Hormonal contraceptives suppress oxytocin-induced brain reward responses to the partner's face

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Title: Hormonal contraceptives suppress oxytocin-induced brain reward responses to the partner's face

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Abstract

The hypothalamic peptide oxytocin (OXT) has been identified as a key modulator of pair-bonding in men, but its effects in women are still elusive. Moreover, there is substantial evidence that hormonal contraception (HC) influences partner preferences and sexual satisfaction, which constitute core domains of OXT function. We thus hypothesized that OXT effects on partner-related behavioral and neural responses could be significantly altered in women using HC. In this functional magnetic resonance imaging study involving 40 pair-bonded women, 21 of whom were using HC, we investigated whether a 24-IU nasal dose of OXT would modulate brain reward responses evoked by the romantic partner's face relative to the faces of familiar and unfamiliar people. Treatment with OXT increased the perceived attractiveness of the partner relative to other men, which was paralleled by elevated responses in reward-associated regions, including the nucleus accumbens. These effects of OXT were absent in women using HC. Our results confirm and extend previous findings in men that OXT interacts with the brain reward system to reinforce partner value representations, indicating a common OXT-dependent mechanism underlying partner attraction in both sexes. This mechanism may be disturbed in women using HC, suggesting that gonadal steroids could alter partner-specific OXT effects.

Introduction

Romantic love is one of the most powerful motivators of human behavior and has been linked to physical and mental health (Holt-Lunstad *et al.*, 2010). Grief over the loss of a partner is considered a major adverse life event associated with an elevated risk for stress-related psychiatric disorders (Lundin, 1984; Keyes *et al.*, 2014), acute myocardial infarction in men (Mostofsky *et al.*, 2012), and breast cancer in women (Lillberg *et al.*, 2003; Lin *et al.*, 2013). Multiple lines of evidence suggest that the hypothalamic peptide oxytocin (OXT) is a key factor modulating pair-bonding behaviors (Young and Wang, 2004; Hurlemann and Scheele, 2015). For instance, monogamous prairie voles exhibit higher OXT receptor (OXTR) densities in the medial prefrontal cortex and nucleus accumbens (NAcc) compared to polygamous montane voles (Shapiro and Insel, 1992) and exogenous administration of OXT into the cerebrospinal fluid of male and female prairie voles induces their partner preferences (Cho *et al.*, 1999). In humans, peripheral OXT concentrations were significantly higher in new lovers compared to singles (Schneiderman *et al.*, 2012) and OXT stimulated men in a monogamous relationship, but not single men, to keep a greater distance between themselves and an attractive woman during a first social encounter (Scheele *et al.*, 2012). Furthermore, we have previously shown that the intranasal administration of OXT biases men towards perceiving their female partners as more attractive and elicits increased activity in neural reward regions, including the NAcc, when viewing the faces of their partners (Scheele *et al.*, 2013). Likewise, OXT reduced jealousy ratings and neural responses in an imagery task of sexual partner infidelity (Preckel *et al.*, 2015). However, in light of accumulating evidence implying sexual-dimorphic effects of OXT on reproductive behavior, social cognition, and emotional processing (Domes *et al.*, 2010; Fischer-Shofty *et al.*, 2013; Lischke *et al.*, 2012; Lynn *et al.*, 2014; Preckel *et al.*, 2014; Rilling *et al.*, 2014; Scheele *et al.*, 2014b; Yao *et al.*, 2014), whether OXT has a similar function in women is still elusive.

Previous OXT studies with female participants have mainly focused on the domain of parenting and child-care. Specifically, it has been shown that OXT increased the arousal induced by infant photos in nulliparous women (Rupp *et al.*, 2013) and promoted responsiveness to infant crying and laughter by reducing activation in anxiety-related neural circuits (Riem *et al.*, 2011; Riem *et al.*, 2012). Clearly, OXT effects in women may vary as a function of steroid hormones, which influence plasma levels of the peptide (Chiodera *et al.*, 1991), regulate the expression of the OXTR gene (Quinones-Jenab *et al.*, 1997), and

affect OXTR function through a non-genomic mechanism (Grazzini *et al.*, 1998). In fact, millions of women around the world use steroid-based hormonal contraception (HC) as an effective way of birth-control (Alkema *et al.*, 2013). Importantly, HC use alters women's pair-bonding behavior, evident in decreased attractiveness ratings of masculine faces (Little *et al.*, 2013), reduced neural response to the expectation of erotic stimuli (Abler *et al.*, 2013), a preference shift towards olfactory cues of genetic similarity (Roberts *et al.*, 2008), and increased sexual jealousy (Geary *et al.*, 2001). Furthermore, women who use HC while choosing partners are more likely to initiate an eventual separation (Roberts *et al.*, 2012) and wives who discontinue HC use tend to be less satisfied with marriage if they perceive their husband's face to be less attractive (Russell *et al.*, 2014).

Given that OXT proximally interacts with striatal circuits to create a rewarding neural representation of the partner's face, we hypothesized that this core mechanism would be blunted in women using HC. To test this intriguing hypothesis, we devised a double-blind, within-subject, randomized study in which we measured endogenous OXT concentrations and administered OXT (24 IU) or placebo (PLC). Specifically, the rationale was to acquire functional magnetic resonance imaging (fMRI) scans from 40 pair-bonded women while they viewed facial photographs of their male partners, non-related but familiar people, or unknown men. Subsequently, face stimuli were rated for attractiveness and emotional arousal.

Methods and Materials

Participants

Forty healthy, right-handed adult females (mean age ± SD: 24.38 ± 3.26 years) participated in the study after giving written, informed consent. The study was approved by the institutional review board (IRB) of the Medical Faculty of the University of Bonn and was carried out in compliance with the latest revision of the Declaration of Helsinki. Subjects received monetary compensation for study participation. Screening of the subjects was conducted prior to the test sessions (see ***Supplemental Table S1***). Subjects were free of current and past physical or psychiatric illness, as assessed by medical history and the Mini-International Neuropsychiatric Interview (MINI) (Sheehan *et al.*, 1998). In addition, they were naive to prescription-strength psychoactive medication, and had not taken any over-the-counter psychoactive medication in the past four weeks. Twenty-one women used HC (cf. ***Supplemental Information***). The other 19 women who did not use HC were tested in the follicular or luteal phase of their menstrual cycle as validated by blood assays obtained on the days of fMRI scanning (see ***Supplemental Table S2***). Due to technical problems, fMRI data were not available for one subject in the no HC group and behavioral data were not available for three participants in each group.

All subjects were in a romantic heterosexual relationship (mean duration: 33.58 ± 28.09 months, min: 6 months, max: 143 months). The subjects reported to be passionately in love, and the time intervals since they last saw their partners and had intimate contact were comparable between the OXT and PLC sessions (see ***Supplemental Table S3***). We also controlled for whether the subjects had had an argument with their partners in the week before both test sessions and if anything important in their relationships changed between the two test sessions.

Experimental Design

We applied a randomized, placebo-controlled, double-blind, within-group design. Subjects were randomly assigned to intranasal administration of either OXT (24IU; Syntocinon spray, Sigma Tau Pharmaceuticals, Inc., Pomezia, Italy; three puffs per nostril, each with 4IU OXT) or PLC (containing all ingredients except the peptide) 30 minutes before the start of the fMRI. The mean interval between the two fMRI sessions was 29 days (min: 1, one subject was tested after one day; max: 127, another subject

was tested after four month). Saliva samples were collected before nasal spray administration and after the experiment using commercial sampling devices (Salivettes, Sarstedt, Germany). Details on the tasks, fMRI procedure, and analyses can be found in the ***Supplemental Information*** section.

FMRI paradigm

We adopted a modified version of an established face perception task (Goossens *et al.*, 2009) involving passive viewing of photographs of the partner, matched unknown men, a familiar woman, and matched unknown women. In the present study, we used female friends as a control for familiarity since the oldest and therefore most familiar friends are usually of the same sex (Arndorfer and Stormshak, 2008). Furthermore, the sexual attraction of an opposite-sex friend would be different from that of the romantic partner. As additional control stimuli, a photograph of an unknown man matched to the partner and another photograph matched to the familiar woman were used. Subjects were asked to send several photographs of their partners and of a familiar, but non-related woman, in a passport photograph format/layout, with a neutral but friendly facial expression. The selected photographs were equated for facial expression, size, and luminance. An independent sample of 10 heterosexual women (age: 27.2 ± 4.66 years) rated the attractiveness of and the arousal induced by all women and men as well as the quality of the photographs on a visual analog scale (0 = minimum, 100 = maximum) prior to the first fMRI session. Based on these three dimensions, the Euclidian distances between all photographs were computed, and the photograph with the lowest Euclidian distance was chosen as control stimulus for a partner photograph. The same procedure was applied to find matched photographs for the familiar women. We ensured that no subject knew the individuals whose photographs were used as control stimuli. To avoid confounding factors in the photographs, the backgrounds were masked in black and the photographs were gray-scaled.

Stimuli were presented on a 32-inch MRI compatible TFT LCD monitor (NordicNeuroLab, Bergen, Norway) placed at the rear of the magnet bore using Presentation 14 (Neurobehavioral Systems, Albany, CA). In total, there were six blocks for each stimulus category, and each block included four repetitions of the same stimulus (e.g., the photograph of the partner appeared four times). Stimuli were presented for 2625 ms on-screen, and the interstimulus interval varied between 250 ms and 1500 ms to create jitter, resulting in a mean block length of 14.5 s. The sequence of blocks was randomized and blocks were

separated from each other by a low-level baseline period lasting 14.5 s, during which a fixation cross was depicted in the center of the screen. To assure attentive stimulus processing, subjects were asked to press a keypad button whenever a stimulus was presented (percent correct responses: OXT 99.13 ± 1.60, PLC 98.11 ± 9.22, $P = 0.47$). In addition, the subjects were asked to look attentively at the pictures. To avoid cognitive processing, there were no further instructions.

Behavioral task

After scanning, subjects were seated in front of a computer and were asked to rate all photographs presented during scanning on a visual analog scale for attractiveness (ranging from 0, most unattractive, to 100, most attractive) and arousal (ranging from 0, not arousing, to 100, most arousing). The sequence of the photographs was randomized.

Acquisition and analysis of fMRI data

MRI data were acquired with a Trio MRI system (Siemens, Erlangen, Germany) (cf. **Supplemental Information**) and fMRI data were preprocessed and analyzed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab 7 (The MathWorks Inc., Natick, MA). On the first level, eight conditions ('$Partner_{OXT}$', '$Control\_Partner_{OXT}$', '$Familiar_{OXT}$', '$Control\_Familiar_{OXT}$', '$Partner_{PLC}$', '$Control\_Partner_{PLC}$', '$Familiar_{PLC}$', '$Control\_Familiar_{PLC}$') were modeled by a boxcar function convolved with a hemodynamic response function. The movement parameters were included as confounds in the design matrix. Main effects of between-subject variables, that is HC (HC, no HC) and sex (female, male from our previous study (Scheele *et al.*, 2013)), were analyzed by comparing the above mentioned conditions on the first level and submitting the parameter estimates to two-sample *t*-tests on the second level for the whole-brain with a significance threshold of $P < 0.05$ corrected for multiple comparisons (family-wise error (FWE)). To specifically examine the modulatory effects of OXT, a flexible factorial model was designed with the factors 'treatment' (OXT vs. PLC) and 'bond' (partner, unfamiliar, and familiar) to test the contrasts $[Partner_{OXT}>Control_{OXT}] > [Partner_{PLC}>Control_{PLC}]$, $[Familiar_{OXT}>Control\_Familiar_{OXT}] > [Familiar_{PLC}>Control\_Familiar_{PLC}]$ and $[Partner_{OXT}>Familiar_{OXT}] > [Partner_{PLC}>Familiar_{PLC}]$. The Wake Forest University (WFU) Pickatlas (Version 3.0) was used to generate region-of-interest (ROI) masks (10

mm radius) for the NAcc and VTA (cf. **Supplemental Information**) and the threshold for significance was set at $P < 0.05$, FWE-corrected for multiple comparisons based on the size of the ROI.

Results

Women who used HC and who did not use HC did not differ with regard to sociodemographic variables, baseline neuropsychological performance, or relationship characteristics (cf. **Supplemental Table S1 and S3***).* As expected, baseline serum levels of estradiol and progesterone were significantly higher in freely cycling women than in women using HC (cf. **Supplemental Table S2**). However, since none of the women were tested during ovulation, the concentrations of the follicle-stimulating and luteinizing hormones were similar in both groups.

We defined a positive partner bias as the difference between attractiveness / arousal ratings of the partner and of other non-familiar men. A positive partner bias based on the comparison with the familiar person yielded the same pattern of results (cf. **Supplemental Results**). A repeated-measures analysis of variance (ANOVA) with treatment (OXT, PLC) as within-subject factor, HC (HC, no HC) as between-subject variable, and the positive partner bias for attractiveness as dependent variable revealed a main effect of treatment ($F_{(1, 32)}$ = 5.53, P = 0.03, η^2 = 0.15). There was no main effect of HC ($F_{(1, 32)}$ = 0.07, P = 0.79, η^2 < 0.01) and the interaction of treatment and HC did not reach significance ($F_{(1, 32)}$ = 2.18, P = 0.15, η^2 = 0.06). Nevertheless, an exploratory analysis with paired *t*-tests revealed that OXT enhanced this positive attractiveness bias in freely cycling women ($t_{(16)}$ = 2.56, P = 0.02, d = 0.37, cf. **Figure 1**), but not in women using HC ($t_{(16)}$ = 0.66, P = 0.52, d = 0.11). Interestingly, there was a trend for a positive correlation between baseline endogenous OXT concentrations and the OXT effect on the attractiveness-based positive partner bias in women using HC (r = 0.44, P = 0.08), but not in freely cycling women (r = 0.31, P = 0.23). As in our previous study (Scheele *et al.*, 2013), the OXT effect on the arousal-based positive partner bias did not reach statistical significance, yet it was also more pronounced in freely cycling women ($t_{(15)}$ = 1.16, P = 0.26, d = 0.27) than in women with HC ($t_{(16)}$ = -0.25, P = 0.80, d = -0.06). Importantly, OXT did not alter the positive bias for the familiar person (all Ps > 0.56, cf. **Figure 1**), suggesting that, in line with our previous findings in men (Scheele *et al.*, 2013), OXT specifically influenced perception related to pair-bonding. Furthermore, we observed that OXT had no effect on vital signs (cf. **Supplemental Information**) or mood and anxiety ratings (cf. **Supplemental Table S4***).*

On the neural level, photographs of the partner compared to the familiar person elicited activations in a broad neurocircuit involving frontal areas and the anterior cingulate cortex (cf.

Supplemental Tables S5 and S6*).* Interestingly, on the whole-brain level, we detected positive correlations between baseline endogenous OXT concentrations and partner-specific neural responses [$Partner_{PLC}$ > $Familiar_{PLC}$] in the left (peak MNI x, y, z: -24, 14, -8, $t_{(36)}$ = 4.81, and -21, -7, -8, $t_{(36)}$ = 4.03, k = 302, P_{FWE} < 0.01) and right (30, 5, -11, $t_{(36)}$ = 3.95, and 39, 11, -11, $t_{(36)}$ = 3.69, k = 212, P_{FWE} = 0.02) lentiform nucleus. A ROI-analysis also revealed a similar positive correlation with activation in a striatal cluster including the left (-9, 8, -2, $t_{(36)}$ = 3.50, P_{FWE} = 0.03) and right (15, 11, -5, $t_{(36)}$ = 3.48, P_{FWE} = 0.03) NAcc (cf. **Figure 2**). There was no main effect of HC and we observed no significant differences between freely cycling women and women using HC in the partner-specific contrast [$Partner_{PLC}$ > $Familiar_{PLC}$]. The correlation between peripheral OXT concentrations and neural responses to the partner in the NAcc was also comparable between women using and not using HC.

Across all stimuli, OXT enhanced activity in the left (-15, -55, 46, $t_{(38)}$ = 3.94, and -48, -61, 31, $t_{(38)}$ = 3.76, k = 348, P_{FWE} < 0.01) and right (24, -70, 37, $t_{(38)}$ = 4.01, and 30, -61, 46, $t_{(38)}$ = 3.99, k = 223, P_{FWE} = 0.03) precuneus. There were no significant deactivations under OXT compared to PLC. To determine whether OXT modulated partner-specific responses, we computed the contrast [($Partner_{OXT}$ > $Familiar_{OXT}$) > ($Partner_{PLC}$ > $Familiar_{PLC}$)]. There were no significant OXT effects for the whole sample. However, when we conducted the analysis separately for freely cycling women and women using HC, we observed that OXT elicited stronger activations in the bilateral NAcc ROI (left: -6, -4, -8, $t_{(136)}$ = 3.60, P_{FWE} < 0.01; right: 6, -1, -11, $t_{(136)}$ = 3.68, P_{FWE} < 0.01, and 15, 2, -11, $t_{(136)}$ = 2.99, P_{FWE} = 0.04) and VTA ROI (left: -6, -4, -11, $t_{(136)}$ = 3.71, P_{FWE} < 0.01; right: 6, -4, -11, $t_{(136)}$ = 4.06 P_{FWE} < 0.01) in the former group. Intriguingly, this OXT-induced activity increase was significantly stronger in freely cycling women than in women using HC both in the NAcc ROI (left: -12, -1, 4, $t_{(37)}$ = 3.20, P_{FWE} = 0.04; right: 18, 8, -5, $t_{(37)}$ = 4.32, P_{FWE} < 0.01, and 15, 2, -11, $t_{(37)}$ = 3.51, P_{FWE} = 0.02, cf. **Figure 3A**) and VTA ROI (left: -3, -4, -11, $t_{(37)}$ = 3.24, P_{FWE} = 0.04; right: 6, -4, -11, $t_{(37)}$ = 3.12, P_{FWE} = 0.057, cf. **Figure 3B**). In fact, a comparison of the OXT effects in women with our previous data obtained in men (Scheele *et al.*, 2013) showed that the OXT-induced augmentation of partner-specific responses in the NAcc (left: -9, 2, 1, $t_{(39)}$ = 3.22, P_{FWE} = 0.04 and -15, 11, -2, $t_{(39)}$ = 3.19, P_{FWE} = 0.04; right: 18, 8, -2, $t_{(39)}$ = 4.35, P_{FWE} < 0.01) was significantly stronger in men than in women who used HC. By contrast, the OXT effects did not significantly differ between freely cycling women and men.

Furthermore, OXT had no effect on the neural response to the familiar person [(Familiar$_{OXT}$ > Control$_{OXT}$) > (Familiar$_{PLC}$ > Control$_{PLC}$)] in women not using HC, but for the same contrast we detected significant increases in activity in the bilateral medial frontal gyrus (left: -12, 38, 40, $t_{(160)}$ = 4.10, k = 910, P_{FWE} < 0.01; right: 12, 17, 31, $t_{(160)}$ = 3.99, k = 910, P_{FWE} < 0.01), right superior frontal gyrus (9, 50, 37, $t_{(160)}$ = 3.91, k = 910, P_{FWE} < 0.01), and left NAcc (-18, 5, -8, $t_{(160)}$ = 3.24, P_{FWE} = 0.02) in women using HC. There were also trend significant effects in the right NAcc (18, 8, -8, $t_{(160)}$ = 2.68, P_{FWE} = 0.09) and right VTA (12, -13, -8, $t_{(160)}$ = 2.78, P_{FWE} = 0.08). Interestingly, this OXT effect in the NAcc (left: -18, 5, -8, $t_{(37)}$ = 3.25, P_{FWE} = 0.04; right:18, 8, -8, $t_{(37)}$ = 3.67, P_{FWE} = 0.01) and VTA (left: -3, -4, -11, $t_{(37)}$ = 3.43, P_{FWE} = 0.03; right: 0, -4, -11, $t_{(37)}$ = 3.29, P_{FWE} = 0.04) was even significantly more pronounced in women using HC than in freely cycling women.

Discussion

Our results indicate that endogenous OXT concentrations at baseline positively predicted striatal responses to the romantic partners' faces in all female participants. The intranasal administration of OXT selectively enhanced attractiveness ratings of the partners relative to other men. This behavioral effect of OXT was paralleled by increased NAcc and VTA responses, suggesting that OXT interacts with the brain reward system to reinforce partner value representations. Strikingly, however, this mechanism was disturbed in those women using HC, indicating that the partner-specific modulatory effects of OXT are antagonized by gonadal steroids. Both the NAcc and VTA have previously been identified as reward-associated brain regions activated by viewing the face of a romantic partner while recalling shared experiences with him or her (Bartels and Zeki, 2004; Aron *et al.*, 2005; Acevedo *et al.*, 2012).

In contrast, OXT had no significant partner-specific effect in women using HC. Importantly, this differential OXT effect cannot be attributed to a-priori differences between women using and not using HC because both groups were comparable regarding demographics and relationship characteristics. Consistent with OXT's well-established reproductive functions, including the induction of uterine contraction during labor and milk ejection during lactation (Gimpl and Fahrenholz, 2001), its role as a key underlying factor of the mother-infant attachment system has often been documented (Rilling and Young, 2014; Feldman, 2015). Thus, our observation that OXT contributes to pair-bonding behavior in both men and women lends support to the notion that human pair-bonding behavior may have evolved from the evolutionarily older mother-infant bonding (Numan and Young, 2015). Interestingly, baseline endogenous OXT concentrations were associated with stronger striatal responses to the partner in all women, indicating that – irrespective of HC use – OXT is part of the neurobiological mechanism mediating the maintenance of long-term pair-bonds. As such, it seems likely that the increased incidence for major depression, panic disorder, and posttraumatic stress disorder (Keyes *et al.*, 2014), as well as the substantially increased risk of acute myocardial infarction (Mostofsky *et al.*, 2012) linked to bereavement and profound grief after the loss of a partner, are also at least partially mediated by a disruption of OXT signaling (Hurlemann and Scheele, 2015). Along these lines, longitudinal studies are warranted to determine the predictive value of peripheral OXT levels as a biomarker for these severe 'sequelae' of emotional disequilibrium. In the present study, OXT also increased activations in the bilateral precuneus

across all stimulus types in both groups of women. Given that the precuneus is heavily implicated in mediating interoceptive awareness and consciousness (Lou *et al.*, 2004), this finding resonates well with our hypothesis that OXT induces a self-referential processing bias (Scheele *et al.*, 2014a; Hurlemann and Scheele, 2015), thus increasing the conscious perception of partner value.

Intriguingly, the partner-related effects of elevated OXT concentrations following nasal spray administration were restricted to normally cycling women. Previous research suggests that partner preferences vary across the menstrual cycle in an evolutionarily adaptive way. For instance, women prefer masculine faces (Little and Jones, 2012) and exhibit higher levels of intrasexual competition related to attractiveness (Fisher, 2004) at peak fertility in the menstrual cycle. In line with our own data, these cyclical shifts were found to be diminished in women using HC (Cobey *et al.*, 2013; Little *et al.*, 2013). In a natural setting, several partner-related activities such as massage (Morhenn *et al.*, 2012) or coitus (Carmichael *et al.*, 1994) elicit the release of OXT, and a decrease in OXT effectiveness could explain why HC users report reduced sexual functioning (Wallwiener *et al.*, 2010) and increased sexual jealousy (Geary *et al.*, 2001). In fact, OXT has been found to increase the intensity of orgasm and contentment after copulation (Behnia *et al.*, 2014) and to reduce arousal induced by the imagination of sexual infidelity (Preckel *et al.*, 2015). Peripheral estradiol and progesterone levels were found to be altered in women using HC and therefore a potential mechanism could be related to the direct binding of progesterone to OXTRs, thereby inhibiting OXTR functioning (Grazzini *et al.*, 1998). Of note, an estradiol treatment in mice and rats results in a several-fold increase in OXTR mRNA in the brain (Quinones-Jenab *et al.*, 1997; Young *et al.*, 1998). However, the acute effects of single-dose administration may differ from the chronic consequences of a long-term treatment such as HC. We did not detect any differences in OXT baseline levels between women using and not using HC, and correlations between OXT levels and neural responses were evident in all women. Furthermore, we observed a trend for stronger effects of OXT in women with higher endogenous baseline OXT levels in the HC group, possibly suggesting that higher doses are required to produce the same effects as in women not using HC.

Previous conflicting findings concerning the effects of exogenously administered OXT in women and men (Preckel *et al.*, 2014) could be reconciled by taking the hormonal status of the female participants into account. Importantly, however, there are also studies in which OXT has produced social effects in women using HC. For example, OXT altered neural responses to infant crying (Riem *et al.*,

2011) and facilitated spontaneous anthropomorphism (Scheele *et al.*, 2015) in samples in which the majority of women used HC.

We observed partner-specific OXT effects rather than general familiarity-related effects in women not using HC. By contrast, OXT effects on responses in the NAcc and VTA to the familiar person were only evident in women using HC. We did not observe any OXT effect on attractiveness or arousal ratings of the familiar person, which could mean that an OXT-mediated modulation of the bond to a female friend requires the assessment of different constructs. For instance, in women using HC, which to some extent mimics hormone profiles during pregnancy, OXT may increase the reward value of a female friend as a potential source of social support. We speculate that the OXT-induced increase in prefrontal cortex activity may also reflect social support seeking (Sherman *et al.*, 2009). Interestingly, an OXTR polymorphism has been linked to social support seeking, but the direction of this effect was dependent on cultural norms (Kim *et al.*, 2010). Likewise, the interaction between OXT and gonadal steroids could cause increased or decreased OXT effects depending on the social domain. Of note, the partner-specific neural OXT effect was significantly more pronounced in women not using HC, but on the behavioral level the interaction of treatment and HC did not reach significance. It is thus conceivable that larger sample sizes are required to detect behavioral partner-specific OXT effects in women using HC. Nevertheless, future clinical trials should carefully monitor possible differential treatment effects as a function of HC use.

Although the present study is the first to provide substantial evidence that OXT may contribute to pair-bond behavior in women in a similar manner as in men, we have to acknowledge several limitations. The sample size does not allow us to differentiate between different types (e.g. generations) of HC and we did not test women during ovulation. In an exploratory analysis, we did not detect a differential OXT effect depending on the menstrual cycle phase (i.e. follicular vs. luteal phase), but these results should be interpreted cautiously given the small sample size in the subgroups. A future study should therefore follow-up and compare OXT effects during different phases of the menstrual cycle. Furthermore, the time interval between treatment sessions was not constant in all participants (e.g. two test sessions in the same phase of one menstrual cycle or in the same phase of different cycles) and this may have increased the variance in the data.

In conclusion, the present findings, in conjunction with our previous study, indicate that OXT interacts with the brain reward system to reinforce partner value representations in both sexes, a

mechanism which may significantly contribute to stable pair-bonding in humans and appears to be altered in women using HC.

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Author Contributions

D.S. and R.H. designed the experiments; D.S. and J.P. conducted the experiments; D.S., J.P. and R.H. analyzed the data. All authors contributed to writing the paper. All authors read and approved the manuscript in its current form.

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Figure Legends

Figure 1. Oxytocin (OXT) effects on attractiveness and arousal ratings of the partner

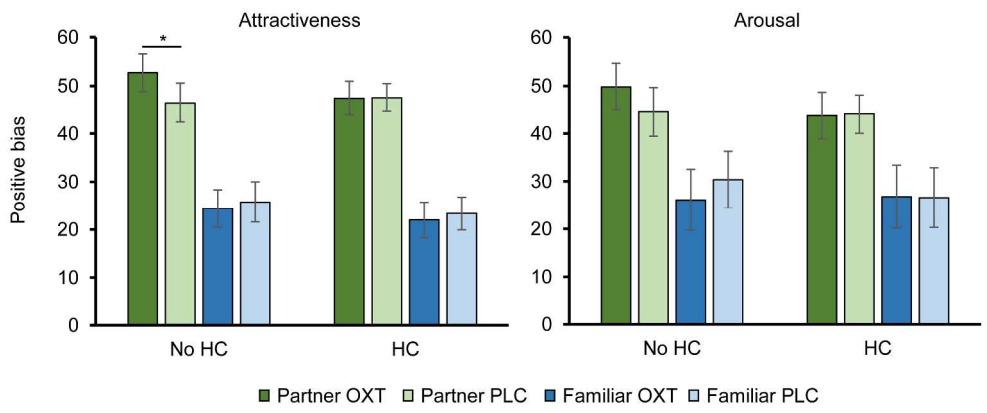
The intranasal administration of OXT yielded no behavioral effect in women using hormonal contraception, but it significantly increased the positive partner bias for attractiveness in women not using hormonal contraception (i.e. the absolute difference in ratings of the male partner versus other non-familiar men). Error bars indicate the standard error of the mean (SEM). Abbreviations: HC, hormonal contraception; OXT, oxytocin; PLC, placebo; *$P < 0.05$.

Figure 2. Baseline salivary oxytocin (OXT) concentration and neural response to the partner

Baseline salivary OXT concentrations of all participants irrespective of hormonal contraception positively correlated with neural response to the partner compared to the familiar person in the left and right striatum (left peak MNI coordinates -9, 8, -2; $t_{(36)} = 3.50$, $P_{FWE} = 0.03$; right peak MNI coordinates 15, 11, -5; $t_{(36)} = 3.48$, $P_{FWE} = 0.03$). The curves next to the regression lines indicate 95% confidence intervals. Abbreviations: OXT, oxytocin; PLC, placebo; **$P < 0.01$.

Figure 3. Oxytocin (OXT) effects on neural response to the partner

The effect of intranasal OXT on neural response to the partner's face compared to a familiar person in the bilateral striatum (left peak MNI coordinates -12,-1, 4; $t_{(37)} = 3.20$, $P_{FWE} = 0.04$; right peak MNI coordinates 18, 8, -5; $t_{(37)} = 4.32$, $P_{FWE} < 0.01$ and 15, 2, -11; $t_{(37)} = 3.51$, $P_{FWE} = 0.02$; **A**) and ventral tegmental area (left peak MNI coordinates -3, -4, -11; $t_{(37)} = 3.24$, $P_{FWE} = 0.04$; right peak MNI coordinates 6, -4, -11; $t_{(37)} = 3.12$, $P_{FWE} = 0.057$; **B**) was significantly stronger in women not using hormonal contraception than in women using hormonal contraception. Error bars indicate the standard error of the mean (SEM). Abbreviations: HC, hormonal contraception; L, left hemisphere; OXT, oxytocin; PLC, placebo; R, right hemisphere.



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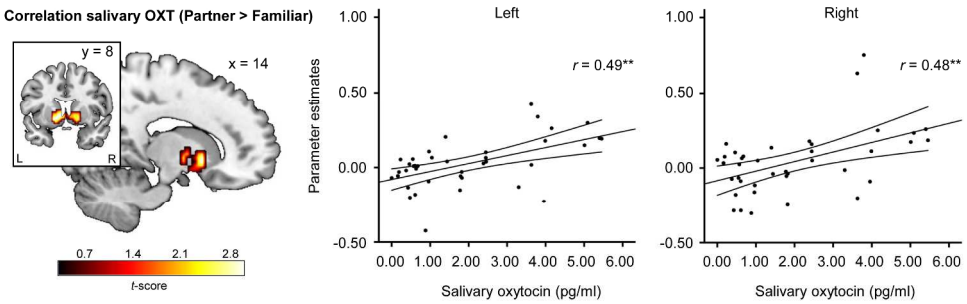

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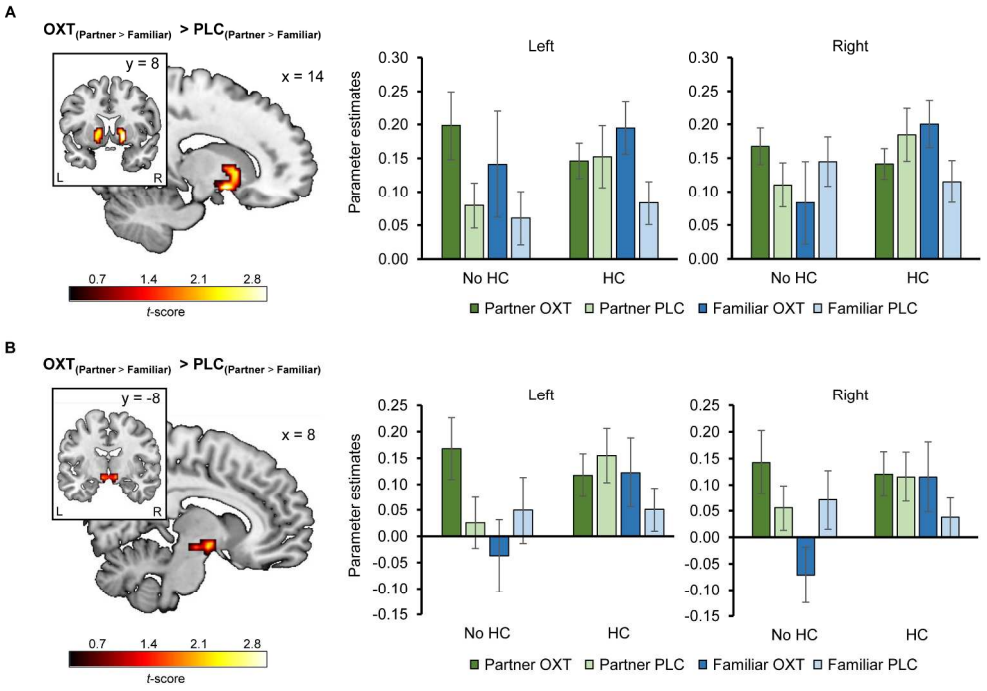

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