Neuro-Psychopharmacology Biol. Psychiatry 49, 47–52. 10.1016/j.pnpbp.2013.11.006

Yatawara CJ, Einfeld SL, Hickie IB, Davenport TA, Guastella AJ, 2016. The effect of oxytocin nasal spray on social interaction deficits observed in young children with autism: A randomized clinical crossover trial. Mol. Psychiatry 21, 1225–1231. 10.1038/mp.2015.162 [PubMed: 26503762]

Highlights

- Chronic use of IN OXT represents a closer approach to its intended use as a life-long treatment for social deficits in ASD.
- Animals treated with IN OXT showed a moderate increase in social behavior during treatment.
- We found important sex differences in the direction of social behaviors when given the choice between parents and strangers.
- IN OXT increased brain activity across the social salience network as a whole after one month of daily treatment.
- OXT-treated males displayed more anxiety-like behavior during an anxiety test, no significant effects were found in females.

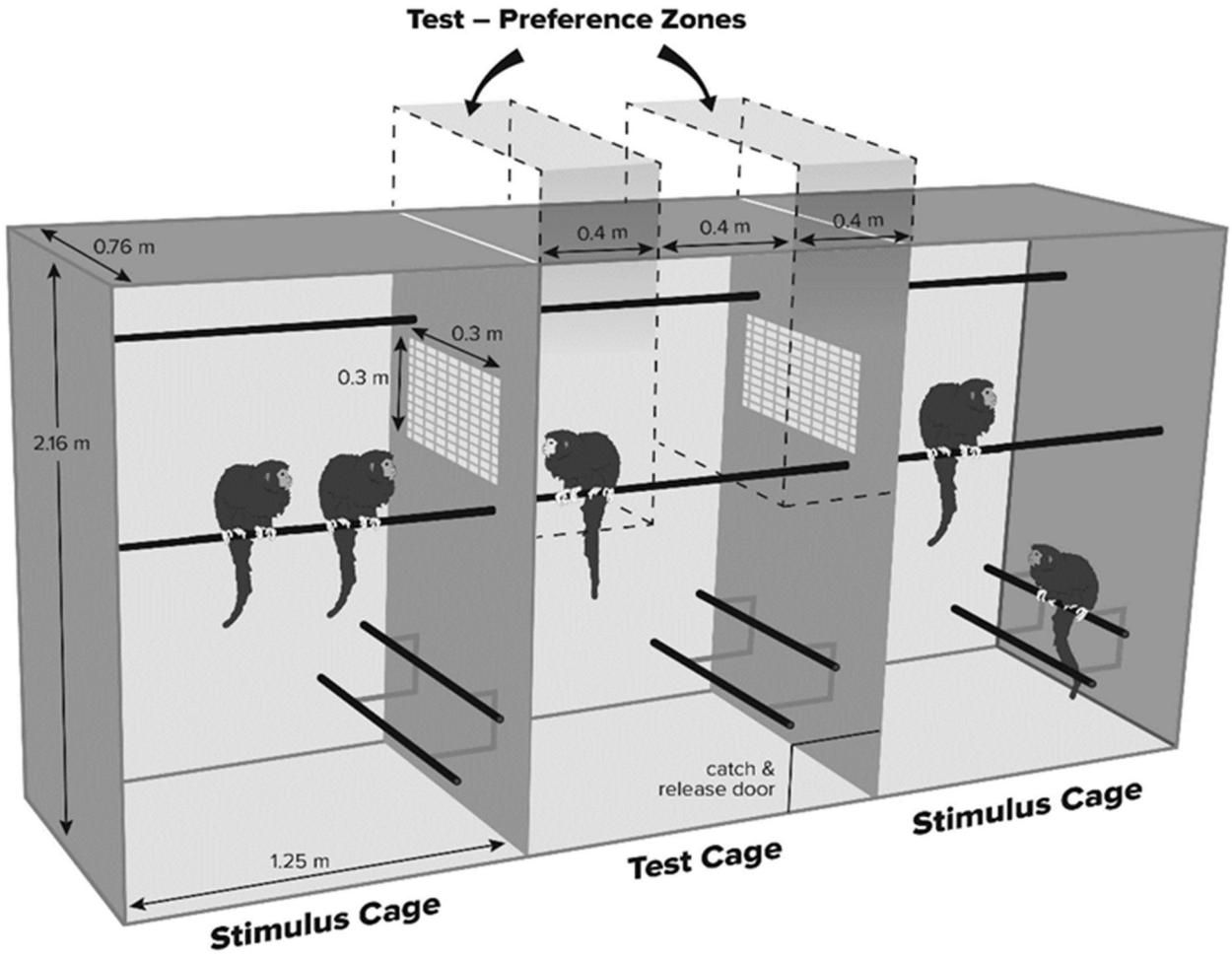

Fig. 1.

Parent Preference Test. The test apparatus consisted of three adjacent metal cages with mesh windows connecting the side cages with the center cage. The stimulus animals were released into one of the side cages and the subject into the center cage. In the center cage, the 2 upper perches were divided into three equal segments and classified as either a preference zone right, left, and neutral zone (the middle third). The physical location of the subject was live-scored and included time spent in each preference zone.

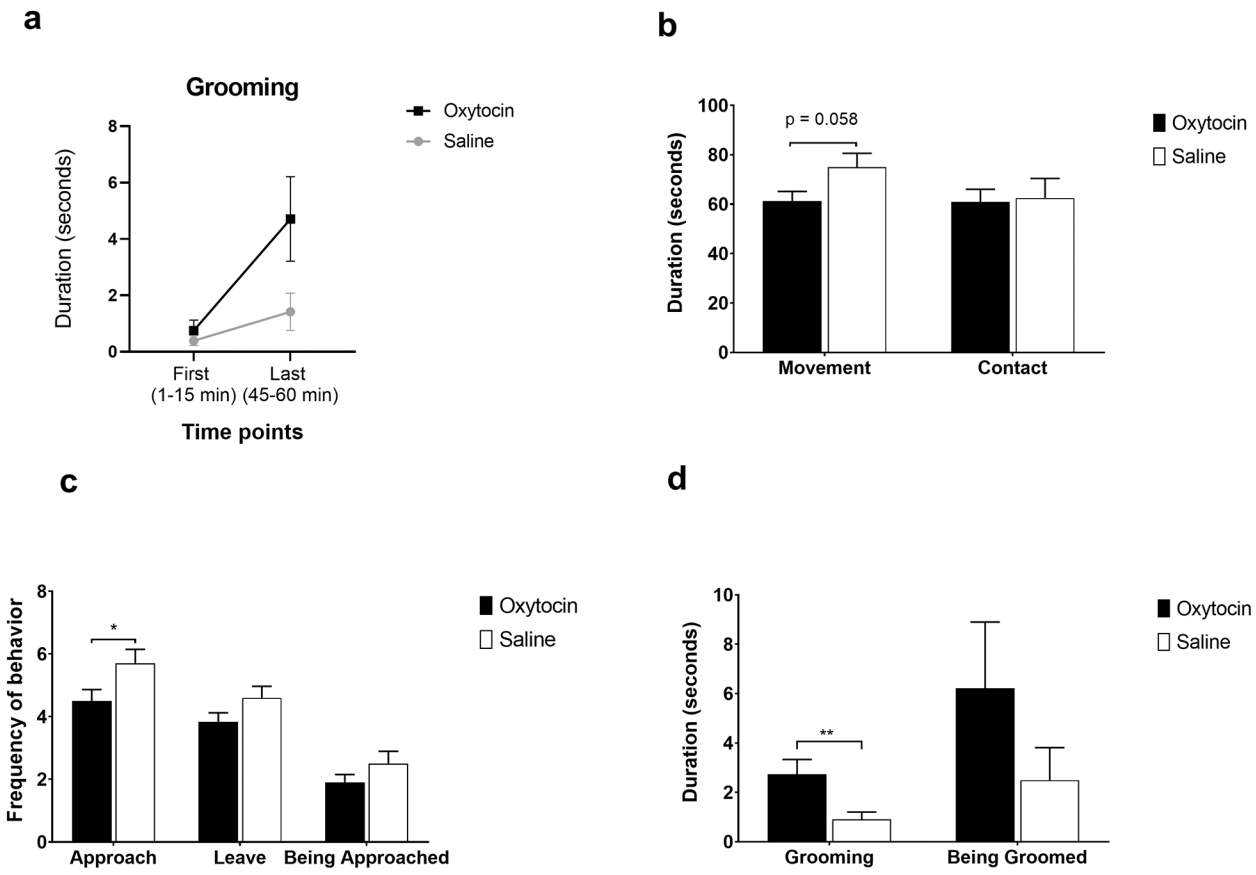

Fig. 2. Behavior within the family group.

Data shows averages for behaviors during the 6 months of treatment. **Fig. 2a.** Subjects spent more time grooming other family members during the second time point, compared to the first time point (F_1=8.03, p=0.0049). **Fig. 2b.** OXT-treated subjects tended to locomote less (F_1 = 3.94, p=0.058) but spent a similar amount of time in contact with their family members (F_1 = 0.02, p = 0.890). **Fig. 2c.** SAL-treated subjects approached other monkeys more than OXT-treated subjects (F_1= 4.31, p=0.048). **Fig. 2d.** OXT-treated subjects spent significantly more time grooming other animals (F_1 = 8.97, p = 0.006); other animals did not groom OXT-treated subjects more than SAL-treated subjects (F_1 = 1.61, p = 0.216). Height of the bars or symbols indicate means; error bars indicate standard errors.

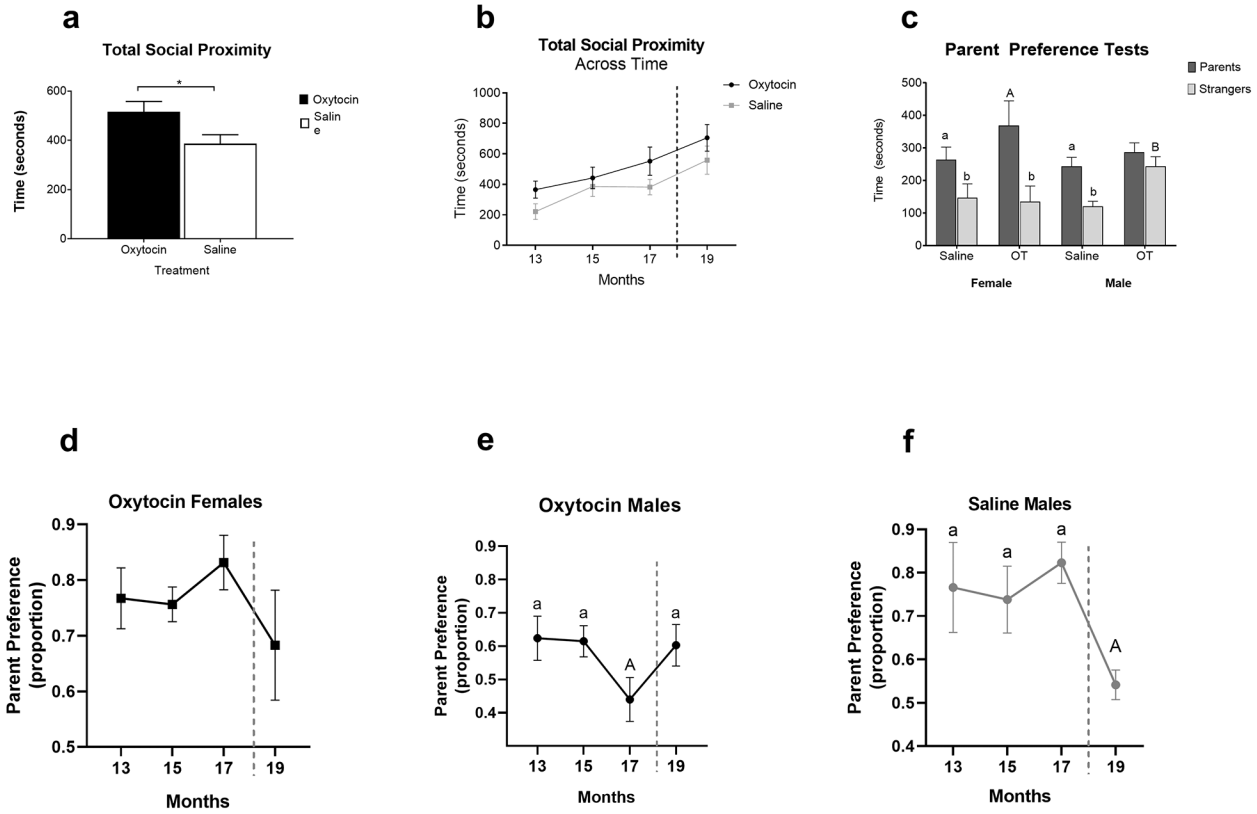

Figure 3. Social proximity during the parent preference tests.

Data shows average for all parent preference tests. **Fig. 3a**. OXT-treated subjects spent more time in total social proximity (parent zone + strange pair zone) during the tests ($F_1 = 8.35$, $p = 0.005$). **Fig. 3b.** Total social proximity increased as animals got older ($F_3 = 10.37$, $p < 0.0001$). **Fig. 3c.** OXT-treated females' time in proximity to their parents tended to be higher than OXT-treated males ($t = 1.770$, $p = 0.080$) and was higher than SAL-treated females ($t = 2.265$, $p = 0.026$) and SAL-treated males ($t = 2.506$, $p = 0.014$). OXT-treated males spent significantly more time in the strange pairs' preference zone than female OXT-treated animals or SAL-treated animals of either sex (all p's < 0.01). **Fig. 3d.** Parent preference in OXT-treated and SAL-treated females did not change significantly across months of treatment. **Fig. 3e**. OXT-treated males at 17 months spent significantly less time in the parent preference zone compared to their previous tests at 13 and 15 months (p's< 0.005). This effect was lost at 19 months of age (after treatment). **Fig. 3f.** SAL-treated males showed a preference for their parents over the unfamiliar pair during treatment (13, 15, 17 months) but not after treatment (19 months). Significant differences are indicated by a capital letter as compared to the bars with the same lower-case letter ($p<0.05$). Height of the bars or symbols indicate means; error bars indicate standard errors.

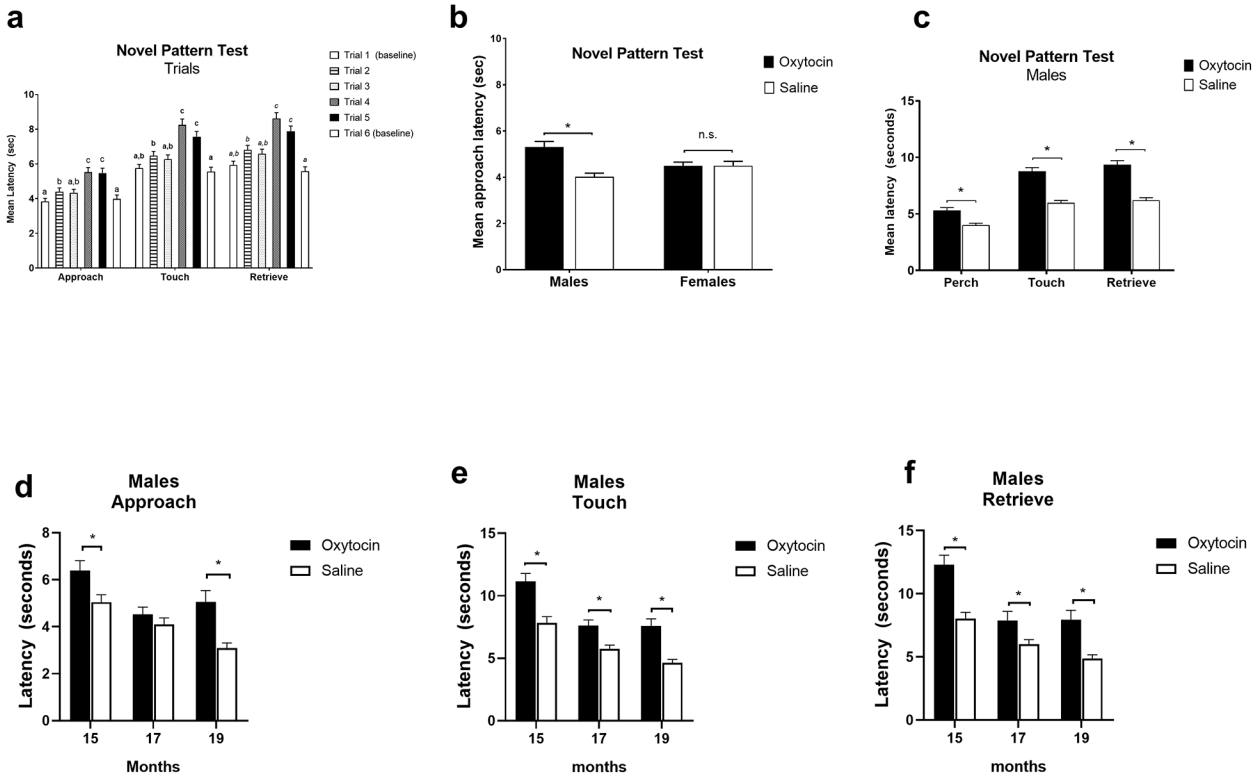

Fig. 4. Novel Pattern Test.

Figures 4a-c show average for all novel pattern tests. **Fig. 4a.** Trial number 1 and 6 are white screen conditions and 2 to 5 represent increasing complexity of the pattern (shown with balks included). Latencies for trials 1 and 6 were not significantly different for any of the variables (all p's >.5), trials 4 and 5 were the most complex and had significantly higher latencies compared to the rest of the trials (all p's <.0001). **Fig. 4b.** OXT treatment did not affect female latencies for any of the variables; however, OXT-treated males had higher latencies than SAL-treated males for all the variables (approach, $t = -4.791$, $p < 0.0001$) (shown with balks excluded). **Fig. 4c.** OXT-treated males had higher latencies than SAL-treated males (approach $t = -4.791$, $p < 0.0001$, $d=0.30$; touch, $t = -7.040$, $p < 0.0001$, $d=0.47$; retrieve, $t = -6.643$, $p < 0.0001$, $d=0.50$) (shown with balks excluded). **Fig. 4d-f.** OXT-treated males showed higher latencies compared to SAL-treated males during treatment (15 and 17 months) and after treatment (19 months) for touch and retrieve variables (* all p's <.0001) (shown with balks excluded). Height of the bars indicate means; error bars indicate standard errors.

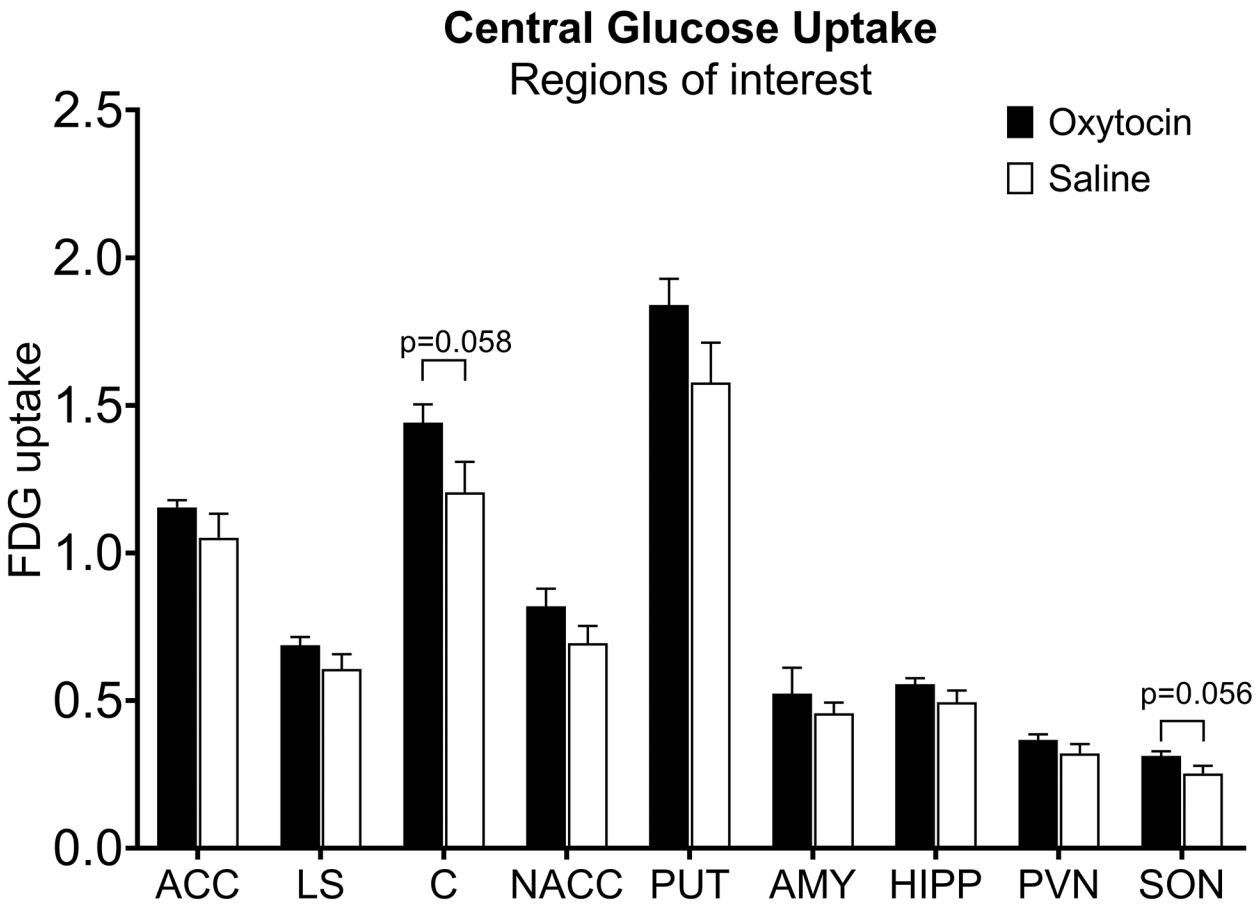

Fig. 5. Central glucose uptake.

OXT-treated animals had higher glucose uptake across targeted brain areas after one month of treatment (Wilks' lambda = 0.6092, $F_{1,9} = 1.07$, p = 0.042). There were non-significant trends for increased uptake in the SON and C. Height of the bars indicate means; error bars indicate standard errors.