Hormones and Behavior 75 (2015) 150–153

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Intranasal oxytocin increases social grooming and food sharing in the common vampire bat *Desmodus rotundus*

Gerald G. Carter *, Gerald S. Wilkinson

Department of Biology, University of Maryland, College Park, MD, USA

ARTICLE INFO

Article history:
Received 18 February 2015
Revised 17 September 2015
Accepted 9 October 2015
Available online 22 October 2015

Keywords:
Allogrooming
Desmodus rotundus
Food sharing
Inhaled oxytocin
Intranasal oxytocin
Vampire bat

ABSTRACT

Intranasal oxytocin (OT) delivery has been used to non-invasively manipulate mammalian cooperative behavior. Such manipulations can potentially provide insight into both shared and species-specific mechanisms underlying cooperation. Vampire bats are remarkable for their high rates of allogrooming and the presence of regurgitated food sharing among adults. We administered intranasal OT to highly familiar captive vampire bats of varying relatedness to test for an effect on allogrooming and food sharing. We found that intranasal OT did not have a detectable effect on food-sharing occurrence, but it did increase the size of regurgitated food donations when controlling for dyad and amount of allogrooming. Intranasal OT in females increased the amount of allogrooming per partner and across all partners per trial, but not the number of partners. We also found that the peak effect of OT treatments occurred 30–50 min after administration, which is consistent with the reported latency for intranasal OT to affect relevant brain areas in rats and mice. Our results suggest that intranasal OT is a potential tool for influencing dyadic cooperative investments, but measuring prior social relationships may be necessary to interpret the results of hormonal manipulations of cooperative behavior and it may be difficult to alter partner choice in vampire bats using intranasal OT alone.

Introduction

Social mammals face decisions regarding when, where, and how much to invest in cooperative behaviors with various partners. These decisions integrate past social experience, depend on an interacting suite of internal and external factors, and often culminate in complex long-term social relationships. To determine the consequences of variation in cooperative investments, one must be able to experimentally manipulate the size of social investments from one animal to another (Carter, 2014). The neuropeptide oxytocin (OT) is one promising tool for this purpose. Peripheral OT administration can experimentally alter the cooperative investments of targeted individuals without highly invasive procedures (e.g. Madden and Clutton-Brock, 2011), and it affects expression of a wide variety of species-specific cooperative behaviors (e.g. humans: Bartz et al., 2011, Veening and Olivier, 2013; macaques: Simpson et al., 2014; marmosets: Smith et al., 2010; meerkats: Madden and Clutton-Brock, 2011; naked mole-rats: Mooney et al., 2014; rats: Calcagnoli et al., 2015; dogs: Romero et al., 2014). Oxytocin manipulation also provides a method for identifying mammalian social behaviors that share a common hormonal mechanism.

We tested for effects of intranasal OT on food sharing and allogrooming in the highly social common vampire bat (*Desmodus rotundus*). These bats feed only on a single meal of blood per night, can starve in less than 72 h, and often fail to obtain meals (18% of 598 bats failed to feed on a given night), but bats that fail to feed often solicit regurgitations of blood from familiar conspecifics (Wilkinson, 1984). Common vampire bats form stable and symmetrical networks of regurgitated food sharing and allogrooming, both in the wild (Wilkinson, 1984, 1985) and in captivity (Carter and Wilkinson, 2013a, 2013b). Even under conditions of frequent roost switching, free-ranging female bats are known to associate for more than a decade (Wilkinson, 1985). Allogrooming and food sharing rates are correlated across dyads and over time (Carter and Wilkinson, 2013a). Allogrooming is more common in this species than in some other bats (Wilkinson, 1986, Carter & Leffer, 2015) and often occurs immediately before food sharing (Wilkinson, 1986), suggesting that allogrooming may serve in facilitating social recognition, assessing ability to give, or signaling intention to share or receive (i.e. begging; Wilkinson, 1986, Carter and Wilkinson, 2013a). Using familiar captive vampire bats, we asked the following questions. Does intranasal OT increase allogrooming and food sharing? And if so, does OT increase cooperative investments with established sharing and grooming partners, broaden investments to more partners, or promote increased investments to fewer partners?

* Corresponding author.
E-mail address: gerry@socialbat.org (G.G. Carter).

http://dx.doi.org/10.1016/j.yhbeh.2015.10.006
0018-506X/

Methods

Food sharing

We tested if OT affects food sharing in four female and one male common vampire bats on 39 trial days from Sept. 17, 2013 to Dec. 16, 2013 (Supplemental Table S1). We housed these five bats together on a 12/12 h partly lit/dark cycle at 25–28 °C and >40% humidity in a 1.7 × 2.1 × 2.3 m flight cage that allowed them to freely associate. These adults (age 4–13 years) were born at one of two zoos and had been housed together previously for several years.

We prepared OT treatments by mixing OT (Bachem, USA) into saline solution at a concentration of 0.45 μg/μl and treated bats by slowly pipetting 5 μl of solution into each nostril of a bat with a micropipettor (10 μl total) allowing 5–15 s between each intranasal administration. If bats sneezed during the treatment, we administered another 5 μl of solution (15 μl total pipetted). Given the range of bat mass (27–35 g) this resulted in a possible dose range of 0.13—0.25 μg/g. We chose this dose to be comparable to a previous intranasal OT dosage (0.15 μg/g) that elicited behavioral responses in 300 g marmosets (Smith et al., 2010).

For each trial day, we first removed and fasted a subject bat while the other four bats were fed blood ad libitum in a 1.7 × 2.1 × 2.3 m cage for 24 h. We then randomly treated two fed bats with OT and two fed bats with saline, then returned the fasted bat to the four treated fed bats in their home cage, and observed interactions for 2 h with a Nightshot camera (DCR-SR85, Sony, USA) and infrared spotlight (IRlamp6, Wildlife Engineering, USA). For each min, we scored the presence (>5 s) or absence of mouth-licking and allogrooming (defined as one bat licking, chewing, sniffing, or nuzzling another bat's body). We measured the subject's mass to the nearest 0.01 g (model L125 digital scale, Escali, Burnsville, MN, USA) immediately before and after observation. We defined "donation size" as the number of minutes with the presence of mouth-licking between two bats in a trial that led to subsequent mass gain in the fasted subject.

Subjects were not fasted again until all other bats served as subjects, but otherwise bats chosen for fasting or OT treatment were selected at random. We stopped our experiment at 35 trials due to suspected illness in the one of the bats. This led to each bat serving an unequal number of times as a fasted subject (7, 7, 5, 8, 8) or potential donor treated with saline/OT (13/15, 13/15, 14/16, 15/12, and 14/13). We estimated pairwise kinship among bats from genotypes of 19 polymorphic microsatellite markers as described in Carter & Wilkinson (in review).

The distributions of donation size and allogrooming both deviated from normality with a positive skew (Shapiro Wilk's $W = 0.84$ and 0.82, $p < 0.0001$). Therefore, we natural log (ln)-transformed both donation size and allogrooming duration so that neither deviated significantly from normality (Shapiro Wilk's $W = 0.98$ and 0.95, $p > 0.05$). We used Chi-square tests in JMP 12 (SAS Institute Inc., 2014) to test the effect of subject, donor, and treatment on the presence or absence of a donation across all opportunities. For all observed donations, we tested which factors (allogrooming, treatment, dyad, kinship, and the interaction between kinship and treatment) predicted donation size using minimum AIC and backward stepwise regression for model selection in JMP 12. Our best model for donation size included treatment and allogrooming (fixed factors), and dyad (random factor).

Allogrooming

To determine if intranasal OT influences allogrooming, we conducted a double-blind study where 13 adult females were treated with intranasal OT or saline at the same hour on two consecutive days. We housed these bats together in a 1.5 × 2 × 3 m home cage with 22 adult males, one other adult female, and one juvenile male (under the same captive conditions described above). Treatment with OT (1 μg/μl) or saline solutions was randomly scheduled and labeled with numbers to conceal identity during application. Between 1600–2100 h, we intranasally administered a bat with 15 μl of either saline or OT solution, or 20 μl if the bat sneezed. Given the range of bat weights (28–40 g), this resulted in a possible dose range of 0.38–0.71 μg/g. We used a higher concentration and dose to increase the effect size, and to make our results comparable with those in mice (0.4–0.6 μg/g in 20–27 g mice; Neumann et al., 2013). For intranasal pipetting methods, see food sharing section above. Immediately after intranasal administration, we returned the subject to the home cage, and recorded interactions for 1 h with a Sony Nightshot camera and infrared spotlight (described above). For each min, we scored the presence (>5 s) of allogrooming (defined above), the identity of the allogrooming partner, and the presence (>5 s) of physical contact with others without allogrooming. The same bat received the opposite treatment at the same time on the next day.

We focused on female allogrooming, because most allogrooming in the wild occurs among adult females and juveniles of both sexes (Wilkinson, 1986) and many of the males had been castrated. In pilot trials, we also failed to detect an effect of oxytocin on young males isolated with their mothers (see Supplementary Information). We used Wilcoxon Signed Rank tests (and report the statistic, S) to compare the effect of treatment on each response paired within bat. We report effect size using Cohen's d.

As above, we estimated pairwise kinship from genotypes of 19 polymorphic microsatellite markers and from maternal pedigree (Carter & Wilkinson, in review). To test if oxytocin influenced the extent to which bats preferentially groomed genetic kin, we first multiplied the duration of allogrooming by the recipient's kinship, and then averaged those values across partners in that trial to calculate a nepotism index for each trial. A greater nepotism index indicates that more allogrooming was targeted towards kin. For all cases where allogrooming partners in both trials were identified, we compared the nepotism index by treatment.

All procedures involving animals adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the University of Maryland Institutional Animal Care and Use Committee (Protocol R-13-30).

Results

Donation occurrence

Food sharing was evident in fasting trials because total mouth-licking time predicted a recipient's subsequent weight gain ($R^2 = 0.63$, $p < 0.001$). However, food sharing occurred in only 16 of 35 fasting trials (36 donations out of 140 possible dyad-trial cases). We detected no effect of OT treatment on the presence of food donations, which occurred 18 times in each treatment condition. The occurrence of food sharing instead varied largely by subject ($\chi^2 = 39.84$, $p < 0.0001$), donor ($\chi^2 = 14.25$, $p < 0.0065$), and subject–donor dyad ($\chi^2 = 62.68$, $p < 0.0001$). Pairwise kinship ranged from 0 to 0.44 and was not strongly linked to occurrence of food sharing (t-test, $t = 1.67$, $df = 18$, $p = 0.11$; Supplemental Table S1).

Donation size

For the 36 confirmed donations, we found that donation size increased with OT treatment ($R^2 = 0.61$, $F_{1,32.8} = 10.78$, $p = 0.0024$; least squares means ± standard error: saline treatment = 0.97 ± 0.23, OT treatment = 1.97 ± 0.27) and allogrooming (ln-transformed; $F_{1,21.4} = 54.24$, $p < 0.001$). These treatment effects were only detectable when controlling for dyad (Fig. 1) and allogrooming (Fig. 2). Kinship did not increase donation size (ln-transformed, $R^2 = 0.002$; $F_{1,34} = 0.074$, $p = 0.79$), and when we included kinship and the kinship by treatment interaction in our model, neither factor was significant.

Allogrooming

OT treatment of females did not change the number of groomers ($S = 2.6$, $p = 0.22$), the number of grooming recipients ($S = 0.5$,

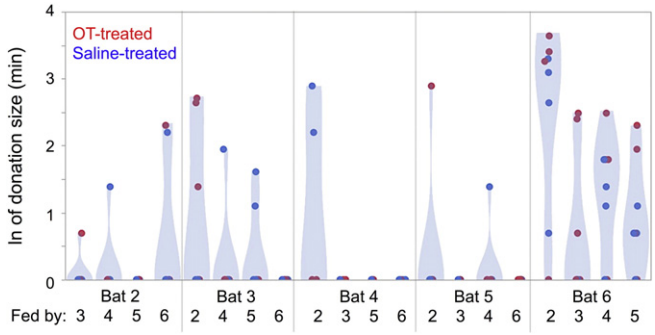

Fig. 1. Donation sizes across all possible dyads. Points show the number of trial minutes that had food sharing (ln-transformed). Probability density functions of donation size are shown in light blue. Food donations occurred independently of treatment (oxytocin: red, n = 18; saline: blue, n = 18).

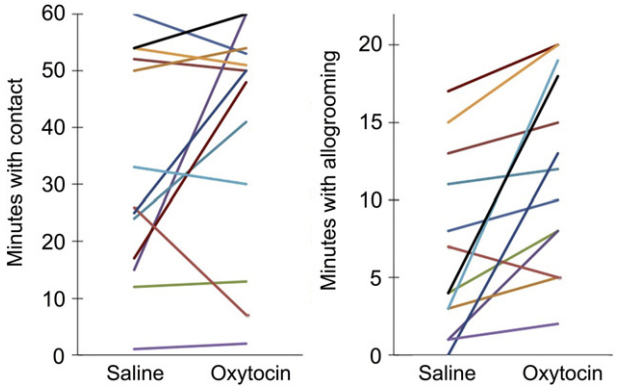

Fig. 3. Oxytocin increases allogrooming. Lines show changes in time spent in physical contact without allogrooming (A) or with allogrooming (B) in female vampire bats treated with either intranasal saline or oxytocin. The effect size was large (95% confidence interval of difference = 1.8–8.6 min samples; Cohen's d = 0.88).

p = 0.48), or the amount of physical contact (S = 15.5, p = 0.30, Fig. 3A), but it did increase the number of minutes a treated animal engaged in allogrooming overall (S = 41, n = 13, p = 0.002, Fig. 3B) and per partner (S = 5.7, p = 0.034). By analyzing the number of grooming bats and the effect at each trial minute, we found that the peak effect of OT treatments occurred 30–50 min after administration (Fig. 4).

There were 8 subjects with known grooming partners in both treatments. In these cases, we failed to detect an OT effect on the nepotism index (paired t-test, t = 0.98, df = 7, p = 0.36). Most grooming occurred between unrelated bats (55% of grooming dyads in trials had estimated kinship values of <0.05, mean kinship = 0.135), and there was only one dependent pup present at the time. For the five females that groomed this pup, there was a trend towards more grooming when treated with OT (paired t = 2.59, n = 5, one-sided p = 0.031; differences in number of grooming observations of OT − saline treatments: −1, +3, +5, +9, +10).

Discussion

Oxytocin (OT) increased two kinds of cooperative investments within dyads. Over a period of three months, we tracked food-sharing donations among five vampire bats (20 dyads), and found that OT treatment increased donation sizes within dyads, but did not affect the probability of a given dyad to share food. Our second study showed that OT increased female allogrooming within dyads, but did not alter the number of grooming partners. Allogrooming in female vampire bats was elevated 30–50 min after intranasal OT exposure (Fig. 4), which is consistent with studies on rats and mice showing that intranasal OT

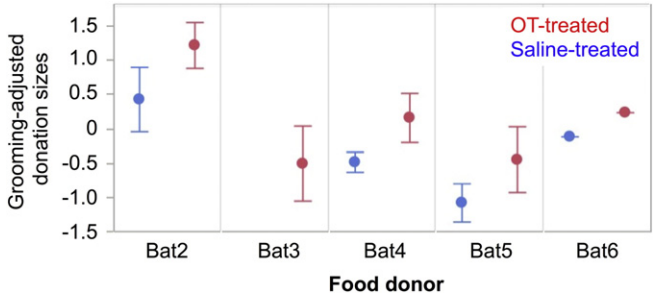

Fig. 2. Mean donation sizes (controlling for grooming) when donors were treated with oxytocin (red) or saline (blue). Y-axis shows the residuals obtained from regressing ln (donation size) against ln (allogrooming), to control for the amount of allogrooming per trial. The effect size of oxytocin on ln donation size (Cohen's d = 0.70) was larger after controlling for allogrooming (Cohen's d = 0.85). See text for full model results. Error bars show one standard error. All five bats donated more when treated with oxytocin. Bat3 only donated when treated with oxytocin.

administration first increases plasma levels, and then within the next 30 min leads to increased OT in behaviorally relevant brain areas (Neumann et al., 2013).

The positive effect of OT on food donation size was only detectable after accounting for dyad-specific variation in food sharing, and OT treatment did not increase the probability of food sharing across dyads. Likewise, OT increased allogrooming per partner, but it did not increase the number of grooming partners. Intranasal oxytocin manipulations therefore seem more likely to influence the strength of cooperative investments rather than extend an individual bat's investments to new partners or limit social investments to fewer partners.

Increasing evidence suggests that the link between cooperative behavior and OT depends on the subject's prior relationship with the partner, exaggerating pre-existing social predispositions to particular individuals or categories of individuals (Crockford et al., 2014). The link between prior social bonds and OT response is complicated by the fact that OT can induce cooperative behavior but can also be released by it (Crockford et al., 2014). When OT-induced behaviors or cognitive states lead to further OT release, the result is a positive feedback loop that may be important in the development of social bonds. Interpreting the relationship between oxytocin and behavior can therefore also be limited by lack of knowledge of prior social relationships. For example, elevated urinary OT in wild chimpanzees is caused by both the giving and receiving of allogrooming and food sharing, but the effect of allogrooming on subsequent urinary OT depends on the strength of the existing social bond (Crockford et al., 2013, Wittig et al., 2014). Given this complex interaction between OT and social experience, much can be learned by further studies pairing OT manipulation with long-term observations of marked individuals with known social histories, social bonds, and kinship relationships.

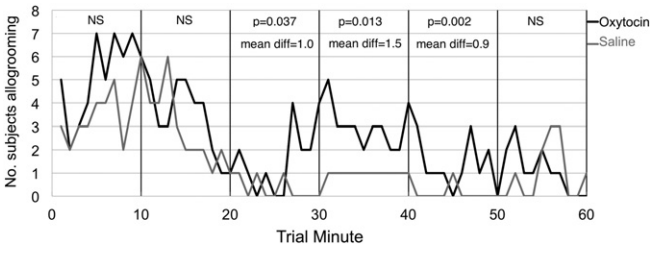

Fig. 4. Latency of effect of intranasal oxytocin on allogrooming. Lines plot the number of subject bats (across all trials) that groomed others during that minute of the trial, when subjects were treated with oxytocin (black) or saline (gray). For each 10-min segment, the statistical significance (two-tailed p-value or NS) and difference between bat-centered treatment means is shown. Time zero marks the treated bat's release into the cage, and grooming rates are greatest in the period soon after.

Acknowledgments

We thank K. Thompson, the editor, and two anonymous reviewers for suggestions that improved the manuscript, L. Leffer for help with data collection, and the Organization for Bat Conservation for animal care. This work was supported by a National Science Foundation Doctoral Dissertation Improvement grant (IOS-1311336) to GSW and GGC.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh.2015.10.006.

References

Bartz, J.A., Zaki, J., Bolger, N., Ochsner, K.N., 2011. Social effects of oxytocin in humans: context and person matter. Trends Cogn. Sci. 15, 301–309.

Calcagnoli, F., Kreutzmann, J.C., de Boer, S.F., Althaus, M., Koolhaas, J.M., 2015. Acute and repeated intranasal oxytocin administration exerts anti-aggressive and pro-affiliative effects in male rats. Psychoneuroendocrinology 51, 112–121.

Carter, G., 2014. The reciprocity controversy. Anim. Behav. Cogn. 1, 368–386. http://dx.doi.org/10.12966/abc.08.11.2014.

Carter, G., Leffer, L., 2015. Social grooming in bats: are vampire bats exceptional? PLoS One 10, e0138430. http://dx.doi.org/10.1371/journal.pone.0138430.

Carter, G., Wilkinson, G., 2015. Social benefits of non-kin food sharing by female vampire bats. Proc. R. Soc. B (in review).

Carter, G., Wilkinson, G., 2013b. Does food sharing in vampire bats demonstrate reciprocity? Commun. Integr. Biol. 6, e25783. http://dx.doi.org/10.4161/cib.25783.

Carter, G.G., Wilkinson, G.S., 2013a. Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. Proc. R. Soc. B 280, 20122573. http://dx.doi.org/10.1098/rspb.2012.2573.

Crockford, C., Deschner, T., Ziegler, T.E., Wittig, R.M., 2014. Endogenous peripheral oxytocin measures can give insight into the dynamics of social relationships: a review. Front. Behav. Neurosci. 8, 68.

Crockford, C., Wittig, R.M., Langergraber, K., Ziegler, T.E., Zuberbühler, K., Deschner, T., 2013. Urinary oxytocin and social bonding in related and unrelated wild chimpanzees. Proc. R. Soc. B 280, 20122765.

Madden, J.R., Clutton-Brock, T.H., 2011. Experimental peripheral administration of oxytocin elevates a suite of cooperative behaviors in a wild social mammal. Proc. Natl. Acad. Sci. U. S. A. 278, 1189–1194.

Mooney, S.J., Douglas, N.R., Holmes, M.M., 2014. Peripheral administration of oxytocin increases social affiliation in the naked mole-rat *Heterocephalus glaber*. Horm. Behav. 65, 380–385.

Neumann, I.D., Maloumby, R., Beiderbeck, D.I., Lukas, M., Landgraf, R., 2013. Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. Psychoneuroendocrinology 38, 1985–1993. http://dx.doi.org/10.1016/j.psyneuen.2013.03.003.

Romero, T., Nagasawa, M., Mogi, K., Hasegawa, T., Kikusui, T., 2014. Oxytocin promotes social bonding in dogs. Proc. Natl. Acad. Sci. U. S. A. 111, 9085–9090.

SAS Institute Inc., 2014. Jmp® 12. SAS Institute Inc., Cary, NC.

Simpson, E.A., Sclafani, V., Paukner, A., Hamel, A.F., Novak, M.A., Meyer, J.S., Suomi, S.J., Ferrari, P.F., 2014. Inhaled oxytocin increases positive social behaviors in newborn macaques. Proc. Natl. Acad. Sci. U. S. A. 111, 6922–6927.

Smith, A.S., Ågmo, A., Birnie, A.K., French, J.A., 2010. Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, *Callithrix penicillata*. Horm. Behav. 57, 255–262.

Veening, J.G., Olivier, B., 2013. Intranasal administration of oxytocin: behavioral and clinical effects, a review. Neurosci. Biobehav. Rev. 37, 1445–1465. http://dx.doi.org/10.1016/j.neubiorev.2013.04.012.

Wilkinson, G.S., 1984. Reciprocal food sharing in the vampire bat. Nature 308, 181–184.

Wilkinson, G.S., 1985. The social organization of the common vampire bat. I. Pattern and cause of association. Behav. Ecol. Sociobiol. 17, 111–121.

Wilkinson, G.S., 1986. Social grooming in the common vampire bat, *Desmodus rotundus*. Anim. Behav. 34, 1880–1889.

Wittig, R.M., Crockford, C., Deschner, T., Langergraber, K.E., Ziegler, T.E., Zuberbühler, K., 2014. Food sharing is linked to urinary oxytocin levels and bonding in related and unrelated wild chimpanzees. Proc. R. Soc. B 281 (1778), 20133096.