Supplemental Information

Oxytocin enhances cognitive control of food craving in women

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Supplemental Experimental Procedures

1. Study subjects

All subjects completed a comprehensive neuropsychological test battery. Neuropsychological testing before study enrollment included the DST (digit-span test), derived from the revised Wechsler adult intelligence scale (Wechsler, 1997), to assess working memory performance, the LPS 4 ('Leistungspruefsystem Subtest 4') (Horn, 1983) to assess nonverbal reasoning IQ, and the MWT-B ('Mehrfach-Wortschatz-IntelligenztestTeil B') (Lehrl et al., 1995) to assess verbal IQ based on lexical decisions. 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) (Spielberger et al., 1970) and the Positive and Negative Affective Scale (PANAS) (Watson et al., 1988) immediately before the OXT/placebo administration and after the experimental task. Three repeated-measure analyses of variance (ANOVA) with 'measurement' (before and after the experiment) and 'treatment' (OXT and placebo) as within-subjects factors and 'state anxiety', 'positive affect', or 'negative affect' as dependent variables revealed no significant main or interaction effects (all *P*s > 0.05; cf. *Supplemental Table S3*). Thus, OXT did not influence subjective anxiety or mood ratings. After completing the task, subjects were debriefed and asked to guess whether they had received OXT or PLC. Neither in the OXT (correct estimates 39 %, $\chi^2_{(1)} = 1.58$, $P = 0.21$) nor in the PLC session (correct estimates 52 %, $\chi^2_{(1)} = 0.93$, $P = 0.34$) did the correct estimation of the received treatment significantly differ from chance, showing that the subjects were unaware of whether they had received OXT or PLC.

2. FMRI paradigm

Pictures of candies and desserts were collected from the International Affective Picture System (Lang et al., 2005) and from public online sources. An independent sample of six healthy women and four healthy men (mean age ± S.D.: 26.9 ± 2.12) rated the palatability and calorie content of 102 pictures on a Likert scale (1 = minimum, 9 = maximum). Sixty pictures with the highest palatability ratings were selected and two picture sets A (for NOW trials) and B (for LATER trials) were created, each with 30 pictures. These picture sets were matched for palatability (A mean ± SD: 6.99 ± 0.41, B: 6.97 ± 0.44) and calorie content (A: 6.57 ± 0.96, B: 6.71 ± 0.99). All participants completed four test trials before the start of the experiment to familiarize them with the craving task. In total, the task lasted approximately 19 minutes.

3. 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 re-realigned 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 using the unified segmentation function in SPM8. This algorithm combines image registration, tissue classification,

and bias correction within the same generative model. All images were thereby transformed into standard stereotaxic space and resampled at 3 x 3 x 3 mm voxel size. The normalized images were spatially smoothed using a 6-mm FWHM Gaussian kernel. Raw time series were detrended using a high-pass filter (cut-off period 128 s). A two-level random effects approach based on the general linear model as implemented in SPM8 was used for statistical analyses.

4. Hormonal assessment

Serum FSH, LH, and estradiol were analyzed by fully automated homogeneous sandwich chemiluminescent immunoassays based on the LOCI™ technology on a Dimension Vista™ System according to the manufacturer´s instructions (Siemens Healthcare Diagnostics, Eschborn, Germany). The detection limits of each assay were 0.2 IU/l for LH and FSH and 11 pg/ml for estradiol, respectively. The coefficients of variation for intra-assay and inter-assay precision were <1.8 % and <2.1 % for LH, <1.9 % and <2.2 % for FSH, and <5.5 % and <5.9 % for estradiol, 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- and inter-assay precision were <4.2 % and <5.5 %. The cross-reactivity of all assays with other related compounds was minimal.

5. Statistical analysis

Demographical, neuropsychological, and behavioral data were analyzed using SPSS 22 (SPSS Inc., Chicago, IL, USA). Quantitative behavioral data were compared by repeated measures analysis of variance (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*s > 0.05). The assumption of sphericity was assessed with Mauchly's test, and Greenhouse-Geisser's correction was applied for significant violations. Pearson's chi-squared tests were used for qualitative variables. All reported *P*-values are two-tailed, if not otherwise noted, and *P*-values < 0.05 were considered significant.

6. Salivary oxytocin collection and analysis

Saliva samples were collected before the nasal spray administration and after the experiment using commercial sampling devices (Salivettes, Sarstedt). Salivettes were immediately centrifuged at 4,180 g for 2 min and aliquoted samples were stored at -80°C until assayed. Saliva OXT was extracted and quantified by a highly sensitive and specific radioimmunoassay (RIAgnosis, Munich, Germany) (Kagerbauer et al., 2013). The limit of detection was 0.1 - 0.5 pg depending on the age of the tracer. Intra- 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.

Supplemental Results

1. Behavioral Results

A repeated measures ANOVA with the salivary OXT concentrations as dependent variable and the within-subject factors 'treatment' (OXT, PLC) and 'time' (before the fMRI paradigm, after the fMRI paradigm) yielded significant main effects of treatment ($F_{(1, 23)} = 30.22$, $P < 0.01$, $\eta^2 = 0.57$) and time ($F_{(1, 23)} = 40.19$, $P < 0.01$, $\eta^2 = 0.64$) as well as an interaction of treatment and time ($F_{(1, 21)} = 32.61$, $P < 0.01$, $\eta^2 = 0.59$). As expected, intranasal OXT significantly increased the salivary OXT concentrations (OXT session: before = 2.05 ± 2.74 pg/ml, after = 12.71 ± 6.78 pg/ml, $t_{(25)} = 8.12$, $P < 0.01$), but the mere presentation of food stimuli did not alter OXT concentrations (PLC session: before mean ± SD = 1.87 ± 1.84 pg/ml, after = 3.38 ± 4.90 pg/ml; $t_{(27)} = 1.20$, $P = 0.24$). Furthermore, neither baseline OXT concentrations nor the difference between OXT concentrations (before minus after the paradigm) were correlated with the BMI or food craving ratings (all Ps > 0.10).

In a supplemental analysis, we also explored whether the eating style measured with the Restraint Scale (Dinkel et al., 2005) moderated the OXT effect on food craving. However, there was no significant correlation ($P = 0.27$) between the eating style and the food craving ratings ($[Now_{OXT} - Later_{OXT}] - [Now_{PLC} – Later_{PLC}]$).

2. Imaging Results

In a correlational analysis, we explored whether baseline OXT concentrations were associated with neural responses to food stimuli (LATER and NOW) or the cognitive down-regulation of craving (LATER > NOW) in the PLC session. Neither in the whole brain analysis nor in the ROI-based analysis of the middle frontal gyrus did we detect any significant correlations.

Supplemental References

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Tables

Table S1. Demographics and neuropsychological performance

	Mean (± SD)
Age (years)	25.35 (4.37)
Education (years)	16.02 (3.25)
Weight (kg)	62.94 (9.02)
Height (cm)	168.13 (6.30)
LPS-4 [a]	29.94 (4.05)
MWT-B [b]	26.88 (6.48)
Digit-span, forward	9.59 (2.11)
Digit-span, backwards	9.03 (2.15)
Trait anxiety [c]	36.90 (7.88)
BDI [d]	4.81 (4.83)

Notes. Nonverbal reasoning IQ was assessed by the [a] LPS (Leistungsprüfsystem) subtest 4 (maximum possible score 40). Verbal IQ based on lexical decisions was assessed by the [b] MWT-B (Mehrfachwahl-Wortschatz-Intelligenz-Test, Teil B) (maximum possible score 37), working memory performance was assessed using the digit-span forward and backward test (maximum possible score 14). Anxiety symptoms were assessed by the [c] State Trait Anxiety Inventory and depressive symptoms by the self-report [d] BDI (Beck´s Depression Scale, Version II).

Table S2. Baseline hunger rating, sleep data and measurement of endocrine factors and blood sugar

	OXT Mean (± SD)	PLC Mean (± SD)	*t*	*P*
Hunger rating [1]	1.58 (1.03)	1.81 (1.05)	-0.93	0.36
Blood sugar (mg/dl) [1]	93.52 (8.36)	97.22 (13.74)	-1.44	0.16
Progesterone (ng/ml) [1]	3.28 (4.79)	1.96 (3.03)	1.55	0.13
Estradiol (pg/ml) [1]	67.03 (62.72)	64.57 (67.60)	0.27	0.79
FSH (U/l) [1]	4.26 (2.93)	3.64 (2.77)	1.13	0.27
LH (U/l) [1]	6.72 (11.39)	5.43 (8.52)	1.36	0.19
Sleep duration last night (h)	7.66 (1.22)	7.22 (1.53)	1.25	0.22
Time between getting up and onset of the experiment (h)	6.76 (3.40)	6.90 (3.09)	-0.15	0.88

Notes. Abbreviations: [1] Hunger ratings were obtained on a scale from 1 (minimum) to 6 (maximum). FSH, follicle-stimulating hormone; LH, luteinizing hormone; OXT, oxytocin; PLC, placebo.

Table S3. State measurement of anxiety and mood

	OXT session Mean (± SD)	PLC session Mean (± SD)	*t*	*P*
STAI – pre [a]	31.58 (4.99)	32.16 (5.42)	-0.71	0.49
STAI – post [a]	33.06 (6.61)	31.23 (5.71)	1.52	0.14
PANAS – positive – pre [b]	30.77 (6.18)	30.77 (4.40)	<0.01	0.99
PANAS – positive – post [b]	29.52 (6.64)	30.48 (5.73)	-0.75	0.46
PANAS – negative – pre [b]	11.68 (3.13)	11.16 (1.49)	1.06	0.30
PANAS – negative – post [b]	11.77 (3.40)	10.71 (1.32)	1.98	0.06

Notes. State anxiety before and after the experiment was assessed using the [a]STAI = State Trait Anxiety Inventory. Mood before and after the experiment was assessed using the [b] PANAS = Positive and Negative Affect Schedule. Abbreviations: OXT, oxytocin; PLC, placebo.

Table S4. Areas showing significantly greater activation for LATER compared to NOW trials

Region	Right/left	Cluster size (voxels)	*t*	*MNI-coordinates*		
				x	*y*	*z*
OXT						
Inferior Parietal Lobule*	L	844	5.77	-50	-56	48
Precuneus*	L		4.01	-36	-68	34
Inferior Parietal Lobule*	L		3.79	-40	-56	40
Middle Frontal Gyrus*	L	302	4.65	-32	48	-8
Inferior Frontal Gyrus*	L		4.44	-42	36	-10
Supramarginal Gyrus	R	276	4.52	42	-56	36
Supramarginal Gyrus	R		3.72	50	-56	36
Superior Temporal Gyrus	R		3.54	56	-62	28
Precental Gyrus	R	252	3.97	46	-14	50
Precental Gyrus	R		3.89	42	-18	40
Precental Gyrus	R		3.62	34	-20	46
Middle Temporal Gyrus*	L	327	3.93	-62	-40	2
Superior Temporal Gyrus*	L		3.53	-52	-38	4
Middle Frontal Gyrus*	L	274	3.85	-34	10	44
Middle Frontal Gyrus	L		3.73	-42	12	52
Middle Frontal Gyrus	L		3.63	-36	26	46
PLC						
Inferior Frontal Gyrus	L	195	5.66	-50	20	16
Inferior Frontal Gyrus	L		3.13	-42	16	12
Inferior Frontal Gyrus	L	221	5.29	-48	36	-8
Middle Frontal Gyrus	L		4.25	-46	48	-6
Middle Frontal Gyrus	L		3.93	-38	48	-6
Middle Temporal Gyrus*	L	412	5.25	-64	-36	-6
Middle Temporal Gyrus*	L		4.75	-48	-38	0
Middle Temporal Gyrus*	L		4.41	-62	-42	0
Inferior Parietal Lobule*	L	544	4.90	-44	-68	44
Superior Temporal Gyrus*	L		4.80	-42	-58	26
Supramarginal Gyrus*	L		4.76	-40	-56	34
Middle Frontal Gyrus	L	157	4.31	-38	12	52
Supramarginal Gyrus	R	129	4.31	58	-60	32
Supramarginal Gyrus	R		3.41	60	-54	24
Superior Frontal Gyrus	L	84	4.18	-12	52	30
Superior Frontal Gyrus	L		3.68	-18	54	20
Medial Frontal Gyrus	L	114	3.66	-4	38	44
Superior Frontal Gyrus	L		3.55	-14	30	56
Superior Frontal Gyrus	L		3.38	-6	36	52

Notes. The whole-brain analysis was thresholded at an uncorrected $P < 0.005$ with a cluster extent threshold of k = 50 voxels.
*Significant at $P < 0.05$ family wise error corrected. Abbreviations: OXT, oxytocin; PLC, placebo.

Table S5. Areas showing significantly greater activation for NOW compared to LATER trials

Region	Right/left	Cluster size (voxels)	*t*	*MNI-coordinates*		
				x	*y*	*z*
OXT						
Lingual Gyrus*	R	560	7.52	6	-74	-8
Calcarine*	L/R		6.24	0	-86	-4
Lingual Gyrus*	L		4.22	-18	-80	-10
Rolandic Operculum	L	54	3.93	-44	-2	10
Middle Temporal Gyrus	L	53	3.53	-40	-84	16
Middle Occipital Gyrus	L		3.07	-32	-80	12
Superior Occipital Gyrus	L		2.92	-32	-86	22
Postcentral Gyrus	L	82	3.48	-46	-22	48
PLC						
Lingual Gyrus*	3767	R	8.00	8	-76	-6
Calcarine*		R	6.98	2	-84	-2
Lingual Gyrus*		L	6.92	-6	-80	-6
Cuneus*	2740	R	7.48	14	-94	16
Middle Occipital Gyrus*		R	7.28	24	-90	18
Middle Temporal Gyrus*		R	5.42	38	-76	20
Postcentral Gyrus*	1815	L	6.08	-44	-18	58
Precentral Gyrus*		L	5.85	-48	-16	48
Precentral Gyrus*		L	5.45	-36	-24	52
Cingulate Gyrus	208	L	5.48	-10	-10	48
Medial Frontal Gyrus		L	4.52	-4	-14	54
Middle Cingulate Gyrus		L	3.34	-6	0	44
Middle Cingulate Gyrus*	336	R	5.38	2	12	32
Anterior Cingulate Gyrus*		R	4.87	2	22	26
Anterior Cingulate Gyrus*		R	3.83	4	4	28
Midbrain*	388	L	5.10	-8	-22	-12
Midbrain*		L	5.03	-8	-30	-6
Postcentral Gyrus*	267	R	5.04	60	-22	26
Superior Temporal Gyrus*		R	3.89	60	-20	4
Transvere Temporal Gyrus*		R	3.50	66	-16	10
Insula	82	R	4.65	38	0	-6
Superior Temporal Gyrus		R	2.99	38	2	-16
Inferior Frontal Gyrus	67	R	4.31	48	44	16
Inferior Frontal Gyrus		R	3.33	46	40	4
Subcallosal Gyrus	74	R	4.21	18	8	-14
Putamen		R	3.53	22	16	-8
Caudate Nucleus		R	3.31	12	14	-6
Middle Temporal Gyrus	254	L	4.17	-46	-58	0
Middle Temporal Gyrus		L	3.80	-52	-62	-4
Insula	80	L	4.17	-42	-2	14

Precentral Gyrus		L	3.59	-46	-2	20
Postcentral Gyrus		L	3.41	-54	-16	16
Precentral Gyrus	111	R	4.05	48	0	30
Inferior Frontal Gyrus		R	4.01	44	6	26
Midbrain	56	R	3.83	14	-22	-4
Midbrain		R	3.50	6	-28	-4
Calcarine	55	L	3.83	-20	-56	20
Posterior Cingulate Gyrus		L	2.92	-12	-58	12

Notes. The whole-brain analysis was thresholded at an uncorrected $P < 0.005$ with a cluster extent threshold of k = 50 voxels.
*Significant at $P < 0.05$ family wise error corrected. Abbreviations: OXT, oxytocin; PLC, placebo.