Beneficial effects of chronic oxytocin administration and social co-housing in a rodent model of post-traumatic stress disorder

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Post-traumatic stress disorder (PTSD) is in part due to a deficit in memory consolidation and extinction. Oxytocin (OXT) has anxiolytic effects and promotes prosocial behaviors in both rodents and humans, and evidence suggests that it plays a role in memory consolidation. We studied the effects of administered OXT and social co-housing in a rodent model of PTSD. Acute OXT yielded a short-term increase in the recall of the traumatic memory if administered immediately after trauma. Low doses of OXT delivered chronically had a cumulating anxiolytic effect that became apparent after 4 days and persisted. Repeated injections of OXT after short re-exposures to the trauma apparatus yielded a long-term reduction in anxiety. Co-housing with naive nonshocked animals decreased the memory of the traumatic context compared with single-housed animals. In the long term, these animals showed less thigmotaxis and increased interest in novel objects, and a low OXT plasma level. Co-housed PTSD animals showed an increase in risk-taking behavior. These results suggest beneficial effects of OXT if administered chronically through increases in memory consolidation after re-exposure to a safe trauma context. We also show differences between the benefits of social co-housing with naive rats and co-housing with other shocked animals on trauma-induced long-term anxiety. *Behavioural Pharmacology* **27:704–717**

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Introduction

Post-traumatic stress disorder (PTSD) develops in 10% of individuals exposed to traumatic events (Kofoed *et al.*, 1993; Kessler *et al.*, 1995, 2005). One of the major mechanisms believed to be involved in PTSD is a dysfunction of one or several processes tied to memory consolidation or reconsolidation (Parsons and Ressler, 2013).

Physiologically, PTSD is marked by dysregulations of the hypothalamic–pituitary–adrenal axis, the medial prefrontal cortex (mPFC), amygdala, hippocampus, and the ventral tegmental area (Louvart *et al.*, 2006; Williams *et al.*, 2006; Liberzon and Sripada, 2008; Olff *et al.*, 2010; Corral-Frias *et al.*, 2013). Selective serotonin reuptake inhibitors, especially paroxetine and sertraline, are the most commonly prescribed drug treatments for PTSD patients (Berger *et al.*, 2009), with only 20–30% of patients achieving full remission (Stein *et al.*, 2002). Cognitive behavioral therapy (CBT) has had some success in treating the disorder (Bradley *et al.*, 2005; Bisson and Andrew, 2007). There is also evidence to suggest that CBT may be enhanced when used in tandem with pharmacological treatment (Davis *et al.*, 2006; Cloitre, 2009). Recent evidence suggests that oxytocin (OXT) may be an effective drug to use in conjunction with CBT (Olff *et al.*, 2010). OXT also reduces the psychophysiological stress response in veterans with PTSD and results show that an intranasal dose of OXT seems to cause most of the acute PTSD symptoms to subside (Olff *et al.*, 2013).

In addition to pharmacological treatments, social interactions, whether with other PTSD patients (e.g. support group) or with nontraumatized individuals (e.g. friends or family members), seems to be an essential tool to treat and manage PTSD. Social support has the potential to buffer against psychological distress and reduce trauma-related strain, whereas inadequate or lack of support may contribute toward maintenance or aggravation of psychopathological symptoms (Elklit *et al.*, 2012). The most important postwar mediator of risk for developing PTSD is the lack of perceived social support (Charuvastra and Cloitre, 2008). A large body of evidence has linked OXT to social interactions (Ross and Young, 2009; Bartz *et al.*, 2011; Meyer-Lindenberg *et al.*, 2011; Guastella and MacLeod, 2012) and to learning and memory processes (Chini *et al.*, 2013). Recent studies further showed that social bonding among male rats and oxytocin signaling were perturbed by the contextual stress elicited by the odor of a predator (Muroy *et al.*, 2016).

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Most of what is known of the effect of OXT on stress and fear conditioning has relied on acute, single dose experiments, and most studies that have attempted a comparison between single and multiple doses have often shown contradictory results (Stoop, 2012; Macdonald and Feifel, 2013). In rodents, OXT has been shown to inhibit the activity of the hypothalamic–pituitary–adrenal axis in response to an external stressor (Neumann *et al.*, 2000) and to produce amnesic effects on fear conditioning (Bohus *et al.*, 1978a, 1978b) by acting on the hippocampus. Injections of OXT in a stressed animal reduce its ability to cause stress to other cagemates (Agren and Lundeberg, 2002a, 2002b). Low doses of OXT, presumably present in co-housing conditions, have anxiolytic effects (Uvnas-Moberg *et al.*, 1994). OXT has also been shown to acutely reduce background anxiety in a startle paradigm when administered peripherally (Missig *et al.*, 2010), but not centrally (Ayers *et al.*, 2011). Direct injections of OXT into the amygdala and in the medial prefrontal cortex have opposite effects on fear extinction, with enhanced extinction following infralimbic administration and reduced consolidation of extinction following OXT administration into the basolateral amygdala (Lahoud and Maroun, 2013).

The processes of memory consolidation likely follow multiple time scales, from minutes to hours or days (Dudai *et al.*, 2015). Many studies in rodents have shown that the neural activity elicited during a learning task or experience is reactivated during sleep immediately following that experience. This reactivation is considered to be one of the initial mechanisms for memory consolidation and reconsolidation and occurs prominently within 30 min of the learning task during slow wave sleep in several areas including the hippocampus, the prefrontal cortex, and the ventral tegmental area (Kudrimoti *et al.*, 1999; Euston *et al.*, 2007; Valdes *et al.*, 2015). Very little is known of the influence of OXT on this important initial phase of memory formation.

Finally, despite the known relationships between OXT, prosocial behavior, and stress, many studies use co-housed animals without controlling for the effect of co-housing on stress (Toledo-Rodriguez and Sandi, 2011; Eskandarian *et al.*, 2013; Lahoud and Maroun, 2013).

Altogether, these previous studies suggest that peripheral administration of OXT or endogenous release of OXT resulting from social interactions could be used to affect the consolidation of the traumatic memory. Here, we study the effect of acute and chronic administration of OXT and of co-housing on long-term anxiety in a rodent model of PTSD.

Methods

Subjects

All animals had free access to food and water and were housed on a 12-h light–dark (09.00–21.00 h) cycle. All procedures were run during the dark cycle at around the same time (12.00 h ± 1 h). Testing began 6 days after arrival. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Arizona (Tucson, Arizona, USA). The number of animals used was monitored and minimized with respect to statistical significance criteria.

Pharmacological manipulations of oxytocin

A total of 135 male Sprague–Dawley rats aged 3–4 months were used for all injection and osmotic pump experiments (Figs 3 and 4). The numbers in each group are shown in Table 1.

Oxytocin plasma time course

A separate group of animals including male Brown–Norway rats aged 7–9 months ($n = 26$) and male Sprague–Dawley rats aged 3 months ($n = 20$) were used for the time-point experiment (Fig. 2). No difference was noted between the two strains; thus, data were merged.

Social-housing manipulations

A total of 84 male Sprague–Dawley rats aged 2–3 months were used. In these experiments, we found that many of our initial 3–4-month-old co-housed animals were asserting dominance and engaged in fights often resulting in open wound injuries. Slightly decreasing the age of the animals to 2–3 months led to much more stable trios and (perhaps anecdotally) more play and vocalizations in the colony room. The numbers in each group are shown in Table 2.

Experimental design

The experimental designs for trauma induction and behavioral testing have been described elsewhere and are briefly summarized below (Corral-Frias *et al.*, 2013). The time line of the experiments is shown in Fig. 1. The animals were allowed 2–3 days of acclimation and were then run on four open-field tests to obtain baseline data (days −5 to −2). After a day with no procedure, they were run in the trauma induction paradigm (shock and three situational reminders, 7 days). They were then returned to their cage and left unperturbed for another

Table 1 Group sizes for the injection and osmotic pump experiments

Acute OXT	Saline	Shock	12
		Sham	8
	Single injections	Shock	9
		Sham	8
	Multiple injections	Shock	11
		Sham	8
Chronic OXT	Saline	Shock	27
		Sham	16
	7-day OXT pump	Shock	10
		Sham	8
	14-day OXT pump	Shock	10
		Sham	8

OXT, oxytocin.

Table 2 Group sizes for the social-housing manipulation experiments

Single housed	Shock	14
	Sham	10
Co-housed	Solo shocked	14
	All shocked	18
	Sham	28

7 days. They were then tested for long-term anxiety effects on three consecutive days (black and white box, elevated plus maze (EPM), and open field; one test each day; same order for all groups). After 1 day without procedures, rats were decapitated and blood was collected for analysis of OXT plasma levels.

The bottom of the figure shows the four OXT administration conditions that we used: (i) single s.c. injection immediately after shock, which only affected the initial stages of memory consolidation for the shock experience; (ii) multiple injections: s.c. injections immediately after shock and immediately after each situational reminder, affecting the reconsolidation epochs reactivated by the short exposure to the shock context (no shock); (iii) a 7-day osmotic pump group (active during the trauma procedure only); and (iv) a 14-day osmotic pump group (active during trauma and during the no-procedure period).

To complement the pharmacological studies aimed at testing the effect of continuous OXT release on traumatic memory, we studied the effect of endogenously released oxytocin by manipulating the social-housing conditions of the animals. Rats arrived from the vendor and were immediately assigned to one of four groups. Two groups consisted of single-housed shock and single-housed sham rats. Two other groups consisted of co-housed trios: The first included one shock rat and two sham rats (solo shocked), and the other group included three shock rats (all-shocked). These two groups aimed at testing whether differences could be found between shocked animals co-housed with naive cagemates and shocked animals co-housed with other shocked animals under the assumption that bonding, play, and other social interactions may be different in these two conditions. In all cases, the shocked rats in each group were subjected to the same PTSD paradigm as single-housed shocked animals (Fig. 1, top).

Procedures

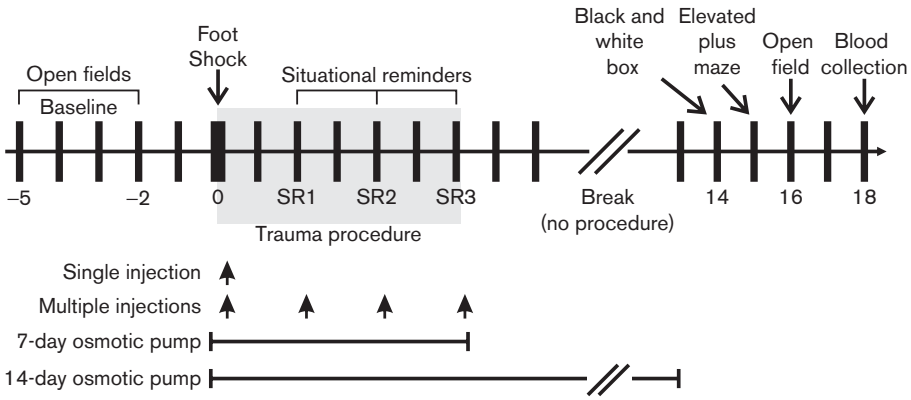
Open field

For the first 4 days of the protocol (days −5 to −2), animals were subjected to an open-field novelty sensitivity task. The open field consisted of a 1.5 m diameter round table with 30 cm high walls around the perimeter and a novel object in the center. The apparatus was lit by overhead white LEDs (2–3 lux, measured with a TES-1337 digital light meter). The rats were tracked with custom tracking software (ZTracker, available from the laboratory website) written in Labview (National Instrument, Austin, Texas, USA) and analyzed using MatLab (The MathWorks, Natick, Massachusetts, USA). The center of the maze was defined by a virtual circle of 70 cm diameter containing the object. The maze was cleaned between rats with 70% ethanol at the end of each day. This apparatus tested the ability of the animals to overcome thigmotactic behavior to explore a novel object (Toledo-Rodriguez and Sandi, 2011).

Trauma procedure

The animals were exposed to an inescapable foot-shock (day 0, Fig. 1). The shock chamber was constructed of wood (54 × 110 × 34 cm) and was divided into two compartments of equal size separated by a guillotine door.

Fig. 1



Time course of the experiment (above) and oxytocin administration experimental groups (below). Subcutaneous injections (closed arrows) occurred immediately after each procedure before returning the animals to their home cage. Osmotic pumps were implanted on day 0 6–8 h before foot shock. The 7-day osmotic pump was active from implantation through the situational reminders, whereas the 14-day osmotic pump was active for an additional 7 days through the break period (days 7–14). Each tick mark represents a day.

One compartment (safe side) had black and white striped walls and thin mesh flooring. The other compartment (shock side) had black walls and an eight-pole shock grid (Coulbourn Instruments, Holliston, Massachusetts, USA). On day 0, animals were placed in the safe side and allowed a 3-min adaptation period. The guillotine door was then opened and a light on the wall opposite the door was turned on for 15 s (about 40 lux) to encourage spontaneous movement to the shock compartment. If rats did not cross on their own after 1 min, they were gently nudged to the shock side by the experimenter, the light was turned off, and the door was closed (2.5 lux in the shock compartment). The rats were given an additional 3-min adaption period in the shock side before receiving a 2 mA inescapable foot-shock for 10 s. Rats were then returned to their home cage. The sham groups were subjected to the same procedure, but received no foot-shock.

Minimal extinction and situational reminders

The shock procedure was followed by three short re-exposures to the apparatus without receiving a shock [situational reminders (SRs)] on days 2, 4, and 6 (Pynoos *et al.*, 1996; Louvart *et al.*, 2006; Corral-Frias *et al.*, 2013). All rats were re-exposed to the apparatus for 2 min on days 2, 4, and 6; an amount of time during which animals are the most active (Eskandarian *et al.*, 2013). The rats were placed in the safe side with the guillotine door removed. These short re-exposures were designed to induce a reactivation of the memory of the shock context and a minimal amount of extinction (common contextual fear extinction procedures last 10 min or more). Because extinction is not complete, this procedure allows for the assessment of the time course of extinction during repeated testing (here three tests over the course of 7 days). The behavior of the animals was recorded and quantified using the AnyMaze software (Stoelting Inc., Wood Dale, Illinois, USA).

Black and white box

The black and white box test is an anxiety test that takes advantage of the rats' natural aversion to bright light (Costall *et al.*, 1989). In our paradigm, it was also chosen to test the animals in an apparatus similar (but not identical) to the shock chamber and to assess their ability to generalize the context in which the trauma occurred. These experiments were conducted in a room adjacent to that of the shock procedure. The apparatus contained two sides: one white ($45 \times 32 \times 40$ cm) and one black ($30 \times 32 \times 40$ cm). As in the shock chamber, the white side had a light shining directly into it from above (910 lux). During testing (day 14), the rats were initially placed in the white side, facing away from the black side (10.5 lux). The latency to enter the black side, the number of white entries, and the total time spent in the white side over the course of 5 min were scored using a four-paw criterion for entries.

Elevated plus maze

Rats were tested on an EPM apparatus to measure long-term general anxiety levels and risk-taking behavior (Toledo-Rodriguez and Sandi, 2011) (day 15). The wooden apparatus consisted of two open arms (50×10 cm) alternating at right angles and two arms enclosed by 40 cm high walls. The four arms delimited a central area of 10 cm^2. The apparatus was placed 60 cm above the floor. Light levels in the closed and open arms were measured at 3.5 and 100 lux, respectively. The center of the maze was measured at about 80 lux. The test began by placing the rat at the center of the maze with its head facing an open arm. The time spent in the open and closed arms was recorded and was expressed as a percentage of the total time spent in the apparatus (Pellow *et al.*, 1985). A four-paw criterion was used for arm entries.

Blood collection

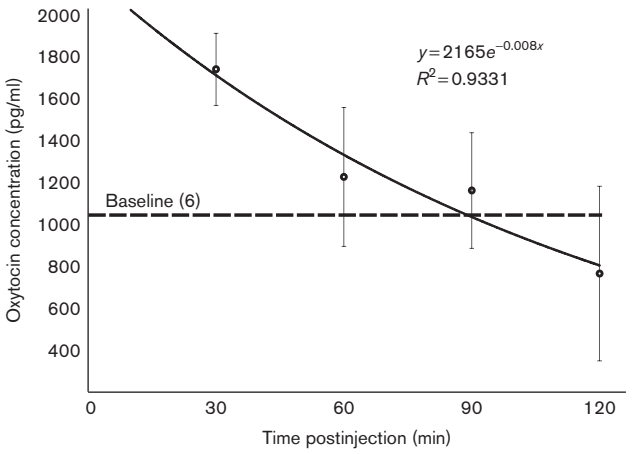
On the last day of the protocol (day 18), rats were anesthetized using 3% isoflurane and quickly decapitated to collect trunk blood. Blood was collected in a chilled vacutainer containing EDTA (1 mg/ml) and 0.130 ml of aprotinin and immediately centrifuged for 20 min at 1200*g*. The plasma was collected and stored at −80°C until processed. Plasma levels of OXT were determined by ELISA (Kit: EKE-051-01; Phoenix Pharmaceuticals, Burlingame, California, USA). The Phoenix Pharmaceuticals kit is specifically designed for extraction-free procedures with a manufacturer-stated minimum detectable level of 0.13 ng/ml and no cross-reactivity with vasopressin. Samples were processed in duplicate. Duplicates with a discrepancy of 100% or more were reprocessed or discarded (9% of the samples).

To determine the decay of OXT in the plasma (Fig. 2), a separate group of rats was injected s.c. with 0.1 mg/kg OXT and euthanized 30, 60, 90, or 120 min later by decapitation to collect trunk blood.

Drugs and drug administration

The pharmacological group was subjected to various drug treatments. No drug was administered to the social-housing groups. S.c. injections were preferred to other forms of administration for several reasons: (i) to precisely control the dosage (unlike intranasal), (ii) to minimize stress to the animals (unlike intraperitoneal or intracerebroventricular injections), and (iii) to provide a long-lasting acute effect likely to influence memory consolidation (>30 min, unlike intracerebroventricular or intravenous). The mechanisms of central actions of peripherally administered OXT are believed to involve a positive feedback loop between plasma OXT and endogenous secretion of OXT (Neumann *et al.*, 1996; Carson *et al.*, 2010; Chini *et al.*, 2013; Macdonald and Feifel, 2013). This can be measured in the amygdala and the hippocampus (Neumann *et al.*, 2013), although

Fig. 2



Time course of oxytocin concentration in plasma after a subcutaneous injection of 0.1 mg/kg oxytocin (time 0). All groups $n = 6$.

indirect actions through the glucocorticoid system may also be at play (Petersson and Uvnas-Moberg, 2003).

S.c. injections consisted of OXT (0.2 mg/kg; Santa-Cruz Biotechnology, Santa Cruz, California, USA) dissolved in a sterile 0.9% saline solution (0.5 ml, total volume). This dosage was below the range known to induce prominent sedative effects that are, in any case, known to be entirely eliminated within 24 h (Uvnas-Moberg *et al.*, 1994). The osmotic pump (7 or 14 days; Alzet Inc., Cupertino, California, USA) groups received the equivalent dosage of OXT throughout the day (0.2 mg/kg/day, or 8.3 ng/kg/h). These dosages are below the dosages known to produce peripheral effects (e.g. cardiac, respiratory, and weight loss) and are unlikely to activate vasopressin receptors (Chini *et al.*, 2013).

Acute oxytocin

Many electrophysiological recording studies in the rodent have shown that neural reactivation (a putative electrophysiological correlate of consolidation and reconsolidation) primarily occurs within the first 20–30 min of rest after a task (Wilson and McNaughton, 1994; Kudrimoti *et al.*, 1999; Valdes *et al.*, 2015). As shown below, this epoch is compatible with the time course of OXT s.c. injections. To test the effect of OXT on consolidation and reconsolidation of the memory for the shock, animals were assigned randomly to two groups. In a first group of manipulations, we tested the effect of OXT at the time of initial memory consolidation, immediately after the shock experience, before the animal was returned to its home cage (single injections, Fig. 1). In a second group, we tested the effect of OXT administered during initial consolidation immediately after shock and during reconsolidation after each situational reminder (multiple injections, Fig. 1). Overall, six groups received s.c. injections as follows: (i) shock and multiple injections of OXT ($n = 12$), (ii) shock and multiple saline injections ($n = 12$), (iii) sham and multiple saline injections ($n = 8$), (iv) sham and multiple injections of OXT ($n = 8$), (5) shock and single injection of OXT ($n = 9$), and 6) sham and single injection of OXT ($n = 7$). To minimize animal use, we did not include single-injection saline shock/sham groups under the assumption that this control was subsumed by the multiple saline injections in groups 2 and 3 above.

Chronic oxytocin

A subset of rats received continuous OXT or saline through osmotic pumps (0.5 µl/h for 7 days or 0.5 µl/h for 14 days). Rats were anesthetized using 3% isoflurane and implanted with the pump s.c. between the shoulder blades. Surgery occurred 6–8 h before the foot-shock on day 0.

In this group of animals, we tested the hypothesis that continuous administration of OXT, as would be in effect during clinical treatments or during natural exposure to social bonding situations, would show a more robust effect than timed acute injections. Two types of osmotic pumps were used: the first was effective continuously during the consolidation and reconsolidation periods (7 days) and the second was effective throughout these periods and through the break period (14 days). Overall, six groups received pumps as follows: (i) Shock and 7-day OXT pump ($n = 11$), (ii) shock and 14-day OXT ($n = 11$), (iii) shock and saline 7-day and 14-day pumps groups were combined ($n = 28$), (iv) sham and saline 7-day and 14-day pumps groups were combined ($n = 16$), (v) sham and 7-day OXT ($n = 8$), and (vi) sham and 14-day OXT ($n = 8$).

Statistics

All statistical analyses were carried out using SigmaStat (Systat Software Inc., San Jose, California, USA) or SPSS (IBM Research, Armonk, New Jersey, USA). Mixed analysis of variance (ANOVA) results are presented only if the data passed the sphericity test (Mauchly's test) and if the variance was homogeneous (Levene's test). Post-hoc Tukey's tests were used. At most, one data point per group was excluded from analysis if it was further than two SDs away from the group mean. ANOVA results were accompanied by Welch tests for homogeneous variance (rejected if $P > 0.01$) and Bonferroni corrections. Only ANOVA results that passed the test are presented. In all graphs, significance is indicated by P less than 0.05 and 0.01, and error bars are the SEM.

Results

Time course of s.c. oxytocin injections

To assess the extent to which acute s.c. injections of OXT were effective at potentially affecting the reactivation process, OXT was administered s.c. and blood was

collected 30, 60, 90, or 120 min after injections ($n=6$, each time point). Another group ($n=6$) was euthanized without being injected to determine the baseline amount of OXT present in the plasma. Without exposure to exogenous OXT, the baseline amount of OXT was 1036 pg/ml (SEM = ± 358, Fig. 2). This value is comparable with that obtained in other studies (Devarajan and Rusak, 2004) and, as expected, is one order of magnitude higher than in studies using an extraction method (Szeto *et al.*, 2011; Neumann *et al.*, 2013). The concentration of blood plasma OXT decreased exponentially and reached the baseline after about 60 min. An exponential fit yielded a half-life of about 35 min, which is well within the time range for neural reactivation.

Effects of administration of oxytocin

The trauma procedure includes three short subextinction re-exposures to the shock context (Pynoos *et al.*, 1996; Valdes *et al.*, 2015). These situational reminders capture, at least in part, the experiences of many PTSD patients who must repeatedly return to the traumatic context shortly after the traumatic event has occurred (e.g. war zone for a soldier, familiar environment for a rape victim). It is hypothesized that these re-exposures reactivate the traumatic memories and hence contribute to their consolidations.

Situational reminders

Acute oxytocin

The shocked animals in the saline groups spent less time in the shock side than their respective saline sham groups for every SR condition (Fig. 3a, hashed bars, one-way ANOVA, SR1: $F(1,17)=23.5$, $P<0.001$, SR2: $F(1,18)=26.0$, $P<0.001$ and SR3: $F(1,18)=73.9$, $P<0.001$. Mixed ANOVA: significant shock × situational reminder interaction, $F(2,34)=3.73$, $P<0.05$, and significant effect of SR $F(2,34)=3.51$, $P<0.05$), as expected (Corral-Frias *et al.*, 2013). A mixed ANOVA of the number of entries to the shock side showed a significant effect of situational reminder [$F(2,32)=5.11$, $P<0.025$, but no significant interaction $F(2,32)=0.91$, NS]. Significant differences between shock and shams were measured in the number of entries to the shock side during SR1 and SR2, but not SR3 [$F(1,18)=8.7$, $P=0.08$; $F(1,17)=7.07$, $P<0.02$ and $F(1,17)=2.93$, NS, respectively]. The sham group showed a marginal increase in the time spent in the shock compartment (hashed white bars) from SR1 to SR2 to SR3, possibly indicating habituation. The rats in the single OXT injection groups showed the same overall shock/sham differences of time spent in the shock side as the saline animals [one-way ANOVA, SR1: $F(1,13)=55.08$, $P<0.001$; SR2: $F(1,14)=10.8$, $P<0.001$; and SR3: $F(1,13)=31.42$, $P<0.001$. Mixed ANOVA: significant effect of SR $F(2,24)=4.57$, $P<0.025$]. An analysis of the entries to the shock side indicated a significant difference between the sham and the shock group in all situational reminders [SR1: $F(1,14)=23.42$, $P<0.001$; SR2: $F(1,13)=19.20$, $P<0.05$; SR3 $F(1,14)=6.19$, $P<0.05$. Mixed ANOVA: no significant effects]. Interestingly, the shocked single-injection animals spent significantly less time in the shock side than the saline shocked group during the first SR, but not during the subsequent trials (one-way ANOVA, $P<0.01$), indicating a potential effect of the single postshock OXT injection on the initial strength of the memory for the traumatic event that returned to control levels after 2 days (tested on SR2, SR3).

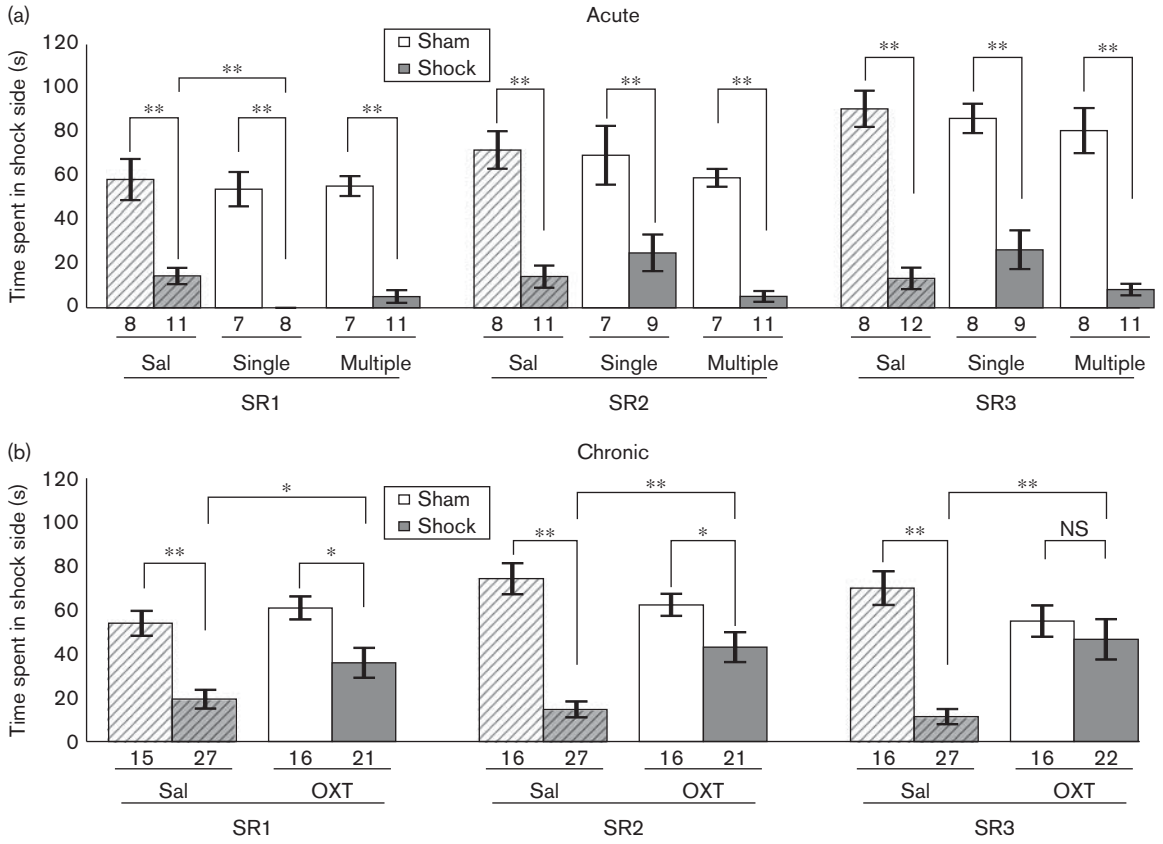
Animals that received multiple injections showed the same overall pattern of sham/shock difference in time spent on the shock side as the saline groups [one-way ANOVA, SR1: $F(1,18)=7.97$ $P<0.02$; SR2 $F(1,18)=19.07$, $P<0.001$; and SR3: $F(1,18)=38.08$, $P<0.001$. Mixed ANOVA: significant shock × SR interaction $F(2,36)=5.21$, $P<0.01$ and significant SR effect $F(2,26)=5.3$, $P<0.01$]. An analysis of the number of entries to the shock side showed a significant difference between shock and sham groups in SR1 and SR2 [$F(1,18)=28.03$, $P<0.005$ and $F(1,18)=10.37$, $P<0.005$, respectively], but not for SR3 [$F(1,17)=3.0$, $P=0.098$. Mixed ANOVA: SR × shock interaction $F(2,30)=3.69$, $P=0.037$].

These results suggest that OXT yields a short-term (2–3 days) increase in the consolidation of the traumatic memory if administered immediately after trauma (tested at SR1), but administration of OXT after re-exposure during periods that would affect extinction learning and/or reconsolidation has no effect on the behavior of the animals (tested at SR2 and SR3, multiple injections group).

Chronic oxytocin

Plasma levels of OXT following acute injections are washed out within 1–2 h (Fig. 2). We aimed to compare the effect of chronic OXT on the memory of the shock episode with that of the acute conditions. Because the 7-day and 14-day osmotic pump groups had received the same amount of OXT during the situational reminders, the groups were combined into one group for analysis (Fig. 3b). The shocked saline group (hashed bars) spent significantly less time in the shock compartment than the sham saline group [one-way ANOVA, SR1: $F(1,40)=23.27$, $P<0.001$; SR2: $F(1,41)=69.47$, $P<0.001$; and SR3: $F(1,41)=62.5$, $P<0.001$. Mixed ANOVA: significant shock × SRs interactions $F(2,78)=4.71$, $P<0.02$]. An analysis of the entries to the shock side also yielded a significant difference in all situational reminders [SR1: $F(1,41)=13.70$, $P<0.001$; SR2: $F(1,41)=8.42$, $P<0.01$; SR3: $F(1,41)=16.0$, $P<0.001$. Mixed ANOVA: main effect of SRs $F(2,82)=3.14$, $P<0.05$]. As in the acute conditions, there was a small increase in the time spent on the shock side (less anxiety, hashed white bar, SR1 vs. SR2/3) in the sham group, showing a small habituation effect. The shocked OXT group avoided the shock side more than the sham OXT group on SR1 and SR2 [Fig. 6b;

Fig. 3



Chronic but not acute oxytocin (OXT) administration reduces contextual fear over multiple exposures. (a) Situational reminders for the subcutaneous injection groups. The Single group received one OXT injection after shock. The multiple group received OXT injections after the shock and after each situational reminder. In all cases, the shocked groups spent less time in the shock compartment than their sham counterparts. (b) Situational reminders for the osmotic pump group (7-day and 14-day groups were merged). OXT animals spent significantly more time in the shock compartment than the Saline shock group. At SR3, the OXT sham and shock groups were not significantly different. Group sizes are indicated below the *x*-axis for each group. In all panels: **P*<0.05 and ***P*<0.01.

one-way ANOVA, SR1: $F(1,36)=5.23$, $P<0.05$; SR2: $F(1,35)=6.66$, $P<0.02$. Mixed ANOVA: no significant effect], but the difference between shock and sham decreased from SR1 to SR2. On SR3, this difference became nonsignificant and the shocked OXT group spent about the same amount of time in the shock side as the sham group [$F(1,36)=1.31$, NS]. This decrease in significance was also observed when analyzing the number of entries into the shock compartment [SR1: $F(1,36)=7.6$, $P<0.01$; SR2: $F(1,35)=2.2$, NS; SR3: $F(1,36)=0.39$, NS. Mixed ANOVA no significant effect). The shocked OXT group spent more time in the shock compartment than the shocked saline group in all situational reminders [SR1: $F(1,47)=6.05$, $P<0.02$; SR2: $F(1,46)=15.45$, $P<0.001$; SR3: $F(1,47)=14.91$, $P<0.001$]. These differences were also present when measuring the number of entries [SR1: $F(1,47)=7.51$, $P<0.01$; SR2: $F(1,46)=20.20$, $P<0.001$; SR3: $F(1,47)=16.75$, $P<0.001$].

These results suggest that low chronic doses of OXT had a positive effect on anxiety throughout the three situational reminders compared with shocked animals receiving saline, and had an additional slowly accumulating anxiolytic effect that became significant after 4 days (comparing OXT sham and shocked animals).

We next tested the effect of post-trauma administration of OXT on long-term anxiety 2 weeks after the shock procedure.

Long-term effects

Acute oxytocin

Two weeks after the foot-shock procedure, animals were tested in the black and white box. In general, this apparatus measures the extent to which animals prefer the black side and avoid the white side. Because this box resembles the shock apparatus (the black side resembles the shock compartment), this test can also be taken, at least in part, as a test of shock context generalization in shocked animals. All sham groups (white bars, Fig. 4a) showed a short latency to cross to the black side and did not differ from each other. The sham saline injection

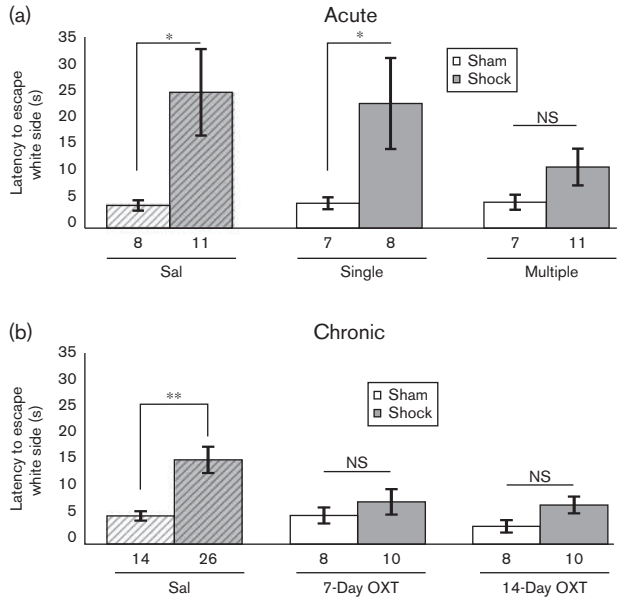
group escaped the white side faster than the saline shocked group [ANOVA, $F(1,17)=4.87$, $P<0.05$]. This difference is also observed in the single-injection group [ANOVA, $F(1,13)=5.23$, $P<0.05$]. Interestingly, this difference was absent in the multi-injection group in which sham and shocked animals were equally likely to cross to the black side within a few seconds of exposure to the apparatus [ANOVA, $F(1,16)=4.37$, NS]. These two groups also failed to show a significant difference in the opened/closed arm ratio in the EPM test (ANOVA, $P>0.05$), with the caveat that the EPM test might not be as sensitive to the sham versus shock manipulation as the black and white box test (see below).

These results suggest that a single injection of OXT postshock does not have long-term effects on generalized anxiety or on the putative ability to generalize the context of the shock. In contrast, repeated injections of OXT to shocked animals after three additional re-exposures to the trauma apparatus (with no shock given) yielded a long-term reduction in anxiety.

Chronic oxytocin

As in the acute conditions, the osmotic pump sham saline group escaped the white side sooner than the saline shock group [Fig. 4b; hashed bars, ANOVA, $F(1,38)=8.5$, $P<0.01$]. Interestingly, neither the 7-day nor the 14-day OXT shock group showed a significant difference in latency to escape the white side compared with the sham groups [$F(1,16)=0.76$, NS and $F(1,16)=3.8$, NS, respectively). These two chronic OXT conditions were also not significantly different from their shams controls in the EPM test when measuring open/closed arm ratios (ANOVA, $P>0.05$), whereas the sham and shock saline groups differed significantly [ANOVA, $F(1,16)=10.66$, $P<0.01$]. There was no significant difference between the two sham groups, or between the two shocked groups. These results are compatible with the multiple-injection acute group and suggest that chronic administration of OXT during the SRs was also effective at decreasing anxiety in this test, and that an additional 7 days of OXT administration had no noticeable effect (although the measurements may have been at the floor level).

Fig. 4



Chronic and multiple acute oxytocin (OXT) administration reduced generalized fear two weeks after shock. (a) Latency to escape the white side in the black and white box for the subcutaneous injection groups. The single-injection groups did not differ significantly from the saline groups. The multiple-injection shocked group did not differ significantly from the sham controls. (b) Latency to escape the white side in the black and white box for the osmotic pump groups. Neither 7-day nor 14-day groups were significantly different from their sham controls. Group sizes are indicated below the *x*-axis for each group. In all panels: **P*<0.05 and ***P*<0.01.

Altogether, these results suggest that acute administration of OXT immediately after re-exposure to the trauma context under 'safe' conditions (SRs, no shocks) was sufficient to reduce long-term anxiety normally shown by shocked animals in this test.

There was no significant difference between the saline shock and saline sham animals in the EPM or open-field measures [acute group – EPM open/closed ratio $F(1,17)=1.25$, NS; open-field, time spent in the center (% baseline) $F(1,17)=1.28$, NS. Chronic group – EPM open/closed ratio: $F(1,26)=3.35$, $P=0.078$; open-field, time spent in the center (% baseline) $F(1,27)=3.20$, $P=0.085$]. Because neither the EPM nor the open-field tests succeed in separating shock from sham saline in our sample, we did not pursue these analyses further.

Effect of co-housing

The causal role of OXT in prosocial behavior has been documented thoroughly. OXT levels increase as a consequence of social bonding, and injections of OXT increase prosocial behaviors. OXT, however, has effects beyond prosocial behaviors, and social bonding can alter the levels of many hormones. We next tested the effect of social bonding (manipulated by co-housing conditions) on postshock behaviors. Two cases of co-housing were studied: the first case consisted of a shocked animal co-housed with two sham cagemates (solo shocked), whereas the second consisted of a shocked animal co-housed with two other shocked animals (all shocked).

Situational reminders

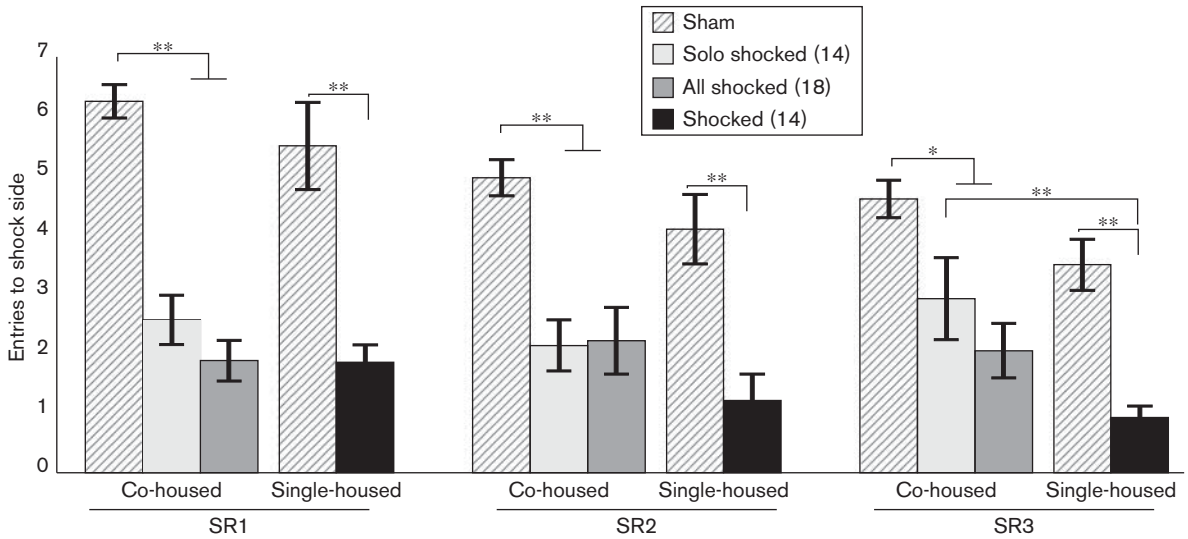
The shock groups in all conditions made significantly fewer entries into the shock side than the shams for each situational reminder [Fig. 5; ANOVA: all shocked versus sham: SR1: $F(1,43)=124.93$, SR2: $F(1,44)=21.82$, SR3: $F(1,44)=22.16$, all $P<0.01$. Solo shocked versus sham: SR1: $F(1,40)=55.71$, $P<0.001$, SR2: $F(1,40)=28.94$, $P<0.001$, SR3: $F(1,40)=4.73$, $P<0.05$. Single-housed

shock versus sham: SR1: $F(1,22) = 26.52$, SR2: $F(1,22) = 15.89$, SR3: $F(1,22) = 36.22$, all $P < 0.01$]. These differences were also found when considering the time spent in the shock compartment [all shocked vs. sham: SR1: $F(1,44) = 125.25$; SR2: $F(1,44) = 80.93$; SR3: $F(1,43) = 21.51$, all $P < 0.001$. Solo shocked vs. sham: SR1: $F(1,39) = 91.3$; SR2: $F(1,39) = 106.87$; SR3: $F(1,38) = 40.6$, all $P < 0.001$. Single-housed shock versus sham: SR1: $F(1,21) = 40.34$; SR2: $F(1,21) = 15.68$; SR3: $F(1,20) = 21.66$. $P < 0.001$]. Co-housed sham animals made generally more entries into the shock side than the single-housed sham animals, reaching statistical significance in SR2 and SR3 [ANOVA: SR2: $F(1,35) = 4.84$, SR3: $F(1,35) = 5.3$, both $P < 0.05$; results not shown for clarity]. There was a significant effect of shock × SRs for the solo-shocked versus sham group [mixed ANOVA, $F(2,78) = 4.74$, $P < 0.05$], for the all-shocked versus sham group [mixed ANOVA, $F(2,86) = 6.94$, $P < 0.01$], and a significant main effect of situational reminders for the single-housed sham versus shock animals [mixed ANOVA, $F(2,44) = 5.26$, $P < 0.01$]. The analysis of time spent in the shock compartment showed a significant SRs × shock interaction for the all-shocked group [$F(2,86) = 6.59$, $P < 0.002$]. The single-housed shocked animals, but not the co-housed animals, showed a significant decrease in entries (increase in anxiety) from SR1 to SR3 [trend analysis, ANOVA, $F(2,37) = 4.7$, $P < 0.012$, Tukey's test]. The difference between the solo-shock group and the single-housed shock group increased from SR1 to SR3, reaching statistical significance at SR3 [Fig. 5a; light gray and black bars, ANOVA SR3: $F(1,25) = 9.86$, $P < 0.01$]. This appeared to be the result of an increase in the entries in the solo-shock group (light gray, compared with the decrease observed in the sham co-housed group, indicating less anxiety) and a decrease in entries in the single-shock groups (black, more anxiety). The all-shocked animals did not show a significant change in entries or time spent in the shock compartment from SR1 to SR3.

These results indicate that co-housing with nonshocked animals (but not with other shocked animals) was effective at decreasing the level of anxiety upon repeated exposure to a safe context, likely through an increase in fear extinction. This result is compatible with the OXT injection and osmotic pump groups, and suggests that the putative increase in natural OXT levels because of co-housing with naive animals may reduce anxiety to the traumatic context upon several re-exposures. Although significant, the effects observed with social housing were weaker than those observed with exogenous administration of OXT.

We next proceeded to test the effects of co-housing on long-term anxiety, as afforded by our protocol (Fig. 1). Co-housing experiments are inherently more variable than well-controlled substance injections. This is because of individual differences between rats, dominance hierarchy composition among cagemates, and other factors beyond the control of the experimenters. For this reason, several tests were taken into consideration when assessing anxiety.

Fig. 5



Co-housed animals showed reduced contextual fear after multiple exposures. All-shocked animals made significantly fewer entries into the shock compartment than shams in all three situational reminders (SRs). The single-housed but not the co-housed shocked rats showed increased avoidance of the shock compartment from SR1 to SR3. The co-housed solo-shocked rats made more entries into the shock compartment than the Single-housed shocked rats (statistically significant in SR3). Co-housed sham: $n = 28$, single-housed sham: $n = 10$. *P<0.05 and **P<0.01.

Long-term effects

Black and white box

No difference was noted in the latency to escape the white side [Fig. 6a; ANOVA, $F(1,33) = 0.1$, NS], in the number of entries to the white side [$F(1,33) = 0.02$, NS], or in the time spent in the white compartment [$F(1,33) = 0.90$, NS] in co-housed and single-housed sham groups. A two-way ANOVA on the influence of housing (single, co-housed) and shock (shock, sham) on the latency to escape the white compartment was carried out. There were significant main effects of the shock treatment [$F(1,73) = 6.75$, $P < 0.02$], of the housing condition [$F(1,73) = 4.52$, $P < 0.05$], and a significant shock × housing interaction [$F(1,73) = 4.1$, $P < 0.05$]. The same analyses on both the number of crossings and time in the white compartment yielded a main effect of shock [$F(1,74) = 12.35$, $P < 0.001$ and $F(1,72) = 4.5$, $P < 0.05$, respectively], but no significant main effect of housing [$F(1,74) = 0.71$, NS and $F(1,72) = 3.2$ $P = 0.076$, respectively] or interaction [$F(1,74) = 0.4$, NS, $F(1,72) = 0.83$, NS]. The single-housed shock and co-housed solo-shock groups showed a significantly greater latency to escape the white side than the sham rats [Fig. 6a; ANOVA, $F(1,46) = 8.99$, $P < 0.005$ and $F(1,45) = 5.09$, $P < 0.05$, respectively]. This effect was also present when measuring the total time spent in the white compartment [$F(1,45) = 8.79$, $P < 0.005$] and the number of crossings [$F(1,46) = 11.25$ $P < 0.002$]. There was no significant difference between the co-housed shock groups [latency to escape: $F(1,27) = 0.78$, NS, time in white: $F(1,27) < 0.005$, NS] or between the all-shocked group and the shams [latency to escape: $F(1,50) = 1.88$, NS, time in white: $F(1,50) = 1.61$, NS, crossings: $F(1,50) = 1.32$, NS]. Interestingly, the single-housed shocked animals showed significantly greater latency to escape the white side compared with the co-housed shock rats [Fig. 6a; $F(1,40) = 5.64$, $P < 0.025$, time in white $F(1,39) = 4.48$, NS]. These results show that both types of co-housing were effective in decreasing long-term anxiety in this test and in decreasing putative generalization of the traumatic context to this apparatus.

Elevated plus maze

No significant difference was noted between the co-housed and the single-housed sham groups [Fig. 6b, ANOVA, $F(1,36) = 0.19$, NS]. A two-way ANOVA on the influence of housing and shock showed a significant main effect of shock [$F(1,65) = 4.77$, $P < 0.05$], but no significant main effect of housing [$F(1,65) = 3.0$, $P = 0.08$] or interaction [$F(1,65) = 2.26$, NS]. There was no significant difference between the solo and the single-housed shocked rats [ANOVA, $F(1,25) < 0.001$, NS]. The co-housed all-shocked rats, however, had a significantly higher open arm to closed arm ratio than the sham rats [ANOVA, $F(1,43) = 7.1$, $P < 0.02$]. The all-shocked rats also had a higher open to closed arm ratio than the combined single-housed and solo-shocked groups [ANOVA, $F(1,41) = 5.23$, $P < 0.05$], but not relative to either of these groups individually [co-housed all shocked vs. solo shocked: ANOVA, $F(1,27) = 2.74$, NS, co-housed all shocked vs. single-housed shocked: ANOVA, $F(1,28) = 3.0$ $P = 0.095$]. This suggests that groups of all-shocked animals, but not animals exposed to no other rats or to naive rats, showed a significant increase in risk-taking behavior (Toledo-Rodriguez and Sandi, 2011).

Open field

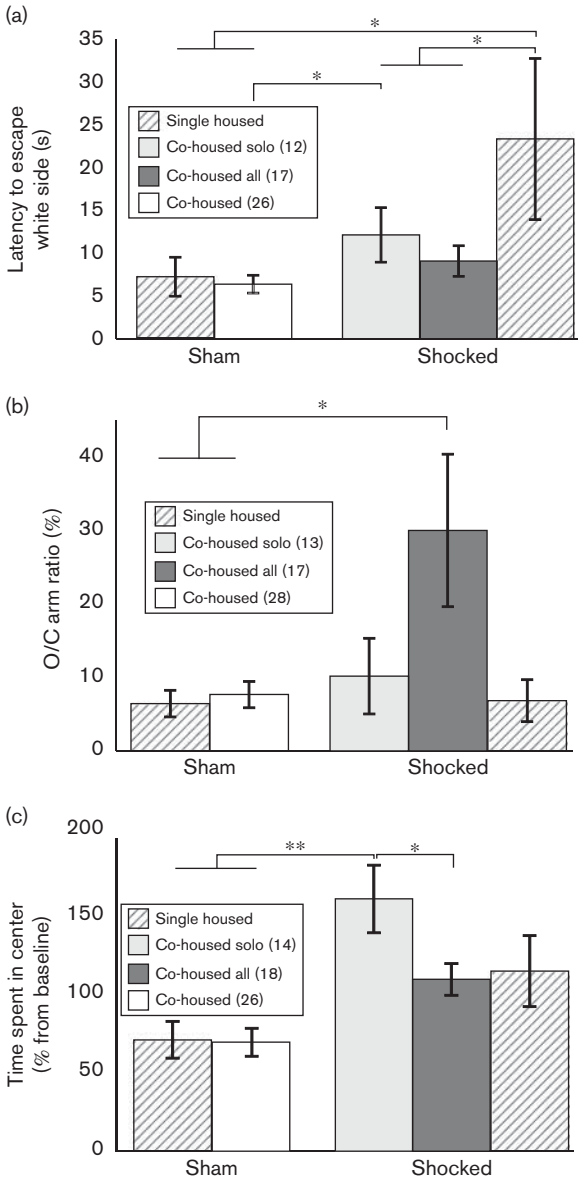
No significant difference was noted between the co-housed and the single-housed sham groups [Fig. 6c; ANOVA, $F(1,35) = 0.07$, NS]. A two-way ANOVA on the influence of housing and shock showed a significant main effect of shock [$F(1,78) = 4.78$, $P < 0.05$], with no significant main effect of housing condition [$F(1,78) = 0.05$, NS] or interaction [$F(1,78) = 0.35$, NS]. The solo-shocked co-housed group spent significantly more time exploring the novel object at the center of the table than the sham groups [ANOVA, $F(1,49) = 12.95$ $P < 0.001$]. The solo-shocked animals also made a higher number of visits to the center than the all-shocked group [ANOVA, $F(1,30) = 5.01$, $P < 0.05$]. As with the SRs, this result showed that the co-housing of a traumatized subject with naive animals (but not with other shocked animals, or alone) was effective at increasing their willingness to explore novel objects at the center of the open field.

Overall, these results show different patterns of test sensitivity when assessing long-term (2 weeks after trauma) anxiety levels. Shocked animals that are single housed or co-housed with naive cagemates are separated from their control in the black and white box test (Fig. 6a), but not in the EPM test (Fig. 6b). The co-housed animals that have all been subjected to shock can only be effectively differentiated from their control in the EPM test. Finally, of the three tests used, the open-field test was the only one able to effectively segregate the solo-shock group from the all-shock group, although the EPM results suggest that this test might become effective with a larger number of animals.

Effect of shock and co-housing on long-term oxytocin plasma level

Finally, we assessed the level of endogenous plasma OXT levels in all groups of animals after all procedures and tests 18 days after trauma. Figure 7 shows the concentration of OXT relative to those of the single-housed sham animals. Single-housed shocked animals showed similar concentrations of OXT [$F(1,21) = 0.34$, NS] to single-housed shams (baseline), indicating that the trauma procedure and subsequent tests did not in and of themselves alter OXT levels. The level of OXT was also not significantly different from the baseline in the co-housed all-shocked group [$F(1,27) = 0.01$, NS]. Interestingly, the solo-shocked animals showed the lowest level of OXT among all groups compared with the baseline

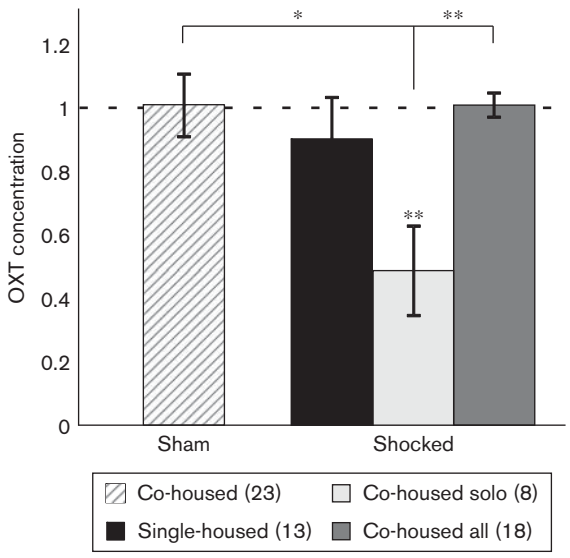
Fig. 6



Different tests were differentially effective at capturing the long-term consequences of the trauma in the different groups of animals. (a) Black and white box: the single-housed shocked animals showed significantly more anxiety than the co-housed shocked and sham rats. single-housed sham: $n=9$, single-housed shocked: $n=12$. (b) Elevated plus maze: the co-housed all-shocked rats showed significantly less anxiety than all other groups. Single-housed sham: $n=10$, single-housed shocked: $n=13$. (c) Open field: the co-housed solo-shocked animals showed significantly less anxiety than the co-housed sham rats. Single-housed sham: $n=9$, single-housed shocked: $n=13$. In all panels: *$P<0.05$ and **$P<0.01$.

[$F(1,15)=12.59$, $P<0.005$], including their cagemates [co-housed shams, $F(1,31)=0.28$, NS]. Finally, the solo-shocked animals had significantly lower OXT levels than the other co-housed groups [sham: $F(1,28)=5.0$, $P<0.05$ and all shocked: $F(1,24)=25.43$, $P<0.001$], but not the single-housed group [$F(1,18)=4.03$, $P=0.06$].

Fig. 7



Oxytocin (OXT) plasma in co-housed and single-housed animals. The co-housed solo-shocked animals showed a significantly lower concentration of OXT. Oxytocin levels were normalized to single-housed sham animals (dashed line). *$P<0.05$ and **$P<0.01$.

This result suggests that if OXT levels were the result of social bonding, this bonding differed significantly between a shocked animal co-housed with naive cagemates and shocked animals co-housed with other shocked animals.

Discussion

Our results show that a single injection of OXT immediately after a traumatic event and before the onset of memory consolidation was effective in increasing memory of the shock compartment 2 days later (SR1). Repeated injections of OXT immediately after memory reactivation mediated by re-exposures had no noticeable effect on the memory for the shock compartment beyond that observed in saline controls (Fig. 3a), but significantly reduced the levels of anxiety of these animals measured 2 weeks after shock (Fig. 4a). These results suggest that acute OXT immediately after a behavioral experience increases memory consolidation for that experience. If the injection occurs after a negative experience (shock), animals are more anxious in the short term, but if the injection repeatedly occurs after positive experiences (minimal contextual fear extinction: safe re-exposure to the shock box), animals become less anxious in the long term, a reduction that may be attributable to an OXT-mediated increase in the consolidation of the memory of the shock apparatus as a safe context across days, and to its generalization to the black and white box. These data are consistent with previous studies that have shown a general benefit of OXT on memory consolidation and fear extinction in rodents when administered shortly after

memory acquisition (de Wied *et al.*, 1993; Chini *et al.*, 2013), possibly through its actions in the central nucleus of the amygdala (Viviani *et al.*, 2011). They are also consistent with the results of studies showing that intranasal OXT administration increases fear extinction in humans (Pitman *et al.*, 1993; Acheson *et al.*, 2013; Eckstein *et al.*, 2015). These results are, however, at odds with a recent study in which postexposure intraperitoneal injections of OXT did not alter fear extinction in shocked animals (Eskandarian *et al.*, 2013). This difference may be the fact that extinction was prolonged in this study (10 min, unlike our minimal extinction procedure lasting 2 min) and may have brought the animals to ceiling levels, such that OXT effects on extinction may not have been noticeable. Our results are also at odds with another study that showed an impairment in cued fear extinction (Toth *et al.*, 2012). In this study, OXT was administered before extinction training (we injected after the exposures, Fig. 1), and hence affected the learning as well as the consolidation phases of the experiment. Our results therefore suggest that the effects that we observed are specific to memory consolidation, and could be washed out (or inverted) by the administration of OXT during learning.

Chronic administration of OXT during the shock and re-exposures appeared to have a cumulative anxiolytic effect, making these animals no different from controls after three re-exposures (7 days, Fig. 3b). This effect persisted for 7 days after the termination of the OXT delivery as these animals were also no different from controls in a general anxiety test delivered at that time (Fig. 4b, 7-day pump). Interestingly, additional delivery of OXT throughout the protocol did not yield a further decrease in anxiety, although these levels may have been too low to be measured (Fig. 4b, 14-day pump). These results confirm the idea that OXT does not need to be administered centrally to have therapeutic effects and to reduce PTSD-induced anxiety (Ayers *et al.*, 2011; Neumann *et al.*, 2013).

Co-housing had a progressive effect on the memory for the shock compartment in groups of animals in which only one animal was shocked and two others were naive. This group was significantly less anxious about the shock compartment than the single-housed counterpart by day 7 after the shock (Fig. 5). This effect was not present in groups of animals that were all shocked, suggesting that there may be important behavioral differences in the home cage interactions between shocked animals and naive cagemates and between those of a shocked animal and other shocked cagemates. This distinction is apparent in long-term tests in which all-shocked cohorts showed more risk-taking behaviors (Fig. 6b) than any other group and in which shocked animals living with naive cagemates showed more willingness to overcome thigmotaxis to explore novel objects than single-housed animals (Fig. 6c). Altogether, these results suggest that long-term exposure of traumatized animals to naive animals, but not to other traumatized animals, may have significant beneficial effects on their memory for the traumatic context and on their long-term anxiety. Using a different paradigm, others have shown that co-housed pairs of stressed animals were less aggressive and interacted more than sham controls (Huzard *et al.*, 2015), suggesting that social interactions in the solo-shocked group in our experiments may be less than that in the all-shocked group. Interestingly, the solo-shocked group of animals showed a significantly reduced amount of plasma OXT than any other group (Fig. 7). These results suggest that solo-shocked animals may have bonded less with their cagemates than all-shocked or co-housed sham animals, but still benefited behaviorally from co-housing. Interestingly, recent studies in voles showed that blocking the OXT receptors in the anterior cingulate cortex or intraventricularly abolished partner-directed consolation behavior toward stressed co-housed animals (Burkett *et al.*, 2016). This study did not manipulate the OXT levels in the stressed animals; thus, no direct comparison is possible, but it suggests that empathy-like behaviors or some forms of social bonding (Arai *et al.*, 2016) may have differed between the all-shocked and solo-shocked trios. Further behavioral and pharmacological work quantifying the nature and amount of OXT-dependent social interactions would be needed to assess this hypothesis.

The neural substrates mediating the effect of peripheral administration of OXT on stress is complex. OXT is a nine amino acid neuropeptide that has been implicated in pair-bonding, attachment, childbirth, regulation of eating and drinking, and stress responses (Gimpl and Fahrenholz, 2001; Carter *et al.*, 2008; Ayers *et al.*, 2011). Animal research has shown a strong link between OXT, social cognition, and prosocial behavior (Lukas and Neumann, 2013; Burkett *et al.*, 2016). The extent to which the behavioral and cognitive effects of OXT generalize to humans is a matter of debate, but is believed to depend on the social context in which OXT is administered (Bartz *et al.*, 2011), compatible with our results. Intranasal OXT in humans increases trust and prosocial behaviors (Kosfeld *et al.*, 2005). In addition to its anxiety-reducing effects, OXT in humans has been suggested to influence affiliative motivation and the perception of social cue salience, both of which were likely at play in our co-housing experiments (Bartz *et al.*, 2011).

The peptide is produced peripherally and centrally from the paraventricular and supraoptic nuclei of the hypothalamus (Gimpl and Fahrenholz, 2001). In both humans and rodents, OXT administration has been shown to reduce activation of the amygdala (Kirsch *et al.*, 2005; Lukas *et al.*, 2013), a region shown recently in rodents to be a possible target for deep-brain stimulation treatments of PTSD (Stidd *et al.*, 2013). Recent studies have shown that intraperitoneal injections of OXT in mice increase central OXT in the amygdala and the dorsal

hippocampus within 30 min of the injection, which is consistent with our data in Fig. 1 (Neumann *et al.*, 2013). Previous studies have also found effects of systemically administered OXT in the central amygdala, hypothalamus, and nucleus of the solitary tract (Carson *et al.*, 2010; Ho *et al.*, 2014). The CA1 area of the rodent hippocampus contains a significant level of OXT (but not vasopressin) receptors in its ventral portion (Elands *et al.*, 1988; Stoop, 2012) and is known to be a key contributor to contextual fear memory, together with the mPFC and the amygdala (Maren *et al.*, 2013). Repeated OXT infusions in the infralimbic region of the mPFC immediately after exposure to the shock context facilitated fear extinction, whereas the same manipulation in the basolateral amygdala had the opposite effect and impaired extinction (Lahoud and Maroun, 2013). Our results show that systemic administration of OXT mimicked the mPFC effects, therefore suggesting that OXT has a more powerful modulatory influence on the mPFC than on the amygdala. Interestingly, this dichotomy between mPFC and the amygdala in fear extinction was also noted by blocking dopamine D1 receptors (Hikind and Maroun, 2008). Ventral tegmental area dopaminergic cells have recently been shown to be chronically depressed by the trauma paradigm used here (Corral-Frias *et al.*, 2013), suggesting that the OXT and the dopaminergic system might work in tandem in the etiology of PTSD.

Limitations and future work: (i) There is a well-known interaction between OXT and vasopressin receptors. Future experiments could be aimed at selecting specific agonists and antagonists to tease apart their respective contributions (Hicks *et al.*, 2012; Neumann and Landgraf, 2012). (ii) There is also a well-documented differential sensitivity of OXT in male and female rats (Neumann, 2008; Slattery and Neumann, 2010). The extent to which these sex differences apply to PTSD and long-term anxiety remains to be elucidated. (iii) Many studies of the effect of OXT on PTSD use co-housed groups of animals, without controlling for the effect of co-housing (Eskandarian *et al.*, 2013; Lahoud and Maroun, 2013). Our results suggest that these experiments should be revisited and extended to differentiate the contributions of OXT and social co-housing following a traumatic event.

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Conflicts of interest

There are no conflicts of interest.

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