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Chronic enhancement of brain oxytocin levels causes enduring anti-aggressive and pro-social explorative behavioral effects in male rats

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ABSTRACT

Oxytocin (OXT) has been implicated in the regulation of social behaviors, including intermale offensive aggression. Recently, we showed that acute enhancement of brain OXT levels markedly suppressed offensive aggression and increased social exploration in resident rats confronted with an intruder in their home territory. Moreover, a different responsivity to the exogenous OXTergic manipulation was observed among individuals based on their baseline aggression. In this study we aimed at evaluating the behavioral response to chronically enhancing or attenuating central OXT levels, and at scrutinizing whether the trait-aggression moderates the treatment-induced behavioral changes. To this end, resident male wild-type Groningen rats were continuously (via osmotic minipumps) intracerebroventricularly infused with synthetic OXT or a selective OXT receptor (OXTR) antagonist for 7 days. Changes in behavior were assessed performing a resident–intruder test before and at the end of the treatment period, as well as after 7 days of withdrawal. Chronic infusion of OXT was found to selectively suppress aggression and enhance social exploration. Chronic blockage of OXTRs instead increased introductory aggressive behavior (i.e. lateral threat), yet without affecting the total duration of the aggression. The magnitude of the anti-aggressive changes correlated positively with the level of baseline aggression. Interestingly, OXT-induced behavioral changes persisted 7 days after cessation of the treatment. In conclusion, these findings provide further evidence that enhanced functional activity of the central OXTergic system decreases social offensive aggression while it increases social explorative behavior. The data also indicate that chronically enhancing brain OXT levels may cause enduring anti-aggressive and pro-social explorative behavioral effects.

Introduction

Oxytocin (OXT) is a cyclic nonapeptide synthesized principally by a relatively small clusters of neurons in the hypothalamic paraventricular, supraoptic and accessory magnocellular nuclei (Stoop, 2013). In addition to its well-known peripheral hormonal functions (i.e., induction of labor and milk ejection), OXT acts as an important neuronal messenger within the brain regulating social and emotional behaviors in a wide variety of animal species including humans (H.J. Lee et al., 2009). Hence, disturbed brain OXTergic signaling has been implicated in several psychiatric disorders where social dysfunction is a core symptom (e.g., autism spectrum disorder, social anxiety, borderline personality disorder, addiction and schizophrenia) (Meyer-Lindenberg et al., 2011). In particular, low cerebrospinal fluid (CSF) OXT (R. Lee et al., 2009), loss of functional polymorphisms of the OXT receptor (OXTR) gene (Beitchman et al., 2012; Malik et al., 2012) and epigenetic silencing (methylation) of the OXTR promoter have been related to impulsive and aggressive temperament, interpersonal violence and callous–unemotional traits in young boys (Kumsta et al., 2013). Similarly, animal research has reinforced the proposed functional role of the central OXTergic system in regulating the behavioral response in conflicting social contexts. For example, genetic knockout studies have demonstrated that abrogating OXTergic signaling results in escalated patterns and dysfunctional forms of intermale offensive aggression (Sala et al., 2011; Winslow et al., 2000). Recent etho-pharmacological studies carried out in our lab on a feral strain (wild-type Groningen, WTG) of male rats have revealed robust dose-dependent anti-aggressive and pro-social exploratory effects of acute intracerebroventricular (icv) administered OXT, especially in animals with high baseline aggression level. Conversely, acute and selective blockade of OXTergic signaling by administering a selective OXTR antagonist tended to potentiate aggressive displays especially in low aggressive individuals (Calcagnoli et al., 2013).

Considering the increasing scientific interest for the brain OXTergic social behavior network, and the increasing exploration of intranasally applied synthetic OXT in humans, it becomes relevant to expand our current psychoneuroendocrine knowledge of this neuropeptide. Before adopting synthetic OXTR agonists as a potential treatment for curbing

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social deficits and abnormal aggressive behaviors in humans, the effects of chronic OXT-treatment and possible long-lasting repercussions on behavior and physiology have to be investigated. To date, the data available on chronic OXT administration are limited to some preclinical studies that are mainly focused on drug addiction processes (Sarnyai and Kovacs, 1994), stress responsivity (Parker et al., 2005), anxiety (Slattery and Neumann, 2010; Windle et al., 1997), or male–female social interaction (Witt et al., 1992). No studies are yet available concerning the effect of chronic OXT on intermale social–aggressive behaviors in particular.

Therefore, in order to extend our recent findings of the acute central OXTergic manipulation effects on offensive and social explorative behaviors (Calcagnoli et al., 2013), a chronic icv administration study has been designed. In particular, we aimed at testing the hypothesis that chronic enhancement or attenuation of the central OXTergic activity would result in the suppression or increase of intermale offensive behavior, respectively. We expected to replicate the selective changes in the social behavioral profile that were observed after acute OXT infusion, i.e., decreased offensive aggression concomitant with increased social exploration, without effects on non-social behaviors. Also, possibly enduring or rebound effects were monitored. Therefore, the behavioral effects of chronic OXTergic manipulation were tested using a standard resident–intruder test, performed before (day −1), immediately after a 7-day period of treatment (day 7), and again after 7 days of withdrawal from chronic treatment (day 14). Moreover, we expected to replicate the observation that the individual's initial aggressive phenotype might moderate the individual responsivity to the OXTergic manipulation. In particular, we hypothesized the most aggressive animals to be more sensitive to the exogenous synthetic OXT, as trained and aggression-experienced highly aggressive wild-type Groningen residents have been recently described to have potentially lower central OXT availability but higher OXTR binding capacities (Calcagnoli et al., 2014). On the other hand, we investigate the possibility that chronic OXTR antagonist infusion may induce pro-aggressive changes, as observed in one of our experiments with acute manipulation.

Material and methods

Animals and housing conditions

Adult male WTG rats (*Rattus norvegicus*) were used as experimental subjects. This strain of rats descended from pairs of wild-trapped individuals that were outbred under conventionalized conditions for over 35 generations now in our laboratory. As compared to commonly used laboratory strains of rats, WTG rats display a much larger variation in the level of intermale offensive aggression (de Boer et al., 2003) and they are therefore a suitable model for clinical aggression research. After weaning (postnatal day 23), the animals were socially housed with five non-sibling conspecifics in Makrolon cages (55 × 34 × 20 cm). Around the age of 120 days, the animals were housed in large observation cages (80 × 55 × 50 cm), each with an oviduct-ligated but gonadally-intact female to avoid social isolation and to allow normal sexual activity, required to stimulate territorial behavior (Albert et al., 1988). Animals had free access to food (Hope Farms, RMH-B) and tap water with a fixed 12 h light/12 h dark photoperiod (lights off at 1300 h) in a temperature- (21 ± 2 °C) and humidity-controlled room (50 ± 5%). All experimental and behavioral procedures were approved by the Animal Ethics Committee on Care and Use of Laboratory Animals (DEC 5824) of the Groningen University and were conducted in agreement with Dutch laws (WoD, 1996) and European regulations (Guideline 86/609/EC).

Behavioral screening: resident–intruder test

After an acclimation period of 7 days in the observation cages, residents (average body weight 400 ± 50 g, 4.5 months old) were tested for their baseline level of offensive aggression using the standard resident–intruder (RI) test paradigm (Koolhaas et al., 1980, 2013). The companion female was always removed approximately 30 min prior to the start of the test and placed back afterwards. Naïve male intruder Wistar rats (Harlan Laboratories, Horst, NL) have been used as intruders (average body weight 300 ± 50 g, 4 months old). The lower range weight of the intruder guaranteed the assessment of dominance from the resident, with only one resident failing the attack during the baseline RI test. The baseline behavioral screening consisted of an Attack Latency Time (ALT) test repeated over three consecutive days, by introducing an unfamiliar intruder into the cage of the experimental animal. The intermale interaction was terminated as soon as the first clinch was recorded. When the resident failed to attack within the first 10 min of testing, the Attack Latency Time was scored as 600 s and the test was terminated. On the fourth day, while videotaped, the interaction was allowed to last for 10 min following the first attack or, in case of no attack, to last for 10 min following the placement of the intruder. A custom-made data acquisition system (E-line) was used to evaluate the video and to determine the duration of the following behavioral categories: (1) offensive behaviors (lateral threat, clinch, keep down, chase, upright posture), (2) social explorative behaviors (moving towards, investigation and ano-genital sniffing of the intruder, crawl over, social grooming), (3) non-social behaviors (ambulation, rearing, exploration of the cage), (4) inactivity (sitting, lying), and (5) self-grooming (washing, scratching) (Koolhaas et al., 1980, 2013). All behavioral tests were performed within the first 3–4 h of the dark (active) phase in a dimmed lights condition, to avoid effects of circadian hormonal fluctuation and light exposure (Devarajan et al., 2005). The baseline behavioral profile was assessed one day prior to the minipump implantation (day −1). The resident animals were then assigned to one of the three experimental groups receiving either vehicle solution (N = 11), synthetic OXT (N = 12) or a selective OXTR antagonist (N = 12). Groups were matched on the baseline offensive behavior level.

Surgical procedures and chronic infusion

All surgical procedures were performed anesthetizing the animals with a mixture of isoflurane and oxygen, using sterile conditions. To minimize pain and risk of infection, directly linked to the surgery, rats were injected subcutaneously with analgesic (Finadyne, 0.1 ml) and antibiotic (Penicillin, 0.25 ml) compounds. For the first 24 h post-operation, rats were singly housed in their home cage and then again housed together with the same female companion as during the pre-surgery period.

A guide cannula (22-gauge stainless steel cannulae, C313; Plastics One, Roanoke, VA, USA) was stereotactically placed according to the brain map of Paxinos and Watson (6th edition, 2007) 2 mm dorsal to lateral ventricle (AP: −1.0 mm from bregma, ML: +1.7 mm, DV: +3.1 mm below the surface of the dura mater, with the tooth bar set at −3.3 mm) and anchored to the skull with two stainless steel screws using dental acrylic cement. To chronically administer the drugs into the brain ventricles, an osmotic minipump (infusion rate 0.5 μl/h for 7 days; Alzet, Model 1007D, DURECT Corporation, CA, USA) was subcutaneously implanted. The pumps were filled with either vehicle (sterile, pyrogen-free saline, 0.9% Versylene®, Fresenius, Kabi, France), or synthetic OXT ($C_{43}H_{66}N_{12}O_{12}S_2$; MW 1007.19; Tocris, Germany) (20 ng/μl) or a selective peptidergic OXTR antagonist (15 ng/μl) {desGly-$NH_{2(9)}$,d$(CH_2)_5$[Tyr$(Me)_2$,Thr_4]OVT} (MW 992.2; kindly donated by Dr. Manning, University of Toledo) (Manning and Sawyer, 1989). The doses were chosen based on effective treatment effects shown in a previous study where the same compounds were chronically infused in adult male rats for the same number of days and using the same model of osmotic minipumps (Slattery and Neumann, 2010). Minipumps were connected to the icv cannula via a 7.5 cm long polyethylene catheter tube (inside diameter of 0.69 mm;

Alzet, brain infusion kit 2, DURECT Corporation, CA, USA). To optimize the operation of the minipump, both minipump and catheter were incubated in sterile saline at room temperature overnight. The 7-day treatment period started immediately after the surgical implantation of the minipump (day 0). Information about the degradation rate of the peptide over 7 days was collected from a pilot study, revealing about 44% destruction of OXT over a 10-day period in vivo (Witt et al., 1992).

Behavioral assessments

At days 7 and 14, the behavioral profile was again assessed using the 10 min RI test and then compared to the baseline ethogram recorded at day −1. In the time period between the RI tests, resident animals were kept housed with their companion female in their home cage.

Exclusion criteria

At the end of the experiment, the animals were rapidly euthanized with CO_2. To check for correct cannula placement and for proper attachment of the cannula to the minipump, blue dye was slowly injected into the guide cannula and through the catheter connecting the cannula to the minipump. Correct cannula placement was scored only when the dye was observed exclusively throughout the entire ventricular system of the brain. Animals that exhibited any indication of dye leakage at the connection sites of the catheter were excluded from the analysis. This led to the following group sizes: vehicle N = 11, OXT N = 10 and OXTR antagonist N = 12.

Statistical analysis

Treatment effects on the various behavioral variables were statistically tested by General Linear Model (GLM) repeated measures analyses of covariance (ANCOVA), while entering the corresponding baseline values as a covariate for the sake of the design's efficiency (power) and validity (Liu et al., 2009; Senn, 2006). We used SPSS for Windows; version 20: SPSS Inc, Chicago, IL, USA. The ANCOVA design consisted of one within-subjects (WS) variable with three measurement levels (time points: days −1, 7, and 14), and one between-subjects (BS) variable with three treatment levels (vehicle, OXT, and OXTR antagonist). If an overall significant interaction between the treatments (BS variable) and time points (WS variable) was found, post hoc pair-wise treatment group comparisons were carried out on the contrasts of the WS variable (day −1 vs. day 7; day −1 vs. day 14, and day 7 vs. day 14). The ability to compare all the three group-dependent time contrasts made us chose to enter the raw scores of the three measurements instead of change scores (Senn, 2006). To account for possible violations of the sphericity assumption for factors with more than two levels, Huynh–Feldt adjusted *p*-values and the epsilon correction factor are reported together with the unadjusted degrees of freedom and F-values.

For all comparisons, next to the *p*-values, Cohen's *d* or eta squared (η^2) are presented as measures of effect size, with $d < 0.5$ and $\eta^2 < 0.06$ reflecting a small effect; $d \geq 0.5$ and $\eta^2 \geq 0.06$ a medium effect; and $d \geq 0.8$ and $\eta^2 \geq 0.14$ a large effect.

Pearson's correlations were computed to find out whether the treatment effects were greater in animals with lower or higher baseline level of offensive behavior. To this end two types of change scores were computed, i.e. the difference scores between the pre-treatment measure (at day −1) and the two post-treatment measures obtained at day 7 and day 14, respectively.

We finally tested whether OXT effects on the time spent in the various behavioral categories might be interdependent. To this end correlations were computed between the above-described change scores referring to aggression and social explorative behavior respectively.

Data are graphically presented as the group means of the time spent in each behavioral category (indicated as percentage of the total 10 min test) ± SEM.

Results

Significant overall time * treatment interactions were found for only offensive behavior [$F_{4,58} = 8.81$, $p < 0.001$, $\eta^2 = 0.17$] and social exploration [$F_{4,58} = 6.75$, $p < 0.001$, $\eta^2 = 0.28$, $\varepsilon = 0.83$]. In particular, the measurements at both day 7 and day 14 of the two behavioral categories significantly differed from their respective baseline measurements [offensive behavior: contrast day −1 vs. day 7 ($F_{2,29} = 11.81$, $p < 0.001$, $\eta^2 = 0.18$) and day −1 vs. day 14 ($F_{2,29} = 15.46$, $p < 0.001$, $\eta^2 = 0.19$); social explorative behavior: contrast day −1 vs. day 7 ($F_{2,29} = 11.91$, $p < 0.001$, $\eta^2 = 0.38$) and day −1 vs. day 14 ($F_{2,29} = 5.48$, $p < 0.01$, $\eta^2 = 0.27$)]. As Figs. 1 and 2 show, the overall effects were due to the fact that chronic OXT infusion (1) significantly attenuated the offensive display as compared to vehicle [$F_{2,36} = 7.10$, $p < 0.01$, $\eta^2 = 0.16$] and OXTR antagonist [$F_{2,38} = 25.40$, $p < 0.001$, $\eta^2 = 0.24$] (Fig. 1) and (2) simultaneously enhanced the social exploration of the resident as compared to vehicle [$F_{2,36} = 6.28$, $p < 0.01$, $\eta^2 = 0.23$] and OXTR antagonist [$F_{2,38} = 13.16$, $p < 0.001$, $\eta^2 = 0.34$, $\varepsilon = 0.75$] (Fig. 2).

Among the elements within the category of offensive behavior, a significant overall time * treatment effect was found in the lateral threat [$F_{4,58} = 5.12$, $p < 0.01$, $\eta^2 = 0.17$, $\varepsilon = 0.79$], the duration of which was lowered by OXT infusion [$F_{2,29} = 5.67$, $p < 0.01$, $\eta^2 = 0.17$] but increased by OXTR antagonist [$F_{2,29} = 15.87$, $p < 0.001$, $\eta^2 = 0.25$] as compared to vehicle (Table 1).

Interestingly, the OXT-induced anti-aggressive effects seen at day 7 [OXT vs. vehicle, contrast day −1 vs. day 7; $F_{1,18} = 10.68$, $p < 0.01$, $\eta^2 = 0.19$. OXT vs. OXTR antagonist, contrast day −1 vs. day 7; $F_{1,19} = 34.29$, $p < 0.001$, $\eta^2 = 0.23$] persisted over time for 7 days post-treatment [OXT vs. vehicle, contrast day −1 vs. day 14; $F_{1,18} = 14.23$, $p < 0.001$, $\eta^2 = 0.20$. OXT vs. OXTR antagonist, contrast day −1 vs. day 14; $F_{1,19} = 36.69$, $p < 0.001$, $\eta^2 = 0.27$] (Fig. 1). Similarly, the pro-social explorative changes seen at day 7 [OXT vs. vehicle, contrast day −1 vs. day 7; $F_{1,18} = 8.35$, $p = 0.01$, $\eta^2 = 0.28$. OXT vs. OXTR antagonist, contrast day −1 vs. day 7; $F_{1,19} = 28.44$, $p < 0.001$, $\eta^2 = 0.45$] appeared to be long-lasting as well [OXT vs. vehicle, contrast day

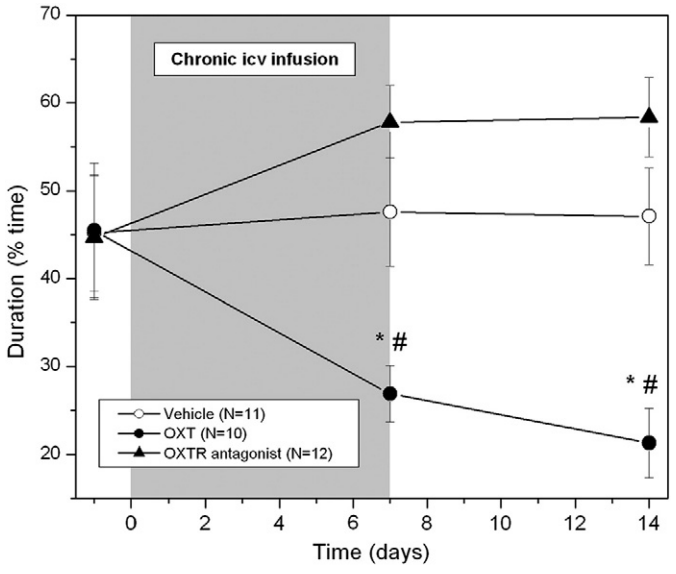

Fig. 1. Offensive behavior displayed by resident male wild-type Groningen rats exposed to an unfamiliar male intruder Wistar rat and chronically infused with vehicle, synthetic OXT or selective OXTR antagonist. The gray area indicates the 7-day treatment period. Procentual duration of offensive behavior is depicted at three time points: baseline measurement (day −1), at the end of the chronic treatment (day 7), and 7 days after the cessation of the treatment (day 14). Data are presented as the mean ± SEM. * denotes significance (day 7: $p = 0.01$, $d = 1.30$ and day 14: $p = 0.001$, $d = 1.63$) between vehicle and OXT-treated groups. # indicates significance (day 7: $p < 0.001$, $d = 2.52$ and day 14: $p < 0.001$, $d = 2.65$) between OXT and OXTR antagonist treatments.

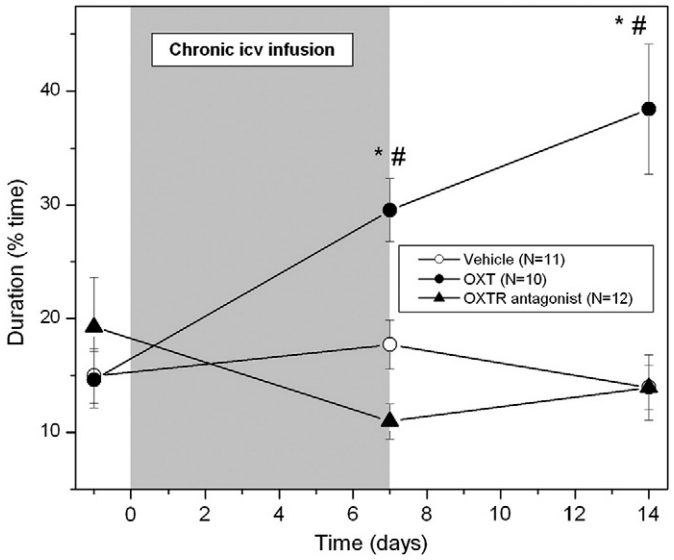

Fig. 2. Social explorative behavior displayed by resident male wild-type Groningen rats exposed to an unfamiliar male intruder Wistar rat and chronically infused with vehicle, synthetic OXT or selective OXTR antagonist. The gray area indicates the 7-day treatment period. Procentual duration of social explorative behavior is depicted at three time points: baseline measurement (day −1), at the end of the chronic treatment (day 7), and 7 days after the cessation of the treatment (day 14). Data are presented as the mean ± SEM. * denotes significance (day 7: $p < 0.01$, $d = 1.48$ and day 14: $p = 0.001$, $d = 1.68$) between vehicle and OXT-treated groups. # indicates significance (day 7: $p < 0.001$, $d = 2.64$ and day 14: $p < 0.001$, $d = 2.05$) between OXT and OXTR antagonist treatments.

−1 vs. day 14; $F_{1,18} = 8.22$, $p = 0.01$, $\eta^2 = 0.29$. OXT vs. OXTR antagonist, contrast day −1 vs. day 14; $F_{1,19} = 9.67$, $p < 0.01$, $\eta^2 = 0.31$] (Fig. 2). Importantly, for both offensive and social explorative behaviors, no differences were found between day 7 and day 14. No significant effects were observed in any of the other behavioral categories of the ethogram, and, equally important, none of the treatments significantly affected the latency of the first attack (Table 2).

In addition, the OXT-induced changes in aggression were found to depend upon the baseline level of offensive behavior, while there was no such baseline dependency for the changes in social explorative behavior. In particular, Fig. 3 shows that most of the OXT-treated rats lowered their offensive aggression and that a greater decrease was observed in animals with the highest baseline aggression scores (day 7 $r = -.93$, $p < 0.001$; day 14 $r = -.86$, $p = 0.001$).

Finally, the effects of OXT on aggression and on social explorative behavior appeared to be highly correlated; change scores at day 7 showed a correlation of $r = -.89$ ($p < 0.001$). Yet for day 14 it was no longer significant ($r = -.31$, $p = 0.38$).

Discussion

This study provides evidence that chronic central infusion of OXT suppresses intermale offensive behavior, particularly in animals with higher baseline level of aggression, while at the same time it enhances social explorative behavior. On the other hand, chronic infusion of the OXTR antagonist could be shown to specifically enhance introductory aggressive behavior (i.e. lateral threat), without affecting the display of agonistic contact or the total duration of the aggression. Surprisingly, the anti-aggressive and pro-explorative effects even persisted 7 days after the cessation of the chronic treatment, indicating protracted behavioral effects after a period of chronic OXT enhancement in the brain. Another interesting observation to note is that synthetic OXT specifically shortened the duration of the aggressive displays without significantly delaying the latency of the first attack. This suggests that OXT does not affect the initiation of an aggressive attack but rather the maintenance and/or termination aspects of offensive aggressive behavior. Moreover, the exogenously administered OXT selectively targeted social behavior components, without altering any of the other non-social behavioral categories evaluated during the resident–intruder test.

To our knowledge, this is the first chronic icv study reporting OXT-induced persistent behaviorally selective anti-aggressive and pro-explorative changes in male rats tested in a social conflicting context.

As extensively reported in the literature, exogenously administering OXT may alter the processing of and responding to social stimuli. In our current study, we defined social behavior as all types of interactive approaches and displays directed by the resident towards the intruder, aiming to either offend or explore. Our results revealed that chronic synthetic OXT infusion qualitatively re-shaped the social behavior profile, with a shift from offensive to social explorative behaviors. These findings are in line with our recent study in WTG male rats where acute pharmacological enhancement of brain OXT levels induced anti-aggressive and pro-social explorative effects that could be blocked by a selective OXTR antagonist (Calcagnoli et al., 2013). Moreover, deficits in social recognition, decreased social investigation, and increased offensive reactions have been reported in male mice when knocking out *oxt* or *oxtr* gene (Ferguson et al., 2000, 2001; Lee et al., 2008; Sala et al., 2011, 2013; Winslow and Insel, 2002; Winslow et al., 2000).

Table 1
Summary of the group means of the time spent in each element constituting the behavioral category of offensive behavior (indicated as percentage of the total 10 min test) ± the respective SEM.

		Day −1	Day 7	Day 14
		Average ± SEM	Average ± SEM	Average ± SEM
Lateral threat	Vehicle	24.62 ± 4.50	26.75 ± 4.81	26.16 ± 4.11
	OXT	24.73 ± 4.59	15.53 ± 3.05*	11.53 ± 2.33*
	OXTR antagonist	22.01 ± 5.21	30.28 ± 4.16*	36.36 ± 3.36*
Clinch	Vehicle	3.11 ± 0.35	3.21 ± 0.63	2.52 ± 0.61
	OXT	3.44 ± 0.69	2.21 ± 0.28	2.08 ± 0.45
	OXTR antagonist	4.63 ± 1.01	3.88 ± 0.44	3.36 ± 0.60
Keep down	Vehicle	14.15 ± 2.74	15.87 ± 4.36	15.49 ± 5.69
	OXT	14.50 ± 4.28	7.65 ± 1.94	6.81 ± 2.05
	OXTR antagonist	15.58 ± 2.64	20.96 ± 4.87	14.28 ± 3.93
Chase	Vehicle	1.10 ± 0.30	0.85 ± 0.31	1.53 ± 0.44
	OXT	1.79 ± 0.93	0.53 ± 0.23	0.61 ± 0.18
	OXTR antagonist	1.38 ± 0.64	1.08 ± 0.21	1.84 ± 0.52
Upright posture	Vehicle	2.22 ± 0.62	0.89 ± 0.34	1.40 ± 0.31
	OXT	1.04 ± 0.21	0.95 ± 0.30	0.27 ± 0.15
	OXTR antagonist	1.09 ± 0.25	1.68 ± 0.30	2.54 ± 1.25

* Denotes significance ($p < 0.05$) between vehicle and OXT or OXTR antagonist treated groups.

Table 2
Summary of the group means of the time (indicated as percentage of the total 10 min test) spent in the behavioral categories evaluated during the intermale encounter (with the exclusion of the categories "offensive behavior" and "social explorative behavior"), and the group means of the latency time to the first attack (ALT; indicated in seconds) ± the respective SEM.

		Day −1	Day 7	Day 14
		Average ± SEM	Average ± SEM	Average ± SEM
Non-social exploration	Vehicle	35.11 ± 4.49	27.76 ± 3.18	28.70 ± 3.41
	OXT	38.45 ± 5.71	37.66 ± 3.66	39.36 ± 3.55
	OXTR antagonist	35.35 ± 4.75	25.93 ± 3.12	25.89 ± 3.20
Inactivity	Vehicle	8.77 ± 2.34	7.93 ± 1.54	11.48 ± 1.99
	OXT	5.80 ± 1.23	6.83 ± 1.20	13.71 ± 3.07
	OXTR antagonist	7.23 ± 1.71	6.05 ± 1.22	6.08 ± 0.89
Self-grooming	Vehicle	4.33 ± 1.30	6.95 ± 2.19	3.25 ± 0.75
	OXT	3.42 ± 1.97	6.18 ± 1.65	2.66 ± 0.89
	OXTR antagonist	2.70 ± 1.52	5.32 ± 1.15	3.33 ± 0.80
ALT	Vehicle	64.27 ± 16.70	54.82 ± 8.54	74.45 ± 13.56
	OXT	53.60 ± 9.46	76.20 ± 12.81	84.60 ± 12.27
	OXTR antagonist	70.58 ± 20.48	48.42 ± 6.85	43.25 ± 7.32

However, there is an evidence for a strong *oxtr* gene-dose dependency in terms of social exploration. A 50% reduction in the expression of the *oxtr* gene led to the same profound deficits in social behavior as that observed in $oxtr^{-/-}$ animals housed and tested under the same experimental conditions (Sala et al., 2011). It is worth noting that this was not observed for other behaviors such as aggression, for which the number of expressed OXTRs in $oxtr^{+/-}$ mice is compatible with normal functioning or is compensated for by other factors (Sala et al., 2013). These findings indicate that in males inactivation of the *oxtr* gene may affect specific behaviors in a dose-dependent manner: social exploration is particularly sensitive to even a partial reduction in *oxtr* gene expression, whereas the emergence of aggression may require complete inactivation of the *oxtr* gene. In our study, the OXT-induced decreased duration of social aggression and increased duration of social exploration clearly appeared to be interdependent. The fact that the prosocial explorative properties of synthetic OXT have been reported more consistently in literature than anti-aggressive effects might favor a primarily pro-explorative action. However, causal relationship cannot be established from our correlational data, hence further studies should be conducted in order to prove whether OXT primarily reduces aggression with the consequence of enhancing social exploration, or vice versa.

To note, although dependent upon species, strain, hormonal state, social experience, as well as the brain region manipulated, many examples in the literature have indeed reported that an acute increase in CSF OXT potentiates social explorative activities (Insel, 1992; Witt et al., 1992), while activation of vasopressin (AVP) receptors, especially in the anterior hypothalamus and lateral septum, promotes intermale aggressive behavior in hamsters and rats, respectively (Albers, 2012; Beiderbeck et al., 2007; Caldwell and Albers, 2004; Ferris et al., 1997). Haller and colleagues have also shown that CSF OXT levels directly correlate with the duration of social investigation, while changes in the measures of aggression significantly correlated only with CSF AVP level (Haller et al., 1996). Hence due to the strong molecular similarities between OXT and vasopressin (AVP) and their potential cross-reactivity, the behavioral profile of the central AVPergic system should also be considered when investigating the primary functional role of OXT in modulating social behaviors. Moreover, dose–response curves, longer pharmacological manipulations and co-administration studies with OXT and OXTR or AVPR antagonist should be performed to verify behavior and receptor specificity, to disprove potential cross-reactivity and to assess the minimal dose needed to modulate a specific behavior, also for clinical translation.

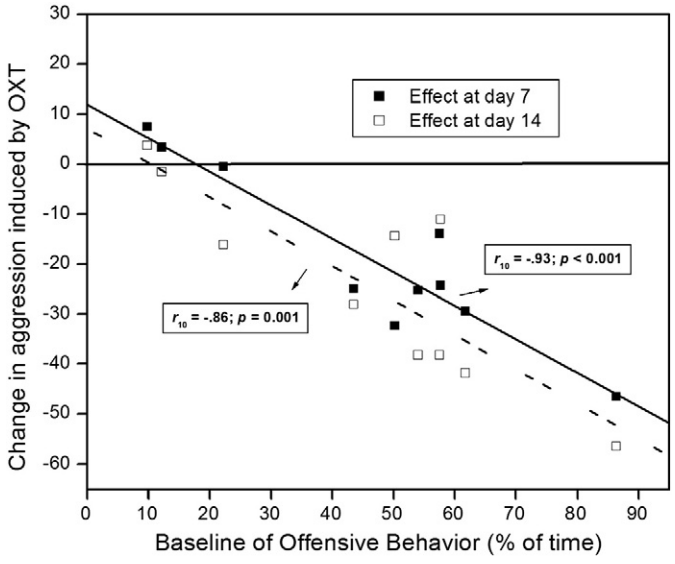

Fig. 3. Correlation between the baseline level of offensive behavior (measured at day −1 and expressed as percentage of time in the total 10 min test) and its relative change induced by OXT at day 7 (filled squares, straight line) and day 14 (open squares, dashed line).

Although our studies on WTG male rats have revealed a significant OXT-induced serenic profile, it is interesting to note that the latency to the first attack was not changed, neither in the present study, nor in our former acute administration study. In other words, synthetic OXT is ineffective in delaying the initiating phase of aggressive behavior, but selectively and potently inhibits the continuation of offensive displays. This selective action on primarily the consummatory phase of aggressive behavior suggests differences between the neuronal mechanism of this nonapeptide and other well-known serenic compounds such as the $5\text{-HT}_{1A/1B}$ receptor agonists. This well-known class of serotonergics generally elicits anti-aggressive actions through both delaying the initiation and accelerating the termination of aggressive attack bouts, often in combination with shortened duration of total social engagements (de Boer and Koolhaas, 2005; Takahashi et al., 2011). Similarly, antagonism on AVP_{1B} receptors during intermale encounters appeared to result in a sharp reduction of the duration of aggressive behavior and olfactory investigation, as well as a significant increase of the latency to attack (Blanchard et al., 2004; Koolhaas et al., 2010). Thus, the anti-aggressive effect of OXT seems to suggest a distinct mechanism of action, in which the reinforcement of positive/explorative social interactions may be responsible for the consequent attenuation of the hostile/offensive behaviors. Two explanations can be considered. (1) After the first aggressive action displayed by the resident, OXT may alter the further processing of social information by, in the first place, reducing the saliency of negative/threatening cues of the intruder, with the consequence that social explorative behavior will increase. This hypothesis finds some support by several human and non-human studies reporting that OXT facilitated social approach behavior as a consequence of reduced amygdala responsiveness to social stimuli in general (Domes et al., 2007; Lukas et al., 2013), and decreased amygdala

reactivity to social threat in particular (Coccaro et al., 2007; Kirsch et al., 2005; Viviani et al., 2011). (2) OXT might on the other hand affect the dopaminergic reward system with midbrain–striatal structures, such as the nucleus accumbens, being activated when social contact comes into play (Aragona et al., 2006). The pathways related to pro-social motivation and reward processing contain high levels of OXTRs (Insel and Shapiro, 1992), while furthermore OXT has been shown to facilitate dopamine release (Pfister and Muir, 1989). The dopaminergic system, when activated by OXT, might potentiate the positive valence of social interaction, directing the social decision-making network towards explorative approaches, rather than being implicated in the "winner effect" development (Schwartzer et al., 2013). Previous research has indeed shown that OXT facilitates affiliation and social attachment by inhibiting defensive behaviors and enhancing the value of social encounters in part by coactivation of dopaminergic circuits that are involved in motivation and reward (Campbell, 2008). As elevated intersynaptic dopamine levels have been previously associated with increased OXT in the amygdala of dams (Johns et al., 2005), central infusion of the nonapeptide might reinforce the serenic effects and increase explorative contact in our rats via activation of dopaminergic reward circuits. Hence further research should locally investigate the interactive role of OXT with other neurotransmitters.

An intriguing and unexpected finding of the present study is that both the direction, magnitude and the specificity of the behavioral effects still persisted 7 days after the cessation of the chronic icv infusion. Considering the very short half-life (about 28 min) of OXT in CSF (Veening et al., 2010) and the relatively short duration of the chronic manipulation (7 days), long-lasting effects as if due to persisting heightened levels of OXT would not have been expected. However, after chronic central infusion of OXT (100 ng/h for 10 days via an osmotic minipump), Insel and colleagues have reported a decrease in OXTR binding of as much as 95% at the time of pump removal, compared to artificial CSF-infused controls (Insel, 1992). As the reduction was observed in every brain region and as it remained for at least 24 h, it is likely that brain OXTRs are profoundly down-regulated as a consequence of sustained stimulation. However, it seems unlikely that such a compensatory neuromolecular change can explain our 7-day persistent anti-aggressive and pro-social effects. In fact, an OXTR down regulation/desensitization, possibly leading to reduced endogenous OXTergic signaling, would rather predict immediate 'withdrawal-like' pro-aggressive and anti-social effects. These rebound effects, however, may actually occur in the immediate withdrawal phase and require testing the animals immediately after cessation of the treatment.

On the other hand, continuous infusion of synthetic OXT might have altered the transcription level of the nonapeptide in the hypothalamic production sites, i.e. the paraventricular and supraoptic nuclei. It might also have elevated the background activity of slow-firing OXTergic neurons involved in the facilitatory control of the nonapeptide release (Freund-Mercier and Richard, 1984). Obviously, the current findings of enduring behavioral effects after a period of sustained enhancement of brain OXTergic signaling prompt future studies of potential treatment-induced alterations in the endogenous OXTergic system, at the level of OXTR expression or binding and mRNA peptide level or release patterns, keeping in mind that simultaneous compensatory alterations are likely to occur also in the AVP system. Moreover, although the different efficacies of the OXTergic manipulations between high and low aggressive animals may be amplified due to a rate-dependency and/or regression to the mean effect, it might be relevant to investigate further the link between individual treatment-induced alterations and the differences in trait-level of aggression and baseline properties of the OXTergic system.

Taken together, we report that a 7-day chronic infusion period with OXT selectively suppressed intermale offensive aggressive and enhanced social explorative behaviors in resident rats confronted with an unfamiliar intruder in their territory. On the other hand, chronic infusion of the OXTR antagonist increased introductory aggressive behavior. Moreover, the previously suggested inverse relationship between the trait-level of aggression and the anti-aggressive effects of exogenously administered OXT seems to be supported by the results of this chronic manipulation experiment. Finally, the persisting behavioral changes observed after OXT-treatment cessation suggest neuronal plasticity and prompt further studies to measure treatment-induced long-term alterations in the endogenous OXTergic system.

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