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Oxytocin enhances attractiveness of unfamiliar female faces independent of the dopamine reward system

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Abstract Evidence from animal studies suggests that the social attraction and bonding effects of the neuropeptide oxytocin (OXT) are mediated by its modulation of dopamine (DA) release in brain reward centers, but this has not yet been demonstrated in humans. DA release can be measured by positron emission tomography (PET) using the radioligand [11C]raclopride. Its binding to DA D2 receptors (D2R) is sensitive and reciprocally related to endogenous DA, especially in the striatum. In a randomized double-blind placebo-controlled within-subjects trial on 18 adult male volunteers we combined [11C]raclopride PET and a facial attractiveness rating task to establish whether intranasal OXT (24 IU) increased both the perceived attractiveness of unfamiliar female faces and striatal DA release compared with placebo administration. While our behavioral data confirmed that subjects rated unfamiliar female faces as more attractive following OXT treatment, and this correlated with an increased perfusion rate in the striatum, there was no evidence for altered [11C]raclopride binding in the striatum or pallidum. Instead under OXT we rather observed an increased [11C]raclopride binding and reduced perfusion rate in subregions of the right dorsomedial prefrontal gyrus and superior parietal

Abbreviations: MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; PET, positron emission tomography.

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gyrus. The absence of OXT effects on dopamine release and D2 receptors in brain reward centers, despite increased striatal activity, implies that the peptide may facilitate perceived attraction via non-dopaminergic actions.

1. Introduction

The neuropeptide oxytocin (OXT) has been shown to promote a variety of social behaviors in humans after intranasal application (Striepens et al., 2011; Bethlehem et al., 2013). In terms of facilitating effects in the domain of partner attraction, OXT has been shown in men to increase the perceived attractiveness of unfamiliar women (Theodoridou et al., 2009) and to strengthen bonds between existing partners, both by increasing the perceived attractiveness of the female partner and the distance men keep between themselves and attractive unfamiliar women (Scheele et al., 2012). In the bonding domain OXT has also been shown to increase trust and cooperation (Baumgartner et al., 2008; Kosfeld et al., 2005) and empathy (Domes et al., 2007; Hurlemann et al., 2010).

Pioneering work using animal models first established that intracerebroventricular OXT promoted the establishment of both maternal-offspring bonds in sheep (Kendrick et al., 1987) and partner bonds in monogamous voles (Williams et al., 1994). Furthermore, evidence from these animal models showed that OXT could exert these modulatory effects on both attraction and bonding by influencing classical neurotransmitter release (Kendrick, 2000). In the domain of partner-bonds, a key initial observation was that the density of OXT receptors (OXTR) was significantly greater in the nucleus accumbens, an important dopaminergic (DA, dopamine) reward center, of monogamous prairie voles as opposed to other species of voles which were promiscuous (Insel and Shapiro, 1992). Subsequently it was shown that the OXT-elicited partner preference in monogamous female prairie voles is blocked by a DA D_2 receptor (D_2R) antagonist. Furthermore, microinjection of a D2R agonist into the nucleus accumbens (NAcc) can promote a partner preference in the absence of mating, an effect blocked by either a D_2R or an OXTR antagonist. Thus co-activation of D_2R and OXTR in the striatum may be important for pair bond formation and its maintenance (Liu and Wang, 2003). Recently D_2R—OXTR heteromers with facilitating receptor—receptor interactions have also been found in the striatum (Romero-Fernandez et al., 2012), further emphasizing the potential functional importance of interactions between D_2R and OXTR.

In humans it has also been proposed that OXT may facilitate both attraction and bonding via interactions with D_2R (Zeki, 2007; Skuse and Gallagher, 2009), with it being proposed that OXT may enhance the hedonic value of social interactions by specifically acting on D_2R in dopaminergic reward centers. Facilitation of interpersonal trust by OXT (Baumgartner et al., 2008; De Dreu et al., 2010; Kosfeld et al., 2005) has been attributed to the inhibition of defensive behaviors and the activation of dopaminergic reward circuits (Skuse and Gallagher, 2009). Similarly, OXT facilitation of face attraction (Theodoridou et al., 2009) has been linked with potential modulation of striatal dopaminergic reward mechanisms since the striatum shows enhanced activation with increasing facial attractiveness (Aharon et al., 2001; Cloutier et al., 2008; Liang et al., 2010; Winston et al., 2007). Furthermore, potential interactions between OXT and dopaminergic signaling in brain reward centers has received support from a study showing effects of OXTR polymorphisms on dopaminergic function (Love et al., 2012).

In view of the growing evidence suggesting that OXT may influence both attraction and bonding behavior via a modulation of DA release our main objective in the current study was to investigate whether OXT facilitation of perceived attractiveness of unfamiliar women by men was associated both with activation of dopaminergic brain reward centers and increased liberation of DA. We decided to focus on OXT effects of attraction first because it is easier to investigate in controlled laboratory conditions than bonding.

In order to quantify changes in both neural and D_2R activity we employed positron emission tomography (PET) and the antagonist radioligand [^{11}C]raclopride to assess changes in the D_2R binding potential (BP_{ND}) providing a sensitive measure of endogenous DA release. While it is commonly recognized that striatal regions are mainly the most reliably measured (Koepp et al., 1998; Laruelle, 2000) using this ligand, recent [^{11}C]raclopride studies also assessed D_2R occupancy in some extra-striatal regions (Garraux et al., 2007; Stokes et al., 2010). By measuring the early phase of [^{11}C]raclopride PET we could also investigate changes in brain perfusion, thereby allowing us to quantify neural activation changes.

In the current study our main objective was to establish whether OXT effects in increasing perceived attractiveness of the faces of unfamiliar women were associated with increased activity in dopamine reward regions and in altered D_2R binding. We therefore used a double-blind, placebo (PLC)-controlled, within-subjects design, with subjects being presented with pictures of unfamiliar, neutral expression female faces during the sensitive phase of [^{11}C]raclopride PET scan. An important consideration in our study design was that since the sensitivity of [^{11}C]raclopride PET to DA changes is lower at resting state and comparatively better at a starting point of low-level activation, and that potential saturation effects can occur with stimuli of maximum reward value, we should aim to raise baseline DA levels by starting to present face pictures slightly before the sensitive phase of the PET scan timed to coincide with the maximum effect of intranasal OXT. We also used neutral expression female faces rated as being of medium attractiveness to avoid possible problems with saturation effects. This design precludes measurement of potential OXT effects on basal dopaminergic activity, but our main objective was to establish changes associated with altered face attraction.

2. Methods

2.1. Participants

Eighteen healthy, male volunteers (age range 21–36 years, mean 26.9 ± 3.4 years) gave written informed consent to participate in this study, in accordance with the Declaration of Helsinki. We only used male subjects in the current study since including female ones would have required additional controls for potential effects of menstrual cycle and contraceptive use. The MRI and PET protocol had been approved by the institutional ethics review board (IRB) of the Medical Faculty of the Heinrich Heine University of Düsseldorf as well as the German Federal Office of Radiation Protection.

All participants completed a comprehensive neuropsychological test battery to control for cognitive performance (Table 1).

2.2. Study design

In this double-blind, randomized, PLC-controlled, counterbalanced cross-over study we compared attractiveness ratings and [^{11}C]raclopride PET measures of DA release in the same human participants after OXT and PLC administration. Each participant completed two sessions of testing separated by at least 4 days (mean 13 days; SD 16.59; Fig. 1). In the first session volunteers self-administered either 24 IU of intranasal OXT (Syntocinon Spray, Sigma-Tau, Pomezia, Italy; 3 puffs per nostril, each puff containing 4 IU OXT) or PLC. In the second session they received the alternate treatment (i.e. OXT or PLC). In both sessions, volunteers completed a face-attraction rating task. In order to obtain two equivalent versions of this task (i.e. one for each PET session), we conducted an independent pilot experiment, in which 364 pictures of female faces were rated by twelve healthy men, none of whom participated in the main experiment. These pictures were taken in house from female volunteers (aged 20–40 years) who gave consent for study use but not for publication. Only pictures rated as medium attractive were entered into the final stimulus pool of 302 faces, which was divided into two sets (A and B) of 151 faces. These two sets did not differ in terms of attractiveness and trustworthiness (attractiveness: $M = 5.36 \pm 1.22$ and $M = 5.39 \pm 1.22$, $p = 0.828$, $t(300) = -0.22$; trustworthiness: $M = 5.13 \pm 1.03$ and 5.11 ± 1.04, $p = 0.897$, $t(300) = 0.13$). Thus, in the main experiment, both drug administration and task performance (Set A or B) were counterbalanced across subjects. In the first session Group 1 was treated with OXT, followed by the behavioral set A ($n = 5$); group 2 received OXT, followed by set B ($n = 4$), group 3 received PLC, followed by set A ($n = 4$); and group 4 received PLC, followed by set B ($n = 5$) (Fig. 1). In line with previous studies, behavioral testing commenced 48 ± 16 min after OXT or PLC was administered (Born et al., 2002; Striepens et al., 2011). The PET scan started 64 ± 18 min following OXT/PLC administration and 18 ± 5 min after starting the attractiveness rating test in order to allow the observation of female faces to promote a degree of pre-activation of the dopaminergic system. Experiments were conducted at the same time of day (morning) for both OXT and PLC sessions (actual times of the intranasal spray application, start of the face-attraction rating paradigm, and start of the scan were: 09h58 ± 27 min; 10h44 ± 34 min and 11h01 ± 35 min, respectively, for OXT and 10h26 ± 42 min; 11h14 ± 44 min and 11h31 ± 45 min, respectively, for PLC; there were no significant differences between the timings in OXT and PLC sessions, $p > 0.05$ in all cases). Blood samples were taken immediately before and

Table 1 Demographics and neuropsychological performance.

	Mean (±SD)	Min	Max
Age, y	26.9 (3.4)	21	36
Body weight, kg	77.7 (14)	63	115
Years of education	18.4 (2.6)	15	24
RAVLT[a]			
Trial 1–5[b]	60.6 (6.5)	49	70
Trial 6 retention[c]	13.3 (1,6)	10	15
Trial 7 delayed recall[d]	14.1 (1.3)	11	15
LPS-4[e]	30.8 (2.8)	26	35
MWT-A[f]	31.2 (1.8)	27	34
d[g]	196.5 (43.0)	124	294
TMT-A[h]	24.3 (8.9)	12	49
TMT-B[h]	63.7 (18.7)	37	100
Digit-span, forward	9.1 (2.5)	4	14
Digit-span, backwards	8.2 (2.0)	5	12
BDI[i]	4.6 (2.9)	0	9

Notes. Verbal declarative memory performance was assessed using a German adaption of the [a]RAVLT (Rey Auditory Verbal Learning Test) and included [b]learning performance across five trials (maximum possible score 75), [c]susceptibility to interference (maximum possible score 15), and [d]delayed recall (maximum possible score 15). Nonverbal reasoning IQ was assessed by the [e]LPS (Leistungsprüfsystem) subtest 4 (maximum possible score 40). Verbal IQ based on lexical decisions was assessed by the [f]MWT-A (Mehrfachwahl-Wortschatz-Intelligenz-Test, Teil A) (maximum possible score 37), visual attention and concentration was assesses using the [g]d2 (Aufmerksamkeits- und Belastungstest d2), visual attention and task-switching was assessed using the [h]TMT-A and TMT-B (trail-making test A, B) (results displayed in seconds), working memory performance was assessed using the digit-span forward and backward test (maximum possible score 14). Depressive symptoms were assessed by the self-report [i]BDI (Beck' Depression Scale, Version II).

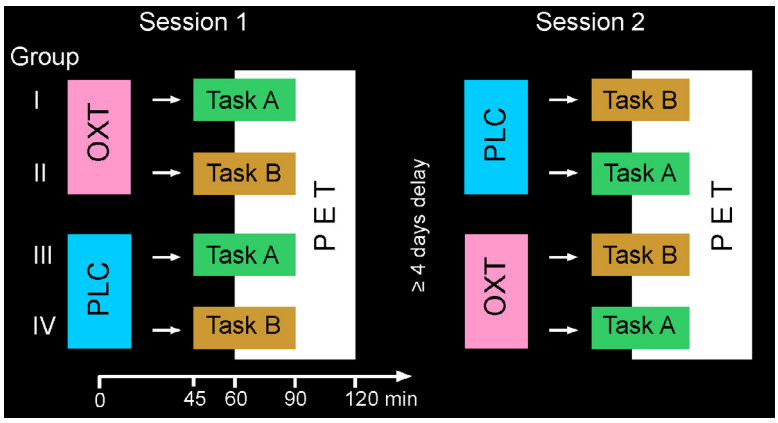

Fig. 1 Study design. Participants self-administered oxytocin (OXT) or placebo (PLC) 45 min prior to the behavioral task and 60 min prior to the start of the PET session. In the first session group I was treated with OXT, followed by the behavioral task A ($n = 5$); group II received OXT, followed by the behavioral task B ($n = 4$); group III received PLC, followed by task A ($n = 4$); and group IV received PLC, followed by task B ($n = 5$). In the second session they received the alternate preparation (OXT or PLC) and the alternate task (set A or B). The two sessions of testing were separated by at least 4 days.

60 min and 120 min after OXT or PLC application. Samples were immediately centrifuged, the plasma mixed with 0.1 mg/ml Aprotinin (Sigma, 3–8 TIU/mg) and then frozen and stored at −80 °C prior to assay. Plasma OXT concentrations were determined by radio-immunoassay as previously described (Neumann et al., 2013). To exclude anatomical abnormalities in the CNS and to carry out co-registration of the anatomical data with the PET results, a high-resolution T1-weighted MRI scan was acquired from each subject after one of the PET scan sessions.

2.3. Behavioral task

Participants were placed inside the scanner and allowed to acclimate to it for at least 10 min (Siemens ECAT EXAT HR+ scanner, see below) before performing the face-attraction rating task. In total, subjects were exposed to 302 stimuli, i.e. 151 in each PET session. Stimuli consisted of size- and luminance-adjusted color pictures of neutral female faces displayed on a neutral background, and were presented in a random order for 3 s each on a computer screen in front of the subject. Participants were instructed to fixate the stimuli and to answer the question "How attractive was this face?" after each picture. The attractiveness ratings were obtained on a 9-point Likert scale (1 = unattractive; 9 = very attractive) by a button press on a moving nine-button device that started at point 1 or point 9 in a random order. The inter-stimulus intervals (ISIs) ranged between 8 s and 22 s. In total, the attractiveness task lasted approximately 45 min. Stimulus presentation and response registration was controlled using Presentation 14.4 (Neurobehavioral Systems, Albany, CA).

2.4. Statistical analyses

Behavioral data were analyzed using SPSS 19 (SPSS Inc., Chicago, IL). Frequency counts of scores, classifications of faces in ascending order of average scores obtained under PLC and random permutations of these for comparison by *T*-statistics were generated using Microsoft Excel 2003.

Behavioral data were analyzed (a) in a within-subject across-faces approach comparing the frequencies of all discrete scores (i.e. 1, 2, 3, 4, 5, 6, 7, 8 and 9) under OXT and PLC, and (b) in a within-face approach by classifying the face images according to their attractiveness valence and assessing the class-wise individual and group OXT response above expectation. In (b) for each of the 302 faces the average scores given under OXT and under PLC were calculated. As each subject saw each face only once, each face was rated by 9 subjects under OXT and 9 other subjects under PLC. To further analyze the OXT effect we binned the 302 faces into 5 classes of 60 or 61 images in ascending order of the attractiveness rating given by the 9 subjects under PLC. In order to assess the consistency of the data, for general comparability and for the determination of cut-off values other classes of faces of variable sizes and delimitations were also analyzed, e.g. classes comprising images rated below 4.5, between 5 and 6 and above 5 under PLC. The raw difference OXT–PLC is not a suitable outcome measure as it depends on the valence of images, especially as here the subjects saw each image only once and the second rating was performed using different stimulus pictures. In the extreme case, an image rated 9 cannot be rated better at the second trial. The trial-re-trial difference of scores per class is not zero at a random distribution (regression to the mean, RTM-effect) but the expected value minus the individual or class value at the first trial. Here, we considered the average score of all ratings for all 302 images under PLC as the expected value. Individual responses to valence-classes of images Δr were calculated for a given subject "*a*" and a face-class "C" as $\Delta r = (OXT_{a,C} - PLC_{a,C}) - (PLC_{Glb} - PLC_{1-18,C})$, with $OXT_{a,C}$, $PLC_{a,C}$ average score given by subject "*a*" to the faces of a class "C" either under PLC or under OXT; $PLC_{Glb} = 4.899$ global average of all ratings under PLC; $PLC_{1-18,C}$, average rating of all 18 subjects for the faces of class "C". For the group average per class the equation simplifies to $\Delta r = OXT_{1-18,C} - PLC_{Glb}$.

All reported *p*-values are two-tailed and $p < 0.05$ was considered significant. Binding potential (BP_{ND}) values obtained by PET were compared using paired *T*-tests for repeated measures. For measures in subregions the *p* threshold was Bonferroni-adjusted. Power estimates, effect sizes and β error probabilities were calculated using G*Power

Version 3.1.7 (Franz Faul, University of Kiel, Germany). All image processing steps were carried out using PMOD (PMOD Technologies LTD., version 3.1, Zurich, Switzerland) and SPM8 software (Wellcome Trust Centre for Neuroimaging, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab 7 (The MathWorks Inc., Natick, MA).

2.5. Positron emission tomography

All participants were placed in supine position in a Siemens ECAT EXAT HR+ scanner with their head fixed in canthomeatal orientation using a vacuum pad. Head position was continuously monitored by a video system and reference skin marks and manually corrected, if necessary. The visual interactive paradigm was started 15 min before tracer injection and continued for 30 min. In parallel, for each of the two scans, a ten-minute transmission scan using a $^{68}Ge/^{68}Ga$ line source was performed for attenuation correction. Subsequently, [^{11}C]raclopride was injected by a motor syringe over 1 min. The tracer was prepared at high specific radioactivity ($>81 \pm 39$ GBq μmol^{-1}) as described previously (Schott et al., 2008). Injected radioactivity amounted to 242 ± 19 MBq and 248 ± 5 MBq, specific radioactivity was $> 42 \pm 23$ GBq μmol^{-1} and $> 47 \pm 26$ GBq μmol^{-1}, and mean mass was $< 2.6 \pm 1.2$ μg and $< 2.3 \pm 1.0$ μg for the first and second scan, respectively. There were no significant differences for any of these radioligand parameters. Dynamic PET scanning was performed for 60 min. PET data were acquired in list mode and reframed into the dynamic sequence of 6×5 s, 3×10 s, 4×60 s, 2×150 s, 2×300 s and 4×600 s (Lammertsma and Hume, 1996). Individual high-resolution MRI scans were acquired using a Siemens Trio 3T scanner in a 3D T1-weighted magnetization-prepared rapid acquisition gradient-echo sequence (192 axial slices, voxel size 1 mm $\times$ 1 mm $\times$ 1 mm; TR = 2200 ms; TE = 3.93 ms; flip angle = 15°; scan time = 8 min) to exclude abnormalities in the CNS and to enable co-registration of the anatomical MRI and PET data.

2.6. Image processing and generation of parametric maps

All image processing steps were carried out using PMOD and SPM8 software packages. MR images were manually realigned to the anterior commissure/posterior commissure line. Dynamic PET series were realigned to the average of 1–10 min p.i. using SPM and for the subsequent steps using PMOD. The averages of 10–60 min p.i. from the realigned series were co-registered to the individual MRI. The calculated transformation was applied to all individual frames. Parametric maps were generated from the series of dynamic frames using the non-invasive Logan model with fixed k_2' (OXT, 0.256 ± 0.02; PLC, 0.253 ± 0.02) implemented in PMOD as multilinear reference tissue model 2 (MRTM2) using the cerebellum as reference region. Modeling was started at 10 min p.i. The outcome parameter (binding potential, BP_{ND}) is defined as ratio of the specifically bound and non-displaceable radioligand in the tissue at equilibrium. While BP_{ND} is essentially derived from the late phase of radioligand distribution, the early phase of a measurement provides information about the relative tracer delivery rate $R1$. As the uptake of [^{11}C]raclopride is quick, $R1$ is a good surrogate measure of regional brain perfusion (Stokes et al., 2010). Parametric images of $R1$, which is the ratio of the influx rate constants into the specific compartment (K1) and the reference compartment (K1′), were also obtained with the MRTM2 model. Modeling started at the earliest possible time point determined individually by the software at 1.15 min (range 0.4–2.5 min) in the OXT and at 1.03 min (range 0.4–1.5) in the PLC scans, respectively.

2.7. Analysis of parametric maps

Parametric maps of scans 1 and 2 were analyzed by a volume of interest (VOI)-based analysis (VBA) and confirmed by voxel-wise statistics using SPM8. Striatal VOIs (Table 2) were manually delineated onto the individual average images of the parametric maps of scan 1 and scan 2. Other VOIs (cortical regions, thalamus, and amygdala; Table 3) were delineated onto individual co-registered MRIs. All VOIs were subsequently read out in the respective individual map. Parametric maps of BP_{ND} and $R1$ were warped into MNI standard space using the transformation determined from the individual MRI (present with isotropic 2 mm^3 voxels) to the Montreal Neurological Institute (MNI)/International Consortium for Brain Mapping (ICBM) 152 T1 template as supplied with SPM. Voxels outside the brain were masked using the binary entire brain mask supplied with SPM. The images were smoothed with a 3 mm^3 Gaussian kernel. A voxel wise T-test for repeated measures was calculated using the following parameters: proportional scaling to a mean of 50; masking at an absolute threshold of 0 for BP_{ND} and at a relative threshold of 0.8 for $R1$; global calculation of mean voxel value within per image full mean/8 masks, without non-sphericity correction. The p threshold was set at $p = 0.001$ and the k threshold at $k = 3$. Individual values in warped space were read out from spherical VOIs of 3 mm radius centered at the significant cluster. The Collin single subject T1 MRI implemented in SPM served for visualization of significant clusters. Changes in sub-regions identified by SPM were further assessed by a confirmatory VBA in individual space. The location of significant clusters within cytoarchitectonically defined anatomical regions was determined using the SPM anatomy toolbox and related maps (Eickhoff et al., 2005).

3. Results

3.1. Oxytocin blood concentrations and behavioral task analysis

Following 24 I.U. of intranasal OXT, plasma concentrations were raised significantly by a factor of 1.5–8.1. Mean $\pm$ SD concentrations under PLC were 7.4 ± 5.6 pg/ml and baseline concentrations prior to OXT were 7.8 ± 4.6 pg/ml. By 60 min after intranasal OXT, concentrations had risen significantly to 23.4 ± 13.7 pg/ml and after 120 min they were 18.2 ± 14.3 pg/ml (vs PLC; $p < 0.003$ and vs baseline; $p < 0.005$) (Fig. 2).

In agreement with previous findings (Theodoridou et al., 2009) participants rated unfamiliar female faces as more attractive under OXT than PLC treatment (mean $\pm$ SD: OXT:

Table 2 Raclopride binding (BP_{ND}) and relative delivery ($R1$, as a measure of perfusion) in striatal regions, substantia nigra, pallidum and in small cortical regions as identified by SPM.

	BP_{ND}					$R1$ (perfusion)				
	OXT	±SD	PLC	±SD	Δ	OXT	±SD	PLC	±SD	Δ
Striatum										
Left	1.911	0.23	1.927	0.25	−0.015	1.739	0.08	1.735	0.08	0.003
Right	1.950	0.25	1.963	0.30	−0.013	1.748	0.12	1.741	0.11	0.006
Ncl. accumbens										
Left	1.701	0.24	1.691	0.27	0.010	1.644	0.10	1.620	0.11	0.023
Right	1.695	0.25	1.702	0.31	−0.008	1.625	0.14	1.616	0.13	0.009
Ncl. caudatus										
Left	1.951	0.27	1.948	0.32	0.003	1.737	0.11	1.723	0.12	0.015
Right	1.967	0.26	1.947	0.37	0.020	1.727	0.16	1.728	0.12	−0.000
Putamen										
Left	2.200	0.27	2.222	0.22	−0.022	1.895	0.11	1.895	0.11	−0.000
Right	2.243	0.27	2.264	0.23	−0.021	1.919	0.12	1.899	0.12	0.020
Anterior putamen										
Left	2.228	0.28	2.243	0.32	−0.015	1.901	0.13	1.900	0.13	0.002
Right	2.285	0.29	2.280	0.37	0.005	1.934	0.14	1.905	0.12	0.029
Posterior putamen										
Left	2.198	0.28	2.224	0.32	−0.027	1.909	0.10	1.911	0.09	−0.001
Right	2.262	0.28	2.296	0.38	−0.035	1.945	0.11	1.917	0.13	0.028
Internal pallidum										
Left	0.208	0.12	0.215	0.10	−0.007	0.838	0.11	0.848	0.09	−0.010
Right	0.200	0.13	0.203	0.12	−0.003	0.845	0.14	0.829	0.14	0.016
External pallidum[a]										
Left	0.645	0.19	0.658	0.18	−0.013	1.097	0.07	1.105	0.11	−0.006
Right	0.645	0.19	0.646	0.20	−0.001	1.086	0.08	1.082	0.09	0.004
Substantia nigra										
Left	0.167	0.09	0.145	0.07	0.022	0.904	0.09	0.881	0.08	0.023
Right	0.165	0.10	0.164	0.10	0.001	0.898	0.12	0.902	0.14	−0.004
Dorsomedial prefrontal subregion[b]										
Right	0.190	0.09	0.087	0.07	0.102	1.062	0.13	1.190	0.12	−0.128
	(+117%; dz = 1.21; β = 0.002; p = 0.0007)					(−11%; dz = 1.01; β = 0.02; p = 0.006)				
Superior parietal subregion[c]										
Right	0.160	0.07	0.080	0.05	0.080	0.559	0.18	0.668	0.12	−0.109
	(+99%; dz = 1.22; β = 0.002; p = 0.0001)					(−16%; dz = 0.67; β = 0.24; p = 0.006)				
Dorsomedial prefrontal subregion[d]										
Right	0.126	0.09	0.189	0.10	−0.063	1.072	0.10	1.191	0.07	−0.119
	(−30%; dz = 0.64; β = 0.28; p = 0.01)					(−10%; dz = 1.31; β = 0.001; p = 0.0002)				
Precentral subregion[e]										
Left	0.104	0.06	0.169	0.09	−0.065	0.995	0.08	1.129	0.07	−0.135
	(−33%; dz = 0.85; β = 0.04; p = 0.0008)					(−12%; dz = 1.78; $\beta < 0.00001$; p = 0.00003)				

Abbreviations: OXT, oxytocin; PLC, placebo; SD, standard deviation; Δ, difference OXT-PLC; x, y, z, coordinates in MNI space (mm) negative x-values are assigned to the left hemisphere; BA, Brodman area; hIP3/SPL, transition between human intraparietal area 3 and superior parietal lobule; dz, effect size; β, β error probability whereby $1 - \beta$ gives the statistical power or sensitivity.

[a] Restricted assessability due to spill over from the adjacent putamen.

[b] BA6, warped space (x = 10, y = 6, z = 52, k = 11 voxel).

[c] hIP3/SPL, warped space (x = 32, y = -44, z = 44, k = 3 voxel).

[d] BA8, warped space (x = 4, y = 40, z = 38, k = 13 voxel).

[e] BA6, warped space (x = −48, y = −6, z = 52, k = 10 voxel).

Table 3 Raclopride binding (BP_{ND}) and relative delivery ($R1$, as a measure of perfusion) in large brain regions.

	BP_{ND}					$R1$ (perfusion)				
	OXT	±SD	PLC	±SD	Δ	OXT	±SD	PLC	±SD	Δ
Superior frontal gyrus (F1)										
Left	0.128	0.02	0.140	0.02	−0.011 *	0.950	0.06	0.976	0.04	−0.026
Right	0.135	0.02	0.134	0.02	0.001	0.971	0.05	0.982	0.05	−0.010
Middle and inferior frontal gyrus (F2 and F3)										
Left	0.165	0.03	0.170	0.03	−0.005	1.000	0.04	1.010	0.04	−0.010
Right	0.164	0.03	0.166	0.03	−0.001	1.012	0.04	1.020	0.05	−0.009
Anterior cingulum										
Left	0.138	0.03	0.145	0.03	−0.007	0.977	0.05	0.974	0.07	0.003
Right	0.147	0.04	0.142	0.03	0.005	0.989	0.07	0.982	0.06	0.007
Orbitofrontal cortex										
Left	0.154	0.03	0.154	0.03	0.000	0.963	0.05	0.947	0.07	0.016
Right	0.159	0.03	0.155	0.04	0.004	0.971	0.06	0.934	0.11	0.037
Insular cortex										
Left	0.250	0.06	0.263	0.06	−0.012	1.123	0.08	1.142	0.05	−0.019
Right	0.258	0.06	0.264	0.06	−0.006	1.134	0.06	1.140	0.07	−0.006
Central region										
Left	0.112	0.02	0.118	0.02	−0.007 **	0.888	0.06	0.908	0.05	−0.020
Right	0.122	0.03	0.128	0.02	−0.006	0.914	0.07	0.929	0.05	−0.015
Parietal cortex										
Left	0.157	0.03	0.160	0.03	−0.003	0.984	0.06	0.989	0.05	−0.002
Right	0.169	0.03	0.170	0.03	−0.001	1.000	0.06	1.007	0.05	−0.001
Temporal cortex										
Left	0.207	0.03	0.208	0.03	−0.001	1.018	0.03	1.013	0.03	−0.002
Right	0.223	0.04	0.223	0.04	0.000	1.041	0.04	1.041	0.04	0.000
Occipital cortex										
Left	0.155	0.02	0.159	0.02	−0.004	0.970	0.05	0.976	0.05	−0.006
Right	0.175	0.03	0.182	0.03	−0.007	1.005	0.07	1.016	0.06	−0.011
Thalamus										
Left	0.288	0.06	0.283	0.08	0.005	1.106	0.06	1.103	0.07	0.003
Right	0.270	0.07	0.284	0.07	−0.014	1.098	0.06	1.106	0.05	−0.009
Amygdala										
Left	0.173	0.06	0.170	0.06	0.003	0.898	0.08	0.884	0.10	0.014
Right	0.185	0.05	0.161	0.05	0.024	0.895	0.07	0.884	0.08	0.011

* $p = 0.008$ not below the Bonferroni threshold of 0.0015, small amplitude of change and no mismatch of BP_{ND} and $R1$.
** $p = 0.048$ not below the Bonferroni threshold of 0.0015, small amplitude of change and no mismatch of BP_{ND} and $R1$.

5.30 ± 0.83; PLC: 4.90 ± 0.71; $t(17)$: -6.13; $p < 0.05$). Indeed, all 18 subjects showed an increased average attractiveness rating under OXT compared to PLC (Fig. 2). To test for possible sequence effects of treatment on attractiveness ratings, we conducted a Wilcoxon rank sum test. There was neither a main effect of behavioral trial sequence (set A then B or set B then A) ($W = 36$; $p = 0.73$) nor of sequence by drug treatment (OXT, PLC) ($W = 45$; $p = 0.73$). Furthermore, we detected no habituation effects during the 45 min duration of the task; neither did ratings given in the last 10 min of the task differ from those given in the first 10 min ($p = 0.59$) nor did any other 10 min subdivisions of different time spans yield any significant differences in ratings.

Rating scores of 6, 7 and 8 were given significantly more frequently under OXT vs PLC and conversely scores of 3 and 4 less frequently ($p < 0.05$ in both cases). Scores of 1 and 2 were only given in 10% of cases under PLC and scores of 9 in only 2%.

Of the 302 faces, 5 were scored in the range 2—3, 23 in the range 3—4, 119 in the range 4—5, 139 in the range 5—6, and 16 in the range 6—7 under PLC (ratings were available from 9 subjects for 282 and 231 faces, from 8 subjects for 20 and 60 faces and from 7 subjects for 0 and 11 faces under OXT and PLC, respectively). The increase in the overall average rating score was mostly explained by a scoring of more attractive faces above expectation ($p < 0.05$ for all classes of 60 face stimuli scored better than 4.4 under PLC, $p < 0.0005$ above a score of 4.9). The least attractive faces (PLC scores of <4.4) tended to be rated worse than expectation although this did not achieve significance ($p > 0.05$; Fig. 2).

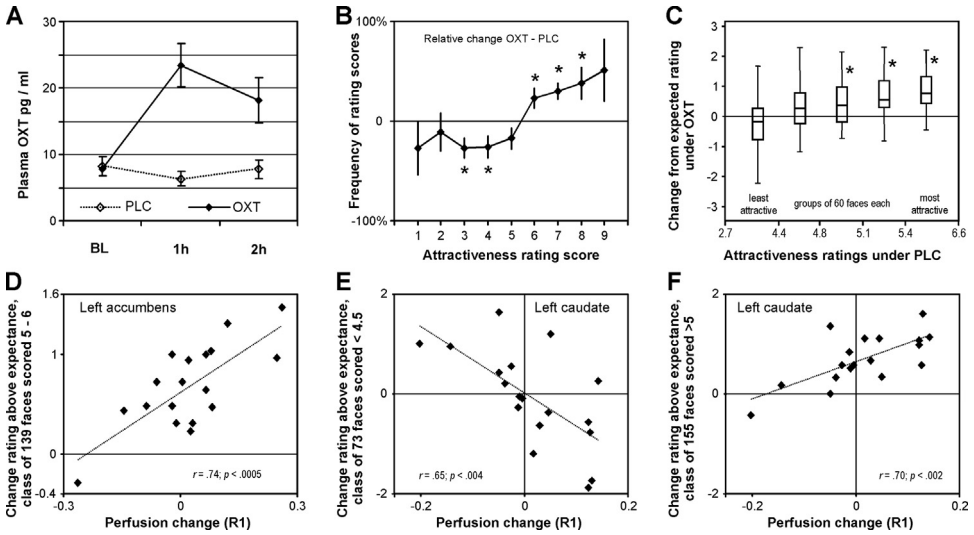

Fig. 2 Effects obtained by intranasal application of 24 IU oxytocin (OXT). (A) Plasma levels raised significantly ($p < 0.0003$) by a factor of 1.5—8.1 vs placebo (PLC) and baseline (BL). (B) and (C) Participants rated unfamiliar female faces as more attractive. In (B) this effect is demonstrated by the frequency of each rating score (between 1 and 9) given under OXT normalized to the frequency of this rating score under PLC. Under PLC on average 20% of faces were scored 5, 18% 4 and 6; 13% 3 and 7; 6% 2 and 8; 4% 1 and 2% 9. Asterisks mark scores where the frequencies differed significantly ($p < 0.05$) between OXT and PLC in this within-subject comparison across all 302 faces. (C) Shows the analysis of OXT effects across the range of rating scores. Faces were classified in ascending order of the mean score obtained from subjects under PLC. It can be seen that only faces rated more attractive than 4.8 under PLC were rated significantly better under OXT (*$p < 0.001$ for 4.8—5.0 and <0.000005 for both 5.1—5.3 and 5.4—6.6). (D)—(F) correlations of the striatal perfusion change (relative tracer delivery rate $R1$) and individual rating changes (Δr) above expectation for classes of more attractive (D, F) and less attractive (E) faces. Δr describes the individual change of rating of a class A of images above the change expected for the group at random distribution; $\Delta r = (OXT_{a,C} - PLC_{a,C}) - (PLC_{Glb} - PLC_{1-18,C})$, with $OXT_{a,C}$, $PLC_{a,C}$ average score given by subject a to, e.g. the faces of a class C either under PLC or under OXT; $PLC_{Glb} = 4.899$ global average of all ratings under PLC; $PLC_{1-18,C}$, average rating of all 18 subjects for the faces of class A. Perfusional activation of the left accumbens (D) was only significantly positively correlated with highly attractive faces (scores of >5). For the left caudate changes in perfusion were negatively correlated with altered ratings of scores considering the class of poorly attractive faces (rated < 4.5 under PLC) and positively correlated with in the class of highly attractive faces (rated > 5).

3.2. [^{11}C]raclopride binding in brain reward regions

Subjects showed no differences in [^{11}C]raclopride BP_{ND} for the whole striatum or any functional striatal subdivision between OXT and PLC treatments (Table 2 and Fig. 3) There were also no differences in pallidum or substantia nigra. Relative changes were −0.4% in the entire striatum and 0.5% in the NAcc (averages of left and right). Respective standard deviations were 3.8% and 4.7% within and 13.1% and 15.5% between subjects. Given these variances in BP_{ND} measures, power analysis showed that the relative change of D_2R BP_{ND} following administration of 24 IU of OXT is $<2.9\%$ in the entire striatum and $<3.6\%$ in the NAcc, respectively, with a 95% confidence level. According to data from combined [^{11}C]raclopride and DA-microdialysis sampling experiments (Tsukada et al., 1999) this would correspond to calculated changes in DA levels of $<290\%$ and $<360\%$, respectively. Regional DA release did not correlate with changes of OXT plasma concentrations nor with rating changes for any class of faces or any frequency of scores. Pearson's r^2 was below 0.1 in all striatal subregions and below 0.01 in the entire striatum.

3.3. [^{11}C]raclopride binding in other brain regions

SPM analysis of BP_{ND} maps revealed no decreases after OXT but two clusters with increases (i.e. less DA release) in the right dorsomedial prefrontal cortex (dmPFC) and in the right superior parietal gyrus (SPG) (Table 2 and Fig. 3). In contrast, perfusion at these sites was significantly reduced. This mismatch speaks against perfusion-related artifacts since areas with higher perfusion usually show increased radioligand uptake and higher apparent BP_{ND} (Logan et al., 1994). However, there were no significant correlations between BP_{ND} in these clusters and blood concentrations ($r^2 < 0.12$) or any behavioral measure ($r^2 < 0.21$).

The VOI-based analysis of large cortical regions showed trends for D_2R BP_{ND} decreases of −0.011 (−7,4%, $\Delta R1 = -0.026$) in the left dmPFC and −0.014 (−5%, $\Delta R1 = -0.009$) in the right thalamus, and an increase of 0.024 (+15%, $\Delta R1 = 0.011$) in the right amygdala, although without reaching significance. Overall these results provide no indication of a local increase of DA release under OXT treatment compared with PLC. Moreover, no correlation of changes of BP_{ND} in extra-striatal regions and attractiveness

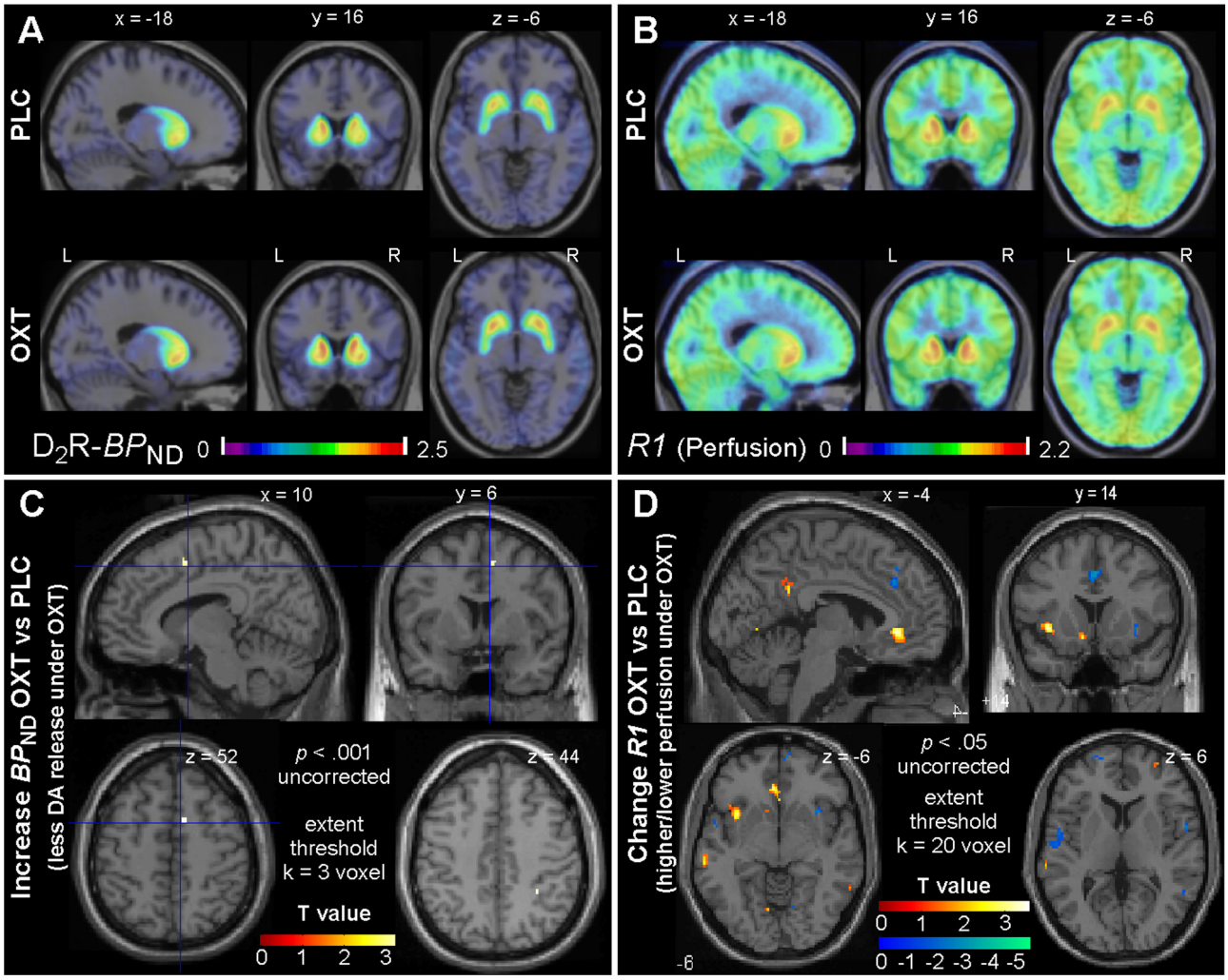

Fig. 3 Results of [^{11}C]raclopride PET imaging. Of the same dynamic scan of 60 min two parameters were extracted per voxel and submitted to SPM and volume of interest based analysis (VBA): from the late specific distribution phase the radioligand D2 receptor (D_2R) binding potential, BP_{ND}, reflecting the availability of D_2R (A) and (C); from the early first-pass tissue extraction phase the relative radioligand delivery rate, $R1$, reflecting perfusion or blood flow, (B) and (D). In the case of higher occupation by endogenous dopamine (DA) and identical perfusion lower BP_{ND} is expected. (A) and (C) show parametric images warped to MNI space and averaged across all 18 participants under OXT and PLC. (C) shows the SPM analysis of BP_{ND}. At the level of $p < 0.001$ no decrease was detected and two clusters of increase, one in the right dorsomedial prefrontal cortex (dmPFC) located within the supplementary motor cortex (BA6) and another one in the right ventral intraparietal area (VIP) (Table 2). (D) SPM analysis of $R1$ at the level $p < 0.001$ yielded no increase and two significant decreases — one in the right dorsomedial prefrontal cortex (BA8) and another one in the left lateral precentral gyrus (BA6). Shown are the data of a comparison at $p < 0.05$. Under OXT higher blood flow occurred in the following regions: left anterior insula, subgenual and posterior cingulum, nucleus accumbens, and fusiform gyrus; right superior parietal gyrus and medial frontal gyrus (BA11). Planum temporale showed higher activation bilaterally. Lower perfusion was observed bilaterally in a large portion of the dmPFC spanning into the dorsal cingulum and bilaterally into the inferior frontal gyrus, pars opercularis. This switch is between patterns with large similarities to what has been described in the fMRI literature as a cognitive network (decrease, blue) and a social network (increase, red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ratings was observed, with the highest r^2 of 0.24 occurring in the left dlPFC.

3.4. Patterns of perfusion changes ($R1$)

When assessed by VOI-based analysis, changes in $R1$ did not exceed the range of −2.6% to 1.7% (or $\Delta R1 = -0.026$ to $\Delta R1 = 0.016$) which was reached in the left superior frontal and left orbitofrontal cortex (OFC), respectively. In the right OFC there was an increase of $R1$ of 3.9% which was close to significance ($\Delta R1 = 0.037$; $p = 0.06$). SPM analysis of $R1$ maps at a level of $p < 0.001$ did not detect any increase but revealed two clusters with significant decreases at the midline in the dmPFC (BA8, −10%, $p = 0.0002$) and in the hand-arm region of the left motor cortex (BA6, −12%, $p = 0.00003$) (Table 2 and Fig. 3). At these sites BP_{ND} was also significantly reduced, albeit to a lower extent. In order to make a wider assessment of activation patterns we also used SPM to

compare *R*1 data at a level of $p < 0.05$ (Fig. 3). Under OXT we observed a higher blood flow in the anterior insula (at the MNI coordinates, *x y z*, −36 14 −4), the rostral cingulate (cingulate-p24/s24, −2 34 −4) extending into the OFC, the posterior cingulate (−6 −44 30), the NAcc (−8 16 −12), and the fusiform gyrus (−34 −30 −24) of the left hemisphere and in the anterior angular gyrus/inferior parietal cortex (area IPC-PG a/p, 46 −64 44) and the medial frontal gyrus (BA10, 32 60 4) of the right hemisphere. Planum temporale showed higher bilateral activations (−62 −30 −4 and 50 −18 −14). Lower perfusion was observed bilaterally in a large portion of the dmPFC (BA8, 4 40 38) extending into the dorsal cingulate (cingulate-32′ −2 34 42) and the inferior frontal gyrus, pars opercularis (OP4, −56 −10 10 and OP4, 56 2 6), as well as in the left frontal pole (FP1, −22 64 10) and the right caudal inferior temporal lobe (54 −54 0 and 54 −50 −16).

Changes in brain perfusion (*R*1) and OXT levels correlated inversely in the left insula ($r = 0.66$, $p < 0.003$), bilaterally in the ACC, right parietal, left temporo-lateral and in the left amygdala (all $r = 0.50$, $p < 0.04$). The above expected increase in ratings of the more attractive faces — for those 159 of 302 faces with scores larger than 5 under PLC — correlated positively with the overall change of *R*1 in all 2 × 6 striatal regions ($r = 0.51$–0.73; $p < 0.03$–<0.0007) with the most significant being the left accumbens ($r = 0.74$, $p < 0.0005$, Fig. 2) and left caudate ($r = 0.70$, $p < 0.002$, Fig. 2). However, there was no positive correlation for the right caudate or the left anterior putamen. On the other hand, rating changes for the less attractive faces — the set of 73 of 302 faces with scores lower than 4.5 under PLC — correlated negatively with *R*1 in the left caudate (Fig. 2). No other significant correlations between attractiveness ratings and regional BP_{ND} or *R*1 were observed.

3.5. Test-retest reliability of BP_{ND}

In order to compare the quality and validity of our data we assessed measures of test-retest reliability in striatum and representative cortical areas. These data provide an estimate for the test—retest reliability — given as average of the relative differences, $rd = |(\text{scan2}/\text{scan1}) - 1|$. Values for *rd* were 5.0% (±4.0%) in the striatum, 7.8% (±6.8%) in the NAcc, 6.9% (±5.4%) in the occipital cortex, and 6.3% (±5.6%) in the temporal cortex. BP_{ND} values in the second scan (retest) were systematically higher in the striatum (1.7%) and lower in NAcc (0.7%), occipital cortex (0.1%), and temporal cortex (0.4%). In the left caudate the increase of retest BP_{ND} reached 3.4% (±6.6%) corresponding to a significance level of $p = 0.04$, which was still above the Bonferroni threshold of $p = 0.005$ for analysis of all 10 striatal subregions. A further measure of test-retest reliability in relation to between-subject variability is the intra-class correlation coefficient (ICC) which was 0.87 for the entire striatum, 0.85 for the NAcc, 0.80 for the occipital cortex and 0.89 for the temporal cortex, respectively.

4. Discussion

In this randomized, double-blind, PLC-controlled, crossover study we have for the first time directly investigated the potential interaction between exogenous OXT application and endogenous DA release in the human brain using [^{11}C]raclopride PET. Our results show that neither OXT per se nor its effect on attractiveness ratings for unfamiliar female faces resulted in altered DA release in the striatum of healthy young male subjects. Similarly, no changes were observed in other reward-related brain areas such as the pallidum. Thus, there was no evidence to support the hypothesis that OXT-induced increased attractiveness ratings for unfamiliar faces in humans are associated with increased dopaminergic activity in reward-related brain areas (Skuse and Gallagher, 2009; Theodoridou et al., 2009). On the other hand, we observed reduced DA release in subregions of the dmPFC and SPG, although this did not correlate with either plasma OXT concentrations or behavioral attraction ratings. The absence of evidence for an OXT-induced increase in DA activity in brain reward systems was obtained despite intranasal OXT treatment firstly significantly increasing plasma OXT concentrations for the entire duration of the PET scan; secondly evoking a robust increase in perceived attraction of the unfamiliar female face stimuli used, and thirdly increasing activity, in terms of brain perfusion, in the striatum (caudate and nucleus accumbens) and many brain regions involved in face stimulus processing. Some of these latter activity changes were also significantly correlated with plasma OXT concentration and/or behavioral rating changes. Overall therefore our findings strongly support a conclusion that intranasal OXT (at a 24 IU dose) does not alter DA release in brain reward regions per se, and also that its facilitation of perceived face attraction can occur in the apparent absence of altered DA release despite increasing striatal activity. Thus, while OXT may still potentially act on striatal brain reward regions to facilitate attraction it may not do so via interactions with DA.

In the current study while OXT-induced overall increases in perceived attractiveness of unfamiliar faces in all 18 subjects, a further analysis revealed that this effect primarily occurred for the faces of the subset of women given higher ratings (>4.5) under PLC treatment. Thus for around 40% of the faces used OXT had no measurable effect on increasing perceived attraction, indeed for the lowest attraction scores there was a trend toward OXT even reducing perceived attraction. Thus one potential explanation for the lack of a significant OXT effect on striatal DA release might be that it did not increase the perceived attractiveness of many of the face-stimuli used. However, we did observe OXT-induced changes in DA activity in other brain regions and there were also perfusion changes in the striatum itself, so it is rather unlikely that this subset of faces with no OXT effect is responsible for the absence of DA changes. Another possible interpretation for the observed lack of OXT effects on the DA reward system is related to the fact that only faces of unfamiliar females were presented in this study. In addition to personal factors such as relationship status (Scheele et al., 2012, 2013) and situational factors such as social threat (Grillon et al., 2012; Striepens et al., 2012), familiarity may thus represent another context-dependent variable that critically influences OXT effects (Bartz et al., 2011). While OXT has been implicated in strengthening existing social bonds rather than creating them de novo (Grillon et al., 2012), the present data argue that OXT could actually play a key role in the de novo formation of male-female bonds via its modulation of perceived attractiveness of unfamiliar

females, however, without engaging the DA reward system. One intriguing yet underexplored possibility reconciling these discrepant views is that the DA reward system is only activated by OXT in males if there is already an established deep bond of affection or romantic love with the female. Furthermore, there is now evidence from experiments in mice that social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin (5-HT) (Dölen et al., 2013), suggesting a key role of serotonergic innervation to the nucleus accumbens in mediating the reinforcing properties of social interaction. It is unclear, though, whether this finding also translates to the domain of human attraction.

The absence of OXT effects on the DA reward system might also derive from the fact that we tested only male subjects. Research using voles has established that OXT primarily influences pair bonding in females via the striatal DA reward system (Liu and Wang, 2003). In males, however, the related neuropeptide arginine vasopressin (AVP), acting primarily on the ventral pallidum, seems to be more important (Lim et al., 2004). There is a receptor cross-affinity between these two neuropeptides whereby the selectivity of OXT for OXT-receptors over VP receptors (V1A, V1B, V2) is at least 20-fold (c.f. PDSP K_i database). Possibly AVP or higher doses of OXT could therefore influence the DA reward system in the human brain — although there is no supporting evidence for increased DA activation in the present study. It is still an unresolved question whether there are OXT or AVP receptors in reward-related or other DA regions of the human brain. A single mapping study on the brains of eight males and four females failed to detect binding of either OXT or AVP in the striatum while some OXT binding was found in substantia nigra and pallidum (Loup et al., 1991). We found no evidence of altered DA release in the pallidum which is in line with previous studies showing that this region is more strongly activated by maternal rather than by romantic attraction (Bartels and Zeki, 2004). In fact, the only direct evidence for OXT receptor mRNA expression in the human forebrain has been described in the temporal cortex (Gregory et al., 2010). It is therefore not surprising that we did not see any OXT-related DA changes in the substantia nigra.

The absence of changes in DA release influencing D_2R binding in the striatum are unlikely to be due to a lack of sensitivity of [^{11}C]raclopride PET. Individual levels of the radioligand binding were in accordance with well-established standard values for [^{11}C]raclopride binding and were highly reproducible. While our task-design precluded an assessment of resting-state radioligand binding, which would have permitted confirmation that exposure to the face stimuli did evoke striatal DA release, mean overall striatal BP_{ND} values (1.94) were lower than those reported by other studies during resting-state conditions (Koepp et al., 1998 — 2.36; Garraux et al., 2007 — 2.7) and also lower than those found in one of our previous studies in similar aged-subjects using neutral valence visual stimuli (a furnished room) (Schott et al., 2008 — 2.00). Thus is seems likely that the face stimuli per se in the present study did evoke striatal DA release under PLC and that OXT did not influence this. Theoretically, the exposure to task-related faces by itself might have increased DA release up to a saturation point which would render it impossible to detect any further enhancement by OXT. However, binding potentials were in the medium range following the face-attraction task suggesting that neither dose ceiling nor threshold effects in the DA D_2R system occurred. Further, the faces used in our study were deliberately chosen to be of medium attractiveness and with neutral emotional expression and therefore any induced DA release would have been substantially lower than that reported in previous studies using highly arousing stimuli (Garraux et al., 2007; Koepp et al., 1998).

The sample size of the present study (n = 18) is in accordance with former pharmacological challenges using [^{11}C]raclopride PET. It was powered to reliably detect a 2.9% difference in [^{11}C]raclopride binding in the striatum. Smaller effects are questionable in biological terms. The calculated test-retest reliability of 5.0% is in the range of previous reports (Hirvonen et al., 2003; Wang et al., 1999; Yoder et al., 2011), supporting the view that technical standards and sample size were suitable to detect a significant DA release in the striatum.

Cortical test-retest reliabilities on a per-subject basis have not been reported so far, although Stokes et al. (2010) reported differences of group averages in the range of 0.020, corresponding to about 20%. While [^{11}C]raclopride is widely used as a striatal tracer, some authors also consider it suitable for quantifying D_2R availability in other brain areas. Stokes et al. (2010), for example, detected a decrease of BP_{ND} in three frontal and temporal clusters under tetrahydrocannabinol and Garraux et al. (2007) reported a decline of BP_{ND} from 0.19 to 0.16 in the premotor cortex following a cognitive learning task. Furthermore, Sawamoto et al. (2008) showed preserved anterior cingulate DA release during a spatial working memory task in early Parkinson's disease (PD) with decrease from 0.28 (negative control task) to 0.21 (target task). Nevertheless, the preferred way for investigating cortical DA would be using another ligand of equal sensitivity for endogenous DA but with greater sensitivity to extra-striatal regions, such as [^{18}F]fallypride (Grunder et al., 2006; Kessler et al., 2005; Mukherjee et al., 1995).

Although we failed to find evidence for OXT-induced DA release in the current study, our secondary outcome measures of correlations between face attractiveness ratings and brain perfusional activation ($R1$) did reveal evidence for changes in both striatal reward and face-processing regions. The perfusion changes observed under OXT involved deactivations in the dorsomedial and dorsolateral PFC and activations in the ACC, the OFC and left insula, left accumbens, left caudatus and right putamen. These perfusion changes were also correlated with plasma OXT concentrations and/or with increased attractiveness ratings of the faces. The finding of increased striatal activation and its correlation with enhanced ratings of attractive faces under OXT, even in the apparent absence of a DA response, potentially suggests that a non-dopaminergic reinforcing mechanism might be involved, although we cannot entirely rule out the possibility that small amounts of DA below the limit of detection were released. However, since we found no evidence for DA release perhaps a more likely possibility is that OXT may have influenced opioid release and μ-opioid receptors which have also been shown to be important for pair-bonding in monogamous voles (Burkett et al., 2011). Interestingly, another recent study has also reported an increase in activity in the left caudate associated with OXT facilitation of mutual cooperation in the Prisoner's dilemma task (Rilling et al.,

2012). Thus OXT facilitation of perceived attraction and social cooperation may share a common mechanism. In addition, there is now evidence to suggest interactions between nucleus accumbens oxytocin and serotonin in mediating social reward (Dölen et al., 2013).

Our findings showing OXT-induced increased perfusion correlated with face attractiveness ratings show broad overlap with brain regions involved in social aspects of face recognition (Todorov et al., 2011), including the subgenual, perigenual and posterior cingulum (−3 −48 29), left anterior insula, pars triangularis of the inferior frontal gyrus (BA44/45), and right inferior parietal cortex (50 −62 38). Previous reports have shown correlations with facial attractiveness ratings in the right OFC and anterior insula (Tsukiura and Cabeza, 2011) and in the rostral cingulum extending into the OFC and left anterior insula (Bray and O'Doherty, 2007). A previous study has reported that OXT increased insula and decreased IFG activation in response to emotional vs neutral expression faces in women (Domes et al., 2010). Together these findings support the view that OXT may be increasing attraction toward female strangers both through reducing cognitive appraisal and negative emotional responses toward faces and also by promoting their social relevance.

The increased plasma OXT concentrations we found following intranasal OXT administration in men are similar to those reported in another study on female subjects (Domes et al., 2010). A number of regions we found with reduced perfusions were negatively correlated with OXT-concentrations. These regions included the bilateral ACC, left insula, right parietal, left temporo-lateral region and the left amygdala. A number of previous studies have reported reduced amygdala activity in response to OXT treatment (Bethlehem et al., 2013; Gamer et al., 2010; Striepens et al., 2011). Deactivations in the right mPFC and right superior parietal gyrus were also associated with increased [^{11}C]raclopride binding suggesting reduced DA release and D_2R activity. Interestingly, deactivations in right medial prefrontal and parietal regions were reported by Bartels and Zeki (2000, 2004) in the context of men and women viewing pictures of their romantic partners or — in a second study — of women viewing their babies. These regions are involved in cognitive aspects of face recognition (Fusar-Poli et al., 2009; Katanoda et al., 2000; Todorov, 2012), responses to negative emotional information (Goldin et al., 2008), and moral judgments (Greene and Haidt, 2002). It has been supposed that individuals experience reduced negative emotion and judgmental responses toward their loved ones (Bartels and Zeki, 2004; Greene and Haidt, 2002).

Thus, OXT may be reducing negative emotional/judgmental responses toward the viewed unfamiliar female faces by reducing frontal and parietal activation and corresponding DA release. This would suggest that OXT is increasing attractiveness ratings by reducing cognitive appraisal of and negative emotional and judgmental responses toward the female faces, rather than enhancing their reward value per se. At this point however our evidence for reduced DA release and D_2R binding following OXT administration should be considered preliminary and await confirmation using another ligand with greater sensitivity for measuring DA release in extrastriatal regions, such as [^{18}F]fallypride (Grunder et al., 2006; Kessler et al., 2005; Mukherjee et al., 1995).

Most of the human studies showing behavioral and brain effects of OXT have applied a single 24 IU OXT dose followed by measurements starting 45 min later (MacDonald et al., 2011; Striepens et al., 2011; Yamasue et al., 2012). The present study followed these protocols giving a single dose of 24 IU OXT 45 min prior to the behavioral task and 60 min prior to the PET measurement. Future studies should evaluate whether higher doses or repeated OXT administrations or different delays between OXT treatment, task performance and PET scans might be necessary to yield an OXT-induced DA release in the striatum. However, the application scheme used in the present study is obviously sufficient to induce a significant facilitating effect on face attractiveness and to evoke activity changes in a number of other brain regions.

In conclusion, the present study shows that the neuropeptide OXT has no significant effect on [^{11}C]raclopride binding in DA reward regions in men despite promoting an increase in the attractiveness ratings for unfamiliar female faces and in striatal activity. Striatal activity changes were also correlated with attractiveness ratings. Thus OXT may be facilitating the attractiveness of unfamiliar female faces by acting on DA-independent striatal reward systems and/or via extra-striatal effects. Indeed, our preliminary evidence for OXT-induced decreased DA release and perfusion in the right dmPFC and the right SPG, suggests that increased face attractiveness ratings may be partly mediated by a DA-related reduction in cognitive appraisal, negative emotion and judgmental responses in cortical brain areas. However, DA-independent activity changes which correlated with increased attractiveness ratings also occurred in a number of other frontal, parietal and temporal regions associated with face recognition and cognitive and emotional processing.

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The authors report no competing role of the funding source with the content of this manuscript.

Conflict of interest

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