Kinetics of oxytocin effects on amygdala and striatal reactivity vary between women and men

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Supplementary Information

SUPPLEMENTARY MATERIALS AND METHODS

Ethics and enrollment

The study was approved by the local ethics committee of the Medical Faculty of the University of Bonn, Germany and carried out in accordance with the latest revision of the Declaration of Helsinki. The study was registered in the ClinicalTrials.gov database (Identifier: NCT03846739) provided by the US National Institutes of Health and data analyses were preregistered (https://osf.io/yxrsf/) prior to conducting any analyses. Participants were recruited from the local population by means of online advertisement and public postings between April 2017 and September 2018. All participants provided written informed consent and received monetary compensation after completion of the study. J.L., T.M., and L.S. enrolled participants and assigned participants to the treatment based on the random allocation sequence (for the double-blind within-subject oxytocin [OXT]/placebo [PLC] treatment and dose group) generated by D.S..

Participants

G*Power 3 [1] was used to conduct an a-priori power analysis for the project based on the effect size obtained in our dose-response study with men [2]. For the effect of OXT (24 international units [IU] and a latency of 45 minutes) on amygdala response to high intensity fearful faces an effect size of $d_z = 0.56$ was observed in a within-subject design. To detect an OXT effect of this size (with $\alpha = .05$ and power = .80), at least 28 participants needed to be tested in a within-subject design (i.e. at least 28 participants in each dose group).

A total of 105 participants were invited to the screening session (exclusively non-smoking, right-handed women, aged 18 to 40, who did not use any kind of hormonal contraceptives). Out of the screened participants, 13 were not eligible for enrollment (for exclusion criteria, see Screening session).

Screening session

Enrollment was preceded by a screening appointment to ensure that all subjects were free of any current physical or psychiatric illness as assessed by self-report and the Mini-International Neuropsychiatric Interview (MINI [3]). Furthermore, we confirmed that participants had not taken any over-the-counter psychoactive medication in the preceding four weeks. Additional exclusion criteria included use of hormonal contraceptives, pregnancy, and contraindications for MRI scanning. To further characterize the sample, we acquired sociodemographic data and neuropsychological questionnaires of each subject. We measured trait anxiety (State-Trait Anxiety Inventory, STAI [4]), depressive symptoms (Becks Depression Inventory, BDI-II [5]), and autistic-like traits (Autism-Spectrum Quotient, AQ [6]).

Intranasal treatment

Participants self-administered 6, 12, or 24 IU of synthetic OXT (depending on the dose group; Sigma-Tau Industrie Farmaceutiche Riunite S.p.A., Rome, Italy) or PLC via nasal spray at the beginning of the testing sessions. Thus, participants administered 3, 6, or 12 puffs balanced across nostrils with 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 (6 IU = 150 mg, 12 IU = 300 mg, 24 IU = 600 mg).

Neuroendocrine parameters

In order to validate the cycle phase and control for baseline differences in gonadal hormone levels, blood samples were collected at the beginning of each testing session. Serum estradiol was 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 limit of each assay was 11 pg/ml. The coefficients of variation for intra-assay and inter-assay precision were < 5.5 % and < 5.9 % respectively. 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 % respectively. The cross-reactivity of all assays with other related compounds was minimal.

Plasma samples for the measurement of OXT concentrations were collected with commercial sampling devices (Vacuette, Greiner Bio-One International, Austria) containing EDTA and aprotinin. Vacuettes were immediately centrifuged at 6000 rpm for 20 min, and aliquoted samples were stored at -80°C until assayed. OXT concentrations were extracted and quantified using a highly sensitive and specific radioimmunoassay (enterprise) [7]. The limit of detection was 0.1 - 0.5 pg, depending on the age of the tracer. Intra-assay and inter-assay coefficients of variability were < 10 %. All samples to be compared were assayed in the same batch, i.e. under intra-assay conditions.

Data analysis

Acquisition of functional MRI data

MRI data were collected using a 1.5-tesla Siemens Avanto MRI system (Siemens AG, Erlangen, Germany). 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 slice 19 and slice 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 [8]. 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: 256 x 256, voxel size: 1 x 1 x 1 mm, flip angle 15°, 160 sagittal slices).

Preprocessing

The first five volumes of each functional time series were discarded to allow for T1 signal equilibration. Images were corrected for head movement between scans by an affine registration. Subjects (n = 9) with excessive head movements (> 4 mm/° in any direction) were excluded from further analysis, as adequate correction could not be guaranteed. Images were initially realigned to the first image of the time-series and then re-realigned to the mean of all images. For spatial normalization, the unified segmentation function in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) was used and the mean EPI image of each participant was normalized to the Montreal Neurological Institute (MNI) template [9,10]. All images were then transformed into standard stereotaxic space and resampled at 2 x 2 x 2 mm voxel size. 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).

fMRI data analysis

A two-stage approach based on the general linear model implemented in SPM8 was used for statistical analyses. Participants' individual data were modeled using a fixed-effect model. Next, their summary data were analyzed at the group level using a random effects model. On the first level, five conditions (neutral, low fearful, high fearful, low happy, and high happy faces) were modeled by a stick function convolved with a hemodynamic response function (HRF) [11]. Movement parameters and button presses were included as confounds in the design matrix. We compared each condition with the baseline condition (white fixation cross). Contrast images were computed by applying linear contrasts to the individual parameter estimates of the response to the experimental conditions. On the first level, the emotional conditions were compared to the neutral face condition for each subject and separately for the two treatment sessions (i.e. [$PLC_{low\ fearful} > PLC_{neutral}$], [$PLC_{high\ fearful} > PLC_{neutral}$], [$PLC_{low\ happy} > PLC_{neutral}$], [$PLC_{high\ happy} > PLC_{neutral}$], [$OXT_{low\ fearful} > OXT_{neutral}$], [$OXT_{high\ fearful} > OXT_{neutral}$], [$OXT_{low\ happy} > OXT_{neutral}$], [$OXT_{high\ happy} > OXT_{neutral}$]). Furthermore, emotion-specific responses were pooled across emotional intensities (i.e. [$PLC_{fearful} > PLC_{neutral}$], [$PLC_{happy} > PLC_{neutral}$], [$OXT_{fearful} > OXT_{neutral}$], [$OXT_{happy} > OXT$

$_{neutral}$]) and treatment effects were calculated (e.g. [OXT $_{low\ fearful\ >\ neutral}$] > [PLC $_{low\ fearful\ >\ neutral}$], [OXT $_{fearful\ >\ neutral}$] > [PLC $_{fearful\ >\ neutral}$]).

On the second level, we conducted planned one sample *t*-tests in SPM across dose groups for treatment effects on emotion-specific contrasts as specified on the first level. For region of interest (ROI) analyses, the amygdala and striatal regions (putamen, caudate nucleus, and globus pallidus) were anatomically defined according to the aal atlas as implemented in the Wake Forest University Pick Atlas (wfu Pick Atlas) [12,13]. Nucleus accumbens was anatomically defined according to the IBASPM71 as implemented in the wfu Pick Atlas. *P* values < .05 after correction for multiple comparisons (family-wise error, FWE) based on the size of the ROIs were considered significant.

To uncover dose-dependent effects, parameter estimates of significant OXT effects on amygdala responses and striatal activation as response to fearful and happy faces were averaged across all voxels of anatomically defined ROIs and extracted using MarsBaR (http://marsbar.sourceforge.net). Parameter estimates were analyzed in SPSS 24 (IBM Corp., Armonk, NY) by calculating 2 x 2 x 3 mixed analyses of variance (ANOVAs) and Bonferroni-corrected (p_{corr}) *t*-tests. Treatment (OXT, PLC) and emotion (e.g. low fearful, neutral) were considered as within-subject factors, and dose group (6 IU, 12 IU, 24 IU) was defined as between-subject factor. Altogether, one ANOVA was conducted for the significant OXT effect on right amygdala responses to low fearful faces and one ANOVA for the significant OXT effect on right putamen reactivity to happy faces pooled across emotional intensities (see main results). Furthermore, ANOVAs were calculated to explore trend-to-significant OXT effects for right amygdala reactivity to fearful faces pooled across emotional intensities, for right putamen activation for both low and high happy faces, for right caudate nucleus responses to low happy faces, and for left nucleus accumbens reactivity to fearful faces pooled across emotional intensities as well as to high fearful faces only (see Supplementary Results). Finally, as an OXT effect was observed for amygdala responses to high fearful faces in healthy men [2], we also conducted an ANVOA to explore amygdala reactivity to high fearful faces. Since no evidence for a possible OXT effect on left amygdala activation was found (see main results), we focused on right amygdala activation.

Whole brain effects of OXT were explored for all emotion-specific contrasts as well as contrasts pooled across emotions. Whole brain analyses were conducted with a height threshold of $p < .001$. Again, FWE corrected p values < .05 were considered significant.

To explore potential moderator variables of observed OXT effects on activation in the amygdala or striatal ROIs, OXT effects were correlated with participants' behavioral, demographic, psychological, and neuroendocrine measurements using Pearson's product-moment correlations. Demographic, psychological variables, and baseline neuroendocrine parameters of both testing sessions as well as OXT effects on behavioral outcomes were correlated with parameter estimates of neural OXT effects (e.g. [OXT $_{\text{low fearful > neutral}}$] > [PLC $_{\text{low fearful > neutral}}$]). Furthermore, correlations with baseline neuroendocrine parameters were calculated separately for OXT and PLC testing sessions (i.e. baseline neuroendocrine parameters of the PLC testing session were correlated with parameter estimates of emotion-specific brain activation in the PLC session and baseline neuroendocrine parameters in the OXT session were correlated with parameter estimates of emotion-specific brain activation after OXT administration). Two-tailed p values < .05 were considered significant.

Finally, to statistically test sexual-dimorphic OXT effects, significant OXT effects in either our current female sample (i.e. OXT effects on right amygdala reactivity to low fearful faces and on right putamen reactivity to happy faces, see main results) or our previous male sample (i.e. OXT effects on left amygdala responses to high fearful faces, see [2]) were compared directly between sexes using the same mixed ANOVAs as described before with the additional between-subject factor sex (female, male). Parameter estimates of the 12-IU- and 24-IU-dose groups with a latency of 45 minutes only were used as dependent variables as OXT effects of these conditions were tested in both samples, resulting in 2 (treatment) x 2 (emotion) x 2 (dose) x 2 (sex) mixed ANOVAs. To disentangle significant interactions with sex, Bonferroni-corrected two-sample t-tests were conducted comparing the four baseline contrasts (e.g. [PLC $_{\text{neutral}}$], [PLC $_{\text{low fearful}}$], [OXT $_{\text{neutral}}$], and [OXT $_{\text{low fearful}}$]) between sexes. Furthermore, differences between the female 24-IU-dose group and the male 48-IU-dose group were analyzed in a 2 x 2 x 2 mixed ANOVA with the within-subject factors treatment and emotion and the between-subject factor sex. Sex differences in OXT plasma levels were examined by using two-sample t-tests for baseline differences across all dose and latency groups. OXT plasma levels after OXT administration (12 IU and 24 IU) were again compared using a

univariate ANOVA with dose and sex as between-subject variables. Two-sample *t*-tests were calculated to compare parameter estimates of significant neural OXT effects on right and left amygdala activity and right putamen responses between the female 6-IU-dose group and the male 12-IU-dose group as well as between the female 24-IU-dose group and the 48-IU-dose group of the male sample.

SUPPLEMENTARY RESULTS

Missing values

Plasma samples for measuring OXT concentrations were missing from two participants due to problems in sample assessment. Furthermore, six blood samples for measuring estradiol levels and 12 samples for measuring progesterone levels were lost because of problems in sample assessment or analysis. Behavioral data from three participants were not recorded in the fMRI sessions due to technical issues in the response grip system.

Measurements of mood and state anxiety

Treatment (OXT, PLC) or time (pre-treatment, post-treatment) had no effect on state anxiety as measured with STAI (see Table S2). However, a main effect of time was found for both the positive ($F_{(1,89)}$ = 46.62, p < .001, η_p^2 = .34) and negative ($F_{(1,89)}$ = 18.12, p <.001, η_p^2 = .17) PANAS (the Positive and Negative Affect Schedule [14]) mood scales. Positive and negative mood significantly decreased from the beginning of the testing sessions (mean ± SD positive: 28.4 ± 5.6; negative: 12.2 ± 3.2) to the end (mean ± SD positive: 25.4 ± 6.9; negative: 11.1 ± 2.1), indicating fatigue over the course of the experiment. No main or interaction effect of treatment was found (see Table S3).

Behavior

Task validation

A mixed ANOVA with hit rates under PLC as dependent variable revealed a main effect of emotion ($F_{(2.43,204.29)}$ = 270.47, p < .001, η_p^2 = .76) but no main or interaction effect with the dose group (all Fs < 0.72, all ps > .60), indicating that emotion recognition did not differ between dose groups. Hit rate was best for high happy faces (mean hit rate ± SD: 97 ± 5 %), followed by high fearful (93 ± 8 %), neutral (90 ± 10 %), low happy (69 ± 21 %), and low fearful faces (41 ± 18 %). Post-hoc tests comparing adjacent face conditions revealed significant differences for all comparisons (all ts > 4.71, all ps$_{corr}$ < .001, all ds > 0.63)

except for high fearful compared to neutral faces ($t_{(86)}$ = 2.10, p_{corr} = .16). Comparing reaction times (RT) for hit runs between emotional conditions yielded a main effect of emotion ($F_{(3.25,272.74)}$ = 128.63, p < .001, η_p^2 = .61), but no significant effects of dose group (all Fs < 1.89, all ps > .07). RT showed the same order across emotions as observed for hit rates (high happy [mean RT ± SD: 1.06 ± 0.20 s], high fearful [1.31 ± 0.23 s], neutral [1.32 ± 0.27 s], low happy [1.39 ± 0.31 s], and low fearful faces [1.64 ± 0.31 s]). Post-hoc tests yielded significant differences in RT between low happy and low fearful ($t_{(86)}$ = -8.15, p_{corr} < .001, d = -0.82) as well as between high happy and high fearful conditions ($t_{(86)}$ = -12.53, p_{corr} < .001, d = -1.17). No other comparisons remained significant after controlling for multiple testing (all ts < 2.21, all ps$_{corr}$ > .11).

OXT effects

Mixed ANOVAs with the additional factor treatment (PLC, OXT) confirmed the main effect of emotion ($F_{(2.29,192.58)}$ = 346.74, p < .001, η_p^2 = .81) on hit rates but showed neither a main effect nor an interaction with treatment or dose group (all Fs < 1.21, all ps > .30). Likewise, an ANOVA with RT as dependent variable showed the main effect of emotion ($F_{(3.33,279.77)}$ = 168.32, p < .001, η_p^2 = .67), but no significant effects of treatment or dose group (all Fs < 1.40, all ps > .21).

fMRI results

Effects of emotional faces of varying intensity on amygdala and striatal response

To explore whether baseline responses to emotional faces in contrast to neutral faces vary as a function of emotional intensity under PLC, two paired t-tests (one for responses to low vs. high fearful faces and one for low vs. high happy faces) were calculated for each anatomically defined ROI and hemisphere using parameter estimates of emotion-specific activation in the PLC condition as dependent variables (i.e. [PLC $_{\text{high fearful > neutral}}$] and [PLC $_{\text{low fearful > neutral}}$] as well as [PLC $_{\text{high happy > neutral}}$] and [PLC $_{\text{low happy > neutral}}$]). Results revealed no significant differences in the emotion-specific reactivity to high intensity faces in comparison to low intensity faces (all ts > -0.87 and < 1.90, all ps > .06) in any ROI.

OXT effects on right amygdala response

Across emotional intensities (i.e. [OXT $_{\text{fearful > neutral}}$] > [PLC $_{\text{fearful > neutral}}$]), a trend-to-significant OXT effect on the reactivity of the right amygdala (MNI peak coordinates *x, y, z*: 28, -2, -16, $t_{(78)}$ = 3.10, *k* = 123, p_{FWE} = .06) was found. Mixed ANOVAs with extracted parameter estimates averaged across all right amygdala voxels as dependent variables revealed main effects of emotion for fearful ($F_{(1,76)}$ = 11.85, *p* = .001, η_p^2 = .14) and low fearful faces ($F_{(1,76)}$ = 5.35, *p* = .02, η_p^2 = .07) in contrast to neutral faces across treatment conditions and dose groups in addition to the reported treatment x emotion interaction (see main results). No main effect of treatment was observed for fearful ($F_{(1,76)}$ = 1.55, *p* = .22) or low fearful faces ($F_{(1,76)}$ = 1.95, *p* = .17). To investigate right amygdala responses to high fearful faces, mixed ANOVAs were conducted that revealed a main effect of emotion ($F_{(1,76)}$ = 10.63, *p* = .002, η_p^2 = .12), indicating heightened reactivity to high fearful in contrast to neutral faces. Importantly, no interaction of treatment and dose group was observed (all other *F*s < 2.17, all *p*s > .12).

OXT effects on striatal response

In addition to OXT effects reported in the main text on right putamen reactivity to happy faces, further trend-to-significant effects of OXT were found on striatal responses. Separate analyses for high and low emotional intensities revealed trend-to-significant OXT effects on right putamen responses to low happy (MNI peak coordinates *x*, *y*, *z*: 32, 12, 2, $t_{(78)}$ = 3.52, *k* = 277, p_{FWE} = .07; see Figure 2C) and high happy faces (MNI peak coordinates *x*, *y*, *z*: 34, -18, -4, $t_{(78)}$ = 3.55, *k* = 28, p_{FWE} = .07). Furthermore, a trend-to-significant OXT effect was observed on the right caudate nucleus responses to low happy faces in contrast to neutral faces (i.e. [OXT $_{\text{low happy > neutral}}$] > [PLC $_{\text{low happy > neutral}}$]; MNI peak coordinates *x*, *y*, *z*: 6, 12, 2, $t_{(78)}$ = 3.53, *k* = 149, p_{FWE} = .07). Interestingly, a trend-to-significant OXT effect on left nucleus accumbens reactivity to fearful (MNI peak coordinates *x*, *y*, *z*: -12, 4, -12, $t_{(78)}$ = 2.50, *k* = 27, p_{FWE} = .07) and high fearful faces (MNI peak coordinates *x*, *y*, *z*: -10, 6, -8, $t_{(78)}$ = 2.59, *k* = 15, p_{FWE} = .06) indicated that OXT effects on striatal reactivity were not limited to positive social cues as happy faces. Analyses of left and right pallidum activation revealed no significant OXT effects (all *t*s < 2.82, all FWE-corrected *p*s > .12).

Further mixed ANOVAs to investigate neural OXT effects on right putamen responses to happy or low happy faces revealed no main or interaction effects apart from the interaction of treatment and emotion as well as the trend-to-significant interaction of treatment and dose group as reported in the main text (all other Fs < 2.35, all ps > .10). Post-hoc tests to further analyze the trend-to-significant dose-dependent OXT effect on right putamen reactivity revealed an enhanced OXT effect on the reactivity to social stimuli after 24 IU and 12 IU compared to 6 IU of OXT, although the comparisons did not reach significance after controlling for multiple testing (24 IU vs. 6 IU: $t_{(36.67)} = 2.24$, $p = .03$ [$p_{corr} = .09$]; 12 IU vs. 6 IU: $t_{(52)} = 2.15$, $p = .04$ [$p_{corr} = .11$]). There were no significant differences between the 24-IU- and 12-IU-dose groups ($t_{(49)} = 0.50$, $p = .62$ [$p_{corr} \approx 1.00$]). The same pattern of results was found for the OXT effect across low happy and neutral faces (24 IU vs. 6 IU: $t_{(34.87)} = 2.19$, $p = .04$ [$p_{corr} = .11$]; 12 IU vs. 6 IU: $t_{(52)} = 2.32$, $p = .02$ [$p_{corr} = .07$]; 24 IU vs. 12 IU: $t_{(49)} = 0.46$, $p = .65$ [$p_{corr} \approx 1.00$]; see Figure 2D). Regarding right putamen responses to high happy faces, a further trend-to-significant interaction of treatment and dose group was detected ($F_{(2,76)} = 2.87$, $p = .06$, $\eta_p^2 = .07$; all other Fs < 2.44, all ps > .12). Exploratory post-hoc tests to disentangle the interaction of treatment and dose group revealed comparable results as reported for happy and low happy faces (24 IU vs. 6 IU: $t_{(38.78)} = 2.24$, $p = .03$ [$p_{corr} = .09$]; 12 IU vs. 6 IU: $t_{(52)} = 1.94$, $p = .06$ [$p_{corr} = .17$]; 24 IU vs. 12 IU: $t_{(49)} = 0.54$, $p = .59$ [$p_{corr} \approx 1.00$]). Mixed ANOVAs with parameter estimates extracted from right caudate nucleus confirmed an OXT effect on responses to low happy compared to neutral faces (interaction of treatment x emotion: $F_{(1,76)} = 4.72$, $p = .03$, $\eta_p^2 = .06$). No other effects of treatment, emotion, or dose group were found for caudate nucleus responses to happy, low happy, or high happy faces (all Fs < 2.36, all ps > .10). Post-hoc tests indicated that OXT enhanced the right caudate nucleus reactivity to low happy compared to neutral faces; however, comparisons did not reach significance when controlling for multiple testing (low happy vs. neutral after OXT administration: $t_{(78)} = 2.13$, $p = .04$ [$p_{corr} = .15$]; for all other comparisons ts < 1.67, all ps > .09 [all ps$_{corr}$ > .39]).

Finally, extracted parameter estimates across all voxels of the left nucleus accumbens were analyzed to further investigate the OXT effects on responses to fearful faces. Mixed ANOVAs revealed a significant interaction of treatment and emotion for responses to fearful faces across emotional intensities ($F_{(1,76)} = 4.30$, $p = .04$, $\eta_p^2 = .05$) as well as a trend-to-significant interaction of treatment and emotion for responses to low fearful faces ($F_{(1,76)} = 3.19$, $p = .08$, $\eta_p^2 = .04$). Furthermore, a trend-to-significant interaction of

emotion and dose group indicated differences in nucleus accumbens reactivity to low fearful faces across treatments depending on dose group ($F_{(2,76)}$ = 2.74, p = .07, η_p^2 = .07). No other effects were detected for nucleus accumbens responses to fearful or low fearful faces (all other Fs < 1.71, all ps > .18) and post-hoc comparisons to disentangle the OXT effects on reactivity to fearful and low fearful faces did not reach significance (all ts < 1.74, all ps > .08 [all ps$_{corr}$ > .34]). Exploratory post-hoc tests to disentangle the interaction of emotion with dose group indicated enhanced reactivity to low fearful compared to neutral faces across treatments in the dose group receiving 12 IU in contrast to 6 IU ($t_{(43.48)}$ = 2.34, p = .02 [p_{corr} = .07]; all other ts < 1.17, all ps > .24 [all ps$_{corr}$ > .16]). No effects of treatment, emotion, or dose group were found for nucleus accumbens responses to high fearful faces (all Fs > 2.78, all ps > .09).

Brain-behavior correlation

To examine whether behavioral outcomes (hit rates, RTs, proportion of faces rated as neutral for low emotional intensity faces) correlated with neural OXT effects, we conducted Pearson's product-moment-correlations. The OXT effect on RTs to fearful faces negatively correlated with the OXT effect on left nucleus accumbens reactivity to fearful ($r_{(75)}$ = -.24, p = .03) and high fearful faces ($r_{(75)}$ = -.25, p = .03), indicating that a greater neural OXT effect was associated with improved (i.e. smaller) RTs compared to the PLC condition. Furthermore, the OXT effect on left nucleus accumbens reactivity to low fearful faces significantly correlated with RTs to neutral ($r_{(75)}$ = -.25, p = .03), fearful ($r_{(75)}$ = -.26, p = .03), and high fearful faces ($r_{(75)}$ = -.30, p = .008), again indicating that improved RTs after OXT compared to PLC were linked to greater neural OXT effects. No other correlations of neural OXT effects with behavioral outcomes were observed (all rs > -.21 and < .19, all ps > .08).

Brain-neuroendocrinology correlation

Across dose groups, emotion-specific amygdala reactivity to fearful faces pooled across emotional intensities and to low fearful faces in the PLC session was significantly greater in participants with higher OXT baseline levels (fearful: $r_{(78)}$ = .32, p = .004; low fearful: $r_{(78)}$ = .32, p = .004; see Figure S1A), indicating that not only exogenously increased OXT levels but also higher endogenous OXT

concentrations are associated with stronger amygdala reactivity to negative stimuli. Baseline OXT levels in the OXT session were not associated with neural reactivity to any kind of emotional faces (all *r*s > -.16 and < .05, all *p*s > .16), but the increase of plasma OXT levels after OXT administration significantly correlated with emotion-specific right putamen responses to happy ($r_{(76)}$ = .27, p = .02), low happy ($r_{(76)}$ = .26, p = .03), and high happy faces ($r_{(76)}$ = .23, p = .04) in the OXT session. Thus, a greater increase in the OXT concentration seems to result in greater increases in putamen responses to positive social stimuli compared to neutral stimuli. Furthermore, estradiol levels negatively correlated with emotion-specific right putamen responses to low happy faces after OXT administration ($r_{(73)}$ = -.23, p = .048), and emotion-specific amygdala reactivity to low fearful faces after OXT administration positively correlated with the progesterone/estradiol ratio ($r_{(72)}$ = .30, p = .009; see Figure S1B), while there was no significant correlation in the PLC session, indicating a possible interaction between OXT and steroid levels.

The correlation of baseline neuroendocrinological measurements with neural OXT effects showed that greater neural OXT effects on amygdala reactivity to fearful faces pooled across emotional intensities ([OXT $_{\text{fearful > neutral}}$] > [PLC $_{\text{fearful > neutral}}$]) and to low fearful faces ([OXT $_{\text{low fearful > neutral}}$] > [PLC $_{\text{low fearful > neutral}}$]) negatively correlated with baseline OXT concentrations of the PLC testing session (fearful: $r_{(78)}$ = -.34, p = .002; low fearful: $r_{(78)}$ = -.30, p = .007) and the OXT testing session (fearful: $r_{(76)}$ = -.30, p = .007; low fearful: $r_{(76)}$ = -.30, p = .008). Likewise, neural OXT effects on right caudate nucleus reactivity to low happy faces ([OXT $_{\text{low happy > neutral}}$] > [PLC $_{\text{low happy > neutral}}$]) negatively correlated with baseline OXT concentrations of the PLC testing session ($r_{(78)}$ = -.23, p = .04). These findings might indicate a ceiling effect in participants with already high endogenous OXT concentrations and further imply that participants with low endogenous OXT concentrations might profit the most from intranasal OXT. Again, a possible interaction between OXT and steroid levels was indicated by a negative correlation of estradiol levels in the PLC testing session with neural OXT effects on right caudate nucleus reactivity to low happy faces ($r_{(77)}$ = -.25, p = .03). Analyses revealed no further correlations for PLC (all *r*s > -.21 and < .14, all *p*s > .07) or OXT sessions (*r*s > -.20 and < .18, all *p*s > .08) or for neural OXT effects (all *r*s > -.21 and < .21, all *p*s > .07).

Further correlation analyses

We found no significant correlations between neural OXT effects and age or body mass index (all *r*s > -.20 and < .18, all *p*s > .07). Regarding psychometric measurements, neural OXT effects on putamen reactivity to high happy faces correlated negatively with AQ scores ($r_{(78)}$ = -.26, p = .02), whereas OXT effects on caudate nucleus responses to low happy faces correlated negatively with BDI scores ($r_{(78)}$ = -.24, p = .03), indicating higher neural OXT effects for participants with lower autistic-like traits or depressive symptoms. No correlations of trait anxiety with neural OXT effects were found (all *r*s > -.13 and < .11, all *p*s > .27).

Blinding of treatment

Chi-squared tests were used to validate blinding of treatment. Correct guess of treatment in the OXT session did not differ from chance for 6 IU (correct estimates: 50.0 %; $\chi^2_{(1)}$ = 0, $p \approx 1$) and the 12-IU-dose group (correct estimates: 43.3 %; $\chi^2_{(1)}$ = 0.53, p = .47). The proportion of correct estimates after 24 IU of OXT was even worse than chance (correct estimates: 30.0 %; $\chi^2_{(1)}$ = 4.80, p = .03). However, dose groups did not significantly differ in their estimates ($\chi^2_{(2)}$ = 2.57, p = .28).

Side effects

No serious side effects occurred and proportion of reported side effects (tiredness, headache, dizziness, unspecific uncomfortableness, stomach pain) did not differ between OXT and PLC sessions (PLC: 6 %; OXT: 8 %; $\chi^2_{(1)}$ = 0.45, p = .50). Importantly, side effects in the OXT session were equally distributed between dose groups ($\chi^2_{(2)}$ = 0.31, p = .86).

Comparison of OXT levels between sexes

In order to examine possible sex differences in endogenous OXT concentrations, we conducted two sample *t*-tests across dose and latency groups with baseline OXT plasma levels as dependent variables separately for each testing session. No significant differences in baseline OXT plasma levels between

sexes were observed for either the PLC ($t_{(136.40)}$ = -0.67, p = .51) or the OXT testing session ($t_{(148.45)}$ = 0.07, p = .95). Furthermore, as baseline peripheral OXT levels do not necessarily correspond to central OXT concentrations [15], concentrations after OXT administration were also compared between sexes using a univariate ANOVA with sex and dose (12 IU, 24 IU) as between-subject factors. Only male participants of the 45-minutes-latency group were included to keep the timing of blood sampling after the administration comparable between male and female participants. Results revealed a main effect of sex ($F_{(1,100)}$ = 8.30, p = .005, η_p^2 = .08), with higher OXT levels in women compared to men (mean OXT concentration ± SD [pg/ml] of women: 6.55 ± 2.00; men: 5.13 ± 3.29). No effects of dose were observed (all Fs < 2.54, all ps > .11).

Comparison of sexual-dimorphic OXT effects between doses

To further test the hypothesized shift of the dose-response function to lower doses in women compared to men, parameter estimates of significant OXT effects (right amygdala: [OXT $_{\text{low fearful > neutral}}$] > [PLC $_{\text{low fearful > neutral}}$]; left amygdala: [OXT $_{\text{high fearful > neutral}}$] > [PLC $_{\text{high fearful > neutral}}$]; right putamen: [OXT $_{\text{happy > neutral}}$] > [PLC $_{\text{happy > neutral}}$]) were compared between the female 6-IU-dose group and the male 12-IU-dose group and between the 24-IU-dose group and the 48-IU-dose group. No significant differences were found for the effects of lower doses in women in contrast to higher doses in men (all ts > -0.81 and < 1.80, all ps > .07). However, the significant treatment x sex interactions and the absence of a treatment x dose x sex interaction for neural OXT effects of 12 and 24 IU (see main text) indicate that these sex-specific effects are not only a byproduct of the hypothesized shifted dose-response function initiated at lower doses in women.

SUPPLEMENTARY DISCUSSION

Biological function of OXT

In women, endogenous OXT is primarily involved in labor and birth [16] but also related to various social behaviors such as affiliative touch or maternal behaviors [17]. A comparison of OXT plasma levels after intranasal administration with levels after endogenous OXT release shows that OXT plasma levels in the current study after OXT nasal spray administration (mean OXT concentration after intranasal administration across dose groups ± SD [pg/ml]: 5.64 ± 2.13) were much lower than OXT concentrations during labor and birth, with the majority of previous studies reporting OXT plasma levels between 17 and 85 pg/ml (cf. [16]). However, the observed OXT concentrations after intranasal application of 12 and 24 IU of OXT (mean OXT concentration ± SD [pg/ml]: 6.55 ± 2.00) are similar to OXT levels measured in breast-feeding mothers (cf. [18]). Given enhanced striatal reactivity to positive social stimuli after OXT administration, our study is in line with the well-known role of OXT for human attachment [19]. From an evolutionary perspective, heightened OXT levels as triggered by maternal behavior might also improve the mother's protective behavior by enhancing amygdala reactivity and thus the salience of threat signals. Importantly, as no dose-dependent effects of OXT on amygdala activation were found in the current study, even a small increase in OXT concentrations as evident in breast-feeding women might facilitate the protection of the defenseless offspring who depends on the mother's alertness.

Clinical implications

Notably, all our participants were naturally-cycling women. Given evidence for diminished OXT effects in women using hormonal contraceptives [20], our study does not allow to extrapolate our results to the kinetics of OXT effects in women using hormonal contraception. This has important implications for clinical research as OXT is considered a promising candidate compound for treating various mental disorders associated with a higher prevalence rate in women such as anxiety disorders [21]. As the use of hormonal contraceptives is widely spread [22], future studies are warranted to compare (clinical) effects of OXT between women with and without hormonal contraception.

Furthermore, any attempts to translate OXT into clinical practice necessitate a strict control of the therapeutic context. Our results indicate that OXT might facilitate avoidance behavior in women due to enhanced salience of negative stimuli. Nevertheless, in a positive context, OXT might also promote prosocial behavior in women [23], which is in line with our findings of enhanced striatal reactivity to positive social stimuli. Thus, the application of OXT in a clinical setting might be used to enhance the salience of a positive context such as social support offered by the therapist [24,25]. Increased perceived social support could further improve the patient-therapist relationship which predicts a better therapy outcome [26-28]. The notion of positive effects of OXT on social functioning in women is in line with favorable outcomes of OXT administration as previously observed in women with posttraumatic stress disorder [29-31] or borderline personality disorder [32,33].

SUPPLEMENTARY TABLES

Table S1. Demographics and psychological screening

	6 IU (n=30)	**12 IU** (n=30)	**24 IU** (n=30)	***F***	***p***
Age (years)	24.2 (3.9)	22.9 (4.3)	24.8 (4.9)	1.42	.25
Education (years)	16.6 (2.8)	15.1 (2.7)	16.1 (2.9)	2.02	.14
AQ[1]	14.2 (5.3)	13.4 (4.6)	13.6 (4.8)	0.22	.80
BDI[2]	2.9 (3.7)	2.2 (3.5)	3.4 (5.0)	0.61	.55
STAI[3]	31.5 (7.2)	28.8 (6.3)	30.5 (7.7)	1.14	.33

Notes. Values are mean and SD. [1]Autistic-like traits were measured with the Autism-Spectrum Quotient (AQ). [2]Depressive symptoms were measured with the Beck Depression Inventory, Version II (BDI). [3]Trait anxiety was measured with the State-Trait Anxiety Inventory (STAI). Abbreviations: AQ, Autism-Spectrum Quotient; BDI, Beck Depression Inventory, Version II; IU, international units; STAI, State-Trait Anxiety Inventory.

Table S2. Mood measurements

	PLC session		OXT session					
					F_{treat}	*p*	$F_{treat*time}$	*p*
	pre	post	pre	post				
STAI[1]	34.7 (7.0)	34.5 (7.3)	34.6 (6.8)	35.0 (7.2)	0.14	.71	0.91	.34
PANAS[2]								
- pos.	28.0 (6.7)	25.6 (7.7)	28.8 (5.8)	25.2 (7.4)	0.05	.82	3.93	.05
- neg.	12.3 (4.7)	11.2 (2.5)	12.1 (3.3)	11.1 (2.1)	0.39	.54	0.01	.91

Notes. Values are mean and SD. [1]State anxiety was measured with the State-Trait Anxiety Inventory (STAI). [2]Mood was measured with the Positive and Negative Affect Schedule (PANAS). *N* = 90. Abbreviations: neg., negative scale of the Positive and Negative Affect Schedule (PANAS); OXT, oxytocin; PLC, placebo; pos., positive scale of the PANAS; STAI, State-Trait Anxiety Inventory; time, pre and post treatment; treat, treatment (PLC or OXT).

Table S3. Baseline neuroendocrine parameters

	PLC session			OXT session						
	6 IU (n = 29)[1]	**12 IU** (n = 30)[2]	**24 IU** (n = 29)[3]	**6 IU** (n = 29)[1]	**12 IU** (n = 30)[2]	**24 IU** (n = 29)[3]	F_{treat}	p	F_{dose}	p
Estradiol (pg/ml)	134.4 (78.9)	103.0 (63.0)	107.7 (57.1)	146.4 (102.6)	122.7 (74.1)	131.4 (103.1)	3.59	.06	1.29	.28
Progesterone (ng/ml)	5.6 (5.4)	7.5 (6.4)	5.9 (5.0)	5.6 (5.8)	6.9 (6.1)	5.5 (5.1)	0.26	.61	0.91	.41
Progesterone/ estradiol-ratio	0.05 (0.04)	0.06 (0.04)	0.05 (0.04)	0.05 (0.04)	0.06 (0.06)	0.05 (0.04)	0.03	.86	1.36	.26
Oxytocin (pg/ml)	1.8 (0.4)	1.6 (0.3)	1.9 (0.5)	1.8 (0.4)	1.7 (0.4)	1.8 (0.4)	0.01	.92	2.36	.10

Notes. Values are mean and SD. [1]n = 28 for analyses of progesterone level and [2]n = 25 for progesterone/estradiol ratio. [3]n = 25 for analyses of both estradiol and progesterone levels as well as progesterone/estradiol-ratio. Abbreviations: dose, dose group (6, 12, or 24 international units, IU); OXT, oxytocin; PLC, placebo; treat, treatment (PLC or OXT).

SUPPLEMENTARY FIGURES

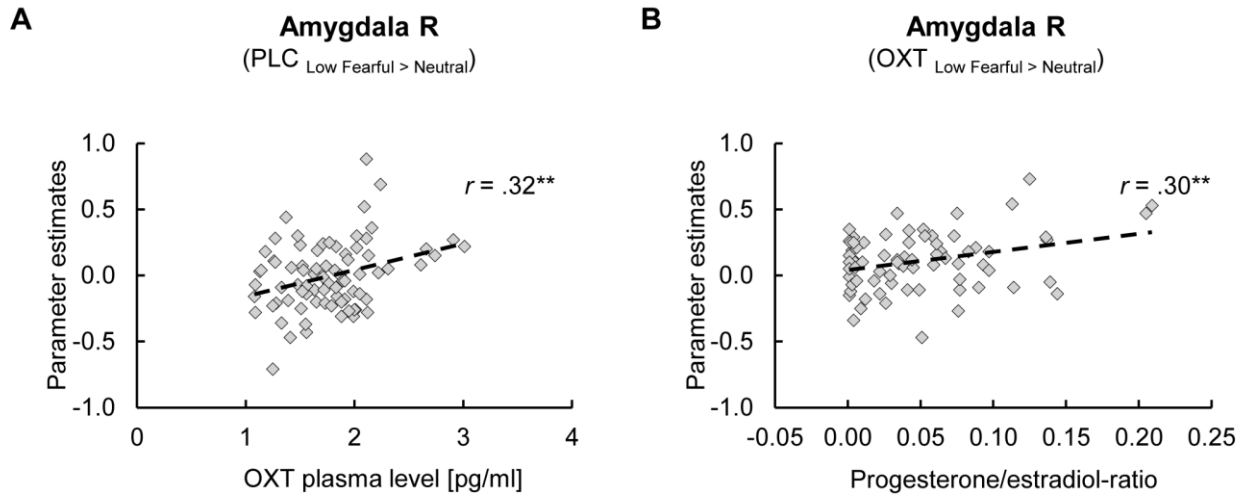

Figure S1. (A) Baseline oxytocin (OXT) plasma levels positively correlated with parameter estimates of enhanced right (R) amygdala responses to low fearful compared to neutral faces under the placebo (PLC) condition ($r_{(78)}$ = .32, p = .004). **(B)** Baseline progesterone/estradiol ratio correlated positively with parameter estimates of enhanced right amygdala reactivity to low fearful compared to neutral faces after OXT administration ($r_{(72)}$ = .30, p = .009). Abbreviations: OXT, oxytocin; PLC, placebo; R, right. **p < .01.

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram

Enrollment

Assessed for eligibility (n= 105)

Excluded (n= 13)
- Not meeting inclusion criteria (n= 11)
- Declined to participate (n= 0)
- Other reasons (n= 2, moved away/no time for fMRI sessions)

Randomized (n= 92)

Allocation to treatment condition

Dose: 6 IU	Dose: 12 IU	Dose: 24 IU
Allocated to intervention (n=31)	Allocated to intervention (n=31)	Allocated to intervention (n=30)
PLC (n= 14)	PLC (n= 16)	PLC (n= 15)
OXT (n= 17)	OXT (n= 15)	OXT (n= 15)
OXT (n= 13) ♦ Excluded (n= 1, no longer met inclusion criteria)	OXT (n= 15) ♦ Excluded (n= 1, no longer met inclusion criteria)	OXT (n= 15)
PLC (n= 17)	PLC (n= 15)	PLC (n= 15)
Analysed (n= 28) ♦ Excluded from analysis - due to head movements (n= 2) - due to technical issues (n= 0)	Analysed (n= 26) ♦ Excluded from analysis - due to head movements (n= 3) - due to technical issues (n= 1)	Analysed (n= 25) ♦ Excluded from analysis - due to head movements (n= 4) - due to technical issues (n= 1)

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