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REVIEW

# Oxytocin facilitates adaptive fear and attenuates anxiety responses in animal models and human studies—potential interaction with the corticotropin-releasing factor (CRF) system in the bed nucleus of the stria terminalis (BNST)

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## Abstract

Despite its relatively well-understood role as a reproductive and pro-social peptide, oxytocin (OT) tells a more convoluted story in terms of its modulation of fear and anxiety. This nuanced story has been obscured by a great deal of research into the therapeutic applications of exogenous OT, driving more than 400 ongoing clinical trials. Drawing from animal models and human studies, we review the complex evidence concerning OT's role in fear learning and anxiety, clarifying the existing confusion about modulation of fear versus anxiety. We discuss animal models and human studies demonstrating the prevailing role of OT in strengthening fear memory to a discrete signal or cue, which allows accurate and rapid threat detection that facilitates survival. We also review ostensibly contrasting behavioral studies that nonetheless provide compelling evidence of OT attenuating sustained contextual fear and anxiety-like behavior, arguing that these OT effects on the modulation of fear vs. anxiety are not mutually exclusive. To disambiguate how endogenous OT modulates fear and anxiety, an understudied area compared to exogenous OT, we survey behavioral studies utilizing OT receptor (OTR) antagonists. Based on emerging evidence about the role of OTR in rat dorsolateral bed nucleus of stria terminalis (BNST) and elsewhere, we postulate that OT plays a critical role in facilitating accurate discrimination between stimuli representing threat and safety. Supported by human studies, we demonstrate that OT uniquely facilitates adaptive fear but reduces maladaptive anxiety. Last, we explore the limited literature on endogenous OT and its interaction with corticotropin-releasing factor (CRF) with a special emphasis on the dorsolateral BNST, which may hold the key to the neurobiology of phasic fear and sustained anxiety.

**Keywords** Oxytocin · Fear · Anxiety · Discrimination · BNST · CRF · CRH · Rat · Human

## Introduction

Oxytocin (OT) is a peptide hormone and a neuromodulator produced by neurons of the paraventricular (PVN), supraoptic (SON), as well as accessory nuclei (AN) of the hypothalamus (Sofroniew 1983; Swanson and Sawchenko 1983). As a hormone, OT is released from the posterior pituitary into general blood circulation, where it mediates a variety of pivotal physiological processes, including uterine contractions during labor and milk ejection reflex (Nickerson et al. 1954; Caldeyro-Barcia and Poseiro 1959). In addition, together with arginine-vasopressin (AVP), OT is a master regulator of water/electrolyte balance (Han et al. 1993; Verbalis et al. 1993). In the central nervous system (CNS), this nine-amino acid neuropeptide has been shown to produce powerful effects on a wide array of social behaviors including but not limited to, pair bond formation, social recognition and the onset of maternal behavior (Pedersen et al. 1992; Bosch and Young 2017). Furthermore, in both female and male rats, OT neurons send considerable projections from the hypothalamus to the

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CNS, targeting many brain structures that are critical for the modulation of fear and anxiety-like behaviors (Dabrowska et al. 2011; Knobloch et al. 2012).

Fear response allows accurate and rapid threat detection that facilitates survival (Liddell et al. 2005; Reinders et al. 2006). Hence, as observed in infants, children and adults, we are innately biased toward rapid detection of threatening vs. non-threatening stimuli (Lobue and DeLoache 2008) and fearful vs. happy or neutral facial expressions (LoBue 2009). In contrast to fear, anxiety occurs in the absence of a threat stimulus or in anticipation of a threat and can be defined as a sustained and maladaptive response to diffuse, less specific, unpredictable, or unsignaled threats (Davis et al. 2010; Goode and Maren 2017). Anxiety can occur as an overgeneralization of learned fear, inability to extinguish conditioned fear and inability to discriminate between threat and safety (Lissek et al. 2014; Dunsmoor and Paz 2015). These characteristics lay the foundation of stress-induced psychiatric disorders including post-traumatic stress disorder (PTSD) and generalized anxiety disorder (GAD).

While a large body of evidence suggests that OT has anxiolytic properties in animal models (Bale et al. 2001; Ring et al. 2006) and human studies (Ellenbogen et al. 2014), the role of OT in the regulation of fear learning appears more complex and multimodal, depending on the time of OT administration, stress history and brain structure studied (Toth et al. 2012; Lahoud and Maroun 2013; Neumann and Slattery 2016; Moaddab and Dabrowska 2017). Similarly, OT has been shown to have contrasting age-dependent effects on the modulation of fear in rats (Lahoud and Maroun 2013; Kritman et al. 2017).

To better understand the distinct roles of OT in the modulation of fear vs. anxiety, this review article separately addresses the effects of OT on fear learning vs. anxiety-like behavior in animal models and human studies, including OT effects in patients suffering from PTSD. We show that although OT appears to exert primarily anxiolytic effects and can reduce sustained contextual fear, both animal models and human studies assert that OT can strengthen fear learning to a discrete cue and facilitate fear recognition. Specifically, OT in the central amygdala (CeA) was shown to reduce sustained contextual fear responses (Huber et al. 2005; Viviani et al. 2011; Knobloch et al. 2012) but OT receptors (OTR) in the bed nucleus of the stria terminalis (BNST) facilitate fear to a short discrete cue (Moaddab and Dabrowska 2017). In humans, OT has been also shown to selectively facilitate recognition of threatening stimuli and fearful facial expressions in healthy male (Fischer-Shofty et al. 2010; Striepens et al. 2012) and female subjects (Domes et al. 2010). In this review, we show that these apparently contrasting effects on anxiety vs. fear are not mutually exclusive. We postulate that OT facilitates fear responses to specific cues or signals and as such promotes rapid and accurate recognition of specific danger, whereas OT reduces responses to sustained, diffuse threats manifested as contextual fear or anxiety-like behavior. We support this interpretation by examining OTR transmission in dorsolateral BNST ($BNST_{dl}$), a critical brain structure that translates stress into anxiety (Davis et al. 2010; Dabrowska et al. 2013b; Sparta et al. 2013; Daniel and Rainnie 2016). $BNST_{dl}$ has been proposed to play a pivotal role in the ability to discriminate between predictable vs. unpredictable stimuli (De Bundel et al. 2016), stimuli representing threat vs. safety (Duvarci et al. 2009), phasic vs. sustained fear responses (Walker et al. 2009a; Lange et al. 2017) and signaled vs. unsignaled threats (for review, see Gungor and Pare 2016, Shackman and Fox 2016 and Goode and Maren 2017). Based on our previously published data on OTR modulation of a fear-potentiated startle (FPS) (Moaddab and Dabrowska 2017), we show that OTR facilitates discrimination between cued fear and background anxiety, such that endogenous OT biases rats' responses toward predictable, signaled fear but it reduces responses to unsignaled and unpredictable threats.

To elucidate the neurobiology of how fear translates into anxiety, we discuss the interaction of OT in the $BNST_{dl}$ with stress hormone-producing, corticotropin-releasing factor (CRF) neurons, which are the main output neurons of the $BNST_{dl}$ (Dabrowska et al. 2016). We also discuss the interaction of local CRF receptors (CRFRs) with OT and show that CRFRs modulate OT release in the $BNST_{dl}$ (Martinon and Dabrowska 2018). The interaction between these two powerful peptidergic systems in the $BNST_{dl}$ may hold more answers about the translation of adaptive phasic fear into sustained maladaptive anxiety.

Finally, we emphasize that a strikingly limited number of studies has examined the role of endogenous OT in the modulation of fear and anxiety by employing OTR antagonists alone in response to fear- or anxiety-promoting stimuli. We propose that given the endogenous OT involvement in the modulation of stress, fear and anxiety, exogenous OT applications might often yield alternative and unexpected effects.

## The role of oxytocin in the regulation of anxiety

Studies examining the role of *endogenous* oxytocin in animal models of fear and anxiety-like behavior are summarized in Table 1.

### Animal models

#### Oxytocin knockout and oxytocin receptor knockout animal models

OT knockout (OTKO) mice are deficient in OT production, which makes them a valuable tool in understanding the role of

**Table 1** Role of endogenous oxytocin in the modulation of fear and anxiety-like behavior in animal models

| Treatment | Dose and Route | Sex and Species | Result and Paradigm | Reference |
|---|---|---|---|---|
| Atosiban | 20 or 100ng, ICV | Female C57BL/6 mice | ↑ anxiety in EPM (100 ng only) | Mantella et al. (2003) |
| - | - | OT knockout (OTKO) female mice | ↑ anxiety in EPM | |
| | | OTKO male mice | ↓ anxiety in EPM | |
| - | - | OTKO female mice | ↑ novelty-induced hyperthermia | Amico et al. (2004) |
| - | - | Oxytocin receptor knockout (OTRKO) female mice | ↓ anxiety in EPM | Wood et al. (2015) |
| - | - | Conditional forebrain OTRKO male mice | ↓ auditory cued fear acquisition (freezing)<br>↓ contextual fear retention<br>↓ cued fear retention | Pagani et al. (2011) |
| Atosiban | 0.3 or 1 mg/kg, IP | Male Wistar rats | ∅ effect on anxiety or locomotion in OF | Klenerova et al. (2010) |
| L-368,899 | 1 mg/kg, IP | | ∅ acute effect on locomotion, grooming, or rearing in OF<br>↑ locomotion 2 days post-injection in OF | |
| cpmProp-D-Tyr-Ile-Thr-Asn-Cys-Pro-Orn-$NH_2$ | 0.3 or 1 mg/kg, IP | | ∅ effect on locomotion, grooming, or rearing in OF | |
| 1-deamino-2-D-Tyr-(Oet)-4-Thr-8-Orn-oxytocin | 0.1 or 1 mg/kg, SC<br>2 or 20 ng, ICV | Male Sprague-Dawley rats | ∅ effect on exploratory locomotion in home cage<br>∅ effect on locomotion in home cage | Uvnas-Moberg et al. (1992) |
| desGly-$NH_2$-d$(CH_2)_5$[D-$Tyr^2$, $Thr^4$]OVT | 0.1 or 0.5 μg, pre-limbic mPFC | Female Sprague-Dawley rats | ↑ anxiety in EPM (postpartum females, 0.1 μg only) | Sabihi et al. (2014a) |
| L-368,899 | 1 mg/kg, IP<br>5 mg/kg, IP | Socially-defeated male and female California mice | ↑ social avoidance in females<br>↑ social approach in females | Duque-Wilckens et al. (2018) |
| | 1 μg, $BNST_{am}$ or NAc core | | ↑ social approach in females ($BNST_{am}$)<br>↓ social vigilance in females ($BNST_{am}$) | |
| desGly-$NH_2$, d$(CH_2)_5$[$Tyr(Me)^2$, $Thr^4$]OVT | 2 μg, ICV | Male CD1 mice | ∅ effect on home cage locomotion or anxiety in EPM<br>↓ social investigation in unconditioned mice | Zoicas et al. (2014) |
| desGly-$NH_2$, d$(CH_2)_5$[$Tyr(Me)^2$,$Thr^4$]OVT | 0.1 or 1 μg, CeA, MeA, or BLA | Male Wistar rats | ∅ effect on social preference | Lukas et al. (2013) |
| | 0.75 μg, ICV | | ↓ social investigation during social preference<br>∅ effect on anxiety in LDB or EPM | |
| | 2 or 20 μg, ICV | Male C57BL/6 mice | ↓ social investigation and induced social avoidance<br>∅ effect on anxiety in EPM | |
| desGly-$NH_2{}^9$, d$(CH_2)_5$[$Tyr(Me)^2$, $Thr^4$]OVT | 0.75 μg, ICV | Sexually-naïve male rats | ↓ mating-induced anxiolysis in EPM and LDB | Waldher and Neumann (2007) |
| desGly-$NH_2{}^9$, d$(CH_2)_5$[$Tyr(Me)^2$, $Thr^4$]OVT | 0.75 μg, ICV | Ovariectomized, estradiol-primed female rats | ∅ effect on paced mating-induced anxiolysis in EPM | Nyuyki et al. (2011) |
| | | Ovariectomized pace- or normally-mated, estradiol-primed female rats | ↓ anxiety in EPM | |
| des-Gly-$NH_2$d$(CH_2)_5$[$Tyr(Me)^2$, $Thr^4$]OVT | 0.75 μg, ICV | Female Wistar females | ↑ forced swim/EPM-induced CORT secretion in virgin but not pregnant or lactating rats<br>↓ anxiety in EPM in pregnant and lactating but not virgin rats | Neumann et al. (1999) |

**Table 1** (continued)

| Treatment | Dose and Route | Sex and Species | Result and Paradigm | Reference |
|---|---|---|---|---|
| desGly-$NH_2^9$, $d(CH_2)_5$ [$Tyr(Me)^2$, $Thr^4$]OVT | 0.75 μg, ICV<br>6 days of 7.5 ng/h, ICV | Male and female LAB or HAB mice | ∅ effect on anxiety in LDB<br>↑ anxiety in LDB (LAB females only) | Slattery and Neumann (2010) |
| Atosiban | 1 mg/kg, IV | Male Sprague-Dawley rats | ↓ anxiety in EPM<br>↓ locomotion in EPM | Mak et al. (2012) |
| $d(CH_2)^1_5$, $Tyr(Me)^2$, $Thr^4$, $Orn^8$, des-Gly-$NH_2^9$ | 2 ng, PVN or CeA | Female LAB and HAB mice | ↓ maternal aggression (but not defensive, exploratory, or maternal behavior) in HAB dams | Bosch (2005) |
| $d(CH_2)^1_5$, $Tyr(Me)^2$, $Thr^4$, $Orn^8$, des-Gly-$NH_2^9$ OVT | 1 μg, prelimbic mPFC | Male and female Sprague-Dawley rats | ∅ effect on anxiety in EPM or OF or on social interaction | Sabihi et al. (2014b) |
| $d(CH_2)_5$, $Tyr(Me)^2$, $Thr^4$, Tyr-$NH_2^9$ OVT | 1, 10, or 100 ng, NAcc shell | Male or female, low or high sociability mandarin voles | ∅ effect on locomotion or anxiety in OF | Yu et al. (2016) |
| Atosiban | 13 days of 600 μg/kg/day, ICV | Male Wistar rats | ↓ anxiety-like behavior in repeatedly restrained rats<br>∅ effect on open arm exploration/entry in EPM | Babic et al. (2015) |
| $d(CH_2)_5$-Tyr(Me)-[$Orn^8$]-vasotocin | 21 ng, CeA | Fear-conditioned female Wistar rats | ∅ effect on freezing | Knobloch et al. (2012) |
| des-Gly-$NH_2$, $d(CH_2)_5$ [$Tyr(Me)^2$, $Thr^4$]OVT | 0.15 μg, PVN or 0.75 μg, ICV | Male Wistar rats | ↓ neuropeptide S (NPS)-induced anxiolysis in LDB<br>↓ NPS-induced OF center exploration | Grund et al. (2017) |
| desGly-($NH_2$, $d(CH_2)_5$ [$Tyr(Me)^2$ , $Thr^4$]OVT | 0.75 μg, ICV | Male Wistar rats and male CD1 mice | ∅ effect on cued fear acquisition or recall (freezing)<br>↓ freezing during fear extinction | Toth et al. (2012) |
| | | Fear-conditioned male mice and rats | ∅ effect on extinction if infused before extinction training | |
| desGly-$NH_2$-$d(CH_2)_5$[D-$Tyr^2$, $Thr^4$]OVT | 3 ng, CeA or BLA | Male Wistar rats | ↓ contextual freezing (CeA only) | Campbell-Smith et al. (2015) |
| 1-$D(CH_2)_5$, $Tyr(ME)^2$, $Thr^4$, Tyr-$NH_2^9$ornithine vasotocin | 10 ng, LS | C57BL/6N male mice | ↓ social defeat-induced freezing to contextual fear | Guzman et al. (2013) |
| - | - | Male mice with downregulated OTR in LS | ↓ social defeat-induced freezing to contextual fear | |
| des-Gly-$NH_2$, $d(CH_2)_5$[$Tyr(Me)^2$, $Thr^4$]OVT | 10 or 100 ng, PVN | Female prairie voles | ↓ social buffering of pair-housed, previously immobilized females<br>↑ anxiety in EPM and serum corticosterone | Smith et al. (2016) |
| $d(CH_2)^1_5$, $Tyr(Me)^2$, $Thr^4$, $Orn^8$, des-Gly-$NH_2^9$ | 200 ng, $BNST_{dl}$ (pre-acquisition)<br>200 ng, $BNST_{dl}$ (post-acquisition) | Male Sprague-Dawley rats | ↓ cued fear acquisition and recall<br>↓ cued fear in low acoustic startle reactive (ASR) responders<br>∅ effect on baseline ASR<br>∅ effect on cued or non-cued fear consolidation | Moaddab and Dabrowska (2017) |
| L-368,899 | 5 or 10 mg/kg, IP | Male and female C57/B6 mice | ↓ socially-transmitted context fear (freezing)<br>∅ effect on context fear acquisition or recall | Pisansky et al. (2017) |
| DREADD-dependent CNO activation of PVN OT neurons | 3 mg/kg, IP | | ↑ freezing in unfamiliar males<br>∅ effect on directly acquired cued fear acquisition or recall | |
| [$d(CH_2)_5$, $Tyr(Me)^2$, $Orn^8$]-Vasotocin | 0.3 μg, IP | Neonatal male and female prairie voles | ∅ effect on anxiety in EPM in adulthood | Bales et al. (2004) |
| Atosiban | 1, 10, 100 or 1000 μg/kg, IP | Male Wistar rats | ↓ contextual freezing when injected post-acquisition<br>∅ effect on short-term fear memory<br>↓ contextual freezing when injected post-recall (only in rats trained using high-shock, 1.5mA) | Abdullahi et al. (2018) |

**Table 1** (continued)

| Treatment | Dose and Route | Sex and Species | Result and Paradigm | Reference |
|---|---|---|---|---|
| ([d(CH$_2$)$_5$, Tyr(Me)$^2$, Thr$^4$, Tyr-NH$_2$$^9$]-OVT | 1, 10 and 100 ng/side, CeA | Female mandarin voles | ↓ exploration in the center of OF (10 and 100 ng only)<br>↓ locomotion in OF (100 ng only)<br>↑ anxiety in EPM (10 and 100 ng only) | Dong et al. (2017) |

Studies utilizing OT or OTR knockout models, oxytocin receptor (OTR) antagonist alone, OTR down-regulation, or inhibition of OT release appear in the order of mentioning. Unless otherwise specified, all animals are adult. *DREADD* designer receptors exclusively activated by designer drugs, *CNO* clozapine-*N*-oxide, *ASR* acoustic startle response, *CRF* corticotropin-releasing factor, *CORT* corticosterone (rodents) or cortisol (humans), *NPS* neuropeptide S, *ICV* intracerebroventricular, *IP* intraperitoneal, *EPM* elevated plus maze, *LAB/HAB* selectively bred low- and high-anxiety animals, *LDB* light-dark box, *OF* open field test, $BNST_{am}$ anteromedial bed nucleus of the stria terminalis, $BNST_{dl}$ dorsolateral bed nucleus of the stria terminalis *PVN* paraventricular nucleus of the hypothalamus, *CeA* central nucleus of the amygdala, *LS* lateral septum, *mPFC* medial prefrontal cortex, *MeA* medial amygdala

endogenous OT in anxiety. OTKO female mice explore the open arms of the elevated plus maze (EPM) less than wild-type females, suggesting OT deficiency to be anxiogenic. An intracerebroventricular (ICV) infusion of OT (2 ng) in OTKO females significantly attenuated their baseline anxiety-like behavior. In wild-type females, ICV infusion of OTR antagonist, atosiban (100 ng), decreased open arm exploration and entry, suggesting that endogenous OT attenuates anxiety-like behavior in open spaces. When atosiban (100 ng) and OT were infused in parallel, the anxiolytic effect of OT (2 ng) was abolished in OTKO females. In contrast, OTKO males tested in the EPM, unlike OTKO females, show decreased anxiety-like behavior, entering and exploring the open arms more (Mantella et al. 2003), implying that OT can be anxiogenic in males. OTKO females transferred to a novel environment showed significantly increased body temperature compared to wild-type females, indicating that OT deficiency increases autonomic activation in response to novel contexts (Amico et al. 2004). Interestingly, deficit in OTR, unlike OT deficit, is not necessarily anxiogenic. For example, OTRKO female mice tested on the EPM made significantly more entries into the open arms and explored them for longer than wild-type females. When OTR knockout was conditionally restricted only to serotonergic neurons in the raphe nucleus, male and female mice did not show alterations in anxiety-like behavior in the EPM (Pagani et al. 2015).

### Acute systemic OT administration

OT can bind to OTR receptors both peripherally and centrally but whether it does readily cross the blood-brain barrier (BBB) remains controversial (Ermisch et al. 1985; Leng and Ludwig 2016). Intraperitoneal (IP) injection of OT (0.02 mg/kg and 0.01 mg/kg) in male rats enhanced their acoustic startle response (ASR) in a novel environment compared to males injected with higher OT dose (0.05 mg/kg) or saline. This result suggests that OT can promote hypervigilance in a novel, unknown territory (King et al. 1985). In contrast, unstressed male rats handled daily and injected IP with OT (0.05 mg/kg) showed increased total movement distance (TMD) in the open field (OF) test, an effect abolished by OTR antagonist (OTR-A), L-368,899 (1.0 mg/kg, IP). In contrast, a higher OT dose (1.0 mg/kg) acutely decreased TMD and the number of rearings while increasing time spent grooming. Rearing, or standing on hind legs, is indicative of exploratory behavior in the absence of an immediate threat (Lever et al. 2006). While L-368,899 reversed all effects of the higher OT dose, atosiban (1.0 mg/kg), another OTR-A, failed to reverse TMD deficit and rearing activity. In comparison, OTR antagonist ornithine vasotocin (0.3 or 1.0 mg/kg, IP) failed to exert an effect on its own or reverse TMD, rearing decrease, or grooming time increase when injected together with OT. These results indicate that systemic OT can dose-

dependently alter locomotor activity in contrasting ways and increase stereotypic behaviors but also that OTR-As differ either in their BBB permeability, mode of action, or both (Klenerova et al. 2009).

A number of studies demonstrate that the anxiolytic and locomotor effects of systemic OT administration are dose-dependent. Unstressed male rats administered carbetocin (deamino-1-monocarba-(2-O-methyltyrosine)-oxytocin, 0.3 mg/kg) or OT (0.05 mg/kg) IP both showed increased TMD and total time mobile in the OF 60 min later (Klenerova et al. 2010). In contrast, male rats injected IP with a higher dose of OT (0.1 or 1 mg/kg) explored the cage less and spent more time in the center of a plastic cage, suggesting anxiolysis and decreased locomotor activity. The OTR-A (l-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin; 0.1 or 1 mg/kg) abolished the locomotor deficit of OT when co-administered together (Uvnas-Moberg et al. 1992). Additionally, male rats injected subcutaneously (SC) with OT (250 and 1000 μg) showed close to no explorative rearing in the OF. Likewise, OT reduced locomotor activity compared to saline-injected rats. These findings suggest that systemic OT injection affects locomotor activity, complicating the dissociation of OT effects on general locomotor and specific anxiety-like behavior (Uvnas-Moberg et al. 1994). Relevantly, IP OT dose of 10 mg/kg but not 3 or 30 mg/kg, in male mice increased the number of punished crossings in the four-plate test, indicative of anxiolytic-like behavior (Ring et al. 2006).

To determine the effect of the endogenous OT system in the social defeat stress-induced anxiety, male and female California mice were exposed to daily social defeat stress over 3 days and injected IP with OTR-A (L-368,899, 1 or 5 mg/kg) 30 min before a social interaction test (Duque-Wilckens et al. 2018). Neither dose of OTR-A affected anxiety-like behavior. However, defeated and OTR-A-injected (5 mg/kg) females investigated more the area around a caged conspecific, suggesting that OTR-A can increase social approach toward a stranger. Interestingly, OTR-A (1 mg/kg) increased but (5 mg/kg) decreased social avoidance in defeated females. Together, these findings highlight the involvement of endogenous OT in social approach and avoidance in repeatedly defeated female mice. Another study reported that in females rats, the anxiolytic effects of OT in females may be modulated by estradiol, evidenced by OT (3 mg/kg, IP) leading to anxiolytic-like behavior only if rats were pre-treated with estradiol before EPM testing (McCarthy et al. 1996).

### Chronic systemic administration of OT

Cardiac dysfunction has been shown to often contribute to symptomatology of anxiety disorders (Cohen et al. 2015). Grippo et al. tested if OT can protect against behavioral and cardiac dysfunction in response to chronic social stressors. Adult female prairie voles (socially monogamous), which are more sensitive to social stressors than males (Grippo et al. 2007), were isolated in single housing for 4 weeks. During weeks 3 and 4, all females received daily SC OT (20 μg for 14 days). Isolated females displayed elevated basal heart rate (HR) and reduced HR variability, suggestive of autonomic disruption observed in affective disorders (Pitzalis et al. 2001). Strikingly, these effects were not observed in isolated voles injected with OT (Grippo et al. 2009). In a separate study, adult female prairie voles were socially isolated for 28 days and supplied with daily OT as above. Here, all isolated females spent less time in the open arms of the EPM, regardless of treatment, such that systemic OT did not affect EPM behavior tested 48 h post-injection. Still, systemic OT reduced isolation-enhanced HR and HR variability before and after EPM testing. These results suggest a dissociative effect of OT on behavioral and autonomic responses to continuous isolation (Grippo et al. 2012).

Male rats subjected to restraint stress (60 min for 3 days) and injected IP with OT (0.05 mg/kg) or carbetocin (0.3 mg/kg) immediately after stress showed both diminished TMD and total time mobile on day 1. On day 3, stressed rats that received carbetocin spent more time in the open arm of the EPM compared to rats that received OT, indicating that carbetocin and OT may differ in their anxiolytic properties (Klenerova et al. 2010). Elsewhere, adolescent male rats received daily IP OT (1 mg/kg for 10 days). An 8-day washout period followed to abolish acute OT effects. During a 5-min emergence test consisting of a brightly illuminated OF with a hide box in the center, previously OT-treated rats displayed increased locomotor activity and spent more time exploring the OF in comparison to vehicle-treated rats. Suggestive of anxiolysis, OT pre-treated rats emerged from the hide box more than vehicle pre-treated rats. Both studies above argue for long-lasting anxiolytic effects of chronic OT treatment in adolescent and adult male rats (Bowen et al. 2011).

### Acute intracerebroventricular administration of OT or OTR-A

ICV infusion of OT ensures CNS-wide action of the drug that is independent of BBB penetration. OT (10 μg)-infused males spent more time in the center of the OF and this effect was blocked by co-administration of ritanserin, a serotonin 5-$HT_{2A/2C}$ receptor antagonist, suggesting that 5-$HT_{2A/2C}$ receptor plays a key role in the modulation of oxytocin's anxiolytic effects (Yoshida et al. 2009).

In line with the abovementioned systemic OT effects on behavior, OT (10 μg) increased the number of foot shock-punished crossings, indicative of anxiolytic behavior. In the same study, male mice administered OT (1 μg) spent twice as much time as males administered vehicle in the open quadrant of the elevated Z-maze, a modification of the EPM, showing a significant anxiolytic effect (Ring et al. 2006). Similarly, in

adult male rats, carbetocin (32 and 100 μg but not 10 μg) acutely reduced anxiety in the EPM 10 min post-infusion (Mak et al. 2012). OT (2 and 20 ng) proved anxiolytic, increasing male rat presence in the center of the home cage (Uvnas-Moberg et al. 1992). These findings argue that ICV OT administration yields similar anxiolytic-like effects to systemic OT. In contrast, in male mice singly housed for a week and infused with OT (0.1 and 0.5 μg), no effects on anxiety measures or locomotion were detected in the EPM. However, OTR-A (ornithine vasotocin, 2 μg) infusion did not affect anxiety-like behavior in the EPM or home cage locomotion, suggesting that endogenous OT does not contribute a baseline level of anxiety observed in the EPM (Zoicas et al. 2014).

Infusion of OTR-A (ornithine vasotocin, 0.75 μg) in unstressed male rats did not alter anxiety-like behavior, measured by time spent in the aversive white compartment in the light-dark box (LDB). However, social defeat-induced social avoidance was prevented by OT administration (0.1 μg), such that defeated males reinstated social preference for their defeater, though local infusion into the CeA or medial amygdala (MeA) failed to replicate this effect (Lukas et al. 2013).

OT is also involved in mating behavior-induced anxiolysis. Sexually naïve adult male rats that successfully mated with an estrogen/progesterone-primed female showed anxiolysis in the EPM and LDB. These anxiolytic effects were significantly reduced by OTR-A (ornithine vasotocin, 0.75 μg). PVN microdialysates collected during mating revealed significantly elevated OT release compared to males exposed to non-receptive females (Waldherr and Neumann 2007). Estradiol priming may have important consequences for endogenous OT release. Here, single-housed, estradiol-primed female rats explored the EPM open arm more and spent more time in the lit compartment of an LDB as well as entered the EMP open arm more often, compared to non-primed females. During mating paced by the female, PVN OT release was increased but OTR-A (ornithine vasotocin, 0.75 μg) infused ICV immediately after mating did not induce observable anxiolytic behavior. However, when all OTR-A-infused females were compared against combined saline-infused females, OTR-A treatment overall resulted in anxiety. These results suggest that paced mating increases PVN OT release in males and females and reduces anxiety-like behavior, which can be antagonized via ICV OT in males only (Nyuyki et al. 2011).

### Chronic intracerebroventricular administration of OT

Selectively bred high- (HAB) and low-anxiety-related behavior (LAB) male and female rats (for review, see Landgraf and Wigger 2002) were infused once ICV with OT (1 μg) or OTR-A (0.75 μg) or chronically ICV with OT (7 days 10 ng/h) or OTR-A (7.5 ng/h). Acute OT or OTR-A administration did not alter time spent in the light compartment of the LDB. However, on the last day of chronic OT (but not OTR-A), HAB females spent significantly more time in the light compartment. In LAB females but not males, OTR-A decreased the time spent in the light compartment on day 7, while chronic OT administration did not yield an effect. These findings suggest that chronic OT infusion reduces anxiety-like behavior in high-anxiety females only. OTR blockade, on the other hand, increased anxiety-like behavior only in low-anxiety females, suggesting that endogenous OT contributes to low-anxiety phenotype in these rats (Slattery and Neumann 2010).

Five-day ICV OT (1, 10, or 100 ng/h) in ovariectomized, estradiol-treated female rats subjected to white noise stress and tested in the EPM did not affect total activity or the number of rearings and no differences were observed during the EPM when rats were acclimatized to the testing environment and treated with saline or OT. In contrast, when on the testing day rats were moved into an unfamiliar environment, saline-treated animals showed anxiety-like behavior in the EPM whereas OT-infused rats (100 ng/h) showed reduced anxiety in the EPM. This finding highlights the role of OT in the regulation of anxiety-like behavior in a novel, stressogenic territory, emphasizing the importance of stress context when studying the effects of OT (Windle et al. 1997).

Chronic carbetocin (32 and 100 μg) infusion repeated over 10 days was anxiolytic when tested in the EPM on day 10. In contrast, systemic IP carbetocin (6.4 and 20 mg/kg) and intravenous (IV) carbetocin (2.5 and 5 mg/kg) both failed to alter behavior in the EPM. OTR-A, atosiban (1 mg/kg, IV), on the other hand increased the time spent exploring the open arms of the EPM, an effect that persisted even when atosiban and carbetocin were administered together. In sum, unlike acute carbetocin, chronic carbetocin exerts anxiolytic effects in the EPM but IV OTR blockade by atosiban alone surprisingly also achieves anxiolysis (Mak et al. 2012).

In opposition to many acute OT findings, chronic, 15-day osmotic minipump ICV infusion of 10 ng/h OT ($OT_{high}$) but not 1 ng/h OT ($OT_{low}$) in male mice induced anxiety-like behavior. On day 16, $OT_{high}$ mice spent less time compared to $OT_{low}$ mice in the lit compartment of the LDB and in the open arm of the EPM. This anxiety-like behavior was not observed in $OT_{low}$ mice, which in contrast showed higher locomotor activity in the LDB than $OT_{high}$ mice. When subjected for 19 days to chronic subordinate colony (CSC) stress, whereby four mice cohabit with a larger, dominant male in the same cage, CSC-$OT_{low}$ animals spent more time in the aversive lit compartment of the LDB compared to vehicle-treated CSC mice. Altogether, these findings suggest bi-directional, dose-dependent effects of chronic ICV infusion that at high dose (10 ng/h) chronic OT ICV administration enhances anxiety-like behavior, while a long-term low dose (1 ng/h) of OT ICV can be protective against chronic stress-induced anxiety (Peters et al. 2014).

### Brain site-specific infusion of OT or OTR-A (acute)

Bilateral OT (1 μg, but not 0.1 μg) infusions into the prelimbic cortex (part of the medial prefrontal cortex, mPFC) in male and female rats reduced anxiety in the EPM. The OT (1 μg) also increased time spent interacting with an unfamiliar conspecific and time spent in the center of the open field in male and female rats. However, intra-mPFC OTR-A (ornithine vasotocin, 1 μg) infusions prior to testing did not exert observable effects in the EPM or OF (Sabihi et al. 2014a). In contrast, socially defeated females given OTR-A (L-368,899) systemically (5 mg/kg) or into the anteromedial BNST (BNSTam) (1 μg) but not nucleus accumbens (NAc) or outside the BNST, exhibited a robust increase in time spent in the social interaction zone, demonstrating an increased social approach behavior (Duque-Wilckens et al. 2018). This suggests that OTRs in the BNSTam underlie social avoidance in socially defeated females.

In the PVN, bilateral OT infusion (10 ng/side) in unstressed, adult male rats reduced anxiety tested in the EPM. OT infusions outside of the PVN did not alter EPM performance, emphasizing the specificity of the observed effect to the PVN (Blume et al. 2008). In support of OT-mediated anxiolysis in the PVN, bilateral OT (10 ng/side) infusion into the PVN of female prairie voles also showed an anxiolytic effect in the EPM. Bicuculline, a $GABA_A$ receptor antagonist, when infused concurrently with OT, blocked the anxiolytic effect of OT in the EPM as well as an OT-mediated decrease of plasma CORT levels. This result suggests that GABA may play a key role in regulating the anxiolytic effects of exogenous OT in the PVN (Smith et al. 2016). Male rats exposed for 10 min to predator scent and infused with OT (10 μg) bilaterally into the dorsal hippocampus either 1 h or 7 days after the stress showed a reversal of anxiety seen in the stressed, vehicle-infused group measured in the EPM (Cohen et al. 2010).

OT (1 μg/side) infused bilaterally into the CeA of ovariectomized, estradiol-treated female rats significantly increased OF activity relative to saline treatment; this effect of OT was blocked by concurrent dopamine D1 receptor antagonist infusion, suggesting that interaction with the dopaminergic system can underlie some of the behavioral effects of OT. In contrast to OT infusion to the CeA, no OF or EPM enhancement was observed upon infusing OT into the ventromedial hypothalamus (Bale et al. 2001).

Male and female mandarin voles were split into high- and low-sociability groups using a social preference test assessing preference toward an empty cage vs. a cage with a same-sex conspecific. Neither locomotor activity nor time spent in the center of the OF was affected by an OTR-A (ornithine vasotocin, 1, 10, or 100 ng) injection into the NAc shell in highly sociable males or females. Similarly, no OT (0.1, 1, or 10 ng) dose affected time spent in the center or general locomotion in less sociable males or females. These findings offer evidence that the NAc shell, despite dense OTR expression, is not involved in OT-specific modulation of anxiety-like behavior (Yu et al. 2016).

### Brain site-specific infusions of OT or OTR-A (chronic)

Gestating females subjected to 2-h restraint stress and injected with 20 or 5 ng OT bilaterally into the PVN for 5 days did not differ in anxiety levels in the OF relative to controls, even though they exhibited less depressive-like behavior (Wang et al. 2018). Similarly, on day 7 of ICV chronic OT (10 ng/h) administration, no significant differences in EPM behavior were detected between groups of male rats (Havranek et al. 2015).

Another study utilized ICV osmotic minipumps to administer atosiban (600 μg/kg/day for 14 days), during which adult male rats were subjected to 2-h restraint stress each day. EPM testing on day 13 argues that chronic OTR blockade did not affect standard exploratory or anxiety-like measures but the frequency of unprotected head dipping over the side of an open arm was significantly reduced in stressed rats given vehicle but not atosiban. Chronic OTR blockade may have therefore attenuated anxiety-like effects of chronic restraint stress in ways not detectable by standard EPM measures (Babic et al. 2015).

Although some of the studies above could suggest the PVN as a major site of anxiolytic OT action, OT neurons from the hypothalamus send massive projections to a variety of limbic brain structures (Dabrowska et al. 2011; Knobloch et al. 2012), including the central amygdala, hippocampus, LS and the BNST, all critically implicated in the regulation of anxiety-like behavior. As OT neurons are regulated via a feedforward mechanism (Owen et al. 2013), application of OT in the PVN would activate OTR and increase somatodendritic release of OT in the hypothalamus, as well as terminal release in the limbic brain structures. Hence, to understand the role of OT in the regulation of anxiety, we need to explore carefully how OT, including the endogenous OT system, affects brain regions underlying anxiety and fear.

## Oxytocin and the regulation of fear memory

### Neurocircuitry of fear

In the neurocircuitry of Pavlovian fear conditioning, the lateral amygdala (LA) is the main point of entry of sensory inputs from the thalamus; the LA then conveys sensory information to the CeA. During fear conditioning in a laboratory setting, an auditory or visual cue (conditioned stimulus, $CS^+$) coterminates with somatosensory information delivered via a

foot shock (unconditioned stimulus, US). The CeA projects to the medial CeA (CeM), the main output structure of the amygdala and source of projections to brainstem fear effector structures, including periaqueductal gray (PAG, which mediates freezing behavior), reticular formation (RF, startle response), lateral hypothalamus (autonomic system cardiovascular and respiratory tone) and PVN (hormone secretion) (Pare et al. 2004). Within this circuitry, CeA has been shown to be critical for acquisition and consolidation of fear memory (Wilensky et al. 2006), whereas the basolateral nucleus of the amygdala (BLA) is the main site of fear memory storage (Gale et al. 2004). Hippocampal formation has been shown to be critical for contextual fear conditioning (Selden et al. 1991; Kim and Fanselow 1992; Phillips and LeDoux 1992), Fig. 1.

In the extended amygdala, the CeA has been primarily associated with fear response to a short-duration, discrete cue (cued fear), while the BNST has associated with contextual fear (Sullivan et al. 2004) as well as long-duration fear responses that resemble anxiety (Davis et al. 2010). Although initial lesion studies did not implicate the BNST in fear conditioning to short, discrete cues (LeDoux et al. 1988; Hitchcock and Davis 1991; Gewirtz et al. 1998), growing evidence suggests that the BNST also modulates a conditioned fear response to a discrete cue (for review, see Gungor and Pare 2016 and Goode and Maren 2017). Specifically, the BNST appears to play a pivotal role in learning to accurately discriminate and differentially respond to stimuli representing threat and safety (Duvarci et al. 2009; De Bundel et al. 2016; Lange et al. 2017; Moaddab and Dabrowska 2017).

During fear memory recall, cued fear is usually measured as duration of freezing behavior (complete immobility except for breathing) in response to a cue ($CS^+$), which has been previously paired with a foot shock (US). Time spent freezing to a cue is usually compared against a baseline freezing level taken in the same session but before $CS^+$ presentation. Alternatively, in the fear-potentiated startle (FPS), employed in rodents, monkeys and humans (Winslow et al. 2008; Walker et al. 2009a; Acheson et al. 2013), cued fear is measured as the enhancement of the ASR amplitude during $CS^+$ presentations as compared to ASR measured in between $CS^+$ presentations. Another component of FPS, non-cued fear or background anxiety, reflects potentiation of the startle amplitude measured between cue presentations in comparison to baseline ASR. This FPS component can only be observed following the $CS^+$ presentation (but not during $CS^+$ presentation) and may represent anticipatory anxiety or slow decay of cued fear (Missig et al. 2010; Yu et al. 2016; Moaddab and Dabrowska 2017). Each ASR is reflexively elicited by a white noise burst. Contextual fear, quantifiable as freezing duration or ASR potentiation, reflects memory of the fear conditioning context (Matus-Amat et al. 2007; Missig et al. 2010). Repeated exposure to $CS^+$ or training context without a US presentation enables animals to learn fear extinction, which is retained and expressed by the mPFC (Milad et al. 2006; Myers and Davis 2007). Finally, fear memory expression can also be measured by the passive avoidance test, where avoidance behavior corresponds to greater latency to enter to a preferable, dark compartment of the apparatus, because it was previously paired with foot shock.

## Animal models

Although substantial evidence suggests that OT has anxiolytic properties (Bale et al. 2001; Ellenbogen et al. 2014; Ring et al. 2006), the role of OT neurotransmission in the regulation of conditioned fear appears more complex. OT has been shown to have diverse effects on fear learning depending on the timing of OT administration, which in a contrasting fashion modulates distinct phases of fear memory formation, e.g., acquisition vs. extinction (Toth et al. 2012). Moreover, the effects of OT appear brain site-specific, such that BLA and CeA OT administration yield opposite effects on fear expression (Lahoud and Maroun 2013). Finally, the effects of OT are also age-dependent, such that OT affects adult and adolescent fear memory in contrasting ways (Kritman et al. 2017). These studies are summarized in Fig. 1.

### The effects of oxytocin on contextual fear conditioning

After contextual fear conditioning sessions over 2 days, a specific and potent OTR agonist ([Thr4,Gly7]-oxytocin (TGOT, 7 ng bilaterally) applied into the CeA before fear testing facilitated fear extinction in male and female rats. And although TGOT-treated rats indeed showed less freezing during recall session, this effect was observed from the beginning and stayed stable of the session. This suggests that TGOT might have also affected initial fear recall and not extinction rate per se (Viviani et al. 2011). In the study, the authors also elegantly combined retrograde labeling of CeA-PAG neurons with in vitro patch-clamp electrophysiology to show that TGOT likely inhibited freezing behavior via OTR-expressing lateral CeA (CeL) neurons that project to and inhibit the main CeA output, CeM (Viviani et al. 2011).

A similar effect on freezing behavior was observed in response to evoked endogenous OT release in the CeA of female rats, achieved by hypothalamic infusion of adeno-associated viral vector (AAV), which encoded Channel Rhodopsin expression (ChR2) under OT promoter in hypothalamic OT neurons and axons in OT neuron projection sites. Optic fiber implantation specifically in the CeA enabled blue light-evoked release of endogenous OT, which suppressed freezing behavior of rats previously exposed to contextual fear conditioning. This freezing suppression was abolished by infusion of OTR antagonist (Knobloch et al. 2012). Incredibly, this study was the first (and so far the only) to demonstrate that evoked axonal

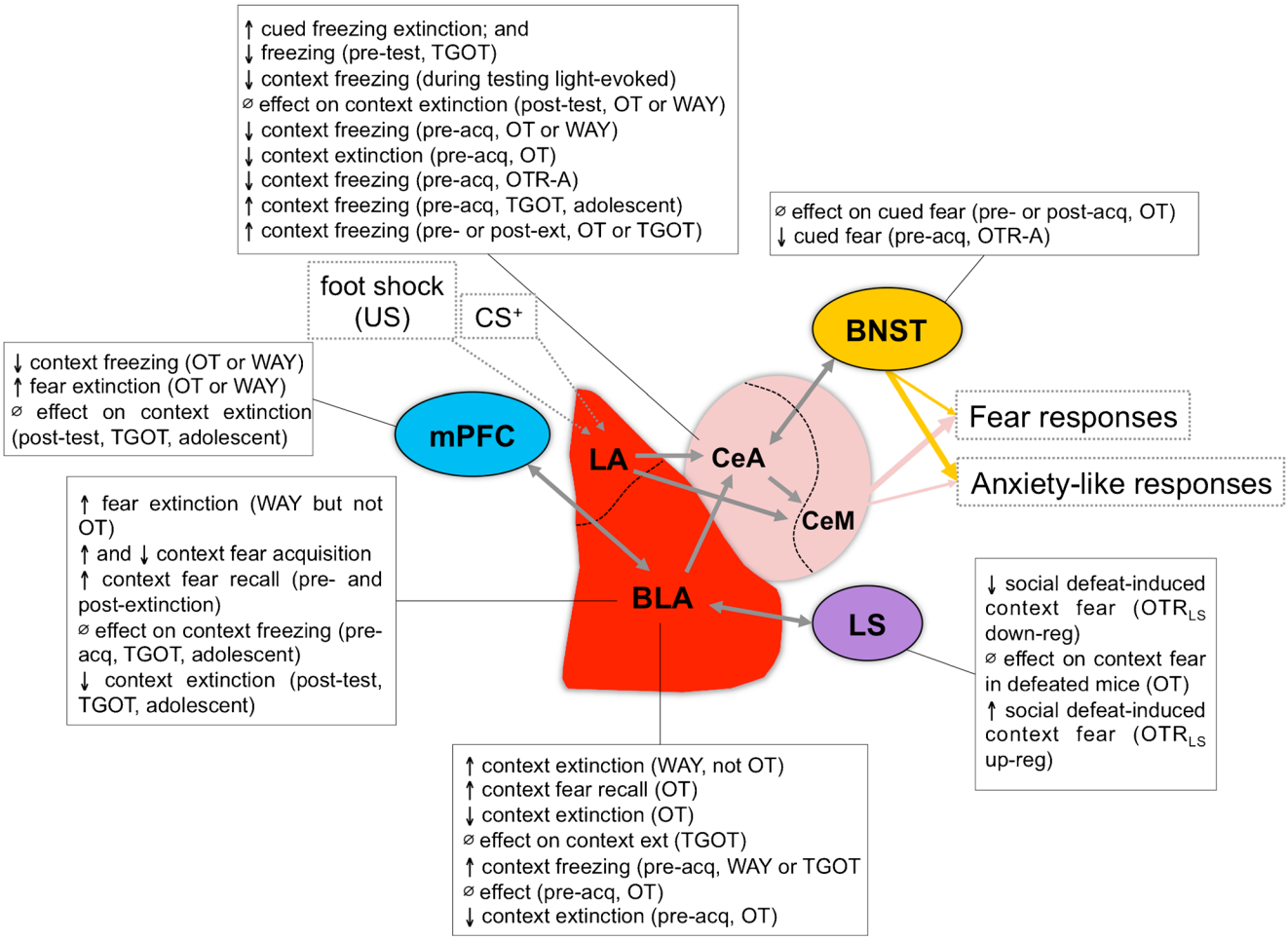


**Fig. 1 Effects of brain-specific exogenous OT or OTR antagonist infusion on fear learning in animal models**. During Pavlovian fear conditioning, somatosensory input (aversive foot shock, US) and visual or auditory thalamic input ($CS^+$, dotted gray line) co-terminate in the lateral amygdala (LA), which conveys information into the central nucleus of the amygdala (CeA). Together, CeA and the BNST, which are reciprocally connected (shown in solid gray) and send projections to the brainstem, modulate key fear and anxiety effector structures. The basolateral nucleus of the amygdala (BLA) is the main site of fear memory storage and also reciprocally projects to both the medial prefrontal cortex (mPFC) and to the lateral septum (LS), which can therefore modulate fear memory. Gray arrows denote projections between key nuclei of the amygdala, while solid black boxes summarize site-specific infusions of the OT or OTR antagonist (OTR-A) and their enhancing (up-pointing arrow), attenuating (down-pointing arrow), or null effects (empty set symbol) on fear learning. Pre-acquisition (pre-acq), post-acquisition (post-acq), pre- and post-testing and pre- and post-extinction time points of drug infusion are noted in parentheses together with the drug used. TGOT, ($Thr^4$,$Gly^7$)-oxytocin is together with WAY a potent OTR agonist; WAY, WAY-267464; OTR-A, OTR antagonist; (BNST, bed nucleus of stria terminalis; PVN, paraventricular nucleus of hypothalamus; CeA, central nucleus of the amygdala; CeM, medial central nucleus of amygdala

release of endogenous OT in the CeA reduces freezing behavior during contextual fear recall. It needs to be noted that these results are long overdue for a replication study, especially considering the low number of rats involved in the original experiment. Overall, these studies suggest that OT can efficiently reduce sustained fear responses, including contextual fear.

More recent studies used a classic in vivo pharmacology approach by infusing OT (10 ng) or selective non-peptide OTR agonist, WAY-267464 (3 μg), first described by Ring et al. (2010), into the CeA of male rats (Lahoud and Maroun 2013). In contrast to the studies above, which manipulated OTR before the first fear recall, here the authors first validated fear memory formation before introducing a treatment. When infused after the first session of contextual fear recall, OT or WAY-267464 had no effect on subsequent extinction (Lahoud and Maroun 2013). However, when OTR agonists, TGOT (7 ng) or WAY-267464, were infused into the CeA before fear conditioning (fear acquisition), they significantly reduced contextual fear expression measured on the next day. Both agonists facilitated subsequent extinction, while synthetic OT infused into the CeA had no effect (Lahoud and Maroun 2013). These results show that activation of OTR in the CeA with selective agonists but not synthetic OT, reduces acquisition and facilitates subsequent extinction of contextual fear. In contrast, in another study, infusion of OT (0.6 to 75 ng) into

the CeA increased expression of conditioned freezing responses and impaired within-session extinction (Campbell-Smith et al. 2015).

Replicating the above design, OT or WAY-267464 infused into the infralimbic cortex (IL), part of the mPFC, was shown to significantly reduce freezing behavior and facilitate fear extinction (Lahoud and Maroun 2013). However, although intra-BLA WAY-267464 infusion was also associated with facilitated extinction, synthetic OT infusion significantly enhanced freezing levels and impaired extinction and TGOT had no effect (Lahoud and Maroun 2013).

Interestingly, when TGOT or WAY-267464 was infused into the BLA before fear conditioning, the rats showed significantly higher freezing levels the next day, suggesting that selective OTR activation in the BLA facilitates formation of contextual fear. Notably, OT by itself had no effect (Lahoud and Maroun 2013), which might potentially be explained by the diverse affinities of the compounds toward OTR vs. vasopressin V1A receptors (V1AR). For example, WAY-267464 was proven a full agonist (with weak affinity) of the OTR and an antagonist at the V1AR, whereas OT is a full agonist with strong affinity for the OTR and a potent V1A agonist (Hicks et al. 2012). Different affinity toward OTR vs. V1AR may explain the variability in these compounds' effects on fear modulation. Explained more generally, OTR transmission may facilitate learning, either initial fear acquisition or extinction learning, depending on when (learning phase) and where (brain site) OTR is manipulated. Therefore, selective OTR activation in the mPFC might facilitate fear extinction learning and therefore reduce freezing, whereas activating OTR in the BLA might facilitate acquisition of fear and therefore increase contextual freezing.

In another study, intra-CeA or intra-BLA OT pretreatment impaired contextual fear acquisition but expression of contextual fear was enhanced by a pre- or post-extinction infusion of OT or TGOT into the CeA, which was blocked by OTR-A (desGly-NH2-d(CH2)5[D-Tyr2,Thr4]OVT). Notably, expression of contextual fear was suppressed by intra-CeA administration of OTR-A alone. Pre-extinction BLA infusion of synthetic OT or TGOT suppressed contextual fear, also abolished by OTR-A (Campbell-Smith et al. 2015).

In male mouse LS, OTR was shown to mediate an enhancement of contextual fear induced by acute social defeat. Here, $OTR^{loxP/loxP}$ mice, in which LS OTR expression was downregulated by septal infusion of AAV encoding Cre-recombinase, prevented the defeat-induced potentiation of contextual fear memory. These results were recapitulated with pharmacological blockade of OTR with an antagonist (1-D(CH2)5,Tyr(ME)2,Thr4,Tyr-NH2(9))ornithine vasotocin). In contrast, when OTR was overexpressed in LS by infusion of AAV-Oxtr-IRES-Venus vector in wild-type mice, this exacerbated defeat-induced contextual fear but infusing exogenous OT had no effect. Six hours after the stress, mice overexpressing LS OTR also approached the aggressive resident significantly less, indicating persisting social memory of the aggressor. Interestingly, a similar effect of OTR on contextual freezing was not observed without a prior social stress exposure (Guzman et al. 2013). The authors suggest that OTR transmission in the LS enhances the salience of emotional stimuli and fear memory in a threatening social context.

The effects of systemic OT (1, 10, 100, 1000 μg/kg IP) administered after each contextual fear extinction session were tested in male rats compared to control rats as well rats exposed to a single prolonged stress (restraint stress, forced swim and ether anesthesia, all in 1 day). Seven days later, the extinction rate was blunted in stressed rats: freezing levels in these rats were significantly higher after the second extinction training. Although systemic OT (at 10, 100, 1000 μg/kg) delayed fear extinction in control rats, it had no effect in stressed rats and the dose of 1 μg did not affect either group (Eskandarian et al. 2013). When male rats received systemic OTR-A, atosiban (1, 10, 100, or 1000 μg/kg, IP), after contextual fear training, their contextual fear recall 48 h later was reduced in a dose-dependent manner. Similarly, atosiban administered post-recall decreased freezing to context but only in the high-shock training group. This evidence suggests that endogenous OT is involved in contextual fear memory consolidation and reconsolidation and can be manipulated systemically (Abdullahi et al. 2018).

Both central and peripheral administration of long-acting OTR agonist (Pfizer compound, BBB non-penetrable) inhibited freezing to the context as well as freezing to the CS in male mice, opening the possibility that some OT effects on fear memory might be mediated via peripheral mechanisms, or rather feedback mechanisms to the hypothalamus. Freezing to the context was measured in the initial part of the recall session, after which cue-induced freezing was recorded. Considering the design of the study, it might be impossible to dissect fear to a discreet cue, for the cue presentation was unusually long (30 s) and cued fear was measured in the conditioning context, allowing contamination with contextual fear (Modi et al. 2016).

Finally, developmental fear memory manipulations via OTR differ from the effects observed in adult rats. In contrast to TGOT effects in adult rats (Lahoud and Maroun 2013), in adolescent male rats (post-natal day 27), pre-conditioning infusion of TGOT (7 or 3.5 ng) into the CeA but not BLA, increased levels of freezing during contextual fear memory recall (Lahoud and Maroun 2013; Kritman et al. 2017) but had no effect on subsequent fear extinction. Thus, in contrast to adult rat CeA, adolescent rat OTR facilitates fear memory acquisition. However, when TGOT was infused into the BLA but not IL, after the retrieval of fear memory, it led to significantly impaired extinction. Although these contrasting effects in adolescent vs. adult rats might be somewhat surprising, it is important to note that OT has repeatedly been shown to be a

potent modulator of inhibitory GABA transmission in the CeA (Huber et al. 2005), mPFC (Nakajima et al. 2014), as well as the hippocampus (Zaninetti and Raggenbass 2000; Owen et al. 2013; Harden and Frazier 2016). As adolescent brain is ongoing an intense maturation of GABA-ergic neurocircuitry (Caballero and Tseng 2016), lack of established inhibitory synapses may inevitably contribute to the drastic OT-modulated differences observed in adolescent vs. adult brain and behavior. Particularly, the mPFC has been shown to undergo a prolonged period of maturation extending toward late adolescence and early adulthood (Caballero et al. 2016; Caballero and Tseng 2016).

### The effects of oxytocin on cued fear conditioning

When applied ICV prior to auditory fear conditioning in mice and rats, OT (1 μg) or OTR-A (desGly-(NH2,d(CH2)5[Tyr(Me)2,Thr4]OVT (Manning et al. 2012) (0.75 μg) did not affect freezing to a tone during the acquisition phase and did not affect fear memory recall, measured on the next day. These results suggest that neither central global activation nor OTR blockade affect fear acquisition. However, OT reduced freezing measured during later blocks of fear memory recall, indicative of facilitated fear extinction. Notably, OTR-A alone had an opposing effect, suggesting that endogenous OT is necessary for the fear extinction learning. However, contrasting results were observed when OT was administered before extinction training (Toth et al. 2012). Here, OT (0.1 or 1.0 μg ICV) increased freezing, leading to delayed fear extinction, whereas OTR-A had no effect. Overall, these findings would suggest that central OT inhibits learning processes, whether it is initial fear memory acquisition or fear extinction learning, resulting in reduced and enhanced freezing, respectively (Toth et al. 2012). Here, the authors conclude that OT-enhanced freezing was tone specific and not generalized as neither rats nor mice froze before tone onset nor did they show increased freezing responses to the tone prior to its association with the shock. Yet, freezing response to a tone usually extends beyond tone presentation, it is often impossible to distinguish if the freezing is truly tone specific. Notably, freezing levels might potentially further increase after the first tone ($CS^+$) presentation. This phenomenon has been repeatedly reported in the FPS paradigm (Missig et al. 2010; Ayers et al. 2011; Moaddab and Dabrowska 2017) and it refers to the non-cued fear (or background anxiety) component of the FPS. As in the FPS, peak of ASR amplitude occurs in less than 200 ms following the white noise burst onset. ASR can be measured during as well as between cue presentations.

In the FPS experiments, when administered before but not after fear conditioning, systemic OT (0.1 μg but not 0.01 μg, SC) appears to reduce background anxiety in male rats but it has no effect on cued or contextual fear (Missig et al. 2010). Similarly, OT (0.1 μg but not 0.01 or 1 μg, SC) administered before the FPS recall session also appears to reduce background anxiety measured in the FPS. These results suggest that OT can reduce acquisition and recall (but not consolidation) of background anxiety. In all the experiments, OT also inhibited ASR in fear-conditioned rats independently of trial type but it had no effect on ASR in control rats (Missig et al. 2010). The authors elegantly demonstrated that the effects of OT on background anxiety are independent from the effects on contextual fear. Here, the effects of OT in rats fear-conditioned to the light ($CS^+$) were then tested in the training context without presenting the $CS^+$. None of the OT doses affected ASR in the conditioning context, suggesting that the effects of OT are specific to the background anxiety. In a follow-up study, ICV administration of OT (0.002, 0.02, 0.1, 0.2 and 2.0 μg) prior to fear recall failed to replicate effects of OT on background anxiety and only the high 2.0-μg OT dose yielded an effect (though it also generally attenuated ASR).

At a first glance, the above findings might suggest that OT modulates background anxiety via peripheral, instead of central, mechanisms (Ayers et al. 2011). However, as shown above, OT appears to modulate fear responses in a multimodal fashion. Hence, ICV administration of OT might activate OTR in brain regions where OTR play contrasting roles in fear memory modulation (CeA vs. BLA), which may facilitate or reduce fear memory formation, potentially resulting in the lack of a net effect. Although a more recent study from the Rosen group did not reproduce the overall effect of OT on background anxiety, it demonstrated that OT can differentially modulate background anxiety in rats with low vs. high baseline ASR (Ayers et al. 2016), similarly to the distinct effects of OT on anxiety in LAB vs. HAB rats discussed above (Slattery and Neumann 2010).

In support, we also showed that blocking OTR transmission in the dorsolateral BNST ($BNST_{dl}$) reduces the acquisition of cued fear differently in rats with low and high baseline ASR. Overall, our results suggest that in male rat $BNST_{dl}$ OTR neurotransmission facilities cued fear acquisition (Moaddab and Dabrowska 2017). In the study, we injected OT (100 ng bilaterally), or specific OTR-A, ($d(CH_2)_5^1$, $Tyr(Me)^2$, $Thr^4$, $Orn^8$, des-Gly-$NH_2^9$)-vasotocin (200 ng bilaterally) (Manning et al. 2012), directly into the $BNST_{dl}$ before fear conditioning. Cued fear recall measured 1 day later showed that although infusion of OT did not affect FPS (either cued or non-cued fear), blocking OTR in the $BNST_{dl}$ significantly reduced acquisition of cued fear. Because we showed that OT or OTR-A had no effect on baseline ASR or shock reactivity during fear conditioning, our results suggest involvement of endogenous OT specifically in the formation of cued fear memory. OT or OTR-A also did not affect consolidation of fear memory when injected after the fear

conditioning session, although a relatively low number of rats used in the consolidation experiment requires a replication study (Moaddab and Dabrowska 2017). Notably, we also observed a consistent (albeit non-significant) trend toward increased acquisition of non-cued fear after OTR blockade, which agrees with the FPS experiments above (Ayers et al. 2011; Missig et al. 2010), where a systemic OT injection reduced the background anxiety. Therefore, it is striking that OTRs in the $BNST_{dl}$ appear to facilitate fear memory to a discreet cue (cued, signaled fear) and at the same time reduce fear memory to unsignaled, diffuse, non-specific threats (non-cued fear). We describe this phenomenon in more detail in a later section.

Similarly, mice with OTR loss restricted to the forebrain had a reduction in freezing behavior during acquisition, as well as during cue and context retention. However, mice with a general loss of OTR (OTR KO) had fear expression equal to their wild-type counterparts (Pagani et al. 2011). These results suggest that OTR transmission in the forebrain is pivotal for fear learning.

In contrast, when OTR-A (L-368,899) was used systemically before fear conditioning, no differences in acquisition of Pavlovian fear were observed in mice. However, blocking OTR produced a dose-dependent reduction in freezing in observer mice that normally show fear-like behavior in response to the distress of fear conditioning of a familiar mouse (observational fear). Simulating global OT release or intranasal OT application had an opposite effect (Pisansky et al. 2017).

#### The effects of oxytocin on passive avoidance behavior

In the passive avoidance test, the effects of OT (200 pg) infused into the CeA were measured in Roman high-avoidance and low-avoidance male adult rats (selected based on shuttle-box acquisition behavior). Rats first acquired avoidance behavior and then received OT before the avoidance testing session, which in low-avoidance rats reduced latency to enter (avoidance) the dark compartment previously paired with foot shock. Furthermore, OT attenuated these rats' bradycardiac responses and shifted their overall behavioral strategies toward active stress coping (Roozendaal et al. 1992). This suggests that either OT reduces fear memory recall and/or OT promotes active avoidance behavior in rats psychogenetically selected for low-avoidance behavior. On the other hand, OT had null effect in rats displaying high-avoidance behavior. These results relate well to the effects of OT on background anxiety (Ayers et al. 2016) and cued fear (Moaddab and Dabrowska 2017), observed predominantly in low-startle responders, overall suggesting that OT can promote active coping behaviors in rats predisposed to low-avoidance behavior. It is exciting to entertain the idea that in the high-avoiding animals endogenous OT already contributes to the active stress coping phenotype.

When injected to hippocampal dentate gyrus (25 pg bilaterally) immediately after a single passive avoidance learning trial (consolidation phase), OT also attenuated passive avoidance behavior in both retention trials (Kovacs et al. 1979), in other words promoting active coping strategies. A similar effect was observed following dorsal raphe injections of OT (50 pg bilaterally) (Kovacs et al. 1979). ICV administration of OT (1 ng) after the learning trial (immediately or 3 h, 23 h but not 6 h after the learning trial) also resulted in an attenuation of passive avoidance behavior in a time-dependent manner. The effect was the strongest when OT was administered immediately after the learning trial (consolidation) or 1 h before the retention test (recall). In contrast, after infusion to dorsal septal nuclei (25 pg), OT potentiated avoidance behavior in two retention tests (Kovacs et al. 1979).

## The role of oxytocin in the modulation of fear memory and anxiety in humans

The effects of intranasal (IN) OT application on the regulation of anxiety and fear response have been tested in humans using functional magnetic resonance imaging (fMRI) studies of brain activity; skin conductance responses measured during fear conditioning or fear memory recall; ASR measured during fear conditioning or fear memory recall; and fear/anxiety questionnaires during/after fear acquisition or fear memory recall.

### The effects of oxytocin on fear responses in healthy subjects

#### Oxytocin, amygdala activity, innate fear and emotional processing

Multiple studies have shown a strong relationship between amygdala activity and the ability to recognize fear (Adolphs et al. 1994; Morris et al. 1996; LeDoux 1998; Whalen et al. 1998). Imaging studies show a differential response of the human amygdala following the presentation of a fearful facial expression as opposed to happiness (Morris et al. 1996) or disgust (Phillips et al. 1998). Patients suffering from a rare disorder of bilateral congenital calcification of the amygdala exhibit a specifically impaired recognition of fearful facial expressions (Adolphs et al. 1994). Notably, humans are biased toward rapid recognition of danger/threat vs. other (happy/neutral) stimuli starting early in the post-natal development (LoBue 2009), highlighting the critical role of rapid and accurate fear recognition in survival.

OT has been shown to acutely reduce amygdala activity in humans (Kirsch et al. 2005; Domes et al. 2007; Quintana et al.

2016). Initial fMRI studies in healthy male subjects found that IN OT (27 international units, IU) reduced activation of the amygdala in response to frightening faces, highlighting a modulatory role of OT during emotional processing. Compared with placebo (PLC), OT also reduced coupling of the amygdala to brainstem regions implicated in autonomic and behavioral manifestations of fear (Kirsch et al. 2005), suggesting that OT can attenuate fear expression by reducing the amygdala activity. Similarly, low dose of IN OT (8 IU) reduced right amygdala activation in response to emotional faces in healthy males. Of note is that these effects may not be specific to social stimuli representing negative valence because OT effects have been observed during presentations of both angry and happy faces, although these effects were not confirmed with 24 IU OT or IV administration of a similar OT dose (Quintana et al. 2016). Other studies showed that IN OT (24 IU) increased functional coupling between medial PFC and the amygdala of healthy male subjects, while having only negligible effects on coupling with other brain regions (Sripada et al. 2013). By strengthening functional coupling between PFC and amygdala, the study suggested that OT could facilitate fear extinction.

However, IN OT (24 IU) was also shown to potentiate ASR in healthy males (measured in humans as eye-blink amplitude) during the presentation of images with negative but not neutral or positive, valence. OT treatment was also shown to facilitate episodic memory for the negative images, such as the OT group showed a subsequent memory bias toward negative information at the cost of neutral information (Striepens et al. 2012). In the study, differences in ASR reactivity during negative vs. neutral stimuli and during negative vs. positive stimuli were significantly larger in the OT-treated group, suggesting that OT facilitates discrimination by biasing episodic memory toward negative vs. neutral or vs. positive stimuli. Interestingly, there was no OT effect for the contrast response (ASR during positive vs. neutral stimuli). In the same study, fMRI analysis revealed that whereas amygdala activity was globally reduced by OT treatment, including during responses to negative stimuli, OT facilitated left insula responses for subsequently remembered items and increased functional coupling between left anterior insula and both the left inferior frontal gyrus and left BLA during the successful encoding of negative stimuli. Notably, the anterior insular cortex has been implicated in the estimation of uncertainty and risk. Interestingly, the OT-induced facilitation of ASR toward aversive images appears to be independent of any physiological arousal or conscious arousal/valence ratings (Striepens et al. 2012). Similarly, Fischer-Shofty et al. showed that single IN dose of OT (24 IU) selectively increased recognition of fear in healthy male subjects by significantly increasing a percentage of correct responses but OT had no effect on recognition of other emotions, including happiness, sadness, anger, disgust, or surprise; no effect on general mood ratings was observed (Fischer-Shofty et al. 2010).

In contrast, Domes et al. reported that a single dose of IN OT (24 IU) attenuated amygdala responses to emotional faces in healthy males, irrespective of emotional valence, although the correct recognition of emotional expressions was not scored in the study (Domes et al. 2007). Still, IN OT (24 IU) was shown to have an opposite effect to the study above and reduce recognition of fearful facial expressions, also diminishing the misclassification of positive emotions as negative ones (Di Simplicio et al. 2009). Overall, meta-analysis of seven studies comprising a total of 381 research participants (including only 71 females) concluded that IN OT enhances emotional recognition of faces overall, with a significant effect of OT on recognition accuracy specifically in fearful and happy faces (Shahrestani et al. 2013). Overall, the facilitating effects of OT on fear recognition are particularly interesting considering studies arguing in favor of fear's high survival or adaptive value allowing rapid detection and avoidance of danger (Liddell et al. 2005; Reinders et al. 2006).

The great majority of studies on the effects of IN OT on fear have been conducted in males. The absence of data in females is problematic as stress-induced mental disorders, including PTSD, are two to three times more prevalent in females than in males (for review, see Olff et al. (2007)). Additionally, the limited number of studies performed in females opens the possibility that OT exerts contrasting sex-dependent effects on amygdala activity. OT (24 IU) was shown to selectively enhance amygdala reactivity to fearful faces and enhance amygdala activity during the processing of fearful facial expressions in females, though had no effect on calmness, alertness and mood scales (Domes et al. 2010). Similarly, although IN OT (24 IU) had no effect on female participants' gazing behavior, it increased amygdala reactivity to scenes depicting both social and non-social threats (e.g., snarling dogs, injured children, or exploding cars) (Demet et al. 1990). Both studies suggest that in women, OT might enhance amygdala activation and increase detection of threatening stimuli in the environment. In contrast, in breastfeeding women, IN OT (24 IU) did not influence amygdala activity, possibly because of OTR saturation (Rupp et al. 2014), further highlighting the involvement of the endogenous OT system in fear modulation and the need to conduct more studies in females.

Moreover, the effects of IN OT on innate fear responses were shown to strongly depend on the exposure to early life adversity. In male participants, OT was shown to improve the ability to recognize avoidance-related emotional faces (fear, sadness, disgust) as compared to approach-related emotional faces (happy, surprise, anger). However, in participants with early life trauma, OT did not affect the performance for

avoidance-related emotions. Independent of OT administration, increased emotion recognition for avoidance-related faces was observed in participants with higher early life stress scores (Feeser et al. 2014). This finding suggests that changes in endogenous OT transmission might contribute to the enhanced recognition of fearful faces in an individual with early life trauma, suggesting enhanced emotional salience processing in individuals exposed to threat and danger from their caregivers. This finding also resembles the pivotal role of septal OTR in mediating social defeat-induced potentiation of the contextual fear in mice (Guzman et al. 2013).

Overall, as argued by Bartels, the interpretation of OT effects on fear via amygdala activity inhibition may be overly simplistic (Bartels 2012). Comparable to OT effects in animal models, OT effects on fear in humans might be stimulus-, sex- and context-specific (e.g., presence of social cues) and they may further depend on personal stress history and especially early life adversity.

### The effects of oxytocin on modulation of conditioned fear responses in healthy humans

Shortly after acquisition of fear (fear conditioning), administration of IN OT (32 IU total) in healthy males was shown to attenuate negative ratings and amygdala reactivity toward faces previously paired with electric shock ($CS^+$), relative to the PLC group. In contrast, OT had no effect on reactivity toward faces unpaired with shock ($CS^-$). Furthermore, a stronger effect was observed toward $CS^+$ paired faces with direct gaze, relative to averted gaze, suggesting specificity of OT effects toward socially relevant cues (Petrovic et al. 2008). Similarly, IN OT (48 IU) in healthy male participants was shown to facilitate the reinforcement of an associative learning task but only when the learning was reinforced by social stimuli, such that positive and negative facial expressions reinforced correct and incorrect answers, respectively. Interestingly, patients with selective bilateral damage to the amygdala (congenital amygdala calcification known as Urbach-Wiethe disease) did not show the OT-induced facilitation of socially reinforced learning but performed normally on non-social learning (Hurlemann et al. 2010).

IN OT (24 IU) was shown to differentially modulate brain activity during cued and contextual fear acquisition in healthy humans when administered before fear conditioning (acquisition). During contextual but not cued fear conditioning, an OT-treated group showed increased activity in the hippocampus but reduced activation of the anterior cingulate cortex (ACC) and the insula compared with the PLC group. However, reduced responses in the nucleus accumbens (NAc) were revealed in the OT group relative to PLC during both cue and context conditioning. Interestingly, the OT group also showed significantly higher arousal in late cued and contextual fear acquisition (Cavalli et al. 2017).

Eckstein et al. (Eckstein et al. 2016) tested the role of IN OT (24 IU) on the acquisition of cued fear in healthy male participants. Here, OT was administered before Pavlovian fear conditioning in a PLC-controlled study with concomitant fMRI and psychophysiological assessments. Fear expression was measured based on reaction times to $CS^+$ vs. $CS^-$ and skin conductance. The OT group demonstrated faster reaction times to the $CS^+$ (fear-associated stimulus paired with electric shock, US) than to the $CS^-$ (safety-associated stimulus, never paired with a shock), suggesting that OT reinforces conditioning effect. OT also enhanced skin conductance to the $CS^+$ in the late conditioning phase. Overall, OT significantly increased the difference in reaction times to fear- ($CS^+$) vs. safety-associated stimuli ($CS^-$), suggesting that OT facilitated discrimination between $CS^+$ and $CS^-$ by biasing learning toward cued fear. OT-induced faster reaction times to a stimulus signaling danger ($CS^+$), which is in agreement with OT enhancing detection of fearful vs. angry or happy faces (Striepens et al. 2012) or facilitating more rapid recognition of fear in general (Fischer-Shofty et al. 2010). Facilitated learning toward $CS^+$ vs. $CS^-$ was also associated with significantly higher activation in the right ACC but not the amygdala. Interestingly, OT effect was observed when both social and non-social stimuli were used as a $CS^+$ (e.g., face vs. house). This suggests that in contrast to the study above (Petrovic et al. 2008), OT effects on fear acquisition might not be restricted to socially relevant cues.

In another study, the same group tested the effects of IN OT (same dose) on extinction of conditioned fear in combination with fMRI in healthy male participants (Eckstein et al. 2015). IN OT administered after Pavlovian fear conditioning increased electrodermal responses (skin conductance) and PFC signals to conditioned fear in the early phase of extinction but it enhanced the decline of skin conductance responses in the late phase of extinction in comparison to PLC. In the study, both fear conditioning and fear recall were performed on the same day, hence OT administered 30 min before the fMRI scan combined with fear memory recall (presentations of $CS^+$ and $CS^-$), might have also affected consolidation of fear memory, rather than extinction alone. Interestingly, the OT group showed increased reactivity specifically to the $CS^+$ in a large cluster located in the right PFC. Treatment with OT also induced higher blood oxygen level-dependent responses to the danger cue ($CS^+$) in right PFC areas during the early phase of extinction and diminished the responses in the amygdala to both danger ($CS^+$) and safety ($CS^-$) cues, regardless of the phase. Overall, the results show that OT specifically increased PFC responses to the fear-associated stimulus ($CS^+$). These data posit that OT initially biases PFC responses to danger-associated stimuli ($CS^+$) and then downregulates amygdala responses (to both $CS^+$ and $CS^-$) (Eckstein et al. 2015).

Acheson et al. (2013) tested the effect of IN OT (24 IU) using FPS in healthy male and female participants. The fear

conditioning protocol consisted of three phases: acquisition and extinction (both on day 1) and fear extinction recall (day 2). OT was administered before extinction training on day 1. Acquisition phase consisted of presentations of cue ($CS^+$) paired with an electric shock, another cue never paired with shock ($CS^-$), as well as presentations of the startle pulses in the absence of any stimuli (noise alone), which served as a measure of baseline ASR. After OT treatment, participants underwent the extinction phase that consisted of presentations of each stimulus type ($CS^+$, $CS^-$ and noise alone), without any shocks. During this session, the OT group showed significantly higher ASR to the $CS^+$ during early extinction relative to PLC but this difference disappeared by the mid and late extinction blocks. Hence, during initial extinction training, OT strengthened fear recall but by the end of extinction training, the OT and PLC groups displayed comparable levels of cued fear. As acquisition and extinction training were performed on the same day, OT might have facilitated initial fear formation and/or consolidation, as shown above (Eckstein et al. 2016), resulting in the observed transient resistance to extinction. These findings also resemble the study above (Eckstein et al. 2015), which showed OT-induced PFC reactivity specifically in response to the $CS^+$ during early extinction. Notably, in the Risbrough study (Acheson et al. 2013), when participants returned for the extinction recall on the next day, the OT group demonstrated significantly facilitated extinction, whereas it had no overall effect on anxiety ratings. No effect of sex was observed at any phase of the conditioning.

In contrast to the above studies, Grillon et al. (2013) demonstrated that during fear conditioning, IN OT (24 IU) increased anxiety rather than fear responses in healthy males and females (data from both sexes was combined). Here, ASR was measured during electric shocks signaled by a cue (predictable shocks) or delivered in an unpredictable fashion (not signaled by a cue). Following OT, ASR measured during unpredictable shocks was significantly increased compared with the PLC group or group administered with AVP. However, OT had no effect on ASR during predictable (signaled) shocks. These results demonstrate that OT can promote defensive responses to unpredictable threats (which resemble anxiety responses) but does not affect acquisition of fear signaled by a cue (cued fear), arguing that OT can potentiate anxiety to unpredictable threats. These findings seem in contrast to previous studies in rats, which proposed that OT reduces ASR during unsignaled threats (background anxiety) measured in the FPS (Missig et al. 2010; Ayers et al. 2011). However, in contrast to the study by Grillon et al. (2013), in the latter studies, OT effects were observed during fear memory recall measured 24 h after fear conditioning.

Multiple studies assert that OT might increase emotional salience during initial fear memory formation. Accordingly, during early stages of fear learning, including acquisition, consolidation and early fear recall, OT appears to facilitate discrimination toward cues predicting danger ($CS^+$) vs. safety cues ($CS^-$). However, in the later stage of extinction training and during the subsequent extinction recall, OT appears to expedite the extinction process. In both stages, OT facilitates learning processes and one might make a stipulation that greater cue discrimination learning during fear acquisition might accelerate later extinction learning.

In regard to anxiety, de Oliveira et al. (2012) recruited healthy men who received either OT (24 IU) or PLC and performed a public speaking task, which significantly elevated anxiety measured on the Visual Analogue Mood Scale. While OT did not affect anxiety levels during or after public speaking, it significantly reduced anticipation anxiety before instructions were given, as discerned using the Visual Analogue Mood Scale. Interestingly, skin conductance in the OT group remained significantly reduced throughout pre-test, anticipation, public speaking and post-test compared to the PLC group. These findings suggest that OT attenuated physiological arousal before, during and after psychosocial stress but this attenuation translated into anxiolysis only during the non-specific anticipatory pre-test phase before participants received instructions or prepared their speech. In other words, OT may attenuate subjective experience of uncertainty-oriented anxiety as opposed to task-oriented anxiety.

## Oxytocin as a potential pharmacotherapeutic for stress-related psychiatric disorders

Multiple studies have found that deficiency in fear extinction is observed in patients suffering from PTSD (for review, see Jovanovic and Ressler 2010). Impaired extinction recall 1 day after extinction learning was observed in PTSD patients compared to their monozygotic twin control (Milad et al. 2008). In addition, in PTSD patients, the original fear response to the traumatic event does not extinguish over time but instead generalizes to safe, unthreatening contexts (Rothbaum and Davis 2003). Using FPS, enhanced fear acquisition (increased ASR to $CS^+$), has been found in PTSD patients compared to healthy controls (Norrholm et al. 2011). In contrast, Grillon et al. demonstrated significantly potentiated ASR in PTSD patients during anticipation of threat administered in an unpredictable (unsignaled) manner (CS unpaired), whereas their ASR during threat signaled by $CS^+$ (predictable, paired, cued fear) were not different from healthy controls (Grillon et al. 2009). Similarly, impaired safety learning has been observed in combat-related PTSD patients who were cognitively aware of their own safety but nevertheless showed increased ASR to safety cues ($CS^-$) in the presence of danger cues ($CS^+$) compared to healthy controls and participants with remitted PTSD (Jovanovic et al. 2009). Overall, this suggests that generalized fear responses due to inability to discriminate between threat

and safety together with impaired extinction of fear are hallmarks of PTSD.

An initial study performed by Pitman et al. (1993) suggested an inhibitory effect of IN OT (20 IU) on fear memory retrieval during combat imagery in male Vietnam War veterans diagnosed with PTSD. In male patients with generalized social anxiety disorder (GSAD), a single administration of IN OT (24 IU) normalized amygdala hyperactivity in response to social cues conveying threat (e.g., fearful faces) to the same level as healthy controls, whereas it had no effect in healthy controls themselves (Labuschagne et al. 2010). This indicates that OT might be most effective in reducing hyperactive amygdala during states of heightened reactivity of stress and fear.

Acceleration of fear extinction is a potential strategy to attenuate exaggerated fear processing in PTSD. Based on this, OT has been proposed as a potential augmentation strategy to the existing evidence-based psychotherapeutic approaches, including cognitive-behavioral therapy or exposure therapy for anxiety disorders (Koch et al. 2014). Guastella et al. (2009) tested the effect of OT as an adjunctive treatment to a short-xposure therapy trial for social anxiety disorder (SAD). OT (24 IU) facilitated extinction of negative self-assessments during public speaking but it had no effect on overall anxiety symptoms. In contrast, IN OT (24 IU) administered before an exposure therapy session for arachnophobia in male and female subjects showed that the OT-treated group self-reported higher spider phobia symptoms 1 and 4 weeks following the session, even though OT did not affect behavioral measures of fear (Acheson et al. 2015). A more recent study by van Zuiden et al. (2017) targeting early intervention in PTSD in recent trauma patients in emergency departments (primarily from accidents) found that IN OT (40 IU, twice daily, for 8 days) did not attenuate clinician-rated PTSD symptoms in trauma-exposed participants 1 month later. However, beneficial effects of OT were observed in participants with high acute clinician-rated PTSD symptom severity, suggesting OT as a promising target for early intervention in individuals with high acute PTSD symptoms.

Prolonged exposure therapy is a highly effective PTSD treatment (Rauch et al. 2009; Weathers et al. 2018). In one study, participants self-administered IN OT (40 IU) or PLC prior to each weekly therapy session, starting at session 2 in order to test the effect of OT on fear extinction learning. While no statistically significant differences between groups emerged on any of the symptoms variables (most likely due to underpowered design), when estimating between-group differences in the trajectory of symptom improvement, PTSD scores during session 3 were significantly higher in the PLC group than scores in the OT group, supporting the hypothesis that OT facilitates extinction learning in PTSD patients (Weathers et al. 2018).

FMRI study in male and female police officers with and without PTSD tested the effect of OT on subjective anxiety and functional connectivity of BLA and CeM with the PFC. In PTSD patients, OT administration (40 IU) resulted in decreased subjective anxiety and nervousness. Under PLC, male PTSD patients showed diminished connectivity between CeM to ventromedial PFC compared with male trauma-exposed controls but OT administration reinstated normal connectivity. Additionally, female PTSD patients showed enhanced connectivity between the BLA and the ACC compared with female trauma-exposed controls and this effect was attenuated after OT administration (Koch et al. 2016). Eidelman-Rothman et al. (2015) examined veterans with PTSD and showed that OT normalized the resting-state brain functioning of these individuals, which became similar to those of controls (veterans not exposed to trauma) (Eidelman-Rothman et al. 2015). Another fMRI study investigated effects of a single IN OT dose (40 IU) on amygdala reactivity to happy, neutral and fearful faces in recently trauma-exposed male and females. Compared with PLC, OT significantly increased amygdala reactivity to fearful faces in males and neutral faces in females. These findings indicate that OT may enhance fearful faces processing in recently trauma-exposed individuals (Frijling et al. 2016).

Sack et al. explored how OT affects provoked PTSD symptoms, whereby healthy women and PTSD patients received IN OT (24 IU) within-subjects and underwent the Trier Social Stress Test (a simulated public speaking challenge) or a trauma-script audiotape challenge, respectively. OT serum levels correlated positively with heart rate before and after stress and OT significantly reduced overall trauma-provoked PTSD symptoms. However, symptom cluster analysis indicated that OT did not significantly affect reduction in dissociative and re-experiencing symptoms and OT-induced avoidance reduction was also short of statistical significance (Sack et al. 2017).

While multiple studies demonstrated promising effects of OT for treatment of PTSD (for review, see Koch et al. 2014, Ragen et al. 2015 and Donadon et al. 2018), OT was also shown to facilitate the initial acquisition of conditioned fear (Acheson et al. 2013; Eckstein et al. 2016), promote alertness to threat and facilitate rapid recognition of fearful facial expressions (Striepens et al. 2012; Eckstein et al. 2016). Although these facets of fear learning might serve as adaptive behaviors in healthy individuals, using exogenous OT for trauma-intervention demands further rigorous control, for OT administered shortly after a traumatic event may yield contra-therapeutic effects in patients recently exposed to trauma. Nonetheless, despite effective evidence-based treatments for PTSD, approximately one third of patients fail to fully recover after psychotherapy (Bradley et al. 2005) or decide to withdraw from psychotherapy. Hence, OT could serve as a pharmacological intervention to augment evidence-based therapies by promoting engagement in behavioral treatments

and reducing avoidance behavior (Preckel et al. 2014), which might in turn help with long-term remission of PTSD (Olff et al. 2010; Koch et al. 2014).

In regard to anxiety disorders, when GSAD and healthy participants received PLC or IN OT (24 IU) and viewed emotional faces, brain fMRI scans revealed that OT significantly increased functional connectivity between the left amygdala and insula and the left amygdala and ACC. These effects were seen only in GSAD participants following exposure to fearful faces (Gorka et al. 2015). Given PLC and presented with sad faces, male GSAD patients showed significantly enhanced activation of several areas, such as clusters in bilateral mPFC and ACC whose activation was abolished by OT (24 IU) treatment. These findings argue that OT modulates non-threatening negative affect processing in GSAD patients (Labuschagne et al. 2012).

In another study, healthy males completed the State-Trait Anxiety Inventory and received IN OT (24 IU) or PLC after which they viewed a series of social and non-social pictures. In participants with elevated trait anxiety, OT increased ASR, particularly when watching non-social pictures, while this was not the case for participants with low trait anxiety. These findings undermine the pro-social hypothesis of OT action but may better relate to the real world as authors used mildly affective neutral, positive and negative images in varying contexts (Schumacher et al. 2018) as opposed to highly affective, single-context stimuli. Furthermore, as the ASR is an avoidance/vigilance reflex, it is possible that OT promotes avoidance behavior primarily in more anxious individuals.

## Role of the bed nucleus of the stria terminalis in the regulation of anxiety and fear

In rodent and human studies, the BNST has emerged as a key brain region translating stress into sustained anxiety (Walker and Davis 1997; Hammack et al. 2004; Hammack et al. 2009; Davis et al. 2010; Ventura-Silva et al. 2012; Dabrowska et al. 2013b; Daniel and Rainnie 2016; Herrmann et al. 2016; Lebow and Chen 2016). BNST lesions have been shown to disrupt the expression of contextual fear (Sullivan et al. 2004) as well as conditioned fear responses to long-lasting (e.g., 8 min) cues (Davis et al. 2010) but not too short, discrete cues (LeDoux et al. 1988; Hitchcock and Davis 1991; Gewirtz et al. 1998) but see Luyck et al. (2017). Following a threat stimulus, the CeA is necessary for eliciting short duration fear responses to a discrete cue (phasic fear), whereas the BNST is critical for long-duration fear responses (sustained fear), which resemble anxiety (Walker and Davis 1997; Walker et al. 2009b; Davis et al. 2010). For example, the BNST is necessary for the ASR potentiation induced by an unconditioned bright light exposure (light-potentiated startle) (Walker et al. 2009b) or by ICV CRF infusion (CRF-potentiated startle) (Lee and Davis 1997). Human fMRI studies have shown potentiation of the BNST activity in conditions of uncertainty (Yassa et al. 2012), during hypervigilant threat monitoring (Somerville et al. 2010) and in anticipatory anxiety in participants suffering from arachnophobia (Straube et al. 2007). The activity of the BNST is further exaggerated in patients suffering from GAD (Yassa et al. 2012) as well as in more anxious individuals during environmental threat monitoring (Somerville et al. 2010).

However, more recent imaging studies in humans exposed discrepancies in the earlier model (Walker and Davis 1997; for review, see Shackman and Fox 2016). For example, activation of the CeA was also demonstrated in response to a sustained, long-term threat stimulus (Andreatta et al. 2015), whereas the BNST showed activation in response to an explicit, previously conditioned cue (Etkin and Wager 2007). Likewise, although initial lesion studies in animal models demonstrated that the BNST is not required for the formation of cued fear, a growing number of studies suggest that the BNST is vital for the modulation of cued fear. BNST has been shown to have an inhibitory influence on cued fear via projections to CeM (Gungor et al. 2015). This is in agreement with an FPS study that observed enhanced cued fear after BNST inhibition with intra-BNST infusions of GABA-A agonist, muscimol (Meloni et al. 2006). In contrast, under stressful conditions, BNST is functionally necessary for stress-induced enhancement (Bangasser and Shors 2008) and stress-induced reinstatement of cued fear (Goode et al. 2015).

In line with the expanded model, we propose the BNST modulates the ability to discriminate between cued (signaled, predictable, phasic) vs. non-cued (unsignaled, unpredictable, sustained) fear (for review, see Gungor and Pare 2016 and Goode and Maren 2017). Lesioning BNST in rats significantly improved ability to discriminate between a cue paired with US ($CS^+$) vs. cue that has not been paired with US ($CS^-$). Lesioning the BNST also abolished profound individual differences observed in discriminatory abilities between $CS^+$ and $CS^-$. Poor discriminatory ability also positively correlated with anxiety-like behavior in the EPM as well as the level of contextual fear (Duvarci et al. 2009). Although the exact mechanism of fear discrimination in the BNST is unknown, dopamine D2 receptors on neurons expressing protein kinase C delta (PKCδ) in the $BNST_{dl}$ have been shown necessary in discriminative learning between stimuli representing threat vs. safety (De Bundel et al. 2016). Similarly, selective serotonin reuptake inhibitors (SSRIs) were shown to enhance cued fear, an effect mediated by BNST neurons expressing serotonin 5-$HT_{2C}$ receptors (Ravinder et al. 2013; Marcinkiewcz et al. 2016; Pelrine et al. 2016). Unlike the apparently similar roles of D2 and 5-$HT_{2C}$ on BNST neurons, presynaptic cannabinoid $CB_1$ receptors on amygdala projections to the BNST facilitate a shift from phasic to sustained fear in response to an unpredictable threat (Lange et al. 2017). These studies

highlight the BNST as an important hub modulating discriminative responses to stimuli representing threat vs. safety.

## Role of OTR in the BNST in the ability to discriminate between threat and safety

One of the highest expression levels of OTR in a rodent brain is in the BNST (Tribollet et al. 1992; Veinante and Freund-Mercier 1997; Dabrowska et al. 2011; Dumais et al. 2013), which receives OT inputs, at least in part, from the PVN (Dabrowska et al. 2011; Knobloch et al. 2012). OTR in the $BNST_{dl}$ facilitates cued fear acquisition as we recently demonstrated using FPS (Moaddab and Dabrowska 2017). By infusing OTR-A directly into the $BNST_{dl}$ before cued fear conditioning (10 pairings of $CS^+$ paired with US), we showed that blocking OTR significantly reduces cued fear recall measured on the next day. In contrast, exogenous OT infused into the $BNST_{dl}$ had no effect and neither treatment affected baseline ASR or shock reactivity during fear conditioning. As we observed the reduction in fear memory recall after blocking OTR but no effect after adding exogenous OT, these results suggest involvement of endogenous OT in the $BNST_{dl}$ in the facilitation of the acquisition of cued fear (Moaddab and Dabrowska 2017).

Moreover, in the same FPS experiments, a consistent, albeit non-significant, trend toward OTR-A-induced enhancement of non-cued fear was observed, hinting at the contrasting role of BNST OTR in the acquisition of cued vs. non-cued fear (Moaddab and Dabrowska 2017). In previous FPS studies, systemic administration of OT reduced the non-cued fear or so-called background anxiety (Missig et al. 2010; Ayers et al. 2011) but see Ayers et al. (2016). Therefore, based on data from our previously published study (Moaddab and Dabrowska 2017), here we determined rats' ability to discriminate between cued (signaled) and non-cued (unsignaled) fear by calculating the discrimination index (DI) of individual rats. We divided rats' cued fear percent change score by their percent change score for non-cued fear responses according to the following formula: Discrimination index (DI) = [(light-noise trials / noise alone trials) / (noise alone trials/pre-shock trials)] (Fig. 2). We demonstrate that control rats (infused into the $BNST_{dl}$ with vehicle) on average show good discrimination between signaled and unsignaled stimuli, such that their percentage change of cued fear is nearly twice as high compared to non-cued fear responses (DI ~ 2). Strikingly, blocking OTR transmission by intra-$BNST_{dl}$ infusion of OTR-A completely disables the discrimination between cued and non-cued fear (DI < 1, Fig. 2). Based on this, we propose that OTRs in the $BNST_{dl}$ enable the formation of adaptive fear responses by biasing fear learning toward cued, signaled fear while preventing the formation of fear to unsignaled, diffuse threats. These results agree with the human studies discussed above, which show OT-induced facilitation of fear responses toward signaled fear ($CS^+$) during initial fear learning or the early consolidation phase as well as faster and more precise recognition of fearful faces or threatening stimuli in general (Fischer-Shofty et al. 2010; Striepens et al. 2012). Hence, OTRs in $BNST_{dl}$ facilitate recognition of discrete, signaled threats (Fig. 2).

## Interaction of OT with CRF neurons of the BNST in the regulation of fear and anxiety

In contrast to the hypothalamus, where OT was shown to regulate CRF neurons' activity and expression (Nomura et al. 2003; Bulbul et al. 2011; Jurek et al. 2015; Jamieson et al. 2017) and CRF receptors were shown to interact with OT secretion (Bruhn et al. 1986; Arima and Aguilera 2000; Dabrowska et al. 2011), very little is known about the functional interaction between OT and CRF in the extra-hypothalamic limbic systems critical for the regulation of fear and anxiety-like behavior.

In the $BNST_{dl}$, OTR mRNA was found on electrophysiologically defined type II neurons, which are putative inhibitory interneurons, as well as on type III, putative CRF neurons (Dabrowska et al. 2011). Notably, CRF-producing neurons constitute the primary $BNST_{dl}$ output to hypothalamic, midbrain and brainstem nuclei (Dabrowska et al. 2016). Similarly to the CeA, they send projections to effector brain structures and are uniquely positioned to mediate coordinated changes in fear and anxiety (Dabrowska et al. 2016). Although the role of the CRF neurons in the $BNST_{dl}$ still remains elusive, these neurons are thought to mediate behavioral and autonomic responses to stressors, including fear and anxiety-like behaviors (Kim et al. 2006; Dabrowska et al. 2013b). For example, repeated stress exposure induces synaptic plasticity measured as enhanced long-term potentiation (LTP) specifically in type III, putative CRF neurons of the $BNST_{dl}$; this stress-dependent effect is associated with long-term potentiation of ASR, reflecting sustained vigilance (Dabrowska et al. 2013b). Interestingly, while overexpression of CRF in the $BNST_{dl}$ does not affect ASR or anxiety-like behaviors as assessed in the EPM in rats (Sink et al. 2013) or mice (Regev et al. 2011), CRF overexpression induced before fear conditioning impairs sustained fear (potentiation of ASR after conditioning to a long-duration cue), whereas CRF overexpression enhances sustained fear when induced after conditioning, measured in the FPS. This suggests that although CRF overexpression in the $BNST_{dl}$ does not modulate unconditioned anxiety, it might be involved in the regulation of sustained fear observed post-conditioning (Sink et al. 2013). In this way, CRF, similarly to OT, seems to also differentially modulate distinct phases of fear learning.

Since OTR mRNA was also found in type III, putative $BNST_{dl}$ CRF neurons (Dabrowska et al. 2011), OT could directly affect the activity of the $BNST_{dl}$ output neurons

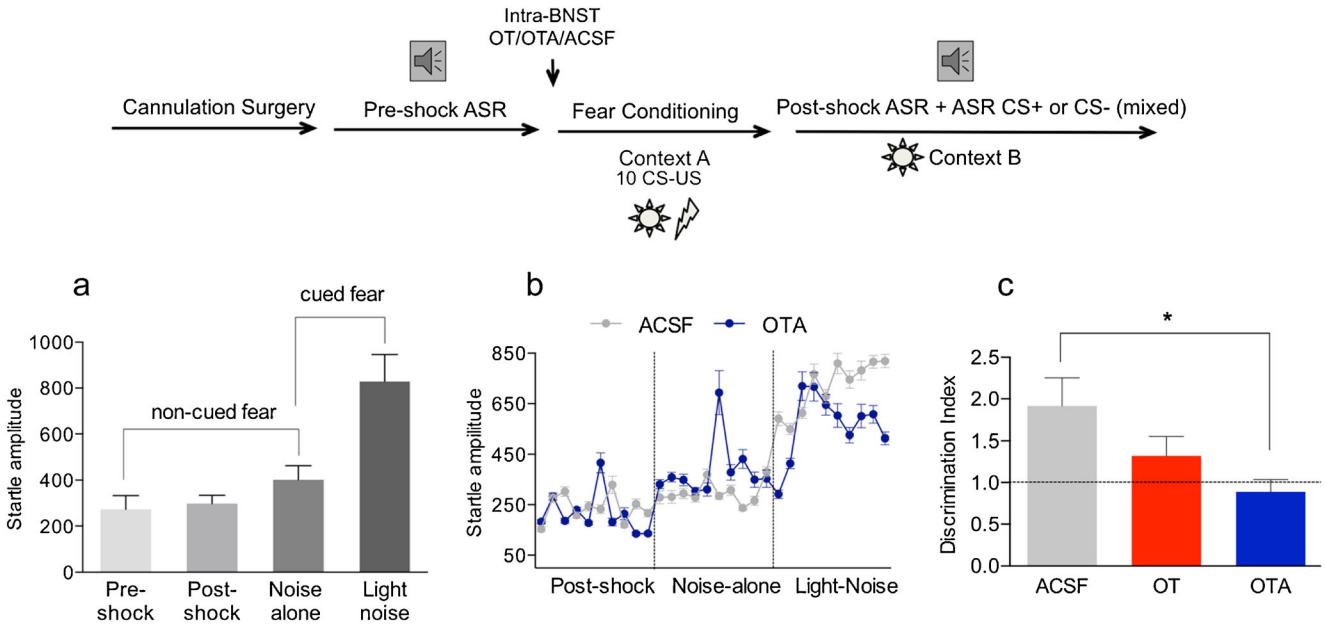


**Fig. 2** OTR transmission in the dorsolateral BNST modulates fear discrimination in the fear-potentiated startle (FPS). Upper panel: schematic representation of the experimental design based on Moaddab and Dabrowska (2017). Rats were habituated to the chambers and tested for an acoustic startle response (ASR). On the next day, rats were subjected to cued fear conditioning (cue light paired with a shock, CS-US). Prior to cued fear conditioning (CS-US), cannulated rats were injected bilaterally into the $BNST_{dl}$ with oxytocin (OT, 100 ng bilaterally), oxytocin receptor antagonist (OTA, 200 ng bilaterally), or artificial cerebrospinal fluid (ACSF) in context A. Twenty-four hours later, rats were tested for the recall of cued and non-cued fear in context B. The recall test consisted of 10 post-shock ASR trials (excluded from analysis), followed by ASR measured during presence ($CS^+$) or absence ($CS^-$) of cue light, mixed in a pseudorandom order. **a** Components of the FPS paradigm. ASR is elicited by white noise bursts. Cued fear represents potentiation of the ASR during presentation of the cue ($CS^+$, previously paired with foot shock, US) in comparison to ASR measured during noise alone trials. The non-cued fear (or background anxiety) represents ASR potentiation during noise alone trials (no $CS^+$), observed following (and not before) the $CS^+$ presentations and measured between $CS^+$ presentations, in comparison to pre-shock startle trials. Although post-shock startle trials only serve as ASR habituation, note that the ASR during noise alone trials are significantly enhanced in comparison to pre- and post-shock ASR. **b** The mean (± SEM) startle amplitude from ACSF-treated (gray) and OTR-A-treated (blue) rats infused into the $BNST_{dl}$ before fear conditioning is shown for grouped 10 post-shocks, 10 noise alone trials and 10 light-noise trials over the FPS test session (memory recall) 24 h later, based on data from Moaddab and Dabrowska (2017). Note that OTR-A increases ASR amplitude during noise alone trials and reduces ASR amplitude during light-noise trials, suggesting contrasting effects on acquisition of non-cued vs. cued fear and reduced discrimination between fear to signaled ($CS^+$) vs. unsignaled stimuli (noise alone). **c** Blocking OTR in the $BNST_{dl}$ (OTA) before fear conditioning disables discrimination between cued (signaled) and non-cued (unsignaled fear). Here, to determine the ability to discriminate between cued (signaled) and non-cued (unsignaled) fear, we calculated the discrimination index (DI) of individual rats by dividing their percent change score of cued fear by their percent change score of non-cued fear responses according to the following formula: Discrimination Index (DI) = [(light-noise trials / noise alone trials) / (noise alone trials / pre-shock trials)] in context B based on data from Moaddab and Dabrowska (2017). DI between the three treatment groups showed a significant effect of treatment ($F$ (2, 45) = 4.320, $P$ = 0.0192, one-way ANOVA) and Bonferroni's multiple comparisons test revealed significant difference between ACSF and OTR-A-treated rats ($^*P < 0.05$, $n = 19$ ACSF, $n = 13$ OT, $n = 18$ OTR-A); the analysis was completed with GraphPad Prism 6.0

and thus regulate the balance between fear and anxiety. However, evidence for direct interaction of OT with the CRF neurons in the BNST has not been yet found (but see Jurek et al. 2015; Jamieson et al. 2017 for OT-CRF interaction in the PVN). Further, a potential mechanism of how OTR in the $BNST_{dl}$ may differentially modulate cued and non-cued fear remains unknown. If the mechanism is indirect, then OT might potentially activate $BNST_{dl}$ neurons mediating cued fear, which in turn inhibit $BNST_{dl}$ output neurons responsible for non-cued, sustained fear responses. A similar dualism was reported in the BNST after auditory fear conditioning, where a population of anterolateral BNST neurons displayed inhibitory responses to $CS^+$, whereas neurons in the BNSTam (both are parts of the $BNST_{dl}$) were excited by $CS^+$ presentation (Haufler et al. 2013).

Although specific cellular targets of OT in the $BNST_{dl}$ remain unknown, OT in the CeA was shown to selectively excite local inhibitory interneurons that in turn silence CeM output neurons projecting to PAG, thus reducing contextual fear (Huber et al. 2005; Viviani et al. 2011). In an independent study, a subset of CeA interneurons expressing PKCδ was shown to inhibit CeA output and inhibit fear. Of interest is that the majority of these PKCδ-positive CeA neurons express OTR mRNA (Haubensak et al. 2010), implying that OT-responsive PKCδ neurons may modulate fear. In the BNST, OT was shown to excite subsets of neurons in lactating females (Ingram et al. 1990) but their phenotype is unknown.

However, in the $BNST_{dl}$, PKCδ mRNA was found in type II, putative inhibitory interneurons (Daniel and Rainnie 2016), entertaining the possibility that OTR-expressing PKCδ neurons impose inhibitory control over $BNST_{dl}$ output neurons and thereby modulate conditioned fear responses.

In support of their potential involvement in fear discrimination, PKCδ neurons in the $BNST_{dl}$ were shown activated in response to cued fear conditioning but not in mice exposed to $CS^+$ alone. Although this finding does not provide proof for the involvement of PKCδ neurons in the modulation of fear memory per se, fear discrimination required D2 receptors, the majority of which were found expressed on PKCδ neurons. Here, intra-BNST infusion of D2R antagonist blocked the differential response to paired $CS^+$ vs. exposure to cue alone (De Bundel et al. 2016). In contrast to the role of D2 in the BNST, lesioning the whole $BNST_{dl}$ was shown to improve discrimination between $CS^+$ vs. $CS^-$ (Duvarci et al. 2009), suggesting that the net activity of BNST output neurons suppresses stimulus discrimination and shifts the balance toward sustained fear, which resembles anxiety. Therefore, although the exact role of PKCδ neurons in modulation of the intrinsic BNST circuitry and conditioned fear remains elusive, OTR-mediated activation of PKCδ neurons might impose inhibitory control over CRF output neurons and hence prevent the sustained anxiety by shifting the response toward signaled, cued fear (Fig. 3).

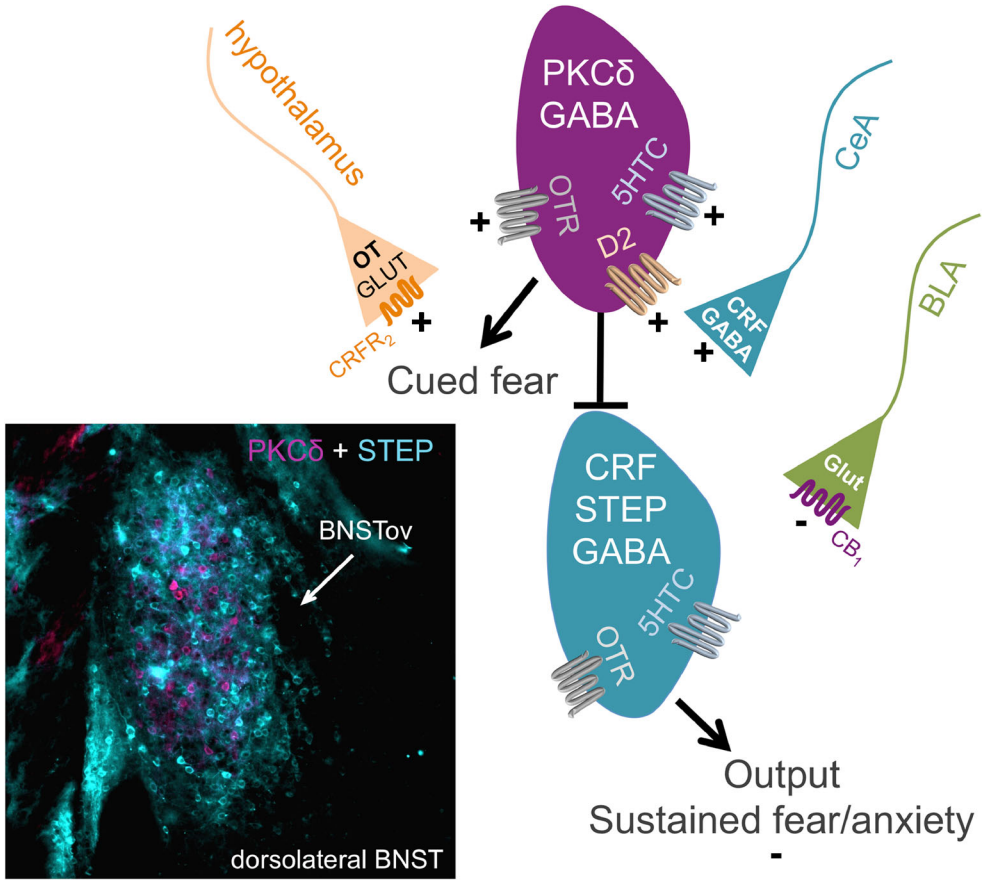


**Fig. 3** Putative model of $BNST_{dl}$ neurocircuitry mediating fear discrimination of stimuli representing threat vs. safety. Left: STEP (blue, striatal enriched protein tyrosine phosphatase), somatodendritic marker of CRF neurons in the $BNST_{dl}$ and PKCδ (pink) show localization on mutually exclusive neurons in the oval nucleus (BNSTov) of the $BNST_{dl}$. Right: BNST neurons modulate ability to discriminate between cued (signaled, predictable, phasic) vs. non-cued (unsignaled, unpredictable, sustained) fear. Lesioning BNST improves discrimination between cues representing threat and safety (Duvarci et al. 2009), suggesting that BNST output neurons (putative CRF neurons, Dabrowska et al. 2016) suppress discrimination and promote sustained fear and/or anxiety responses. In contrast, dopamine D2 receptors on neurons expressing protein kinase C delta (PKCδ) promote discriminative learning between stimuli representing threat vs. safety (De Bundel et al. 2016) and OTR in the $BNST_{dl}$ show a similar role in promoting discrimination (Moaddab and Dabrowska 2017). Similarly, selective serotonin reuptake inhibitors enhance cued fear, an effect mediated by BNST neurons expressing serotonin 5-$HT_{2C}$ receptors (Ravinder et al. 2013; Marcinkiewcz et al. 2016; Pelrine et al. 2016). Overall, this suggests that PKCδ neurons might impose inhibitory control over BNST output, CRF neurons. Unlike the apparently discrimination-promoting roles of D2, OTR and 5-$HT_{2C}$ receptors on $BNST_{dl}$ neurons, presynaptic cannabinoid $CB_1$ receptors on amygdala projections to the BNST facilitate a shift from phasic to sustained fear in response to an unpredictable threat (Lange et al. 2017). (+) facilitation of discrimination between cued fear vs. sustained anxiety and (−) inhibition of discrimination of cued fear vs. sustained anxiety

PKCδ and CRF are indeed expressed in mutually exclusive neuronal populations in the CeA (Haubensak et al. 2010) and the $BNST_{dl}$ (Daniel and Rainnie 2016). In support of the proposed model, using a Cre-CRF transgenic rat model (generously provided by Dr. Robert Messing, University of Texas, Austin) (Pomrenze et al. 2015), we recently showed that Cre-dependent silencing of $BNST_{dl}$ CRF neurons with inhibitory Designer Receptors Exclusively Activated by Designer Drugs (AAV-hSyn-DIO-DREADDs-Gi from Addgene, plasmids deposited by Dr. Bryan Roth, University of North Carolina School of Medicine) before fear conditioning improves discrimination between cued and non-cued fear during FPS recall. Specifically, in rats in which CRF neurons were inhibited, fear responses were biased toward cued fear, whereas their responses to unsignaled, non-cued fear were reduced (Roman et al. 2017). As these effects resemble the role of OTR in the $BNST_{dl}$ in fear modulation, the evidence suggests that OTR might indeed impose inhibitory control over $BNST_{dl}$ output, CRF neurons (Fig. 3).

## Interaction of CRF receptors with OT in the BNST in the regulation of fear and anxiety

In addition to local CRF-producing neurons, $BNST_{dl}$ also expresses CRF receptors (CRFR) (Dabrowska et al. 2011; Henckens et al. 2017). The effects of CRF peptide family are mediated by two receptors, CRFR type 1 (CRFR1) and CRFR type 2 (CRFR2) (Chalmers et al. 1995; Lovenberg et al. 1995), which can be activated by the endogenous peptides, Urocortin (Ucn) 1, 2 and 3, as well as by CRF (Hauger et al. 2003b). Ucn3 is the most potent and selective CRFR2 agonist of all, whereas Ucn1 has high affinity for both CRFR1 and CRFR2 (Lewis et al. 2001; Suda et al. 2004). CRF has approximately a 17-fold greater affinity toward CRFR1 than CRFR2 (Hauger et al. 2003a). Therefore, only elevated levels of CRF (e.g., in response to stress) would be expected to activate both CRFR2 and CRFR1. In addition, a soluble (truncated) splice variant of CRFR2 (CRFR2α-tr) can bind CRF but not CRFR2-specific ligands and CRFR2α-tr has been suggested to serve as a dominant-negative, which might involve binding CRF and decreasing the amount available for full-length receptors (Miyata et al. 1999; Miyata et al. 2001; Chen et al. 2005). Pre-synaptically located truncated CRFR2α-tr has been found in the rat and mouse cerebellum and other brain structures (Tian et al. 2006).

Previously, we showed that OT immunoreactivity in the $BNST_{dl}$ is restricted to fibers characterized by multiple-beaded varicosities, representing possible release sites and axon terminals (Dabrowska et al. 2011). We have also shown that these OT-positive fibers in the $BNST_{dl}$ express CRFR2. Using electron microscopy, we demonstrated presynaptic localization of CRFR2 on axon terminals that contain dense core vesicles in the $BNST_{dl}$ (Dabrowska et al. 2011). More recently, using in vivo microdialysis in freely moving rats, we demonstrated that members of the CRF peptide family modulate OT release in the $BNST_{dl}$ and that CRFRs play distinct roles in the modulation. Specifically, CRFR2 imposes tonic inhibitory control of OT release, revealed by a selective CRFR2 antagonist, Astressin 2B, whose delivery directly into the $BNST_{dl}$ via reverse dialysis significantly increased OT content in $BNST_{dl}$ microdialysates (Martinon and Dabrowska 2018; Fig. 3). Similar modulation was found in the NAc shell of monogamous male prairie voles, where CRFR2 was previously shown to suppress OT release and mediate social loss-induced passive coping behavior (Bosch et al. 2016). We further demonstrated that intact CRFR1 transmission is necessary for the stimulatory effect of Astressin 2B on OT release in the $BNST_{dl}$ but also that CRF by itself causes a delayed increase in OT release in the $BNST_{dl}$ (Martinon and Dabrowska 2018). Activation of CRFR1 has been recently shown to increase OT release in the medial preoptic area in lactating females (Klampfl et al. 2018).

Although these findings clearly suggest that CRFRs modulate OT release, the role of this modulation in the regulation of fear and anxiety remains elusive. In the BNST, CRFR-immunoreactive axons are mostly glutamatergic, whereas CRFR-immunoreactive dendrites are primarily GABA-ergic (Jaferi and Pickel 2009). As $BNST_{dl}$ neurons (including CRF neurons) are GABA-ergic (Sun and Cassell 1993; Dabrowska et al. 2013a), CRFRs are presumably located on dendrites of local GABA neurons, whereas glutamatergic CRFR-expressing terminals in the BNST are extrinsic in origin. Here, it is important to note that the BNST-projecting hypothalamic OT neurons also express glutamatergic markers (Dabrowska et al. 2011; Dabrowska et al. 2013a), offering the possibility that CRFR2-expressing hypothalamic terminals might co-release OT and glutamate in the $BNST_{dl}$. Additionally, presynaptic CRFR2 on these excitatory fibers might modulate the release of OT, or glutamate, or both. Hence, an orchestrated effort of CRFR and OT via OTR-expressing neurons in the $BNST_{dl}$ could control levels of fear and anxiety.

Yet, the source of CRF in the $BNST_{dl}$ is puzzling, since its somatodendritic release has not yet been confirmed. In fact, only a small fraction of CRF receptor-expressing dendrites and terminals in the BNST also contain CRF (Jaferi and Pickel 2009). Accordingly, CRF activates primarily non-CRF neurons in the BNST (Kash and Winder 2006). CeA might be the major source of CRF projection to the $BNST_{dl}$ (Pomrenze et al. 2015). Alternatively, Ucn1 fibers originating from the Edinger Westphal nucleus (Dos Santos Junior et al. 2015) might modulate OT release in the $BNST_{dl}$ via CRFR1 and CRFR2. Therefore, some of the behavioral effects observed after CRF or Urocortin infusions into the $BNST_{dl}$ may be, at least partially, a result of changes in extracellular OT content. In fact, a recent study demonstrated the

importance of CRF neurons in the CeA in fear discrimination (Sanford et al. 2017) but disregarded the fact that CRF released from CeA is acting primarily in the $BNST_{dl}$. Relevantly, optogenetically silencing CRF release from the CeA to the BNST has been shown to disrupt sustained fear responses (Asok et al. 2018). These studies suggest that CRF release within the $BNST_{dl}$ might, akin to OTR, promote adaptive fear discrimination.

However, CRF infusion in the BNST has been also shown to increase vigilance, measured as potentiated ASR. This phenomenon, called CRF-potentiated startle, requires CRFR1 activation in the BNST but not CeA (Lee and Davis 1997; Walker et al. 2009a). Similarly, bright light-potentiated startle is also mediated by CRFR1 in the BNST (Walker et al. 2009a), suggesting involvement of CRFR1 in sustained anxiety rather than fear responses. Similarly, intra-BNST administration of CRF was also shown to produce a dose dependent anxiety-like behavior in the EPM, an effect mediated by CRFR1 but not CRFR2. In contrast, both the CRFR1 and CRFR2 antagonist prevented CRF-induced conditioned place aversion after intra-BNST infusion (Sahuque et al. 2006). Repeated intra-BNST injections of a low dose of Ucn1 elicited long-term anxiety-like behavior reflected in decreased social interaction but not reduced exploration in the EPM (Lee et al. 2008). In contrast, CRFR2 blockade in the BNST was shown to prevent conditioned defeat in Syrian hamsters, whereas CRFR1 blockade had no effect (Cooper and Huhman 2005). This might suggest that whereas CRFR1 activation in the BNST increases unconditioned vigilance measured as ASR and anxiety-like behaviors in the EPM, CRFR2 appear to mediate learned behavioral responses. Modulation of OT release by CRFR2 might also point toward OTR-mediated conditioned fear but not overall vigilance. Indeed, we have shown that OT or OTR-A in the $BNST_{dl}$ do not affect baseline ASR and instead modulate conditioned fear (Moaddab and Dabrowska 2017).

In agreement with that, cued fear response measured in the FPS was not disrupted by intra-BNST administration of CRFR1 antagonist (Davis et al. 2010). However, in rats that have been trained during 20 CS-US pairings, CRFR1 antagonist potentiated cued fear, suggesting inhibitory role of CRFR1 on fear learning when rats are "over-trained" (Walker et al. 2009a). Similarly, in human FPS studies, CRFR1 antagonist GSK561679 was shown to increase cued fear but not conditioned anxiety (non-cued fear) (Grillon et al. 2015). In addition, intra-$BNST_{dl}$ infusion of CRFR1 antagonist disrupted the retention of the contextual fear, another form of sustained conditioned fear but the antagonist did not interfere with the early phases of contextual fear acquisition. Surprisingly, CRFR1 antagonist did not disrupt unconditioned freezing to predator odor (Asok et al. 2016).

In sum, CRF involvement in fear vs. anxiety indicates an interesting dualism, such that its engagement of CRFR1 can promote vigilance, sustained anxiety and contextual fear but at the same time CRFR1 can inhibit cued fear. We have shown that in animal models and human studies, OT appears to play an opposite role to CRF, whereby it facilitates cued fear but reduces contextual fear and anxiety-like behavior. In this light, both OT and CRF emerge as powerful and interactive modulators of emotionally salient behaviors, which is why evidence for their reciprocal signaling should be taken seriously to better understand mechanisms of fear and anxiety-like behaviors in their full complexity that yields clinical relevance.

## Concluding remarks

In this review, we summarized behavioral studies examining exogenous OT to show that in spite of OT reducing anxiety-like behavior (Bale et al. 2001; Ring et al. 2006; Ellenbogen et al. 2014) and attenuating sustained contextual fear responses (Viviani et al. 2011; Knobloch et al. 2012), the OTR system in its complexity escapes this generalization. Notably, in humans, OT was shown to facilitate rapid recognition of environmental threats and fearful facial expressions and as such promote adaptive fear responses, which enable precise detection of danger (Striepens et al. 2012; Acheson et al. 2013). Similarly, in the $BNST_{dl}$, OTR has been shown to bias fear learning toward cued (signaled) fear and reduce fear learning of non-cued (unsignaled) threats, overall promoting discriminatory responses between stimuli representing threat and safety (Moaddab and Dabrowska 2017). These findings have unique translational validity to psychiatric disorders in humans (Grillon et al. 2009). Impaired learning of safety has been observed in PTSD patients who were cognitively aware of safety but nevertheless showed increased reactivity to safety cues in the presence of danger cues (Jovanovic et al. 2009). Furthermore, we show that the effects of OT are mediated by "stress context," such that OT primarily attenuates anxiety in a novel, stressogenic territories (Windle et al. 1997). OT seems to strengthen active stress coping strategies (e.g., active avoidance, cued fear measured in the FPS) and reduce unsignaled non-cued fear responses in animals psychogenetically predisposed to low avoidance behavior (Roozendaal et al. 1992) or rats with low baseline startle responses (Ayers et al. 2016; Moaddab and Dabrowska 2017), suggesting that OT produces the most salient effects in rats with deficiency in avoidance/vigilance behavior. Finally, we emphasize the vast gap in knowledge in studying the role of endogenous OT in fear learning and anxiety in both animal models and human studies by utilizing OTR-A or endogenous OT measures to better understand the role of OTR transmission in the modulation of fear and anxiety. Better understanding of the role of OTR transmission in the neurocircuitry of defensive behaviors will allow to clarify often contrasting or controvercial findings of exogenous OT applications in humans.

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**Abbreviations** 5-HT, Serotonin; ACC, Anterior cingulate cortex; AAV, Adeno-associated viral vector; am, Anteromedial; AN, Accessory nuclei of the hypothalamus; ASR , Acoustic startle response; AVP , Arginine-vasopressin; BBB, Blood-brain barrier; BLA, Basolateral nucleus of the amygdala; BNST, Bed nucleus of the stria terminalis; CeA, Central nucleus of the amygdala; CeL, Lateral central nucleus of the amygdala; CeM , Medial central nucleus of the amygdala; ChR2, Channel Rhodopsin; CNO, Clozapine-*N*-oxide; CNS, Central nervous system; CORT, Corticosterone (rodents) or cortisol (humans); CRF, Corticotropin-releasing factor; CRFR, Corticotropin-releasing factor receptor; $CS^+$, Signaled conditioned stimulus (paired); $CS^-$, Unsignaled conditioned stimulus or unpaired stimulus; CSC, Chronic subordinate colony; dl, Dorsolateral; DI, Discrimination index; DREADD, Designer receptors exclusively activated by designer drugs; EPM, Elevated plus maze; fMRI, Functional magnetic resonance imaging; FPS, Fear-potentiated startle; FST , Forced swim test; GABA, Gamma-aminobutyric acid; GAD, Generalized anxiety disorder; GSAD, Generalized social anxiety disorder; HAB , High-anxiety-related behavior; HPA , Hypothalamic-pituitary-adrenal; HR, Heart rate; ICV , Intracerebroventricular; IN, Intranasal; IP, Intraperitoneal; ITC, Intercalated cell masses of the amygdala; ITI, Inter-trial interval; IU, International unit; IV, Intravenous; LA, Lateral amygdala; LAB, Low-anxiety-related behavior; LDB , Light-dark box; LH, Lateral hypothalamus; LS, Lateral septum; LTP , Long-term potentiation; mPFC, Medial prefrontal cortex; MeA, Medial amygdala; NAc, Nucleus accumbens; NPS, Neuropeptide S; OF, Open field; OT, Oxytocin; OTKO, Oxytocin knockout; OTR, Oxytocin receptor; OTR-A, Oxytocin receptor antagonist; OTRKO, Oxytocin receptor knockout; PAG, Periaqueductal gray; PKCδ, Protein kinase C delta; PLC, Placebo; PND, Post-natal day; PPI, Pre-pulse inhibition; PTSD , Post-traumatic stress disorder; PVN, Paraventricular nucleus of the hypothalamus; RF, Reticular formation; SAD, Social anxiety disorder; SON, Supraoptic nucleus of the hypothalamus; SC, Subcutaneously; STAI, State-Trait Anxiety Inventory; TGOT, ($Thr^4$, $Gly^7$)-oxytocin; potent oxytocin receptor agonist; TMD , Total movement distance; TSST , Trier social stress test; Ucn, Urocortin; US, Unconditioned stimulus; V1AR, Vasopressin V1A receptor; WAY, WAY-267464; oxytocin analog; WNB, White noise burst

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