Supporting Information

Scheele et al. 10.1073/pnas.1314190110

SI Materials and Methods

Study Subjects. All subjects completed a comprehensive neuropsychological test battery. In the discovery (DSC) study, neuropsychological testing before study enrollment included the German version of the Rey Auditory Verbal Learning Test (RAVLT) (1, 2) to assess verbal learning skills, the digit-span test (DST) derived from the revised Wechsler adult intelligence scale (3) to assess working memory performance, the Leistungspruefsystem Subtest 4 (LPS 4) (4) to assess nonverbal reasoning IQ, the Mehrfach-Wortschatz-Intelligenztest Teil B (MWT-B) (5) to assess verbal IQ based on lexical decisions, and the trail-making test (TMT) (6) parts A and B to assess visual attention and task-switching performance. In the replication (RPL) study, cognitive performance was assessed using the Cambridge Neuropsychological Test Automated Battery (CANTAB), a computerized neurocognitive assessment presented through a touch-screen computer (7). For details of the outcome measure, see the CANTABeclipse Test Administration Guide (8). Subjects' speed of response to a visual target, the ability to retain spatial information, and visual memory were measured with the simple and reaction time task (RTI), the spatial working memory task (SWM; eight boxes version), and the paired associates learning task (PAL), respectively. All subjects were within a normal range of cognitive performance (Table S6).

The participants were asked to maintain their regular bed and waking times and to abstain from caffeine and alcohol intake on the day of the experiment. To control for potentially confounding effects of oxytocin (OXT) on state anxiety and mood, all subjects completed the State-Trait Anxiety Inventory (STAI) (9) and the Positive and Negative Affective Scale (PANAS) (10) immediately before the OXT/placebo administration and after the experimental task. Furthermore, all subjects completed the d2 Test of Attention (Aufmerksamkeits- und Belastungstest d2) (11) after the experimental task. For both the DSC and RPL study data, three repeated-measure ANOVAs with measurement (before and after the experiment) and treatment (OXT and placebo) as within-subject factors and state anxiety, positive affect, or negative affect as dependent variables revealed no significant main or interaction effects (all $P > 0.12$). There was also no significant difference between the d2 attention performance of the OXT and placebo (PLC) sessions in the DSC and RPL study (all $P > 0.06$; cf. Tables S4 and S5). Thus, OXT did not influence subjective anxiety, mood ratings, or attention. After completing the task, subjects were debriefed and asked to guess whether they had received OXT or PLC. The estimation of the received treatment was comparable between the OXT and PLC session in the DSC (correct estimates: OXT, $n = 11$; PLC, $n = 9$; $\chi^2_{(1)} = 0.9$; $P = 0.34$) and RPL study (correct estimates: OXT, $n = 9$; PLC, $n = 8$; $\chi^2_{(1)} = 0.12$; $P = 0.73$), showing that the subjects were unaware of whether they had received OXT or PLC.

Functional MRI Paradigm. An independent sample of 10 heterosexual healthy men (mean age ± SD: 25.50 ± 2.99 y; an ANOVA with the factor group yielded no significant age difference between the DSC, RPL, or pilot samples, $P = 0.38$) rated attractiveness of and the arousal induced by partner, familiar, and unfamiliar women, as well as the quality of the photographs on a visual analog scale (0 = minimum, 100 = maximum) before the first functional MRI (fMRI) session. The ratings of each dimension were adequately intercorrelated (DSC: estimated correlation of arousal ratings, $\rho = 0.63$; attractiveness, $\rho = 0.88$; picture quality, $\rho = 0.90$; RPL: arousal, $\rho = 0.56$; attractiveness, $\rho = 0.85$; picture quality, $\rho = 0.93$). In total, the task lasted around 10 min in the DSC study and 12 min (due to the additional trials with the matched controls for highly familiar women) in the RPL study.

Behavioral Task. Before the start of the experiment, the subjects were asked if they knew any of the control persons, and ratings of familiar persons (except for the partner and the familiar woman in the RPL study) were discarded from further analysis (DSC: 3 ratings, RPL: 81 ratings). After the ratings, all subjects completed the Passionate Love Scale (PLS) (12), which had a good internal consistency in both sessions (DSC: OXT Cronbach's $\alpha = 0.85$, PLC $\alpha = 0.91$; RPL: OXT $\alpha = 0.93$, PLC $\alpha = 0.92$). A 1 (minimum) to 9 (maximum) scale was used for the PLS. Example items are "I want ___ to know me—my thoughts, my fears, and my hopes" or "Sometimes I can't control my thoughts; they are obsessively on ___." Furthermore, all subjects completed the Marburg Attitude Scales towards Love Styles (MEIL), which is a German version of love styles developed by Lee (13). It contains three primary styles of loving: the first one is Eros, a romantic love style that is similar to passionate love and is characterized by a powerful attraction to the beloved individual. Interestingly, a dopamine D2 receptor (D2R) polymorphism has been found to be associated with the Eros love scale (14).The second is Ludus, which describes lovers who view love as a game and often have several partners simultaneously. The third is Storge, a slow developing, friendship-based love. These primary love styles can be combined to form secondary styles of love: Pragma (Storge and Ludus combined; pragmatic view on the relationship), Mania (Eros and Ludus combined; obsessive and possessive lover), and Agape (Storge and Eros combined; altruistic love style). In the German version, each love style is assessed with 10 items, and each dimension has an adequate reliability (DSC: Eros $\alpha = 0.86$, Ludus $\alpha = 0.88$, Storge $\alpha = 0.73$, Pragma $\alpha = 0.74$, Mania $\alpha = 0.88$, Agape $\alpha = 0.74$; RPL: Eros $\alpha = 0.94$, Ludus $\alpha = 0.48$, Storge $\alpha = 0.74$, Pragma $\alpha = 0.80$, Mania $\alpha = 0.77$, Agape $\alpha = 0.88$). For the samples of the DSC and RPL study, we observed the highest scores for Eros and the lowest scores for Ludus (cf. Table S1).

Analysis of fMRI Data. The first five volumes of each functional time series were discarded to allow for T1 equilibration. Images were corrected for head movement between scans by an affine registration. For realignment, a two-pass procedure was used, by which images were initially realigned to the first image of the time series and subsequently rerealigned to the mean of all images. For spatial normalization, the mean EPI image of each subject was normalized to the current Montreal Neurological Institute (MNI) template (15, 16) using the unified segmentation function in SPM-8. This algorithm combines image registration, tissue classification, and bias correction within the same generative model. All images were thereby transformed into standard stereotaxic space and resampled at 3 × 3 × 3-mm voxel size. The normalized images were spatially smoothed using an 8-mm full width at half maximum Gaussian kernel. Raw time series were detrended by the application of a high-pass filter (cutoff period, 128 s). A two-level random effects approach based on the general linear model as implemented in SPM-8 was used for statistical analyses.

Based on previous studies investigating the neural correlates of romantic love (17–19), we used 5-mm spheres as regions of interest (ROIs) centered at the coordinates of the reported maximum value for the nucleus accumbens (left: −10, 4, −4; right: 10, 4, −4), ventral tegmental area (left: −2, −12, −8; right: 2,

−12, −8), caudate body (left: −18, −14, 22; right: 18, −14, 22), caudate tail (left: −34, −32, −4; right: 36, −34, 0), putamen (left: −22, 2, 4; right: 24, −18, 10), and globus pallidus posterior (left: −32, 6, −8; right: 24, −8, −8). The Wake Forest University (WFU) Pickatlas (version 3.0) was used to generate ROI masks, and the threshold for significance was set at $P < 0.05$ and familywise error (FWE) corrected for multiple comparisons based on the size of the ROI.

Salivary OXT Collection and Analysis. Saliva samples were collected using prechilled Salivettes (Sarstedt). One sample was collected before administration of the nasal spray both in the OXT and PLC session and another sample was collected after the fMRI task. Salivettes were immediately centrifuged at 4,180 × *g* for 2 min, and aliquoted samples were stored at −80 °C until assayed. Salivary OXT concentrations were determined by using a 96-well commercial OXT-ELISA kit (ENZO). Measurements were performed in duplicate, and samples were treated according to kit instructions. According to the manufacturer, the sensitivity limit of the assay is 11.7 pg/mL, and 15.1% of the samples fell below the lower level of sensitivity. The assay's reported intraassay and interassay coefficients of variability are 9.1–12.4% and 5.2–14.5%, respectively.

Statistical Analysis. Demographical, neuropsychological, and behavioral data were analyzed using SPSS 20 (SPSS Inc.). Quantitative behavioral data were compared by repeated-measures ANOVA, and Pearson's product-moment correlation was used for correlation analysis. Eta-squared and Cohen's *d* were calculated as measures of effect size. The assumption of normality for all target variables was assessed separately for the OXT and PLC sessions using Kolmogorov-Smirnov tests. All target data were derived from normally distributed populations (all $P > 0.06$). The assumption of sphericity was assessed with Mauchly's test, and for significant violations, Greenhouse-Geisser's correction was applied. For qualitative variables, Pearson's χ^2 tests were used. All reported *P* values are two-tailed, if not otherwise noted, and $P < 0.05$ was considered significant.

SI Results

Behavioral Results. In both the DSC and RPL study, the OXT effect on the positive partner bias was dependent on a higher perceived attractiveness of the partner (DSC: $t_{(19)} = 1.75$, $P = 0.048$ one-tailed, $d = 0.27$; RPL: $t_{(19)} = 1.64$, $P = 0.059$ one-tailed, $d = 0.16$) rather than on a derogation of other women (DSC: $t_{(19)} = -1.12$, $P = 0.28$, $d = -0.14$; RPL: $t_{(19)} = -0.78$, $P = 0.44$, $d = -0.12$).

A slightly different definition of the positive partner bias yielded similar results. The positive partner bias defined as the difference between the attractiveness of the partner rated by the partner or other participants was significantly larger in the OXT (DSC: 41.37 ± 15.28; RPL: 44.89 ± 14.06) than in the PLC session (DSC: 36.50 ± 13.89; RPL: 41.25 ± 13.50) (DSC: $t_{(19)} = 2.46$, $P = 0.02$, $d = 0.33$; RPL: $t_{(19)} = 2.53$, $P = 0.02$, $d = 0.26$).

Salivary OXT Concentrations. In the RPL study, we also measured salivary OXT concentrations before the nasal spray administration (pre) and again after the fMRI task (post) to examine potential changes in the endogenous OXT levels in the PLC session due to the repeated presentation of partner and familiar woman photographs. In the OXT session, the concentration rose dramatically (pre: 34 ± 24 pg/mL, post: 589 ± 1022 pg/mL, Z = 3.92, $P < 0.01$), which is consistent with previous studies (20) but may also partly be attributed to OXT leaking from the nasal cavity into the mouth. More importantly, after the fMRI task, the endogenous OXT levels were also increased in the PLC session (pre: 35 ± 24 pg/mL, post: 57 ± 51 pg/mL, Z = 2.58, $P = 0.01$), which may indicate that face pictures of the partner and familiar individuals facilitated endogenous OXT release in line with a report in sheep (21), although we cannot rule out an effect of stress caused by the noisy scanner environment. Peripheral saliva samples have been used in previous studies (20, 22, 23), but the validity of saliva OXT measurement for quantification purposes has been questioned (24, 25), and the association between peripheral and central OXT level in the brain is highly controversial (26–28). Thus, these data should be interpreted as reflecting relative change rather than indicating absolute quantities.

fMRI Results. We found no significant association between the neural response in the nucleus accumbens (NAcc) and ventral tegmental area (VTA) and the OXT-induced changes in attractiveness ratings. This absence of correlation could be related to the absence of behavioral ratings performed during the fMRI scan, but it is also conceivable that the behavioral and neural OXT effects in this study are independent of each other.

Importantly, we also found no evidence for unspecific nonsocial OXT effects because there was no significant effect for the contrast [$\text{House}_{\text{OXT}} > \text{House}_{\text{PLC}}$] in any ROI, even at a very low significance threshold ($P < 0.001$ uncorrected, extent cluster threshold of $k \geq 0$ voxels). The results for the contrast [Partner > Unfamiliar] are shown in Tables S2 and S3 separately for the OXT and PLC sessions.

Furthermore, we were interested in deactivation in response to images of the partner. For the DSC and RPL study, on the whole-brain level, we found no significant deactivation for the contrasts comparing the OXT and PLC sessions ([$\text{Unfamiliar}_{\text{PLC}} > \text{Partner}_{\text{PLC}}$] > [$\text{Unfamiliar}_{\text{OXT}} > \text{Partner}_{\text{OXT}}$], [$\text{House}_{\text{PLC}} > \text{Partner}_{\text{PLC}}$] > [$\text{House}_{\text{OXT}} > \text{Partner}_{\text{OXT}}$]) or for the session-specific contrasts ([$\text{Unfamiliar}_{\text{OXT}} > \text{Partner}_{\text{OXT}}$], [$\text{House}_{\text{OXT}} > \text{Partner}_{\text{OXT}}$], [$\text{House}_{\text{PLC}} > \text{Partner}_{\text{PLC}}$]). Only in the PLC session of the RPL study, but not in the DSC study, did the contrast [$\text{Unfamiliar}_{\text{PLC}} > \text{Partner}_{\text{PLC}}$] yield a significant cluster in the postcentral gyrus (MNI *x, y, z*: −45, −22, 58; $t_{(19)} = 4.70$; $P_{\text{FWE}} = 0.004$).

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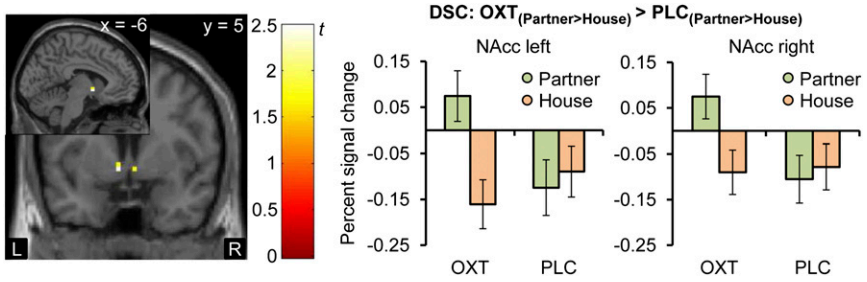

Fig. S1. OXT effects on NAcc responses with houses as control stimuli. The intranasal administration of OXT increased NAcc response to the female partner's face compared with houses ($OXT_{(Partner > House)} > PLC_{(Partner > House)}$; DSC left peak MNI coordinates −6, 5, −5; $t_{(114)} = 2.69$, $P_{FWE} = 0.03$; right peak MNI coordinates 6, 2, −5; $t_{(114)} = 2.05$, $P_{FWE} = 0.098$; display threshold $P < 0.05$ uncorrected). Percent signal change in the bilateral NAcc showed the greatest response to the mate after OXT administration. Error bars indicate SEM. DSC, discovery; L, left hemisphere; NAcc, nucleus accumbens; OXT, oxytocin; PLC, placebo; R, right hemisphere.

Table S1. Relationship characteristics (DSC and RPL study)

Variable	DSC study [mean (±SD)]	RPL study [mean (±SD)]
Relationship duration (months)	28.75 (15.43)	36.35 (25.33)
Age of partner (years)	24.00 (3.87)	24.90 (3.64)
PLS OXT*,†	6.33 (0.96)	6.46 (1.36)
PLS PLC*,†	6.46 (1.21)	6.49 (1.27)
Time (d) since the last time seen OXT*	1.75 (4.52)	1.84 (4.06)
Time (d) since the last time seen PLC*	0.60 (1.27)	1.05 (2.53)
Time (d) since the last intimate contact OXT*	2.55 (4.41)	4.26 (4.31)
Time (d) since the last intimate contact PLC*	3.95 (6.83)	3.32 (3.02)
Love style Eros‡	7.19 (0.99)	6.98 (1.55)
Love style Ludus‡	3.07 (1.58)	3.02 (0.98)
Love style Storge‡	5.49 (1.08)	5.93 (1.18)
Love style Pragma‡	4.68 (1.11)	4.72 (1.34)
Love style Mania‡	3.94 (1.48)	3.88 (1.16)
Love style Agape‡	6.68 (0.87)	6.84 (1.09)

*There was no significant difference in any relationship measure between the OXT and PLC sessions (all $P > 0.30$).
†Love in the relationship was measured with the Passionate Love Scale (PLS).
‡Different love styles were assessed using a German version of Lee's love styles (MEIL).

Table S2. Areas showing significantly greater activation for the partner compared with unfamiliar controls (DSC study)

Region	Right/left	Cluster size (voxels)	Z-score	MNI coordinates		
				x	*y*	*z*
OXT						
Thalamus/hypothalamus*	R	77	4.84	3	−4	−2
Posterior midbrain	L/R	67	4.29	0	−28	1
Middle occipital gyrus	R	169	4.10	33	−85	16
Anterior cingulate cortex	L/R	147	4.09	0	35	10
Cuneus	L	44	3.99	−21	−94	4
Medial frontal gyrus	R	32	3.87	12	53	7
Insula	L	27	3.67	−30	17	−14
Inferior frontal gyrus (tri)	L	25	3.74	−33	32	4
Calcarine	R	11	3.42	18	−91	−2
PLC						
Precuneus	L	56	4.55	−18	−61	40
Middle occipital gyrus	L	235	4.43	−39	−79	−2
Middle occipital gyrus	R	62	4.18	21	−94	4
Inferior frontal gyrus (tri)	R	50	4.10	45	35	10
Precuneus	R	51	4.07	27	−55	49
Middle temporal gyrus	R	23	3.39	36	−73	28

The whole-brain analysis was thresholded at an uncorrected $P < 0.001$ with a cluster extent threshold of $k = 7$ voxels.
*Significant at $P < 0.05$ familywise error corrected. tri, pars triangularis.

Table S3. Areas showing significantly greater activation for the partner compared with unfamiliar controls (RPL study)

Region	Right/left	Cluster size (voxels)	Z-score	MNI coordinates		
				x	*y*	*z*
OXT						
Middle temporal gyrus*	L	282	5.31	−45	−58	−5
Inferior frontal gyrus	R	12	4.14	27	35	−8
Middle occipital gyrus	R	10	3.90	33	−73	1
Cuneus	L	33	3.79	−21	−88	1
Inferior temporal gyrus	R	63	3.73	42	−64	−8
Anterior cingulate cortex	L	37	3.66	−3	47	−2
Anterior cingulate cortex	L/R	14	3.48	0	35	4
Middle occipital gyrus	R	15	3.39	39	−73	22
Inferior frontal gyrus	R	8	3.30	51	38	13
PLC						
Anterior cingulate cortex	L	19	3.96	−9	53	1

The whole-brain analysis was thresholded at an uncorrected $P < 0.001$ with a cluster extent threshold of $k = 7$ voxels.
*Significant at $P < 0.05$ familywise error corrected.

Table S4. State measurement of anxiety, mood, and attention (DSC study)

Variable	OXT group (n = 20) [mean (±SD)]	PLC group (n = 20) [mean (±SD)]	t	P
STAI–pre	32.79 (4.66)	33.26 (6.85)	−0.36	0.71
STAI–post	34.21 (5.89)	34.74 (6.81)	−0.60	0.56
PANAS–positive–pre	29.63 (5.55)	28.32 (5.64)	1.27	0.22
PANAS–positive–post	26.53 (7.03)	25.21 (6.2)	1.52	0.15
PANAS–negative–pre	11.25 (1.25)	12.45 (6.92)	−0.79	0.44
PANAS–negative–post	11.37 (1.54)	11.32 (2.14)	0.14	0.89
D2	224.53 (33.42)	235.21 (34.62)	−1.99	0.06

State anxiety before and after the experiment was assessed using the STAI. Mood before and after the experiment was assessed using the PANAS. Attention performance after the experiment was assessed using the D2 (n = 19).

Table S5. State measurement of anxiety, mood, and attention (RPL study)

Variable	OXT group (n = 20) [mean (±SD)]	PLC group (n = 20) [mean (±SD)]	t	P
STAI–pre	31.65 (5.60)	30.55 (5.29)	1.27	0.22
STAI–post	31.47 (5.59)	31.11 (4.85)	0.39	0.70
PANAS–positive–pre	28.80 (6.00)	28.25 (5.53)	0.48	0.64
PANAS–positive–post	28.20 (5.66)	28.40 (6.55)	−0.22	0.83
PANAS–negative–pre	10.85 (1.23)	11.90 (3.54)	−1.47	0.16
PANAS–negative–post	11.20 (3.09)	10.75 (1.33)	0.96	0.35
D2	215.55 (46.10)	221.25 (47.30)	−0.80	0.43

State anxiety before and after the experiment was assessed using the STAI. Mood before and after the experiment was assessed using the PANAS. Attention performance after the experiment was assessed using the D2 (n = 19).

Table S6. Demographics and neuropsychological performance (DSC and RPL study)

Variable	DSC study [mean (±SD)]	RPL study [mean (±SD)]
Age, y	25.05 (3.25)	26.55 (3.76)
Years of education	17.35 (2.28)	17.20 (2.31)
RAVLT		
Trials 1–5*	62.6 (8.24)	
Trial 6 retention†	13.3 (2.0)	
Trial 7 delayed recall‡	13.35 (1.87)	
LPS-4	30.5 (3.87)	
MWT-B	31.55 (2.56)	
TMT-A (s)	23.47 (6.18)	
TMT-B (s)	55.54 (10.76)	
Digit-span, forward	8.15 (1.39)	
Digit-span, backward	7.9 (2.13)	
RTI		
Simple reaction time (ms)		286.11 (24.56)
Simple movement time (ms)		341.58 (57.54)
Five-choice reaction time (ms)		313.85 (34.25)
Five-choice movement time (ms)		351.30 (60.56)
PAL		
Total errors		8.56 (6.74)
Mean errors to success		2.47 (2.45)
SWM-8		
Between errors		11.89 (13.56)
Strategy score		15.16 (4.21)
Trait anxiety§	31.8 (9.68)	30.85 (7.74)
BDI	3.25 (4.39)	1.90 (3.40)

In the DSC study, verbal declarative memory performance was assessed using a German adaption of the RAVLT and included *learning performance across five trials (maximum possible score 75), †susceptibility to interference (maximum possible score 15), and ‡delayed recall (maximum possible score 15). Nonverbal reasoning IQ was assessed by the LPS subtest 4 (maximum possible score 40). Verbal IQ based on lexical decisions was assessed by the MWT-B (maximum possible score 37), visual attention and task-switching was assessed using the TMT-A and TMT-B (results displayed in seconds), and working memory performance was assessed using the digit-span forward and backward test (maximum possible score 14). In the RPL study, subjects' speed of response to a visual target, visual memory, and the ability to retain spatial information were measured with the RTI, the PAL, and the SWM, respectively. Anxiety symptoms were assessed by the §State Trait Anxiety Inventory and depressive symptoms by the self-report BDI. BDI, Becks Depression Scale, Version II.