Oxytocin enhances resting-state connectivity between amygdala and medial frontal cortex

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Abstract

The neuropeptide oxytocin (OXT) plays an important role in complex socio-affective behaviours such as affiliation, attachment, stress and anxiety. Previous studies have focused on the amygdala as an important target of OXT’s effects. However, the effects of OXT on connectivity of the amygdala with cortical regions such as medial frontal cortex, an important mediator of social cognition and emotion regulation, remain unexplored. In a randomized, double-blind, cross-over design, 15 volunteers received intranasal OXT or placebo prior to resting-state functional magnetic resonance imaging. OXT significantly increased connectivity between both amygdalae and rostral medial frontal cortex (rmFC), while having only negligible effects on coupling with other brain regions. These results demonstrate that OXT is a robust and highly selective enhancer of amygdala connectivity with rmFC, a region critical to social cognition and emotion regulation, and add to our understanding of the neural mechanisms by which OXT modulates complex social and cognitive behaviours.

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Introduction

Oxytocin (OXT), a neuropeptide produced in the hypothalamus, is a key modulator of complex socio-affective responses including affiliation, social approach and attachment, stress and anxiety (Bartz & Hollander, 2006; Meyer-Lindenberg, 2008). Understanding the mechanisms by which OXT exerts these effects promises to shed important light on the brain basis of social behaviours and could also help illuminate abnormalities in social-affective neurocircuitry in psychiatric disorders.

Convergent evidence from animal and human studies points to the amygdala as an important target of OXT effects, consistent with the amygdala’s well-known role in emotion and social behaviour (Adolphs, 2009). OXT receptors are found in high density in the amygdala (Gimpl & Fahrenholz, 2001) and have been shown to selectively regulate specific neuronal populations, especially in central medial amygdala, potentially by stimulating γ-aminobutyric acid (GABA)-ergic inhibitory connections (Huber et al. 2005). In humans, functional imaging studies consistently demonstrate that OXT attenuates amygdala responses to a range of aversive/threatening social stimuli, including faces expressing fear and anger (Kirsch et al. 2005) and failure of one’s partner to reciprocate during social exchange (Baumgartner et al. 2008). A recent study in generalized social anxiety disorder (GSAD; Labuschagne et al. 2010) found that hyperactive amygdala responses to fearful faces present in the GSAD group vs. healthy controls was abolished by acute intranasal administration of OXT.
While previous neuroimaging studies have primarily studied the effects of OXT on activation of amygdala, OXT effects on patterns of amygdala connectivity have been far less investigated. A previous study (Kirsch et al. 2005) found that OXT diminished connectivity during an emotion task between amygdala and brain stem regions implicated in autonomic functions, suggesting a mechanism by which OXT might attenuate emotional arousal. The amygdala is also anatomically and functionally connected to specific areas of the frontal cortex, especially medial frontal cortex (mFC; Ghoshgai et al. 2007; Kim et al. 2011), which is a critical node in the brain’s social cognition network (Adolphs, 2009). Links between amygdala and mFC have been implicated in a number of social and emotional functions, including self-related processing (Blair et al. 2008), fear extinction (Milad et al. 2006) and regulation of emotion (Banks et al. 2007; Kim et al. 2011). Elucidating OXT effects on amygdal-cortical connectivity thus promises to add an important new dimension to our understanding of the mechanisms by which OXT impacts complex social behaviours.

In the present study, we coupled an acute pharmacological challenge with OXT with resting-state functional magnetic resonance imaging (fMRI), a powerful method for characterizing dynamic brain network function (Fox & Raichle, 2007). In a randomized, double-blind, placebo (PBO)-controlled, cross-over design, 15 healthy male volunteers received OXT or PBO 45 min prior to resting-state fMRI. Given prior evidence that connections between amygdala and mFC play a pivotal role in socio-affective processing (Kim et al. 2011), we hypothesized that OXT would enhance amygdala resting-state functional connectivity with mFC regions.

**Method**

**Subjects**

Fifteen right-handed male participants were included in this study and recruited via local newspaper and university advertisements (mean age 30.7 ± 10.6 yr; age range: 19–54 yr). Participants had no history of a psychiatric disorder as verified by the Composite International Diagnostic Interview. All participants were non-smokers, free of head injury, had no allergies and no history of alcohol or substance abuse. None of the participants was on medication at the time of the study or was previously medicated. As part of the clinical screening, all participants went through a brief medical examination with the study physician to assess that they were otherwise fit to take part in the study. The study was conducted in accordance with the guidelines of the Standing Committee on Ethics in Research Involving Humans of Monash University.

**Study design**

The study utilized a randomized, double-blind, PBO-controlled, within-subject design, in which each participant was tested under two acute treatment conditions separated by a 1 wk wash-out period. Treatment conditions were randomized and counter-balanced (by a clinical trials pharmacist, using a computerized randomization program in Windows Excel) with subjects receiving an intranasal spray of either OXT (24 IU or 40.32 mg; Syntocinon-spray; Novartis, Switzerland) or PBO (containing all ingredients except for the peptide) in three puffs of 4 IU or 6.72 mg per nostril. OXT or PBO was administered 45 min before fMRI scanning, consistent with previous studies (Heinrichs et al. 2003; Kirsch et al. 2005). Participants arrived for each treatment session 1 h before the scheduled fMRI scanning session. No caffeine or alcohol intake was allowed on the day of scanning and no food permitted 1 h before arrival. Following a period of rest, participants self-administered either an OXT or a PBO nasal spray as instructed by the investigator. This involved inhaling a full spray per nostril in an alternating order with 45 s wait between each application until total amount (i.e. three sprays per nostril) was reached. The fMRI scanning started 45 min post-treatment (lasting ~30 min). This time window was chosen to coincide with the time of OXT’s predicted maximum pharmacokinetic (Born et al. 2002) and physiological (Heinrichs et al. 2003; Kirsch et al. 2005) effects. In addition to the resting-state scan, subjects performed a series of behavioural tasks that are unrelated to the current report.

**Resting state paradigm**

Subjects were positioned in the MRI scanner and their heads comfortably restrained to reduce head movement. Participants lay supine and were instructed to relax and keep their eyes closed, but not to sleep. The resting-state scan lasted a total of 3 min 20 s. Due to scanner failure, resting-state scans were not performed on two subjects, resulting in 15 subjects available for resting-state analysis.

**fMRI acquisition**

Brain images were acquired with a 3T Siemens Tim Trio scanner using a 12-channel head coil. Functional gradient-echo echo-planar imaging (EPI) data depicting BOLD contrast were acquired during the
resting state paradigm (TE = 40 ms, TR = 2000 ms, flip-angle = 90°, field of view (FoV) = 210 mm, 64x64 matrix, 44 contiguous 3 mm slices parallel to the hippocampus and interleaved). Whole brain T1-weighted anatomical reference images were also acquired from all participants (TE = 2.15 ms, TR = 1900 ms, flip-angle = 9°, FoV = 256 mm, 176 sagittal slices, 1 mm slice thickness, perpendicular to the anterior–posterior commissure line). Data processing and analyses were performed using Statistical Parametric Mapping (SPM5) software.

**Pre-processing of fMRI data**

Images were spatially realigned to correct for head motion, warped to an EPI template in Montreal Neurological Institute (MNI) space, resampled to 2 mm³ voxels and smoothed using an isotropic 8 mm full-width-half maximum Gaussian kernel.

**Data analysis**

The protocol for resting-state analysis is described in detail elsewhere (Weng et al. 2010). In brief, the time-course for each voxel was band-pass filtered (0.01–0.10 Hz band) reflecting our interest in low-frequency spontaneous BOLD oscillations (Fox & Raichle, 2007). Amygdala seed regions of interest (ROIs) were constructed from the Automated Anatomical Labeling system and right and left amygdala masks encompassed 306 and 271 voxels respectively. We extracted the spatially averaged time series from right and left amygdala ROIs for each participant. Correlation coefficients were calculated between average time-courses in the amygdala seed ROIs and all other voxels of the brain resulting in a three-dimensional correlation coefficient image (r-image). These r-images were then transformed to z-scores using a Fisher r-to-z transformation.

Z-score images from the individual functional connectivity analyses were entered into group-level random-effects analyses (one-sample and paired t-tests) implemented in SPM5. Given large positive correlations between seed regions and the rest of the brain in non-globally regressed data, within-session maps were thresholded at a stringent p < 0.00001. Between-session difference maps were thresholded at p < 0.005 uncorrected with a minimum cluster size of 172 voxels. This threshold was chosen using AlphaSim (http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf) to correspond to a false-positive rate of p < 0.05, corrected for multiple comparisons across the whole brain. In addition, clusters were only included if they were located primarily inside grey matter; a threshold of >50% of voxels inside grey matter ROIs from the Automated Anatomical Labeling system was used for inclusion. Connectivity foci were labelled by comparison with the neuroanatomical atlas by Talairach and Tournoux. Reported voxel coordinates correspond to standardized MNI space.

To complement our whole-brain analysis, we also extracted β weights from 5 mm radius spheres centred at peak coordinates from clusters observed in the whole brain map of the OXT vs. PBO contrast. The resulting β weights represent each ROI voxel’s correlation with the amygdala seed averaged across the entire functional ROI. These β weights were plotted to clarify the nature and direction of effects observed in whole-brain maps.

**Results**

Results showed that both right and left amygdala exhibited similar patterns of connectivity under PBO and OXT (see Supplementary Fig. 1 and Table 1). Under PBO, right and left amygdala exhibited functional connectivity with contralateral amygdala, brain stem [encompassing regions of midbrain, periaqueductal grey and reticular formation, consistent with a prior report (Kirsch et al. 2005)], midcingulate gyrus and precuneus. Under OXT, both right and left amygdala showed an additional large region of connectivity with anterior cingulate and adjacent areas of medial prefrontal cortex [hereafter rostral mFC (rmFC)], while connectivity with brain stem was absent.

Difference maps comparing OXT vs. PBO showed that OXT robustly and selectively increased connectivity between right amygdala and rmFC (Fig. 1). ROI analysis with a 5 mm radius sphere centred at the peak voxel in the difference map derived from the right amygdala seed showed that right amygdala was functionally connected with this rmFC region during PBO (p = 0.05), while left amygdala was not (p = 0.34). For both right and left amygdala seeds, OXT significantly increased connectivity with this rmFC region (Fig. 1). OXT had no other effects in enhancing right or left amygdala coupling with any other brain regions or diminishing coupling with other brain regions (Table 1).

**Discussion**

To our knowledge, this is the first study of OXT effects on amygdala–cortical connectivity and the first study to assess OXT connectivity in the resting state. Results showed that OXT is a robust and highly selective enhancer of amygdala connectivity with rmFC and had negligible impact on connectivity with other brain
regions. These results complement prior studies focusing on OXT effects on amygdala activation by showing that OXT effects on the connectivity of the amygdala may also play a key role in explaining its behavioural effects.

At rest, amygdala and rmFC are thought to be key constituents of the brain’s ‘salience’ network, responsible for imparting value to incoming stimuli and cognitive-emotional–somatic integration (Seeley et al. 2007). At the functional level, these results are intriguing given that the mFC, specifically the rmFC, and its connections with amygdala have repeatedly been implicated in a host of social-cognitive-affective functions (Adolphs, 2009), including social cognition and cognitive control of emotion (Kim et al. 2011), with greater amygdala–mFC coupling predicting better regulatory efficiency (Banks et al. 2007).

Resting-state connectivity analysis assesses correlations between spontaneous, slow (<0.1 Hz) fluctuations in brain activation – regions exhibiting high degrees of coherence during the resting state are thought to be functionally interconnected in terms of flow of neural information and/or mutual regulation (Fox & Raichle, 2007). OXT enhancement of connectivity between amygdala and rmFC thus provides evidence of greater cross-talk and/or mutual regulation between these two critical nodes for socio-affective functioning. Given previous studies that show that OXT dampens amygdala activity to a range of aversive/threatening social stimuli (Baumgartner

### Table 1. Results from whole-brain voxel-wise analysis comparing placebo (PBO) vs. oxytocin (OXT) sessions

<table>
<thead>
<tr>
<th>Connectivity map and brain region</th>
<th>Cluster size</th>
<th>MNI coordinates (x, y, z)</th>
<th>Analysis (z)</th>
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<tbody>
<tr>
<td><strong>PBO &gt; OXT</strong></td>
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<td>Right amygdala</td>
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<td>No significant clusters</td>
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<td>Left amygdala</td>
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<td>No significant clusters</td>
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<td><strong>OXT &gt; PBO</strong></td>
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<td>Right amygdala</td>
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<tr>
<td>Anterior cingulate, medial frontal gyrus (rmFC)</td>
<td>209</td>
<td>−4, 32, −4</td>
<td>3.93</td>
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<tr>
<td>Left amygdala</td>
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<td>No significant clusters</td>
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MNI, Montreal Neurological Institute; rmFC, rostral medial frontal cortex.

**Fig. 1.** Effect of oxytocin (OXT) on frontal connectivity with amygdala seed at rest. Time series from a right amygdala anatomical seed (shown at top) was entered into functional connectivity analysis. Whole-brain voxel-wise connectivity t map and extracted weight of connectivity score from rmFC show enhancement (OXT > PBO) of connectivity localized specifically in the rmFC. Functional connectivity t map was thresholded at p < 0.05 corrected for multiple comparisons across the whole brain. Bar represents t score.
et al. 2008; Heinrichs et al. 2003; Kirsch et al. 2005; Labuschagne et al. 2010), the present study suggests a potential mechanism in which enhanced rmFC-amygdala connectivity mediates the observed attenuation of amygdala responses. However, because this study only assessed connectivity in the resting state, future work is required to clarify the implications of enhanced connectivity rmFC-amygdala connectivity in the resting state for regulation during emotion-related tasks.

Our results also add to existing evidence that amygdala–mFC connectivity could serve as a brain-based biomarker of social-affective functioning. For example, abnormal coupling between amygdala and mFC regions, including rmFC, has been observed in psychiatric disorders characterized by compromised social functioning, such as social anxiety disorder (Hahn et al. 2011; Kim et al. 2011) and schizophrenia (Salvador et al. 2010). Additionally, exogenously administered OXT shows promise as a novel therapeutic intervention in autism (Bartz & Hollander, 2008), a disorder of profound social deficits and OXT receptor dysfunction (Young et al. 2002). Our results suggest a specific brain-based mechanism by which OXT might enhance socio-affective functioning in healthy individuals. Further studies are required to investigate the hypothesis that OXT can correct the altered amygdala–mFC coupling observed in patients with psychiatric disorders and thereby ameliorate social-emotional deficits.

This study has several limitations. First, due to scanning length limitations, the duration of the resting state scan was relatively short. However, analysis of limbic connectivity and default mode connectivity with a posterior cingulate seed support the assumption that the scan was long enough to reliably detect study-relevant patterns of functional connectivity. Second, given evidence of differences between males and females in effects of OXT on emotion responding (Domes et al. 2010), we opted to recruit only males for the present study. Additional studies are required to identify whether our findings extend to women. Third, we assessed amygdala connectivity during the resting state. Future studies should ascertain whether OXT effects on resting-state amygdala–rmFC connectivity predict socio-affective functioning during emotion tasks. Fourth, this study does not permit identification of the mechanisms by which intranasal OXT affects resting-state amygdala connectivity. Previous studies have shown that OXT receptors in the amygdala potentiate GABAergic inhibition (i.e. Huber et al. 2005; Viviani et al. 2011). However, whether this mechanism plays a role in OXT modulation of connectivity of the amygdala is not currently known.

In sum, in this fMRI study of OXT effects on amygdala resting-state connectivity, we found that OXT is a robust and highly selective enhancer of connectivity between both amygdalae and medial frontal regions critical for social cognition and emotion regulation. These results add to our understanding of the neural mechanisms by which OXT modulates complex social behaviours.

Supplementary material
For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S1461145712000533.

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Statement of Interest
None.

References


