SUPPLEMENTAL INFORMATION

Trauma disclosure moderates the effects of oxytocin on intrusions and neural responses to fear

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SUPPLEMENTAL METHODS

Pilot study

To validate the experimental trauma paradigm and to explore effects of the trauma movie on the neural processing of fearful and happy faces, 24 healthy women were screened for a pilot study. Due to technical malfunctions, fMRI data were available for 22 participants (mean age ± SD: 23.38 ± 3.28 years). Participants watched either the trauma movie (n = 12) or an equally long control movie (n = 10) consisting of neutral scenes from the same movie. Skin conductance level (n = 24), pupil size (n = 17), and respiration rate (n = 17) were assessed during a 2-min baseline and during the movie. After the movie, all participants were scanned with the same fMRI parameters and tested with the same emotional face matching task as in the main study. Eight participants in the trauma movie group filled out intrusion diaries in the five days following the trauma exposure.

Participants

The study was approved by the local ethics committee of the Medical Faculty of the University of Bonn, Germany. The study was registered in the Clinical Trials.gov database (Identifier: NCT03425929) provided by the US National Institutes of Health. All participants gave written informed consent and the study was conducted in accordance with the latest revision of the Helsinki Declaration. Participants were recruited from the local population by means of online advertisement and public postings. After completion of the study, participants received monetary compensation. The random allocation sequence (for the double-blind, between-subject oxytocin/placebo treatment) was generated by D.S.. J.L. and A.P. enrolled all participants and assigned participants to the treatment based on the random allocation plan. All behavioral and fMRI data were collected in Bonn, Germany.

Given that women are twice as likely to develop PTSD compared to men [1], the present proof-of-concept study was focused on female participants. We screened a total of 94 (70 for the main study) healthy, right-handed women. Subjects were free of current and past physical or psychiatric illness, as assessed by medical history and the Mini-International Neuropsychiatric Interview [34] prior to enrollment. The screening questionnaires were administered by trained research assistants and the

clinical interviews were conducted by MD students who were supervised by an experienced psychiatrist. None of the participants used hormonal contraceptives or were pregnant during the study. Screenings of the participants were conducted prior to the test sessions. Eight participants were excluded (five because they did not fulfill the inclusion criteria and three because they already knew the trauma movie), resulting in 62 participants (mean ± SD age, 23.31 ± 4.20 years) for the behavioral analyses. Trauma disclosure was median-dichotomized, resulting in n = 31 participants with strong trauma disclosure (at least two discussions about the first trauma movie) and n = 31 participants with weak trauma disclosure. Moreover, four participants were excluded due to technical malfunctions (n = 1) or excessive head motion (> 4 mm/º; n = 3) during scanning, leaving 58 participants (mean ± SD age, 23.43 ± 4.20 years) for the fMRI data analyses.

Screening session

Study enrollment was preceded by a screening session to ensure subjects were free of any current or past physical or psychiatric illness as assessed by medical history and the Mini-International Neuropsychiatric Interview (MINI) [2]. Furthermore, participants were lifetime naive to prescribed psychoactive medication. Contraindications for MRI scanning were additional exclusion criteria. To further characterize the sample, we acquired sociodemographic data of each participant. Autistic-like traits were measured with the Autism Spectrum Quotient questionnaire (AQ)[3], childhood trauma was measured with the Childhood Trauma Questionnaire (CTQ) [4, 5], depressive symptoms were assessed by Beck's Depression Scale (BDI, Version II) [6], empathy was assessed by the Saarbrücker Persönlichkeitsfragebogen, a German version of the Interpersonality Reactivity Index (IRI) [7, 8], rumination was assessed by the Rumination-Reflection Questionnaire (RRQ) [9, 10], social anxiety was assessed by the Liebowitz Social Anxiety Scale (LSAS) [11], and the Social Network Index (SNI) questionnaire was used to examine participants' social networks [12] (cf. **Supplemental Tables S1 and S2**).

Experimental design and procedures

We performed a randomized, double-blind, placebo-controlled, parallel-group design study. Participants self-administered a 24-IU daily dose of synthetic oxytocin (OXT; Novartis, Basel, Switzerland) or placebo (PLC) via nasal spray over six consecutive days. The PLC solution contained

identical ingredients except for the peptide itself. On days 1, 2, 4, and 5, participants came to the clinic between 6 and 7 pm and self-administered the nasal sprays. All nasal sprays were administered under the supervision of an experimenter and in accordance with the latest standardization guidelines [13]. On each study day, 12 puffs balanced across nostrils were administered at an inter-puff interval of 45 seconds to allow the solution to be absorbed into the nasal epithelium. The amount of administered substance was weighed and supplemented by an additional puff if it fell below a set minimum (24 IU = 600 mg). On day 0 (before the treatment) and again on day 3 (i.e. after three days of treatment) participants watched the trauma movie (cf. **Supplemental Figure S1** for an overview of the study design). This study design was informed by the results of a preceding observational pilot study showing that most intrusions had vanished within three days post-trauma. Due to this kinetic profile, 32 participants received OXT on the three days post-trauma (i.e. day 0 – 2), whereas 30 participants received PLC during this period. To explore lasting protective effects of the first treatment period, in the three days following the second trauma movie exposure (days 3 to 5), 21 participants continued to receive OXT (i.e. OXT for six days) and 11 participants switched to PLC (i.e. OXT for three days and PLC for three days). FMRI scanning started 20 min after trauma exposure and the nasal sprays were administered after the imaging (i.e. no acute effects could have influenced the imaging results).

It is increasingly recognized that the prosocial and anxiolytic effects of intranasal OXT are moderated by person variables and context factors [14-16]. For instance, a single dose of intranasal OXT reduces the intensity of provoked PTSD symptoms [17, 18] and facilitates fear extinction [19], but depending on the administration time, intranasal OXT also augments fear conditioning [20]. As such, OXT administration before trauma exposure may have detrimental effects by enhancing trauma-related memories. In the present study, we therefore decided to explore the usage of OXT as a secondary prevention after trauma exposure. The repeated OXT administrations did not result in elevated OXT levels (12 h after the last administration), indicating that no acute effects of the third OXT administration (day 2) could have biased the processing of the second trauma exposure (day 3).

The participants completed intrusion diaries at home in the evening on days 0 to 5. In the intrusion diary, the participants stated the number of intrusions (defined as involuntary recollections relating to film events that appear, apparently spontaneously, in consciousness), described the content of the intrusions, and rated the distress caused by these intrusions on a visual analogue scale ranging from 0 (no distress) to 100 (extreme distress). Furthermore, participants were asked to rate how stressful

their day was, their need to discuss the movie, whether and how long they talked to other people, and whether and how long they spontaneously discussed the trauma movie with other people. All online questionnaires were presented with the Qualtrics software (Provo, UT).

During the study, participants were asked to maintain their regular sleep and waking times and to abstain from alcohol intake. This was verified via an informational questionnaire administered at the beginning of each testing session. Additionally, all female participants were required to undergo a urine pregnancy test prior to nasal spray administration.

To measure mood responses to the trauma movie, the Positive and Negative Affective Scale (PANAS) [21] was administered before and after the trauma movie. Furthermore, dissociative symptoms after the trauma movie were assessed with the Dissociation-Tension-Scale acute (DSS acute) [22].

To monitor peripheral OXT levels, saliva samples were collected from each subject before the trauma movie (baseline) and immediately before the MRI scan session (post trauma). To measure cortisol levels, three saliva samples were collected from each subject before the trauma movie (baseline), immediately after the trauma movie (post trauma), and after the MRI scan session (post imaging). Given previous evidence that intrusive memories vary as a function of gonadal hormone concentrations over the menstrual cycle [23, 24], female participants were tested in the luteal phase. This was assessed by self-report and validated by blood assays (FSH, LSH, estradiol, and progesterone concentrations) obtained before the trauma movie on days 0 and 3 (cf. **Supplemental Tables S3 and S4**).

MRI scanning began with functional scans followed by an anatomical scan. Functional scans consisted of a 6-min resting state, the emotional face matching task, and an unrelated reward processing task (resting state and reward processing data are reported elsewhere). The total time of an experimental session on days 0 and 3 was 2.5 h, with each participant approximately 60 min in the scanner. The nasal sprays were administered after the imaging on days 0 and 3. Furthermore, there was a latency of at least 12 h between the nasal spray administration on day 2 and the start of the imaging on day 3. Thus, no acute OXT effects could have influenced the imaging results. Participants were naive to the purpose of the study. At the end of the experimental session, all participants were fully debriefed.

Psychophysiological measurements and experimental trauma

Electrodermal activity (EDA), respiration rate, and pupil sizes were measured during the experimental traumas. Participants were seated in front of a Tobii TX300 binocular eye-tracker with a 23-inch display. The trauma film had a duration of 15 min and consisted of scenes selected from the movie "Irreversible" (France, 2003, directed by Gaspar Noe). These scenes included the assault and brutal rape of a young women on her way home from a party as well as the murder of the alleged offender with a fire extinguisher. A study that compared different aversive movies found that scenes from this movie elicited the most consistent and intense reactions, evident in more distress and vivid intrusive memories [25]. The participants watched the trauma film alone in a darkened room and were instructed to imagine themselves being a close witness at the scene. Furthermore, they were asked to watch the film completely and not look away. The eye-tracking data was used to measure the pupil diameter and to evaluate whether the participant followed these instructions. The same movie was presented on day 0 and again on day 3.

The Tobii TX300 binocular eye-tracker had a maximum resolution of 1920 x 1080 pixels, 0.01° precision, and a sampling rate of 300 Hz. Participants' eye movements were calibrated prior to the experimental trials. Pupil sizes were measured with the Tobii Studio eye-tracking software version 3.2.3. After the calibration procedure, participants were presented with a fixation cross for 2 min in order to obtain a baseline measure of the physiological data (pupil size, electrodermal activity, and respiration). EDA data was acquired at a sampling rate of 1000Hz from Ag/AgCl electrodes filled with isotonic electrolyte gel on the tenar and hypotenar of the left (non-dominant) hand via acquisition module MP150 (Biopac Systems Inc., Goleta CA, USA). EDA data were saved and analyzed with *Acqknowlege 4.3*. software. The EDA data were smoothed (median value smoothing factor: 63) and a low-pass filter (frequency cutoff 1 Hz) was applied. Phasic components were derived from the tonic EDA before the skin conductance level was assessed. Respiratory responses were measured throughout the trauma movie using a breathing belt (RX-TSD221-MRI) affixed to the subject's chest.

fMRI paradigm

During fMRI scanning, participants were exposed to an adapted version of a well-established emotional face matching task [26]. Specifically, participants viewed a trio of faces and houses as a

non-social control condition and selected the face or house in the bottom row that was identical with the target stimulus in the top row by pressing one of two buttons on an MRI-compatible response grip system. Stimuli were presented in 12 blocks (one block with houses and three blocks in each of three emotion categories: neutral, fearful, and happy).

Each block consisted of five face or house trios (duration per trio: 5 s). The sequence of blocks was randomized and blocks were separated from each other by a low-level baseline period lasting 4 – 6 s, during which a fixation cross was depicted in the center of the screen. The stimuli were presented through MRI-compatible goggles (NordicNeuroLab AS**,** Bergen, Norway) using the software Presentation (Neurobehavioral Systems Inc., Berkeley, CA, USA). Visual stimuli were obtained from the stimulus set of Karolinska Directed Emotional Faces (KDEF) database [27]. Given our previous observation that the effect of intranasal OXT on amygdala activation was diminished for ambiguous emotional expressions [28], only highly fearful, happy, and neutral face stimuli were used.

Acquisition of functional MRI data

The MRI data were collected using a 1.5-tesla Siemens Avanto MRI system (Siemens AG, Erlangen, Germany) equipped with a 12-channel head-coil. T2*-weighted echoplanar (EPI) images with blood-oxygen-level dependent contrast were obtained [repetition time (TR) = 3000 ms, echo time (TE) = 50 ms, interleaved slicing, matrix size: 64 x 64, voxel size: 3.3 x 3.3 x 3 mm, distance factor = 10 %, flip angle 90°, 35 axial slices] using an amygdala sensitive sequence, optimized as follows: to refine imaging in subcortical regions, TE was decreased linearly by 10 ms in a transition zone between slices 19 and 14, resulting in a final TE of 40 ms in the lower slices, as previous studies have shown largest amygdala activations at an echo time of 40 ms [29]. In addition, high-resolution anatomical images were acquired on the same scanner using a T1-weighted 3D MPRAGE sequence (imaging parameters: TR = 1660 ms, TE = 3.09 ms, matrix size: 256x256, voxel size: 1 x 1 x 1 mm, flip angle 15°, 160 sagittal slices).

fMRI data analysis

Preprocessing

The MRI data were preprocessed and analyzed using SPM12 software (Wellcome Trust Centre for Neuroimaging, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB R2010b (MathWorks, Natick, Massachusetts). 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, a two pass procedure was used by which images were initially realigned to the first image of the time series and subsequently re-realigned to the mean of all images. For normalization, a two-step procedure was applied. Normalization parameters were first determined by segmenting the T1-image using the default tissue probability maps. Next, normalization parameters were applied to normalize the functional images to the standard anatomical Montreal Neurological Institute (MNI) space resampled at 2 × 2 × 2 mm voxel. The normalized images were spatially smoothed using a 6-mm FWHM Gaussian kernel. Raw time series were detrended using a high-pass filter (cut-off period, 128 s).

First-level analysis

On the first level, eight ('Fearful_First', 'Happy_First', 'Neutral_First', 'House_First', 'Fearful_Second', 'Happy_Second', 'Neutral_Second', 'House_Second') regressors were modeled by a boxcar function convolved with a hemodynamic response function (duration per condition block: ~25 s). The six movement regressors (realignment parameters) were included as confounds in the design matrix. A two-level random effects approach based on the general linear model as implemented in SPM12 was used for statistical analyses.

Second-level analysis

On the second level, effects of OXT were analyzed by employing a 2 x 2 flexible factorial design with treatment (OXT, PLC) as between-subject variable and time (first, second) as within-subject factors and the BOLD response of the contrasts [Fearful > Neutral], [Fearful < Neutral], [Happy > Neutral], [Happy < Neutral], and [House > Baseline] as dependent variables. Based on the observation of

differential OXT effects on intrusions in participants with strong and weak trauma disclosure, all analyses were repeated and conducted separately for participants with strong and weak trauma disclosure. Unspecific, domain-general effects of OXT (i.e. the main effect of treatment) were analyzed by comparing all conditions with the low level baseline ([OXT>PLC]) and ([PLC>OXT]). For correlational analyses, the number of intrusions was included as covariate in the second level and examined using one-sample *t*-tests in SPM.

Based on previous studies indicating modulatory effects of OXT on neural responses in the amygdala, middle frontal gyrus, and ventromedial prefrontal cortex [19, 28, 30], we focused the second level analyses on three a priori bilateral regions of interest (ROIs): the amygdala and the medial and middle frontal gyrus. ROIs were anatomically defined according to the Wake Forest University Pick Atlas (Version 3.0). In addition, an exploratory whole-brain analysis was performed applying a height threshold of $P < 0.001$. *P*-values were corrected for multiple comparisons (family-wise error (FWE)) based on the size of the ROI and $P < 0.05$ was considered significant. To disentangle the specificity of OXT effects, parameter estimates were extracted from significant clusters of the BOLD level analysis.

Connectivity analysis

A generalized psychophysiological interaction (gPPI; http://www.nitrc.org/projects/gppi) in SPM8 was used to examine the effect of OXT on functional connectivity between the amygdala and the prefrontal cortex. Compared with standard PPI implementation in SPM, gPPI methods allow for a more efficient investigation of task-dependent connectivity between identified seed regions and chosen ROIs when there are more than two task conditions [31]. Seed regions were identified as 8-mm radius spheres centered at the locus of maxima of significant clusters in our a priori ROIs (amygdala: -26, 0, -16; middle frontal cortex: -36, 46, 0; medial frontal cortex: 10, 54, -6). On the first level, mean time series for each condition were extracted from these spheres and deconvolved with the hemodynamic response function (HRF). The resulting time series were multiplied with the task condition regressors and reconvolved with the HRF to obtain the PPI interaction variables. For this purpose, the same task regressors specified for the BOLD level analysis were modeled in the first level models. Separate PPI models were estimated for each seed for each participant. On the second level, obtained contrast images were entered in a 2 × 2 flexible factorial design with treatment (OXT, PLC) as between-subject variable and time (first, second) as within-subject factors. We examined the modulatory effect of OXT

on connectivity between seeds and ROIs using planned SPM dependent *t*-tests for the contrasts [Fearful > Neutral], [Fearful < Neutral], [Happy > Neutral], [Happy < Neutral], and [House > Baseline] in the flexible factorial design. Results were considered significant at $P_{FWE} < 0.05$ (peak-level inference) adjusted to the size of the ROIs. Parameter estimates were extracted from significant clusters, indicating condition-specific functional connectivity of the seed region to a target region.

Statistical analyses

Behavioral, demographic, and psychometric data were analyzed using SPSS Version 24.0 (IBM Corp., Armonk, NY, USA). OXT effects on intrusions after the first trauma exposure were analyzed with mixed-design analyses of variance (ANOVAs) with the within-subject factor “days” (three days), the between-subject variables “treatment” (OXT, PLC) and “trauma disclosure” (strong, weak), and the number of intrusions as dependent variable. OXT effects on intrusions after the second trauma exposure were analyzed with an additional ANOVA with the same variables except for treatment, which comprised three groups after the second trauma exposure (OXT-OXT, OXT-PLC and PLC-PLC). The same ANOVAs were used to test possible effects on other diary measurements (stress ratings and time spent talking to other people).

Furthermore, psychophysiological responses (EDA, pupil size, and respiration), mood effects (positive and negative affect), and effects on hormonal levels (cortisol, oxytocin) were examined with the same ANOVAs with the factor “time” (for psychophysiological responses: baseline, during trauma movie; for mood effects: before trauma, after trauma; for hormonal effects: before trauma, after trauma, (for cortisol also: after imaging)). Post hoc analyses to delineate higher order effects were calculated using dependent *t*-tests. Potential demographical and neuropsychological a priori differences between both treatment groups were explored using independent *t*-tests. Chi-squared tests were used to compare categorical variables (e.g. side effects) between treatment groups. The assumption of sphericity was assessed with Mauchly's test, and for significant violations Greenhouse-Geisser's correction was applied. All reported *P*-values are two-tailed and *P*-values of $P < 0.05$ were considered statistically significant. Measures of effect sizes are reported using partial eta squared values (η_p^2) for ANOVAs and Cohen’s d for dependent and independent *t*-tests.

Brain behavior associations

To further explore associations between intrusions and neural responses, parameter estimates were extracted from significant clusters from the BOLD level and the connectivity analysis using MarsBaR [32]. Associations were examined using Pearson's product-moment correlation and considered significant at $P < 0.05$.

Hormonal assessment

On days 0 and 3, saliva samples were collected at three different time points: immediately before the trauma movie, after the trauma movie, and after the imaging using pre-chilled Salivettes (Sarstedt, Rommelsdorf, Germany). Salivettes were centrifuged at 6000 rpm for 2 min and aliquoted samples were then 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 coefficient of variation for the assays were 3.42% and 12.2%, respectively.

In order to validate the cycle phase and to control for baseline differences in gonadal hormones levels, blood samples were collected on days 0 and 3 before the trauma movie. Serum FSH, LH, and estradiol were analyzed by fully automated homogeneous sandwich chemiluminescent immunoassays based on the LOCI™ technology on a Dimension Vista™ System according to the manufacturer´s instructions (Siemens Healthcare Diagnostics, Eschborn, Germany). The detection limits of each assay were 0.2 IU/l for LH and FSH and 11 pg/ml for estradiol. The coefficients of variation for intra-assay and inter-assay precision were < 1.8 % and < 2.1 % for LH, < 1.9 % and < 2.2 % for FSH and < 5.5 % and < 5.9 % for estradiol. Serum progesterone was determined by a fully automated solid-phase competitive chemiluminescent enzyme immunoassay on an Immulite™ 2000xpi System according to the manufacturer´s instructions (Siemens Healthcare Diagnostics). The detection limit of the assay was 0.1 ng/ml. The coefficients of variation for intra-assay and inter-assay precision were < 4.2 % and < 5.5 %. The cross-reactivity of all assays with other related compounds was minimal.

Salivary OXT concentrations were determined by using two 96-well commercial OXT-ELISA kit (ENZO). Measurements were performed in duplicate, and samples were treated according to kit instructions. According to the manufacturer, the sensitivity limit of the assay is 15 pg/ml, and 10.3 % of

the samples fell below the lower level of sensitivity. Five outlier OXT values ($z > 3.5$) were discarded from the analysis. The assay's reported intraassay and interassay coefficients of variability are 10.2-12.6% and 11.8-20.9%, respectively.

SUPPLEMENTAL RESULTS

Pilot study

The results of our pilot study showed that the trauma movie increased neural responses to fearful faces in the amygdala compared to a neutral control movie (cf. **Supplemental Figure S2**).

The number of intrusions declined over time and the number of intrusions was no longer significantly different from zero on day 3 ($P = 0.37$). The trauma movie was rated as significantly more unpleasant ($t_{(9.57)} = 6.81$, $P < 0.01$, $d = 3.34$) and arousing ($t_{(12.20)} = 5.71$, $P < 0.01$, $d = 2.73$) than the neutral control movie. A mixed-design ANOVA with the between-subject factor "movie" (trauma movie, control movie), the within-subject variable "time" (baseline, movie), and the pupil sizes as dependent variable revealed a significant interaction between movie and time ($F_{(1, 15)} = 3.36$, $P = 0.04$, $\eta_p^2 = 0.25$), indicating a significantly stronger pupil size increase in the trauma movie group. The interactions between movie and time did not reach significance for either the skin conductance level ($P = 0.12$) or respiration rate ($P = 0.12$). Furthermore, participants in the trauma movie group exhibited significantly stronger responses to fearful faces compared to neutral faces in the right amygdala than participants who had watched a neutral control movie (peak MNI coordinates x, y, z: 36, 2, -26; $t_{(80)} = 3.37$, $P_{FWE} = 0.04$; cf. **Supplemental Figure S2**). There were no significant differences in other brain regions and no significant differences for the opposite contrast (Control > Trauma). Interestingly, an exploratory whole brain analysis for the contrast [Happy > Neutral] revealed significantly increased activations after the trauma movie in a cluster including the posterior cingulate cortex (-30,-70, 10; $t_{(80)} = 5.87$, $k = 43$, $P_{FWE} < 0.01$) and a cluster entailing the middle cingulate cortex (6,-40, 46, $t_{(80)} = 4.23$), precuneus (-2, -38, 55, $t_{(80)} = 3.81$), and paracentral lobule (14, -38, 54, $t_{(80)} = 3.55$; $k = 126$, $P_{FWE} < 0.01$, cf. **Supplemental Figure S2**). There were no significant group differences for the opposite contrast (Control > Trauma).

Main study

There were no a-priori differences in demographic and trait variables between treatment groups (cf. **Supplemental Tables S1 and S2**).

Intrusions

The number of intrusions declined over time ($F_{(1.76, 77.44)} = 4.77$, $P = 0.01$, $\eta_p^2 = 0.10$) after the second trauma exposure and we observed a significant interaction between time, treatment, and trauma disclosure ($F_{(3.52, 77.44)} = 2.75$, $P = 0.04$, $\eta_p^2 = 0.11$). In OXT-treated participants with strong trauma disclosure, the number of intrusions in the first day was significantly reduced compared to participants who received PLC for six days ($t_{(15.00)} = -2.21$, $P = 0.04$, $d = -0.82$) (cf. **Supplemental Figure S3**), but there was a non-significant increase in participants with weak trauma disclosure ($t_{(22)} = -1.08$, $P = 0.29$, $d = 0.47$). The total number of intrusions in the three days after the second trauma reported by participants with strong trauma disclosure who had received OXT in the three days following the first trauma exposure and then switched to PLC after the second trauma exposure did not differ from intrusions reported by participants who received OXT for six days ($P = 0.10$) and were significantly lower compared to the number of intrusions reported by participants who had received PLC for six days ($t_{(14)} = 2.43$, $P = 0.03$, $d = 0.75$), suggesting that the initial treatment had a lasting protective effect. Neither the trauma disclosure (first: $F_{(1, 47)} = 0.01$, $P = 0.91$, $\eta_p^2 < 0.01$; second: $F_{(1, 17)} = 1.66$, $P = 0.22$, $\eta_p^2 = 0.09$) nor the treatment (first: $F_{(1, 47)} = 0.72$, $P = 0.40$, $\eta_p^2 = 0.02$; second: $F_{(2, 17)} = 0.05$, $P = 0.95$, $\eta_p^2 < 0.01$) altered how stressful the intrusions were.

To further explore the specificity of the observed interaction between trauma disclosure and treatment, we median-dichotomized the valence ratings of the trauma movie as well as dissociative symptoms and pupillary responses induced by the first trauma exposure. These variables were positively associated with the number of intrusions in the PLC group (cf. Further correlations with intrusions). However, additional mixed-design ANOVAs with the median-dichotomized variables and treatment as between-subject factors and the number of intrusions in the two days after the first trauma exposure as dependent variable did not reveal any significant interactions (all Ps > 0.65) indicating that OXT specifically reduced intrusions in the subgroup with strong trauma disclosure rather than decreasing intrusive memories in all participants exhibiting more pronounced responses to the trauma movie.

Physiological and psychological responses to the trauma movie

Watching the trauma movie induced strong sympathetic responses, evidenced by increases in skin conductance level (first: $F_{(1, 48)} = 19.27$, $P < 0.01$, $\eta_p^2 = 0.29$; second: $F_{(1, 48)} = 5.43$, $P = 0.02$, $\eta_p^2 =$

0.10), respiratory rate (first: $F_{(1, 55)} = 22.23$, $P < 0.01$, $\eta_p^2 = 0.29$; second: $F_{(1, 55)} = 37.80$, $P < 0.01$, $\eta_p^2 = 0.41$), and pupil diameter (first: $F_{(1, 58)} = 9.87$, $P < 0.01$, $\eta_p^2 = 0.15$, second: $F_{(1, 58)} = 4.86$, $P = 0.03$, $\eta_p^2 = 0.08$; cf. **Supplemental Figure S4**). We also observed a trend-to-significant interaction between trauma disclosure and treatment for pupil dilation during the trauma movie compared to baseline ($F_{(1, 58)} = 3.34$, $P = 0.07$, $\eta_p^2 = 0.05$). Post-hoc t-tests showed a significantly reduced pupillary response in OXT-treated participants with strong trauma disclosure compared to PLC during the second trauma exposure ($t_{(29)} = -2.49$, $P = 0.02$, $d = -0.93$), but no significant treatment effect for participants with weak trauma disclosure ($t_{(29)} = 0.52$, $P = 0.60$, $d = 0.19$). There were no main or interaction treatment effects for skin conductance level or respiration rate (all Ps > 0.52).

The trauma movie was rated as highly unpleasant after the first and second exposure (cf. **Supplemental Figure S5A**). Interestingly, OXT-treated participants with weak trauma disclosure experienced the trauma movie as more unpleasant compared to PLC ($t_{(29)} = 3.28$, $P < 0.01$, $d = 1.22$), while there was no significant treatment effect in participants with strong trauma disclosure ($t_{(29)} = 1.35$, $P = 0.19$, $d = 0.50$). Furthermore, participants reported dissociative symptoms after the first (1.42 ± 1.23) and second trauma exposure (0.93 ± 1.02; cf. **Supplemental Figure S5B**). The magnitude of this dissociative experience was similar to the scores reported for patients with posttraumatic stress disorder (1.87 ± 1.40; healthy controls without trauma exposure: 0.11 ± 0.12; [22]). A mixed-design ANOVA with the between-subject variables “treatment” (OXT, PLC) and “trauma disclosure” (strong, weak), the within-subject factor “time” (first exposure, second exposure), and the dissociative symptoms as dependent variable yielded a main effect of time ($F_{(1, 58)} = 33.00$, $P < 0.01$, $\eta_p^2 = 0.36$) and an interaction between treatment, trauma disclosure, and time ($F_{(1, 58)} = 7.09$, $P = 0.01$, $\eta_p^2 = 0.11$). The dissociative symptoms were weaker after the second trauma exposure and this decrease in dissociative symptoms was diminished in OXT-treated participants with strong trauma disclosure. However, post-hoc *t*-tests did not show any significant difference between OXT and PLC groups after the first or second trauma exposure (all Ps > 0.21). Of note, dissociative symptoms positively correlated with the total number of intrusions after the first and second trauma exposure in the PLC group (cf. SI Further correlations with intrusions), while there was no significant correlation with intrusions after the second trauma exposure in the OXT group ($P = 0.66$). Taken together, these findings suggest that the OXT effect on intrusions is not driven by altered dissociative symptoms.

The trauma movie also had a significant impact on mood such that positive affect decreased (first: $F_{(1, 58)} = 154.98$, $P < 0.01$, $\eta_p^2 = 0.73$; second: $F_{(1, 58)} = 36.72$, $P < 0.01$, $\eta_p^2 = 0.39$) and negative affect increased (first: $F_{(1, 58)} = 12.03$, $P < 0.01$, $\eta_p^2 = 0.17$; second: $F_{(1, 58)} = 8.66$, $P < 0.01$, $\eta_p^2 = 0.13$; cf. **Supplemental Figure S6**). The deteriorating effect of the trauma movie on positive affect was more pronounced in participants with strong trauma disclosure compared to participants with weak trauma disclosure (interaction between trauma disclosure and movie; first: $F_{(1, 58)} = 3.33$, $P = 0.07$, $\eta_p^2 = 0.05$; second: $F_{(1, 58)} = 8.53$, $P < 0.01$, $\eta_p^2 = 0.13$), but the OXT treatment did not alter the immediate mood response to the trauma movie (all *P*s > 0.17).

The trauma movie was also rated as highly arousing (first: 78.03 ± 16.32; second: 68.23 ± 18.11) and the arousal decreased over time ($F_{(1, 58)} = 33.96$, $P < 0.01$, $\eta_p^2 = 0.37$), but there were no further significant main or interaction effects (all *P*s > 0.05).

Further correlations with intrusions

Under PLC, trait rumination positively correlated with the total number of intrusions in the three days after the first ($r_{(30)} = 0.45$, $P = 0.01$) and second trauma exposure ($r_{(30)} = 0.37$, $P = 0.046$). Childhood trauma was positively associated with the total number of intrusions in the three days after the second trauma exposure ($r_{(30)} = 0.38$, $P = 0.04$), but not with intrusions after the first trauma exposure ($r_{(30)} = 0.09$, $P = 0.66$). Furthermore, a more complex social network was negatively associated with the total number of intrusions after the first ($r_{(30)} = -0.45$, $P = 0.01$) and second trauma exposure ($r_{(30)} = -0.43$, $P = 0.02$). There were no other significant correlations with demographic variables or personality traits (all *P*s > 0.24).

Furthermore, the total number of intrusions in the three days after the first trauma exposure positively correlated with arousal ($r_{(30)} = 0.61$, $P < 0.01$) and unpleasantness ($r_{(30)} = 0.50$, $P < 0.01$) ratings of the movie, dissociative symptoms after the movie ($r_{(30)} = 0.49$, $P < 0.01$), and the increase in pupil diameter induced by the movie ($r_{(30)} = 0.37$, $P = 0.04$). The total number of intrusions in the three days after the second trauma exposure were positively associated with arousal ($r_{(30)} = 0.42$, $P = 0.02$) and unpleasantness ($r_{(30)} = 0.34$, $P = 0.06$) ratings of the movie and dissociative symptoms after the movie ($r_{(30)} = 0.42$, $P = 0.02$). There were no significant correlations between intrusions and mood ratings,

skin conductance response, respiratory rate, or endocrine measurements (i.e. OXT, cortisol, estradiol, and progesterone levels) (all *P*s > 0.07).

Under OXT, trait empathy positively correlated with the total number of intrusions in the three days after the first ($r_{(32)}$ = 0.52, P < 0.01) and second trauma exposure ($r_{(21)}$ = 0.37, P = 0.097). There were no other significant correlations with demographic variables or personality traits (all *P*s > 0.08). Furthermore, the total number of intrusions in the three days after the first trauma exposure positively correlated with arousal ($r_{(32)}$ = 0.53, P < 0.01) and unpleasantness ($r_{(32)}$ = 0.39, P = 0.03) ratings of the movie, dissociative symptoms after the movie ($r_{(32)}$ = 0.54, P < 0.01), and the movie-induced increase in negative affect ($r_{(32)}$ = 0.51, P < 0.01). There were no significant associations with the total number of intrusions in the three days after the second trauma exposure (all *P*s > 0.37). In addition, the total number of intrusions in the three days after the first trauma exposure positively correlated with cortisol levels after the movie ($r_{(31)}$ = 0.42, P = 0.02) and after the imaging ($r_{(30)}$ = 0.38, P = 0.04). Likewise, positive correlations were evident for the total number of intrusions in the three days after the second trauma exposure (cortisol levels after the movie: $r_{(20)}$ = 0.60, P < 0.01; after the imaging: $r_{(20)}$ = 0.78, P < 0.01). There were no significant correlations between intrusions and other mood ratings, skin conductance changes, respiratory rate, and endocrine measurements (i.e. OXT, estradiol, and progesterone levels) (all *P*s > 0.14).

fMRI results

In the PLC group, the total number of intrusions in the three days following the first trauma exposure was negatively associated with stronger responses to fearful faces compared to neutral faces in the anterior cingulate (-2, 36, 10, $t_{(26)}$ = 4.99; 0, 44, 15, $t_{(26)}$ = 4.30; -8, 48, 12, $t_{(26)}$ = 4.10; k = 126, P_{FWE} < 0.01) and medial frontal cortex (-10, 56, -2, $t_{(26)}$ = 4.78 and -2, 54, 2, $t_{(26)}$ = 3.88; k = 54, P_{FWE} = 0.04; cf. **Supplemental Figure S7A**). By contrast, increased amygdala activation in response to fearful faces positively correlated with the total number of intrusions (-26, -6, -18; $t_{(26)}$ = 3.64, P_{FWE} = 0.05; cf. **Supplemental Figure S7B**).

In the total OXT-treated sample compared to PLC, we observed a trend-to-significant reduction of activations in response to fearful faces in the medial frontal cortex (0, 60, 18, $t_{(112)}$ = 4.90, P_{FWE} = 0.07) and a trend-to-significant increase in activations in response to happy faces in the left amygdala (-24,

0, -12, $t_{(112)}$ = 3.03, P_{FWE} = 0.07). Furthermore, OXT significantly reduced activation in the left amygdala in response to house stimuli compared to PLC (-26, 2, -18; $t_{(112)}$ = 3.20, P_{FWE} = 0.03). Interestingly, this OXT effect was evident in both participants with strong trauma disclosure (-22, -2, -14; $t_{(56)}$ = 3.03, P_{FWE} = 0.06) and participants with weak trauma disclosure (-28, 2, -18; $t_{(52)}$ = 3.26, P_{FWE} = 0.06). There were no further significant treatment effects for house stimuli. A main treatment effect across stimuli was significant neither in the whole sample nor in the subgroups.

In participants with strong trauma disclosure, we also detected a trend-to-significant OXT-induced increase in amygdala responses to happy faces (-22, 0, -14, $t_{(56)}$ = 3.08, P_{FWE} = 0.07). In participants with weak trauma disclosure, OXT significantly decreased activations in the left amygdala in response to happy faces (-14, 0, -16, $t_{(52)}$ = 4.32, P_{FWE} < 0.01; cf. **Supplemental Figure S8B**).

A comparison of participants with strong and weak trauma disclosure in the PLC group revealed no significant whole brain or ROI-specific difference in the response to fearful and happy faces. Interestingly, however, participants with strong trauma disclosure exhibited significantly increased functional connectivity between the middle frontal cortex as seed region and the left amygdala after the first trauma exposure ($t_{(26)}$ = 3.11, P < 0.01, d = 1.22). There were no significant differences between participants with strong and weak trauma disclosure in the OXT group.

Behavioral responses in the fMRI paradigm

To explore potential treatment effects on response time (RT), we performed separate mixed-design ANOVAs for each category (fearful, happy, neutral, house) with treatment (OXT, PLC) and trauma disclosure (strong, weak) as between subject factors and days (day 0, day 3) as within-subject variable. For fearful faces, the ANOVA yielded a significant main effect of days ($F_{(1, 57)}$ = 7.25, P < 0.01, η_p^2 = 0.11) and trend-to-significant interaction between days and trauma disclosure ($F_{(1, 57)}$ = 3.18, P = 0.08, η_p^2 = 0.05) as well as treatment and trauma disclosure ($F_{(1, 57)}$ = 3.36, P = 0.07, η_p^2 = 0.06). RTs decreased over time and this effect was more pronounced for participants with weak trauma disclosure. Furthermore, post-hoc t-tests showed a significant treatment effect only for participants with weak trauma disclosure. There were no significant group differences on day 0 (all P > 0.33), but OXT significantly increased RTs in participants with weak trauma disclosure compared to PLC ($t_{(28)}$ = 2.78, P = 0.01, d = 1.05). Given a positive correlation between social anxiety and RTs for

fearful faces on day 0 (r = 0.26, P = 0.04), an increase in RTs could indicate that OXT enhanced the interference induced by fearful stimuli in participants with weak trauma disclosure. There were no treatment effects for participants with strong trauma disclosure (all Ps > 0.34). RTs also decreased over time for happy faces ($F_{(1, 57)}$ = 9.66, P < 0.01, η_p^2 = 0.15) and houses ($F_{(1, 57)}$ = 5.54, P = 0.02, η_p^2 = 0.09), but there were no treatment effects in either category (all Ps > 0.08).

The number of correct responses was very high (> 95% in all conditions) and there were no significant main or interaction effects (all Ps > 0.09).

Hormonal assessments

A mixed design ANOVA with salivary OXT levels as dependent variable showed main effects of trauma disclosure (strong, weak; $F_{(1, 50)}$ = 4.34, P = 0.04, η_p^2 = 0.08) and time (before trauma, after trauma; $F_{(1, 50)}$ = 4.58, P = 0.04, η_p^2 = 0.08) on day 0. The trauma movie induced an increase in OXT levels. The interaction between trauma disclosure and time was not significant (P = 0.25), but post-hoc t-tests showed that participants in the total sample with weak trauma disclosure exhibited significantly lower salivary OXT concentrations than participants with strong trauma disclosure only after the trauma movie (before: $t_{(53)}$ = -1.19, P = 0.24, d = -0.33; after: $t_{(36.13)}$ = -2.24, P = 0.03, d = -0.63).

A mixed design ANOVA with the salivary OXT levels on day 3 as dependent variable again revealed significantly lower OXT levels in participants with weak trauma disclosure ($F_{(1, 48)}$ = 5.11, P = 0.03, η_p^2 = 0.10). Furthermore, we found a significant interaction between treatment, trauma disclosure, and time ($F_{(1, 48)}$ = 5.29, P = 0.03, η_p^2 = 0.10). Participants with weak trauma disclosure in the OXT group had significantly lower OXT baseline levels (before the trauma) compared to the PLC group ($t_{(14.62)}$ = -2.53, P = 0.02, d = -0.98), while OXT-treated participants with strong trauma disclosure showed a trend-to-significant reduced level of salivary OXT after the trauma movie ($t_{(25)}$ = -1.81, P = 0.08, d = -0.73). Furthermore, participants in the PLC group with weak trauma disclosure showed significantly reduced OXT levels after the trauma movie (before: $t_{(25)}$ = 0.53, P = 0.60, d = 0.17; after: $t_{(25)}$ = -2.18, P = 0.04, d = -0.87), while OXT-treated participants with weak trauma disclosure exhibited decreased OXT concentrations before the trauma movie (before: $t_{(15.56)}$ = -2.45, P = 0.03, d = -0.90; after: $t_{(23)}$ = -0.96, P = 0.35, d = -0.40).

Mixed design ANOVAs with salivary cortisol levels as dependent variable yielded a trend-to-significant effect of time (before trauma, after trauma, after imaging) on day 0 ($F_{(1.36, 76.13)}$ = 3.20, P = 0.07, η_p^2 = 0.05) and a significant effect of time on day 3 ($F_{(1.13, 63.47)}$ = 31.03, P < 0.01, η_p^2 = 0.36). Surprisingly, the cortisol levels decreased on both days, possibly indicating high anticipatory stress immediately before the trauma movie when the first saliva samples were collected. There were no other significant main or interaction effects (all *P*s > 0.19; cf. **Supplemental Tables S3 and S4**).

Further diary measurements

The day of the first trauma exposure (day 0) was rated as most stressful and stress ratings declined in the following two days ($F_{(2, 106)}$ = 7.02, P < 0.01, η_p^2 = 0.12). Stress ratings did not change after the second trauma exposure (P = 0.79), but we observed an interaction between days, trauma disclosure, and treatment ($F_{(4, 88)}$ = 3.42, P = 0.01, η_p^2 = 0.14). However, neither in the strong trauma disclosure group nor in the weak trauma disclosure group did post-hoc *t*-tests show any significant difference between OXT and PLC groups (all *P*s > 0.16).

We also assessed whether the participants spoke with another person during the study (but not necessarily discussed the trauma movie). The majority of participants spoke with at least one other person during the study, but there were no treatment effects (total sample: OXT = 96%, PLC = 94%; strong trauma disclosure; OXT = 98%, PLC = 99%; weak trauma disclosure: OXT = 95%, PLC = 89%; all *P*s > 0.14). Furthermore, a mixed-design ANOVA with the between-subject factors “treatment” (OXT, PLC) and “trauma disclosure” (strong, weak) and the time spent talking to other people (sum across the three days after the first trauma exposure) showed a main effect of trauma disclosure ($F_{(1, 57)}$ = 9.92, P < 0.01, η_p^2 = 0.15), indicating that participant with strong trauma disclosure spent more time talking to other people (652 ± 266 min) than participants with weak trauma disclosure (429 ± 294 min). There were no further significant main or interaction effects after the first or second trauma exposure (all *P*s > 0.20). In a next step, we median-dichotomized the variable “time spent talking to other people” and computed an additional mixed-design ANOVA with the “time spent talking to other people” and treatment (OXT, PLC) as between-subject variables and the number of intrusions in the two days following the first trauma as dependent variable. There were no significant main or interaction effects (all Ps > 0.45). The fact that the OXT effect is dependent upon trauma disclosure

(i.e. time spent discussing the movie), but is not influenced by the total time spent talking to another person, indicates that it is not social interaction *per se* that moderates the effects of OXT.

Side effects

No serious side effects occurred. On day 0, significantly more participants in the OXT group (28%) than in the PLC (0%) reported a slight headache ($\chi^2_{(1)} = 8.96$, $P < 0.01$). The same pattern was evident on day 1 (OXT: 41%, PLC: 11%, $\chi^2_{(1)} = 6.83$, $P < 0.01$), but there were no significant differences on day 2 (OXT: 22%, PLC: 17%, $\chi^2_{(1)} = 0.27$, $P = 0.60$) or on the following days (all *P*s > 0.12). Furthermore, significantly more participants in the OXT group (63%) than in the PLC group (36%) reported tiredness as a side effect ($\chi^2_{(1)} = 4.29$, $P = 0.04$) on day 1, but there were no significant differences on the other days (all *P*s > 0.25). Importantly, these side effects cannot explain the differential OXT effects in participants with strong and weak trauma disclosure because side effects were equally distributed between these groups (all *P*s > 0.69).

Missing values

Across the six days of the study, n = 19 participants (OXT: n = 4; PLC: n = 15) failed to complete the intrusion diaries (of n = 372 total diary entries). Six blood samples and 17 saliva samples were lost due to problems in sample assessment or analysis, resulting in 59 hormonal blood measurements and 182 cortisol / 121 OXT measurements for day 0 and 59 hormonal blood measurements and 183 cortisol / 117 OXT measurements for day 3. EDA data from n = 8 subjects (OXT: n = 6; PLC: n = 2) could not be analyzed due to acquisition failure. Respiration data of three participants (OXT: n = 2; PLC: n = 1) on day 0 and of three participants (OXT: n = 1; PLC: n = 2) on day 3 were not recorded due to technical issues.

SUPPLEMENTAL DISCUSSION

It is now well established that intranasal administration of synthetic OXT can be used as a pharmacological means to alter central OXT signaling because intranasal OXT leads to elevated OXT concentrations in the cerebrospinal fluid in rodents [33], macaques [34, 35], and humans [36]. Importantly, a recent positron emission tomography (PET) study developed a radiolabeled tracer for the OXT receptor and demonstrated direct nose-to-brain uptake of OXT [37]. Intranasal OXT may reach the brain via different routes: OXT may travel to the olfactory nerve via olfactory sensory neurons located in the mucous layer or reach the trigeminal nerve via trigeminal ganglion cell fibers [38]. In addition, OXT is absorbed into the systemic blood circulation and peripherally circulating peptides may produce central effects [39]. In a recent fMRI kinetic study, we found evidence for an inverted U-shaped dose-response relationship between OXT and amygdala responses to fearful faces in healthy men [28]. The most pronounced OXT effect was evident with a dose of 24 IU, but so far, no study examined the kinetics of intranasal OXT in women. As such, it is conceivable that a different dose or frequency of OXT administration would produce stronger effects.

Attachment organization and social support are increasingly recognized as central factors determining the recovery from traumatic experiences [40]. For instance, verbal revelation of emotional events can reduce distress by promoting integration and extinction of the traumatic memories [41]. Trauma disclosure facilitates social support, which is a robust resilience factor protecting one from developing PTSD [42] and our results allude to a beneficial impact of OXT combined with positive social contacts on the processing of traumatic experiences.

A blunting of medial PFC activation after a single dose of OXT has been reported for self and other judgments [43], while the opposite effect was evident in a task assessing cognitive control of food craving [44]. Thus, our study extends previous findings by showing that during a prolonged treatment, not only task features but also social interaction behavior exhibited over the course of the study can moderate OXT effects. In contrast to previous studies exploring the acute effects of a single dose of OXT administration (24 IU) in healthy women [45, 46], we found no evidence for an upregulation of amygdala activation in response to fearful faces, which may reflect different effects of a prolonged treatment regimen or could be due to stress-related differences following trauma exposure. In fact, dampened amygdala reactivity towards emotional faces following a single dose of 40 IU OXT has also been observed in female PTSD patients [47]. Furthermore, it is noteworthy that a single dose

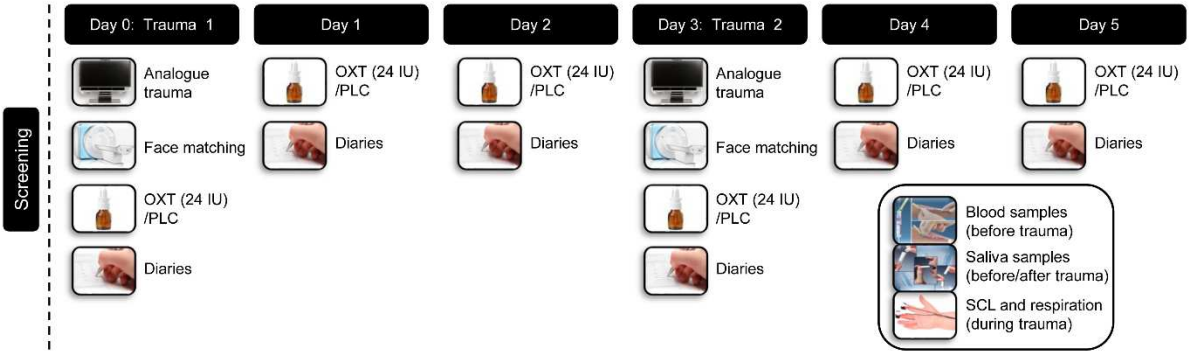
administration of OXT had no effect on emotional and cognitive empathy in women with PTSD [48]. However, negative alterations in cognition, mood, and intrusive symptoms are distinct diagnostic criteria of PTSD and there was no association between empathic ability and the number of intrusions in our study. Hence, OXT-induced changes in intrusive memories may be independent of possible OXT effects on empathy.

Furthermore, we found that participants with weak trauma disclosure under OXT rated the trauma movie as more unpleasant, while the OXT-treated participants with strong trauma disclosure showed diminished pupillary responses to the second trauma movie, indicating that OXT-augmented trauma disclosure may facilitate stress habituation [49]. We did not detect a significant treatment effect on other psychophysiological measurements, mood ratings, dissociative symptoms, or cortisol levels after the trauma movie. The juxtaposition of reduced intrusions in participants with strong trauma disclosure and only moderate effects on the proximate stress response to the trauma movie (that participants watched alone) indicates that OXT has more pronounced anxiolytic effects in conjunction with positive affiliative contacts.

Our study has some limitations. First, we focused on female participants because women are twice as likely to develop PTSD [1]. There is the accumulating evidence for sexual-dimorphic effects of OXT [50-52], which may be due to differences in OXT receptor distribution or interactions with sex steroids. In the present study, we controlled for hormonal fluctuations during the menstrual cycle and the use of hormonal contraception, but our findings need to be replicated in men. Second, while the use of an experimental trauma model enabled us to control numerous potential confounds, we did not systematically vary trauma disclosure and social support. It is also conceivable that OXT facilitates the integration and processing of traumatic memories in participants with strong trauma disclosure. Along these lines, the stress-buffering effects of OXT may be mostly modulated by the individual's perception or experience of social support, which we did not assess in the current study. Thus, future randomized studies are warranted to explore the effects of OXT-augmented social support interventions in the aftermath of trauma.

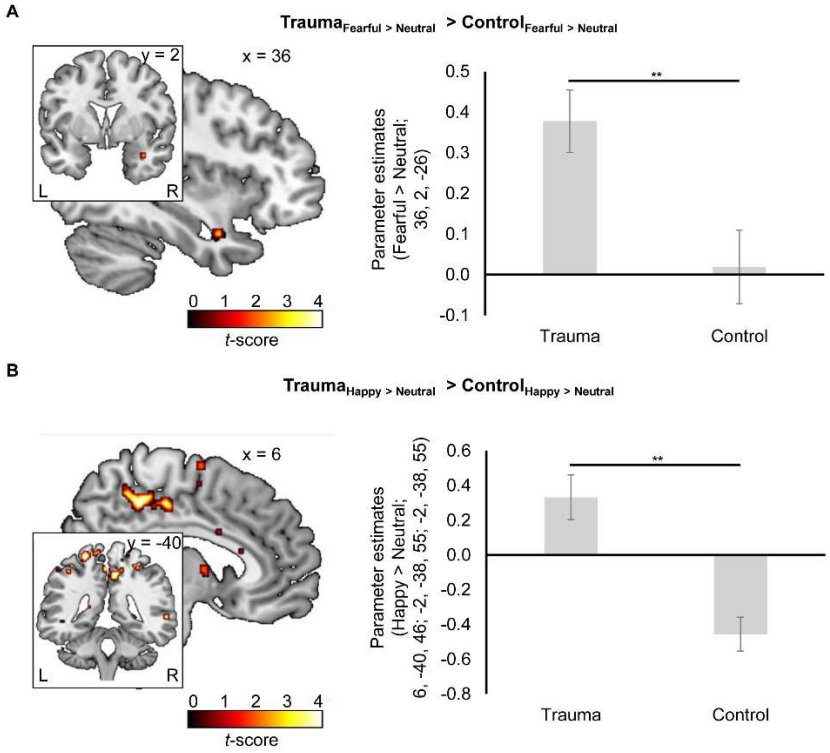
SUPPLEMENTAL FIGURES

Figure S1.



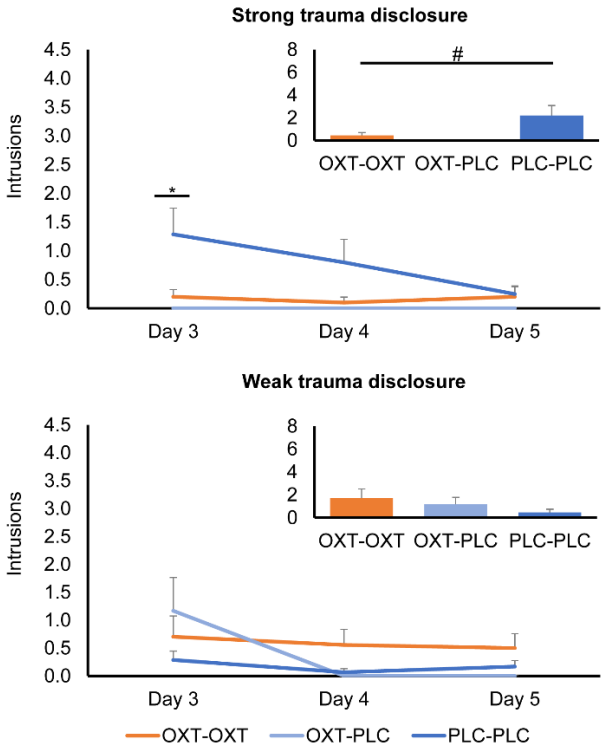
Study design. Participants self-administered 24 IU synthetic oxytocin (Novartis, Basel, Switzerland) or placebo via nasal spray for six days. Participants were confronted with the same trauma movie on day 0 and day 3. At the beginning of test days 0 and 3, blood samples were collected to measure hormone concentrations (estradiol, progesterone, follicle-stimulating hormone, luteinizing hormone). Before and after the trauma movie, saliva samples were collected to measure cortisol and oxytocin concentrations. During the trauma movie, changes in pupil diameter, skin conductance level, and respiration rate were measured. The participants completed intrusion diaries at home on the evenings of the days 0 to 5. Abbreviations: OXT, oxytocin; PLC, placebo; SCL, skin conductance level.

Figure S2.



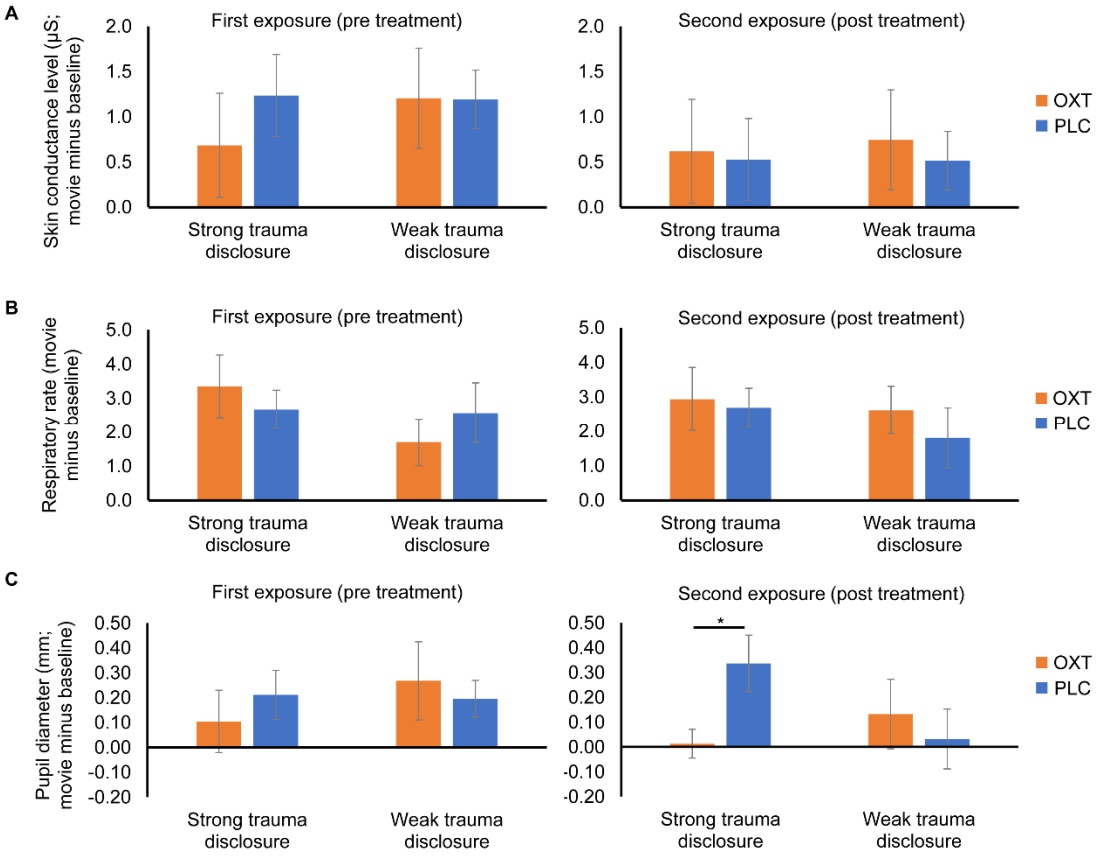
Results of the fMRI pilot study. Participants who watched the trauma movie showed a significantly stronger amygdala response to fearful faces compared to neutral faces than participants who had watched a neutral control movie (**A**). Participants in the trauma movie group also exhibited stronger responses to happy faces compared to neutral faces in a cluster including the middle cingulate cortex, precuneus, and paracentral lobule (**B**). Error bars indicate the standard error of the mean (SEM). Abbreviations: $^{**}P < 0.01$.

Figure S3.



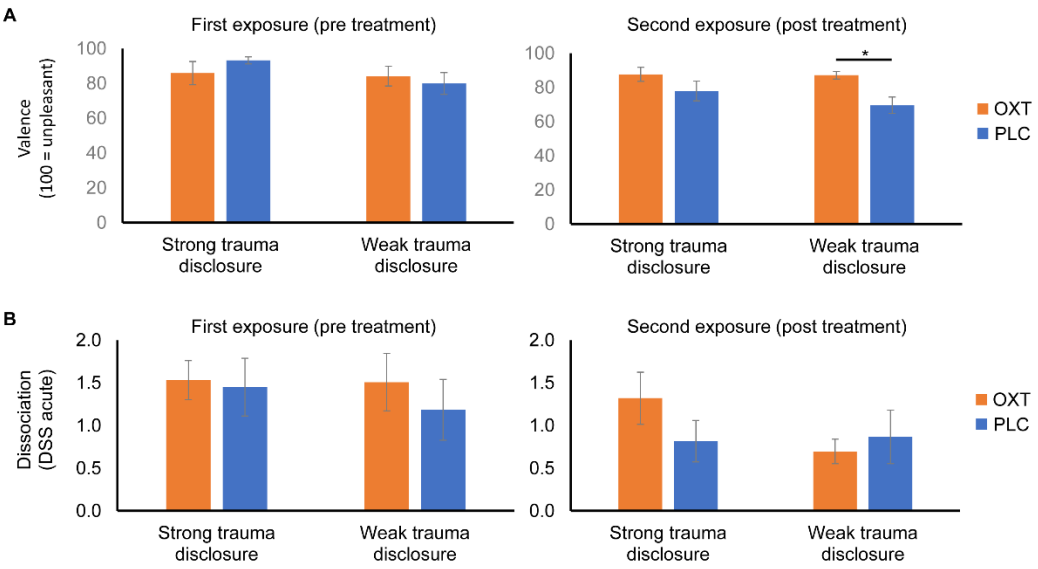
Intranasal oxytocin (OXT) compared to placebo (PLC; group with PLC for six days) significantly reduced the number of trauma-induced intrusions in participants with strong trauma disclosure after the second trauma exposure. By contrast, OXT non-significantly increased the number of intrusions after the second trauma exposure in participants with weak trauma disclosure. Inlays display the average sum of intrusions in the three days after trauma exposure. Error bars indicate the standard error of the mean (SEM). Abbreviations: OXT-OXT, oxytocin treatment on days 0 to 5; OXT-PLC, oxytocin treatment on days 0 to 2; PLC, placebo; PLC-PLC, placebo treatment on days 0 to 5. * $P < 0.05$; # $P < 0.10$.

Figure S4.



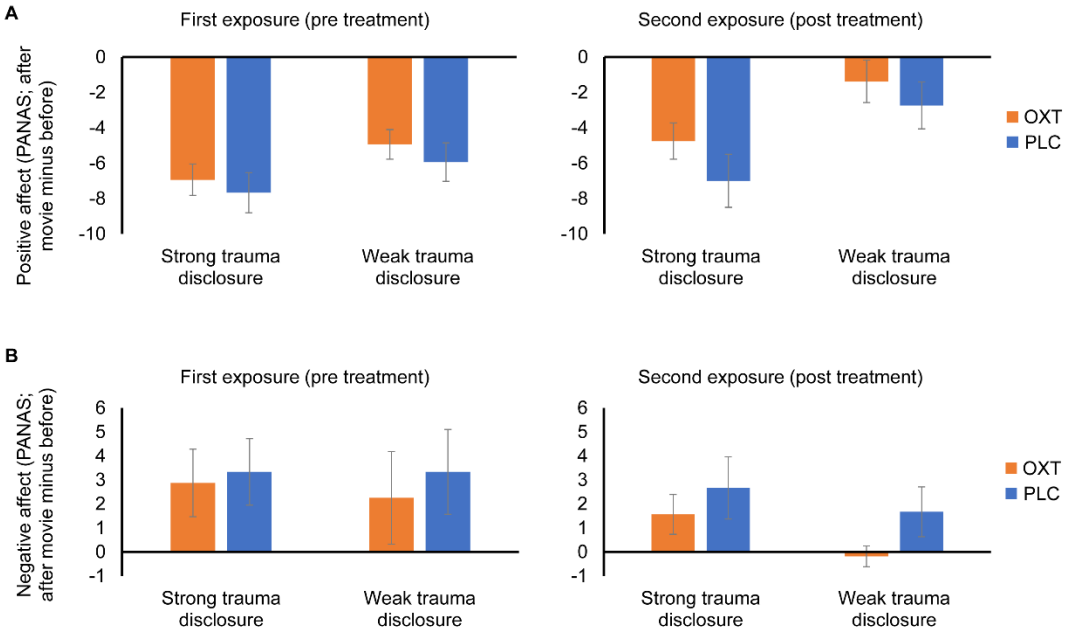
The trauma movie elicited increases in skin conductance level (**A**) and respiratory rate (**B**), but there were no significant differences between treatment and trauma disclosure groups. The trauma movie also induced pupil dilation (**C**). Pupillary responses to the trauma movie were significantly diminished after three days of intranasal oxytocin (OXT) treatment compared to placebo (PLC) in participants with strong trauma disclosure, but not in participants with weak trauma disclosure. Error bars indicate the standard error of the mean (SEM). Abbreviations: OXT, oxytocin; PLC, placebo; Post, trauma exposure post treatment; Pre, trauma exposure before treatment; * $P < 0.05$.

Figure S5.



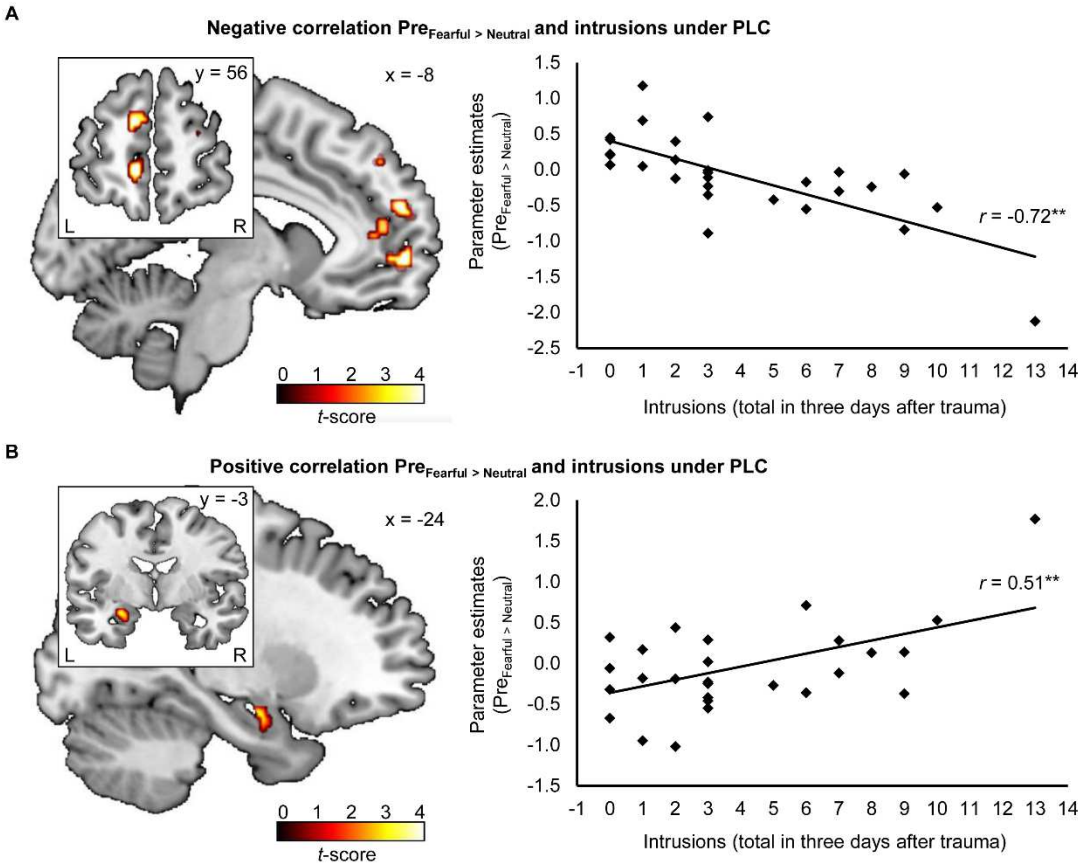
The trauma movie was rated as highly unpleasant (**A**). Oxytocin (OXT)-treated participants with weak trauma disclosure rated the movie as more unpleasant than participants in the placebo group. Furthermore, the trauma movie induced dissociative symptoms (**B**). Dissociative symptoms were weaker after the second trauma exposure and this decrease in dissociative symptoms was diminished in OXT-treated participants with strong trauma disclosure. Error bars indicate the standard error of the mean (SEM). Abbreviations: DSS acute, Dissociation-Tension Scale acute, OXT, oxytocin; PLC, placebo; Post, trauma exposure post treatment; Pre, trauma exposure before treatment; * $P < 0.05$.

Figure S6.



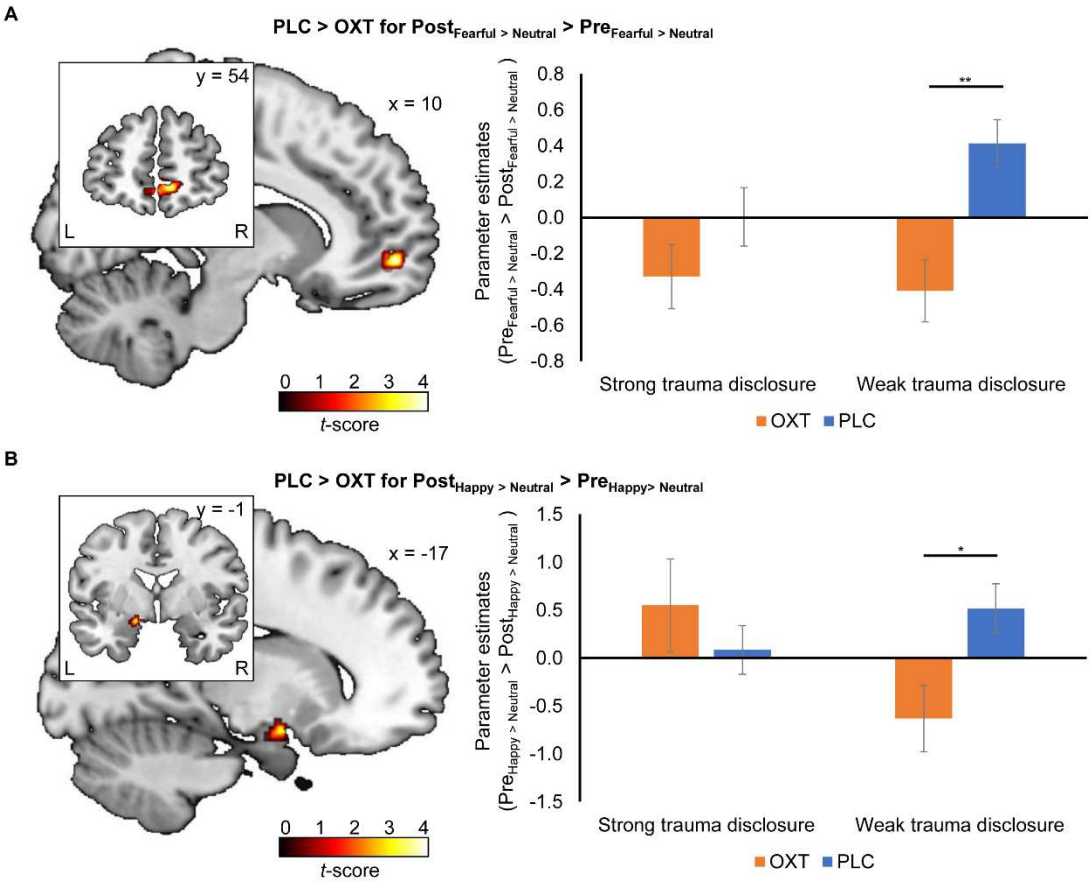
The trauma movie decreased positive affect (**A**) and increased negative affect (**B**). The effects of the trauma movie on affect were more pronounced in participants with strong trauma disclosure, but there were no significant differences between treatment groups. Error bars indicate the standard error of the mean (SEM). Abbreviations: OXT, oxytocin; PANAS, Positive and Negative Affective Scale; PLC, placebo; Post, trauma exposure post treatment; Pre, trauma exposure before treatment.

Figure S7.



Stronger responses to fearful faces compared to neutral faces in the anterior cingulate cortex and middle frontal cortex negatively correlated with the total number of intrusions in the three days following the first trauma exposure in the placebo group (**A**). By contrast, stronger responses in the amygdala were positively associated with the total number of intrusions (**B**). Abbreviations: PLC, placebo; Pre, fMRI assessment before treatment; * $P < 0.05$; ** $P < 0.01$.

Figure S8.



Intranasal oxytocin (OXT) over three days significantly reduced responses to fearful faces compared to neutral faces in the medial frontal cortex in participants with weak trauma disclosure (**A**). OXT also decreased responses to happy faces compared to neutral faces in the left amygdala in participants with weak trauma disclosure (**B**). Error bars indicate the standard error of the mean (SEM). Abbreviations: OXT, oxytocin; PLC, placebo; Post, fMRI assessment after treatment; Pre, fMRI assessment before treatment; * $P < 0.05$; ** $P < 0.01$.

SUPPLEMENTAL TABLES

Table S1. Demographics and psychometric trait data in participants with strong trauma disclosure

	Oxytocin (n = 16) Mean (± SD)	Placebo (n = 15) Mean (± SD)	*t*	*P*
Age (years)	23.75 (2.21)	23.20 (3.97)	0.47	0.64
Autistic-like traits [a]	13.75 (5.11)	11.73 (3.95)	1.22	0.23
Childhood trauma [b]	32.06 (8.15)	30.73 (6.94)	0.49	0.63
Depressive symptoms [c]	2.69 (2.84)	3.27 (6.36)	-0.33	0.74
Education (years)	16.31 (2.00)	15.67 (2.69)	0.76	0.45
Empathy [d]	44.25 (4.63)	44.53 (4.67)	-0.17	0.87
Rumination [e]	35.81 (9.47)	36.60 (11.20)	-0.21	0.83
Social anxiety [f]	20.56 (21.09)	15.73 (14.24)	0.74	0.46
Social network complexity [g]	2.50	1.93	1.55	0.13
Social network size [g]	34.44 (26.46)	24.07 (15.60)	1.32	0.20

Notes. [a] Autistic-like traits were assessed by the AQ (Autism Spectrum Quotient). [b] Childhood trauma was assessed by the CTQ (Childhood Trauma Questionnaire.) [c] Depressive symptoms were assessed by the BDI (Beck's Depression Scale, Version II). [d] Empathy was assessed by the Saabrücker Persönlichkeitsfragebogen, a German version of the IRI (Interpersonality Reactivity Index). [e] Rumination was assessed by the RRQ (Rumination-Reflection Questionnaire). [f] Social anxiety was assessed by the LSAS (Liebowitz Social Anxiety Scale). [g] Social networks were examined with the SNI (Social Network Index questionnaire).

Table S2. Demographics and psychometric trait data in participants with weak trauma disclosure

	Oxytocin (n = 16) Mean (± SD)	Placebo (n = 15) Mean (± SD)	*t*	*P*
Age (years)	22.44 (3.60)	23.87 (6.38)	-0.78	0.45
Autistic-like traits [a]	12.50 (4.38)	14.07 (3.84)	-1.06	0.30
Childhood trauma [b]	29.00 (4.40)	29.07 (4.03)	-0.04	0.97
Depressive symptoms [c]	0.94 (0.93)	1.67 (2.29)	-1.15	0.27
Education (years)	15.41 (2.44)	15.33 (2.45)	0.08	0.93
Empathy [d]	45.50 (5.59)	44.67 (6.45)	0.39	0.70
Rumination [e]	33.31 (8.20)	35.13 (9.11)	-0.59	0.56
Social anxiety [f]	18.31 (14.47)	22.07 (18.03)	-0.64	0.53
Social network complexity [g]	2.44 (1.03)	2.60 (1.35)	-0.38	0.71
Social network size [g]	28.88 (17.25)	30.07 (34.58)	-0.12	0.90

Notes. [a] Autistic-like traits were assessed by the AQ (Autism Spectrum Quotient). [b] Childhood trauma was assessed by the CTQ (Childhood Trauma Questionnaire.) [c] Depressive symptoms were assessed by the BDI (Beck's Depression Scale, Version II). [d] Empathy was assessed by the Saabrücker Persönlichkeitsfragebogen, a German version of the IRI (Interpersonality Reactivity Index). [e] Rumination was assessed by the RRQ (Rumination-Reflection Questionnaire). [f] Social anxiety was assessed by the LSAS (Liebowitz Social Anxiety Scale). [g] Social networks were examined with the SNI (Social Network Index questionnaire).

Table S3. Measurement of endocrine factors in participants with strong trauma disclosure

	Oxytocin (n = 16) Mean (± SD)	Placebo (n = 15) Mean (± SD)	*t*	*P*
Day 0 (Baseline)				
Cortisol baseline (µg/dl)	0.25 (0.18)	0.19 (0.17)	0.89	0.38
Cortisol post trauma (µg/dl)	0.15 (0.08)	0.20 (0.15)	-1.23	0.23
Cortisol post imaging (µg/dl)	0.14 (0.08)	0.16 (0.09)	-0.42	0.68
Estradiol (pg/ml)	127.09 (61.28)	127.07 (86.66)	<0.01	>0.99
FSH (U/l)	4.24 (2.70)	4.93 (2.84)	-0.66	0.51
LH (U/l)	9.15 (9.84)	11.37 (21.42)	-0.35	0.73
Oxytocin baseline (pg/ml)	46.80 (22.12)	57.17 (35.63)	-0.93	0.36
Oxytocin post trauma (pg/ml)	65.86 (54.62)	88.28 (74.19)	-0.90	0.38
Progesterone (ng/ml)	4.82 (6.44)	5.81 (6.53)	-0.41	0.69
Day 3 (after treatment)				
Cortisol baseline (µg/dl)	0.28 (0.28)	0.19 (0.12)	1.10	0.28
Cortisol post trauma (µg/dl)	0.18 (0.16)	0.17 (0.14)	0.14	0.89
Cortisol post imaging (µg/dl)	0.14 (0.11)	0.14 (0.09)	0.07	0.94
Estradiol (pg/ml)	113.83 (73.71)	107.46 (68.97)	0.24	0.81
FSH (U/l)	5.03 (4.32)	5.15 (3.10)	-0.09	0.93
LH (U/l)	13.64 (16.57)	12.74 (22.56)	0.13	0.90
Oxytocin baseline (pg/ml)	49.71 (40.56)	42.85 (22.03)	-0.54	0.59
Oxytocin post trauma (pg/ml)	46.76 (27.39)	73.14 (46.50)	-1.81	0.08
Progesterone (ng/ml)	5.18 (5.43)	5.68 (6.03)	-0.24	0.81

Notes. FSH, follicle-stimulating hormone; LH, luteinizing hormone; OXT, oxytocin; PLC, placebo.

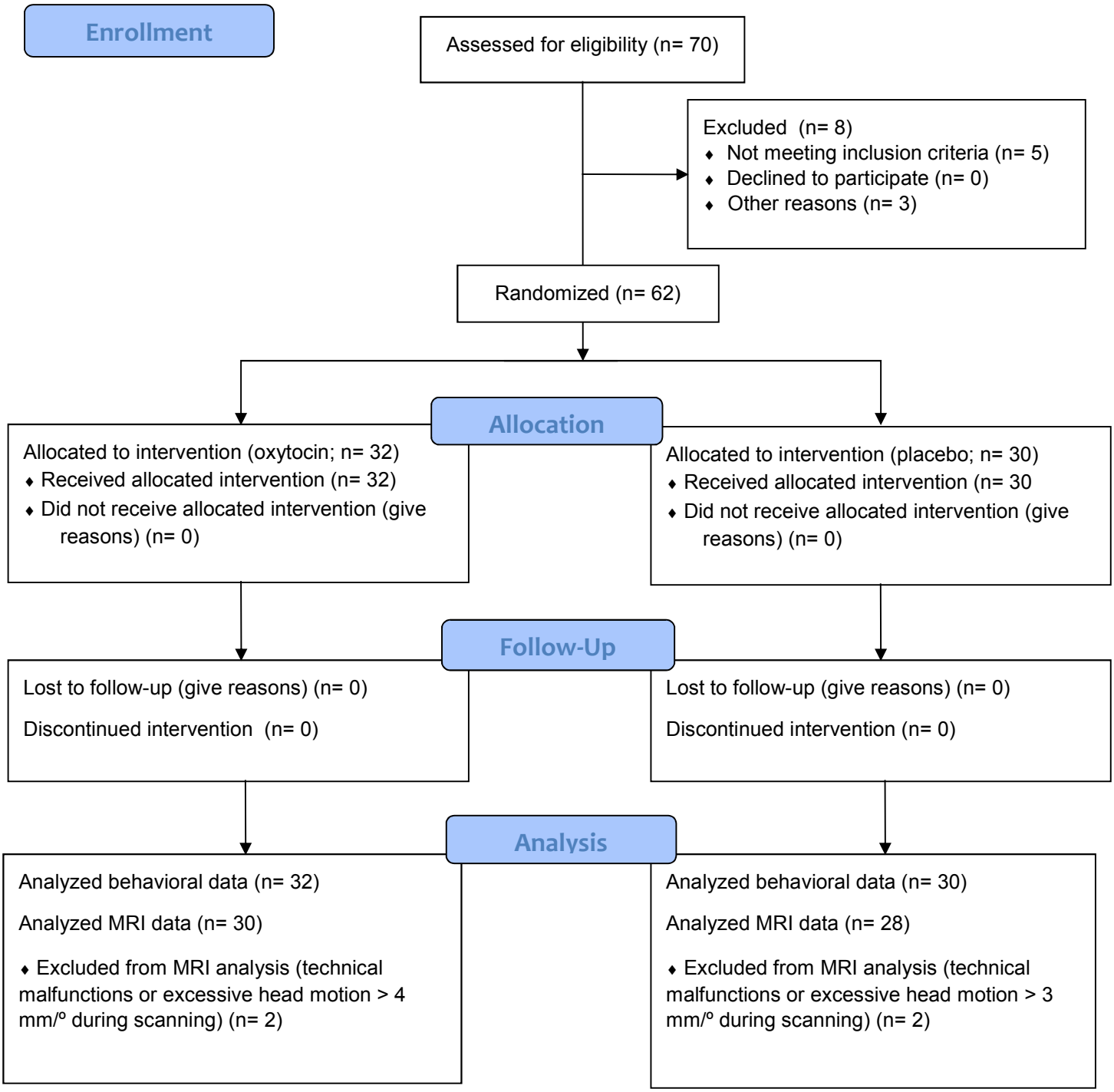
Table S4. Measurement of endocrine factors in participants with weak trauma disclosure

	Oxytocin (n = 16) Mean (± SD)	Placebo (n = 15) Mean (± SD)	*t*	*P*
Day 0 (Baseline)				
Cortisol baseline (µg/dl)	0.25 (0.33)	0.23 (0.21)	0.15	0.89
Cortisol post trauma (µg/dl)	0.25 (0.23)	0.17 (0.11)	1.25	0.23
Cortisol post imaging (µg/dl)	0.22 (0.24)	0.18 (0.17)	0.55	0.59
Estradiol (pg/ml)	129.78 (82.39)	145.69 (106.21)	-0.47	0.64
FSH (U/l)	5.19 (2.40)	5.26 (3.50)	-0.07	0.95
LH (U/l)	11.71 (10.54)	13.65 (15.87)	-0.40	0.69
Oxytocin baseline (pg/ml)	32.80 (17.46)	47.44 (48.41)	-1.03	0.31
Oxytocin post trauma (pg/ml)	45.04 (31.94)	47.20 (29.04)	-0.19	0.85
Progesterone (ng/ml)	5.74 (5.87)	7.18 (6.51)	-0.65	0.52
Day 3 (after treatment)				
Cortisol baseline (µg/dl)	0.25 (0.20)	0.20 (0.11)	0.88	0.38
Cortisol post trauma (µg/dl)	0.19 (0.15)	0.13 (0.07)	1.33	0.20
Cortisol post imaging (µg/dl)	0.15 (0.12)	0.11 (0.05)	1.29	0.21
Estradiol (pg/ml)	114.51 (76.34)	108.82 (41.31)	0.25	0.80
FSH (U/l)	4.05 (1.89)	3.95 (2.29)	0.12	0.90
LH (U/l)	6.67 (3.84)	8.59 (8.67)	-0.78	0.44
Oxytocin baseline (pg/ml)	23.32 (8.96)	49.18 (37.12)	-2.53	0.02
Oxytocin post trauma (pg/ml)	36.52 (25.48)	40.45 (30.42)	-0.34	0.73
Progesterone (ng/ml)	8.67 (7.90)	7.07 (5.01)	0.65	0.52

Notes. FSH, follicle-stimulating hormone; LH, luteinizing hormone; OXT, oxytocin; PLC, placebo.

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram



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