Chronic Intranasal Oxytocin Causes Long-Term Impairments in Partner Preference Formation in Male Prairie Voles

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Background: Oxytocin (OT) is a hormone shown to be involved in social bonding in animal models. Intranasal OT is currently in clinical trials for use in disorders such as autism and schizophrenia. We examined long-term effects of intranasal OT given developmentally in the prairie vole (*Microtus ochrogaster*), a socially monogamous rodent, often used as an animal model to screen drugs that have therapeutic potential for social disorders.

Methods: We treated voles with one of three dosages of intranasal OT, or saline, from day 21 (weaning) through day 42 (sexual maturity). We examined both social behavior immediately following administration, as well as long-term changes in social and anxiety behavior after treatment ceased. Group sizes varied from 8 to 15 voles ($n = 89$ voles total).

Results: Treatment with OT resulted in acute increases in social behavior in male voles with familiar partners, as seen in humans. However, long-term developmental treatment with low doses of intranasal OT resulted in a deficit in partner preference behavior (a reduction of contact with a familiar opposite-sex partner, used to index pair-bond formation) by male voles.

Conclusions: Long-term developmental treatment with OT may show results different to those predicted by short-term studies, as well as significant sex differences and dosage effects. Further animal study is crucial to determining safe and effective strategies for use of chronic intranasal OT, especially during development.

Key Words: Autism, intranasal, oxytocin, schizophrenia, social behavior, vasopressin

Oxytocin (OT), a neuropeptide hormone found exclusively in mammals, is associated with maternal behavior (1,2) and adult pair-bond formation (partner preference) behavior (3,4) in rodents. Born *et al.* (5) showed that many neuropeptides crossed the blood-brain barrier when given intranasally. Although Born *et al.* (5) did not actually examine OT, this study has led to an expansion of studies examining intranasal OT actions on human social behavior. Generally, in healthy subjects, prosocial feelings, including generosity and trust (6–8), social communication and emotional recognition (9,10), self-perception (11), and social interactions with offspring are altered by OT (12).

Intranasal OT, currently the subject of multiple clinical trials (clinicaltrials.gov), has been identified as a treatment for developmental disorders involving social dysfunction, including autism spectrum disorders (13), social anxiety (14), and schizophrenia (15). In individuals with autism, intranasal OT was shown to increase emotion recognition (13) and to increase feelings of trust and willingness to interact socially (16). Acute OT administration reveals few safety concerns (17). However, no studies have examined long-term effects of intranasal OT exposure. With sustained stimulation, OT receptors can undergo desensitization and internalization (18) leading to physiological tolerance. In other words, it is possible that long-term exposure, especially during development, may lead to different effects than those predicted by short-term results. Given that OT is not a controlled substance, is in clinical trials, and is already being prescribed off-label by health practitioners in the United States (written communication to K.L.B., April 25, 2011), animal studies of long-term effects are overdue and should be pursued in a coordinated strategy with human studies.

In addition, few human intranasal OT studies have examined dose-response curves. Oxytocin (like other peptides) can produce opposing effects at different dosages (19–22). In schizophrenic patients, intranasal OT increased emotion recognition at one dose (20 IU) and decreased emotion recognition at a different dose (10 IU) (23). In Fragile X patients, one dose (24 IU) increased eye gaze but did not affect cortisol, while a higher dose (48 IU) affected cortisol but not eye gaze (14). In voles, we found a single intraperitoneal OT administration on postnatal day 1 led to long-term effects on partner preference in both male and female prairie voles, which differed depending on dose (19,20).

The prairie vole (*Microtus ochrogaster*), a socially monogamous rodent native to the American Midwest (24), is the premier animal model for the neurobiology of social bonding (25) and increasingly used to screen drugs that have therapeutic potential for social disorders such as autism (26,27). Pair-bond formation is a social cognitive process that involves both social recognition and social reward (27–29) and models a human attachment relationship far more closely than do the social interactions of adult mice or rats. Prairie voles are evolutionarily adapted for this type of social behavior and therefore have neural substrates for social bonding that nonmonogamous species might lack.

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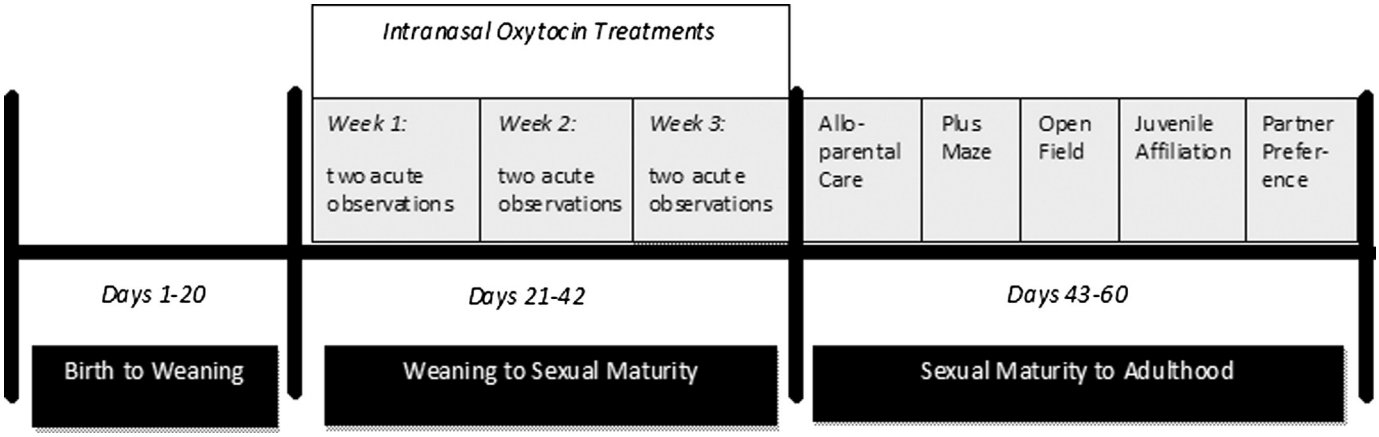

Figure 1. Timeline of study procedures.

In prairie voles, OT has been shown to have extensive sex-specific effects, although in both sexes it is intimately involved in social behavior. Oxytocin is primarily responsible for pair-bonding in female voles, with the related peptide arginine vasopressin (AVP) responsible for pair-bonding in male voles (3,30–33). Adult male voles are also responsive to OT but require higher dosages than female voles to induce a partner preference (4). Male voles appear to facilitate infant care behavior through either the OT or AVP system (34). In single developmental manipulations of the OT system, male voles appear to be more responsive to lower doses of OT, which induce changes in partner preference (20,35), sexual behavior, and reproductive potential (36); responses to infants (37); and AVP receptors (38). Female voles seem more resilient to developmental manipulations, typically responding only at higher dose of OT (19,39). Some data suggest that women may be more responsive to OT than men [40], but a recent meta-analysis indicated data are insufficient to analyze gender differences, even in the best studied areas, trust and facial recognition (41).

In this study, we administered three dosages of intranasal OT, or a saline control, daily to prairie voles from age 21 days to 42 days. This age range represents the period from weaning through sexual maturity, roughly equivalent to the developmental span being used in at least one of the clinical trials (Clinicaltrials.gov identifier: NCT01256060, Principal Investigators E. Anagnoustou and S. Jacob). We examined the acute effects of OT administration on social interactions with a familiar cage mate. After the end of OT administration, we ran a series of adult tests on social and anxiety behavior to examine the long-term effects of chronic OT administration. We hypothesized that the long-term effects of chronic OT would be sex- and dosage-dependent and not always prosocial. Specifically, we predicted that chronic developmental exposure to OT would result in disruption of critical, species-specific social behaviors such as formation of a partner preference, display of alloparenting, and interactions with juveniles, especially at the highest dosage. We predicted that anxiety-related behaviors would be associated with lower normative social behavior. We also predicted that disruptions of social behavior would occur at lower dosages in male voles than in female voles.

Methods and Materials

Subjects

Subjects were prairie voles (*Microtus ochrogaster*) from the breeding colony in the Department of Psychology of the University of California, Davis. This colony was originally started with animals obtained from Dr. C. Sue Carter at the University of Illinois, Chicago. Voles are maintained in breeding pairs in polycarbonate cages (44 × 22 × 16 cm), with water and food (Purina High Fiber Rabbit Chow, PMI Nutrition International, Brentwood, Missouri) ad libitum. They are on a 14:10 light cycle and maintained at approximately 70°F. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

At 20 days of age, subjects were weaned and marked with nontoxic Nyanzol D dye (American Color and Chemical Corporation, Charlotte, North Carolina) for identification. They were then housed in same-sex pairs in smaller cages (27 × 16 × 13 cm), with a sibling when available and a similarly aged nonsibling when not available. Sixteen percent of male subjects and 21% of female subjects were housed with a nonsibling animal. All subjects were thus sex- and pairing-naive.

Intranasal OT Treatments

Starting on day 21, subjects received intranasal treatments for 21 days (Figure 1). Treatments were sterile saline or oxytocin (Bachem, Torrance, California) at one of three dosages: low (.08 IU/kg), medium (.8 IU/kg), or high (8.0 IU/kg). The medium dosage was based on publicly available information regarding clinical studies in progress that were testing the effects of OT on social deficits in autism. The medium dosage was roughly equivalent to a weight-adjusted dose used in cited human studies. Specifically, it would be equivalent to a 40 IU dosage given to a 110-lb subject. Group sizes varied from 8 to 15 voles ($n = 89$ voles total). Intranasal treatments were administered once per day, in the morning between 7:00 AM and 12:00 PM. A blunt cannula needle (33 gauge, 2.8 mm length; Plastics One, Roanoke, Virginia) was attached to cannula tubing, flushed, and filled with the compound, then attached to an airtight Hamilton syringe (Bachem, Torrance, California). The animal was held still and 25 uL of compound was expelled slowly through the cannula needle and allowed to absorb into the nasal mucosa (divided between the two nostrils). Following administration, the animal was returned to its home cage and familiar companion. Initial order of treatment for cage mates was randomized and then alternated on subsequent test days. Administration was rapid (less than 30 seconds) and handling was consistent across treatment groups.

Acute Behavioral Observations

Twice per week of treatment, behavioral observations were conducted following OT administration for each animal (30

minutes of acute behavioral data per animal). Following OT treatment, animals were returned to their home cage and allowed 5 minutes to resume normal activities; then, a 5-minute focal observation of each cage mate was performed. The last treatment was administered on day 42. The treatments thus spanned from weaning (and the earliest known age of sexual maturity) to full sexual maturity (42,43).

Adult Tests

Within the 2 weeks following the end of treatment, each vole received five behavioral tests as detailed in Figure 1. This time span (approximately 42 days to 60 days of age) is still squarely within the time period of young adulthood for a prairie vole (43).

All behavioral scorers (for this and other tests) were trained against one primary observer (or in the case of recorded tests, against a recording previously scored by a primary observer). All observers were trained to 95% or greater reliability on all behaviors before they were allowed to score actual sessions. Tests were either scored live or recorded and scored later, in either case using Behavior Tracker 1.5 (www.behaviortracker.com).

Alloparental Care Testing

Alloparental care tests are 10-minute tests in which the vole has access to two cages, an empty cage and a cage containing a 0- to 4-day old pup (34). Many nulliparous rodents find pups to be an aversive stimulus (44); however, male prairie voles are overwhelmingly alloparental with approximately 70% to 80% acting parentally upon first exposure to a pup (37,45). Virgin female prairie voles, on the other hand, are less likely to be alloparental and more likely to attack pups (37,45,46).

Elevated Plus-Maze Testing

The elevated plus-maze (EPM) tests anxiety and exploration (47,48) by exploiting preference to remain in dark, enclosed places. The EPM consisted of two open and two closed opaque arms, each 67 cm long and 5.5 cm wide (48), elevated 1 m above the floor. Vole behavior in the maze has been shown to be responsive to early experience (49) and pharmacologic manipulations (50). Each vole was placed in the center of the EPM and its behavior scored for 5 minutes.

Open Field Testing

The open field test also tests anxiety and exploration in prairie voles (51) and other rodents (47,52) . Time spent in the center of the open field is interpreted as exploratory behavior, while time along the edges is interpreted as escape or anxiety behavior. The open field consisted of a 40 × 40 × 40 cm Plexiglas box with grids marked on the floor. The vole was placed in the center of the arena and behavior was digitally recorded for 10 minutes and scored as per Olazabal and Young (51).

Juvenile Affiliation Testing

A 15- to 20-day old juvenile vole placed in a two-chamber arena provides a friendly, nonthreatening stimulus (53,54) to evaluate social motivation. Behavior toward the juvenile was digitally recorded for 10 minutes.

Partner Preference Testing

This is an operational index of pair-bond formation (55), used extensively with prairie voles to investigate the effects of hormones and early experience on pair-bonding (4,35,56,57). The test vole was given a cohabitation period with an opposite-sex partner (6 hours for female voles, 24 hours for male voles) previously shown to be sufficient for formation of a partner preference (58). Prairie voles are induced ovulators that normally start displaying lordosis between 42 and 68 hours after exposure to a strange, naive male vole, with a median of 52 hours (59). For female subjects, therefore, we did not expect mating during the 6-hour cohabitation period or subsequent preference testing. Only one female subject mated during the partner preference test (a low-dose OT-treated subject that mated with the strange male). Male subjects were paired with intact, nonestrogen primed stimulus female subjects, which also should theoretically not have entered behavioral estrus during the cohabitation period, but should have been a much more attractive social partner for a normal male prairie vole than a strange female not in estrus.

Following cohabitation, test and stimulus voles (partner and stranger) were placed in a three-chamber apparatus. The partner animal, as well as a stranger of the same sex, age, and approximate size, were loosely tethered within the two end cages, while the test animal was placed, untethered, in the empty middle cage. The test animal could choose to spend time in either the partner's cage, the stranger's cage, or a third, empty cage. Tests were digitally recorded. Durations spent in each location (time spent in partner, stranger, and empty cages) were assessed, as well as duration of time spent in side-to-side contact with stimulus animals.

Data Analysis

Social behaviors measured for each social test (alloparenting, juvenile affiliation, and partner preference) differed somewhat (Tables 1–5). Throughout the testing, we were most interested in side-to-side contact, both because OT is intimately involved in gentle touch across many species and social bonds (60) and because this behavior has been classically measured to assess social bond formation in prairie voles (3,55). We also focused on approach behaviors as reflecting the motivation to interact socially; underlying neural substrates may involve both dopamine and OT (61,62). Anxiety was evaluated by frequency of auto-grooming from each test, the number of line crosses and time spent in the center squares in the open field, and time spent in the open arm/(time spent in the open arm + time spent in the closed arm) of the plus-maze. Other behavioral variables are presented in Tables 1–5 but not statistically analyzed.

Initially, male and female saline-treated animals were compared for baseline sex differences via mixed model analyses of variance (ANOVAs) (63) in SAS 9.2 (SAS Institute, Cary, North Carolina). Sexes were then considered separately with treatment as the fixed factor. For acute observations, ID was a random factor, thus accounting for the multiple observations on each animal. For other tests, litter within pair was a random factor. All significance levels were set at $p < .05$ and all tests were two-tailed.

Data from partner preference testing were analyzed in two different ways. First, time spent with partner and time spent with stranger were analyzed in separate mixed model ANOVAs as described above. A second two-way ANOVA was also performed with stimulus animal (partner or stranger) as one factor, treatment as the second factor, and a stimulus animal by treatment interaction.

Results

Acute Behavioral Observations

Behavioral observations starting 5 minutes following administration of OT showed acute, positive effects on interactions with a familiar cage mate in male voles (Figure 2, additional data in

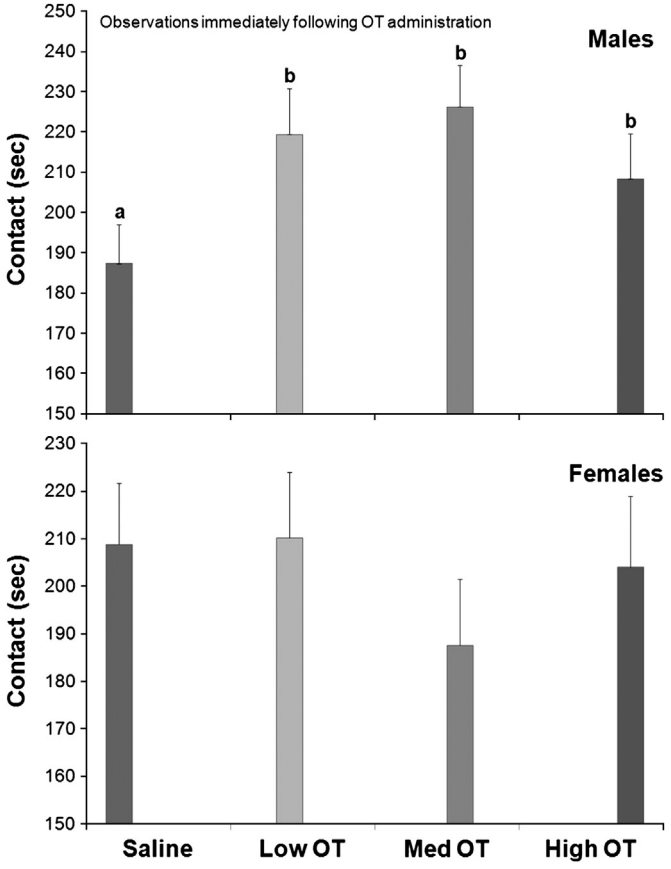

Figure 2. After acute administration of intranasal oxytocin (OT), voles were observed with familiar cage mates. **(A)** Male voles increased contact at all dosages of OT [$F(3,227) = 3.48$, $p = .017$], while **(B)** female voles did not respond to OT [$F(3,237) = .76$, $p = .516$]. Different letters indicate significant differences between means.

Table 1). When considering the saline treatment only, there was a sex difference in autogrooming [$F(1,181) = 5.08$, $p = .026$] and a trend for a sex difference in contact [$F(1,181) = 3.64$, $p = .058$; Table 1]; saline-treated male voles autogroomed more and spent less time in social contact than saline-treated female voles. Male voles increased contact with the cage mate when they received OT of any dosage compared with saline [$F(3,227) = 3.48$, $p = .017$]. Social approach was also significantly affected by OT in male voles, although effects varied by dosage, with low dosages inhibiting approach [$F(3,227) = 2.97$, $p = .033$; Table 1]. Autogrooming following administration was significantly and dose-dependently reduced in male voles [$F(3,227) = 2.74$, $p = .044$], with male voles receiving the highest dosage of OT autogrooming the least. Oxytocin administration had no effects on acute social behavior in female voles (Table 1).

Behavioral Testing Following Long-Term Treatments

Alloparental care tests were carried out on approximately day 43, after intranasal treatment was completed. There were no sex differences in saline-treated animals (Table 2). The overall ANOVAs for female contact and approach were not significant. However, a suggestive pattern emerged in which chronic intranasal exposure to low-dose OT may have affected female interactions with unrelated pups (Table 2), causing a reduction in total contact with pups when compared with saline control subjects. While there were no significant treatment effects on male contact with pups or on autogrooming (Table 2), there was a trend for treatments to affect approach startles [$F(3,24) = 2.42$, $p = .091$].

Elevated plus-maze tests did not indicate any effects of intranasal OT on anxiety (Table 3), nor were there sex differences in saline-treated animals. In the test of interactions with a strange juvenile, there were sex effects in saline-treated animals on autogrooming [$F(1,21) = 7.68$, $p = .039$] with female voles autogrooming more than male voles during this test (Table 4). There were no treatment effects on contact or approaches with a strange juvenile (Table 4).

In the open field test (Table 5), there was a trend in saline-treated animals for female voles to autogroom more than male voles [$F(1,22) = 5.19$, $p = .072$]. Female voles showed a treatment effect of OT on line crosses [$F(3,26) = 3.66$, $p = .025$], a measure of emotionality (47). In post hoc tests, female voles treated with either low or medium OT treatments crossed fewer lines than female voles treated with saline (Table 5).

In the partner preference test, there were sex differences in saline-treated animals in time spent in contact with the partner [$F(1,21) = 11.09$, $p = .045$], with male voles spending more time with the partner (Figure 3). Male voles that were treated with low

Table 1. Results of Acute Behavioral Observations Following OT Administration

Behavior	Saline	Low OT	Medium OT	High OT
Male Voles	$n = 14$	$n = 10$	$n = 10$	$n = 10$
Social contact	187.35 ± 16.31^{a}	219.61 ± 17.0^{b}	225.24 ± 14.62^{b}	210.97 ± 19.97^{b}
Sniff	$1.87 \pm .47$	$1.00 \pm .28$	$1.10 \pm .37$	$2.49 \pm .64$
Groom	5.76 ± 1.32	9.50 ± 2.74	8.31 ± 2.08	6.26 ± 1.49
Approach	$1.76 \pm .23^{a}$	$1.15 \pm .19^{b}$	$1.16 \pm .21^{a,b}$	$2.05 \pm .33^{a,c}$
Total play	$.41 \pm .11$	$.25 \pm .10$	$.52 \pm .18$	$.29 \pm .10$
Autogroom	51.1 ± 5.7^{a}	$38.8 \pm 6.4^{a,b}$	$39.3 \pm 6.0^{a,b}$	30.6 ± 6.1^{b}
Female Voles	$n = 15$	$n = 11$	$n = 10$	$n = 10$
Social contact	208.83 ± 12.88	210.23 ± 13.73	187.52 ± 13.93	204.03 ± 14.88
Sniff	$1.50 \pm .37$	$2.45 \pm .67$	$2.13 \pm .59$	$1.81 \pm .56$
Groom	4.70 ± 1.06	6.58 ± 1.57	6.08 ± 1.70	6.46 ± 1.79
Approach	$1.63 \pm .24$	$2.04 \pm .32$	$1.63 \pm .20$	$1.67 \pm .27$
Total play	$.41 \pm .11$	$.29 \pm .16$	$.24 \pm .10$	$.39 \pm .12$
Autogroom	34.7 ± 4.7	37.1 ± 5.8	47.1 ± 6.9	34.1 ± 5.9

Differing letters indicate significant differences between treatments.
OT, oxytocin.

Table 2. Results of Alloparental Care Testing in Male and Female Voles

Behavior	Saline	Low OT	Medium OT	High OT
Male Voles	$n = 14$	$n = 9$	$n = 10$	$n = 10$
Sniff pup	.64 ± .2	1.33 ± .44	2.0 ± .77	.5 ± .27
Lick pup	182.64 ± 41.13	212.67 ± 63.21	185.0 ± 31.46	193.7 ± 53.64
Retrieve	1.0 ± .56	.67 ± .33	3.1 ± 2.56	.4 ± .22
Contact	57.86 ± 22.63	88.11 ± 62.45	54.3 ± 23.9	80.5 ± 36.51
Startle	1.64 ± .4	1.33 ± .6	4.9 ± 3.18	.5 ± .31
Autogroom	13.29 ± 3.65	11.33 ± 2.69	25.6 ± 9.58	7.5 ± 2.57
Female Voles	$n = 15$	$n = 11$	$n = 10$	$n = 9$
Sniff pup	2.07 ± .62	.55 ± .37	1.30 ± .62	1.56 ± .77
Lick pup	185.1 ± 32.45	94.5 ± 31.0	139.0 ± 35.68	168.2 ± 45.44
Retrieve	2.40 ± .87	.64 ± .31	1.50 ± .5	1.33 ± .71
Contact	68.87 ± 28.18	38.0 ± 25.72	118.6 ± 40.45	139.7 ± 41.93
Startle	1.73 ± .88	1.00 ± .38	1.30 ± .88	.67 ± .24
Autogroom	9.33 ± 2.49	7.45 ± 3.77	29.50 ± 14.11	11.56 ± 4.23

OT, oxytocin.

or medium dosages of chronic intranasal OT later showed deficits in formation of a partner preference, tested approximately 2 weeks after the cessation of OT treatment (Figure 3), while female voles did not. Male voles showed a significant reduction in time spent with the female pair-mate [$F(3,20) = 3.12$, $p = .048$]. Time spent with the stranger did not differ significantly by treatment, nor did time spent in the empty cage.

When analyzed with treatment as one factor and stimulus animal (partner or stranger) as the second factor, female voles showed a significant effect of stimulus animal [$F(1) = 15.97$, $p < .001$] but no treatment effect [$F(3) = .10$, $p = .961$] and no treatment by stimulus interaction [$F(3) = .59$, $p = .622$] (Figure 3). Male voles, in contrast, showed a significant effect of stimulus animal [$F(1) = 6.60$, $p = .013$], and no treatment effect [$F(3) = .68$, $p = .568$], but a significant treatment by stimulus animal interaction [$F(3) = 3.03$, $p = .037$].

Discussion

The acute effects of intranasal OT that we found here resemble those found in many human studies, consisting of an increase in prosocial behavior and engagement (specifically time spent in contact in male subjects) (13,15,64–66). However, in this study, we were able to study long-term developmental effects of repeated intranasal treatments with OT. The picture that emerges is one in which dosage and sex effects are extremely important. In particular, our medium and low dosages (medium = similar to that used in human studies, while low = an order of magnitude lower) changed social behavior, primarily partner preferences in male subjects, in a potentially detrimental fashion (Figure 3).

Specifically, male voles treated with low or medium doses of OT displayed impaired formation of a pair-bond, shown by a reduction of time spent with a familiar partner. Twenty-four hours is considered more than sufficient time for male voles to form a partner preference (67). Lack of partner preference formation could indicate a deficit in social memory formation (68), a lack of motivation to interact with the mate (69,70), a lack of hedonic reward (71) experienced from contact with the mate, or potentially other processes that would need to be disentangled by further research. The observed changes in social behavior do not reflect a difference in general social motivation, as time spent alone did not differ between treatment groups.

While not statistically analyzed here, other behaviors measured suggest that male voles treated with low OT also showed less interest in a strange juvenile (Table 4) (and more interest in a strange pup, Table 2), indicating perhaps altered social behavior in multiple social contexts. Similar patterns of behavior with low-dose OT in female interactions with pups (Table 2), which do suggest lower motivation to socially interact, also deserve further investigation and replication in a future study. It is also worth considering whether increased interactions with unfamiliar animals might be viewed as negative or positive, for instance, as a more general urge to affiliate. Better social interactions with strangers might be a desirable goal in human treatments (although reduced social interactions with family and friends would not be). In this case, however, these changes in partner

Table 3. Results of Elevated Plus-Maze Testing (Means ± Standard Errors)

Behavior	Saline	Low OT	Medium OT	High OT
Male Voles	$n = 14$	$n = 10$	$n = 10$	$n = 10$
Open arm	94.4 ± 16.79	94.1 ± 26.85	92.1 ± 20.93	104.9 ± 15.41
Closed arm	171.86 ± 17.11	177.6 ± 27.67	172.2 ± 24.52	160.7 ± 13.71
Center	32.0 ± 4.9	30.4 ± 3.15	37.8 ± 9.32	33.1 ± 3.68
Ratio	.355 ± .062	.347 ± .097	.361 ± .088	.392 ± .055
Female Voles	$n = 15$	$n = 11$	$n = 10$	$n = 9$
Open arm	113.3 ± 18.34	112.9 ± 32.0	108.2 ± 26.92	86.67 ± 30.16
Closed arm	145.57 ± 18.0	166.9 ± 28.46	141.5 ± 26.56	195.67 ± 27.15
Center	45.87 ± 12.67	28.64 ± 5.34	49.0 ± 13.3	25.56 ± 5.3
Ratio	.437 ± .068	.389 ± .107	.435 ± .095	.298 ± .095

OT, oxytocin.

Table 4. Results of Juvenile Affiliation Testing (Means ± Standard Errors)

Behavior	Saline	Low OT	Medium OT	High OT
Male Voles	$n = 14$	$n = 10$	$n = 10$	$n = 10$
Rear	51.21 ± 9.9	62.6 ± 11.78	51.7 ± 7.94	43.0 ± 6.26
Sniff	68.36 ± 9.94	49.6 ± 12.44	74.1 ± 12.06	69.7 ± 15.47
Withdraw	19.43 ± 4.94	12.1 ± 3.55	9.4 ± 3.34	12.6 ± 2.25
Lunge	1.21 ± .53	.6 ± .27	.5 ± .17	.3 ± .15
Autogroom	19.07 ± 6.15	15.5 ± 5.3	40.6 ± 18.25	13.6 ± 4.35
Contact	.43 ± .25	7.4 ± 7.4	3.3 ± 1.8	.6 ± .5
Wrestle	1.36 ± .71	1.3 ± .99	1.3 ± 1.01	1.2 ± .73
Female Voles	$n = 14$	$n = 11$	$n = 9$	$n = 9$
Rear	37.71 ± 6.52	33.91 ± 8.08	33.78 ± 7.45	53.44 ± 13.23
Sniff	77.0 ± 10.44	72.45 ± 11.61	61.33 ± 8.49	59.11 ± 11.27
Withdraw	5.64 ± 2.02	4.82 ± 1.66	2.0 ± .44	2.44 ± .71
Lunge	.57 ± .44	1.0 ± .75	.33 ± .24	.00 ± .00
Autogroom	34.64 ± 15.21	13.3 ± 7.55	26.44 ± 8.67	1.67 ± 9.13
Contact	24.07 ± 13.55	10.55 ± 8.66	10.11 ± 4.30	1.67 ± 1.07
Wrestle	10.29 ± 4.93	.64 ± 1.44	3.55 ± 2.11	3.11 ± 1.89

OT, oxytocin.

preference behavior are clearly species-atypical, and the apparent direction of changes in female behavior toward pups is clearly less nurturing.

Interestingly, the impaired social behavior we observed in tests does not appear to be secondary to an increase in anxiety. In fact, acutely OT-treated male voles actually showed lower autogrooming (one measure of anxiety). Multiple measures of anxiety across the adult tests indicated either no difference between OT- and saline-treated animals or a reduction in emotionality in OT-treated animals (such as in the lower number of line crosses in the open field in OT-treated female voles). The detrimental changes thus appear to be relatively specific to social behavior.

While sex differences in responses to OT are pervasive in both adult voles (3,72) and in single-dose developmental studies (37,38,73), the human literature is still lacking in sufficient information to assess the impact of gender on OT response. It will be important in future human research to assess both genders at the same time and at multiple dosages. It is also worth emphasizing that our intranasal OT treatments were given to developing animals, for a time span chosen to approximate humans aged 12 to 17 years. Developmental treatments can have particular ramifications to receptor binding and upregulation or downregulation of peptide production (38,54,74,75). Animal research on additional developmental ages, as well as treatment effects on adults, will be important in the future. Finally, we also used a between-subjects design in this study and used different tests that were developmentally appropriate for each age. While this avoided design issues associated with retesting, it also did not allow us to assess changes in the same behaviors longitudinally. Future research could concentrate on tests that are well validated throughout development and that can be used multiple times.

The possible mechanism for these changes is intriguing. If the main behavioral results had been at high dosages, a potential culprit would be secondary binding to vasopressin receptors. In other words, high-dose OT might saturate OT receptors and subsequently bind to vasopressin receptors, to which OT also has binding affinity and which can cause differing and sometimes opposite behavioral effects (18,38,76,77). However, this explanation is less likely for the results seen here, as the low-dose OT would not flood the receptors to the extent that the higher doses would. It is possible that the intranasal OT has 1) acted to

Table 5. Results of Open Field Testing (Means ± Standard Errors)

Behavior	Saline	Low OT	Medium OT	High OT
Male Voles	$n = 14$	$n = 10$	$n = 10$	$n = 10$
Center	71.21 ± 11.84	111.1 ± 49.3	84.3 ± 18.96	114.2 ± 38.42
Periphery	524.29 ± 11.47	530.1 ± 14.32	476.0 ± 44.21	507.6 ± 16.84
Line crosses	359.5 ± 41.88	446.6 ± 95.61	483.4 ± 84.64	423.1 ± 62.77
Autogroom	18.21 ± 5.91	13.6 ± 6.69	46.8 ± 28.87	15.6 ± 5.04
Rear	55.64 ± 7.2	49.6 ± 10.04	57.1 ± 10.75	55.0 + 10.33
Female Voles	$n = 15$	$n = 11$	$n = 10$	$n = 9$
Center	138.2 ± 46.8	73.64 ± 14.55	45.5 ± 10.0	77.55 ± 13.23
Periphery	535.6 ± 9.38	524.27 ± 14.58	552.3 ± 10.41	520.11 ± 13.19
Line crosses	563.2 ± 62.38[a]	361.91 ± 44.58[b]	279.4 ± 57.42[b]	486.22 ± 70.74[a]
Autogroom	23.6 ± 7.04	20.09 ± 8.01	30.4 ± 16.03	15.88 ± 3.78
Rear	64.87 ± 6.82	54.18 ± 6.56	37.7 ± 8.64	55.78 ± 10.85

Differing letters indicate significant differences between treatments.
OT, oxytocin.

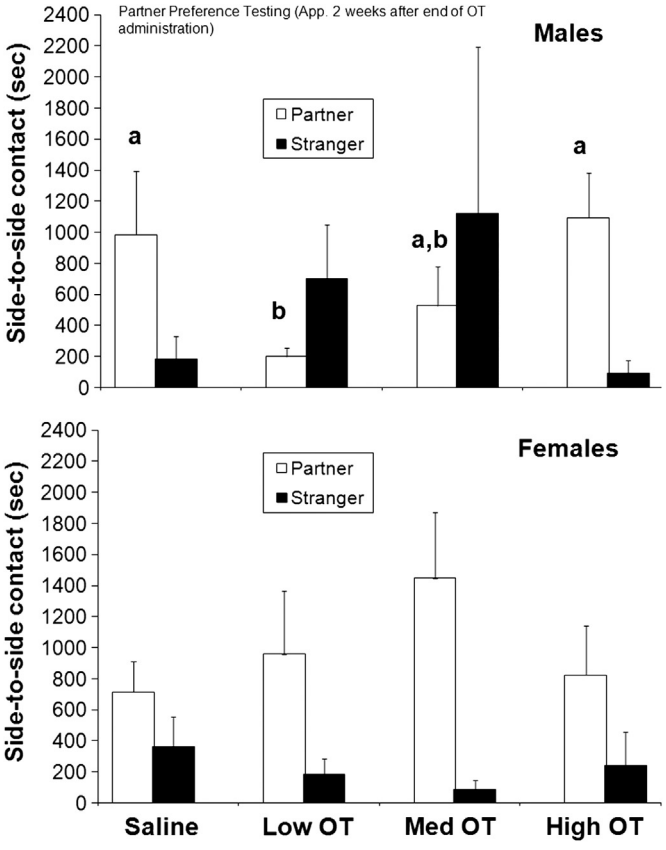

Figure 3. The effects of chronic intranasal oxytocin (OT) on partner preferences in prairie voles. Chronic intranasal OT significantly affected the duration of time that male voles spent in contact with a familiar partner [$F(3,20) = 3.12$, $p = .048$]. There was no treatment effect on the duration of time that female voles spent in contact with a familiar partner [$F(3,25) = .14$, $p = .933$] or an effect on time spent with the stranger for either sex.

upregulate or downregulate endogenous OT release; 2) upregulated or downregulated the closely related peptide arginine vasopressin (78); or 3) desensitized and downregulated the OT receptor (79,80). It is also possible that low doses could have negatively impacted behavior through modulating peripheral OT receptors, while at the highest dose, sufficient OT might have entered the brain and rescued behavioral deficits caused by chronic peripheral stimulation. These possibilities require further investigation and may significantly inform treatment decisions.

While manipulations of OT hold great promise for treatment of disorders involving social deficits, the results of this study should sound a cautionary note. In particular, many parents may believe that starting their children off at lower dosages of any treatment, including intranasal OT, is safer. The results of the current study suggest otherwise. Moreover, long-term changes in social behavior induced by chronic OT treatment may include effects that diminish rather than promote social bonding and these apparently detrimental social consequences of OT treatment persist long beyond the treatments themselves. The need for animal studies that examine the dosages, timing of administration, sex differences in efficacy, and developmental timing of potential OT therapeutics is clear.

There are several ways in which these and future animal studies can be used to inform human treatment options. It is important to note that acute administration did have prosocial effects in the context of interactions with a familiar partner. Context may be important for long-term effects of OT (for example, pairing OT administration with specific environments or social learning tasks) to generate specific effects in humans and animals (81). Short-term administration may be safer or more effective than chronic administration, although ideally long-term learning effects would be demonstrated. Finally, in this study, the detrimental effects were only observed after OT treatment was stopped. Future investigations should determine if OT administration continued into adulthood, rather than stopped at some developmental time point, would have similar or different effects.

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