Neuropsychopharmacology (2014) 39, 1102–1114

www.neuropsychopharmacology.org

Chronic and Acute Intranasal Oxytocin Produce Divergent Social Effects in Mice

Huiping Huang[1], Caterina Michetti[2,3], Marta Busnelli[4,5], Francesca Managò[1], Sara Sannino[1], Diego Scheggia[1], Luca Giancardo[6], Diego Sona[6], Vittorio Murino[6], Bice Chini[5], Maria Luisa Scattoni[3] and Francesco Papaleo*[,1,7]

[1]Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Genova, Italy; [2]Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy; [3]Behavioural Neuroscience Section, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy; [4]Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Milan, Italy; [5]CNR, Institute of Neuroscience, Milan, Italy; [6]Pattern Analysis and Computer Vision, Istituto Italiano di Tecnologia, Genova, Italy; [7]Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, Padova, Italy

Intranasal administration of oxytocin (OXT) might be a promising new adjunctive therapy for mental disorders characterized by social behavioral alterations such as autism and schizophrenia. Despite promising initial studies in humans, it is not yet clear the specificity of the behavioral effects induced by chronic intranasal OXT and if chronic intranasal OXT could have different effects compared with single administration. This is critical for the aforementioned chronic mental disorders that might potentially involve life-long treatments. As a first step to address these issues, here we report that chronic intranasal OXT treatment in wild-type C57BL/6J adult mice produced a selective reduction of social behaviors concomitant to a reduction of the OXT receptors throughout the brain. Conversely, acute intranasal OXT treatment produced partial increases in social behaviors towards opposite-sex novel-stimulus female mice, while on the other hand, it decreased social exploration of same-sex novel stimulus male mice, without affecting social behavior towards familiar stimulus male mice. Finally, prolonged exposure to intranasal OXT treatments did not alter, in wild-type animals, parameters of general health such as body weight, locomotor activity, olfactory and auditory functions, nor parameters of memory and sensorimotor gating abilities. These results indicate that a prolonged over-stimulation of a 'healthy' oxytocinergic brain system, with no inherent deficits in social interaction and normal endogenous levels of OXT, results in specific detrimental effects in social behaviors.
Neuropsychopharmacology (2014) **39,** 1102–1114; doi:10.1038/npp.2013.310; published online 4 December 2013

Keywords: behavior; oxytocin receptors; social interaction; intranasal treatment; schizophrenia; autism

INTRODUCTION

The neuropeptide oxytocin (OXT) has been strongly implicated in social behaviors including social recognition, social approach, pair bonding, paternal care and maternal behavior (Lim *et al*, 2005; Sala *et al*, 2011; Winslow and Insel, 2002). This has led to the proposal of its use as an adjunctive therapeutic treatment in neuropsychiatric diseases characterized by impaired social behaviors, such as social anxiety disorder, borderline personality disorder, autism spectrum disorders and schizophrenia (Meyer-Lindenberg *et al*, 2011; Striepens *et al*, 2011). This would be particularly important as social alterations are considered among the most debilitating core features of these mental illnesses, and currently available treatments are not effective in ameliorating these symptoms.

*Correspondence: Dr F Papaleo, Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Genova, Italy, Tel: +39 010 71781786, E-mail: francesco.papaleo@iit.it
Received 6 July 2013; revised 25 October 2013; accepted 28 October 2013; accepted article preview online 4 November 2013

Intranasal administration of neuropeptides holds promising potential for the treatment of brain diseases as it might deliver biologically effective concentrations to the brain without eliciting potent systemic hormone-like side effects (Born *et al*, 2002). Intranasal administration of vasopressin (AVP) and of OXT have been reported, in rats and mice, to increase the intracerebral concentration of these peptides, not only in the cerebrospinal fluid but also in selected brain regions (Born *et al*, 2002; Neumann *et al*, 2013). However, central effects might be also due to AVP/OXT endogenous release mediated by peripheral mechanisms (Ludwig *et al*, 2013). Whatever the mechanism(s) involved, there is no doubt that central effects are observed in humans after intranasal administration (Veening and Olivier, 2013).

Acute intranasal OXT seems to produce no negative side effects in both healthy volunteers and subjects with developmental or mental health difficulties when delivered in doses of 18–40 IU (MacDonald *et al*, 2011). Moreover, in subjects diagnosed with autism, a single intranasal administration of OXT might be able to improve, at least temporarily, emotion recognition abilities in the Reading the Mind in the Eyes test and the patients' ability to process

socially relevant cues in a computer-based game (Andari *et al*, 2010; Green and Hollander, 2010; Guastella *et al*, 2010). Furthermore, in patients with schizophrenia, an adjunctive 3-week treatment with intranasal OXT has been found to significantly improve Positive and Negative Syndrome Scale (PANSS) total scores (Feifel *et al*, 2010) and verbal memory measures of the California Verbal Learning Test (Feifel *et al*, 2012a).

Despite these promising initial results, it is not yet clear if chronic intranasal OXT might affect psychiatric-relevant behavioral measures (eg, in social and memory domains), and if chronic intranasal OXT has different effects compared with single administration. This is critical for the aforementioned chronic mental disorders that might potentially involve life-long treatments. Unfortunately, ethical issues, genetic, environmental and clinical heterogeneity in human studies might dampen the resolution of these questions and thus, slow down the progress towards this new promising therapeutic treatment. In this context, preclinical studies in rodents might be critical to dissect OXT-dependent behavioral effects and hold the potential to better understand the impact of intranasal OXT in brain functioning. Finally, the possibility to test intranasal OXT treatments in mice could pave the way for large genetic and pharmacological screenings aiming to develop more selective and personalized therapeutic strategies for human pathologies, such as autism and schizophrenia.

The role of the OXT system has been investigated in rodent models, but uniquely using OXT-related mutant mice and giving OXT through intracerebroventricular (ICV), intraperitoneal (IP) and subcutaneous (SC) injections (Bielsky and Young, 2004; Jin *et al*, 2007; Popik *et al*, 1996; Sala *et al*, 2012; Sala *et al*, 2011; Takayanagi *et al*, 2005; Winslow and Insel, 2002). Only one recent study in prairie voles has initiated to investigate the behavioral effects of intranasal OXT administration before adulthood (ie, from weaning through sexual maturity; (Bales *et al*, 2012)). Thus, the present study is aimed to elucidate the behavioral effects of intranasal administration of OXT in adult mice. In order to set the ground for future studies with genetically modified mice, disease-related mouse models and facilitate inter-laboratory comparisons, we tested C57BL/6J mice. This strain of mice is classically used as the background strain for genetically modified mice and animal models of neurodegenerative and neurodevelopmental disorders. In line with clinical settings where intranasal OXT treatments would be mainly administered to address chronic symptoms in psychiatric disorders, we first performed behavioral and molecular studies in mice undergoing chronic intranasal treatment. Thereafter, we separately tested the effects of acute intranasal OXT treatment.

MATERIALS AND METHODS

Subjects

All procedures were approved by the Italian Ministry of Health (permit n. 230/2009-B) and strictly adhere to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. C57BL/6J male mice between 12 and 20 weeks of age used in this study were obtained from Charles River Laboratories (France). Animals were housed two to four per cage, in a climate-controlled animal facility (22 °C ± 2 °C) and maintained on a 12-hour light/dark cycle, with food and water available *ad libitum* throughout the experiments. All behavioral testing and procedures were conducted during the light phase of the cycle. The experimenter handled the mice on alternate days during the week preceding the first behavioral test. Experimenters were blind to the mouse treatments during testing and behavioral scoring.

Intranasal OXT Administration

OXT (Novartis Pharma AG, Switzerland) was dissolved in saline (0.9% NaCl) and administered intranasally in a volume of 5 µl to each mouse in doses of 0.15 IU/5 µl (OXT 0.15 IU) or 0.3 IU/5 µl (OXT 0.3 IU). An amount of 1 IU of our solution contained 1.667 µg of synthetic OXT. Thus, 0.15 and 0.3 IU corresponded to 0.25005 µg ($\approx 2.48^{e-10}$ mol) and 0.5001 µg ($\approx 4.96^{e-10}$ mol) of OXT, respectively, for each administration of 5 µl (ie OXT 0.15 IU ≈ 9.6 µg/kg or 5 IU/kg; OXT 0.3 IU ≈ 19 µg/kg or 11 IU/kg). These doses were chosen in order to be much lower than subcutaneous OXT doses (ie 250 µg/kg) used in mice that could have produced peripheral effects (Sala *et al*, 2011). The doses we used are also similar to the higher range of intranasal OXT doses recently given to adolescent prairie voles (Bales *et al*, 2012); even though in the previous study they used a much higher volume of injection (ie 25 µl) compared with our study (ie, 5 µl). Control mice received the same volume of saline (VEH). A 200-µl Eppendorf pipette with gel-loading tips (Costar) were used for administration. Drops of the 5 µl solution were gently placed equally on both nostrils of each mouse, which were taken in when they reflexively inhaled. Administration was rapid (less than 30 s) and handling was consistent across treatment groups. The detailed timelines of each procedural manipulation and OXT treatments are reported in Supplementary Figure 1. Briefly, for the chronic intranasal treatments, mice were administered two times a day, once in the morning and once in the evening, for 7–21 consecutive days and tested as described below, 1 hour after the last administration. The time of testing was based on preliminary pilot studies showing that chronic intranasal OXT treatments of 7, 15 and 21 days produced the same decrease in social interactions (data not shown). For the acute intranasal treatments, mice were administered with OXT only once, 5 min before the test. The delay of only 5 min was chosen based on evidence indicating that intranasal administration of OXT has very rapid pharmacokinetics with effects expected to appear already within a few minutes (Veening and Olivier, 2013).

Behavioral Testing

Body weight and gross physical appearance. Body weights and general appearance of the mice were recorded before each VEH or OXT administration.

Male–female social interaction test. The test was conducted in 2150E Tecniplast cages (35.5 × 23.5 × 19 cm) lightly illuminated (5 ± 1 lux) and video-recorded using a Unibrain Fire-i digital camera. The video camera was mounted facing the front of the cage to record the session

for subsequent scoring of social investigation parameters as previously described (Scattoni *et al*, 2011). Unfamiliar female stimulus mice in estrus were matched to the subject male mice by age and maintained in social groups of four per home cage. In a subgroup of animals, ultrasonic vocalizations (USVs) were also recorded during the test. See Supplementary Materials and Methods for a detailed description.

Male–male cagemate social interaction test. A day after the male–female interaction test, each cage of male mice was placed in a grey, opaque open-field box ($40 \times 40 \times 40$ cm) evenly illuminated with overhead red lighting (5 ± 1 lux) in the room. Interactions between cagemates were recorded using an infrared thermal camera (FLIR A315, FLIR Systems) mounted 1.5 m above the arena for 1 hour. Social interaction was scored by an automatic videosystem as previously described (Giancardo *et al*, 2013).

Olfactory abilities. Mice were individually tested for time spent sniffing sequential presentations of different odors: water, two nonsocial odors (ie almond extract and vanilla extract, Belbake 1 : 20 dilution), and two social odors as similarly reported previously (Papaleo *et al*, 2012). The test was performed on a separate cohort of naïve mice at the same time-point at which the social interaction test would have been performed to verify their olfactory abilities after chronic intranasal OXT administration.

Locomotor activity and temporal order object recognition (TOR) test. Mice were tested in an experimental apparatus consisting of an opaque open-field box ($40 \times 40 \times 40$ cm) with even, overhead red-light illumination as mentioned above. Each session was video-recorded using an overhead camera from ANY-maze (Stoelting). Each mouse was monitored for its locomotor activity in the empty open-field boxes for 1 hour. The next day, in the TOR test, the subjects' ability to differentiate between two objects presented at different intervals was assessed as previously described (Barker *et al*, 2007). See Supplementary Materials and Methods for more details.

Acoustic startle response and prepulse inhibition (PPI). Mice were tested in Startle Response/PPI test system chambers (TSE Systems GmbH, Germany) using standard methods as previously described (Papaleo *et al*, 2008; Papaleo *et al*, 2012). In short, a sudden acoustic stimulus elicits the startle response, while an acoustic, non-startling prepulse preceding the startle stimulus inhibits the startle response (PPI). The startle response elicited by sudden sensory stimuli and its PPI are among some of the most widely studied phenotypes that are highly conserved across mammalian species.

Habituation/dishabituation social interaction test. Naïve mice were tested as similarly reported previously (Ferguson *et al*, 2000; Scearce-Levie *et al*, 2008) in 2150E Tecniplast cages ($35.5 \times 23.5 \times 19$ cm) lightly illuminated (5 ± 1 lux) and video-recorded using a Unibrain Fire-i digital camera. Male mice were individually placed in the testing cage 1 h prior to the testing. No previous single-housing manipulation was adopted to avoid any instauration of home-cage territory and aggressive behaviors. Testing began 5 min after the intranasal treatment when a stimulus male mouse was introduced into the testing cage for a 1-min interaction. At the end of the 1-min trial, we removed the stimulus animal and returned it to an individual holding cage. We repeated this sequence for three trials with 3-min inter-trial intervals introducing the same stimulus mouse in all three trials. In a fourth 'dishabituation' trial, we introduced a new unfamiliar stimulus mouse in the testing cage. Videos of behaviors were recorded and subsequently scored offline.

Brain Autoradiography

A separate cohort of naïve mice was handled and intranasally treated with VEH or OXT as described above and, at the same time-point when the social interaction test would have been performed, their brains were rapidly dissected and frozen in isopentane at $-25\,^{\circ}$C and then stored at $-80\,^{\circ}$C. Brains were cut in coronal sections of 14-μm thickness and mounted on chrome-alum-gelatin-coated microscope slides. All slides were stored at $-80\,^{\circ}$C until used in receptor autoradiography. The binding procedure was performed as previously described (Tribollet *et al*, 1997). For a detailed description, see Supplementary Materials.

Statistical Analyses

Results are expressed as mean ± standard error of the mean (SEM) throughout. One- or two-way analyses of variance (ANOVAs) were used for each single parameter measured. Newman–Keul's *post-hoc* test was used for making comparisons between groups when the overall ANOVA showed statistical significant differences for the main factors. The accepted value for significance was $p \leqslant 0.05$. All statistical analyses were performed using the Statistica version 11 software (Statistica, StatSoft).

RESULTS

Chronic Intranasal OXT Treatment Decreased Male–Female Social Interaction

The OXT system has been extensively involved in the modulation of social-related behaviors (Bielsky and Young, 2004; Sala *et al*, 2012; Sala *et al*, 2011; Winslow and Insel, 2002). Thus, to assess possible effects of chronic intranasal OXT treatment in social abilities, we first tested mice chronically treated with intranasal OXT or VEH in a classical male–female social interaction test (Scattoni *et al*, 2011).

A significant OXT-treatment effect was evident for body sniffing (frequency: $F_{(2,43)} = 5.69$, $p < 0.05$; duration: $F_{(2,43)} = 10.14$, $p < 0.0005$) and anogenital sniffing (frequency: $F_{(2,43)} = 4.79$, $p < 0.05$; duration: $F_{(2,43)} = 3.86$, $p < 0.05$). In particular, compared with the VEH-treated group, male mice treated with 0.15 or 0.3 IU OXT showed decreased frequency and duration of body sniffing events ($p < 0.05$; Figure 1a). In addition, for the 0.3-IU OXT group, there was also a significant decrease in the number of anogenital sniffing events ($p < 0.05$; Figure 1a) and in their duration

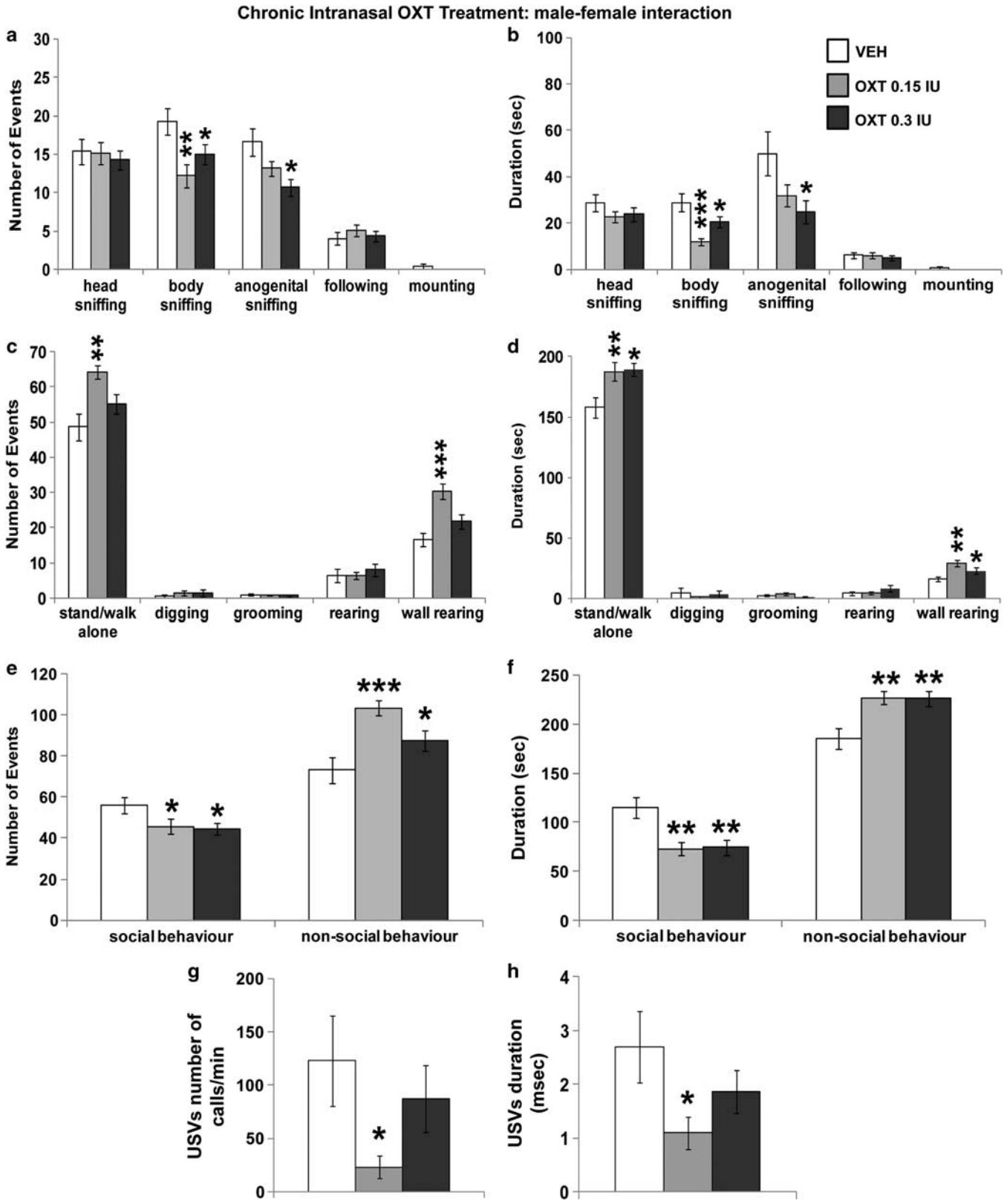

Figure 1 Chronic intranasal OXT treatment decreased male–female social interaction. (a) Frequency of occurrence of various social behaviors such as head sniffing, body sniffing, angogenital sniffing and following. (b) Duration of events of above-mentioned social behaviors. (c) Frequency of occurrence of various nonsocial behaviors such as standing/walking alone, digging, grooming, rearing and wall rearing. (d) Duration of events of above-mentioned nonsocial behaviors. (e) Sum of the frequency of all events of social and nonsocial behaviors. (f) Sum of the duration of all events of social and nonsocial behaviors. Ns = 14 VEH, 16 OXT 0.15 IU/5 μl, 16 OXT 0.3 IU/5 μl. (g) Mean number of USV calls per minute, and (h) mean duration of USVs in milliseconds emitted by VEH-, OXT 0.15- or OXT 0.3-treated mice. Ns = 9 VEH, 16 OXT 0.15 IU/5 μl, 10 OXT 0.3 IU/5 μl. *$p<0.05$, **$p<0.005$, ***$p<0.0005$ *vs* VEH group. Values represent mean ± SEM throughout all Figures.

($p<0.05$; Figure 1b) relative to the VEH group. An effect of OXT treatment was also observed in nonsocial behaviors such as standing/walking alone (frequency: $F_{(2,43)}=7.68$, $p<0.005$; duration: $F_{(2,43)}=5.95$, $p<0.05$) and wall rearing (frequency: $F_{(2,43)}=10.98$, $p<0.0005$; duration: $F_{(2,43)}=6.70$, $p<0.005$). Specifically, *post-hoc* tests revealed an increase in the duration of standing/walking alone ($p<0.05$), increases in the frequency and duration of wall rearing in the OXT 0.15-IU group ($p<0.005$), and an increase in the duration of wall rearing in the OXT 0.3-IU group ($p=0.05$; Figure 1c and d).

No significant effect of OXT treatment was observed in other measures of social interaction such as head sniffing (frequency: $F_{(2,43)}=0.17$, $p=0.84$; duration: $F_{(2,43)}=1.11$, $p=0.34$), following (frequency: $F_{(2,43)}=0.47$, $p=0.63$; duration: $F_{(2,43)}=0.36$, $p=0.70$), and mounting (frequency: $F_{(2,43)}=2.41$, $p=0.14$; duration: $F_{(2,43)}=2.33$, $p=0.11$; Figure 1a and b). Similarly, in comparison with the VEH group, both OXT 0.15 and 0.3 IU groups showed no significant differences in nonsocial behaviors such as digging (frequency: $F_{(2,43)}=0.45$, $p=0.64$; duration: $F_{(2,43)}=0.27$, $p=0.77$), grooming (frequency: $F_{(2,43)}=0.04$, $p=0.96$; duration: $F_{(2,43)}=1.43$, $p=0.25$) and rearing (frequency: $F_{(2,43)}=0.38$, $p=0.68$; duration: $F_{(2,43)}=1.57$, $p=0.22$; Figure 1c and d).

Overall, there was a significant effect of OXT in social behaviors (frequency: $F_{(2,43)}=3.10$, $p<0.05$; duration: $F_{(2,43)}=8.08$, $p<0.005$) and in nonsocial behaviors (frequency: $F_{(2,43)}=9.13$, $p<0.0005$; duration: $F_{(2,43)}=7.95$, $p<0.005$). In particular, *post-hoc* tests showed a decrease in the frequency and duration of social behaviors in both OXT 0.15 and 0.3 IU OXT-treated groups ($p<0.05$) and an increase in the frequency and duration of nonsocial behaviors of both OXT-treated groups ($p<0.005$; Figure 1e and f) compared with VEH-treated mice.

During the male–female social interaction test, it is also possible to record USVs emitted by the male mice that are considered facets of social communication among rodents (Scattoni *et al*, 2011). OXT treatment showed a significant effect in the mean number of vocalizations emitted ($F_{(2,32)}=4.16$, $p<0.05$) and the duration of the USVs ($F_{(2,32)}=3.45$, $p<0.05$). In particular, the OXT 0.15-IU group of mice showed a decrease in the number ($p<0.05$) and duration ($p<0.05$; Supplementary Figure 2) of vocalizations emitted compared with the VEH group. There were no differences in the peak frequencies ($F_{(2,32)}=1.56$, $p=0.23$) and amplitudes ($F_{(2,32)}=1.54$, $p=0.23$; Figure 1g and h, and Supplementary Figure 2) between the three groups. Overall, these findings indicate that chronic intranasal OXT treatment in normal C57BL/6J mice reduces social interaction behaviors towards opposite-sex female mice.

Chronic Intranasal OXT Treatment Decreased Male–Male Social Interaction

To test whether chronic intranasal OXT treatment might also impact same-sex social interactions, we used a novel, automatic system algorithm which is able to precisely track and simultaneously characterize social and nonsocial behavioral interactions of multiple mice (Giancardo *et al*, 2013).

OXT treatment produced a significant effect in the duration of body sniffing ($F_{(2,23)}=4.90$, $p<0.05$) and following ($F_{(2,23)}=6.82$, $p<0.005$). Specifically, the OXT 0.3-IU group showed a decrease in the duration of body sniffing ($p<0.05$) and following ($p<0.05$; Figure 2a) as compared with the VEH group. There was also a decrease in the duration of following behavior in the OXT 0.15 IU relative to the VEH group ($p<0.005$; Figure 2a). No significant effects of OXT treatment were observed in the duration of other social behaviors such as head sniffing ($F_{(2,23)}=1.01$, $p=0.38$), anogenital sniffing ($F_{(2,23)}=1.70$, $p=0.21$), and above ($F_{(2,23)}=1.02$, $p=0.38$). On the other hand, in nonsocial behaviors, a significant effect of OXT treatment was observed in the duration of walking alone ($F_{(2,23)}=5.96$, $p<0.05$) and standing alone ($F_{(2,23)}=3.35$, $p=0.05$). Primarily, the OXT 0.15-IU group presented a decrease in the duration of walking alone and standing alone compared with the VEH group ($p<0.05$; Figure 2b).

In summary, there was a significant OXT treatment effect in the total duration of social behaviors ($F_{(2,23)}=3.32$, $p=0.05$; Figure 2c) while no significant treatment effect was found in the total duration of nonsocial behaviors ($F_{(2,23)}=3.02$, $p=0.07$). Notably, the OXT 0.3-IU group presented a decrease in the total duration of social behaviors ($p<0.05$; Figure 2c) in comparison with the VEH group. Overall, in agreement with the male–female interaction test, these findings strongly suggest that chronic intranasal OXT treatment in normal C57BL/6J male mice reduces social behavioral measures.

Chronic Intranasal OXT Treatment did not Affect Body Weight, General Olfactory and Locomotor Abilities

In order to rule out possible confounding factors that might have produced the social deficits found in OXT-chronically-treated mice, we analyzed body weight, olfactory abilities and gross locomotor activity following chronic intranasal OXT treatment.

There was no significant effect of OXT treatment on body weight ($F_{(2,37)}=0.85$, $p=0.44$) and no significant effect of treatment × time interaction ($F_{(6,111)}=1.62$, $p=0.15$). Thus, chronic intranasal OXT treatment did not alter the body weight of mice, up to 21 days of administration (Supplementary Figure 3).

Olfactory abilities were tested by presenting the mice with different nonsocial and social odors after the same OXT intranasal treatment given in the social interaction tests. No effect of chronic intranasal OXT treatment was detected between the VEH- and OXT-treated groups ($F_{(2,14)}=0.01$, $p=0.99$), neither there was an effect of treatment × odor interaction ($F_{(8,56)}=0.69$, $p=0.70$). There was an effect of the odor stimulus ($F_{(4,56)}=118.90$, $p<0.0005$) with the mice spending more time sniffing the social odors as compared with the nonsocial odors ($p<0.0005$; Supplementary Figure 4).

Gross locomotor functions were assessed in an empty open-field arena. For the measure of total distance traveled, there was an effect of treatment ($F_{(2,43)}=3.77$, $p<0.05$), an effect of time ($F_{(11,473)}=47.35$, $p<0.0005$) and a treatment × time interaction ($F_{(22,473)}=1.62$, $p<0.05$). In particular, OXT 0.15 IU-treated mice showed slightly reduced total distance traveled at time-points 20, 30, 55

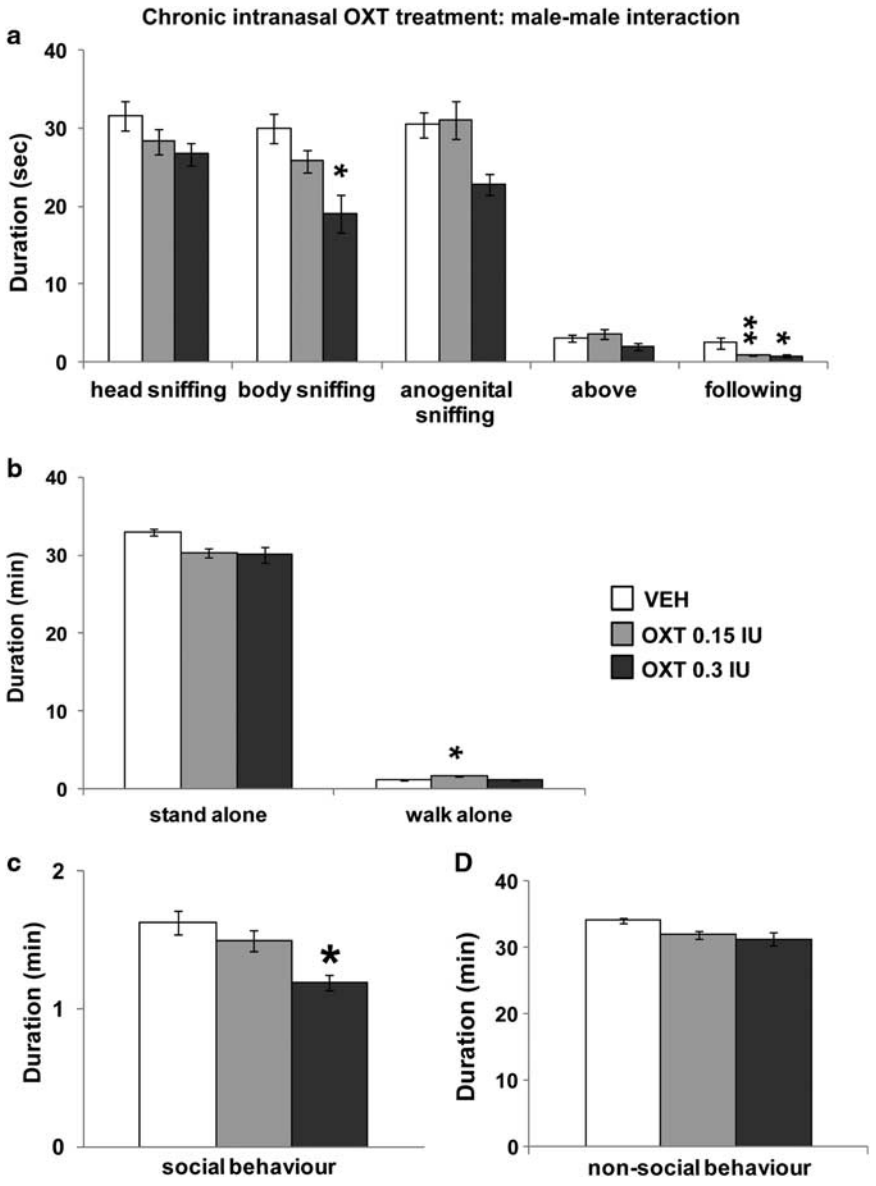

Figure 2 Chronic intranasal OXT treatment decreased male–male social interaction. (a) Duration of occurrence of various social behaviors such as head sniffing, body sniffing, anogenital sniffing, going on top of another mouse (above) and following. (b) Duration of occurrence of various nonsocial behaviors such as walking alone and standing alone. Sum of the duration of all events of above-mentioned (c) social behaviors and (d) nonsocial behaviors. Note that in this experiment we used cagemates in order to avoid any possible aggressive behavior. Ns = 6 VEH, 16 OXT 0.15 IU/5 μl, 4 OXT 0.3 IU/5 μl. *$p<0.05$, **$p<0.005$ *vs* VEH.

and 60 min compared with VEH mice ($p<0.05$; Supplementary Figure 5). OXT 0.3 IU-treated mice showed a slight decrease in distance traveled only at the 5-min time-point relative to VEH mice ($p<0.05$; Supplementary Figure 5). All three groups of mice decreased the total distance traveled over time ($p<0.0005$), indicating normal habituation to the novelty of the open-field arena. For the measure of time spent being mobile, there was neither an effect of treatment ($F_{(2,43)}=0.21$, $p=0.81$), nor a treatment × time interaction ($F_{(2,473)}=1.4$, $p=0.11$). All three groups of mice showed a decrease in the time they were mobile over the 1-hour test ($F_{(11,473)}=23.42$, $p<0.0005$; Supplementary Figure 5). For the measure of time spent being immobile, no effect of treatment ($F_{(2,43)}=0.21$, $p=0.81$) or treatment × time interaction was observed ($F_{(2,473)}=1.4$, $p=0.11$). All three groups of mice showed an increase in the time they spent immobile over the 1-hour test ($F_{(11,473)}=23.41$, $p<0.0005$; Supplementary Figure 5). Hence, these results indicate that the social behavioral deficits found in C57BL/6J mice chronically treated with intranasal OXT were not dependent on alterations of body weight, olfactory abilities or gross locomotor activity.

Chronic Intranasal OXT Treatment did not Alter Temporal Order Object Recognition Memory Functions

OXT administration has been suggested to affect also nonsocial recognition memory functions with generally detrimental effects in healthy subjects and perhaps advan-

tageous effects in schizophrenia (Feifel *et al*, 2012a; Heinrichs *et al*, 2004; Herzmann *et al*, 2012). To evaluate whether chronic intranasal OXT treatment might affect recognition memory, we tested VEH- and OXT-intranasally-treated mice in a temporal order object recognition task. Lesion and disconnection studies in rodents (Barker *et al*, 2007; Barker and Warburton, 2011) have demonstrated that memory functions measured in this task are critically dependent on an integrated information flow between the medial prefrontal cortex (mPFC), the perirhinal cortex (PRH) and the hippocampus.

The performances of VEH-treated and OXT-treated mice in this task were equal ($F_{(2,41)} = 0.14$, $p = 0.87$; Supplementary Figure 6). Similar to VEH-treated mice, OXT 0.15-IU and 0.3-IU groups showed positive discrimination between the object presented least recently (from sample phase 1) and the object presented most recently (from sample phase 2). These results suggest that chronic intranasal OXT treatment did not alter object memory abilities that depend on intact mPFC, hippocampus and PRH in wild-type C57BL/6J mice.

Acoustic Startle and Prepulse Inhibition are Unaltered by Chronic Intranasal OXT Treatment

Systemic subcutaneous injection of exogenous OXT might affect startle responses and prepulse inhibition (PPI) of an acoustic startle in rats (Feifel *et al*, 2012b). PPI is a measure of inhibitory sensorimotor gating abilities with high translational validity, as it can be studied in both mice and humans, and is found to be attenuated in patients with schizophrenia and autism spectrum disorders (Barker and Warburton, 2011; Braff *et al*, 2001; Kumari *et al*, 2005; Papaleo *et al*, 2008; Papaleo *et al*, 2012). Thus, we explored the startle response and PPI in mice chronically treated with intranasal VEH or OXT.

There was no effect of treatment in either acoustic startle reactivity to the 120-dB stimulus or basal activity in the apparatus when no stimulus was presented ($F_{(2,77)} = 0.69$, $p = 0.50$; Supplementary Figure 6). There was no effect of interaction of treatment and acoustic startle reactivity ($F_{(2,77)} = 0.77$, $p = 0.47$). Similarly, analysis of PPI with a 120-dB acoustic startle stimulus showed no effect of treatment ($F_{(2,77)} = 0.07$, $p = 0.93$) or treatment × PPI interaction ($F_{(8,308)} = 0.78$, $p = 0.62$; Supplementary Figure 6). These results indicate that chronic intranasal OXT treatment did not alter reactivity to stressful events or sensorimotor gating abilities in healthy C57BL/6J mice.

Chronic Intranasal OXT Treatment Decreased OXT Receptors in the Brain

Upon agonist stimulation, OXT receptors (OXTRs) can undergo desensitisation and internalization (Conti *et al*, 2009). To evaluate whether chronic intranasal OXT treatment might affect the number of OXTRs in the brains of mice, we then quantified them using an autoradiography binding assay. As the functionally related vasopressin V1a receptor (V1aR) can be potentially up-/downregulated following OXT treatment, we also quantified V1aR expression by autoradiography.

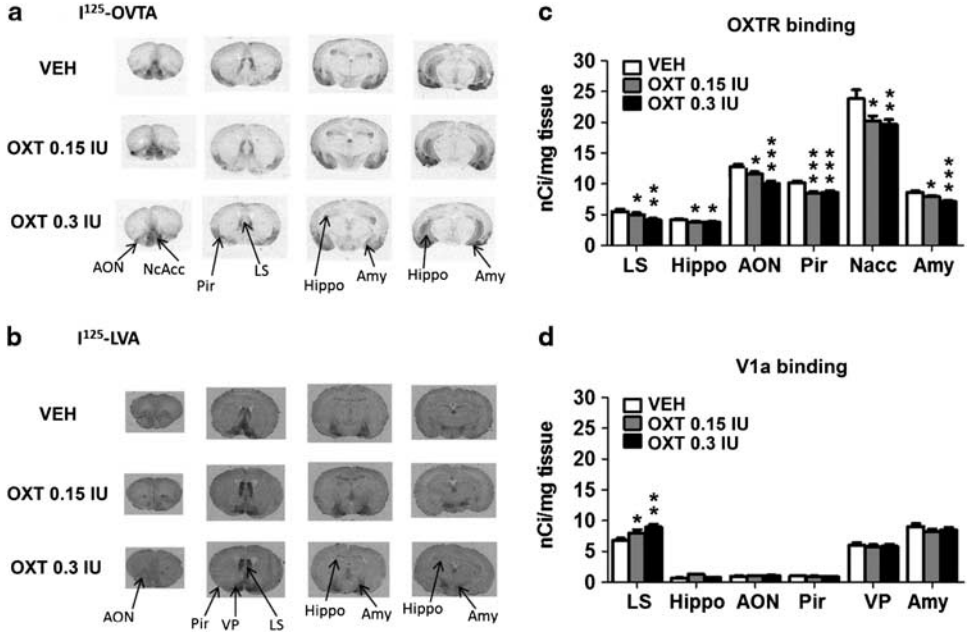

Figure 3 Chronic intranasal OXT treatment decreased OXT receptors in various brain areas. Representative autoradiographs showing the rostro-caudal ligand binding (a) of 20 pmol/l I^{125}-labeled OVTA, a potent and selective ligand for OXTR and (b) of 20 pmol/l I^{125}-labeled linear vasopressin antagonist (LVA), a potent and selective ligand for V1aR. Autoradiograms were obtained from coronal sections of 3-month-old brains of mice chronically treated with intranasal OXT 0.15 IU/5 μl (OXT 0.15), OXT 0.3 IU/5 μl (OXT 0.3) or vehicle (VEH). (c and d) Quantification of the autoradiographic I^{125-} receptors was obtained using NIH ImageJ- Software. Data is expressed as nCi/mg tissue equivalent. Amy, amygdala; AON, anterior olfactory nucleus; Hippo, hippocampus; LS, lateral septum; NAcc, nucleus accumbens; PIR, piriform cortex; VP, ventral pallidum. Ns = 7 for each group; *$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$ *vs* VEH.

As shown in Figure 3, mice treated with OXT had a significant decrease in OXTR-binding sites in all of the regions considered. The higher dose, OXT 0.30 IU, caused a significantly ($p<0.005$) greater reduction in OXTR binding than the lower OXT 0.15 IU dose in the lateral septum (-25 *vs* -9%), anterior olfactory nucleus (-21 *vs* -9%) and amygdala (-18 *vs* -8%); whereas the two doses had comparable effects in hippocampus (-10 *vs* -9%), piriform cortex (-15 *vs* -17%) and nucleus accumbens (-17 *vs* -15%).

Interestingly, we observed that chronic intranasal OXT treatment increased the V1aR-binding sites in the lateral septum, that is, $+18\%$ in OXT 0.15 IU-treated mice and $+31\%$ in OXT 0.3 IU-treated mice (Figure 3b). In contrast, there was no effect of the OXT treatment on V1aR-binding sites in all the other regions considered, including hippocampus, anterior olfactory nucleus, piriform cortex, ventral pallidum and amygdala.

These results clearly show that chronic intranasal OXT treatment reduced OXTRs throughout the brain whereas having much less impact on V1aR vasopressin receptors.

Acute Intranasal OXT Treatment Increased Male–Female Social Interaction

Chronic intranasal OXT selectively altered social behavioral measures. To investigate whether a single acute intranasal administration of OXT would have different effects as chronic treatments, we intranasally administered VEH, OXT 0.15 IU or OXT 0.3 IU to male mice, just 5 min before performing the same social interaction tests as performed after chronic intranasal OXT.

There was a significant effect of OXT treatment in the frequency of anogenital sniffing events ($F_{(2,14)}=8.05$, $p<0.005$). Male mice treated with a single administration of 0.15 and 0.3 IU OXT before the test showed increases in the frequency of anogenital sniffing events as compared with the VEH group ($p<0.05$; Figure 4a). The number of body-sniffing events display a trend of OXT-dependent dose-response increase ($F_{(2,14)}=8.05$, $p=0.09$; Figure 4a). No significant effect of OXT treatment was observed in the frequency of other measures of social interaction such as head sniffing ($F_{(2,14)}=0.12$, $p=0.89$) and following ($F_{(2,14)}=0.92$, $p=0.42$; Figure 4a). Similarly, there was no significant effect of OXT treatment in the duration of social behaviors, such as head sniffing ($F_{(2,14)}=0.09$, $p=0.91$), body sniffing ($F_{(2,14)}=1.34$, $p=0.29$), anogenital sniffing ($F_{(2,14)}=0.56$, $p=0.58$) and following events ($F_{(2,14)}=2.62$, $p=0.11$; Figure 4b). Both OXT 0.15 and 0.3 IU groups showed no significant differences in the frequency and duration of all nonsocial behaviors including standing/walking alone (frequency: $F_{(2,14)}=0.65$, $p=0.54$; duration: $F_{(2,14)}=0.51$, $p=0.61$), digging (frequency: $F_{(2,14)}=1.24$, $p=0.32$; duration: $F_{(2,14)}=1.24$, $p=0.32$), grooming (frequency: $F_{(2,14)}=0.51$, $p=0.61$; duration: $F_{(2,14)}=0.20$, $p=0.82$), rearing (frequency: $F_{(2,14)}=2.35$, $p=0.13$; duration: $F_{(2,14)}=1.34$, $p=0.29$), wall rearing (frequency: $F_{(2,14)}=1.98$, $p=0.17$; duration: $F_{(2,14)}=1.12$, $p=0.35$), in comparison with the VEH group (Figure 4c and d).

Overall, there was a significant OXT treatment effect in the frequency of total social behaviors ($F_{(2,14)}=4.09$, $p<0.05$) but no treatment-dependent differences in the frequency of total nonsocial behaviors ($F_{(2,14)}=0.69$, $p=0.52$). The OXT 0.3-IU group showed increased total social behaviors compared with the VEH group ($p<0.05$; Figure 4e).

No significant difference was observed in the duration ($F_{(2,14)}=2.25$, $p=0.14$), mean number ($F_{(2,14)}=0.67$, $p=0.53$), peak frequencies ($F_{(2,14)}=0.42$, $p=0.67$) and amplitudes ($F_{(2,14)}=0.36$, $p=0.70$) of the USVs emitted by the OXT-treated groups compared with the VEH group (Figure 4g and h and Supplementary Figure 7).

Overall, these findings indicate that, in contrast to chronic treatments, acute intranasal OXT administration in normal C57BL/6J mice increases social interaction behaviors towards opposite-sex female mice.

Acute Intranasal OXT Treatment did not Affect Social Interaction between Familiar Males

To check if acute intranasal OXT treatment might also impact same-sex social interactions, 5 min after a single intranasal administration of VEH, OXT 0.15 IU or OXT 0.3 IU, male mice were evaluated for interactions with their cagemates in an open-field arena as done after chronic intranasal OXT (Figure 5).

No significant effects of acute intranasal OXT administration were evident in any of the measures of social behaviors between the male–male cagemates, such as head sniffing ($F_{(2,15)}=0.60$, $p=0.56$), body sniffing ($F_{(2,15)}=1.68$, $p=0.22$), anogenital sniffing ($F_{(2,15)}=0.94$, $p=0.41$) going on top of another mouse—'above' ($F_{(2,15)}=2.31$, $p=0.13$) and following ($F_{(2,15)}=0.57$, $p=0.58$). Similarly, there were no significant effects of a single intranasal OXT administration in any of the nonsocial behavior measures, namely, standing alone ($F_{(2,15)}=0.46$, $p=0.64$) and walking alone ($F_{(2,15)}=0.31$, $p=0.74$). Thus, in contrast to the chronic treatments, acute intranasal OXT produced no major social interaction changes between male–male familiar cagemates.

Acute Intranasal OXT Treatment Reduced Social Interaction between Unfamiliar Males

To test if the lack of effects in the previous male–male social interaction test following acute intranasal OXT might have been dependent on the familiarity of the social stimulus, 5 min after a single intranasal administration of VEH, OXT 0.15 IU or OXT 0.3 IU, male mice were evaluated in a habituation/dishabituation social memory test.

A significant OXT treatment $\times$ trial interaction effect was evident for the duration of social ($F_{(6,111)}=3.49$, $p=0.003$; Figure 5e) and nonsocial behaviors ($F_{(6,111)}=2.95$, $p=0.01$; Figure 5f). As expected, VEH-treated mice showed a characteristic decline ($p<0.01$) in the time spent investigating the same stimulus male, with a full recovery following the introduction of a new unfamiliar male ($p<0.001$). In contrast, both OXT 0.15- and 0.3 IU-treated groups showed no changes (p's >0.80) in the exploration of unfamiliar males. Almost no aggressive/fighting behaviors were observed (ie, VEH group: 1 mouse out of 13; OXT 0.15-IU group: 1 out of 14; OXT 0.3-IU group: 1 out of 13) with no OXT treatment ($F_{(2,37)}=1.14$, $p=0.33$) or OXT treatment $\times$ trial interaction effects ($F_{(6,111)}=1.15$, $p=0.34$). Thus, in agreement with the previous experiment, acute intranasal

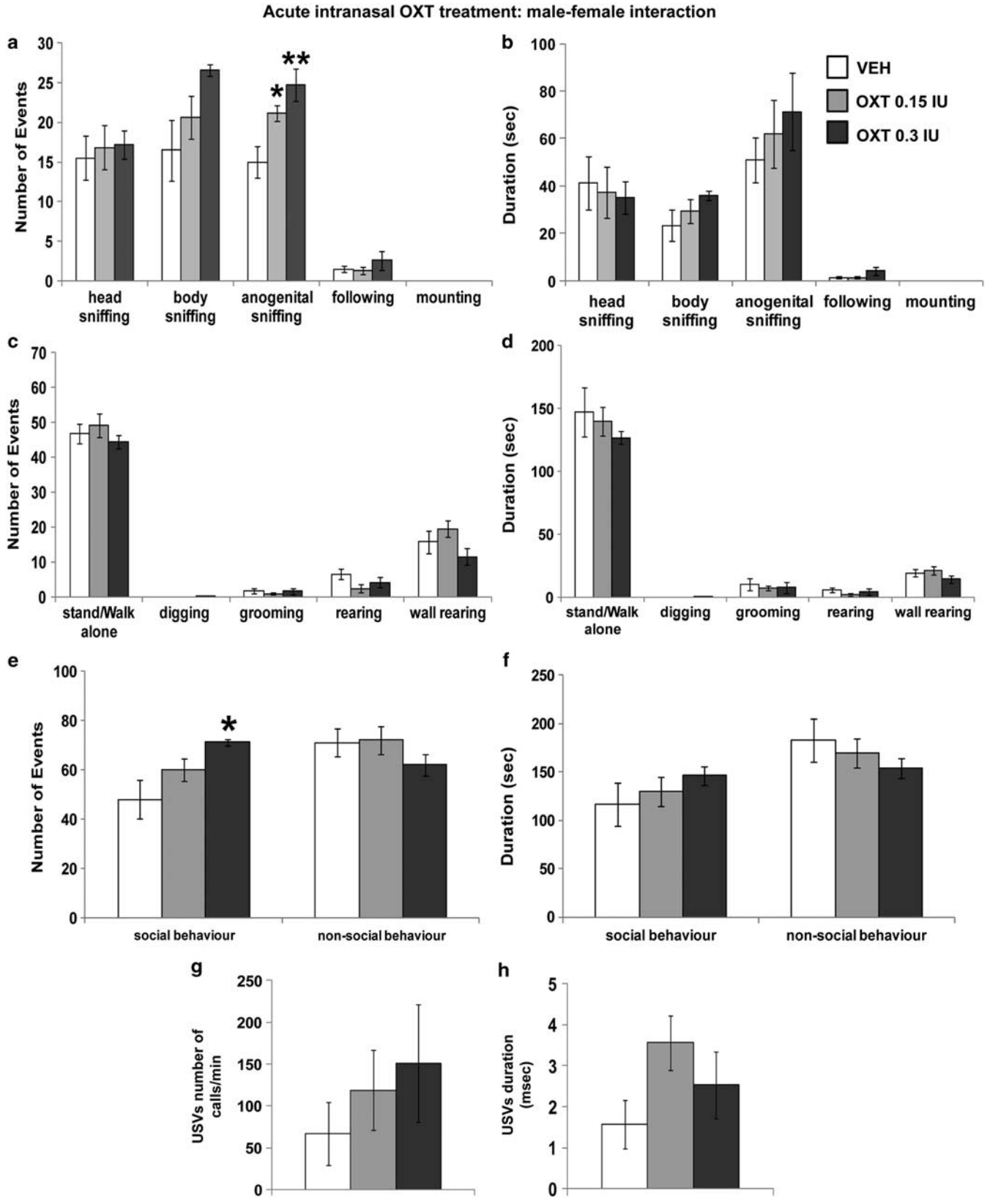

Figure 4 Acute intranasal OXT treatment increased male–female social interaction. (a) Frequency of occurrence of various social behaviors such as head sniffing, body sniffing, angogenital sniffing and following in male mice towards female stimulus mice after a single acute dose of saline (VEH), OXT 0.15 IU/5 μl or 0.3 IU/5 μl. (b) Duration of events of above-mentioned social behaviors. (c) Frequency of occurrence of various nonsocial behaviors such as standing/walking alone, digging, grooming, rearing and wall rearing. (d) Duration of events of above-mentioned nonsocial behaviors. (e) Frequency of all events of social and nonsocial behaviors. (f) Duration of all events of social and nonsocial behaviors. (g) Mean number of USV calls per minute, and (h) mean duration of USVs in milliseconds. Ns = 6 acute VEH, 6 acute OXT 0.15 IU/5 μl, 6 acute OXT 0.3 IU/5 μl. *$p < 0.05$, **$p < 0.005$ *vs* VEH.

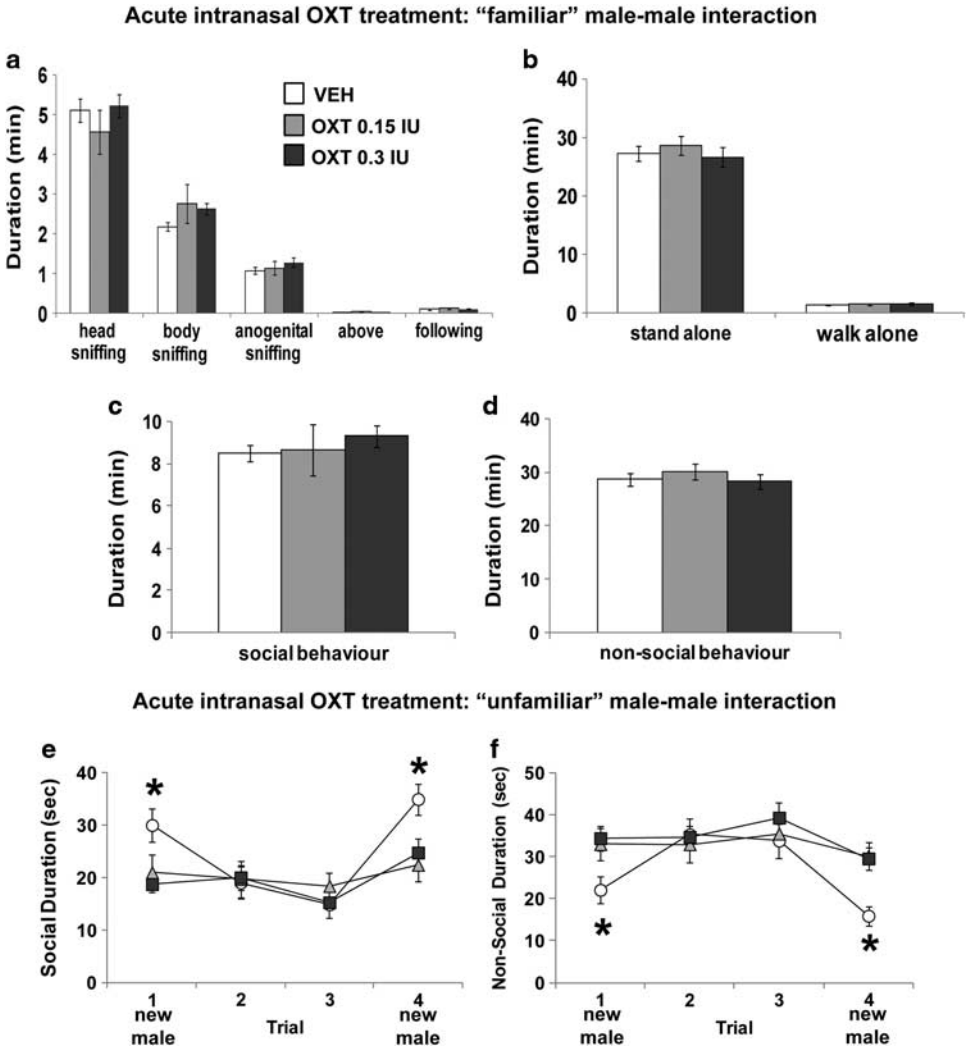

Figure 5 Acute intranasal OXT treatment did not alter social interaction between familiar males but reduced it between unfamiliar males. (a) Duration of occurrence of various social behaviors such as nose-to-body, nose-to-nose, nose-to-back sniffing, going on top of another mouse—'above' and following in male mice towards male cagemates after a single acute dose of saline (VEH), OXT 0.15 IU/5 μl or 0.3 IU/5 μl. (b) Duration of occurrence of various nonsocial behaviors such as walking alone and standing alone. Sum of the duration of all events of the above-mentioned (c) social and (d) nonsocial behaviors. Ns = 6 acute VEH, 6 acute OXT 0.3 IU/5 μl, 6 acute OXT 0.15 IU/5 μl. (e and f) Data depict the amount of time in seconds allocated to (e) investigation of the same unfamiliar male and (f) other nonsocial behaviors in the cage but not towards the stimulus mouse during each of three successive 1-min trials. A fourth 'dishabituation' trial depicts the response of the subject males to the presentation of a new unfamiliar male in a 1-min pairing 3 min after the third trial. Ns = 13 acute VEH, 14 acute OXT 0.15 IU/5 μl, 13 acute OXT 0.3 IU/5 μl. *$p < 0.05$ *vs* trials 2 and 3.

OXT treatment did not alter social exploration of a familiar male whereas; in contrast, it reduced exploration of an unfamiliar male.

DISCUSSION

The main finding of the present study was that, in wild-type C57BL/6J mice, chronic and acute intranasal OXT produced selective but divergent effects in social behavior. In particular, chronic intranasal OXT treatment produced a selective reduction of social behaviors indiscriminately towards same or opposite sex and towards familiar or unfamiliar subjects. Chronic intranasal OXT concomitantly produced a reduction of OXT receptors throughout the brain. Conversely, acute intranasal OXT treatment increased social behaviors towards opposite-sex novel-stimulus females, did not alter social interactions in same-sex familiar males, and reduced social behaviors towards same-sex novel-stimulus male mice. Finally, prolonged exposure to intranasal OXT treatments did not alter, in wild-type animals, neither parameters of general health, such as body weight, gross locomotor activity, olfactory and auditory functions, nor parameters of memory and sensorimotor gating abilities.

C57BL/6J male mice chronically treated with intranasal OXT decreased social interaction behaviors towards opposite-sex novel-stimulus female mice as well as towards same-sex familiar cagemates. Concomitantly, chronic intranasal OXT treatment decreased the number of USVs, revealing that this treatment consistently reduced both social behaviors and communication. Intranasal OXT

administration in healthy humans have also indicated OXT's modulation of psychosocial functions, such as reducing endocrinal and behavioral responses to social stress, enhancing stress-buffering effects of social support (Heinrichs *et al*, 2003), attenuating activation of amygdala to social stimuli (Baumgartner *et al*, 2008; Domes *et al*, 2007a; Gamer *et al*, 2010; Kirsch *et al*, 2005), improving social and emotion recognition (Domes *et al*, 2007b; Guastella *et al*, 2008; Rimmele *et al*, 2009), attachment (Buchheim *et al*, 2009) and heterosexual couples interaction (Ditzen *et al*, 2009). Despite this, there is a lack of information on long-term effects of intranasal OXT in healthy subjects; as only recently, one study has started to investigate the effects of chronic intranasal OXT administration in prairie voles (Bales *et al*, 2012). Thus, a possible reason for the apparent discrepancies between our findings and current evidence from human studies regarding OXT-induced effects in social-related behaviors might be that a prolonged over-stimulation of a 'healthy' oxytocinergic system, with no inherent deficits in social interaction and normal endogenous levels of OXT, might result in detrimental effects. Indeed, human studies have prevalently used acute administration of OXT in healthy subjects (Baumgartner *et al*, 2008; Buchheim *et al*, 2009; Ditzen *et al*, 2009; Domes *et al*, 2007a; Domes *et al*, 2007b; Gamer *et al*, 2010; Guastella *et al*, 2010; Heinrichs *et al*, 2003; Kirsch *et al*, 2005; MacDonald *et al*, 2011; Rimmele *et al*, 2009), whereas prolonged OXT treatments have been adopted only in pathological states presenting social deficits (Feifel *et al*, 2012a; Feifel *et al*, 2010; Tachibana *et al*, 2013). Thus, intranasal administration of OXT might act to increase the concentration of OXT beyond endogenous levels in a normal-functioning brain resulting in desensitisation and hence, down-regulation of OXTRs. Indeed, the oxytocin receptor is a G protein-coupled receptor and can undergo desensitisation and internalization after agonist application (Conti *et al*, 2009; Insel *et al*, 1992). Accordingly, we have shown that chronic intranasal OXT treatment reduced the number of OXTRs in various brain regions, namely: lateral septum, hippocampus, piriform cortex, anterior olfactory nucleus, nucleus accumbens and amygdala.

The brain areas in which we found alterations of OXT receptors are involved in the regulation of social recognition. In particular, the amygdala is the site of convergence of socially-relevant olfactory inputs from the main and accessory olfactory systems; the anterior olfactory nucleus and the piriform cortex exert a key role in integrating olfactory information (Choleris *et al*, 2009); the lateral septum, hippocampus and ventral pallidum are areas where the forebrain OXTR knockout mice that present social recognition impairments, showed reduced OXTR expression (Lee *et al*, 2008; Macbeth *et al*, 2009). Furthermore, chronic intranasal OXT treatment increased V1aR-binding sites in the lateral septum, a key area for V1aR-mediated regulation of social recognition (Bielsky and Young, 2004; Landgraf *et al*, 1995). These evidence correlate with the reduction of social behaviors following chronic intranasal OXT treatment, and suggest that intranasal OXT treatments effectively influence and modulate the brain's oxytocinergic and vasopressinergic systems. The significance of these brain modulations and the underlying neurobiological mechanisms represents an important subject for future study. However, these findings corroborate the hypothesis that a prolonged over-stimulation of a 'healthy' oxytocinergic system might result in detrimental effects, at least in social behavior.

In contrast to the indiscriminate decrease in social behaviors following chronic intranasal OXT, a single acute intranasal dose of OXT in normal C57BL/6J male mice modulated social behaviors differently, depending on the sex (opposite- *vs* same-sex), and on the different social value (novel *vs* familiar) of the interacting mouse. In particular, acute intranasal OXT increased social behaviors towards a novel female stimulus mouse did not change social interaction between male–male familiar cagemates, but reduced social behaviors towards a novel unfamiliar male mouse. A wealth of evidence indicates that behavioral and histological measures in both the OXT system and OXT-mediated effects are sex-dependent (Macdonald, 2012). Although our experimental setting was not specifically designed to investigate sexual behaviors but that of a male's approach towards a sexually-receptive female, our results agree with previous works showing that either intraperitoneally or intracerebroventricularly acutely-injected OXT facilitate reproductive and sociosexual behaviors in male rodents (Arletti *et al*, 1985; Neumann, 2008; Witt, 1995). Moreover, intranasal OXT might be involved in the stress-protective and health-promoting role of positive heterosexual couples interactions (Ditzen *et al*, 2009). Furthermore, in agreement with our results showing selective reduced social behaviors towards a novel unfamiliar male subject following acute OXT, an acute intracerebroventricular injection of OXT in wild-type male mice reduced the sociability towards a stranger male subject (Sala *et al*, 2011). Similarly, our data agree with and might also explain recent findings showing that acute OXT aerosol in male monkeys reduced social vigilance for unfamiliar dominant faces (Ebitz *et al*, 2013). Altogether, our results show that the OXT intranasal route of administration in mice might be a valuable tool to preclinically test novel compounds and to better investigate the neural mechanisms of OXT-mediated behaviors in different social settings (eg, differentiating between novel *vs* familiar and opposite- *vs* same-sex social interactions).

In conclusion, we demonstrated that intranasal OXT treatment in normal C57BL/6J mice can reliably modulate the oxytocinergic system to selectively alter social behavior; most importantly, different effects on specific social behaviors are obtained upon chronic or acute intranasal administration. These preclinical findings constitute a starting point in unraveling the mechanistic impact of intranasal OXT. Moreover, the feasibility of administering OXT intranasally in mice pave the way towards the development of more selective and personalized use of OXT intranasal treatments based on genetic and pharmacological screenings. These bear important clinical implications for the life-long management of intranasal OXT treatments to be applied to the human population.

FUNDING AND DISCLOSURE

This research was supported by the Istituto Italiano di Tecnologia, the Marie Curie FP7-Reintegration-Grant N°268247, the Italian Ministry of Health Grants (GR3)-

Young Researcher 2008 and GR-2010-2315883, and the Telethon Foundation Grant (GGP12207). The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Dr M Morini, R Pelizzoli, D Cantatore, R Navone, T Luchetta and V Rea for their excellent technical assistance. We also thank Novartis for their generous gift of OXT.

REFERENCES

Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A (2010). Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proc Natl Acad Sci USA* **107**: 4389–4394.

Arletti R, Bazzani C, Castelli M, Bertolini A (1985). Oxytocin improves male copulatory performance in rats. *Horm Behav* **19**: 14–20.

Bales KL, Perkeybile AM, Conley OG, Lee MH, Guoynes CD, Downing GM *et al* (2012). Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles. *Biol Psychiatry* **15**: 00800–00801.

Barker GR, Bird F, Alexander V, Warburton EC (2007). Recognition memory for objects, place and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *J Neurosci* **27**: 2948–2957.

Barker GR, Warburton EC (2011). When is the hippocampus involved in recognition memory? *J Neurosci* **31**: 10721–10731.

Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E (2008). Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron* **58**: 639–650.

Bielsky IF, Young LJ (2004). Oxytocin, vasopressin and social recognition in mammals. *Peptides* **25**: 1565–1574.

Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL (2002). Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci* **5**: 514–516.

Braff DL, Geyer MA, Swerdlow NR (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)* **156**: 234–258.

Buchheim A, Heinrichs M, George C, Pokorny D, Koops E, Henningsen P *et al* (2009). Oxytocin enhances the experience of attachment security. *Psychoneuroendocrinology* **34**: 1417–1422.

Choleris E, Clipperton-Allen AE, Phan A, Kavaliers M (2009). Neuroendocrinology of social information processing in rats and mice. *Front Neuroendocrinol* **30**: 442–459.

Conti F, Sertic S, Reversi A, Chini B (2009). Intracellular trafficking of the human oxytocin receptor: evidence of receptor recycling via a Rab4/Rab5 'short cycle'. *Am J Physiol Endocrinol Metab* **296**: E532–E542.

Ditzen B, Schaer M, Gabriel B, Bodenmann G, Ehlert U, Heinrichs M (2009). Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biol Psychiatry* **65**: 728–731.

Domes G, Heinrichs M, Glascher J, Buchel C, Braus DF, Herpertz SC (2007a). Oxytocin attenuates amygdala responses to emotional faces regardless of valence. *Biol Psychiatry* **62**: 1187–1190.

Domes G, Heinrichs M, Michel A, Berger C, Herpertz SC (2007b). Oxytocin improves 'mind-reading' in humans. *Biol Psychiatry* **61**: 731–733.

Ebitz RB, Watson KK, Platt ML (2013). Oxytocin blunts social vigilance in the rhesus macaque. *Proc Natl Acad Sci USA* **110**: 11630–11635.

Feifel D, Macdonald K, Cobb P, Minassian A (2012a). Adjunctive intranasal oxytocin improves verbal memory in people with schizophrenia. *Schizophr Res* **7**: 7.

Feifel D, Macdonald K, Nguyen A, Cobb P, Warlan H, Galangue B *et al* (2010). Adjunctive intranasal oxytocin reduces symptoms in schizophrenia patients. *Biol Psychiatry* **68**: 678–680.

Feifel D, Shilling PD, Belcher AM (2012b). The effects of oxytocin and its analog, carbetocin, on genetic deficits in sensorimotor gating. *Eur Neuropsychopharmacol* **22**: 374–378.

Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT (2000). Social amnesia in mice lacking the oxytocin gene. *Nat Genet* **25**: 284–288.

Gamer M, Zurowski B, Buchel C (2010). Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans. *Proc Natl Acad Sci USA* **107**: 9400–9405.

Giancardo L, Sona D, Huang H, Sannino S, Manago F, Scheggia D *et al* (2013). Automatic visual tracking and social behavior analysis with multiple mice. *PLoS One* **8**: e74557.

Green JJ, Hollander E (2010). Autism and oxytocin: new developments in translational approaches to therapeutics. *Neurotherapeutics* **7**: 250–257.

Guastella AJ, Einfeld SL, Gray KM, Rinehart NJ, Tonge BJ, Lambert TJ *et al* (2010). Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. *Biol Psychiatry* **67**: 692–694.

Guastella AJ, Mitchell PB, Dadds MR (2008). Oxytocin increases gaze to the eye region of human faces. *Biol Psychiatry* **63**: 3–5.

Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry* **54**: 1389–1398.

Heinrichs M, Meinlschmidt G, Wippich W, Ehlert U, Hellhammer DH (2004). Selective amnesic effects of oxytocin on human memory. *Physiol Behav* **83**: 31–38.

Herzmann G, Young B, Bird CW, Curran T (2012). Oxytocin can impair memory for social and non-social visual objects: a within-subject investigation of oxytocin's effects on human memory. *Brain Res* **1451**: 65–73.

Insel TR, Winslow JT, Witt DM (1992). Homologous regulation of brain oxytocin receptors. *Endocrinology* **130**: 2602–2608.

Jin D, Liu HX, Hirai H, Torashima T, Nagai T, Lopatina O *et al* (2007). CD38 is critical for social behavior by regulating oxytocin secretion. *Nature* **446**: 41–45.

Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S *et al* (2005). Oxytocin modulates neural circuitry for social cognition and fear in humans. *J Neurosci* **25**: 11489–11493.

Kumari V, Das M, Zachariah E, Ettinger U, Sharma T (2005). Reduced prepulse inhibition in unaffected siblings of schizophrenia patients. *Psychophysiology* **42**: 588–594.

Landgraf R, Gerstberger R, Montkowski A, Probst JC, Wotjak CT, Holsboer F *et al* (1995). V1 vasopressin receptor antisense oligodeoxynucleotide into septum reduces vasopressin binding, social discrimination abilities and anxiety-related behavior in rats. *J Neurosci* **15**: 4250–4258.

Lee HJ, Caldwell HK, Macbeth AH, Tolu SG, Young WS 3rd (2008). A conditional knockout mouse line of the oxytocin receptor. *Endocrinology* **149**: 3256–3263.

Lim MM, Bielsky IF, Young LJ (2005). Neuropeptides and the social brain: potential rodent models of autism. *Int J Dev Neurosci* **23**: 235–243.

Ludwig M, Tobin VA, Callahan MF, Papadaki E, Becker A, Engelmann M *et al* (2013). Intranasal application of vasopressin fails to elicit changes in brain immediate early gene expression, neural activity and behavioral performance of rats. *J Neuroendocrinol* **25**: 655–667.

Macbeth AH, Lee HJ, Edds J, Young WS 3rd (2009). Oxytocin and the oxytocin receptor underlie intrastrain, but not interstrain, social recognition. *Genes Brain Behav* **8**: 558–567.

MacDonald E, Dadds MR, Brennan JL, Williams K, Levy F, Cauchi AJ (2011). A review of safety, side-effects and subjective reactions to intranasal oxytocin in human research. *Psychoneuroendocrinology* **36**: 1114–1126.

Macdonald KS (2012). Sex, receptors, and attachment: a review of individual factors influencing response to oxytocin. *Front Neurosci* **6**: 194.

Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M (2011). Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat Rev Neurosci* **12**: 524–538.

Neumann ID (2008). Brain oxytocin: a key regulator of emotional and social behaviors in both females and males. *J Neuroendocrinol* **20**: 858–865.

Neumann ID, Maloumby R, Beiderbeck DI, Lukas M, Landgraf R (2013). Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology* **38**: 1985–1993.

Papaleo F, Crawley JN, Song J, Lipska BK, Pickel J, Weinberger DR *et al* (2008). Genetic dissection of the role of catechol-O-methyltransferase in cognition and stress reactivity in mice. *J Neurosci* **28**: 8709–8723.

Papaleo F, Yang F, Garcia S, Chen J, Lu B, Crawley JN *et al* (2012). Dysbindin-1 modulates prefrontal cortical activity and schizophrenia-like behaviors via dopamine/D2 pathways. *Mol Psychiatry* **17**: 85–98.

Popik P, Vetulani J, Van Ree JM (1996). Facilitation and attenuation of social recognition in rats by different oxytocin-related peptides. *Eur J Pharmacol* **308**: 113–116.

Rimmele U, Hediger K, Heinrichs M, Klaver P (2009). Oxytocin makes a face in memory familiar. *J Neurosci* **29**: 38–42.

Sala M, Braida D, Donzelli A, Martucci R, Busnelli M, Bulgheroni E *et al* (2012). Mice heterozygous for the oxytocin receptor gene (Oxtr(+/−)) show impaired social behavior but not increased aggression or cognitive inflexibility: evidence of a selective haploinsufficiency gene effect. *J Neuroendocrinol* **11**: 1365–2826.

Sala M, Braida D, Lentini D, Busnelli M, Bulgheroni E, Capurro V *et al* (2011). Pharmacologic rescue of impaired cognitive flexibility, social deficits, increased aggression, and seizure susceptibility in oxytocin receptor null mice: a neurobehavioral model of autism. *Biol Psychiatry* **69**: 875–882.

Scattoni ML, Ricceri L, Crawley JN (2011). Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. *Genes Brain Behav* **10**: 44–56.

Scearce-Levie K, Roberson ED, Gerstein H, Cholfin JA, Mandiyan VS, Shah NM *et al* (2008). Abnormal social behaviors in mice lacking Fgf17. *Genes Brain Behav* **7**: 344–354.

Striepens N, Kendrick KM, Maier W, Hurlemann R (2011). Prosocial effects of oxytocin and clinical evidence for its therapeutic potential. *Front Neuroendocrinol* **32**: 426–450.

Tachibana M, Kagitani-Shimono K, Mohri I, Yamamoto T, Sanefuji W, Nakamura A *et al* (2013). Long-term administration of intranasal oxytocin is a safe and promising therapy for early adolescent boys with autism spectrum disorders. *J Child Adolesc Psychopharmacol* **23**: 123–127.

Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T *et al* (2005). Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc Natl Acad Sci USA* **102**: 16096–16101.

Tribollet E, Barberis C, Arsenijevic Y (1997). Distribution of vasopressin and oxytocin receptors in the rat spinal cord: sex-related differences and effect of castration in pudendal motor nuclei. *Neuroscience* **78**: 499–509.

Veening JG, Olivier B (2013). Intranasal administration of oxytocin: behavioral and clinical effects, a review. *Neurosci Biobehav Rev* **37**: 1445–1465.

Winslow JT, Insel TR (2002). The social deficits of the oxytocin knockout mouse. *Neuropeptides* **36**: 221–229.

Witt DM (1995). Oxytocin and rodent sociosexual responses: from behavior to gene expression. *Neurosci Biobehav Rev* **19**: 315–324.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)