Published in final edited form as:

Psychopharmacology (Berl). 2014 May ; 231(10): 2097–2105. doi:10.1007/s00213-013-3356-6.

Role of Oxytocin Receptors in Modulation of Fear by Social Memory

Yomayra F Guzmán¹, Natalie C Tronson^{1,2}, Keisuke Sato³, Ivana Mesic¹, Anita L. Guedea¹, Katsuhiko Nishimori³, and Jelena Radulovic¹

¹Department of Psychiatry and Behavioral Sciences, Northwestern University, Chicago, Illinois, USA

²Department of Psychology, University of Michigan, Ann Arbor, Michigan, USA

³Department of Molecular and Cell Biology, Graduate School of Agricultural Science, Tohoku University, Miyagi, Japan

Abstract

Rationale—Oxytocin receptors (Oxtr) are important mediators of social learning and emotion, with bidirectional effects on fear and anxiety. Contrary to the anxiolytic actions of Oxtr in the amygdala, we recently showed that Oxtr in the lateral septum mediate the enhancement of fear conditioning by social defeat in mice.

Objectives—Using positive social interactions, which impair fear conditioning, here we attempted to delineate whether the role septal Oxtr in fear regulation depends on the valence of the social memory.

Methods—Pharmacological and genetic manipulations of lateral septal Oxtr were combined with the social buffering of fear paradigm, in which pre-exposure to nonfearful conspecifics reduces subsequent contextual fear conditioning, as revealed by decreased freezing behavior.

Results—Antagonism and down-regulation of Oxtr in the lateral septum abolished, while oxytocin (Oxt) administration before pre-exposure to nonfearful conspecifics facilitated the decrease of freezing behavior.

Conclusions—The septal oxytocin system enhances memory of social interactions regardless of their valence, reducing fear after positive and enhancing fear after negative social encounters. These findings explain, at least in part, the seemingly bidirectional role of Oxt in fear regulation.

Keywords

Oxytocin; Oxytocin Receptors; Social interaction; Memory; Valence; Fear conditioning; Lateral septum; Hippocampus; NMDA receptors

Introduction

Social factors potentially exacerbate or ameliorate neuropsychiatric disorders by modulating emotional processes (Allen and Badcock 2003; Barrett and Mizes 1988). Positive social interactions and social support decrease anxiety (Egliston and Rapee 2007; Heinrichs et al. 2003; Thorsteinsson et al. 1998; Ullman and Filipas 2001), whereas negative, stress-inducing social interactions are anxiogenic (Solomon et al. 1986). Accordingly, anxiety disorders, such as post-traumatic stress disorder, (Heim and Nemeroff 2009) are exacerbated by lack of social support (Boscarino 1995).

Animal models have been successfully employed to identify the various mechanisms by which social interactions affect emotional learning processes (Lipina and Roder 2013; Panksepp 2011). In some cases, observers mimic the behavioral state of a demonstrator experiencing pain (Langford et al. 2006), fear (Kim et al. 2010) or lack of fear (Davitz and Mason 1955; Kikusui et al. 2006), whereas in others, subjects learn stimulus contingencies (Bandura 1969a, 1969b; Kavaliers et al. 2001; Mineka et al. 1984; Olsson and Phelps 2007). For example, observing a conspecific receiving a shock paired with a cue leads to successful fear conditioning in the observers (Bruchey et al. 2010; Jeon et al. 2010; Riess 1972). Similarly, contextual fear conditioning following positive and negative social interactions respectively reduces and enhances conditioned freezing (Guzman et al. 2009; Guzman et al. 2013). Thereby, learning processes lead to persistent changes of behavior after the social interactions have ceased (Knapska and Maren 2009; Knapska et al. 2010; Nowak and Knapska, 2013).

Despite the well-characterized behavioral effects of social interactions on fear and anxiety, the neurobiological mechanisms involved in this type modulation remain largely unexplored. At a neuroanatomical level, brain regions involved in direct and vicarious fear learning are thought to overlap (Olsson et al. 2007; Olsson and Phelps 2007). The hippocampus, a structure essential for the acquisition of contextual fear conditioning (Ahi et al. 2004; Kim and Fanselow 1992; Radulovic et al. 2000) has been implicated in social recognition learning (Kogan et al. 2000), whereas the amygdala responds with immediate early gene activity in rodents directly experiencing fear as well as in those that observe the fearful models (Knapska et al. 2006). Similarly, regions of the broader fear circuit, such as the anterior cingulate cortex (Jeon et al. 2010) and lateral septum (Guzman et al., 2013) also contribute to vicarious fear learning.

At a molecular level, social interactions and their effect on emotional behavior have been most extensively linked to the oxytocin system. Oxt is best known for its anxiolytic actions (Ayers et al. 2011; Carter 1998; Ishak et al. 2011; Waldherr and Neumann 2007; Windle et al. 1997) mediated by the hypothalamus (Blume et al. 2008) or amygdala (Huber et al. 2005; Knobloch et al. 2012; Viviani et al. 2011). Nevertheless, an increasing number of findings also reports anxiogenic actions of Oxt (Grillon et al. 2012; Striepen et al. 2012; Tabak et al. 2011). In line with the latter observations, we recently demonstrated that Oxtr in the lateral septum mediate the enhancement of fear by social defeat stress in mice (Guzman et al. 2013). The lateral septum is a cytoarchitecturally diverse region dense in Oxtr (Barberis and Tribollet 1996; Loup et al. 1991; Yoshida et al. 2009), which also mediates

social learning (Dantzer et al., 1987; Popik and Vetulani 1991a; Popik et al. 1992a; Popik et al. 1992b). Thus it has remained unclear whether lateral septal Oxt mediated the coding of negative valence, social memory of the defeat, or both. In order to delineate between these possibilities, here we investigated the role of the septal oxytocin system in modulation of fear by positively valenced social interactions. We employed the model of social buffering of fear, in which Interactions with non-fearful conspecifics reduce subsequent fear conditioning (Guzman et al. 2009). We hypothesized that, if Oxt subserved encoding negative valence and fear, activation of these receptors would abolish the reduction of freezing by positive social interactions. Alternatively, if Oxt subserved encoding the social memory, their activation would be required for reduction of freezing. Using pharmacological antagonism and genetic down-regulation of lateral septal Oxt, we demonstrated that Oxt mediated the reduction fear conditioning by positive social interactions, which was further facilitated by local Oxt infusion. The lateral septal Oxt system thus enhances social memory regardless of its valence, and thereby exerts bidirectional regulation of fear.

Materials and methods

Animals

Experimental subjects consisted of wild type male C57Bl/6N mice obtained from Harlan Laboratories, *Oxtr^{LoxP/LoxP}* mice (Takayanagi et al. 2005) backcrossed for 9 generations to C57BL/6N mice, and Oxt reporter mice that expressed Venus under the control of the Oxt promoter (Yoshida et al. 2009) backcrossed for 6–8 generations to C57BL/6N mice. Mice were individually housed in a satellite facility adjacent to the behavioral equipment. The satellite facility was maintained at $20 \pm 2^\circ\text{C}$ and 40%–50% humidity under a 12/12 dark light cycle (7am–7pm). All studies were approved by the Animal Care and Use Committee at Northwestern University and are in compliance with the National Institute of Health standards.

Surgery

Mice were anesthetized with an intraperitoneal injection of 240 mg/kg of Avertin (1.2% solution of 2, 2, 2-Tribromoethanol dissolved in 2-methyl-2-butanol) and implanted with bilateral 26-gauge cannulae using a stereotaxic apparatus, as previously described (Radulovic et al. 1999). Lateral septum was targeted using coordinates: AP+0.2 mm, ML ± 0.5 mm, DV–3.0 mm, where we observed the highest level of Oxt (Figure 1a). In a neuroanatomical control experiment we targeted the dorsal hippocampus using coordinates: AP+1.5 mm, ML ± 1.0 mm, DV–3.0 mm from bregma (Paxinos and Franklin 2001). Mice were allowed to recover for one week before injection of peptides or viral vectors.

Drugs and Injections

Oxytocin (Sigma), [1-D(CH₂)₅, Tyr(ME)₂, Thr₄, Tyr-NH₂(9)]ornithine vasotocin (OTA, Bachem) and 2-amino-5-phosphonovaleric acid (APV, Sigma) were injected at a rate of 0.5 μl / per minute. A total volume of 0.2 μl /side (lateral septum) or 0.25 μl /side (dorsal hippocampus) was injected while the mice were under isoflurane anesthesia for approximately one minute. The viral vectors rAAV-Cre (driven by a synapsin promoter and

obtained from Pavel Osten), rAAV-GFP (driven by a synapsin promoter, University of Pennsylvania Viral Vector Core) had a titer of $1-2 \times 10^{12}$ particles/ml. Oxytocin (lateral septum: 1 ng/0.4 μ l/mouse, hippocampus: 1 ng/0.5 μ l/mouse), OTA (lateral septum: 10 ng/0.4 μ l/mouse, hippocampus: 10 ng/0.5 μ l/mouse) and APV (lateral septum: 4 ng/0.4 μ l/mouse, hippocampus: 5 ng/0.5 μ l/mouse) were dissolved in artificial cerebrospinal fluid (aCSF) and aCSF alone served as vehicle. The dose of Oxt was selected from pilot studies as the minimal effective dose.

Animals were injected 15 min (OTA, APV), 2 min (Oxt), or 14 days (rAAV-Cre, rAAV-GFP) before pre-exposure to the non-fearful model or the control condition (Figure 1b). The time point for OTA and APV administration was selected to ensure maximal receptor blockade at the time of the pre-exposure sessions. Oxt administration was given 2 min before pre-exposure, because at this time-point Oxt was shown to enhance social learning in other paradigms (Ferguson et al. 2000). As previously described, a single mouse was used as a non-fearful model for the NF group to minimize variability of individual interactions (Guzman et al. 2009).

RNA extraction and quantitative PCR (qPCR) for determination of *Oxtr* mRNA levels

Selected mice was used to validate the genetic knockdown of *Oxtr* by viral vectors. Mice were injected with methylene blue to visualize the viral injection site and then killed by cervical dislocation. Brains were dissected and the lateral septum was collected using a brain matrix. Tissue was homogenized in lysis buffer with -mercaptoethanol and frozen in liquid nitrogen. RNA was extracted using miRCURY total RNA isolation kit (Exiqon), reversely transcribed and subjected to real-time PCR using SYBR Green master mix (Applied Biosystems) and primers for *Oxtr* or mouse *hypoxanthine phosphoribosyl transferase 1* (*mHprt1*) as an internal control. For *Oxtr* the forward primer was 5'-GGA GCG TCT GGG ACG TCA AT-3' and the reverse primer was 5'-AGG AAG CGC TGC ACG AGT T-3'. For *mHprt1* the forward primer was 5'-GGG CTT ACC TCA CTG CTT TC -3' and reverse 5'-TCT CCA CCA ATA ACT TTT ATG TCC-3'. The level of *Oxtr* expression in *Oxtr^{LoxP/LoxP}* mice injected with rAAV-Cre or was normalized to *mHprt1* and shown relative to the rAAV-GFP control.

Experimental Apparatus

A transparent Plexiglas partition was used to diagonally bisect a conditioning chamber (35 cm long x 20 cm wide x 20 cm high) into equally-sized regions with an area of 350 cm². The floor of the conditioning chamber consisted of a removable shock grid made of stainless steel rods (diameter 4 mm; bars spaced 0.9 cm apart). The chamber was cleaned between mice with 1% ethanol.

Social buffering of fear paradigm

Social buffering of fear generally refers to the reduction of fear by positive social interactions. In our paradigm, social buffering refers to the decrease of context freezing in mice pre-exposed to non-fearful conspecific prior to conditioning (Guzman et al. 2009). Non-fearful models were habituated to the divided conditioning chamber for 90 min, 24 hrs prior to pre-exposure sessions as previously described (Guzman et al. 2009). Experimental groups

consisted of observer mice pre-exposed to a non-fearful model once (NF1) or twice (NF2). Typically, two pre-exposures are required to observe significant reduction of freezing behavior, therefore, we used the NF2 paradigm to determine whether Oxt mediate this effect. In order to establish whether Oxt can further enhance social buffering, we used the NF1 paradigm that normally does not result in reduced freezing. Control groups consisted of mice pre-exposed to a toy mouse only (Controls). We previously showed that mice pre-exposed to a toy mouse or the empty box freeze similarly (Guzman et al. 2009); thus we used these groups interchangeably as controls. Pre-exposures consisted of placing the observer animals and the model animals in the divided conditioning chamber for 3 min. The first time a pharmacological or genetic manipulation was used, we scored the number of times the animals interacted at the partition during pre-exposure to ensure that treatments did not affect social interactions. An interaction was scored when mice approached the partition, and faced or reared toward one another. After each pre-exposure the grid floor in the observer area and the tray underneath was cleaned with 70% ethanol. After the end of each experiment, all brains were collected for verification of cannula placements following methylene blue injection. Only mice with correct cannula placement were included in the data analysis.

Contextual fear conditioning training of observers

Observer mice were trained on contextual fear conditioning 24 hr after the last pre-exposure session with an automated system (TSE Inc.). Fear conditioning consisted of placing the observer animals in the side of the box originally occupied by the model (Guzman et al. 2009). After a 3-min exploration period the mice were given a 2 s 0.7 mA foot shock. The fear response was measured 24 hours after training by scoring freezing behavior, defined as lack of movement except that needed for respiration (Blanchard and Blanchard 1969) every 10th second for 3 min by a trained observer and expressed as a percentage of the total number of observations.

Statistical Analysis

Data were analyzed by Student's t-test for comparison of two groups, or one- or two-way ANOVA with Group or Group and Treatment as main factors, for comparison of three or more groups. Post-hoc comparisons were performed using Tukey's HSD test. Data are presented as mean $\pm$ the standard error of the mean (SEM). Statistically significant differences are marked as * $p < 0.05$ and ** $p < 0.01$ when compared to the indicated control group.

Results

Effect of lateral septal Oxt on social buffering of fear

Consistent with our earlier observations (Guzman et al. 2009), observer mice of the NF2 ($n = 7$) group showed significantly reduced contextual freezing (Figure 2a) when compared to the vehicle control ($n = 7$). Two-way ANOVA with factors Group (Control, NF2) and Treatment (Vehicle, OTA) revealed significant effect of Group ($F_{3,28} = 3.549$, $p = 0.027$) and Group $\times$ Treatment interaction [$F(1,31) = 8.639$, $p = 0.007$], suggesting that Oxt in the lateral septum were required for this type of modulation. Control mice not pre-exposed to a

non-fearful model and injected with OTA ($n = 8$) or vehicle ($n = 10$) froze similarly suggesting that the role of septal Oxt on modulation of fear is specific to social interactions rather than a generalized effect on fear or anxiety (Fig. 2a).

To further characterize the role of septal oxytocin on social modulation of fear, we administered Oxt intraseptally 2 min before a single pre-exposure session (NF1 paradigm). Two way ANOVA with factors Group (Control, NF2) and Treatment (Vehicle, Oxt) revealed significant effects of Group [$F(3,26) = 4.926, p = 0.008$] and Drug [$F(1,29) = 7.752, p = 0.01$] but no Group $\times$ Drug interaction. A single pre-exposure to a non-fearful model is not sufficient to decrease fear responses (Guzman et al. 2009), however, Oxt administration into the lateral septum prior to NF1 ($n = 9$) decreased freezing when compared to the NF1 Vehicle group, $n = 7$) or non-pre-exposed control mice injected with vehicle ($n = 7$) or Oxt ($n = 7$) (Fig. 2b, $F(3,26) = 4.927, p < 0.01$). These results suggest that Oxt administration into the lateral septum accelerated social buffering of fear by enhancing social learning, and that these effects are modulatory rather than instructive because the non-pre-exposed control groups were unaffected by Oxt treatment.

Effect of genetic inactivation of lateral septal Oxt on social buffering of fear

To corroborate that the effects of Oxt on social modulation of fear are mediated by Oxt, we used a conditional knockout approach to down-regulate these receptors in the lateral septum (Guzman et al. 2013; Takayanagi et al. 2005). Oxt^{LoxP/LoxP} mice were injected with a replication defective adeno-associate virus (rAAV) expressing Cre recombinase (Cre) directly in the lateral septum or with a control rAAV expressing green fluorescent protein (GFP). This approach led to successful down-regulation of septal Oxt mRNA of mice injected with rAAV-Cre when compared to rAAV-GFP controls (Figure 3a), as confirmed by Student's t-test comparison of qPCR data in a sample of the rAAV-GFP ($n = 3$) and rAAV-Cre ($n = 4$) groups ($t(6) = 5.272, p < 0.01$). Consistent with a role of septal Oxt in the modulation of fear after positive social interactions, mice with septal Oxt down-regulation did not exhibit social modulation of fear (NF2 Cre group, $n = 26$) and froze similar to non-pre-exposed control mice injected with rAAV-Cre [$n = 25, t(49) = -0.369, p > 0.05$; Fig. 3b]. This effect was not due to non-specific effects of viral injections since mice pre-exposed to non-fearful models but injected with rAAV-GFP ($n = 21$) showed decreased freezing when compared to non-pre-exposed control mice injected with rAAV-GFP [$n = 25, t(44) = 2.098, p < 0.05$; Fig. 3c]. Importantly, down-regulation of septal Oxt (NF2 Cre group, $n = 7$) did not affect the number of interactions between the observer and the non-fearful model during pre-exposure as they did not differ from NF2 mice injected with rAAV GFP [$n = 10; t(15) = -0.735, p > 0.05$; Fig. 3c]. This suggests that the lack of modulation seen in mice with septal Oxt down-regulation was not due to decreased social behavior during the interactions but by a social learning deficit common to Oxt knockout models (Takayanagi et al. 2005).

Regional specificity of Oxt effects on social buffering of fear

To determine whether the fear-reducing effect of Oxt was specific for the lateral septum or also involves other brain regions containing Oxt and implicated in social memory and fear conditioning, we compared the effects of OTA injected intraseptally versus

intrahippocampally using the NF2 paradigm. We also introduced groups that received the NMDAR antagonist APV. This approach was selected because NMDAR in the hippocampus and lateral septum regulate contextual fear conditioning (Radulovic et al., 2000), and can thus be used to further delineate the neurotransmitter involvement in social buffering versus direct fear learning. Thus, the experimental groups consisted of control vehicle (n = 8), NF2 vehicle (n = 13), NF APV (n = 12), and NF OTA (n = 9) for the lateral septum and control vehicle (n = 8), NF2 vehicle (n = 16), NF APV (n = 8), and NF OTA (n = 10) for the hippocampus. One way ANOVA revealed significant group differences in the lateral septum ($F(3,41) = 3.086, p < 0.05$; Figure 4a). Post-hoc analyses showed that the NF2 vehicle groups froze significantly less than their corresponding controls injected with vehicle, an effect that was significantly reversed only by OTA ($p < 0.05$). Freezing of the NF2 APV-injected mice did not differ significantly from any other group. This result suggested that Oxt are key septal mediators of social buffering of fear.

Significant group differences were also found among groups with hippocampal manipulations ($F(3,41) = 2.989, p < 0.05$; Figure 4b) with NF2 vehicle groups freezing significantly less than their controls ($p < 0.05$). However, freezing behavior of neither OTA- nor APV-injected groups differed from vehicle control or vehicle NF2, suggesting that in the hippocampus, these neurotransmitters did not play a major role in social buffering of fear, although the overall tendencies might suggest a partial contribution.

Discussion

We have identified the Oxt system in the lateral septum as a key mediator of the lasting reduction of freezing caused by positive social interactions. This was concluded from pharmacological antagonism and genetic knockdown approaches of Oxt, which abolished the decrease of fear after pre-exposure to non-fearful demonstrators. We also showed that Oxt administration into the lateral septum accelerated social buffering of fear by reducing the number of pre-exposures required to decrease contextual freezing. It is important to note that the effect of Oxt manipulations was specific to mice exposed to the social context (NF groups) and did not extend to those exposed to non-social contexts prior to contextual fear conditioning (controls), demonstrating the specificity of septal Oxt in modulation of fear by social stimuli but not directly acquired fear by classical conditioning. The number of social interactions was not affected by Oxt down-regulation in this study, which is in line with the normal social approach behavior of Oxt knockouts (Crawley et al. 2007). This result is important because it allows delineating the role of Oxt in social memory from effects on acute social behaviors.

The lateral septum exerts an important inhibitory control over fear responses (Thomas et al. 1991; Vouimba et al. 1998; Winslow and Insel 2004; Yadin et al. 1993) and plastic changes in this region have a long lasting impact on fear modulation (Vouimba et al. 1998). For example, induction of LTP in the lateral septum impairs fear conditioning performed 24 hours later (Vouimba et al. 1998). This remarkably correlates with our data on social buffering of fear, where pre-exposure to the non-fearful model 24 hours before fear conditioning significantly dampened the fear response. Thus, we speculate that pre-exposure to non-fearful conspecifics induces Oxt-mediated increases in septal neuronal activity that

lead to social memory and exogenous Oxt administration during a single pre-exposure to the non-fearful model can facilitate social memory and lead to stronger social buffering of fear. Identifying a fear-reducing role for septal Oxt is relevant for interpretation of human findings showing decreased fear and amygdala responses after Oxt administration (Kirsch et al. 2005). To date, evidence for the presence of Oxt in the amygdala has been controversial (Loup et al. 1991; Boccia et al., 2013) and there is no evidence for *Oxt* mRNA in this region. Thus, Oxt might reduce fear and amygdala output indirectly, by regulating other interconnected regions (Calderazzo, et al. 1996; Gallagher et al. 1995), such as the lateral septum, which is rich in Oxt (Barberis and Tribollet 1996; Loup et al. 1991; Yoshida et al. 2009).

Septal manipulations, however, do not always decrease fear. In fact, muscimol-induced inactivation of lateral septum neurons inhibits fear learning of aversive odor cues (Endres and Fendt 2008). Additionally, cyclin-dependent kinase 5 in the lateral septum mediates the anxiogenic effects of restraint stress in mice (Bignante et al. 2008). Still, the question remains whether the lateral septum bidirectionally modulates fear and anxiety responses or if it primarily enhances memory of certain stimuli to subsequently modulate responses. In line with the latter idea, the lateral septum promotes fear learning by enhancing conditioning of discrete cues to the detriment of indiscrete cues (Desmedt et al. 1999), thus rather than unilaterally affecting anxiety it enhances memory of certain cues over others. In the case of the social stimuli presented in our model, we argue that septal Oxt mediate social learning rather than generally influencing emotion based on several lines of evidence. First, Oxt did not affect fear conditioning or emotional responses to nonsocial contexts. Second, administration of Oxt into the lateral septum significantly enhances social learning in rodents (Popik et al. 1992b), suggesting that septal neurons are equipped to process social information into lasting social memory. Finally, the lateral septum is thought to modulate anxiety by integrating diverse sensory information to evaluate the valence of an event and transmitting this information to areas directly guiding behavioral output (Sheehan et al. 2004). Thus, in our model Oxt in the lateral septum could enhance social learning independently of their valence, so that improved memory of positive social interactions would decrease fear while improved memory of negative social interactions would increase fear.

It is notable that the role of Oxt showed neuroanatomical and neurotransmitter specificity for the lateral septum when compared to the hippocampus and to NMDAR. This reveals region- and receptor-specific mechanisms in processing social when compared to other environmental stimuli. Alternatively, the significance of this specificity may be to ensure a mechanistic differentiation between observational versus direct learning. It is very likely that other neuroanatomical and neurotransmitter mechanisms contribute to the modulation of fear or anxiety by social factors. For example, oxytocin improves social memory by acting via the medial preoptic and not only lateral septal area (Popik et al., 1991ba). In addition, the oxytocin-related peptide vasopressin also enhances social learning through the lateral septum (Dantzer et al., 1988; Popik et al., 1992b), so contribution of the vasopressinergic system in social modulation of fear would not be surprising. Finally, the glutamatergic system cannot be ruled out yet, because type 1a metabotropic glutamate receptors are

important mediators of Oxt-induced changes of Ca²⁺ fluxes (Kuo et al. 2009). It remains to be determined whether engagement of distinct brain regions and neurotransmitter systems is required for processing different components of the social memory, such as memory of the social interaction and association of social and environmental contexts.

In summary, Oxt has recently been associated with bidirectional modulation of fear and anxiety. Whereas, earlier studies revealed anxiolytic roles of Oxt (Ayers et al. 2011; Carter 1998; Heinrichs et al. 2003; Ishak et al. 2011), recent work found anxiogenic effects for this peptide (J. Bartz et al. 2011; Grillon et al. 2012; Striepens et al. 2012; Tabak et al. 2011). A general fear-provoking or anxiogenic action of lateral septal Oxt and Oxtr was excluded in our study because instead of Oxtr-mediated enhancement of freezing, as observed after negative social interactions (Guzman et al. 2013), we found a significant Oxtr-mediated reduction of freezing after positive social interactions. A similar role of Oxt in memory has also been established in humans, where Oxt enhanced the intensity rating of both positive and negative social memories at recall (Bartz et al. 2010). Oxt actions thus seem to be predominantly targeted toward the enhancement of social learning independent of the valence (positive versus negative) of the social interactions, thus reconciling seemingly bidirectional effects on fear and anxiety.

Acknowledgments

This research was supported by the National Institutes of Health grants R01 MH078064 (J.R.) and MH092065 (Y.F.G.). Part of this study carried out by N.K. and K.S. was the result of “Integrated research on neuropsychiatric disorder” in the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- Ahi J, Radulovic J, Spiess J. The role of hippocampal signaling cascades in consolidation of fear memory. *Behav Brain Res.* 2004; 149(1):17–31. [PubMed: 14739006]
- Allen NB, Badcock PBT. The Social Risk Hypothesis of Depressed Mood: Evolutionary, Psychosocial, and Neurobiological Perspectives. *Psychological Bulletin.* 2003; 129(6):887–913. [PubMed: 14599287]
- Ayers LW, Missig G, Schulkin J, Rosen JB. Oxytocin reduces background anxiety in a fear-potentiated startle paradigm: peripheral vs central administration. *Neuropsychopharmacology.* 2011; 36(12):2488–2497. [PubMed: 21796104]
- Bandura, A. *Principles of Behavior Modification.* New York Holt: Rinehart & Winston; 1969a.
- Bandura, A. *Vicarious and self-reinforcement processes.* New York Holt: Rinehart & Winston; 1969b.
- Barberis C, Tribollet E. Vasopressin and oxytocin receptors in the central nervous system. *Crit Rev Neurobiol.* 1996; 10(1):119–154. [PubMed: 8853957]
- Barrett TW, Mizes JS. Combat level and social support in the development of posttraumatic stress disorder in Vietnam veterans. *Behav Modif.* 1988; 12(1):100–115. [PubMed: 3223883]
- Bartz J, Simeon D, Hamilton H, Kim S, Crystal S, Braun A, et al. Oxytocin can hinder trust and cooperation in borderline personality disorder. *Soc Cogn Affect Neurosci.* 2011; 6(5):556–563. [PubMed: 21115541]
- Bartz JA, Zaki J, Ochsner KN, Bolger N, Klevzon A, Ludwig N, et al. Effects of oxytocin on recollections of maternal care and closeness. *Proc Natl Acad Sci U S A.* 2010; 107(50):21371–21375. [PubMed: 21115834]
- Bignante EA, Rodriguez Manzanares PA, Mlewski EC, Bertotto ME, Bussolino DF, Paglini G, et al. Involvement of septal Cdk5 in the emergence of excessive anxiety induced by stress. *Eur Neuropsychopharmacol.* 2008; 18(8):578–588. [PubMed: 18406108]

- Blanchard RJ, Blanchard DC. Crouching as an index of fear. *J Comp Physiol Psychol*. 1969; 67(3): 370–375. [PubMed: 5787388]
- Boccia ML, Petrusz P, Suzuki K, Marson L, Pedersen CA. Immunohistochemical localization of oxytocin receptors in human brain. *Neuroscience*. 2013 in press.
- Blume A, Bosch OJ, Miklos S, Torner L, Wales L, Waldherr M, et al. Oxytocin reduces anxiety via ERK1/2 activation: local effect within the rat hypothalamic paraventricular nucleus. *Eur J Neurosci*. 2008; 27(8):1947–1956. [PubMed: 18412615]
- Boscarino JA. Post-traumatic stress and associated disorders among vietnam veterans: the significance of combat exposure and social support. *Journal of Traumatic Stress*. 1995; 8(2):317–336. [PubMed: 7627446]
- Bruchey AK, Jones CE, Monfils MH. Fear conditioning by-proxy: social transmission of fear during memory retrieval. *Behav Brain Res*. 2010; 214(1):80–84. [PubMed: 20441779]
- Calderazzo L, Cavalheiro EA, Macchi G, Molinari M, Bentivoglio M. Branched connections to the septum and to the entorhinal cortex from the hippocampus, amygdala, and diencephalon in the rat. *Brain Res Bull*. 1996; 40(4):245–251. [PubMed: 8842407]
- Carter CS. Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology*. 1998; 23(8):779–818. [PubMed: 9924738]
- Crawley JN, Chen T, Puri A, Washburn R, Sullivan TL, Hill JM, et al. Social approach behaviors in oxytocin knockout mice: comparison of two independent lines tested in different laboratory environments. *Neuropeptides*. 2007; 41(3):145–163. [PubMed: 17420046]
- Dantzer R, Koob GF, Bluthé RM, Le Moal M. Septal vasopressin modulates social memory in male rats. *Brain Res*. 1988; 457(1):143–147. [PubMed: 3167559]
- Dantzer R, Bluthé RM, Koob GF, Le Moal M. Modulation of social memory in male rats by neurohypophyseal peptides. *Psychopharmacology (Berl)*. 1987; 91(3):363–368. [PubMed: 3104959]
- Davitz JR, Mason DJ. Socially Facilitated Reduction of a Fear Response in Rats. *Journal of Comparative and Physiological Psychology*. 1955; 48(3):149–151. [PubMed: 13242680]
- Desmedt A, Garcia R, Jaffard R. Vasopressin in the lateral septum promotes elemental conditioning to the detriment of contextual fear conditioning in mice. *Eur J Neurosci*. 1999; 11(11):3913–3921. [PubMed: 10583480]
- Egliston K-A, Rapee RM. Inhibition of fear acquisition in toddlers following positive modelling by their mothers. *Behaviour Research and Therapy*. 2007; 45(8):1871–1882. [PubMed: 17448441]
- Endres T, Fendt M. Inactivation of the lateral septum blocks fox odor-induced fear behavior. *Neuroreport*. 2008; 19(6):667–670. [PubMed: 18382283]
- Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT. Social amnesia in mice lacking the oxytocin gene. *Nat Genet*. 2000; 25(3):284–288. [PubMed: 10888874]
- Gallagher JP, Zheng F, Hasuo H, Shinnick-Gallagher P. Activities of neurons within the rat dorsolateral septal nucleus (DLSN). *Prog Neurobiol*. 1995; 45(5):373–395. [PubMed: 7617889]
- Grillon C, Krimsky M, Charney DR, Vytal K, Ernst M, Cornwell B. Oxytocin increases anxiety to unpredictable threat. *Mol Psychiatry*. 2012
- Guzman YF, Tronson NC, Guedea A, Huh KH, Gao C, Radulovic J. Social modeling of conditioned fear in mice by non-fearful conspecifics. *Behav Brain Res*. 2009; 201(1):173–178. [PubMed: 19428631]
- Guzman YF, Tronson NC, Jovasevic V, Sato K, Guedea AL, Mizukami H, et al. Fear-enhancing effects of septal oxytocin receptors. *Nat Neurosci*. 2013
- Heim C, Nemeroff CB. Neurobiology of Posttraumatic Stress Disorder. *CNS Spectrum*. 2009; 14(1): 13–24.
- Heinrichs M, Baumgartner T, Kirschbaum C, Ehler U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry*. 2003; 54(12): 1389–1398. [PubMed: 14675803]
- Huber D, Veinante P, Stoop R. Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. *Science*. 2005; 308(5719):245–248. [PubMed: 15821089]

- Ishak WW, Kahloon M, Fakhry H. Oxytocin role in enhancing well-being: a literature review. *J Affect Disord.* 2011; 130(1–2):1–9. [PubMed: 20584551]
- Jeon D, Kim S, Chetana M, Jo D, Ruley HE, Lin SY, et al. Observational fear learning involves affective pain system and Cav1.2 Ca²⁺ channels in ACC. *Nat Neurosci.* 2010; 13(4):482–488. [PubMed: 20190743]
- Kavaliers M, Choleris E, Colwell DD. Learning from others to cope with biting flies: social learning of fear-induced conditioned analgesia and active avoidance. *Behavioral Neuroscience.* 2001; 115:661–674. [PubMed: 11439455]
- Kikusui T, Winslow JT, Mori Y. Social buffering: relief from stress and anxiety. *Philosophical Transactions of the Royal Society.* 2006; 361:2215–2228.
- Kim EJ, Kim ES, Covey E, Kim JJ. Social transmission of fear in rats: the role of 22-kHz ultrasonic distress vocalization. *PLoS One.* 2010; 5(12):e15077. [PubMed: 21152023]
- Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. *Science.* 1992; 256(5057):675–677. [PubMed: 1585183]
- Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, et al. Oxytocin modulates neural circuitry for social cognition and fear in humans. *J Neurosci.* 2005; 25(49):11489–11493. [PubMed: 16339042]
- Knapaska E, Maren S. Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. *Learn Mem.* 2009; 16(8):486–493. [PubMed: 19633138]
- Knapaska E, Mikosz M, Werka T, Maren S. Social modulation of learning in rats. *Learn Mem.* 2010; 17(1):35–42. [PubMed: 20042480]
- Knobloch HS, Charlet A, Hoffmann LC, Eliava M, Khrulev S, Cetin AH, et al. Evoked axonal oxytocin release in the central amygdala attenuates fear response. *Neuron.* 2012; 73(3):553–566. [PubMed: 22325206]
- Kogan JH, Frankland PW, Silva AJ. Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus.* 2000; 10(1):47–56. [PubMed: 10706216]
- Kuo J, Hariri OR, Micevych P. An interaction of oxytocin receptors with metabotropic glutamate receptors in hypothalamic astrocytes. *J Neuroendocrinol.* 2009; 21(12):1001–1006. [PubMed: 19807846]
- Langford DJ, Crager SE, Shehzad Z, Smith SB, Sotocinal SG, Levenstadt JS, et al. Social Modulation of Pain as Evidence for Empathy in Mice. *Science.* 2006; 312(5782):1967–1970. [PubMed: 16809545]
- Lipina TV, Roder JC. Co-learning facilitates memory in mice: a new avenue in social neuroscience. *Neuropharmacology.* 2013; 64:283–293. [PubMed: 22776545]
- Loup F, Tribollet E, Dubois-Dauphin M, Dreifuss JJ. Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study. *Brain Res.* 1991; 555(2):220–232. [PubMed: 1657300]
- Mineka S, Davidson M, Cook M, Keir R. Observational conditioning of snake fear in rhesus monkeys. *J Abnorm Psychol.* 1984; 93(4):355–372. [PubMed: 6542574]
- Nowak A, Werka T, Knapaska E. Social modulation in extinction of aversive memories. *Behav Brain Res.* 2013; 238:200–205. [PubMed: 23103405]
- Olsson A, Nearing KI, Phelps EA. Learning fears by observing others: the neural systems of social fear transmission. *Soc Cogn Affect Neurosci.* 2007; 2(1):3–11. [PubMed: 18985115]
- Olsson A, Phelps EA. Social learning of fear. *Nat Neurosci.* 2007; 10(9):1095–1102. [PubMed: 17726475]
- Panksepp J. Toward a cross-species neuroscientific understanding of the affective mind: do animals have emotional feelings? *Am J Primatol.* 2011; 73(6):545–561. [PubMed: 21319205]
- Paxinos, G.; Franklin, KB. The mouse brain in stereotaxic coordinates. San Diego: San Diego Academic Press; 2001.
- Popik P, Vetulani J. Opposite action of oxytocin and its peptide antagonists on social memory in rats. *Neuropeptides.* 1991a; 18(1):23–27. [PubMed: 2046886]

- Popik P, van Ree JM. Oxytocin but not vasopressin facilitates social recognition following injection into the medial preoptic area of the rat brain. *Eur Neuropsychopharmacol.* 1991b; 1(4):555–560. [PubMed: 1668316]
- Popik P, Vetulani J, van Ree JM. Low doses of oxytocin facilitate social recognition in rats. *Psychopharmacology (Berl).* 1992a; 106(1):71–74. [PubMed: 1738795]
- Popik P, Vos PE, Van Ree JM. Neurohypophyseal hormone receptors in the septum are implicated in social recognition in the rat. *Behav Pharmacol.* 1992b; 3(4):351–358. [PubMed: 11224137]
- Radulovic J, Fischer A, Katerkamp U, Spiess J. Role of regional neurotransmitter receptors in corticotropin-releasing factor (CRF)-mediated modulation of fear conditioning. *Neuropharmacology.* 2000; 39(4):707–710. [PubMed: 10728892]
- Radulovic J, Ruhmann A, Liepold T, Spiess J. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. *J Neurosci.* 1999; 19(12):5016–5025. [PubMed: 10366634]
- Radulovic J, Fischer A, Katerkamp U, Spiess J. Role of regional neurotransmitter receptors in corticotropin-releasing factor (CRF)-mediated modulation of fear conditioning. *Neuropharmacology.* 2000; 39(4):707–710. [PubMed: 10728892]
- Riess D. Vicarious conditioned acceleration: Successful observational learning of an aversive pavlovian stimulus contingency. *Journal of the Experimental Analysis of Behavior.* 1972; 18(1):181–186. [PubMed: 5078091]
- Sheehan TP, Chambers RA, Russell DS. Regulation of affect by the lateral septum: implications for neuropsychiatry. *Brain Res Brain Res Rev.* 2004; 46(1):71–117. [PubMed: 15297155]
- Solomon Z, Mikulincer M, Hobfoll SE. Effects of social support and battle intensity on loneliness and breakdown during combat. *Journal of personality and social psychology.* 1986; 51(6):1269–1276. [PubMed: 3806362]
- Striepen N, Scheele D, Kendrick KM, Becker B, Schafer L, Schwalba K, et al. Oxytocin facilitates protective responses to aversive social stimuli in males. *Proc Natl Acad Sci U S A.* 2012; 109(44):18144–18149. [PubMed: 23074247]
- Tabak BA, McCullough ME, Szeto A, Mendez AJ, McCabe PM. Oxytocin indexes relational distress following interpersonal harms in women. *Psychoneuroendocrinology.* 2011; 36(1):115–122. [PubMed: 20688437]
- Takayanagi Y, Yoshida M, Bielsky IF, Ross HE, Kawamata M, Onaka T, et al. Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc Natl Acad Sci U S A.* 2005; 102(44):16096–16101. [PubMed: 16249339]
- Thomas E, Yadin E, Strickland CE. Septal unit activity during classical conditioning: a regional comparison. *Brain Res.* 1991; 547(2):303–308. [PubMed: 1884205]
- Thorsteinsson EB, James JE, Gregg ME. Effects of video-relayed social support on hemodynamic reactivity and salivary cortisol during laboratory-based behavioral challenge. *Health Psychology.* 1998; 17(5):436–444. [PubMed: 9776002]
- Ullman SE, Filipas HH. Predictors of PTSD symptom severity and social reactions in sexual assault victims. *J Trauma Stress.* 2001; 14(2):369–389. [PubMed: 11469163]
- Viviani D, Charlet A, van den Burg E, Robinet C, Hurni N, Abatis M, et al. Oxytocin selectively gates fear responses through distinct outputs from the central amygdala. *Science.* 2011; 333(6038):104–107. [PubMed: 21719680]
- Vouimba RM, Garcia R, Jaffard R. Opposite effects of lateral septal LTP and lateral septal lesions on contextual fear conditioning in mice. *Behav Neurosci.* 1998; 112(4):875–884. [PubMed: 9733193]
- Waldherr M, Neumann ID. Centrally released oxytocin mediates mating-induced anxiolysis in male rats. *Proc Natl Acad Sci U S A.* 2007; 104(42):16681–16684. [PubMed: 17925443]
- Windle RJ, Shanks N, Lightman SL, Ingram CD. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology.* 1997; 138(7):2829–2834. [PubMed: 9202224]
- Winslow JT, Insel TR. Neuroendocrine basis of social recognition. *Curr Opin Neurobiol.* 2004; 14(2):248–253. [PubMed: 15082332]
- Yadin E, Thomas E, Grishkat HL, Strickland CE. The role of the lateral septum in anxiolysis. *Physiol Behav.* 1993; 53(6):1077–1083. [PubMed: 8346290]

Yoshida M, Takayanagi Y, Inoue K, Kimura T, Young LJ, Onaka T, et al. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *J Neurosci*. 2009; 29(7):2259–2271. [PubMed: 19228979]

Fig. 1.

Experimental design for Oxt manipulations in the employed paradigm for social buffering of fear. a Localization of Oxt in the lateral septum, as determined by a Oxt Venus reporter mouse; b Timing of injections for the three employed treatments [1- antagonists (OTA or APV), 2-oxytocin, and 3-viral vectors (rAAV-Cre or rAAV-GFP)]. # Animals in the oxytocin experiments were only pre-exposed once to the non-fearful model (NF1).

Fig. 2.

Effect of pharmacological manipulations of the septal oxytocin system on social buffering of fear. a Significantly decreased contextual fear conditioning after two pre-exposures to nonfearful conspecifics (NF2) and abolishment of this effect by infusion of OTA into the lateral septum. OTA did not produce a significant effect in the control group. b NF1 alone did not reduce freezing, however such effect emerged after Oxt administration into the lateral septum. Data represent the mean $\pm$ SEM.

Statistically significant differences: $*p < 0.05$, $**p < 0.01$ when compared to the indicated group.

Fig. 3.

Effect of genetic down-regulation of septal Oxtr on social buffering of fear. a Down-regulation of Oxtr after injection of rAAV-Cre but not rAAV-GFP in floxed Oxtr mice, as determined by qPCR. b In NF2 mice injected with rAAV-Cre 14 days before the social buffering paradigm, freezing behavior was similar to controls. c Intraseptal injection of rAAV-GFP did not interfere with freezing of the NF2 group, which was significantly lower than control littermates. d Oxtr down-regulation did not affect social interactions during pre-exposure. Data represents the mean ± SEM. Statistically significant differences: $*p < 0.05$ when compared to the indicated control.

Fig. 4.

Region-specific effects of Oxtr on social buffering of fear. a Pharmacological antagonism of septal Oxtr or NMDAR with OTA and APV, respectively, revealed that OTA was able to completely abolish the effect of Oxt. This was shown by a significant difference between NF2 mice injected intraseptally with OTA when compared to NF2 mice injected intraseptally with vehicle. b

Representative pictographs of cannula placements (left) and an example of a methylene-blue trace validating lateral septal cannula placements. c Hippocampal treatments only partially attenuated the reduction of freezing in the NF2 group, as revealed by the finding that mice injected with OTA or APV did not differ significantly either from the control or NF2 mice injected with vehicle. d Representative pictographs of cannula placements (left) and an example of a methylene-blue trace (right) validating dorsal hippocampal cannula placements. Data represents the mean $\pm$ SEM. *Statistically significant differences: * $p < 0.05$, when compared to the indicated control.