Supporting Information

Exogenous estradiol and oxytocin modulate sex differences in hippocampal reactivity during the encoding of episodic memories

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Supplementary Methods

Pharmacokinetic pre-study

We conducted a prestudy involving 10 healthy participants (5 women; mean age ± SD = 24.10 ± 4.07 years), to examine the pharmacokinetics of estradiol gel (Estramon 2 mg estradiol, Hexal AG, Holzkirchen, Germany) administration. Blood samples were taken prior to estradiol administration (i.e., baseline) and in 1-hour intervals after drug application up to 5 hours post administration. An additional blood sample was taken the next day (after 18 hours). Serum estradiol levels peaked 3-4 hours after gel administration, but a significant increase relative to baseline was already evident after 2 hours ($t_{(9)} = 2.44$, $p = 0.04$, $d = 1.10$; **cf.** (Coenjaerts et al., 2021)). Estradiol levels remained significantly elevated throughout the last measurement. A previous study tested the topical administration of a different drug (Divigel, Orion Pharma AG, Zug, Switzerland) containing 2 mg estradiol and found significantly increased estradiol serum concentrations as soon as 1 hour after administration and maximum average levels after 2 hours (Eisenegger et al., 2013).

Experimental design

Questionnaires assessing mood (Positive and Negative Affect Schedule [PANAS] (Watson et al., 1988)) and state anxiety (State-Trait Anxiety Inventory [STAI] (Spielberger, 1970)) were administered twice, first directly following the EST_{tra} or PLC_{tra} treatment at the beginning of the testing session and after the fMRI session. The study was carried out at the University Hospital Bonn. The data collection started in October 2018 and was completed in January 2020. Data collection was completed before the start of the COVID-19 pandemic. M.C., I.T. and B.A. enrolled participants and assigned participants to the treatment based on the random allocation sequence (double-blind) generated by D.S.. To generate the random allocation sequence, an integer generator was used (https://www.random.org/).

Memory performance

To quantify memory performance, a combined score involving the number of correct responses (hits) and number of false alarms is often used, since neither one should be interpreted without the other (Haatveit et al., 2010). Simply calculating hits minus false alarms would lead to heavily skewed scores in the case of many false alarms. Thus, both scores were z-standardized allowing for comparisons of measures with different ranges of absolute values (Macmillan and Creelman, 1990).

Statistical analyses

Demographic and psychological data (i.e., age, autistic-like traits, depressive symptoms, alexithymia, trait anxiety and social anxiety) were considered covariates in the main analyses with significant behavioural (*d'*) and neural outcomes (i.e., parameter estimates of significant contrasts of interests). As an additional explorative measure, confidence ratings were used to calculate *Meta d'* for each participant, which reflects the participants' metacognitive sensitivity and thus the efficacy in which the confidence ratings of the participants discriminate between correct and incorrect judgements. *Meta d'* can be directly compared to *d'*. If *meta d'* equaled *d'*, the participant was acting in a metacognitively ideal manner. If *Meta d'* was not equal to *d'*, the participants either underperformed or outperformed the expectation (Maniscalco and Lau, 2012; Rouault et al., 2018). To compute *Meta d'*, we used code provided by Maniscalco and Lau (2012, http://www.columbia.edu/~bsm2105/type2sdt/).

fMRI data acquisition and analysis

Functional data were obtained using a T2*-weighted echoplanar (EPI) sequence [repetition time (TR) = 2690 ms, echo time (TE) = 30 ms, ascending slicing, matrix size: 96 x 96, voxel size: 2 x 2 x 3 mm^3, slice thickness = 3.0 mm, distance factor = 10%, field of view (FoV) = 192 mm, flip angle = 90°, 41 axial slices]. High-resolution T1-weighted structural images were collected on the same scanner (TR = 1660 ms, TE = 2.54 ms, matrix size: 256 x 256, voxel size: 0.8 x 0.8 x 0.8 mm^3, slice thickness = 0.8 mm, FoV = 256 mm, flip angle = 9°, 208 sagittal slices). To control for inhomogeneity of the magnetic field, fieldmaps were obtained for each T2*-weighted EPI sequence and included during preprocessing of the fMRI data (TR = 392 ms, TE [1] = 4.92, TE [2] = 7.38, matrix size: 64 x 64, voxel size: 3 x 3 x 3, slice thickness = 3.0 mm, distance factor = 10%, FoV = 192 mm, flip angle 60°, 37 axial slices).

Preprocessing

The first five volumes of each functional time series were discarded to allow for T1 equilibration. Functional images were corrected for head movements between scans by an affine registration. Images were initially realigned to the first image of the time series before being re-realigned to the mean of all images. To correct for signal distortion based on B0-field inhomogeneity, the images were unwarped by applying the voxel displacement map (VDM file) to the EPI time series (Realign & Unwarp). Normalization parameters were determined by segmentation and nonlinear warping of the structural scan to reference tissue probability maps in Montreal Neurological Institute (MNI) space. Normalization parameters were then applied to all functional images, which were resampled at 2 x 2 x 2 mm^3 voxel size. For spatial smoothing, a 6-mm full width at half maximum (FWHM) Gaussian kernel was used. Raw time series were detrended using a high-pass filter (cut-off period 128 s).

fMRI data analyses

In addition to the model described in the main text, we used another model with twelve conditions (valence (3) x memory (2) x sociality (2)) which were modelled by a stick function convolved with a haemodynamic response function. Button presses were included as regressors of no interest. Furthermore, to exclude scans of participants with excessive head movements, the artefact detection toolbox was used (ART; http://www.nitrc.org/projects/artifact_detect) to identify high motion volumes using a volume-to-volume shift of >1.5 mm and a volume-to-volume change in mean signal intensity of >3 standard deviations. Artefacts were treated as regressors of no interest in the following analysis. Participants with >20% volumes identified as outliers by ART were excluded (n = 1 participant had to be excluded, but this person did not complete the surprise recognition task either).

On the first level, task-specific effects were modelled (e.g.: [Remembered > Forgotten], [Negative and Positive $_{\text{remembered}}$ > Negative and Positive $_{\text{forgotten}}$], [Negative $_{\text{remembered}}$ > Negative $_{\text{forgotten}}$], [Neutral $_{\text{remembered}}$ > Neutral $_{\text{forgotten}}$], [Positive $_{\text{remembered}}$ > Positive $_{\text{forgotten}}$], [Negative and Positive $_{\text{remembered > forgotten}}$ > Neutral $_{\text{remembered > forgotten}}$], [Negative $_{\text{remembered > forgotten}}$ > Neutral $_{\text{remembered > forgotten}}$], and [Positive $_{\text{remembered > forgotten}}$ > Neutral $_{\text{remembered > forgotten}}$]).

To further examine the potential influence of sex, estradiol, and oxytocin on task-based functional connectivity, a generalized psychophysiological interaction (gPPI) analysis was conducted with the anatomical regions-of-interest (ROIs) as seeds. The analysis was operated with the same preprocessed data, regressors, and contrasts that were used in the SPM analyses. The CONN toolbox (v19.c., www.nitrc.org/projects/conn, RRID:SCR_009550, Whitfield-Gabrieli and Nieto-Castanon, 2012) was used to analyse task-based functional connectivity.

Questionnaires

To characterize the sample, we assessed depressive symptoms (Becks Depression Inventory ([BDI], Beck et al., 1996)), alexithymia (Toronto Alexithymia Scale ([TAS], Taylor et al., 1985)), social anxiety (Liebowitz Social Anxiety Scale ([LSAS], Liebowitz, 1987)), autistic-like traits (Autism Spectrum Quotient ([AQ], Baron-Cohen et al., 2001)) and trait anxiety (State Trait Anxiety Inventory ([STAI], Spielberger, 1970). For the presentation of the questionnaires, Qualtrics software was used (Provo, USA). To measure baseline memory performance, the participants completed an adapted version of the Verbal Learning and Memory Test ([VLMT], Helmstaedter and Durwen, 1990). In line with our preregistration, we planned to exclude participants with an abnormal memory (i.e., learning performance ± 3 SD different from the mean). None of the participants showed abnormal memory performance, thus resulting in no further exclusions.

Neuroendocrine parameters

1.8.1. Estradiol and other gonadal hormones

Serum estradiol, testosterone and progesterone were determined by fully automated electrochemiluminescent immunoassays (ECLIA, Elecsys test) on a cobas e801 analyzer (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions (Roche Diagnostics). The coefficients of variation for intra-assay and inter-assay precision were 1.63 % and 2.51 % for estradiol, 2.27 % and 3.71 % for testosterone, and 2.28 % and 2.83 % for progesterone, respectively.

Oxytocin

Plasma samples for the measurement of oxytocin (OXT) concentrations were collected with commercial sampling devices (Vacuette, Greiner Bio-One International, Austria) containing ethylenediaminetetraacetic acid (EDTA) and aprotinin. Vacuettes were immediately centrifuged at 3250 rpm for 10 minutes, and aliquoted samples were stored at -80°C until assayed. OXT concentrations were extracted and quantified using a highly sensitive and specific radioimmunoassay (RIAgnosis, Munich, Germany). 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.

fMRI picture set

The stimuli in the fMRI task were selected based on a pilot study. A sample of 36 healthy participants (16 females) was shown 360 stimuli, divided into six blocks, each containing 60 pictures. The valence of the pictures was positive, neutral, or negative, and the content of the pictures was either social (defined as the presence of a depicted human) or nonsocial. The pictures were selected from the IAPS database (Lang, 2005) and from the internet. The participants were asked to rate the valence and arousal of each stimulus on a 7-point Likert scale. Based on their ratings, the picture set for the fMRI task (n = 120) and 60 distractor pictures for the surprise recognition task were chosen such that negative and positive stimuli produced comparable arousal ratings and sex differences were absent for all ratings.

The final picture set for the fMRI task consisted of 120 pictures, with 20 pictures in each category (examples are displayed in Figure **S1**). Valence ($F_{(2,68)}$ = 260.84, p < 0.001, η_p^2 = 0.89, BF_{incl} = 5.603 x 10^{41}) and arousal ratings ($F_{(2,68)}$ =51.21, p < 0.001, η_p^2 = 0.60, BF_{incl} = 2.569 x 10^{12}) differed significantly between the three valence categories, but there were no main or interaction effects of sex (p > 0.05; all BF_{incl} < 0.4). Post-hoc comparisons of the valence ratings revealed significant differences between all valence categories (negative and neutral: $t_{(35)}$ = -13.94, p < 0.001, d = -2.32, BF_{10} = 8.152 x 10^{12}; negative and positive: $t_{(35)}$ = -17.37, p < 0.001, d = 2.90, BF_{10} = 5.430 x 10^{15}; positive and neutral: $t_{(35)}$ = 16.53, p < 0.001, d = -2.75, BF_{10} = 1.212 x 10^{15}). Further post hoc comparisons revealed that the arousal ratings between negative and positive stimuli were comparable ($t_{(35)}$ = 0.09, p = 0.93, d = 0.01; BF_{10} = 0.18), whereas the neutral valence category was perceived as less arousing than both the positive ($t_{(35)}$ = -11.88, p < 0.001, d = -1.98, BF_{10} = 9.405 x 10^{10}) and negative ($t_{(35)}$ = 8.18, p < 0.001, d = 1.36, BF_{10} = 9.263 x 10^{6}) valence categories. The 60 distractor pictures in the surprise recognition task, with 10 pictures in each category, were carefully selected to match the arousal and valence ratings of the picture set used in the fMRI task.

Supplementary Results

Behavioural results

Across sex and treatment groups, we also observed a significant interaction between valence and sociality ($F_{(2,388)} = 5.89$, $p < 0.01$, $\eta_p^2 = 0.03$) such that the effect of sociality was significantly more pronounced for positive items ($t_{(201)} = 4.24$, $p < 0.001$, $d = 0.30$; $BF_{10} = 388.63$), than for neutral ($t_{(201)} = 1.90$, $p = 0.06$, $d = 0.13$; $BF_{10} = 0.46$) and negative ($t_{(201)} = -0.46$, $p = 0.64$, $d = -0.03$; $BF_{10} = 0.09$) stimuli. Given that the effect of sociality was only evident for positive items, it seems unlikely that the behavioral task induced an attentional bias towards social stimuli *per se*. Furthermore, we repeated the behavioural analyses with a more liberal recognition criterion in which an item was classified as remembered if the participants correctly identified the item in the recognition task irrespective of the confidence of the classification. These analyses yielded a similar pattern of results.

The mixed ANOVA with the between-subject factors sex, OXT_{int}, and EST_{tra} and d' as dependent variable also revealed a significant main effect of sex ($F_{(1,194)} = 7.67$, $p < 0.01$, $\eta_p^2 = 0.04$) in addition to the reported three-way interaction. Across treatment groups, women showed a better memory performance than men (mean ± SD women: 1.43 ± 0.51; men: 1.26 ± 0.49). There were no further main or two-way interaction effects on the memory performance (all *p*s > 0.05).

Meta D'

We observed a significant three-way interaction of sex * EST_{tra} treatment * OXT_{int} treatment on *Meta d'* (cf. **Table S1**; $F_{(1,189)} = 5.23$, $p = 0.02$, $\eta_p^2 = 0.03$; $BF_{incl} = 2.72$). However, there were no significant sex differences in either treatment type (all $ps_{cor} > 0.05$; all $BF_{10} < 2.5$) and the interaction between the EST_{tra} and OXT_{int} treatments was not significant in either men ($F_{(1,92)} = 4.99$, $p = 0.03$ [$p_{cor} = 0.06$], $\eta_p^2 = 0.05$; $BF_{incl} = 2.22$) or women ($F_{(1,97)} = 1.55$, $p = 0.22$, $\eta_p^2 = 0.02$; $BF_{incl} = 0.52$).

Difference score D' and Meta D'

As *Meta d'* can be directly compared to d', we calculated a difference score between *meta d'* and *d'* for each participant. Therefore, we calculated a mixed-design ANOVA with EST$_{tra}$ treatment, OXT$_{int}$ treatment, and sex as between-subject factors and the difference score as dependent variable. However, analyses revealed no significant main or interaction effects of sex and treatment type (all *p*s > 0.05; all BF$_{10}$ < 0.7). Further analyses of the male and female subgroups revealed that in each of the four treatment conditions, the difference score was not significantly different from zero (all *ps* > 0.05; all BF$_{10}$ < 0.6). Thus, in conclusion, participants acted metacognitively ideal, and neither sex nor the treatment types significantly changed meta-cognition.

Whole brain

Analyses revealed that there were neither significant whole-brain main effects of sex or treatment type (EST$_{tra}$ or OXT$_{int}$ treatment), nor a significant three-way interactions (sex * EST$_{tra}$ treatment * OXT$_{int}$ treatment) for the contrasts of interests (1. [Remembered > Forgotten] and 2. [Emotional $_{\text{Remembered > Forgotten}}$ > Neutral $_{\text{Remembered > Forgotten}}$]; all *p*s$_{cor}$ > 0.05). For whole brain task effects see Supplementary **Tables S2-3**.

Neural emotional memory effect

A significant sex * EST$_{tra}$ treatment * OXT$_{int}$ treatment interaction emerged for the emotional memory effect in the left hippocampus ([Emotional $_{\text{Remembered > Forgotten}}$ > Neutral $_{\text{Remembered > Forgotten}}$]; MNI peak coordinates [x, y, z]: -14, −40, 10, $F_{(1,194)}$ = 16.66, on peak level p_{FWE} = 0.019).

Sex differences

Post hoc tests to unravel the significant sex * EST$_{tra}$ treatment * OXT$_{int}$ treatment interaction for the emotional memory effect in the left hippocampus revealed that women following placebo administration exhibited nonsignificantly larger left hippocampus responses to emotional remembered stimuli than men (i.e., contrast of interest: [Emotional $_{\text{Remembered > Forgotten}}$ > Neutral $_{\text{Remembered > Forgotten}}$]; PLC$_{\text{tra}}$ & PLC$_{\text{int}}$; $t_{(42)}$ = -2.42, p = 0.02 [p_{cor} = 0.08], d = -0.74; BF$_{10}$ = 2.88). This sex difference was reversed (i.e. male > female) after OXT$_{\text{int}}$ treatment (PLC$_{\text{tra}}$ & OXT$_{\text{int}}$; $t_{(54)}$ = 2.69, p = 0.01 [p_{cor} = 0.04], d = 0.72; BF$_{10}$ = 4.91) and EST$_{\text{tra}}$ treatment (EST$_{\text{tra}}$ & PLC$_{\text{int}}$: $t_{(52)}$ = 1.92, p = 0.06, [p_{cor} = 0.24], d = 0.52; BF$_{10}$ = 1.23). Interestingly, sex differences were absent in the combined treatment group ($t_{(46)}$ = -1.27, p = 0.21, [p_{cor} = 0.84], d = -0.37; BF$_{10}$ = 0.55).

Treatment effects

We found a significant interaction between the OXT$_{\text{int}}$ and EST$_{\text{tra}}$ treatments for the emotional memory effect in the left hippocampus in men ([Emotional $_{\text{Remembered > Forgotten}}$ > Neutral $_{\text{Remembered > Forgotten}}$]; $F_{(1,95)}$ = 9.54, p < 0.01 [p_{cor} < 0.01], η_p^2 = 0.09; BF$_{\text{incl}}$ = 14.29) and in women ($F_{(1,99)}$ = 6.86, p = 0.01 [p_{cor} = 0.02], η_p^2 = 0.07; BF$_{\text{incl}}$ = 4.70). Thus, given these data, the Bayes factors indicated that models including the interaction between OXT$_{\text{int}}$ and EST$_{\text{tra}}$ were 14.29 and 4.70 times more likely than models without it. In men, OXT$_{\text{int}}$ nonsignificantly increased hippocampal responses after PLC$_{\text{tra}}$ (PLC$_{\text{tra}}$ & OXT$_{\text{int}}$ vs. PLC$_{\text{tra}}$ & PLC$_{\text{int}}$ $t_{(44)}$ = -2.53, p = 0.02 [p_{cor} = 0.06], d = -0.76; BF$_{10}$ = 3.57*)*, but nonsignificantly reduced the response after EST$_{\text{tra}}$ treatment (EST$_{\text{tra}}$ & OXT$_{\text{int}}$ vs. EST$_{\text{tra}}$ & PLC$_{\text{int}}$ $t_{(51)}$ = 1.97, p = 0.05 [p_{cor} = 0.2], d = 0.54; BF$_{10}$ = 1.34). EST$_{\text{tra}}$ had no significant effect on hippocampal activation in men after PLC$_{\text{int}}$ (EST$_{\text{tra}}$ & PLC$_{\text{int}}$ vs. PLC$_{\text{tra}}$ & PLC$_{\text{int}}$: $t_{(46)}$ = -1.59, p = 0.12 [p_{cor} = 0.48], d = -0.47; BF$_{10}$ = 0.81), but significantly decreased the hippocampus activation in the combined group (EST$_{\text{tra}}$ & OXT$_{\text{int}}$ vs. PLC$_{\text{tra}}$ & OXT$_{\text{int}}$: $t_{(49)}$ = 2.89, p < 0.01 [p_{cor} = 0.02], d = 0.81; BF$_{10}$ = 7.49). In women, OXT$_{\text{int}}$ nonsignificantly decreased hippocampal responses after PLC$_{\text{tra}}$ (PLC$_{\text{tra}}$ & OXT$_{\text{int}}$ vs. PLC$_{\text{tra}}$ & PLC$_{\text{int}}$ $t_{(52)}$ = 2.54, p = 0.01 [p_{cor} = 0.06], d = 0.69; BF$_{10}$ = 3.67), but had the opposite effect after EST$_{\text{tra}}$ treatment (EST$_{\text{tra}}$ & OXT$_{\text{int}}$ vs. EST$_{\text{tra}}$ & PLC$_{\text{int}}$ $t_{(47)}$ = -1.17, p = 0.25 [p_{cor} ≈ 1], d = -0.34 ; BF$_{10}$ = 0.50). Likewise, EST$_{\text{tra}}$ significantly decreased hippocampal activation in women after PLC$_{\text{int}}$ (EST$_{\text{tra}}$ & PLC$_{\text{int}}$ vs. PLC$_{\text{tra}}$ & PLC$_{\text{int}}$: $t_{(48)}$ = 2.84, p < 0.01 [p_{cor} = 0.03], d = 0.80; BF$_{10}$ = 6.74) and had no effects in the combined group (EST_{tra} & OXT_{int} vs. EST_{tra} & PLC_{int} $t_{(51)} = -0.88$, $p = 0.38$ [$p_{cor} \approx 1$], $d = -0.24$; $BF_{10} = 0.38$).

Simple effect analyses of the oxytocin treatment at the neural level

To examine a possible treatment effect of OXT_{int}, two-sample *t*-tests were calculated to compare the neural responses in the PLC_{tra} & PLC_{int} and PLC_{tra} & OXT_{int} groups separately for women and men. There were no significant OXT_{int} treatment effects in women or men for the two main contrasts ([Remembered > Forgotten]) at the whole brain level, or in the amygdala or insula ROIs (all *p*s > 0.05).

Simple effect analyses of the estradiol treatment at the neural level

To examine a possible treatment effect of EST_{tra}, two-sample *t*-tests were calculated to compare the neural responses in the PLC_{tra} & PLC_{int} and EST_{tra} & PLC_{int} groups separately for women and men. There were no significant EST_{tra} treatment effects in women or men for the contrast ([Remembered > Forgotten) at the whole brain level, or in the amygdala or insula ROIs (all *p*s > 0.05).

Simple effect analyses of sex at the neural level

To examine possible sex effects, two-sample *t*-tests were calculated to compare the neural responses of women and men separately for the four treatment groups. In the combined treatment group (EST_{tra} &OXT_{int}), men showed an increased activation in the left insula in contrast to women for the memory effect ([Remembered > Forgotten]; MNI peak coordinates [x, y, z]: -32, 18, -4, $t_{(46)} = 4.59$, on peak level $p_{FWE} = 0.013$). Further comparisons at the whole brain level and the remaining ROIs were not significant for the Remembered > Forgotten contrast (all *p*s > 0.05).

Social vs. nonsocial stimuli

To investigate social-specific neural treatment effects, we calculated a mixed-design ANOVA with EST_{tra} treatment, OXT_{int} treatment, and sex as between-subject factors and the neural responses to social compared to non-social stimuli as dependent variables (i.e., [Social $_{\text{Remembered > Forgotten}}$ > Nonsocial $_{\text{Remembered > Forgotten}}$]). We found a significant two-way interaction of sex and OXT_{int} treatment in the left and right amygdala responses (left: MNI peak coordinates [x, y, z]: -24, −4, -16, $F_{(1,194)} = 11.31$, on peak level $p_{\text{FWE}} = 0.048$; right: MNI peak coordinates [x, y, z]: 20, −4, -16, $F_{(1,194)} = 18.98$, on peak level $p_{\text{FWE}} = 0.002$). We found no further significant main or interaction effects of sex and treatment types for the hippocampus, insula and amygdala responses in this contrast (all *p*s > 0.05).

Functional connectivity

Three-way interactions

To address the effects of the two treatment types on task-based functional connectivity, we conducted a generalized psychophysiological interaction (gPPI) analysis using the CONN Toolbox. The hippocampus, insula and amygdala were used as seeds in whole-brain seed-to-voxel analyses. To investigate a potential three-way interaction, the memory-related connectivity values (i.e., contrast of interest: [Remembered > Forgotten] and [Emotional $_{\text{Remembered > Forgotten}}$ > Neutral $_{\text{Remembered > Forgotten}}$]) were used as dependent variables in a mixed-design ANOVA. The analyses did not reveal significant three-way interactions with sex and treatment types for either contrast.

Simple effect analyses of the treatments and sex for [Remembered > Forgotten]

In addition, exploratory analyses and post-hoc *t*-tests revealed no simple effects of treatment or sex (e.g. men vs. women in the placebo group (PLC_{tra} & PLC_{int})) for the contrast [Remembered > Forgotten].

Supraphysiological EST levels

Women exhibited a significantly larger increase in blood EST levels than men. To address that difference, we included the EST increase as a covariate in our analyses. To further probe the impact of this difference, we used a median-dichotomization and excluded $\mathrm{EST}_{\mathrm{tra}}$-treated women with large EST increase (n = 23). In this subsample, the treatment-induced increases in EST levels were comparable between women and men within the treatment groups (all *p*s > 0.05; $\mathrm{BF}_{10} < 0.6$) We repeated the main behavioral and neural analyses with this subsample and observed a similar pattern of results (see **Figure S2**). In line with our reported main findings, we also found a significant three-way interaction of sex, $\mathrm{OXT}_{\mathrm{int}}$, and $\mathrm{EST}_{\mathrm{tra}}$ treatment in the left hippocampus responses to remembered stimuli compared to forgotten stimuli (MNI peak coordinates [x, y, z]: -12, −38, 8, $F_{(1,165)} = 15.01$, $p < 0.01$ [$p_{\mathrm{cor}} < 0.01$], $\eta_p^2 = 0.08$; $\mathrm{BF}_{\mathrm{incl}} = 208.56$), as well as a significant three-way interaction for the recognition memory ($F_{(1,165)} = 6.24$, $p = 0.01$, $\eta_p^{2} = 0.04$; $\mathrm{BF}_{\mathrm{incl}} = 5.05$). Further analyses of the extracted parameter estimates revealed results that were comparable to our reported main findings. In line with our main analyes, there were no significant sex differences after $\mathrm{EST}_{\mathrm{tra}}$ treatment ($\mathrm{EST}_{\mathrm{tra}}$ & $\mathrm{PLC}_{\mathrm{int}}$; recognition memory: $t_{(41)} = -0.04$, $p = 0.97$, $d = -0.01$; $\mathrm{BF}_{10} = 0.32$; hippocampal response: $t_{(41)} = 1.28$, $p = 0.21$, $d = 0.42$; $\mathrm{BF}_{10} = 0.60$). After the combined treatment ($\mathrm{EST}_{\mathrm{tra}}$ & $\mathrm{OXT}_{\mathrm{int}}$), similar, non-significant, sex differences as in the placebo group ($\mathrm{PLC}_{\mathrm{tra}}$ & $\mathrm{PLC}_{\mathrm{int}}$) were evident for hippocampal responses (women > men; $t_{(29)} = -2.13$, $p = 0.04$ [$p_{\mathrm{cor}} = 0.16$], $d = -0.84$; $\mathrm{BF}_{10} = 1.83$) and recognition memory ($t_{(29)} = -1.49$, $p = 0.15$, $d = -0.59$; $\mathrm{BF}_{10} = 0.81$). Therefore, the significantly larger EST increase in women than men has no major impact on the results.

Further hormonal assessments

In addition to a significant main effect of sex ($F_{(1,180)} = 771.19$, $p < .001$, $\eta_p^2 = .81$; $\mathrm{BF}_{\mathrm{incl}} = 5.65 \times 10^{64}$), we observed a significant interaction between sex, time, and $\mathrm{EST}_{\mathrm{tra}}$ treatment ($F_{(1.73,311.46)} = 3.61$, $p = .03$, $\eta_p^2 = .02$; $\mathrm{BF}_{\mathrm{incl}} = 2.93$) for testosterone levels. Separate post-hoc *t*-tests for sex and time points revealed that estradiol-treated participants ($\mathrm{EST}_{\mathrm{tra}}$ & $\mathrm{PLC}_{\mathrm{int}}$ and $\mathrm{EST}_{\mathrm{tra}}$ & $\mathrm{OXT}_{\mathrm{int}}$) showed non-significantly decreased post-treatment testosterone levels compared to the placebo groups (PLC$_{tra}$ & PLC$_{int}$ and PLC$_{tra}$ & OXT$_{int}$,: women $t_{(100)}$ = -2.39, p = 0.02, [p_{cor} = 0.11], d = 0.47; BF$_{10}$ = 2.55; men $t_{(96)}$ = -2.23, p = 0.03, [p_{cor} = 0.17], d = 0.45; BF$_{10}$ = 1.88). We did not observe any main or interaction effects for the progesterone levels. Furthermore, we examined whether EST levels three days after the treatment (i.e. the day when we tested recognition memory) were comparable to the baseline values within the sexes. In women, EST levels three days after the treatment were significantly higher compared to the baseline values (main effect of time: $F_{(1,92)}$ = 28.53, p < 0.001, η_p^2 = .24; BF$_{incl}$ = 17380.64). Importantly no further significant main or interaction effect of time and EST$_{tra}$ and OXT$_{int}$ treatments were found (all ps > 0.05; all BF$_{incl}$ < 1.2). The increased EST levels reflect the progressing menstrual cycle, which is naturally accompanied by increasing EST levels. In men, no significant main or interaction effects of time, EST$_{tra}$, and OXT$_{int}$ treatments were found (all ps > 0.05; all BF$_{incl}$ < 0.91), indicating that the EST levels three days following the treatment were comparable to the baseline levels. Thus, treatment effects on recognition memory are most likely driven by acute effects on memory encoding.

Demographic and psychometric baseline characteristics

Demographics and baseline psychometric assessments of the participants are displayed in **Table S7**. There were no significant differences between treatment groups within sexes (all ps$_{cor}$ > 0.05; all BF$_{10}$ < 0.7). Across treatments men were older than women (age, $t_{(200)}$ = 2.29, p = 0.02, d = 0.3; BF$_{10}$ = 1.74). In addition, women reported significantly higher social anxiety (Liebowitz scale, $t_{(195)}$ = -3.57 , p < 0.001, d = -0.51; BF$_{10}$ = 52.37) and increased trait anxiety (STAI Trait, $t_{(186.31)}$ = -2.60, p = 0.01, d = -0.37; BF$_{10}$ = 3.45) than men. Alexithymia, depressive symptoms, and autistic-like traits were not significantly different between the sexes (all ps > 0.05; all BF$_{10}$ < 0.9).

Demographic and psychometric variables were included as covariates in the main analyses of behavioural (d') and neural effects ([Remembered > Forgotten] and [Emotional $_{\text{Remembered > Forgotten}}$ > Neutral $_{\text{Remembered > Forgotten}}$]. All reported effects remained significant and the covariates were not significant with the exception of a significant effect of autistic-like traits (AQ) in the hippocampal response for the contrast [Emotional $_{\text{Remembered > Forgotten}}$ > Neutral$_{\text{Remembered > Forgotten}}$] ($F_{(1,176)}$ = 7.42, p < 0.01, η_p^2 = 0.04). Increased AQ scores were associated with increased activation (r = 0.203, p < 0.01; BF_{10} = 5.21). However, the significant three-way interaction in the hippocampus remained significant despite the significant AQ covariate ($F_{(1,176)}$ = 11.83, p = 0.001, η_p^2 = 0.06; BF_{incl} = 70.46).

Weight, body mass index and hormonal levels

Neither the weight nor the body mass index (BMI) of the participants did significantly correlate with the baseline EST or OXT levels in men or women (all ps > 0.05; all BF_{10} < 0.2) or with treatment-induced EST_{tra} increases in either sex (all ps > 0.05; BF_{10} < 1.0). Thus, the EST_{tra} treatment did not result in different peripheral levels depending on weight or BMI. While OXT_{int} treatment did not produce different peripheral OXT levels depending on the weight or BMI in men (all ps > 0.05; BF_{10} < 0.4), both BMI and weight positively correlated with the OXT increase in OXT_{int}-treated women (weight: $r_{(51)}$ = 0.29, p = 0.04; BF_{10} = 1.39; BMI: $r_{(51)}$ = 0.30, p = 0.03; BF_{10} = 1.63). Thus, OXT_{int} treatment resulted in higher peripheral OXT levels in women with a higher BMI and increased weight.

Side effects

Three days following the treatment, the participants were asked to report the side effects of their treatment. No participant experienced severe side effects, but 10.3% of the participants reported side effects consisting of light headache, tiredness, dizziness, sleep disturbance, hilarity, lack of concentration, or circulatory problems. Importantly, the proportion of participants who reported side effects did not significantly differ between the treatment groups (PLC_{tra} & PLC_{int}: 16.3%, PLC_{tra} & OXT_{int}: 7.5%, EST_{tra} & PLC_{int}: 9.6%, EST_{tra} & OXT_{int}: 8.5%; $\chi^2_{(3)}$ = 2.30, p = .51).

Blinding of treatment

Both the participants and the experimenters who conducted the experiment were blinded to treatments. Following the MRI scan, the participants were asked to guess which treatment they received. Out of the 104 participants (99 with available estimates) in the OXT groups (PLC_{tra} & OXT_{int} and EST_{tra} & OXT_{int}), 23 (23.2%; 9 men) believed that they had received OXT, while 23 (24.7%; 11 men) participants in the placebo groups (PLC_{tra} & PLC_{int} and EST_{tra} & PLC_{int}; *n* = 98, 93 with available estimates) believed that they had received verum treatment. Out of the 102 participants in the EST groups (EST_{tra} & PLC_{int} and EST_{tra} & OXT_{int}, 100 with available estimates), 22 (22.0%; 12 men) estimated that they had received verum treatment, while 17 (18.5%; 2 men) participants in the placebo groups (PLC_{tra} & PLC_{int} and PLC_{tra} & $OXT_{int;}$ *n* = 100, 92 with available estimates) assumed that they had received EST. In general, the participants who believed in an OXT treatment also guessed an EST treatment ($r_{(190)} = 0.39$, $p < 0.001$; all $BF_{10} = 397232.22$). However, the treatment estimates did not significantly correlate with the actual treatments (all *p*s > 0.05; all $BF_{10} < 0.2$).

Measurements of mood

A main effect of time was found for the positive ($F_{(1,181)} = 72.35$, $p < 0.001$, $\eta_p^2 = .29$; $BF_{incl} = 1.943 \times 10^{12}$) and negative ($F_{(1,181)} = 8.80$, $p < 0.01$, $\eta_p^2 = .05$; $BF_{incl} = 4.23$) affect measured with the PANAS (the Positive and Negative Affect Schedule) for both sexes (cf. **Table S6**). Positive and negative mood significantly decreased from the beginning of the testing session (mean ± SD positive: 28.69 ± 5.91; negative: 12.15 ± 3.19) to the end (mean ± SD positive: 25.15 ± 6.24; negative: 11.55 ± 2.23), suggesting an increasing fatigue over the time course of the experiment. No further significant main or interaction effects of time and EST_{tra} and OXT_{int} treatments were found for positive or negative mood.

Confidence ratings

Confidence ratings were significantly higher for correct than incorrect responses (fMRI stimuli: $t_{(201)} = 11.11$, $p < 0.001$, $d = 0.78$; $BF_{10} = 3.566 \times 10^{19}$; distractors: $t_{(197)} = 35.51$, $p < 0.001$, $d =$

2.52; $BF_{10} = 6.807 \times 10^{83}$). A mixed-design ANOVA with stimulus type (fMRI remembered, fMRI forgotten, distractor correct rejection, distractor false alarm) yielded a significant main effect of sex ($F_{(1,190)} = 6.50$, $p = 0.01$, $\eta_p^2 = .03$; $BF_{incl} = 0.66$) and a significant interaction of sex * stimulus type ($F_{(1.88,358.67)} = 3.99$, $p = 0.02$, $\eta_p^2 = 0.02$; $BF_{incl} = 1.08$), with women reporting higher confidence than men for remembered fMRI items and correctly rejected distractors, but there were no further main or interaction effects of sex and treatment groups on the confidence ratings (all *p*s > 0.05; all $BF_{10} < 0.3$).

Missing values

The following blood samples were missing due to problems in sample assessment or analysis: baseline (oxytocin, n = 4; estradiol, n = 4; progesterone, n = 5; testosterone, n = 4), post-treatment (estradiol, n = 2; progesterone, n = 1; testosterone, n = 2), and three days after the treatment (estradiol, n = 8; progesterone, n = 8; testosterone, n = 9). Connection issues and technical errors resulted in the loss of questionnaires evaluating depressive symptoms (n = 8), autistic-like traits (n = 4), alexithymia (n = 4), trait anxiety (n = 5), social anxiety (n = 5), treatment guesses (n = 10), pretreatment negative and positive mood (n = 3), and posttreatment negative and positive mood (n = 10). In addition, five *Meta d'* values were missing because five participants did not have any false alarms in the memory recognition task.

Attentional control

The results of the attention control in the fMRI task showed a high rate of correct responses (mean percent ± SD correct responses: 96.90% ± 8.80) which were not influenced by treatment type and sex (all *p*s > 0.05). In combination with a high response count (mean percent ± SD total responses: 98.76 ± 5.36), this demonstrated that the participants were paying attention and understood the task.

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Supplementary Figures

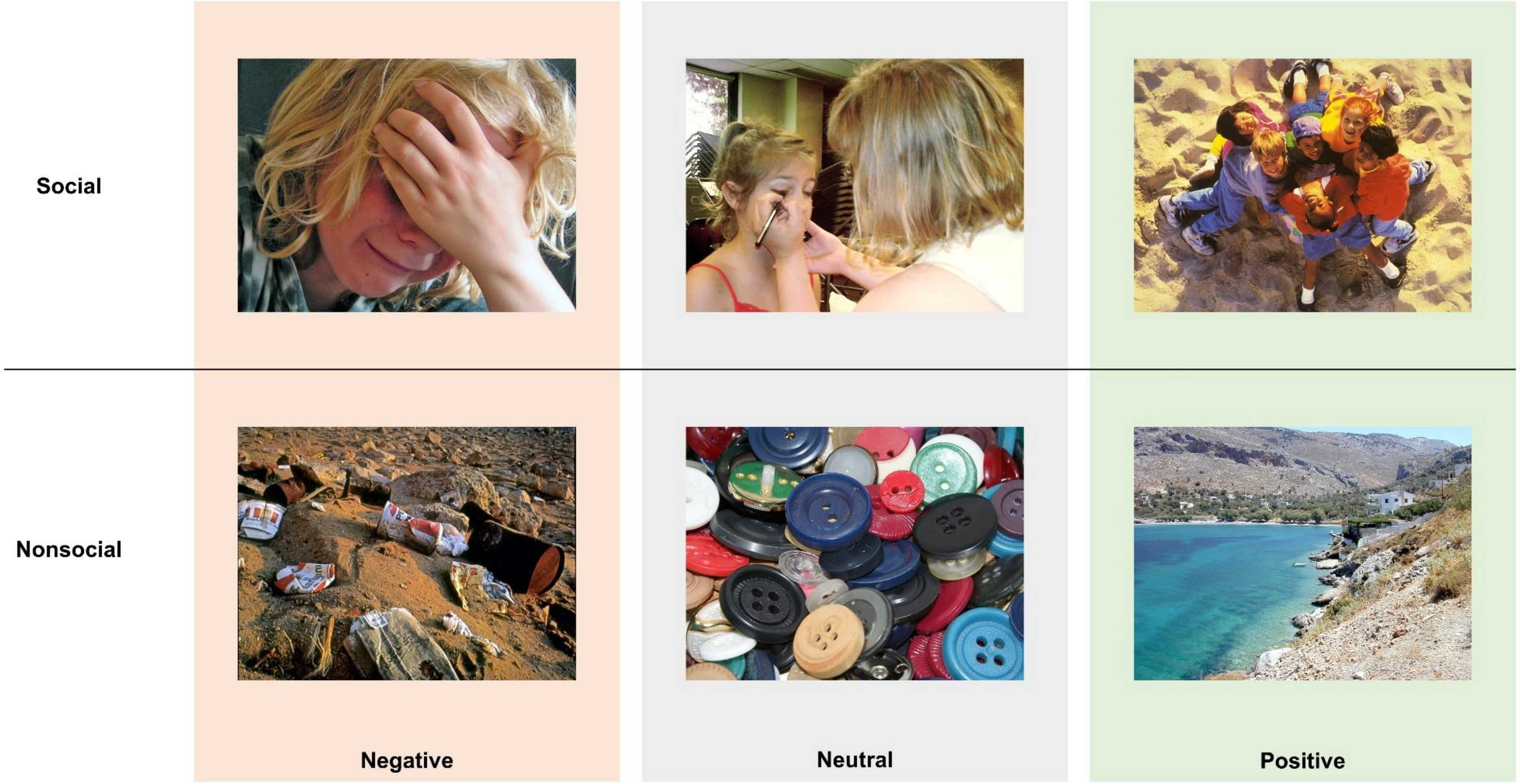


Figure S1. fMRI picture set. The valence of the pictures in was positive, neutral, or negative, and the content of the pictures was either social (defined as the presence of a depicted human) or nonsocial. The displayed pictures are from the IAPS database (Lang, 2005).

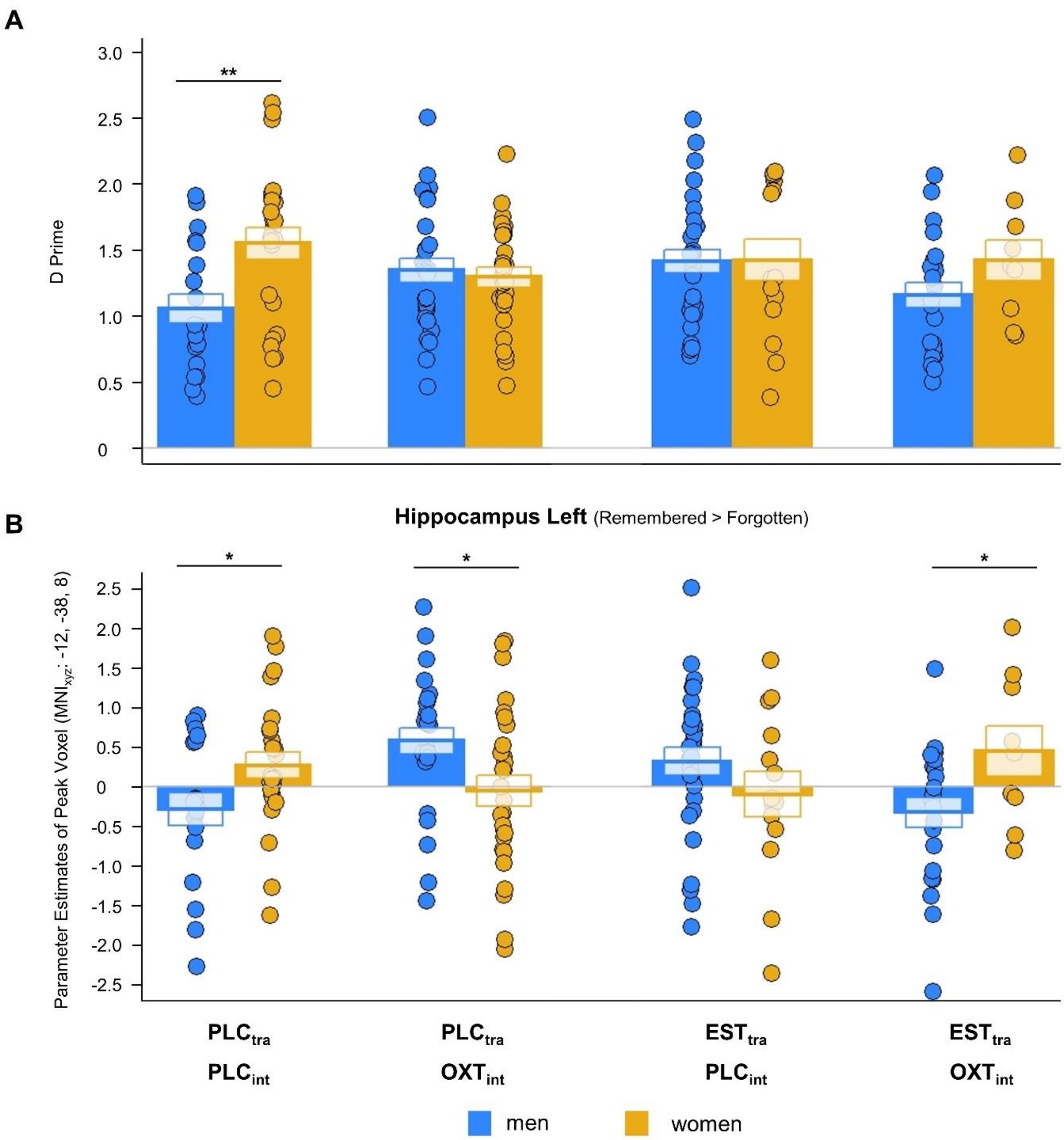


Figure S2. Treatment effects on mnemonic and hippocampal sex differences in a subsample with comparable blood estradiol (EST) increase in women and men. **A** In line with the results of the total sample, there were no significant sex differences after the single trandermal estradiol treatment (EST$_{tra}$)**.** After the combined treatment, a non-significant sex difference similar to that observed in the placebo group was evident. **B** Further analyses of the parameter estimates for the left hippocampal responses revealed a similar pattern as in the total sample. Intriguingly, the same pattern as that observed in the placebo group was again evident in the combined treatment group. PLC$_{tra}$ = transdermal placebo gel; PLC$_{int}$ = intranasal placebo; OXT$_{int}$ = intranasal oxytocin; EST$_{tra}$ = transdermal estradiol. **p* < 0.05, ***p* < 0.01.

Supplementary Tables

Table S1. Hit rate, false alarm rate, *D’*,*Meta D’* values and *D*’ for each valence category in the memory recognition task

	Females				Males			
	PLC$_{tra}$ & PLC$_{int}$ Mean (n, ± SD)	PLC$_{tra}$ & OXT$_{int}$ Mean (n, ± SD)	EST$_{tra}$ & PLC$_{int}$ Mean (n, ± SD)	EST$_{tra}$ & OXT$_{int}$ Mean (n, ± SD)	PLC$_{tra}$ & PLC$_{int}$ Mean (n, ± SD)	PLC$_{tra}$ & OXT$_{int}$ Mean (n, ± SD)	EST$_{tra}$ & PLC$_{int}$ Mean (n, ± SD)	EST$_{tra}$ & OXT$_{int}$ Mean (n, ± SD)
Hit rate (%)	0.62 (25, 0.12)	0.56 (29, 0.15)	0.63 (25, 0.15)	0.55 (24, 0.13)	0.56 (19, 0.17)	0.57 (27, 0.15)	0.57 (29, 0.12)	0.51 (24, 0.21)
False alarm rate (%)	0.14 (25, 0.11)	0.13 (29, 0.08)	0.15 (25, 0.10)	0.12 (24, 0.08)	0.20 (19, 0.08)	0.15 (27, 0.09)	0.13 (29, 0.09)	0.15 (24, 0.11)
D‘	1.55 (25, 0.60)	1.32 (29, 0.41)	1.46 (25, 0.52)	1.42 (24, 0.49)	1.05 (19, 0.48)	1.34 (27, 0.48)	1.41 (29, 0.48)	1.16 (24, 0.47)
Meta D‘	1.55 (24, 0.73)	1.21 (29, 1.51)	1.36 (25, 1.50)	1.65 (23, 1.07)	1.06 (19, 0.62)	1.29 (26, 0.98)	1.55 (29, 0.87)	0.96 (22, 0.94)
Negative D‘	1.64 (25, 0.70)	1.46 (29, 0.59)	1.61 (25, 0.71)	1.42 (24, 0.56)	1.17 (19, 0.60)	1.39 (27, 0.63)	1.40 (29, 0.56)	1.23 (24, 0.68)
Neutral D‘	1.38 (25, 0.63)	1.15 (29, 0.49)	1.35 (25, 0.54)	1.31 (24, 0.50)	0.83 (19, 0.54)	1.26 (27, 0.58)	1.34 (29, 0.54)	1.06 (24, 0.63)
Positive D‘	1.49 (25, 0.59)	1.25 (29, 0.49)	1.39 (25, 0.51)	1.34 (24, 0.53)	1.16 (19, 0.53)	1.28 (27, 0.53)	1.43 (29, 0.61)	1.09 (24, 0.51)

Notes. PLC$_{tra}$ = transdermal placebo gel; PLC$_{int}$ = intranasal placebo; OXT$_{int}$ = intranasal oxytocin; EST$_{tra}$ = transdermal estradiol.

Table S2. Whole brain activation for remembered items (i.e., [Remembered > Forgotten]) across sexes and treatments

Region	Right/Left	Cluster size (voxels)	Peak *F*-score	MNI coordinates		
				x	y	z
Inferior temporal gyrus	L	4889	236.45	-44	-56	-10
Middle occipital gyrus	R	5161	184.97	36	-74	26
Middle frontal gyrus	R	4676	180.07	36	52	6
Precuneus	R	3862	174.27	8	-68	40
Inferior parietal gyrus	R	1382	171.08	52	-48	50
Middle frontal gyrus	L	1705	148.42	-32	48	8
Inferior frontal gyrus, orbital part	L	1209	133.09	-36	30	-14
Inferior frontal gyrus	R	761	121.10	48	32	12
Inferior frontal gyrus, orbital part	R	239	105.94	26	28	-12
Hippocampus	R	230	105.12	20	-6	-14
Amygdala	L	186	86.47	-20	-8	-16
Gyrus rectus	L	203	77.99	-2	22	-18
Precuneus	R	166	74.54	16	-48	6
Inferior parietal gyrus	L	611	68.12	-52	-54	42
Middle temporal gyrus	R	222	64.28	66	-28	-14
Calcarine fissure	L	165	64.25	-12	-48	6
Superior frontal gyrus, medial	L	67	49.39	-6	52	32
Superior temporal gyrus	R	36	42.32	52	-8	-14
Superior frontal gyrus	L	16	39.53	-18	10	62
Middle frontal gyrus	R	9	33.77	50	-4	52
Thalamus	L	6	32.70	-22	-26	6
Superior temporal gyrus	L	38	31.96	-58	-26	12
Superior temporal gyrus	R	30	30.92	62	-20	10
Insula	R	6	30.34	34	18	-10
Hippocampus	L	3	28.81	-32	-18	-14
Median cingulate and paracingulate gyrus	R	2	27.49	6	2	30
Middle temporal gyrus	L	1	26.77	-54	-32	-14
Median cingulate and paracingulate gyrus	L	1	26.04	-14	-50	36

Notes. Only clusters with FWE-corrected *p*s < 0.05 on peak level are listed (cluster-forming threshold p_{FWE} < 0.05).

Table S3. Whole brain activation for the emotional memory effect (i.e., [Emotional $_{\text{Remembered > Forgotten}}$ > Neutral $_{\text{Remembered > Forgotten}}$]) across sexes and treatments

Region	Right/Left	Cluster size (voxels)	Peak *F*-score	MNI coordinates		
				x	y	z
Occipital lobe	L	69	42.64	-40	-56	-8
Inferior frontal gyrus, triangular part	R	48	40.23	48	34	6
Fusiform gyrus	R	14	34.93	42	-46	-14
Inferior frontal gyrus, orbital part	L	10	29.54	-38	30	-14
Middle temporal gyrus	R	2	26.21	40	-62	18

Notes. Only clusters with FWE-corrected *p*s < 0.05 on peak level are listed (cluster-forming threshold $p_{\text{FWE}} < 0.05$).

Table S4. Estradiol, progesterone and testosterone concentrations at baseline, immediately post treatment and three days after the treatment

		Females				Males			
		PLC$_{tra}$ & PLC$_{int}$ Mean (n, ± SD)	PLC$_{tra}$ & OXT$_{int}$ Mean (n, ± SD)	EST$_{tra}$ & PLC$_{int}$ Mean (n, ± SD)	EST$_{tra}$ & OXT$_{int}$ Mean (n, ± SD)	PLC$_{tra}$ & PLC$_{int}$ Mean (n, ± SD)	PLC$_{tra}$ & OXT$_{int}$ Mean (n, ± SD)	EST$_{tra}$ & PLC$_{int}$ Mean (n, ± SD)	EST$_{tra}$ & OXT$_{int}$ Mean (n, ± SD)
Estradiol (pg/ml)	pre	34.12 (25, 18.97)	49.02 (28, 47.04)	46.60 (24, 25.62)	44.12 (23, 38.67)	24.27 (19, 8.04)	24.94 (27, 11.13)	22.02 (29, 11.78)	20.35 (23, 9.77)
	post	39.12 (25, 20.39)	50.70 (29, 42.15)	982.36 (25, 598.66)	965.09 (23, 381.80)	27.34 (19, 9.28)	30.30 (27, 10.82)	542.93 (29, 439.07)	623.43 (23, 363.60)
	3 days post	45.34 (24, 28.76)	59.10 (28, 32.74)	77.66 (23, 58.98)	58.10 (23, 28.96)	24.53 (18, 6.47)	24.12 (26, 8.28)	23.00 (28, 10.35)	20.50 (24, 8.11)
Progesterone (ng/ml)	pre	1.05 (25, 3.54)	0.71 (28, 2.45)	0.30 (23, 0.28)	0.17 (23, 0.09)	0.20 (19, 0.17)	0.23 (27, 0.15)	0.17 (29, 0.11)	0.13 (23, 0.08)
	post	1.17 (25, 4.39)	0.45 (29, 1.48)	0.19 (25, 0.22)	0.12 (24, 0.06)	0.13 (19, 0.10)	0.13 (27, 0.06)	0.12 (29, 0.09)	0.10 (23, 0.05)
	3 days post	0.68 (24, 2.58)	0.23 (28, 0.33)	0.14 (23, 0.11)	0.23 (23, 0.44)	0.16 (18, 0.11)	0.16 (26, 0.09)	0.16 (28, 0.13)	0.14 (24, 0.10)
Testosterone (ng/ml)	pre	0.28 (25, 0.15)	0.27 (28, 0.11)	0.20 (24, 0.14)	0.24 (23, 0.14)	4.45 (19, 1.23)	5.35 (27, 1.85)	4.70 (29, 1.45)	4.28 (23, 1.75)
	post	0.23 (25, 0.13)	0.25 (29, 0.10)	0.17 (25, 0.13)	0.20 (23, 0.12)	4.67 (19, 1.66)	5.50 (27, 2.00)	4.36 (29, 1.69)	4.38 (23, 1.56)
	3 days post	0.27 (24, 0.14)	0.24 (28, 0.09)	0.19 (23, 0.14)	0.24 (23, 0.14)	4.65 (18, 1.32)	4.69 (26, 2.11)	4.71 (28, 1.42)	4.19 (23, 1.49)

Notes. pre; pretreatment; post, 4.5 hours after the gel administration; 3 days post, 3 days post treatment; PLC$_{tra}$ = transdermal placebo gel; PLC$_{int}$ = intranasal placebo; OXT$_{int}$ = intranasal oxytocin; EST$_{tra}$ = transdermal estradiol.

Table S5. Oxytocin baseline and post treatment concentrations

	Females				Males			
	PLC_{tra} & PLC_{int} Mean (n, ± SD)	PLC_{tra} & OXT_{int} Mean (n, ± SD)	EST_{tra} & PLC_{int} Mean (n, ± SD)	EST_{tra} & OXT_{int} Mean (n, ± SD)	PLC_{tra} & PLC_{int} Mean (n, ± SD)	PLC_{tra} & OXT_{int} Mean (n, ± SD)	EST_{tra} & PLC_{int} Mean (n, ± SD)	EST_{tra} & OXT_{int} Mean (n, ± SD)
Oxytocin pre (pg/ml)	1.99 (25, 0.70)	1.57 (28, 0.66)	1.66 (24, 0.66)	1.60 (23, 0.47)	1.91 (19, 0.82)	2.01 (27, 0.65)	1.99 (28, 0.64)	1.92 (24, 0.67)
Oxytocin post (pg/ml)	2.11 (25, 0.69)	5.08 (29, 2.04)	1.83 (25, 0.61)	4.79 (24, 1.34)	2.11 (19, 0.99)	6.20 (27, 2.78)	2.27 (29, 0.59)	6.49 (24, 3.22)

Notes. pre; pretreatment; post, 4.5 hours after the gel administration; PLC_{tra} = transdermal placebo gel; PLC_{int} = intranasal placebo; OXT_{int} = intranasal oxytocin; EST_{tra} = transdermal estradiol.

Table S6. Mood measurements

	Females				Males			
	PLC_{tra} & PLC_{int} Mean (n, ± SD)	PLC_{tra} & OXT_{int} Mean (n, ± SD)	EST_{tra} & PLC_{int} Mean (n, ± SD)	EST_{tra} & OXT_{int} Mean (n, ± SD)	PLC_{tra} & PLC_{int} Mean (n, ± SD)	PLC_{tra} & OXT_{int} Mean (n, ± SD)	EST_{tra} & PLC_{int} Mean (n, ± SD)	EST_{tra} & OXT_{int} Mean (n, ± SD)
Positive affect pre	27.46 (24, 6.77)	28.00 (28, 6.45)	29.28 (25, 5.70)	27.63 (24, 5.30)	30.28 (18, 5.17)	29.33 (27, 6.65)	27.83 (29, 5.31)	30.33 (24, 5.48)
Positive affect post	23.74 (23, 6.98)	24.81 (27, 6.21)	23.71 (24, 5.47)	25.00 (24, 5.98)	27.53 (17, 5.82)	24.68 (25, 6.43)	25.52 (29, 6.23)	26.91 (23, 6.58)
Negative affect pre	13.50 (24, 5.13)	11.57 (28, 1.55)	12.64 (25, 4.80)	11.71 (24, 1.78)	12.56 (18, 2.41)	11.89 (27, 3.31)	11.62 (29, 1.95)	12.00 (24, 2.52)
Negative affect post	12.48 (23, 2.17)	11.00 (27, 1.86)	11.71 (24, 2.26)	12.13 (24, 2.31)	10.94 (17, 1.39)	11.48 (25, 2.33)	11.14 (29, 1.85)	11.52 (23, 3.13)

Notes. Mood was assessed with the Positive and Negative Affect Schedule (PANAS). Abbreviations: pre; pretreatment; post, 4.5 hours after the gel administration; PLC_{tra} = transdermal placebo gel; PLC_{int} = intranasal placebo; OXT_{int} = intranasal oxytocin; EST_{tra} = transdermal estradiol.

Table S7. Demographic and psychometric baseline characteristics

	Females				Males			
	PLC_{tra} & PLC_{int} Mean (n, ± SD)	PLC_{tra} & OXT_{int} Mean (n, ± SD)	EST_{tra} & PLC_{int} Mean (n, ± SD)	EST_{tra} & OXT_{int} Mean (n, ± SD)	PLC_{tra} & PLC_{int} Mean (n, ± SD)	PLC_{tra} & OXT_{int} Mean (n, ± SD)	EST_{tra} & PLC_{int} Mean (n, ± SD)	EST_{tra} & OXT_{int} Mean (n, ± SD)
Age (years)	23.68 (25, 4.40)	24.10 (29, 4.52)	24.72 (25, 5.16)	24.38 (24, 5.02)	24.63 (19, 4.76)	25.07 (27, 3.49)	26.90 (29, 4.10)	25.71 (24, 5.07)
Depressive symptoms (BDI[b])	4.17 (24, 3.41)	2.11 (28, 2.57)	3.30 (23, 3.72)	3.00 (24, 3.09)	2.89 (18, 3.92)	1.65 (26, 2.42)	1.97 (29, 2.41)	2.86 (22, 2.92)
Autistic-like traits (AQ[c])	17.29 (24, 6.69)	13.67 (27, 3.21)	14.64 (25, 5.69)	16.71 (24, 6.60)	16.22 (18, 4.90)	14.26 (27, 5.54)	14.76 (29, 4.70)	15.50 (24, 5.79)
Alexithymia (TAS[d])	45.67 (24, 9.98)	41.04 (27, 9.98)	44.08 (25, 12.90)	43.96 (24, 11.12)	42.33 (18, 7.66)	43.74 (27, 9.32)	42.07 (29, 7.71)	40.46 (24, 9.32)
Trait anxiety (STAI[e])	38.88 (24, 8.25)	35.63 (27, 8.65)	34.24 (25, 7.38)	36.08 (24, 9.38)	33.06 (18, 8.00)	32.37 (27, 6.58)	34.66 (29, 6.11)	33.13 (23, 6.20)
Social anxiety (Liebowitz Total[f])	22.22 (23, 9.75)	18.48 (27, 13.08)	16.88 (25, 11.91)	19.46 (24, 12.73)	15.89 (18, 13.77)	11.70 (27, 11.13)	13.17 (29, 11.20)	12.67 (24, 12.04)

Notes. Participants rated their depressive symptoms with the [b] BDI (Becks Depression Inventory, Beck et al., 1996). Autistic-like traits were measured with the [c] AQ (Autism Spectrum Quotient, Baron-Cohen et al., 2006). Alexithymia was assessed with the [d] TAS (Toronto Alexithymia Scale, Taylor et al., 1985). The [e] STAI-Trait (State-Trait-Anxiety Inventory, Spielberger, 1970) was used to assess trait anxiety and the [f] Liebowitz questionnaire was used to measure social anxiety. PLC_{tra} = transdermal placebo gel; PLC_{int} = intranasal placebo; OXT_{int} = intranasal oxytocin; EST_{tra} = transdermal estradiol.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

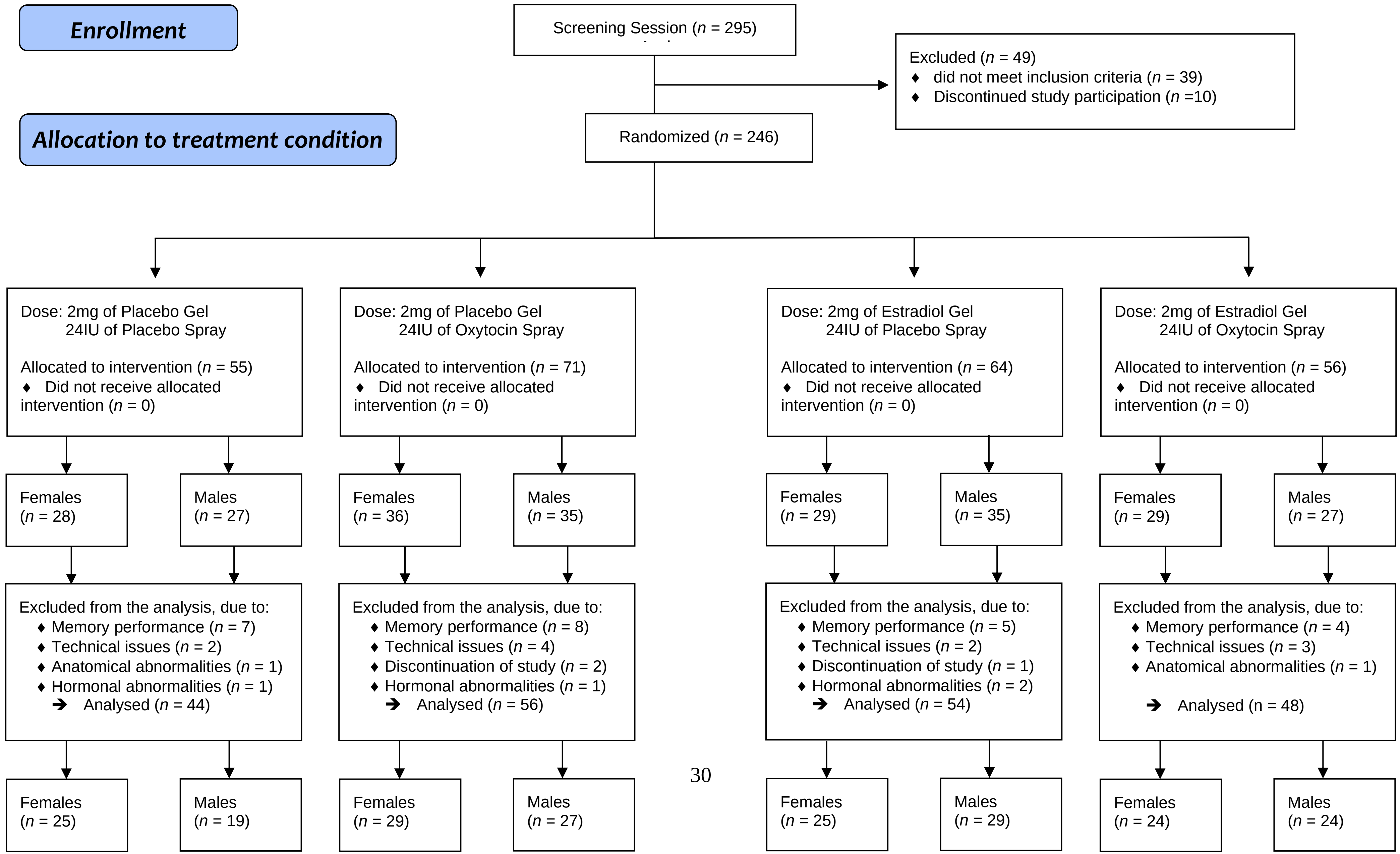
Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	n.a.
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	pg. 2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	pg. 3-6
	2b	Specific objectives or hypotheses	pg. 5-6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	pg. 7, 8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n.a.
Participants	4a	Eligibility criteria for participants	pg. 8 SI. pg. 5, 6
	4b	Settings and locations where the data were collected	SI. pg. 3
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	pg. 8, 9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	pg. 9, 10 SI. pg. 3-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n.a.
Sample size	7a	How sample size was determined	SI. pg. 2
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n.a.
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	SI. pg. 3
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	n.a.
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	n.a.
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	SI. pg. 3
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	pg. 8 SI. pg. 3

	11b	If relevant, description of the similarity of interventions	n.a.
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	pg. 10-12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	SI. pg. 3-8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	pg. 7; SI. pg. 30
	13b	For each group, losses and exclusions after randomisation, together with reasons	pg. 7; SI. pg. 30
Recruitment	14a	Dates defining the periods of recruitment and follow-up	SI. pg. 3
	14b	Why the trial ended or was stopped	n.a.
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	SI. pg. 27
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	pg. 7; SI. pg. 30
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	pg. 13-17
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	n.a.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	SI. pg. 9-18
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	SI. pg. 15-16
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	pg. 21
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	pg. 21
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	pg. 18-21
Other information			
Registration	23	Registration number and name of trial registry	pg. 7
Protocol	24	Where the full trial protocol can be accessed, if available	pg. 7
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	pg. 22

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram



Enrollment

Allocation to treatment condition

Screening Session (*n* = 295)

Excluded (*n* = 49)
- ♦ did not meet inclusion criteria (*n* = 39)
- ♦ Discontinued study participation (*n* =10)

Randomized (*n* = 246)

Dose: 2mg of Placebo Gel
24IU of Placebo Spray

Allocated to intervention (*n* = 55)
♦ Did not receive allocated intervention (*n* = 0)

Females (*n* = 28)

Males (*n* = 27)

Excluded from the analysis, due to:
- ♦ Memory performance (*n* = 7)
- ♦ Technical issues (*n* = 2)
- ♦ Anatomical abnormalities (*n* = 1)
- ♦ Hormonal abnormalities (*n* = 1)

➔ Analysed (*n* = 44)

Females (*n* = 25)

Males (*n* = 19)

Dose: 2mg of Placebo Gel
24IU of Oxytocin Spray

Allocated to intervention (*n* = 71)
♦ Did not receive allocated intervention (*n* = 0)

Females (*n* = 36)

Males (*n* = 35)

Excluded from the analysis, due to:
- ♦ Memory performance (*n* = 8)
- ♦ Technical issues (*n* = 4)
- ♦ Discontinuation of study (*n* = 2)
- ♦ Hormonal abnormalities (*n* = 1)

➔ Analysed (*n* = 56)

Females (*n* = 29)

Males (*n* = 27)

Dose: 2mg of Estradiol Gel
24IU of Placebo Spray

Allocated to intervention (*n* = 64)
♦ Did not receive allocated intervention (*n* = 0)

Females (*n* = 29)

Males (*n* = 35)

Excluded from the analysis, due to:
- ♦ Memory performance (*n* = 5)
- ♦ Technical issues (*n* = 2)
- ♦ Discontinuation of study (*n* = 1)
- ♦ Hormonal abnormalities (*n* = 2)

➔ Analysed (*n* = 54)

Females (*n* = 25)

Males (*n* = 29)

Dose: 2mg of Estradiol Gel
24IU of Oxytocin Spray

Allocated to intervention (*n* = 56)
♦ Did not receive allocated intervention (*n* = 0)

Females (*n* = 29)

Males (*n* = 27)

Excluded from the analysis, due to:
- ♦ Memory performance (*n* = 4)
- ♦ Technical issues (*n* = 3)
- ♦ Anatomical abnormalities (*n* = 1)

➔ Analysed (n = 48)

Females (*n* = 24)

Males (*n* = 24)