Archival Report

Biological Psychiatry

Oxytocin Facilitates the Extinction of Conditioned Fear in Humans

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ABSTRACT

BACKGROUND: Current neurocircuitry models of anxiety disorders posit a lack of inhibitory tone in the amygdala during acquisition of Pavlovian fear responses and deficient encoding of extinction responses in amygdala–medial prefrontal cortex circuits. Competition between these two responses often results in a return of fear, limiting control over anxiety. However, one hypothesis holds that a pharmacologic strategy aimed at reducing amygdala activity while simultaneously augmenting medial prefrontal cortex function could facilitate the extinction of conditioned fear.

METHODS: Key among the endogenous inhibitors of amygdala activity in response to social fear signals is the hypothalamic peptide oxytocin. To address the question whether oxytocin can strengthen Pavlovian extinction beyond its role in controlling social fear, we conducted a functional magnetic resonance imaging experiment with 62 healthy male participants in a randomized, double-blind, parallel-group, placebo-controlled design. Specifically, subjects were exposed to a Pavlovian fear conditioning paradigm before receiving an intranasal dose (24 IU) of synthetic oxytocin or placebo.

RESULTS: Oxytocin, when administered intranasally after Pavlovian fear conditioning, was found to increase electrodermal responses and prefrontal cortex signals to conditioned fear in the early phase of extinction and to enhance the decline of skin conductance responses in the late phase of extinction. Oxytocin also evoked an unspecific inhibition of amygdalar responses in both phases.

CONCLUSIONS: Collectively, our findings identify oxytocin as a differentially acting modulator of neural hubs involved in Pavlovian extinction. This specific profile of oxytocin action may open up new avenues for enhancing extinction-based therapies for anxiety disorders.

Keywords: Fear extinction, fMRI, Oxytocin, Psychophysiology, Skin conductance

http://dx.doi.org/10.1016/j.biopsych.2014.10.015

An ability to detect and avoid danger is essential for all species. It is likewise essential to adapt flexibly to new life circumstances in which a situation previously predicting danger no longer has this association. Facilitating this process has strong therapeutic implications for anxiety disorders (1), which are among the most common mental illnesses with a lifetime prevalence of up to 25% (2). Together with supportive pharmacotherapy, exposure-based behavioral interventions currently represent the "gold standard" for treating anxiety disorders (3). However, a substantial percentage of patients do not benefit from established therapeutic approaches (4,5).

Procedurally, exposure therapy is very similar to Pavlovian extinction (6), which can be modeled experimentally by the progressive decrement of a conditioned fear response (CR) when a conditioned stimulus (CS) is repeatedly presented in the absence of a noxious unconditioned stimulus (US) with which it has previously been paired (7,8). The return of fear after extinction owing to reinstatement, renewal, or spontaneous recovery serves as behavioral evidence that extinction does not erase the original fear, but rather involves new and independent inhibitory learning that competes with the original CS-US association (6). Current neurocircuitry models suggest that Pavlovian extinction is orchestrated by the medial prefrontal cortex (PFC) and surrounding areas, the amygdala, and their functional interactions (9,10). Consistent with the assumption that deficient extinction may contribute to the development and preservation of pathologic anxiety, patients with anxiety disorders typically present a neural pattern of medial PFC hypoactivation paralleled by amygdala hyperactivation, which normalizes after exposure therapy (11,12).

Informed by translational research on the neurocircuitry of extinction, innovative approaches for augmenting exposure therapy with pharmacologic agents have evolved (3,13,14). Specifically, animal studies have identified the oxytocin (OXT) system as a promising pharmacologic target for therapeutic interventions aimed at attenuating anxiety disorders (15–17). Investigators have shown OXT to modulate key nodes implicated in both anxiety disorders and Pavlovian extinction, including the amygdala and prefrontal areas, which is consistent with rich OXT receptor expression in these regions (18,19). Intranasal OXT administration has been found to reduce amygdala responses to social fear stimuli and to increase

ISSN: 0006-3223

amygdala–medial PFC functional interplay (20–23). When administered after Pavlovian fear conditioning, intranasal OXT attenuates the negative evaluation of previously conditioned faces (24) and enhances the recall of extinction assessed with fear potentiated startle responses (25). Taken together, there is substantial evidence implicating OXT in the inhibition of anxiety; however, it remains unclear whether these effects or additional mechanisms might promote extinction learning.

We report a randomized, double-blind, parallel-group, placebo-controlled proof-of-concept study using a Pavlovian fear conditioning and extinction procedure with concomitant functional magnetic resonance imaging (MRI) and psychophysiologic assessments in 62 healthy men to examine the potential of OXT to modulate extinction learning. First, we hypothesized that OXT would specifically increase reactivity to the fear-conditioned stimulus in prefrontal regions implicated in extinction learning (7). Second, we predicted that neural activity in fear-associated brain areas such as the amygdala as well as electrodermal responses to the fear-associated stimulus (CS+) would be diminished. Given previous findings of phase-dependent modulation of fear extinction (26–28) and a selective effect of OXT on fear-potentiated startle during the earliest stage of extinction training (25), we also expected OXT specifically to modulate early extinction learning.

METHODS AND MATERIALS

Participants

Participants included 62 healthy, right-handed men (mean age ± SD, 24.61 ± 4.28 years) who gave written, informed consent. The study was approved by the institutional review board (Identifier: 329/12) and carried out in compliance with the latest revision of the Declaration of Helsinki. The study was registered in the ClinicalTrials.gov database (Identifier: NCT02156661) provided by the U.S. National Institutes of Health. Subjects were free of current and past physical or psychiatric illness, as assessed by medical history and the Mini-International Neuropsychiatric Interview (30); were nonsmokers, were naïve to prescription-strength psychoactive medication, and had not taken any over-the-counter psychoactive medication in the past 4 weeks. Subjects were not told the aim of the study. At the end of the experiment, they received a detailed debriefing and monetary compensation.

Experimental Design

We applied a randomized, placebo-controlled, double-blind, between-subject design. We preferred a parallel-group design over a crossover within-subject design to avoid potentially confounding effects of repetitive fear conditioning. Volunteers were randomly assigned to either intranasal administration of OXT (Syntocinon Spray; Novartis, Basel, Switzerland), 3 puffs per nostril, each with 4 IU OXT for a total dose of 24 IU, or placebo (PLC), sodium chloride solution, in accordance with current guidelines (31). Screening of the subjects was conducted before the test sessions. Participants completed a comprehensive neuropsychological test battery to control for possible pretreatment differences in cognitive performance, Beck Depression Inventory, and the Anxiety Sensitivity Index (32,33). The experimental groups did not differ in demographic variables or neuropsychological performance (Table 1).

Functional MRI Conditioning and Extinction Paradigm

We used an adapted version of a validated functional MRI fear-conditioning procedure (34). Briefly, during the procedure, neutral conditioned stimuli (CS+) were paired with an aversive US (electric shock) in 70% contingency, whereas other neutral stimuli were never paired with the US (non–fear-associated stimulus [CS−]). To account for previous findings suggesting that OXT specifically modulates processing of social stimuli, we included a social CS pair (two neutral faces from the Karolinska face database; face CS+, face CS−) and a

Table 1. Demographics and Neuropsychological Performance

	OXT Group, Mean (SD)	PLC Group, Mean (SD)	*t*	*df*	*p*
Age (Years)	25.20 (4.46)	24.03 (4.08)	1.068	59	.290
Education (Years)	16.77 (2.47)	16.23 (2.34)	.833	54	.409
SST[a]					
Median reaction time (msec)	491.65 (158.68)	443.00 (115.16)	1.381	60	.172
Stop signal reaction time (msec)	198.71 (143.99)	197.06 (137.48)	.046	60	.963
Proportion of correct stops	.52 (.14)	.51 (.08)	.088	60	.930
PAL[a]					
Total errors	23.52 (19.40)	17.87 (15.77)	1.257	60	.214
Mean errors to success	2.10 (3.76)	1.35 (1.68)	1.002	60	.320
SWM 8[a]					
Between errors	5.16 (7.49)	6.48 (9.31)	−0.616	60	.540
Strategy score	13.26 (4.25)	12.45 (3.14)	.878	60	.383
ASI[b]	15.10 (8.36)	17.13 (10.66)	−.832	59	.409
BDI[c]	2.71 (3.54)	2.65 (3.14)	.080	57	.937

ASI, Anxiety Sensitivity Index; BDI, Beck Depression Inventory; OXT, oxytocin; PAL, paired associates learning task; PLC, placebo; SST, stop signal task; SWM, spatial working memory task.

[a]Used to measure subjects' ability to inhibit a reaction, their visual memory, and their ability to retain spatial information, using the Cambridge Neuropsychological Test Automated Battery.

[b]Used to assess anxiety sensitivity.

[c]Used to measure depressive symptoms.

nonsocial CS pair (house CS+, house CS−) (29,35,36). The same face and house stimuli were used for all subjects, but CS+ and CS− assignment within the two stimuli pairs was counterbalanced across treatment groups.

Subjects were first habituated to all four stimuli by presenting the stimuli outside the scanner. Throughout the following conditioning procedure, all CS+ and CS− stimuli were presented 30 times for 4000 msec each in a randomized order (restriction: there were no more than two consecutive presentations of any one type of CS). The CS+ and CS− stimuli were separated by a variable interstimulus interval ranging from 8–11 sec, during which subjects viewed a central fixation cross (low-level baseline). To ensure attentive processing, 50% of the subjects were instructed to press the right response button for a face and the left button for a house, and the other 50% of subjects were instructed vice versa.

After the conditioning procedure, there was a break during which OXT or PLC nasal spray was administered and the T1 anatomic MRI scan was acquired. The extinction functional MRI session started 30 min after inhalation of the nasal spray, presenting identical numbers and durations of trials and interstimulus intervals as the conditioning session but with no shocks applied at all. In addition, participants completed the Spielberger State–Trait Anxiety Inventory and the Positive and Negative Affective Scale before and after the paradigm (37,38).

Psychophysiologic Measurement and Electrical Stimulation

The US consisted of brief electrical shocks of 2 msec duration that were individually adapted to be "highly annoying yet not painful." During both the conditioning and extinction procedure, the skin conductance responses (SCRs) on the thenar and hypothenar of the left (nondominant) hand were sampled simultaneously with functional MRI scans. Detailed information on the setup is provided in Supplement 1.

Processing of Psychophysiologic Data

Participants were selected for the final analysis by the number of valid responses they produced during the extinction procedure. A valid response was defined as >.02 S, which has been previously used as a deflection criterion (27). For both the OXT group and the PLC group, participants with the fewest number of responses were excluded. Data from 36 subjects entered the final analysis (OXT, $n = 18$; PLC, $n = 18$). We assume that participants who failed to show an adequate SCR in the present study might be SCR nonresponders or, alternatively, that electrodes might have run dry or loosened because they were attached 1–1.5 hours before the extinction paradigm. In line with previous investigations, the SCR was defined as the maximum SCR signal during a time window of 5 sec after CS onset minus the SCR baseline value (34). To account for interindividual differences in physiologic reactivity, SCR data were *z*-transformed, and outliers of ±2 SD were excluded from each subject's analysis (39).

Acquisition and Analysis of Functional MRI Data

The MRI data were collected using a 1.5-tesla Siemens Avanto MRI system (Siemens AG, Erlangen, Germany). T2*-weighted echoplanar images with blood oxygen level–dependent (BOLD) contrast were obtained (repetition time = 3000 msec; echo time = 35 msec; matrix size 64 × 64; pixel size 3 mm × 3 mm × 3 mm; slice thickness = 3.0 mm; distance factor = 10%; field of view = 192; flip angle = 90°; 36 axial slices). High-resolution anatomic images were acquired using a T1-weighted three-dimensional magnetization prepared rapid acquisition gradient-echo sequence (repetition time = 1570 msec; echo time = 3.42 msec; matrix size 256 × 256; pixel size 1 mm × 1 mm × 1 mm; slice thickness = 1.0 mm; field of view = 256; flip angle = 15°; 160 sagittal slices).

The MRI data were preprocessed and analyzed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB 7 (MathWorks, Natick, Massachusetts). Images were first preprocessed using a standardized procedure including reorientation, affine registration, re-alignment, and spatial normalization to the current Montreal Neurological Institute (MNI) template using the unified segmentation function in SPM8 (40,41). The normalized images were spatially smoothed using an 8-mm full-width at half maximum gaussian kernel. A detailed description of the preprocessing and analysis procedure is provided in Supplement 1. On the first level, the four CS+ and CS− stimuli were modeled as separate conditions. To examine the effects of OXT on neural changes during extinction learning, condition-specific regressors for the early phase (spanning trials 1–15) and late phase (spanning trials 16–30) of extinction were defined (face $CS+^{early}$, face $CS-^{early}$, house $CS+^{early}$, house $CS-^{early}$, face $CS+^{late}$, face $CS-^{late}$, house $CS+^{late}$, house $CS-^{late}$) and modeled in an epoch design convolved with a hemodynamic response function (29,42). Random effects group analyses were conducted in SPM8 and focused on main and interaction effects of treatment (OXT, PLC) on extinction learning. Extinction-specific effects of OXT were assessed using a repeated measures analysis of variance (ANOVA) with the within-subject factor "phase" (early, late) and the between-subject factor "treatment" (OXT, PLC), and the contrast (CS+ > CS−) as dependent variable.

Differential effects of OXT on extinction learning for social and nonsocial stimuli were assessed using a repeated measures ANOVA with the within-subject factor "sociality" (face, house) and the between-subject factor "treatment" (OXT, PLC). Unspecific, domain-general effects of OXT were assessed using a repeated measures ANOVA with the within-subject factor "phase" (early, late) and the between-subject factor "treatment" (OXT, PLC).

Based on our a priori hypothesis, the analysis of treatment effects focused on prefrontal regions and the amygdala as key neural substrates of extinction learning. To this end, the initial analysis was restricted to atlas-based regions of interest (ROIs) for the amygdala, the medial PFC, and the middle frontal cortex (mid-PFC) (significance threshold $p < .05$, family-wise error [FWE] corrected). In addition, an exploratory whole-brain analysis was performed (significance threshold $p < .05$, FWE corrected; cluster defining threshold $p = .005$) (42). To disentangle the direction and specificity of OXT effects, parameter estimates were extracted from regions showing significant treatment effects using MarsBar toolbox (http://marsbar.sourceforge.net/). Anatomic classification was

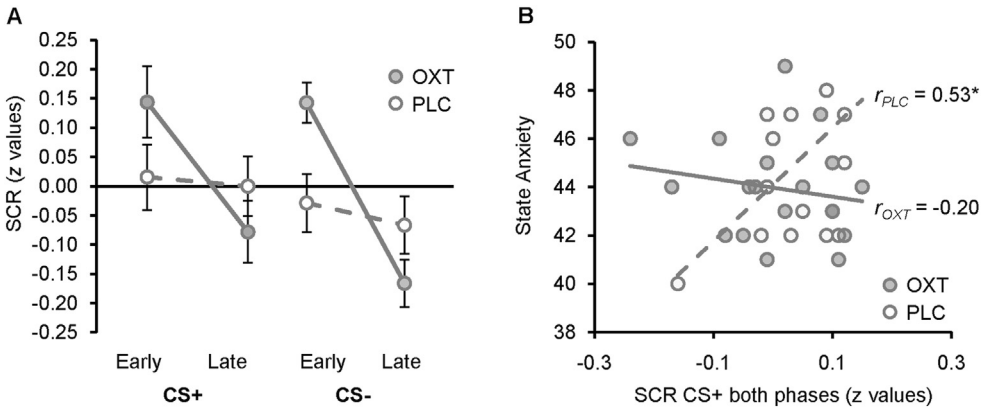

Figure 1. Oxytocin effects on skin conductance response. **(A)** Oxytocin facilitated the decrease of skin conductance responses to the fear-associated stimulus and non–fear-associated stimulus between the early and the late extinction phases. **(B)** In the placebo group, skin conductance responses to the fear-associated stimulus in both phases correlated significantly with state anxiety, whereas this association was uncoupled under oxytocin. Error bars indicate SEM. *$p < .05$. CS+, fear-associated stimulus; CS−, non–fear-associated stimulus; OXT, oxytocin; PLC, placebo; SCR, skin conductance response.

completed using WFU Pick atlas, automatic anatomic labeling (aal) or Talairach Daemon (TD) labels (43–45).

To address further the effects of OXT on the functional interplay of regions involved in extinction, a generalized form of context-dependent psychophysiologic interactions analysis was conducted (46). Specifically, we examined modulatory effects of OXT on the functional connectivity between the regions showing significant treatment effects in the BOLD analysis and the structurally defined left and right amygdala. Following data quality assessments, two subjects were excluded from the generalized form of context-dependent psychophysiologic interaction analysis of the left amygdala, and three subjects were excluded from the analysis of the right amygdala.

RESULTS

Physiologic Parameters

Results of the conditioning session before OXT treatment are reported in Supplement 1. For the extinction procedure, a 2 × 2 × 2 repeated measures ANOVA with the within-subject factors "stimulus type" (CS+, CS−) and "phase" (early phase, late phase), the between-subject variable "treatment" (OXT, PLC), and the SCR as dependent variable was performed. The analysis revealed significant main effects of stimulus type [$F_{1,34} = 2.99$, $p = .046$ one-tailed, $\eta^2 = .08$] and phase [$F_{1,34} = 7.08$, $p = .01$, $\eta^2 = .17$] and an interaction of phase × treatment [$F_{1,34} = 4.75$, $p = .04$, $\eta^2 = .12$] (Figure 1A). There were no further significant main or interaction effects (all $p > .30$). The CS+ provoked stronger electrodermal responses than the CS−, and responses were larger in the early than in the late phase of extinction for both types of stimuli. The interaction of phase and treatment indicates that the reduction of electrodermal responses over time was more pronounced in the OXT than in the PLC group. Post hoc unpaired *t* tests revealed that in the early phase of extinction the OXT group exhibited larger electrodermal responses (for both CS+ and CS−) than the PLC group [$t_{34} = -2.35$, $p = .03$, $d = -.81$], whereas this pattern was reversed in the late extinction phase [$t_{34} = 1.45$, $p = .08$ one-tailed, $d = .50$].

A correlational analysis showed a significant association between SCRs to the CS+ (both early and late extinction phase) and the pre-extinction state anxiety ratings for the PLC group ($r = .56$, $p < .05$) (Figure 1B), but not the OXT group ($r = .29$, $p = .9$). Fisher's *r*-to-*z* transformation confirmed a significant difference between the correlation coefficients ($z = 1.64$, $p = .05$, one-tailed), suggesting that OXT uncouples the association between SCR and state anxiety. We detected no correlations between the SCRs in the early and late extinction phase (all $p > .05$).

Functional MRI

The functional MRI results from the conditioning session carried out before OXT treatment document successful conditioning (Supplement 1). The previously CS (CS+) was associated with widespread activity in extinction-related networks, including the insula and prefrontal areas (Table 2), across both phases of the subsequent extinction session and both treatment groups. Auxiliary analysis yielded no differential effects of OXT on social and nonsocial stimuli during extinction (Supplement 1). Consequently, the social and nonsocial stimuli were pooled together

Table 2. Activation Table for GLM Analysis of Extinction (CS+ > Baseline)

	Region	Cluster Size[a]	Peak *Z*	MNI Coordinates x	y	z
L	Insula	8443	7.12[b]	−42	−1	10
L	Postcentral gyrus			−54	−22	28
L	Middle frontal gyrus			−42	31	43
R	Middle Occipital Gyrus	1016	6.76[b]	30	−79	22
R	Parahippocampal gyrus			27	−61	−8
R	Occipital superior gyrus			21	−67	46
L	Fusiform Gyrus	398	6.60[b]	−30	−58	−8
L	Subgyral			−36	−64	−8
L	Lingual gyrus			−21	−82	−8
L	Thalamus	246	6.38[b]	−6	−22	−2
R	Thalamus			6	−19	1
L	Thalamus			−18	−25	−2
L	Middle Frontal Gyrus	2247	6.15[b]	−27	47	10
L	Middle frontal gyrus			−27	41	22
L	Superior frontal gyrus			−24	38	34

CS+, fear-associated stimulus; GLM, general linear model; L, left; MNI, Montreal Neurological Institute; R, right.

[a]Height threshold = .0001.

[b]$p < .01$, family-wise error–corrected.

during the subsequent analyses to increase the power to examine the effects of OXT on extinction.

The ROI analysis of specific effects of OXT on extinction revealed no significant main effect of treatment across both phases. However, a significant treatment × phase interaction effect in the right middle frontal gyrus (peak MNI x, y, z = 24, 29, 40, $t_{118} = 3.80$, $p_{FWE} < .05$, $k = 17$) indicated that the OXT group exhibited increased prefrontal reactivity during early, but not late, extinction learning. A subsequent whole-brain analysis for the early extinction phase further revealed that the OXT group showed increased reactivity specifically to the CS+ in a large cluster located in the right mid-PFC extending into the right medial PFC (peak MNI x, y, z = 24, 26, 43, $t_{118} = 4.22$, $p_{FWE} < .05$, $k = 136$) (Table 3 and Figure 2A–D).

The ROI analysis of unspecific effects of OXT revealed no significant treatment × phase interaction effect; however, a significant main effect of treatment in the right amygdala ROI (peak MNI x, y, z = 24, 2, −17, $t_{60} = 4.13$, $p_{FWE} < .05$, $k = 4$) (Figure 3A, B) indicated that OXT reduced amygdala responsiveness regardless of the extinction phase. This reduction was histoprobabilistically mapped to the superficial amygdala (47).

Connectivity Analysis

For the medial PFC as a seed region, a repeated measures ANOVA with the contrast (CS+ > CS−) as the dependent variable and the between-subject factor "treatment" (OXT, PLC) as well as the within-subject variable "phase" (early, late) yielded no significant main or interaction effects. However, because the results of the BOLD analysis point to phase-specific effects, we performed the connectivity analysis separately for the early and late extinction phases. In the early extinction phase, OXT increased functional coupling of the right PFC to a cluster extending from the left posterior cingulate cortex (PCC) (peak MNI x, y, z = −3, −55, 3) to the right precuneus (peak MNI x, y, z = 12, −31, −8, $t_{60} = 4.14$, $p_{FWE} < .05$, $k = 136$) (Figure 2E). No OXT-facilitated interactions were present during the late phase of extinction.

With the right amygdala as a seed region, we detected a significant treatment × phase interaction for a cluster located at the left precuneus (peak MNI x, y, z = −6, −40, 67, $t_{118} = 3.7$, $p_{FWE} < .05$). The OXT-induced increase in functional coupling was evident only during the late, but not the early, phase of extinction. We found no significant main or interaction effects with the left amygdala as seed region.

Table 3. Activation Table for GLM Analysis of Treatment Effects

Region				MNI Coordinates		
Extinction		Cluster Size	Peak *Z*	x	y	z
$PLC^{[All\ Stimuli > Baseline]} > OXT^{[All\ Stimuli > Baseline]}$						
R	Amygdala[a]	4	3.86[b]	24	2	−17
Early Phase of Extinction						
$PLC^{[CS+ > CS-]} < OXT^{[CS+ > CS-]}$						
R	Middle frontal gyrus	136	4.06[b]	24	26	43
R	Middle frontal gyrus			18	38	37
R	Middle frontal gyrus			12	47	40

CS+, fear-associated stimulus; CS−, non–fear-associated stimulus; GLM, general linear model; MNI, Montreal Neurological Institute; OXT, oxytocin; PLC, placebo; R, right.

[a]Analysis based on predefined anatomic regions of interest with a height threshold = .001.

[b]$p < .05$, family-wise error–corrected.

DISCUSSION

The present study was designed to examine the modulatory effects of intranasal OXT on the neural and psychophysiologic substrates of Pavlovian extinction. Analysis of the SCR profiles revealed that in the early phase of extinction, the sensitivity to both danger (CS+) and safety (CS−) cues was increased by OXT, and this was followed by a greater decline in regard to the late phase. Treatment with OXT also induced higher BOLD responses to the danger cue (CS+) in right prefrontal areas during the early phase of extinction and diminished BOLD responses in the superficial subregion of the amygdala to both danger (CS+) and safety (CS−) cues, regardless of the phase. Also, OXT increased the functional connectivity between the PFC and left PCC and right precuneus in the early phase and facilitated functional coupling between the left amygdala and the right precuneus in the late phase.

The initial increase in electrodermal responses strongly resembles the increased fear-potentiated startle magnitude during the earliest stage of extinction learning observed in other studies and may be interpreted in terms of higher sensitivity to potentially averse cues (20,28,48). Mechanistically, stronger SCRs may reflect the discrepancy between the participants' expectancies and the absence of any shock and contribute to the later facilitation of extinction learning, but we did not find corresponding behavioral evidence for this interpretation (i.e., correlations between SCRs in the early and late phase). The psychophysiologic effect of OXT was not specific to the CS+ but also affected the safety cue (CS−). One possible explanation for heightened electrodermal responses to a CS− can be a generalization of fear or negative affect. This generalization could be important because clinical manifestations of fear are also often generalized.

In rodents, OXT effects on extinction memory are region-dependent (49), with microinjection of OXT into the dorsal raphe nucleus (50) or intracerebroventricular administration (51) impairing fear extinction and infusions into the central amygdala (52) or the dorsolateral septum (53) facilitating extinction. Our observation of increased medial PFC recruitment in healthy humans after OXT treatment might suggest that OXT acts to promote fear extinction by strengthening activity-dependent synaptic plasticity in the medial PFC (54). However, OXT boosted neural activity in a dorsal part of the medial PFC, which has previously been found in human fear extinction studies (27,55,56), but which is also clearly distinct from the ventromedial PFC, the human homologue of the rat infralimbic cortex, where most studies consistently detected extinction-related activations (57). The dorsolateral PFC has often been implicated in mediating cognitive emotion-regulation strategies (58), and lateral PFC regions are engaged by the regulation of conditioned fear in particular (59). An alternative interpretation for our pattern of results is that OXT enhances fear extinction in the late phase of extinction training by initiating the deployment of PFC-associated regulatory

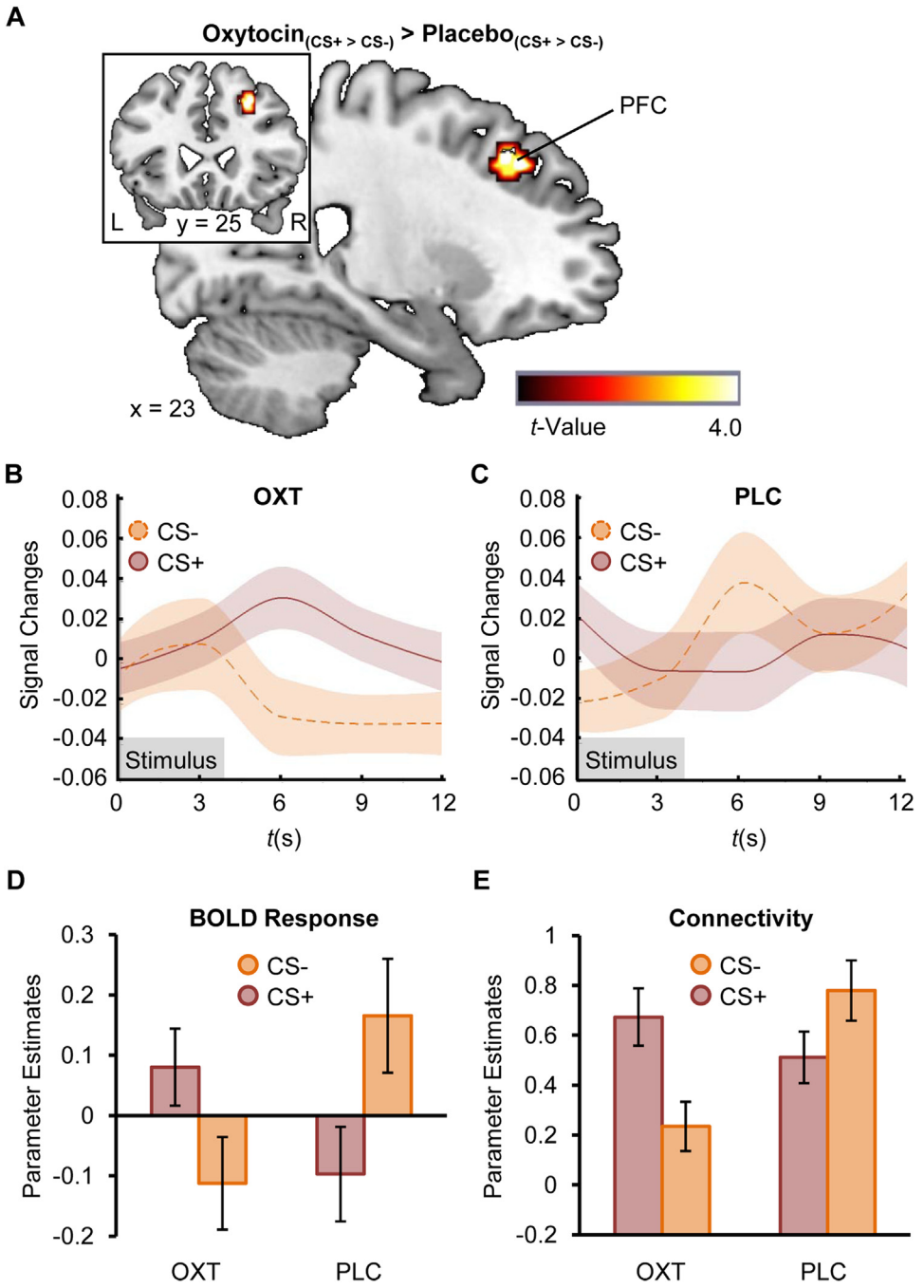

Figure 2. Oxytocin effects on prefrontal cortex activity during early fear extinction. **(A)** Oxytocin specifically increased right prefrontal cortex activity during the early extinction phase. **(B–D)** Percent signal changes and extracted parameter estimates revealed that oxytocin specifically increased prefrontal cortex responses to the fear-associated stimulus. **(E)** An additional functional connectivity analysis showed that oxytocin specifically enhanced functional coupling of the prefrontal cortex with the precuneus and posterior cingulate cortex for the fear-associated stimulus during the early extinction phase. Error bars and the shaded area represent SEM, and the gray area indicates the duration of the fear-associated stimulus/non–fear-associated stimulus presentation. BOLD, blood oxygen level–dependent; CS+, fear-associated stimulus; CS−, non–fear-associated stimulus; L, left; OXT, oxytocin; PFC, prefrontal cortex; PLC, placebo; R, right.

strategies to control fear responses. This line of reasoning also resonates well with the increased functional crosstalk between the PFC and precuneus and PCC after OXT treatment. Both the PFC and the PCC have been linked to the neural representation of SCR during fear extinction (26), and their enhanced interplay may indicate an improved self-referential processing and cognitive evaluation (60,61). In this context, the medial PFC has been identified as a key target region of the beneficial effects of OXT on social communication skills in individuals with autism spectrum disorders (62), whereas the precuneus is involved in the oxytocinergic modulation of psychosocial stress sensation (48).

Effects of OXT on the amygdala are supported by a broad range of human and animal studies (15,20–22,63). In the present study, OXT diminished the neural response to both danger (CS+) and safety (CS−) cues, which is plausible because the amygdala is known to code not only signals of imminent danger but also of safety (64,65). The superficial subregion of the amygdala is a key area for the processing of social stimuli, and we have previously shown that OXT suppresses the activity in the superficial

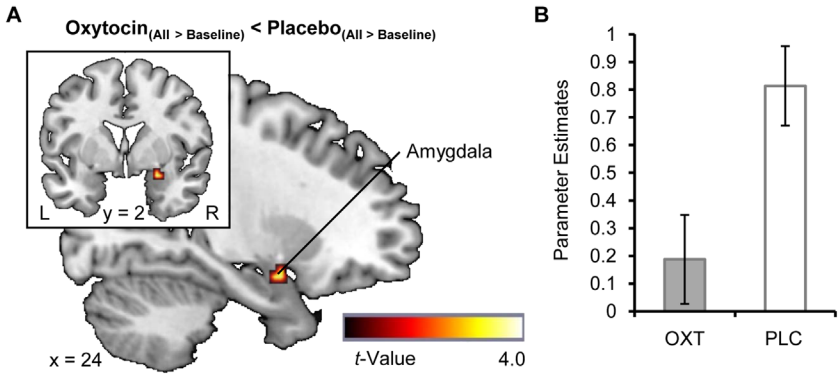

Figure 3. Oxytocin effects on amygdala activity. **(A, B)** Oxytocin generally diminished neural responses in the right amygdala. L, left; OXT, oxytocin; PLC, placebo; R, right.

amygdala during the presentation of neutral and aversive stimuli (20,66–68). Our data suggest that OXT influences the neural substrates of fear conditioning on dual pathways by initially upregulating PFC responses to fear-associated stimuli and by unspecifically downregulating amygdala responses. Given the complementary effect profile of OXT compared with the medial PFC hypoactivation and amygdala hyperactivation typically observed in anxiety disorders (69,12), the neuropeptide may qualify as a treatment option for the pharmacologic augmentation of exposure therapies in clinical trials.

The present study has several limitations. Although our results may provide preliminary evidence for a clinical use of OXT, many questions concerning patient samples need to be addressed in future research. Studies with patients with social anxiety disorder already indicate therapeutic potential for OXT; however, evidence for other types of phobia and generalized anxiety disorder is still lacking (63,70). In depressed patients, subjective fear was increased by OXT in a first session of psychotherapy, which could be related to enhanced self-referential processing (71). Other studies documented differential effects of OXT in individuals who experienced early life trauma (72) or exhibited low social-emotional abilities associated with autistic traits (73). The facilitating effect of OXT on fear extinction needs to be carefully examined in these patient populations.

In our study, fear acquisition and extinction were done on the same day. In clinically relevant applications, the learning phases would be clearly distinct from each other and occur over separate days and sessions. From our data, we cannot deduce an effect of OXT on extinction alone. Before clinical use, effects on processes such as overnight memory reconsolidation and extinction recall need to be evaluated (28). The paradigm of Pavlovian fear conditioning used in the present study addresses only one typical learning mechanism, and it is currently unknown whether the effects of OXT can be extrapolated to other types of emotional learning, such as evaluative conditioning (74). In addition, we included only healthy men in the present experiment because the study was primarily designed for proof-of-concept purposes to assess the general efficacy of OXT in human fear extinction. Given increasing evidence for gender dimorphisms in OXT effects (75,76), our current findings need to be replicated in female samples.

In conclusion, the results of our study indicate that administration of a single 24-IU dose of OXT increases frontal brain activity and connectivity related to fear extinction and dampens amygdalar activity related to fear and safety along with electrodermal responses. To prevent more efficiently the return of conditioned fear in patients with anxiety and posttraumatic stress disorder, one future strategy might be to use OXT to augment extinction-related medial PFC processing, while diminishing amygdalar responses to conditioned fear during cognitive-behavioral intervention.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by a Starting Independent Researcher Grant (Neuromodulation of Emotion research group) jointly provided by the Ministry of Innovation, Science, Research & Technology of the German State of North Rhine–Westphalia and the University of Bonn (RH) and the National Natural Science Foundation of China Grant No. 91132720 (KMK).

Author contributions are as follows: ME, BB, VG, and RH designed the experiments; ME, CS, and KP conducted the experiments; ME, CS, BB, DS, and RH analyzed the data; ME, BB, DS, TES, KP, VG, KMK, WM, and RH wrote the paper.

The authors report no biomedical financial interests or potential conflicts of interest.

Clinical Trials.gov: Oxytocin and Emotion Processing; http://www.clinicaltrials.gov/show/NCT02156661.

ARTICLE INFORMATION

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Received Jun 30, 2014; revised Oct 8, 2014; accepted Oct 16, 2014.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2014.10.015.

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