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Oxytocin Facilitates the Sensation of Social Stress

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Abstract: Essentially all social species experience social stress which can be a catalyst for detriments in mental and physical health. The neuropeptide oxytocin (OXT) has been shown to produce anxiolytic and antistress effects, thereby qualifying the OXT system as a promising drug target in the treatment of stress-related disorders. However, recently it has been shown that OXT can have anxiogenic effects as well. In the present study, we used functional magnetic resonance imaging to scan the brains of 60 healthy men while they were exposed to social stress after they received either intranasal OXT (24 IU) or placebo treatment. Although OXT administration did not alter salivary cortisol levels as a surrogate marker of stress axis activity, our participants initially reported an increment in perceived social stress. This behavioral effect was paralleled on the neural level by increased activity in the precuneus and cingulate cortex. Taken together, our results support the hypothesis that OXT can induce a self-referential processing bias which facilitates the sensation of social stress in the absence of altered endocrine responses. *Hum Brain Mapp 35:4741–4750, 2014.*

Key words: fMRI; oxytocin; social; stress

INTRODUCTION

A common denominator of animal and human behavior is a tendency to strive for a dynamic equilibrium (homeostasis) in order to promote survival and reproductive success (de Kloet et al., 2005). By threatening homeostasis, psychosocial stress not only interferes with subjective well-being but also potentiates the risk for various physical and mental disorders including coronary heart disease (Kivimaki et al., 2012), cancer (Cohen et al., 2007), or major depression (MD; Krishnan and Nestler, 2008). If the organism's response to psychosocial stress (allostatic load) is excessive and/or prolonged, homeostasis may collapse (McEwen, 2012). On the neural level, psychosocial stress has been associated with signal changes in a broad neurocircuitry involving the cingulate and insular cortices, precuneus, hypothalamus as well as frontotemporal areas (Dedovic et al., 2009, 2013; Soliman et al., 2011). The direction of these changes seems to depend on task as well as sample characteristics, with both activation (Lederbogen

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et al., 2011) and deactivation (Pruessner et al., 2008) of the limbic system being observed.

Research into resilience to psychosocial stress has identified social support as a key protective factor promoting successful adaption to psychosocial stress (Feder et al., 2009). In addition, there is mounting evidence that the hypothalamic neuropeptide oxytocin (OXT) can augment the efficacy of social support in suppressing the organism's stress response (Heinrichs et al., 2003). Consequently, the OXT system is regarded as a promising pharmacological target for therapeutic strategies aimed at attenuating maladaptive responses to psychosocial stress (Meyer-Lindenberg et al., 2011; Striepens et al., 2011; Yamasue et al., 2012). Indeed, further studies have found decreased cortisol levels after intranasal OXT administration (Cardoso et al., 2013; Ditzen et al., 2009; Meinlschmidt and Heim, 2007), but there are also reports of unaltered cortisol concentrations (Burri et al., 2008; de Oliveira et al., 2012a, 2012b; McRae-Clark et al., 2013; Simeon et al., 2011) and even enhanced cortisol reactivity in fathers within a parenting context (Weisman et al., 2013). As yet, it thus remains elusive whether OXT influences stress-induced neuroendocrine responses via direct modulation of the stress axis, the activity of which is reflected in bodily fluid concentrations of corticosteroid hormones. Alternatively, anti-stress effects of OXT may depend on context-dependent factors (Olff et al., 2013; Scheele et al., 2012). Current perspectives on the neuromodulatory effects of OXT emphasize its role in facilitating social cognition, especially in the domain of facial emotion recognition, thereby allowing individuals to improve their insight into the intentions, desires, and mental states of others (Eckstein and Hurlemann, 2013; Hurlemann et al., 2010; Shahrestani et al., 2013). Several studies also showed that OXT can enhance social approach and bonding behavior (Scheele et al., 2013; Striepens et al., 2014). This prosocial profile of OXT contrasts sharply with recent observations of anxiogenic effects of OXT treatment (Grillon et al., 2013; Guzman et al., 2013; Striepens et al., 2012). Transient anxiogenesis has also been reported as an adverse effect of OXT when it was administered as an adjunct to psychotherapy for MD (Macdonald et al., 2013). One possible explanation for these findings is that OXT may enhance self-referential processing, thereby promoting an increased perception and awareness of one's own negative feelings (Bryant et al., 2012; Liu et al., 2013). To test this hypothesis experimentally, the rationale of the present pharmaco-functional MRI study was to compare indices of psychosocial stress in 60 healthy male volunteers treated with either OXT or placebo (PLC). Outcome measures were verbal reports as well as endocrine and neural responses to psychosocial stress. We predicted that if OXT acts directly upon psychosocial stress, this should be evident in a global reduction of stress indices (Hypothesis 1). In contrast, an OXT-induced self-referential processing bias may be associated with increased sensation of psychosocial stress, evidenced by altered stress ratings and neural responses (Hypothesis 2).

MATERIALS AND METHODS

Participants

Sixty-five healthy male adults (mean age ± S.D.: 24.67 ± 3.89) participated in the study after giving written, informed consent. The study was approved by the institutional review board of the Medical Faculty of the University of Bonn and carried out in compliance with the latest revision of the Declaration of Helsinki. Subjects were free of current and past physical or psychiatric illness, as assessed by medical history and a Mini-International Neuropsychiatric Interview. In addition, they were naive to prescription-strength psychoactive medication and had not taken any over-the-counter psychoactive medication in the past 4 weeks. The subjects were kept naive with respect to the aim of the study. At the end of the experiment, they received a detailed debriefing and received monetary compensation for study participation. Functional magnetic resonance imaging (fMRI) data of one subjects could not be analyzed due to technical problems ($n = 1$). Further subjects were excluded from analysis due to failure to follow the instructions ($n = 1$), and blocked noses due to sinusitis ($n = 3$). Two saliva samples could not be analyzed because the sample did not contain enough saliva.

Experimental Design

In this study, we applied a randomized, placebo-controlled, double-blind, between-subject design. Subjects were randomly assigned to either intranasal administration of OXT (24 IU; Syntocinon-Spray, Novartis; three puffs per nostril, each with 4 IU OXT) or PLC (sodium chloride solution) 30 min before the start of the fMRI. In the vast majority of previous studies, experiments started 30 or 45 min after nasal delivery of OXT (e.g., de Oliveira et al., 2012a, 2012b; De Dreu et al., 2010; Scheele et al., 2013). The rationale for this timeline is supported by observations in human subjects that intranasal administration of a closely related nonapeptide, arginine vasopressin (AVP), produced a significant increase in lumbar cerebrospinal fluid (CSF) concentration of AVP after 10 min (Born et al., 2002). In principle, Striepens et al. (2013) replicated this finding for OXT; in their study, OXT peak plasma concentrations were reached 15 min after intranasal administration, whereas significant increases in lumbar CSF levels were measured no earlier than 75 min. The fact that the neural and/or behavioral effects of OXT usually occur at much shorter latencies suggests that transnasally delivered OXT rapidly penetrates into the brain, but needs to accumulate in (lumbar) CSF before a reliable signal over baseline can be detected. Possible reasons for the observed discrepancies in CSF peak concentration latencies between studies may be different CSF concentrations of AVP and OXT at baseline, different dosing protocols, and perhaps also different sensitivities of the administered assays, yielding a much better signal-to-noise ratio for AVP (Born

TABLE I. Demographics and neuropsychological performance at baseline

	Oxytocin group, mean (SD)	Placebo group, mean (SD)	*t*	*df*	*P*
Age (years)	24.28 (4.37)	24.90 (3.47)	0.63	59	0.53
Years of education	16.52 (4.56)	17.00 (2.82)	0.50	59	0.62
RTI[a]					
Simple reaction time (ms)	336.84 (55.90)	335.49 (60.66)	0.09	56	0.93
Simple movement time (ms)	285.04 (37.11)	274.17 (25.71)	1.31	56	0.20
Five-choice movement time (ms)	313.54 (57.88)	312.10 (41.25)	0.15	58	0.89
Five-choice reaction time (ms)	362.54 (87.51)	351.91 (63.78)	0.67	58	0.50
PAL[b]					
Total errors	11.62 (19.66)	7.72 (6.56)	1.06	59	0.29
Mean errors to success	2.40 (3.66)	2.03 (6.56)	0.50	56	0.62
SWM-8[c]					
Between errors	5.72 (5.76)	5.66 (5.69)	0.05	59	0.96
Strategy score	13.24 (3.28)	12.88 (2.39)	0.50	59	0.62
Trait anxiety[d]	34.52 (4.82)	34.63 (6.67)	0.07	53	0.94
BDI[e]	3.88 (3.62)	2.81 (3.34)	1.15	54	0.26
Current urbanicity score[f]	2.97 (0.19)	2.87 (0.43)	1.10	58	0.28
Early life urbanicity score[g]	31.75 (11.23)	28.66 (12.34)	1.02	59	0.31

[a]Speed of performance as measured with the RTI task implemented in the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Robbins et al., 1994).
[b]Episodic memory and new learning as assessed with the PAL task (CANTAB) (Robbins et al., 1994).
[c]Spatial working memory performance as measured with the SWM task (CANTAB) (Robbins et al., 1994).
[d]Trait anxiety as assessed with the STAI (State Trait Anxiety Inventory) (Spielberger et al., 1983).
[e]Depressive symptom load as assessed with the BDI-II (Beck Depression Inventory-II) (Beck et al., 1996).
[f]Urbanicity score ranging from 1 to 3, with 1 indicating rural residence and 3 indicating city dweller (Lederbogen et al., 2011).
[g]Urbanicity score ranging from 15 to 45, with 15 indicating life in rural areas and 45 indicating life in a city (until the age of 15 years) (Lederbogen et al., 2011).

et al., 2002) than for OXT (Striepens et al., 2013). The screening of the subjects was conducted prior to the test sessions. All subjects completed a comprehensive neuropsychological test battery to control for possible pretreatment differences in cognitive performance (cf. Table I). Subjects' ability to perform mental arithmetic was assessed by recording the average time needed to solve problems on a computer-based training version of the fMRI task (outside the imaging unit). The first baseline saliva sample was also collected at this time. Additionally, we measured mood and state anxiety before and after the experimental tasks.

All sessions were commenced between 4 pm and 8 pm to avoid confounds due to the circadian rhythm of cortisol. All subjects had to rest for 30 min in a quiet room before a second baseline saliva sample was collected. Then, the participants intranasally administered OXT or PLC under supervision according to current guidelines (Guastella et al., 2013). Thirty minutes after the nasal spray application, a third saliva sample was collected and the functional MRI was conducted. Following the MRI, the last saliva sample was obtained and a working memory (HAWIE subtest digit span, Wechsler, 1997) task was completed. The participants completed follow-up questionnaires and were debriefed.

FMRI Paradigm

The Montreal Imaging Stress Task (Dedovic et al., 2005) is an adaptation of the Trier Social Stress Test and induces social stress by having the subjects perform mental arithmetics and giving them feedback that their performance is very bad compared to other participants. Before the start of the session, the participants were informed about the need for both speed and correctness. The experimenters further established an evaluative threat by informing the participants that all experimenters are following the participants' performance on a monitor in the control room. Between the task blocks, the experimenters communicated with the participants via headphones, criticized their "insufficient" results, and reminded the participants that their individual performance must be close to the average performance of all subjects if their data are to be used in the study. Using Presentation 14 (Neurobehavioral Systems, Albany, CA), stimuli were presented block wise, via liquid crystal display video goggles (Nordic NeuroLab, Bergen, Norway). In total, we conducted three 6-min blocks with time-limit and negative social feedback ("stress" condition) and three 6-min blocks without any time-pressure and social evaluation ("no stress" condition).

These blocks were presented alternating, always starting with a no stress block. In the nonstress condition, participants performed random arithmetic tasks for 60 s (four repetitions), introduced by a 5-s attention announcement and followed by an interstimulus interval of 20 s where a fixation cross was depicted in the center of the display. Subjects had to select a number on a rotary dial as an answer for the arithmetic task by pressing a button. This response is then compared with the correct answer for the task, and the appropriate feedback ("correct" or "incorrect") is presented. In the stress condition, a time limit is enforced by a progress bar moving from the left to right. If no response is recorded within the time limit, the response "timeout" is displayed. Additionally, performance indicators (for the subject's own performance and the average performance of all subjects) are shown. At the beginning of the stress condition, the time limit is set to the participant's average response time recorded in the training session. Subsequently, the time limit is adapting such that the subject fails on 60% of the trials. After 340 s of calculation, the participant is allowed to finish the running task before the block ends. At the end of each block subjects gave a rating of their perceived stress on a scale from 1 ("very low") to 8 ("very high"). Including the ratings and the short verbal feedback between blocks, the task lasted approximately 40 min.

Neuroendocrine Parameters

Saliva samples were collected with commercial sampling devices (Salivetten, Sarstedt) and Salivettes were immediately centrifuged at 4,180*g* for 2 min and aliquoted samples were stored at −80°C until assayed. Cortisol concentrations were determined using an electrochemiluminence immunoassay (Elecsys Cortisol Test, Roche, Mannheim). The sensitivity of the assay was set at 0.018–63.4 μg/dl. The mean inter- and intra-assay coefficients of variation for the assays were 3.42% and 12.2%, respectively. Assays were conducted in the laboratory of author BS.

Acquisition of fMRI Data

The MRI data were acquired with a Siemens Avanto MRI system (Siemens, Erlangen, Germany) operating at 1.5 T. T2*-weighted echoplanar (EPI) images with blood-oxygen-level-dependent contrast were obtained [retention time (TR) = 3,000 ms, echo time (TE) = 35 ms, matrix size: 64 × 64, pixel size: 3 mm × 3 mm × 3 mm, slice thickness = 3.0 mm, distance factor = 10%, field of view (FoV) = 192, flip angle = 90°, 36 axial slices]. In addition, high-resolution anatomical images were acquired on the same scanner using a T1-weighted 3D MPRAGE sequence (imaging parameters: TR = 1,570 ms, TE = 3.42 ms, matrix size: 256 × 256, pixel size: 1 mm × 1 mm × 1 mm, slice thickness = 1.0 mm, FoV = 256, flip angle = 15°, 160 sagital slices).

Analysis of fMRI Data

The MRI data were preprocessed and analyzed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab 7 (The MathWorks, Natick, MA). The first five volumes of each functional time series were discarded to allow for T1 equilibration. Images were corrected for head movement between scans by an affine registration. For realignment, images were initially realigned to the first image of the time-series and subsequently rerealigned to the mean of all images. For spatial normalization, the mean EPI image of each subject was normalized to the current Montreal Neurological Institute (MNI) template (Evans et al., 1992; Holmes et al., 1998) using the unified segmentation function in SPM8. This algorithm combines image registration, tissue classification, and bias correction within the same generative model. All images were thereby transformed into standard stereotaxic space and resampled at 3 mm × 3 mm × 3 mm voxel size. The normalized images were spatially smoothed using an 8-mm full width at half maximum Gaussian kernel. Raw time series were detrended by the application of a high-pass filter (cutoff period, 128 s). A two-level random effects approach based on the general linear model as implemented in SPM8 was used for statistical analyses. On the first level, the two conditions (stress, no stress) of the block design were defined and modeled by a boxcar function convolved with a hemodynamic response function (Friston et al., 1994). The movement parameters were included as confounds in the design matrix. Each experimental condition was compared relative to the low-level baseline and differences between each condition were computed separately for the OXT and PLC group. To examine effects of OXT, parameter estimates of the "stress," "no stress," and "stress > no stress" contrasts were submitted to two-sample *t*-tests on the second level. For the whole-brain a significance threshold of $P < 0.05$, corrected for multiple comparisons [family-wise error (FWE)], was used. In order to further examine the specificity of the OXT effect, the parameter estimates were extracted from the activated clusters in the cingulum and precuneus. Data for the activations during the blocks versus baseline were extracted using the MarsBaR toolbox (Brett et al., 2002; see also http://marsbar.sourceforge.net/).

Based on a previous study investigating the neural correlates of stress (Lederbogen et al., 2011), we used 6-mm spheres as regions of interest (ROI) centered at the coordinates (peak MNI mm *x, y, z*: 10, 4, −4 and 21, −9, −15) of the reported maximum value for the anterior cingulate cortex (ACC) and the amygdala. ROI-based two-sample *t*-tests were computed with a threshold of $P < 0.05$ and FWE-corrected for multiple comparisons based on the size of the ROI. Anatomical classification was done using WFU pick atlas, automatic anatomic labeling or Talairach Daemon (TD) labels (Lancaster et al., 2000; Maldjian et al., 2003).

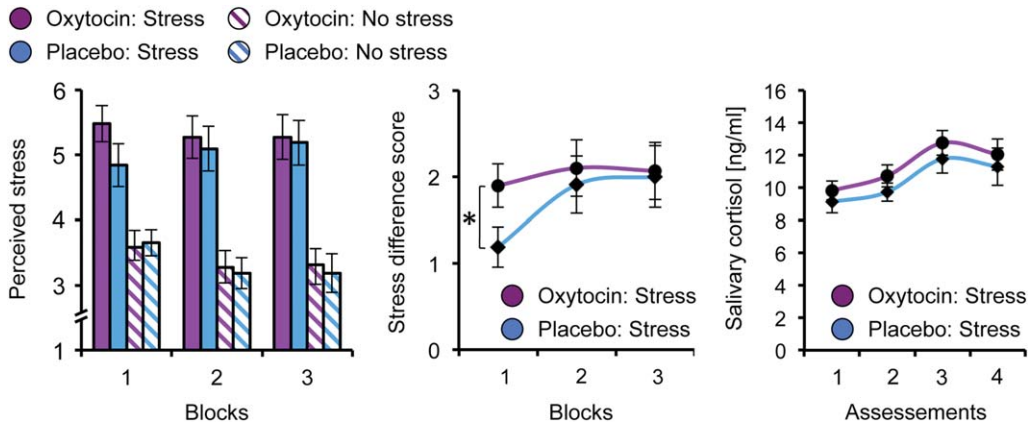

Figure 1.

Displayed are verbal ratings of and salivary cortisol responses to social stress. In the first block of the stress task ratings of the stress condition, compared to the nonstress condition, were significantly higher under oxytocin (OXT) than under placebo. Elevated cortisol levels demonstrated that the task was successful in inducing an endocrine stress response; however, OXT had no modulatory effect on cortisol levels.

RESULTS

Experimental groups did not differ in demographic variables or neuropsychological performance (cf. Table I). The estimation of the received treatment was comparable between the OXT and PLC group ($\chi^2_{(1)} = 0.65$, $P > 0.05$), showing that the subjects were unaware of whether they had received OXT or PLC.

Behavioral Results

A repeated-measures analysis of variance (ANOVA) with "condition" (stress vs. no stress) and "time" (three blocks) as within-subject factors, "treatment" (OXT vs. PLC) as between subject factor and the stress ratings as dependent variable yielded a significant main effect of condition ($F_{(1,59)} = 94.33$; $P < 0.01$) with significantly more reported stress in the "stress" condition (5.19 ± 1.71) compared to "no stress" (3.72 ± 1.37), but no further interactions. However, an exploratory analysis revealed that the OXT group (difference between "stress" and "no stress": 1.97 ± 1.37) already perceived more stress in the first block compared to PLC (1.19 ± 1.33; $t_{(59)} = 2.05$, $P = 0.04$), while the stress ratings in the PLC group increased over time (cf. Fig. 1).

Furthermore, we also examined OXT effects on anxiety measures and the working memory performance. While OXT did not significantly influence the global anxiety state or working memory as assessed with the STAI and HAWIE digit span (all *P*s > 0.05), in the OXT group, the stress task led to a significantly more pronounced increase in concern (pre minus post-STAI item "concerned": 0.30 ± 0.79) than in the PLC group (−0.04 ± 0.33; $t_{(56)} = 2.13$; $P = 0.04$).

Endocrines Parameters

A repeated-measures ANOVA with "time" (screening, baseline, pre- and post-stress) as within-subject factors, "treatment" (OXT vs. PLC) as between-subject factor, and the salivary cortisol levels as dependent variable showed a main effect of time ($F_{(3,59)} = 13.68$, $P < 0.01$; cf. Fig. 1), indicating that our task was successful in inducing an endocrine stress response. However, there was no main or interaction effect of treatment (all *P*s > 0.05). We also tested possible moderator variables (trait anxiety, athleticism and early parental separation) by incorporating median dichotomized variables into the ANOVA, but no significant main or interaction effects were detected (all *P*s > 0.05). As childhood traumata are known to alter central OXT concentrations (Heim et al., 2009), we identified subjects ($n = 5$) with traumatic childhood experience and repeated all analyses in a reduced sample, but we obtained no different results.

fMRI Results

Stress related neural activations [main effect of task: (stress > no stress)] were evident in a broad neurocircuitry involving the precuneus, superior temporal gyrus, cingulate gyrus and middle frontal gyrus (cf. Table II). OXT had no unspecific global effects, that is there was no main effect of treatment [(OXT > PLC) and (PLC < OXT)]. To examine the specific effects of OXT on neural substrates of stress, we contrasted the stress associated activations in the OXT and the PLC group [OXT $^{\text{stress > no stress}}$ > PLC $^{\text{stress > no stress}}$]. The whole-brain analysis of this contrast revealed that the stress-related activity in a large cluster ranging from the posterior parts of the cingulate cortex

TABLE II. Activation table for the GLM analysis

Hemisphere	Region[a]	Cluster size	Peak Z	MNI coordinates		
				x	*y*	*z*
Contrast: Oxytocin[stress > no stress] > Placebo[stress > no stress]						
R	Cingulate gyrus	116[b]	3.98[c]	3	−43	40
L	Cingulate gyrus		3.73[c]	−6	−40	37
L	Precuneus		3.71[c]	−18	−46	31
Contrast stress > baseline for all subjects						
R	Cingulate gyrus	14,414[d]	*Inf*[e]	27	−67	37
R	Precuneus			12	−67	52
L	Middle occipital gyrus			−27	−88	16
R	Superior temporal gyrus	92[d]	6.44[e]	60	−37	19
R	Middle frontal gyrus	20[d]	6.63[e]	27	56	−8
R	Middle frontal gyrus	4[d]	5.03[e]	45	53	−2
L	Cingulate gyrus	1[d]	4.87[c]	−15	−25	40
L	Middle frontal gyrus	1[d]	4.76[c]	−21	53	−8

Abbreviations: L, left; R, right.
[a]Talairach Demon (TD) labels.
[b]Cluster threshold set at $P < 0.001$.
[c]$P < 0.05$, *family wise error (FWE)* corrected.
[d]Cluster threshold $P < 0.0001$.
[e]$P < 0.01$, *FWE* corrected.

to the precuneus was significantly higher under the influence of OXT than PLC (peak MNI mm *x, y, z*: 3, −43, −6, $Z_{(58)} = 3.98$; −6, −40, 37, $Z_{(58)} = 3.73$; and −18, −46, 31, $Z_{(58)} = 3.71$; $k = 116$, family-wise error corrected: $P_{FWE} < 0.01$; cf. Fig. 2a and Table II). An additional analysis based on the ACC as predefined ROI demonstrated that OXT also increased stress related activity in this region (peak MNI mm *x, y, z*: 9, 20, 22, $Z_{(58)} = 3.10$, $P = 0.02$, *cf.* Fig. 2a). By contrast, no significant effects were observed in the ROI-based analysis of the amygdala. To examine possible time-dependent neural effects of OXT, we submitted the contrasts (stress > no stress) to a repeated measures ANOVA with treatment (OXT vs. PLC) as between-subject factor and block (first, second, and third) as within-subject factor. This analysis yielded no significant interaction between treatment and block (all P_{FWE}s > 0.05).

Examination of the extracted parameter estimates confirmed that all three significant regions exhibited the strongest neural response to social stress under OXT (cf. Fig. 2b). Paired *t*-tests showed highly significant differences between the estimates of the "stress" and "no stress" conditions for the peaks of the precuneus ($t_{(28)} = -2.56$, $P < 0.01$), the PCC ($t_{(28)} = -5.00$, $P < 0.01$), and the ACC ($t_{(28)} = -3.12$, $P < 0.01$) in the OXT group. Under PLC, only the estimates for the precuneus were significantly different ($t_{(30)} = 3.05$, $P < 0.01$).

Furthermore, we conducted a correlational analysis and detected significant associations between the stress ratings of the first "stress" block in the OXT group and the corresponding parameter estimates of the ACC (peak MNI mm *x, y, z*: 12, 17, 25, $r = 0.39$, $P < 0.05$) and precuneus (peak MNI mm *x, y, z*: 3, −43, 40, $r = 0.48$, $P < 0.01$). These results show that the neural stress responses under OXT were more pronounced in subjects who reported a stronger sensation of stress. There were no significant correlations in any other condition (all *P*s > 0.05).

Taken together, participants under OXT showed augmented activity in the precuneus as well as the anterior and posterior parts of the cingulate and the neural changes are correlated with stress ratings.

DISCUSSION

In the present study, we examined the modulatory effects of intranasally administered OXT on the behavioral ratings and physiological substrates of social stress. Our results show that treatment with OXT induced an increase in the initial sensation of stress, which was paralleled by elevated stress-related responses in the cingulate and precuneus. Moreover, the level of perceived stress was positively correlated with anterior cingulate and precuneus activity. These findings support our hypothesis 2 that OXT can induce a self-referential processing bias associated with increased perception and awareness of psychosocial stress. Interestingly, the putative enhancement of self-referential processing was not followed by increased cortisol levels, suggesting that an enhanced sensation of psychosocial stress is not automatically linked to an increase in stress axis activation. Our observation of unchanged cortisol levels is compatible with the view that OXT effects on psychosocial stress are context-dependent (Heinrichs et al., 2003; Olff et al., 2013).

While there is evidence from animal studies that stressful events can trigger an endogenous OXT release and that OXT can have salubrious effects by altering stress-related

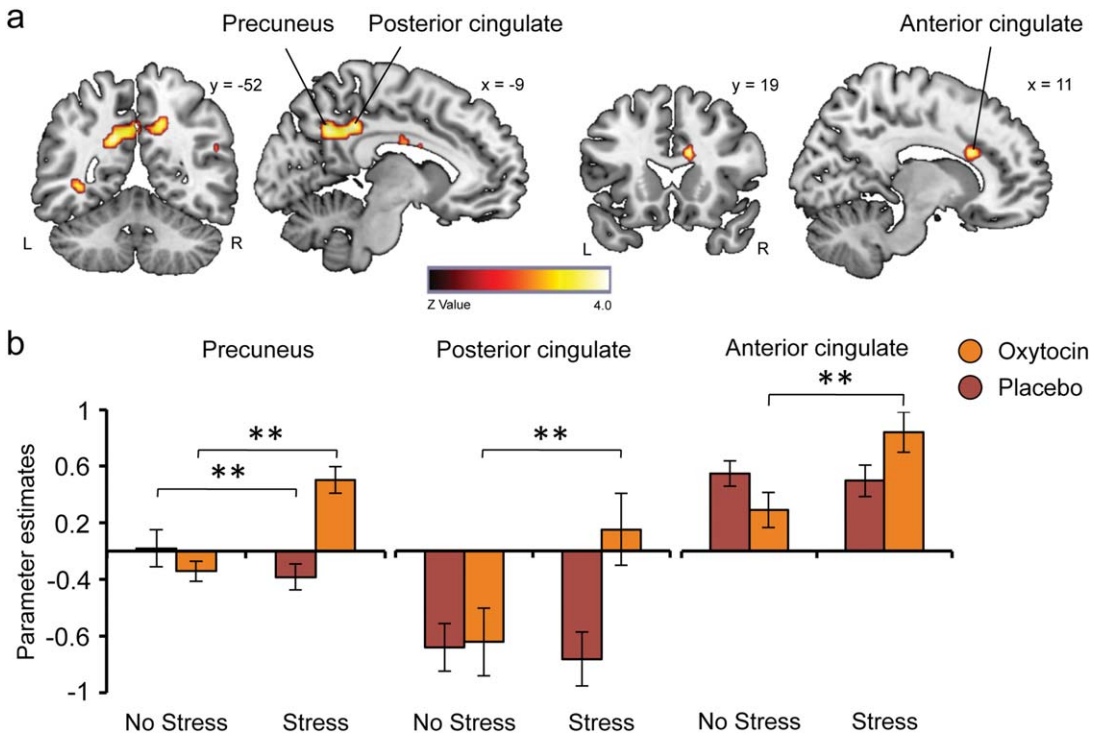

Figure 2.

Illustrated are OXT effects on the neural correlates of psychosocial stress. (**a**) OXT specifically enhanced stress-related activation in the posterior cingulate cortex, precuneus, and also in a region of interest, the anterior cingulate cortex. (**b**) Parameter estimates of the peak activations for the stress and nonstress condition confirm the observed response changes under OXT. Error bars indicate the standard error of the mean (SEM). Abbreviations: L, left; R, right; $^{**}P < 0.01$.

stress axis activity (Shahrestani et al., 2013), findings in humans are much more ambiguous. It has been reported, for instance, that acute stress stimulates OXT secretion at least in specific subsamples (Pierrehumbert et al., 2010; Sanders et al., 1990; Seltzer et al., 2013), whereas two studies failed to find any OXT response to a psychosocial stressor (Grewen et al., 2005; Light et al., 2005). Recently, Cardoso et al. (2013) demonstrated that OXT attenuates the cortisol response elicited by physical stress. Our finding that a single dose of intranasally administered OXT does not influence the cortisol response to psychosocial stress is in line with previous studies using similar behavioral paradigms (de Oliveira et al., 2012a, 2012b; Simeon et al., 2011). In situations where the experimental manipulation does not increase salivary cortisol levels, OXT may attenuate stress axis activity (Ditzen et al., 2009; Linnen et al., 2012) but this modulatory influence may itself depend on developmental experiences (Meinlschmidt and Heim, 2007), and there are also negative studies in which OXT had either no modulatory effect (Burri et al., 2008; Weisman et al., 2013) or increased salivary alpha-amylase, a proxy of stress-related sympathetic activity (Ditzen et al., 2013). The absence of an OXT effect on the cortisol level suggests that the heightened subjective awareness of the stressor abolished anti-stress effects of OXT that can be observed if social support is available (Heinrichs et al., 2003).

Our fMRI findings document increased activity in a neural circuitry encompassing the precuneus as well as the posterior and anterior portions of the cingulate cortex under OXT. ACC and precuneus have been previously implicated in self-processing (Cabanis et al., 2013) and interoceptive awareness (Lou et al., 2004), suggesting that the peptide may facilitate a more self-focused processing and improve conscious awareness of the negative social feedback administered in our stress task. Increased cingulate responses may reflect an enhanced emotional sensation of psychosocial stress, since this region has often been found to be sensitive to signals of social stress (Lederbogen et al., 2011). Initially higher stress ratings are also consistent with the notion that OXT augments the salience of social information (Prehn et al., 2013), thus enabling a stronger task engagement and emotional involvement of the subjects. By contrast, anterior cingulate and precuneus also constitute key regions of the default-mode network and thus the OXT-induced neural responses could be related to task-independent mind-wandering (Christoff et al., 2009). However, given the observed association with the behavioral stress ratings, we consider it more likely

that the neural activations reflect an altered stress experience.

Furthermore, the precuneus belongs to a wide-spread neurocircuitry of higher association cortical and subcortical areas and may subserve a variety of behavioral functions ranging from simple goal-directed hand movements (Grefkes et al., 2004) to consciousness (Lou et al., 2004). We thus cannot infer a particular cognitive process, although our behavioral results substantiate the idea of an enhanced stress perception. Cavanna and Trimble (2006) have put forward a functional subdivision of the precuneus with the anterior parts being involved in self-centered mental imagery strategies and the posterior parts mediating successful episodic memory retrieval. Consistent with our interpretation, the cluster peak of the OXT effect in our study is in the anterior subdivision. We did not observe an OXT effect on other cortical midline-structures which have been found to be involved in self-referential processing (Northoff et al., 2006), but quite recently it has been suggested that the engagement of specific structures may depend on various features such as task difficulty (Yaoi et al., 2013).

Task features could also explain why we did not observe responses in regions for which stress-related activations have been previously reported, such as the right temporoparietal junction, the posterior cingulate cortex, the insula and the hypothalamus. For example, Lederbogen et al. (2011) incorporated a mental rotation task in their version of the MIST in addition to the mental arithmetics and the neural signature of stress can vary across different stressors (Dedovic et al., 2009). Lederbogen et al. (2011) also enforced a higher failure rate (60–75%) compared to our study (60%). Considering the individual performance in the task is modulated by time pressure, it is very likely that this proxy for the induced cognitive load contributes to the neural activation pattern. Although social stress can interact with cognitive processing (Dickerson and Kemeny, 2004), there was no OXT effect evident in the no-stress control condition suggesting that OXT specifically augmented the stress component. Initially, the absence of an OXT effect on amygdala reactivity may be surprising. In fact, anxiolytic actions of OXT have been often derived from diminished amygdala responses (Kirsch et al., 2005), but amygdala activations in the MIST depend on current urbanicity. The majority of our participants lived in cities and in our sample the MIST did not elicit amygdala responses, thereby hindering any pharmacological down-regulation.

Our results are consistent with the notion that OXT effects are context-dependent and we speculate that OXT may require concomitant social support to precipitate in a reduction of cortisol levels. Our idea resonates well with recent findings in prairie voles (Smith and Wang, 2013), but since we did not experimentally manipulate social support, future studies are warranted to directly test this hypothesis. Our results cannot be explained in terms of altered cognitive abilities, since OXT had no effect on a subsequently administered attention and working memory test. Furthermore, we controlled for possible confounds due to between-group differences in developmental experiences or attachment style, however, our results did not change when these variables were additionally incorporated in the analysis. We also controlled for possible influences of city living and urban upbringing, which have been shown to affect neural substrates of psychosocial stress in the same task as used in the present study (Lederbogen et al., 2011). Given the preponderance of subjects living in a nonrural environment, we observed no variation in OXT effects as a function of habitation. Of note, we only tested male volunteers in the present study and since OXT appears to have sex-dimorphic effects (Lischke et al., 2012), an extrapolation of our findings to women is hampered. Furthermore, another limitation pertains to the PLC nasal spray which only contained sodium chloride solution but none of the pharmacologically inactive compounds found in the OXT spray. Hence, we cannot exclude the possibility that the vehicle itself might have contributed to the present results.

In conclusion, the results of our study indicate that a 24-IU single-dose administration of OXT may facilitate the sensation of psychosocial stress, while leaving stress axis activity and endocrine measures unaltered. Our interpretation of an OXT-induced self-referential processing bias of negative context could explain why previous clinical trials using the same dosing schema have failed to find a beneficial effect of OXT administered as an adjunct to initial psychotherapy of stress-related disorders.

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