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Oxytocin's Effect on Resting-State Functional Connectivity Varies by Age and Sex

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Abstract

The neuropeptide oxytocin plays a role in social cognition and affective processing. The neural processes underlying these effects are not well understood. Modulation of connectivity strength between subcortical and cortical regions has been suggested as one possible mechanism. The current study investigated effects of intranasal oxytocin administration on resting-state functional connectivity between amygdala and medial prefrontal cortex (mPFC), as two central regions involved in social-cognitive and affective processing. Going beyond previous work that largely examined young male participants, our study comprised young and older men and women to identify age and sex variations in oxytocin's central processes. This approach was based on known hormonal differences among these groups and emerging evidence of sex differences in oxytocin's

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Disclosures

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effects on amygdala reactivity and age-by-sex-modulated effects of oxytocin in affective processing. In a double-blind design, 79 participants were randomly assigned to self-administer either intranasal oxytocin or placebo before undergoing resting-state functional magnetic resonance imaging. Using a targeted region-to-region approach, resting-state functional connectivity strength between bilateral amygdala and mPFC was examined. Participants in the oxytocin compared to the placebo group and men compared to women had overall greater amygdala mPFC connectivity strength at rest. These main effects were qualified by a significant three-way interaction: while oxytocin compared to placebo administration increased resting-state amygdala mPFC connectivity for young women, oxytocin did not significantly modulate connectivity in the other age-by-sex subgroups. This study provides novel evidence of age-by-sex differences in how oxytocin modulates resting-state brain connectivity, furthering our understanding of how oxytocin affects brain networks at rest.

Keywords

Oxytocin; Aging; Sex; Resting-State Functional Connectivity; Amygdala; Medial Prefrontal Cortex

1. Introduction

The neuropeptide oxytocin has received increasing attention, especially in its role in modulating different aspects of social-cognitive and affective processing and treatment of disorders characterized by social and affective deficits (Bartz et al., 2011; Meyer-Lindenberg et al., 2011). Oxytocin has been shown to influence complex socio-affective behaviors such as affiliation, stress, and anxiety, possibly by increasing salience of socio-affective information and augmenting reward neural circuits (De Dreu, 2014). However, the mechanistic process in the brain by which oxytocin modulates these behaviors is not well understood yet. One region that has received particular attention regarding oxytocin-mediated effects is the amygdala, an oxytocin receptor dense area in the brain (Gimpl and Fahrenholz, 2001) that serves as a hub in a neural network involved in a wide range of emotional and social behaviors (Sergerie et al., 2008). Amygdala medial prefrontal cortex (mPFC) coupling constitutes a key component of the brain's “salience” network, involved in evaluating incoming information and integrating it cognitively, affectively, and somatically (Pessoa, 2008).

Neuroimaging studies allow evaluation of the neurophysiology of oxytocin in vivo (Bethlehem et al., 2013). Only a limited number of functional neuroimaging studies have looked at oxytocin's effects on brain connectivity between amygdala and other brain regions. This is surprising given the dense interconnectivity of the amygdala and possible widespread effects of oxytocin on the brain's intrinsic functioning due to its relatively slow degradation in the brain and interactions with other systems (e.g., the dopaminergic system, gonadal hormones; Bos et al., 2012). Rather than acting on a single target region, by modulating amygdala activity, neuropeptides can shift neural output towards other brain regions within the interactive cognitive-affective network, impacting functional connectivity of particularly those regions associated with the “social brain” (e.g., mPFC; Dunbar, 1998).

The few functional connectivity studies that have investigated the dynamics of oxytocin in the human brain support this mechanism (Bethlehem et al., 2013). In particular, even a single-dose administration of intranasal oxytocin, by ways of which it taps into central functions (Born et al., 2002), has a notable influence on the stimulus-induced response and connectivity of the amygdala. Some studies that looked at functional connectivity during task engagement suggest an attenuating role of oxytocin on amygdala response to a variety of aversive stimuli (Baumgartner et al., 2008) as well as reduced amygdala coupling (Kirsch et al., 2005). Of note, however, as will be discussed further below, these studies focused on young male participants, while effects in female participants as well as in older adults were not addressed.

Resting-state functional magnetic resonance imaging (rsfMRI) permits the study of interactions in a network of multiple brain regions at rest. Task-free assessment of brain connectivity can be particularly useful for investigation of the fundamental neurocircuitry of the brain without the confounding influence of task load or learning effects. Still little is known about oxytocin's effects on neural connectedness at rest and the existing evidence is mixed (see Table 1): some studies suggest increased resting-state coupling between amygdala and cortical regions (Dodhia et al., 2014; Kovács and Kéri, 2015; Sripada et al., 2013) while other studies suggest decreased coupling (Kumar et al., 2015), or no modulation among these regions (Fan et al., 2014; Riem et al., 2013). Sripada et al. examined resting-state functional connectivity in healthy men after a single-dose of oxytocin vs. placebo administration in a cross-over design. Oxytocin relative to placebo enhanced bilateral amygdala connectivity with rostral medial frontal cortex but did not affect amygdala coupling with any other brain region. Kovács and Kéri investigated long-term (non-prescription) use of oxytocin in individuals who self-selected for oxytocin use and found that higher cumulative doses of oxytocin were associated with enhanced resting-state connectivity between the right amygdala and the dorsal anterior cingulate cortex (ACC). In Dodhia et al., patients with generalized social anxiety disorder showed greater resting-state functional connectivity of bilateral amygdala with rostral ACC/mPFC after a single-dose oxytocin administration, suggesting “normalization” of the reduced amygdala-frontal connectivity typically observed in this disorder. However, Kumar et al. found that oxytocin reduced resting-state functional connectivity between left and right amygdala and between bilateral amygdala and precuneus. Furthermore, results from a study in women by Riem et al. suggested no effects of oxytocin on amygdala's functional connectivity at rest.

This latter finding is particularly relevant given that most current research on oxytocin in the domain of social cognition and affective processing examined young men. Only a very small number of studies have addressed age and sex variations. For example, Campbell et al. (2014) found improved emotion recognition skills by oxytocin in older men but not older women or young adults. Similarly, Ebner et al. (2015a) showed that oxytocin administration enhanced self-reported attention to own feelings (i.e., meta-mood) only in older men and young women. There also is evidence suggesting sex differences in oxytocin's effects on amygdala reactivity, supporting varied neurophysiological effects of the neuropeptide across sex (MacDonald, 2013; MacDonald and Feifel, 2012). Domes et al. (2010; see also Lischke et al., 2012) demonstrated increased amygdala response to emotional stimuli in young women, while previous studies had shown reduced amygdala reactivity in young men

(Domes et al., 2007; Kirsch et al., 2005). Somewhat in contrast, Rilling et al. (2014) reported increased amygdala response to mutual cooperation with human partners in men but decreased amygdala response in women. However, there also are studies that do not support sex differences such as in oxytocin's effect on dampening basic physiological arousal (i.e., acoustic startle reflex; Ellenbogen et al., 2014).

This emerging evidence of age and sex differences in the actions of the oxytocin system raise the possibility of a regulation of oxytocin's effects by gonadal steroids (e.g., testosterone, estrogen, progesterone) or other age- and sex-specific biological factors (e.g., brain anatomy, endogenous oxytocin levels; Bos et al., 2012; Carter et al., 2007; Ebner et al., 2015b; Kanat et al., 2014; MacDonald, 2013), as proposed in our recent Age-Related Genetic, Neurobiological, Sociobehavioral Model of Oxytocin (AGeNeS-OT) model (Ebner et al., 2013). Further, evidence that oxytocin's social-cognitive and affective effects differ by level of proficiency, in that more impaired compared to less impaired individuals appear to benefit more from oxytocin administration (Bartz et al., 2010) also supports the idea that oxytocin's effects may vary across age and sex. Based on these considerations, there have been recent calls for systematic examination of age and sex influences on oxytocin' level and function in brain and behavior (Bethlehem et al., 2013; Ebner et al., 2013, 2015b; Huffmeijer et al., 2013). The present paper constitutes a first response to these calls.

In sum, to date nothing is known about differential effects of intranasal oxytocin on brain networks, task-related or at rest, among young and older men and women. There is evidence of age (Huang et al., 2015) and sex (Gur et al., 1995) effects on resting-state functional connectivity. Going beyond previous work, the aim of the present study was to determine effects of intranasal oxytocin administration on resting-state functional connectivity between amygdala and mPFC, as two central regions involved in social cognition and affective processing, in an age- and sex-heterogeneous sample. Leveraging prior evidence (Sripada et al., 2013), we focused on the functional relationship between bilateral amygdala and mPFC in a double-blind, placebo-controlled randomized between-group design. Randomization to assign participants to the oxytocin vs. placebo group was applied to minimize the chance of pre-existing group differences. In particular, we predicted an age-by sex-varied effect of oxytocin on functional resting-state connectivity between amygdala and mPFC based on theoretical considerations (Ebner et al., 2013) and first empirical evidence (Campbell et al., 2014). However, given the mixed evidence pertaining to oxytocin's effects on amygdala mPFC functional resting-state connectivity (see Table 1 for a summary), we refrained from formulating hypotheses regarding the direction of the oxytocin vs. placebo treatment comparisons between young and older men and women, respectively.

2. Material and Methods

2.1. Participants

In total, we had resting-state scan data from 83 of the 102 volunteers who participated in the larger project conducted in the Department of Psychology, at the Institute on Aging, and at the McKnight Brain Institute at University of Florida between August 2013 and October 2014.[1] We excluded one participant due to corrupted images, and another three participants because they had a large extent of head motion. This resulted in a total of 79 for the analysis

presented in this paper, comprising 40 young (M = 22.7 years, SD = 3.02) and 39 older (M = 71.2 years, SD = 5.19) white, English-speaking adults. All older participants scored ≥ 30 on the Telephone Interview for Cognitive Status (Brandt et al., 1988). All older women were postmenopausal, all young women were premenopausal. Ten young women were in the follicular phase of their menstrual cycle. One older man was on hormone replacement therapy (HRT). Seven young women were on oral contraception.

Participants were recruited through mailouts and fliers in the community and on campus and were screened for physical and cognitive health via self-report during an initial phone contact and an on-campus visit. Among the exclusion criteria were pregnancy, breastfeeding, psychological disorder, severe or progressive medical illness, known allergies to the preservatives in the nasal spray, any contraindication to MRI, and excessive smoking or drinking.

2.2. Procedure

Test sessions were conducted by trained study staff. Participants were instructed to stay well-hydrated before their visit but to abstain from smoking, caffeine, alcohol, and use of recreational drugs in the 24 hours, and from food, exercise, or engagement in sexual activity in the two hours, leading up to their appointment. All test sessions took place in the mornings, typically starting around 8AM.

During the screening phase, participants completed the Digit Symbol Substitution task (sensorimotor speed; Wechsler 1981) and the Rey Verbal Learning Memory task (short-term verbal memory; Rey, 1964). They also responded to the Experiences in Close Relationship Scale (ECR)-Short Form (Wei et al., 2007) and the Ten-Item Personality Inventory (TIPI; Gosling et al., 2003).[2] Participants also underwent a blood test and a health review covering all major bodily systems. For the 79 participants whose resting-state functional connectivity data was analyzed, 22 young (50% female) and 18 older (56% female) participants were randomly assigned to self-administer via a nasal spray 24 IUs (one puff per nostril) of oxytocin. Eighteen young (50% female) and 21 older (62% female) participants self-administered a placebo that contained all ingredients with the exception of the oxytocin at the start of the full study visit. Nasal spray administration followed recommendations for the standardized administration of intranasal oxytocin (Guastella et al., 2013). Randomization was overseen by the study PI.

Right before spray administration, participants indicated their current mood via the brief Positive Affect Negative Affect Scale (PANAS; Watson et al., 1988).[3] The rsfMRI scan took

[1]Several participants did not complete the resting-state functional scan, which was the last scan in the sequence, due to time restrictions (e.g., technical difficulties earlier on in the session, late arrival of participant). For two participants an incorrect image acquisition was used and thus their data was dropped.

[2]The ECR-Short Form comprises the dimensions attachment anxiety (6 items; e.g., *I worry that romantic partners won't care about me as much as I care about them*) and attachment avoidance (6 items; e.g., *I want to get close to my partner, but I keep pulling back*). The TIPI comprises five dimensions: emotional stability (2 items; e.g., *calm, emotionally stable*), extraversion (2 items; e.g., *extraverted, enthusiastic*), openness (2 items; e.g., *conventional, uncreative*; reversed), agreeableness (2 items; e.g., *critical, quarrelsome*; reversed), and conscientiousness (2 items; e.g., *disorganized, careless*; reversed). Both the ECR and the TIPI were assessed on a scale ranging from 1 = *disagree strongly* to 7 = *agree strongly*.

[3]The brief PANAS comprises a list of adjectives (e.g., *excited, happy, afraid, alert*) that participants evaluate regarding their current mood on a scale ranging from 1 = *very slightly or not at all* to 5 = *extremely*.

place between 70–90 minutes after spray administration. Participants were placed in the MRI scanner with their heads comfortably positioned and stabilized with cushions to reduce head motion. Participants lay supine and were instructed to relax and to look at a white fixation cross on a black screen. The study protocol was approved by the Institutional Review Board at University of Florida. Written informed consent was obtained following a study description. No adverse side effects were reported.

2.3. fMRI Acquisition

Brain images were acquired with a 3T Philips Achieva MR Scanner (Philips Medical Systems, Best, The Netherlands) using a 32-channel head coil. Functional gradient-echo-planar imaging (EPI) data were acquired during the resting-state paradigm (38 interleaved slices, TR 2 sec, TE 30 msec, FOV 252 × 252 × 133 mm, 80 × 80 × 38 mm matrix, flip angle 90°, in plane resolution of 3.15 × 3.15 mm, slice thickness 3.5 mm, 0 mm skip). The resting-state scan lasted about 8 minutes, and 240 time points were acquired. Whole-brain high-resolution three-dimensional T1-weighted anatomical reference images were also acquired using an MP-RAGE sequence (sagittal plane, FOV = 240 mm × 240 mm × 170; 1 × 1 × 1 mm isotropic voxels). Processing and analysis of brain images were performed using Statistical Parametric Mapping (SPM8) software (www.fil.ion.ucl.ac.uk/spm).

2.4. fMRI Data Preprocessing

Standard preprocessing procedures were employed including slice time correction, motion correction with artifact rejection (scan-to-scan motion threshold 2 mm), spatial normalization, and smoothing with an 8 mm Gaussian kernel as implemented in the Functional Connectivity Toolbox (CONN; http://www.nitrc.org/projects/conn/; Whitfield-Gabrieli and Nieto-Castanon, 2012). Prior to analysis, data was denoised using "aCompCor," an anatomically informed component-based noise correction, to correct for physiological and other sources of noise from white matter and cerebral spinal fluid (Behzadi et al., 2007).

2.5. Data Analysis

The study comprised two treatment groups (oxytocin, placebo), two age groups (young, older), and two sexes (male, female) in a factorial between-group design. Participants were randomly assigned to self-administer either intranasal oxytocin or placebo. Data from 40 young (age-range: 18–31 years, 50% female) and 39 older (age range: 63–81 years, 59% female) participants were analysed using a region-to-region resting-state connectivity approach with amygdala and mPFC as regions of interest (ROIs; see Figure 1A).

As depicted in Figure 1A, we conducted region-to-region resting-state functional connectivity analysis with amygdala and mPFC as ROIs. Amygdala was defined based on the maximum likelihood subcortical FSL Harvard-Oxford Atlas (Desikan et al., 2006; Frazier et al., 2005; Goldstein et al., 2007; Makris et al., 2006).[4] mPFC was defined as a 10 mm sphere around the MNI coordinates $x = -1$, $y = 49$, $z = -5$ (i.e., frontal node of the

[4]FSL Harvard-Oxford Atlas: The CMA provided data for an atlas distributed with FSL. We are very grateful for the training data for FIRST, particularly to David Kennedy at the CMA, and also to: Christian Haselgrove, Centre for Morphometric Analysis, Harvard;

default mode network; Makris et al., 2006). The time-course for each voxel was band-passed filtered (0.01–0.10 Hz band), reflecting our interest in low-frequency spontaneous BOLD oscillations (Fox and Raichle, 2007). Correlation coefficients were calculated between average time-courses in the bilateral amygdala and mPFC ROIs. For normal distribution, these correlations were Fisher-transformed to *z*-scores before data analysis. Also, we examined the data for outliers before proceeding with statistical tests.

Using the statistical software SAS 9.4., *z*-scores were fitted to the following three models: (i) linear model with treatment, age, and sex main effects only (M1: $R^2 = 0.199$); (ii) linear model with treatment, age, sex, together with all two-way interactions (treatment × age, treatment × sex, age × sex; M2: $R^2 = 0.210$); and (iii) linear model with treatment, age, sex, together with all two-way interactions (treatment × age, treatment × sex, age × sex) and the three-way interaction among them (treatment × age × sex; M3: $R^2 = 0.283$). We used a stringent head-motion correction, by adjusting for mean frame-wise displacement in the models to control for excessive head motion (Power et al., 2012). We used *F*-tests to determine significance for all main and interaction effects in the model (Table 2). M3 explained most of the variance in the data (see also Figure 1B). As our subgroup sample size was small, we limited our model-based follow-up contrasts to four specific within-subgroup comparisons regarding treatment effects (i.e., oxytocin vs. placebo in (1) young males, (2) young females, (3) older males, and (4) older females).

3. Results

The treatment groups reported comparable pre-treatment processing speed (oxytocin: $M = 55.35$, $SD = 11.66$; placebo: $M = 55.67$, $SD = 13.61$; $F(1,77) = 0.012$, $p = .912$, $\eta_p^2 < .001$) and short-term verbal memory (oxytocin: $M = 8.22$, $SD = 2.52$; placebo: $M = 8.51$, $SD = 2.36$; $F(1,77) = 0.275$, $p = .602$, $\eta_p^2 = .004$). Also, the groups did not differ in positive (oxytocin: $M = 3.03$, $SD = 0.65$; placebo: $M = 3.25$, $SD = 0.75$; $F(1, 77) = 1.860$, $p = 0.177$, $\eta_p^2 = 0.024$) or negative (oxytocin: $M = 1.19$, $SD = 0.268$; placebo: $M = 1.24$, $SD = 0.308$; $F(1, 77) = 0.550$, $p = 0.461$, $\eta_p^2 = 0.007$) mood, in self-reported attachment anxiety (oxytocin: $M = 2.60$, $SD = 1.00$; placebo: $M = 2.92$, $SD = 1.08$; $F(1, 76) = 1.858$, $p = 0.177$, $\eta_p^2 = 0.024$) or attachment avoidance (oxytocin: $M = 2.38$, $SD = 1.21$; placebo: $M = 2.28$, $SD = 1.07$; $F(1, 76) = 0.158$, $p = 0.692$, $\eta_p^2 = 0.002$), and in emotional stability (oxytocin: $M = 5.38$, $SD = 1.06$; placebo: $M = 5.67$, $SD = 1.09$; $F(1,77) = 1.45$, $p = .232$, $\eta_p^2 = .019$) or extraversion (oxytocin: $M = 4.63$, $SD = 1.22$; placebo: $M = 4.76$, $SD = 1.43$; $F(1,77) = 0.194$, $p = .661$, $\eta_p^2 = .003$). These scores suggested that our sample was cognitively fit and mentally healthy as well as comparable among the two treatment conditions and comparable to other samples in these age ranges and sex composition used in healthy aging research.

Further confirming comparability of the two randomly assigned groups pre-treatment, proportions of young women in the follicular (oxytocin: 46%; placebo: 44%) compared to the luteal (oxytocin: 54%; placebo: 56%) phase of their menstrual cycle did not significantly differ for the two treatment groups ($\chi^2 = 0.002$, $p = 0.964$). Also, the two groups were

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statistically comparable with respect to proportions of young women on oral contraception (oxytocin: 36%; placebo: 33%) compared to those not on oral contraception (oxytocin: 64%; placebo: 67%; $\chi^2 = 0.020$, $p = 0.888$).

Results from our final model M3 are summarized in Table 2. The main effects for treatment ($F(1,70) = 4.31$, $p = .042$, $\eta_p^2 = .058$) and sex ($F(1,70) = 10.59$, $p = .002$, $\eta_p^2 = .131$) were significant. In particular, participants in the oxytocin ($M = 0.60$, $SE = 0.04$) compared to the placebo ($M = 0.47$, $SE = 0.04$) group, and male ($M = 0.64$, $SE = 0.05$) compared to female ($M = 0.43$, $SE = 0.04$) participants, had greater amygdala mPFC functional connectivity strength at rest. The main effect for age approached significance ($F(1,70) = 3.69$, $p = .059$, $\eta_p^2 = .050$). That is, young ($M = 0.60$, $SE = 0.05$) compared to older ($M = 0.47$, $SE = 0.05$) participants showed a trend towards stronger resting-state amygdala mPFC functional connectivity. None of the two-way interactions were significant but, as depicted in Figure 1B, the three-way interaction of treatment × age × sex was significant ($F(1,70) = 7.08$, $p = .010$, $\eta_p^2 = .092$).

We followed up on this significant three-way interaction in a conceptually and empirically grounded, targeted fashion (i.e., one comparison for each of the four age × sex subgroups). This showed that oxytocin compared to placebo administration increased amygdala mPFC functional connectivity strength at rest for young female participants (amygdala: $t = 2.69$, $p = .009$). An effect in the same direction approached significance for older male participants (amygdala: $t = 1.91$, $p = .061$; note that this effect was significant when tested one-sided, but did not survive multiple comparison correction). In contrast, older female participants showed no enhanced resting-state functional connectivity after oxytocin administration (amygdala: $t = -0.67$, $p = .507$). Also, young male participants' resting-state functional connectivity in the two treatment conditions was comparably strong (amygdala: $t = 0.02$, $p = .985$; see Figure 1B).[5]

Controlling for mood, attachment variables, and personality (emotional stability, extraversion) did not change the pattern of findings, neither did exclusion of the one older participant who was on HRT. Further, the treatment effects in young women remained the same after control for phase of menstrual cycle and use of oral contraception.

4. Discussion

We examined effects of intranasal oxytocin administration in young and older men and women on resting-state functional connectivity between amygdala and mPFC, as two regions crucially connected in social cognition and affective processing. Our study is the first to test an age- and sex-heterogeneous sample in determining oxytocin's role on neural connectivity at rest, and our findings importantly qualify previous research on brain mechanisms of oxytocin dynamics.

Largely converging with previous evidence (Dodhia et al., 2014; Kovács and Kéri, 2015; Sripada et al., 2013), our results support enhanced amygdala mPFC resting-state functional

[5]The pattern of results was comparable when analyzing left and right amygdala mPFC connectivity separately.

coupling after oxytocin spray. However, most intriguingly, we provide novel evidence of age-by-sex differences in how oxytocin modulates resting-state brain connectivity strength between amygdala and mPFC. This finding importantly advances the currently limited literature regarding oxytocin's role on brain networks at rest and suggests variations in the neurophysiological mechanism in young and older men and women.

Resting-state functional connectivity analysis assesses functional interconnectedness between regions of the brain in terms of flow of neural information (Fox and Raichle, 2007). Thus, our finding of greater amygdala mPFC resting-state functional coupling supports growing evidence that centrally administered oxytocin (Born et al., 2002) can alter functional connections between subcortical and cortical regions while at rest. This promotes the idea of enhanced cross-talk and/or mutual regulation by oxytocin between amygdala and mPFC, as two central regions in socio-affective-cognitive integration (Pessoa, 2008).

The process of “priming” may underlie the enhanced resting-state functional connectivity after oxytocin administration. In particular, prolonged priming promoted by oxytocin can result in functional neuronal rewiring, with possible effects on how the system responds to stimuli (Bethlehem et al., 2013). Functional connectivity between areas of the network can be elicited through priming for oxytocin release. A signal, such as exogenous oxytocin administration, can prime endogenous oxytocin release from the large dense-core vesicles that oxytocin is stored in within a cell. When oxytocin is released from these stores it causes a cascade that leads to more stores being primed for release, creating a loop. This priming effect will initially occur at the regions of the brain that are sensitive to oxytocin such as the amygdala and its connecting areas (e.g., mPFC). It will help to temporarily change the functional connections between these areas. Oxytocin administration may start this priming effect, changing the functional connectivity between areas to predispose the individual towards affiliative response. However, this response will also depend upon the stimulus presented, the social context, and individual variation (Bartz et al., 2010; 2011; Bethlehem et al., 2013).

Most intriguingly, our findings suggest age-by-sex variations in the effect oxytocin exerts on brain mechanisms at rest. This result it is in line with the emerging notion in the literature that oxytocin function may change with age (Campbell et al., 2014; Ebner et al., 2013, 2015a,b; Huffmeijer et al., 2013) as well as differ between men and women (Bethlehem et al., 2013; Domes et al., 2010; Lischke et al., 2012; MacDonald, 2013; Rilling et al., 2014). In particular, we found a significant three-way interaction between treatment, age, and sex. Results from targeted follow-up analyses within the age-by-sex subsamples suggested a pattern according to which oxytocin administration enhanced functional connectivity between amygdala and mPFC at rest in young women, while there were no significant effects in young men or older adults. However, it is worth noting that the connectivity-enhancing effect of oxytocin approached significance in older men. This finding in older males is largely in line with the only two other studies in the literature on oxytocin's age-by-sex effects, evidencing an oxytocin administration effect on older men's affective processing (Campbell et al., 2014; Ebner et al., 2015a). Overall, the pattern of findings stemming from the significant three-way interaction and the targeted follow-up contrasts in our study is quite convincing in suggesting an age-by-sex modulated role of intranasal oxytocin. These

findings did survive various control analyses but given the small sample size for our subgroup-analyses, even though relatively large for a neuroimaging drug administration study, these results need to be considered as preliminary until confirmed in larger independent samples.

Study designs, particularly those with small samples sizes and aiming to test a heterogeneous sample (e.g., participants of different age and sex), can benefit from use of more powerful within-subject designs. Thus, the between-subject design of our study constitutes a limitation as participants could not serve as their own control. However, control analyses confirmed that affective, attachment-related, and personality measures were comparable pre-treatment across the two randomly assigned treatment groups. Also, control for mood, attachment, and personality as well as exclusion of one older participant on HRT did not change the pattern of findings of oxytocin on age and sex effects on resting-state functional connectivity. In addition, the treatment effects in young women remained the same after control for phase of menstrual cycle and use of oral contraception.

Small sample size may have been one of the reasons why previous studies did not target age and sex variations in oxytocin action. Furthermore, studying oxytocin's effects in females or across different age groups compared to an exclusive focus on young males is challenging because of sex differences in gonadal hormone levels as well as changes in these hormones across the menstrual cycle and with age. For example, it is known that estradiol induces oxytocin receptors (Champagne et al., 2001; Pedersen et al., 1994), stimulates oxytocin release from hypothalamic neurons (Akaishi and Sakuma, 1985), and promotes oxytocin receptor gene expression (Bale and Dorsa, 1997; Insel, 2010; Quinones-Jenab et al., 1997) as well as oxytocin binding in the amygdala (Young et al., 1998). These processes presumably change responsivity to oxytocin. Thus, one possible explanation for the present study's age-by-sex variations in the effects of oxytocin at resting brain connectivity may be the influence of gonadal steroid hormone environment.

There is evidence from pre-clinical studies that females have higher plasma oxytocin levels (Carter, 2007) and higher cerebrospinal fluid oxytocin levels (Altemus et al., 1999) than males. Knowledge about possible oxytocin level changes across the adult lifespan is scarce and mixed (Ebner et al., 2013). While our understanding of oxytocin receptor expression is nascent, it is possible that age- and sex-related alterations in oxytocin receptor expression contribute to the effects observed in our study. As mentioned, estrogen upregulates oxytocin production and oxytocin receptor expressivity (Bale and Dorsa, 1997; Vasudevan et al., 2001). In particular, supporting developmental effects on the oxytonergic system, there is evidence of a role of oxytocin receptor gene (*OXTR*) methylation in the development of psychopathy across childhood and adolescence (Dadds et al., 2014). Given the comparatively higher estrogen levels in young compared to older (post-menopausal, not hormonally treated) women, *OXTR* expression in our young women was probably greater than in our older women. Also, older compared to young males exhibit reduced release of androgens and this may result in an increased responsivity to endogenous estrogen and in turn upregulation of *OXTR*. The net result of these estrogen-dependent modulations of *OXTR* would be an increased responsivity to oxytocin administration in young females (and trendwise in older males), in line with the reported pattern of results.

Discussion of our findings is also fruitful in the context of Bos et al. s (2012) model for the neuroendocrine regulation of human social-affective behavior. According to this model, steroid hormones and neuropeptides interact in their effect on brain function and behavior in a dynamic fashion that also considers contextual variations (e.g., perception of the environment as socially safe versus challenging). Neuroendocrine factors (e.g., associated with endogenous central and/or peripheral oxytocin levels, or neurotransmitters levels such as dopamine), in interaction with differences in gonadal hormones appear to influence malleability (such as via exogenous oxytocin) of neural connectedness between subcortical and cortical brain structures and thus may underlie the observed age- and sex-related differences in the present study.

It also appears relevant to reflect on our findings of enhanced amgydala mPFC coupling at rest in young women, while not observed in young men, in the context of results from task-related fMRI. These prior studies suggest increased amygdala reactivity to affective threatening stimuli in females (Domes et al., 2010; Lischke et al., 2012) but attenuated amygdala activity in males (Kirsch et al., 2005). It is possible that higher levels of estradiol and progesterone and lower levels of testosterone in women underlies oxytocin's effect on processing affective information and particularly threat-detection in young women, by increasing amygdala activity and its communication with connected regions, e.g., mPFC. Such brain mechanisms would be suggested by our data.

However, at this point specific hormone explanations for the observed age-by-sex variations in oxytocin's effect on amygdala mPFC coupling are speculative. The present study did not directly control for the influence of levels of gonadal hormones on the reported effects. Future studies are warranted that specifically investigate the impact of gonadal hormone levels and neurotransmitter levels for a targeted analysis of neurobiological factors underlying oxytocin's age- and sex-specific modulation on amygdala mPFC connectivity at rest. Note however, that a recent study by Rilling et al. (2014) could not confirm modulation of plasma estradiol levels on behavioral or brain effects of oxytocin. Also, our control analyses did not suggest an effect of self-reported phase of menstrual cycle or intake of oral contraception.

The non-significant finding in young men in our study differed from a previous report of increased resting-state functional connectivity between amygdala and mPFC in this group (Sripada et al., 2013). However, in line with previous findings of greater resting-state functional connectivity in young men than women (Gur et al., 1995), and in young compared to older adults (Huang et al., 2015), young men in our study had the highest resting-state functional connectivity in the placebo condition, compared with the other age-by-sex groups. Thus, functional connectedness at rest in young men was already at a high level under placebo, and oxytocin administration in this group did not result in further increase in amygdala mPFC connectedness. This may suggest little malleability by oxytocin of the socio-affective brain system in young men, as it is already very active and strongly connected “at resting default”. In contrast, in older men exogenous oxytocin administration may contribute to a “ramping up” effect of their social and affective system, which, at resting default, is less activated and connected than in young men.

Importantly, resting-state functional connectivity as analyzed in the present context cannot speak to the direction of communication between brain regions. It will be particularly interesting in future analyses to determine effective connectivity between amygdala and mPFC in the context of oxytocin administration at rest, that is the causal influences that amygdala and mPFC exert over another. It is, for example, possible that mPFC upregulates amygdala activity, or that enhanced amygdala activity from oxytocin feeds back into mPFC. It will be crucial to determine the direction of these communication pathways in future research and explore possible age and sex variations therein.

Our study did not permit identification of the neurochemical mechanisms by which intranasal oxytocin affects resting-state amygdala mPFC connectivity (e.g., interactions between the dopaminergic and the oxytonergic systems; Bos et al., 2012). That is, while our acute placebo-controlled pharmacological approach provides insight into the effects oxytocin has on resting-state functional connectivity between amygdala and mPFC, our potential for mechanistic explanation is limited. For example, oxytocin receptors in the amygdala potentiate γ-aminobutyric acid (GABA)ergic inhibition (Huber et al., 2005). Oxytocin administration may inhibit subcortical (e.g., amygdala) activity and activate cortical regions (e.g., mPFC) as well as alter connectivity within and among these subcortical and cortical regions (Bethlehem et al., 2013). In particular, a “dampening effect” of oxytocin on amygdala coupling may be related to oxytocin receptors located in the amygdala that stimulate GABA-ergic inhibitory connections and thus only selectively regulate specific neuronal populations (Huber et al., 2005). This may then result in increased approach motivation by either reducing negative reactions to social cues and/or reducing (aversive) associative learning in response to socially relevant cues (Petrovic et al., 2008). Oxytocin may also enhance cognitive control from prefrontal regions that have been shown to exert an inhibitory influence on the amygdala to regulate emotionality (Banks et al., 2007).

Although main effects have to be interpreted with caution in the context of significant interactions, our findings also inform the currently sparse and mixed literature regarding age group and sex differences on functional connectivity at rest. In accord with some previous work, we found trendwise enhanced amygdala mPFC resting-state functional connectivity for young compared to older adults (Huang et al., 2015), which may be affected by both reduced integrity of white matter tracts as well as neurotransmitter changes with age. We also found significantly greater amygdala mPFC resting-state functional coupling for men compared to women (Gur et al., 1995).

We used an 8-min scan sequence, with all images acquired during one single session. This scan duration is within the range of previous studies on oxytocin's effect on resting-state functional connectivity (see Table 1). Also, it is very similar to the current standard of resting-state data more generally (Birn et al., 2013; Braun et al., 2012). There is evidence that test–retest reliability, especially for scans acquired during the same session and across-session similarity of functional connectivity estimates greatly improve for longer imaging durations (Birn et al., 2013). However, longer scan durations may impact validity of the measures such as associated with greater heterogeneity in thought processes or might lead to fatigue or even a sleep state in some participants. Newer exciting developments in resting-

state research propose examination of smaller time units of analysis to elucidate the variety of thought processes and promote investigation of temporal dynamics (i.e., quantification of changes in functional connectivity metrics over time to provide greater insight into fundamental properties of brain networks; Hutchison et al., 2013) It will be very interesting to pursue these newer trends in future targeted studies on oxytocin's effects within and across age and sex groups. However, given the relatively small subgroup sample in the current study and the conceptually novel approach of our research, we have chosen to use a standard index of rsfMRI, namely the averaged measure from a scan of a duration that has widely been reported to be stable.

The present study focused on functional connectivity at rest. Future extensions should ascertain whether oxytocin's effect of enhanced cross-talk between amygdala and mPFC during resting, and age-by-sex variations therein, extends to functional connectivity between these regions during socio-affective tasks (e.g., emotion regulation, emotion perception, face processing). Further warranted is examination of the extent to which oxytocin effects on amygdala–mPFC coupling during social and affective tasks predict behavioral levels of socio-affective functioning and clinical outcomes (Pessoa, 2008). In this context, possible tonic and phasic alterations in the amygdala–frontal cortex network need to be considered (Prater et al., 2013). Also, three distinct amygdala networks have been proposed that need to be targeted in future research on oxytocin's brain mechanisms in the attempt to further delineate diverse functionalities (Bickart et al., 2012): connectivity between dorsal amygdala and dorsal ACC, a network implicated in social aversion; connectivity between medial amygdala and rostral ACC, implicated in social affiliation, and the ventrolateral amygdala network associated with social perception.

5. Conclusion

To conclude, the current study is highly innovative and unique in its comparison of oxytocin effects on brain mechanisms at rest in a sample comprising young and older men and women. The majority of previous social-cognitive and affective research on oxytocin's effects has focused on young male participants. Only a very small number of studies have addressed age (Campbell et al., 2014; Ebner et al., 2015a) and sex variations in these effects, some of which have supported sex differences (Lischke et al. 2012; Domes et al., 2010; Rilling et al., 2014) and some of which have not (Ellenbogen et al., 2014). Our study is the first to suggest age-by-sex variations in how oxytocin modulates resting-state functional connectivity between amygdala and mPFC. Several of the obtained results are in line with the theory and related research while some findings invite new perspectives. Our findings provide new insight into the network-level mechanism of oxytocin effects, adding to the current understanding of how oxytocin can modulate brain networks at rest. These observations of enhanced resting-state functional connectivity may be pertinent in disorders associated with amygdala mPFC disruption and associated social dysfunction. This suggests the possibility for a wider role for oxytocin in disorders of connectivity involving the core hubs of the “social brain” network (Dunbar, 1998), like schizophrenia (MacDonald and Feifel, 2012), autism spectrum disorder (Andari et al., 2010; Farmer and Reupert, 2013), and social anxiety or depression (Dodhia et al., 2014; Prater et al., 2013). Together with other work in the literature, our findings support the emerging notion in the literature that

generalization of findings from studies done with only males and young adults to females and older subjects is not justified. Rather, it is important to achieve a full characterization of effects of intranasal oxytocin in both males and females and across the age range to determine use and benefit in clinical applications. We hope that the present study's findings will spur future replication of oxytocin's modulatory function in age- and sex-heterogeneous samples, with a particular focus on identification of interactions with other neurobiological factors (e.g., gonadal hormones, neurotransmitters), as well as behavioral and genetic influences that change with age and among men and women, and towards increased understanding of oxytocin's therapeutic benefits via improved amygdala and mPFC coupling in social and affective disorders with relevance across the lifespan.

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Highlights

- We examined effects of intranasal oxytocin on resting-state functional connectivity
- Young and older men and women either self-administered oxytocin or placebo
- Oxytocin increased amygdala mPFC coupling at rest for young women
- No significant modulatory effect of oxytocin in the other age-by-sex subgroups
- Age- and sex-specific neurobiological change impact oxytocin's effect at rest

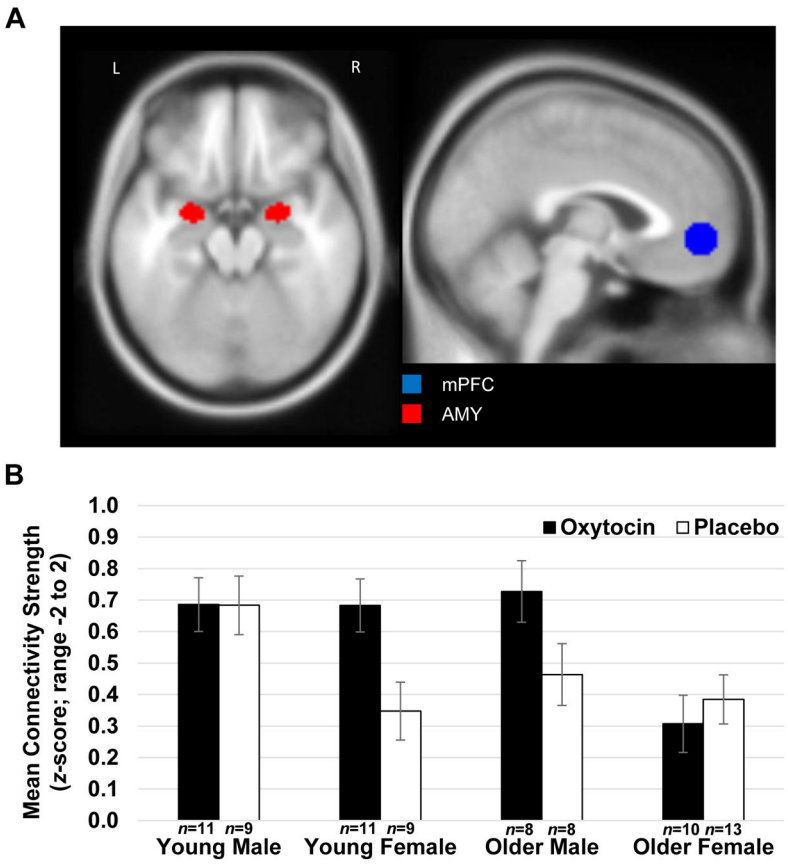

Figure 1.

Resting-state functional connectivity between bilateral amygdala and medial prefrontal cortex (mPFC; N = 79). **A** Region-to-region approach between amygdala (FSL Harvard-Oxford Atlas) and mPFC (10 mm sphere around MNI coordinates x = −1, y = 49, z = −5). **B** Variations in functional connectivity strength between amygdala and mPFC as a function of treatment (oxytocin, placebo), age (young, older), and sex (male, female).

AMY = Amygdala, mPFC = Medial prefrontal cortex. Bars indicate standard errors.

Table 1

Overview of Current Evidence of Oxytocin's Effects on Amygdala Resting-State Functional Connectivity

Reference	Design	Sample	Treatment	rsFC Paradigm	Main Findings
Dodhia et al. (2014)	WS	**N:** 18 patients with generalized social anxiety disorder (GSAD) vs. 18 healthy control (HC) **Age:** 19–55 yrs. **Sex:** males	**Conditions:** OT vs. P **Dose:** 24 IUs	**ROI:** Amygdala seed, whole brain **Instructions**: eyes closed, relax and let minds wander, not to fall sleep **Duration**: ~3 min	**OT > P:** OT administration in GSAD patients resulted in enhanced rsFC of bilateral amygdala with rostral ACC/mPFC relative to HC who administered P
Fan et al. (2014)	WS	**N:** 18 **Age:** 21–36 yrs. **Sex:** male	**Conditions:** OT vs. P **Dose:** 24 IUs	**ROI:** amygdala seed, whole brain **Instructions**: rest silently, watch a white fixation cross against a black background, remain relaxed and awake **Duration**: ~8 min	**OT = P:** OT administration did not modulate the association between increment in severity of emotional abuse and rsFC of right amygdala with pregenual/subgenual ACC and dorsal mPFC
Kovács and Kéri (2015)	BS	**N:** 41 OT vs. 41 C (matched for age, education, gender) **Age:** 19–42 yrs. **Sex:** male, female	**Conditions:** OT chronic use vs. no OT use (C) **Dose:** mean of 9 weeks, mean of 4.6 applications per week, mean dose of 35.2 IUs per application	**ROI:** amygdala seed, whole brain **Instructions**: fixate on point on screen, let mind wander without falling asleep **Duration**: ~5 min	**OT > C:** OT chronic use resulted in increased rsFC of right amygdala and dorsal ACC relative to C
Kumar et al. (2015)	WS	**N:** 15 **Age:** 18–33 yrs. **Sex:** male	**Conditions:** OT vs. P **Dose:** 24 IUs	**ROI:** amygdala seed, whole brain **Instructions**: keep eyes open, focus on fixation cross on screen. **Duration**: ~5 min	**OT < P:** OT administration resulted in reduced rsFC of bilateral amygdala with right precuneus relative to P
Riem et al. (2013)	BS	**N:** 22 OT vs. 20 C **Age:** 22–49 yrs. **Sex:** female	**Conditions:** OT vs. P **Dose:** 16 IUs	**ROI:** amygdala, insula, and PCC seed, whole brain **Instructions**: close eyes during entire resting state scan **Duration**: ~6 min	**OT = P:** OT administration did not modulate rsFC of amygdala and insula (OT administration resulted in increased rsFCs of PCC with cerebellum and brainstem relative to P)
Sripada et al. (2013)	WS	**N:** 15 **Age:** 19–54 yrs. **Sex:** male	**Conditions:** OT vs. P **Dose:** 24 IUs	**ROI:** amygdala seed, whole brain **Instructions**: relax and keep eyes closed, without falling asleep **Duration**: ~3 min	**OT > P:** OT administration resulted in increased rsFC of bilateral amygdala with rostral medial frontal cortex (ACC and mPFC) relative to P

Note. WS = Within-subject design, BS = Between-subject design, OT = Oxytocin, P = Placebo, C = Control group, rsFC = Resting-state functional connectivity, ROI = Region of interest, ACC = anterior cingulate cortex, mPFC = medial prefrontal cortex, PCC = posterior cingulate cortex

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Table 2

Main and Interaction Effects of Treatment, Age, and Sex on Resting-State Functional Connectivity Between Bilateral Amygdala and Medial Prefrontal Cortex

Effect	*Mean Square*	*F Value*	*p*
Treatment	0.33	4.31	0.042 *
Age	0.28	3.69	0.059
Sex	0.81	10.59	0.002 *
Treatment ×Age	0.03	0.36	0.551
Treatment × Sex	0.00	0.00	0.972
Age × Sex	0.03	0.40	0.527
Treatment × Age × Sex	0.54	7.08	0.010 *

Notes. Model parameters: $R^2 = 0.283$, $F(8, 70) = 3.45$; $p = .002$;

* $p < .05$.