NeuroImage 149 (2017) 458–467

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Oxytocin differentially alters resting state functional connectivity between amygdala subregions and emotional control networks: Inverse correlation with depressive traits

Monika Eckstein[a,1], Sebastian Markett[b,c,1], Keith M. Kendrick[d,1], Beate Ditzen[a], Fang Liu[e], Rene Hurlemann[f], Benjamin Becker[d,*]

[a] Institute of Medical Psychology, Center for Psychosocial Medicine, University Hospital Heidelberg, D-69115 Heidelberg, Germany
[b] Department of Psychology, University of Bonn, D-53127 Bonn, Germany
[c] Center for Economics and Neuroscience, University of Bonn, D-53127 Bonn, Germany
[d] Key Laboratory for NeuroInformation of Ministry of Education, Center for Information in Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan 611731, China
[e] Department of Radiology, University of Wisconsin-Madison, Madison, WI 53705-2275, USA
[f] Department of Psychiatry and Division of Medical Psychology, University of Bonn, D-53127 Bonn, Germany

ARTICLE INFO

Keywords:
Basolateral amygdala
Superficial amygdala
Centromedial amygdala
Emotion
Oxytocin

ABSTRACT

The hypothalamic neuropeptide oxytocin (OT) has received increasing attention for its role in modulating social-emotional processes across species. Previous studies on using intranasal-OT in humans point to a crucial engagement of the amygdala in the observed neuromodulatory effects of OT under task and rest conditions. However, the amygdala is not a single homogenous structure, but rather a set of structurally and functionally heterogeneous nuclei that show distinct patterns of connectivity with limbic and frontal emotion-processing regions. To determine potential differential effects of OT on functional connectivity of the amygdala subregions, 79 male participants underwent resting-state fMRI following randomized intranasal-OT or placebo administration. In line with previous studies OT increased the connectivity of the total amygdala with dorso-medial prefrontal regions engaged in emotion regulation. In addition, OT enhanced coupling of the total amygdala with cerebellar regions. Importantly, OT differentially altered the connectivity of amygdala subregions with distinct up-stream cortical nodes, particularly prefrontal/parietal, and cerebellar down-stream regions. OT-induced increased connectivity with cerebellar regions were largely driven by effects on the centromedial and basolateral subregions, whereas increased connectivity with prefrontal regions were largely mediated by right superficial and basolateral subregions. OT decreased connectivity of the centromedial subregions with core hubs of the emotional face processing network in temporal, occipital and parietal regions. Preliminary findings suggest that effects on the superficial amygdala-prefrontal pathway were inversely associated with levels of subclinical depression, possibly indicating that OT modulation may be blunted in the context of increased pathological load. Together, the present findings suggest a subregional-specific modulatory role of OT on amygdala-centered emotion processing networks in humans.

Introduction

The hypothalamic neuropeptide oxytocin (OT) plays an important role in modulating social-cognitive and emotional behavior. Accumulating evidence from intranasal-OT (IN-OT) administration studies in healthy individuals suggests modulatory effects on emotional processing, including not only basal functional domains such as attention and emotional learning (Bartz et al., 2011; Eckstein et al., 2015a), but also complex emotion-cognition interactions, such as emotion regulation (Preckel et al., 2015), pair bonding and social interaction (Ditzen et al., 2012).

The amygdala, a subcortical structure with a pivotal role in emotion processing, has been defined as a key neural target of IN-OT effects. Studies that combined the administration of IN-OT with functional neuroimaging techniques consistently observed modulatory effects on neural activity in this region following OT (Wigton et al., 2015;

* Correspondence to: Center for Information in Medicine, No. 2006, Xiyuan Ave., West Hi-Tech Zone, Chengdu, Sichuan 611731, China.
E-mail address: ben_becker@gmx.de (B. Becker).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neuroimage.2017.01.078
Received 16 June 2016; Accepted 31 January 2017
Available online 01 February 2017

Rocchetti et al., 2014). Increasing evidence further suggests that IN-OT influences the functional interplay between the amygdala and frontal, striatal and brainstem regions during emotional task challenges (Kirsch et al., 2005; Striepens et al., 2012; Eckstein et al., 2015a). More recent studies employed functional MRI-based resting state functional connectivity (rsFC) and established effects of IN-OT on the intrinsic connectivity networks of the amygdala, with increased coupling between the amygdala and top-down regulatory hubs, particularly the medial prefrontal cortex (mPFC) and the anterior cingulate cortex (ACC), being most consistently reported (Riem et al., 2012; Sripada et al., 2013; Fan et al., 2014).

These previous rsFC studies generally treated the amygdala as a single homologous structure, while convergent neuroanatomical evidence from animal (Huber et al., 2005; Adhikari et al., 2015) and human (Amunts et al., 2005) studies, as well as accumulating human functional neuroimaging findings (Ball et al., 2007; Roy et al., 2009) emphasize the structural and functional heterogeneity of the amygdala. The human amygdala comprises at least three broad subdivisions (basolateral, superficial and centromedial amygdala subregion) with distinct functions and connectivity patterns (Roy et al., 2009; Bzdok et al., 2013). Prior neuroimaging research in humans suggests that the superficial subregion is particularly sensitive to social information (Goossens et al., 2009) and emotional tension (Lehne et al., 2014), whereas the basolateral subregion plays a pivotal role in higher-level sensory processing (Bzdok et al., 2013) and evaluation of potential threat (Onur et al., 2009). Initial findings have linked the centromedial subregion with motor responses and attentional allocation (Bzdok et al., 2013). The functional subdivision of the human amygdala is further corroborated by resting state functional MRI studies demonstrating distinct connectivity patterns across the three amygdala subregions: whereas spontaneous activity in the superficial subregion predicts activity in limbic regions, the basolateral subregion associates with temporal and frontal regions and the centromedial subregion primarily associates with striatal regions (Roy et al., 2009; Bzdok et al., 2013). The functional relevance of the distinct connectivity patterns is further emphasized by reports on subregion-specific associations with trait dimensions related to emotional processing such as harm avoidance (Li et al., 2012).

Studies in rodents have begun to examine selective effects of OT on the amygdala subregions and suggest differential effects in the domains of emotional learning and social interaction (Calcagnoli et al., 2015; Campbell-Smith et al., 2015). In humans, differential effects of IN-OT on the amygdala subregions have not been systematically examined. Initial evidence for subregion-specific effects of IN-OT on amygdala functioning in humans was provided by a task-based fMRI study reporting that the effects of IN-OT in the domains of valence and attention relate to subregion-specific activity changes in the anterior and posterior amygdala (Gamer et al., 2010). Moreover, a recent clinical study reported that IN-OT produced sex- and subregion-specific effects of the rsFC networks of the centromedial and basolateral amygdala in patients with post-traumatic stress disorder (PTSD) (Koch et al., 2016).

Together, these findings emphasize that examining the effects of IN-OT on the level of the whole amygdala might not fully account for the complex modulatory influence of OT on amygdala functioning. Given that a growing number of studies have begun to link subregion-specific amygdala rsFC networks with specific emotional functions (Papini et al., 2016) and neuropsychiatric disorders characterized by emotional deficits (Kleinhans et al., 2015; Aghajani et al., 2016), the examination of IN-OT effects on the subregion-specific amygdala networks might help to further disentangle the complex modulatory role of OT and inform future studies exploring its potential therapeutic application.

Against this background the present study combined IN-OT administration with fMRI-based rsFC and probabilistic amygdala subdivisions (Amunts et al., 2005) in healthy male participants to (1) characterize distinct effects of IN-OT on the subregion-specific amygdala networks, and to (2) evaluate whether the subregional analysis reveals more specific insights into the neural effects of IN-OT in comparison to the analysis on the level of the whole amygdala. Given the growing interest in the therapeutic application of OT in psychiatric disorders characterized by marked emotional dysfunctions, including depression and anxiety (McQuaid et al., 2014), the present study additionally explored associations between effects of IN-OT on the amygdala networks and sub-clinical levels of alexithymia, depression and trait anxiety. Previous findings in healthy individuals suggest that individual differences in these pathology relevant dimensions may moderate the effects of IN-OT (Alvares et al., 2012; Ellenbogen et al., 2013; Luminet et al., 2011). Moreover, individual variations in the sub-clinical range of these dimensions have been associated with both, impaired emotional functioning (e.g. Wiebking and Northoff, 2015) and amygdala integrity (Goerlich-Dobre et al., 2015).

Material and methods

Subjects and procedure

We recruited N=79 male participants (mean age M=24.27 years, SD=4.16 years) for the study that was registered as clinical trial (identifier NCT02689596) and conducted in accordance with the latest declaration of Helsinki. All participants were non-smokers and gave written consent (IRB Identifier 329/12). Intranasal oxytocin (24 IU; Syntocinon-Spray, Novartis; three puffs per nostril, each with 4 IU OT) or placebo (PL; 0.9% sodium chloride solution) was administered in a randomized double-blind between-group design according to current guidelines (Guastella et al., 2013). N=27 participants received OT, and N=52 participants received PL. Groups did not differ in age (t(76) =−.111, p=.912), height (t(75)=−.595, p=.553), weight (t(76)=.062, p=.951), years of education (t(71)=.269, p=.788), sexual orientation (Chi square=1.591, p=.451), relationship status (Fisher's exact test, p=.340), or parental status (none of the participants had children, detailed group charateristics are given in Table 1). During a first screening session, participants were interviewed to ensure that none of the following exclusion criteria were met: Chronic physical or mental illness, regular nicotine or alcohol use, current or regular use of medication. In addition, the Beck's Depression Inventory (BDI;

Table 1
Demographics and questionnaires.

	OT	PL	T	df	p	N(OT)	N(PL)
Age (years)	24.22 (±3.89)	24.29 (±4.32)	−.067	77	.947	27	52
Weight (kg)	79.11 (±11.02)	78.94 (±11.79)	.062	76	.951	27	51
Height (mm)	181.08 (±6.83)	182.09 (±7.05)	−.595	75	.553	26	51
Education (years)	16.33 (±2.18)	16.20 (±2.07)	.247	71	.788	27	46
BDI	3.17 (±4.02)	2.90 (±3.51)	.293	71	.771	24	49
TAS	48.50 (±8.35)	50.48 (±12.43)	−.704	70	.484	24	48
Trait Anxiety	43.58 (±3.13)	44.06 (±2.60)	−.689	70	.493	24	49

Abb.: OT, oxytocin; PL, placebo; BDI, Beck's Depression Inventory; TAS, Toronto Alexithymia Scale.

Kühner et al., 2007), Toronto Alexithymia Scale (TAS; Taylor et al., 2003) and the STAI (State-Trait Anxiety Inventory; Spielberger, 1983) were administered to all participants to assess levels of depression, alexithymia and anxiety. Because of incompletely filled in questionnaires, we had to exclude n=6 subjects (OT, n=3; PL, n=3) from the BDI analysis and n=8 subjects (OT, n=3; PL, n=5) from TAS and STAI analysis, resulting in a total of n=24 subjects in the OT and n=48 or n=46 in the PL group for the analysis of associations between symptom load on these scales and functional connectivity of the amygdala networks. Participants in both groups had comparable symptom scores (t(71)=.293, p=.77 for BDI, t(70)=−.704, p=.484 for TAS, t(70)=−.689, p=.493 for STAI trait anxiety) and variances for the three measures were comparable in the treatment groups (BDI, F=.381, p=.539; TAS, F=.956, p=.332; trait anxiety F=.917, p=.342). The differences in the number of degrees of freedom in the analysis of associations between questionnaire data and neural indices results from the missing questionnaire data from some participants.

Resting-state fMRI was performed in a subsequent experimental session 30 minutes after OT/PL administration. Scans were acquired using a 1.5 T Avanto system (Siemens AG, Erlangen, Germany). Each functional scan was 5.75 min in length and acquired with the same echo planar imaging (EPI) sequence (110 volumes, each volume consisted of 37 slices, Time of Repetition (TR)=3 s, Time of Echo (TE)=45 ms, flip angle=90°, Field of View (FOV)=192 mm, voxel size=3×3×3 mm^3, slice thickness=3 mm). Participants were instructed to lie still with closed eyes but without falling asleep and without thinking of anything in particular. The participants performed a task-based fMRI experiment following the resting state fMRI acquisition (Eckstein et al., 2015b).

Preprocessing of resting-state time series data

The fMRI data processing was carried out using FMRI Expert Analysis Tool (FEAT) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The following preprocessing was applied: motion correction using MCFLIRT (Jenkinson et al., 2002), slice-timing correction using Fourier-space time-series phase-shifting, non-brain removal using BET (Smith, 2002), spatial smoothing using a Gaussian kernel of 5mm full width at half maximum, and multiplicative mean intensity normalization of the volume at each time point. Registration of functional data to high resolution structural images was carried out using FLIRT (Jenkinson et al., 2002). Registration from high resolution structural to standard space was then further refined using FNIRT nonlinear registration (Andersson et al., 2007).

Additional preprocessing of functional images included an independent component analysis for automatic removal of motion artifacts (ICA-AROMA; Pruim et al., 2015), removal of mean signals from white matter and cerebrospinal fluid by means of linear regression, and bandpass filtering (.01−.1 Hz). Masks for white matter and cerebrospinal fluids signals were created using SPM's tissue probability maps thresholded at .9 (white matter) and .7 (CSF) as in a previous report (Markett et al., 2015). All preprocessing steps were carried out in native space.

As a quality control measure for motion artifacts, mean framewise displacement (FD) was calculated according to Power et al. (2012). One participant from the PL group was classified as an outlier (mean FD=.9) and excluded from the main analysis. The two groups (OT and PL) did not differ in mean FD (OT M=.151, SD=.06, range=.29, minimum=.08, maximum=.37; PL M=.135, SD=.06, range=.20, minimum=.05, maximum=.25). There was no significant group difference in motion (t(76)= 1.181, p=.241.

Functional connectivity analysis

Whole brain functional connectivity maps were created by extracting mean time series from a set of regions of interest (ROIs). To examine subregional effects of OT on amygdala functional connectivity masks for the three amygdala subregions centromedial (CM), basolateral (BLA) and superficial (SF) amygdala were created using probabilistic maps from the anatomy Toolbox (Eickhoff et al., 2005) that provides cytoarchitectonic probabilistic maps based on Amunts et al. (2005). Masks are displayed in Supplementary Fig. S1. Three non-overlapping seed regions were defined according to the methods outlined in Roy et al. (2009). To determine distinct seed regions with a high probability for the respective subregions these maps were initially thresholded at >50% probability for the corresponding subregion. In case of overlap between thresholded maps, each voxel was assigned to the subregion for which it had the highest probability. In addition, whole amygdala subregions based on cytoarchitecture were subsequently created by combining the three non-overlapping masks into a single probabilistic amygdala ROI for each hemisphere. This procedure yielded a total of four probabilistically defined seed regions for each hemisphere: whole amygdala, basolateral, centromedial, and superficial amygdala subregions.

Separate functional connectivity maps were created for each participant and each ROI by computing Pearson correlations between the mean time-course extracted from the ROI and all other voxels in the brain. Prior to statistical analysis, correlation coefficients were transformed into z-scores (Fisher's r-to-z transform) to approximate normality. Differences in functional connectivity between the OT and PL group were assessed by means of SPM8 two-sample t-tests. Age, hours after waking up and state anxiety were controlled for in the group comparisons. The latter two nuisance regressors were added to the model because *a priori* group differences were observed in hours after waking up (t(77)=−2.422, p=.018) and state anxiety (t(77) =2.182, p=.032), despite random allocation of participants to the two treatment conditions. The resulting t-maps were initially thresholded at $p < .005$, and subsequently corrected for the family-wise error at the cluster level. All reported results are significant at a level of $p \leq .05$ at the whole brain level. In line with previous studies (e.g. Sripada et al., 2013) only clusters that were predominantly located in grey matter (i.e. >50% grey matter) are reported. For this purpose, a grey matter mask was created by thresholding SPM's grey matter tissue probability map at .5.

Given the growing interest in the therapeutic application of OT (see e.g. Striepens et al., 2011) we additionally explored associations with depression (as assessed by the BDI), alexithymia (as assessed by the TAS), and trait anxiety (as assessed by the STAI). To this end mean parameter estimates from significant clusters were extracted and correlated with the BDI, TAS, and STAI scores. To evaluate between-group differences in the associations between the scores and neural indices correlation coefficients were converted to z-statistics. Spearman's rank correlations were computed to account for the non-normal distribution of the scores in the present samples.

Results

Initial data quality assessments

As quality check for the probabilistic ROIs and to facilitate comparison with a previous paper examining amygdala subregional connectivity during rest (Roy et al., 2009), we explored the connectivity patterns in the placebo group in a separate analysis. Importantly, this analysis revealed a pattern of overlapping as well subregion-specific connectivity patterns, suggesting that the probabilistic masks used in the present study correspond to functionally distinct amygdala subregions. Findings in the placebo group largely resembled previous findings from Roy and colleagues (2009) (details see Supplementary Results). In a subsequent step we validated the pharmacological modulation by additionally examining the effects of IN-OT using a structurally defined total amygdala mask as provided by the Automated Anatomical Labeling Atlas (AAL). This allowed the replication of

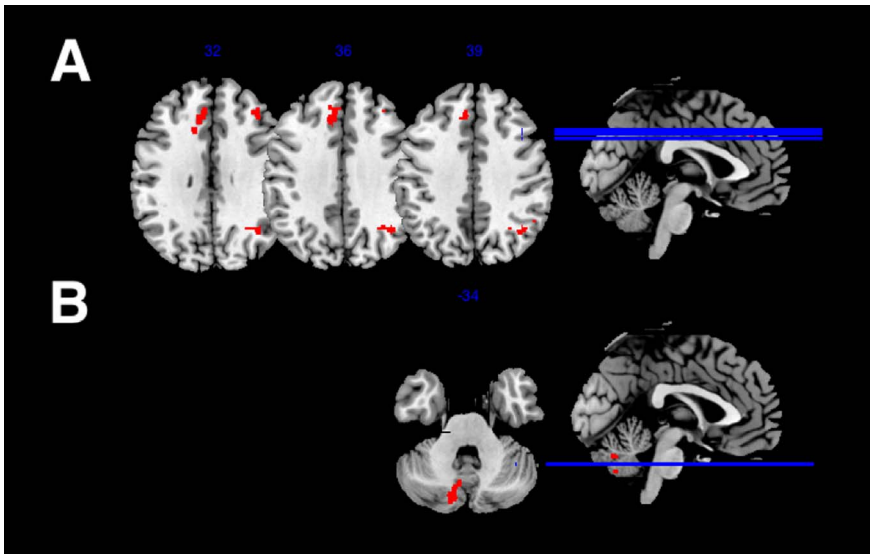

Fig. 1. Oxytocin modulation of whole amygdala functional connectivity. Increased functional connectivity following OT relative to PL on the level of the whole amygdala as defined by the probabilistic cytoarchitectonic maps. A shows results for the left, and B for the right amygdala. Transversal slices were placed upon the peak *z*-coordinates of significant clusters. The blue lines on the sagittal image show the positions of the transversal slices for anatomical reference.

results from a previous IN-OT rsFC study (Sripada et al., 2013) (details see Supplementary results).

Functional connectivity analysis

Analyzing the influence of IN-OT on the functional connectivity of the whole amygdala as defined by the combined probabilistic seed regions revealed that, relative to PL, OT increased functional connectivity between the right probabilistically defined amygdala and the left cerebellum, and between the left probabilistically defined amygdala and the left ACC, the bilateral mPFC, the right cerebellum and the right angular gyrus (see Fig. 1 and Table 2).

Next, we examined the influence of IN-OT on functional connectivity of the amygdala subregions. Significant clusters are shown in Fig. 2, statistics and details are given in Table 2. The right BLA subregion increased its connectivity to the bilateral cerebellum whereas the left BLA increased its connectivity to the contralateral cerebellum, as well as the right middle frontal cortex and the right inferior parietal lobule. The left and right CM amygdala subregions increased their functional connectivity to the bilateral cerebellum during OT relative to PL. The right SF amygdala increased its functional connectivity after OT administration as compared to PL, particularly to anterior regions of the prefrontal cortex. Examining the left SF subregion revealed no significant differences between the treatment groups.

Decreases in functional connectivity after OT administration relative to PL were less widespread and constrained to the CM subregion. More specifically, decreased connectivity was observed between the right CM and the bilateral lingual gyri, the left post central gyrus, and the right middle occipital gyrus following OT administration. For the left CM amygdala, connectivity decreased with the left lingual gyrus and the precuneus as well as the right fusiform and middle temporal gyrus following OT administration.

In order to rule out the possibility that our results were affected by the unequal number of participants in both groups, we matched a subsample of the larger PL group to the OT group and reran our analyses and found similar results (see Supplementary results). We therefore conclude that any potential bias introduced by the unequal study groups is negligible.

Correlational analyses with alexithymia, depression, and trait anxiety

Finally, we explored associations between mean parameter estimates from significant clusters and alexithymia (TAS total scores), depression (BDI total scores), and trait anxiety (STAI trait total scores) within each group using Spearman's rank correlations.

Relationships between BDI scores and functional connectivity were only observed between the right SF amygdala and the left mPFC (MNI coordinates $x=-6$ $y=50$ $z=-4$): in this cluster, BDI scores correlated inversely with functional connectivity after OT administration ($r=-.438$, $p=.032$) but not after PL administration ($r=.077$, $p=.602$). The two coefficients differed significantly between the two conditions ($z=-2.069$, $p=.039$) suggesting that a higher subclinical depressive symptom load is associated with a blunted increase in functional connectivity in this pathway. For trait anxiety, associations with functional connectivity of the left and right CM amygdala were found: left CM – left precuneus connectivity was inversely correlated with levels of trait anxiety after OT administration ($r=-.407$, $p=.049$) but not after PL ($r=.128$, $p=.395$). The difference between correlation coefficients was significant ($z=-2.1062$, $p=.035$). In addition, right CM – right lingual gyrus connectivity was positively associated with levels of trait anxiety during PL ($r=.323$, $p=.028$) but not OT ($r=-.304$, $p=.149$). The difference between correlation coefficients was significant ($z=-2.4375$, $p=.0148$). During PL, however not OT, functional connectivity between the left CM and the left cerebellum as well as the right middle temporal gyrus correlated significantly with trait anxiety (cerebellum, $r=-.295$, $p=.047$ vs. $r=.048$, $p=.823$; middle temporal gyrus, $r=.349$, $p=.017$ vs $r=-.079$, $p=.713$). However, the differences failed to reach statistical significance between experimental groups ($z=1.3225$, $p=.186$; $z=-1.6658$, $p=.0958$). No associations were found with respect to alexithymia.

Given a high overlap between state anxiety and trait anxiety as well as depression, we further explored whether including state anxiety might have biased the association between neural indices and BDI or trait anxiety scores. Examining associations on the levels of the scales did not reveal significant associations between the BDI scores and state anxiety (rho=−.131, $p=.242$) nor between trait and state anxiety (rho=.094, $p=.441$). To further exclude confounding effects we re-estimated the model testing group differences while omitting the covariates. We then extracted the parameter estimates from the three clusters showing significant correlations with levels of depression and trait anxiety and compared the correlation coefficients between functional connectivity and BDI and trait anxiety scores in the OT group ($n=24$) and a state anxiety matched sub-group of the PL group. A total of 10,000 samples of $n=24$ participants from the PL group were

Table 2
Connectivity OT > PL.

Right amygdala (whole amygdala probabilistic)			
Structure	Cluster size	MNI coordinate	*t*-Value
Left cerebellum	146	−8 −70 −34	4.1531
Left amygdala (whole amygdala probabilistic)			
Structure	Cluster size	MNI coordinate	*t*-Value
Left mPFC/ACC	176	−8 30 36	5.2187
Right cerebellum	200	32 −58 −44isms@2015	4.5252
Right mPFC	164	36 36 32	3.9902
Right angular gyrus	264	40 −62 34	4.1235
Right basolateral amygdala			
Structure	Cluster size	MNI coordinate	*t*-Value
Left cerebellum	172	−46 −54 −32	4.4479
Right cerebellum	173	16 −72 −20	4.7654
Right centromedial amygdala			
Structure	Cluster size	MNI coordinate	*t*-Value
Left Cerebellum	888	−28 −72 −46	4.8708
Left cerebellum	487	−2 −66 −40	5.3171
Right cerebellum	312	40 −62 −26	5.8974
Rigt cerebellum	467	4 −84 −28	4.6283
Right superficial amygdala			
Structure	Cluster size	MNI coordinate	*t*-Value
Left mPFC	200	−6 50 −4	4.5928
Right superior frontal gyrus	155	20 58 18	5.0292
Left basolateral amygdala			
Structure	Cluster size	MNI coordinate	*t*-Value
Right middle frontal gyrus	691	36 36 34	5.06
Right cerebellum	300	38 −54 −34	4.5537
Right inferior parietal lobule	353	46 −58 44	4.5389
Left centromedial amygdala			
Structure	Cluster size	MNI coordinate	*t*-Value
Left Cerebellum	1581	−4 −48 −32	5.1182
Right Cerebellum	230	6 −60 −12	5.1463
Connectivity **PL > OT**			
Right centromedial amygdala			
Structure	Cluster size	MNI coordinate	*t*-Value
Left post central gyrus	144	−50 −34 56	4.1645
Left lingual gyrus	259	12 −54 −4	4.8943
Right middle occipital gyrus	676	34 −84 22	5.4985
Right lingual gyrus	794	8 −70 0	4.6954
Left centromedial amygdala			
Structure	Cluster size	MNI coordinate	*t*-Value
Left lingual gyrus	345	−18 −84 −16	4.8837
Left precuneus	244	−2 −68 18	4.1272
Right fusiform gyrus	138	36 −74 −20	4.7204
Right middle temporal gyrus	193	52 −74 −2	4.3396

Abb: OT, oxytocin; PL, placebo; ACC, anterior cingulate cortex; mPFC, medial prefrontal cortex.

selected randomly with the constrain that the two groups should not differ in state anxiety. Z-test revealed group differences in correlation strength in 61.46% of the bootstrap samples for the BDI finding (SF amygdala - left mPFC), 36.47% of the bootstrap samples for the left CM trait anxiety finding (left precuneus), and 41.04% for the right CM trait anxiety finding (right lingual gyrus). The results from the bootstrapping procedure put some constraints on the reported behavioral association. The fact that the reported behavioral associations were only replicated in a subset of the analysis of state anxiety matched samples, indicates that the observed associations between neural indices and trait anxiety in the full sample might be influenced by between-group differences in state anxiety. Associations with the BDI showed more stable results across the bootstrapping procedure and thus will be discussed as exploratory findings in the discussion section.

Discussion

The present study explored subregion-specific effects of OT on the resting state connectivity of the probabilistically defined CM, BLA and SF amygdala subregions as well as on the probabilistically defined total amygdala that combined the three probabilistic subregions.

On the level of the combined total amygdalae OT increased connectivity with ipsilateral cerebellar regions, with the left total amygdala additionally demonstrating increased connectivity with the bilateral dorso-medial PFC and the right inferior parietal lobue following IN-OT. Examining the amygdala subregions provided a more

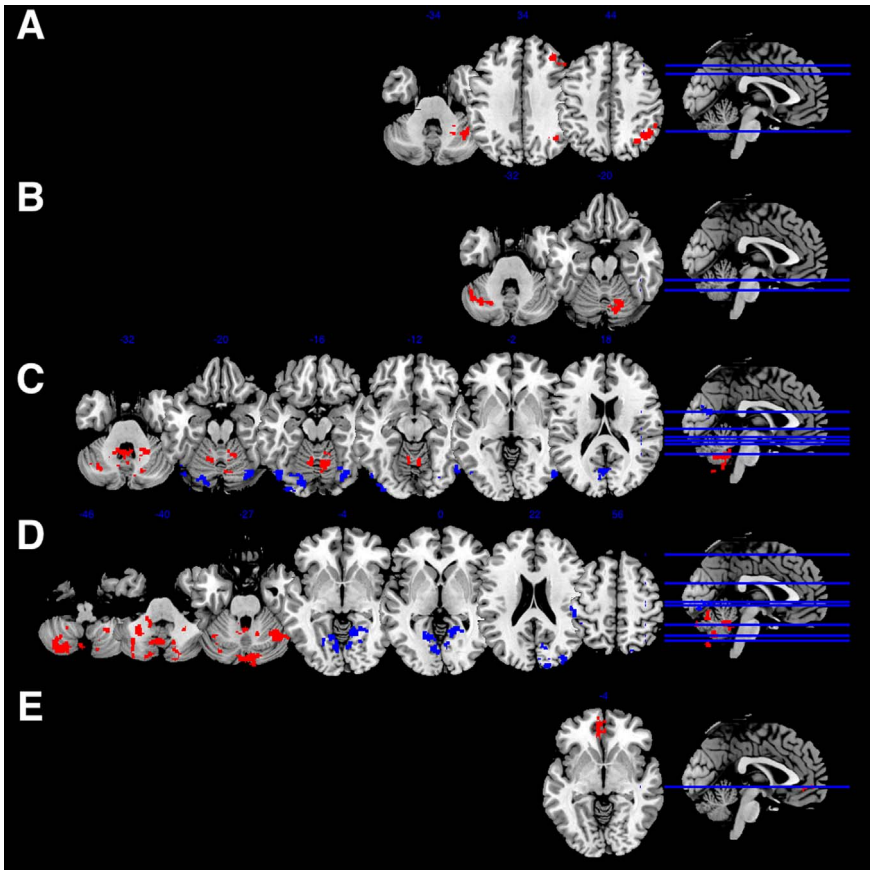

Fig. 2. Oxytocin modulation of amygdala subregion connectivity. Red indicates an increase in functional connectivity after oxytocin administration, blue indicates a decrease. A and B show results for left and right basolateral amygdala, C and D for the left and right centromedial amygdala. E shows results for the right superficial amygdala. No results were observed for the left superficial amygdala. Transversal slices were placed upon the peak *z*-coordinates of significant clusters. The blue lines on the sagittal image show the positions of the transversal slices for anatomical reference.

specific pattern of IN-OT effects on the amygdala networks, indicating that increased connectivity with cerebellar regions following IN-OT were mainly driven by effects on the bilateral CM and BLA subregions, whereas increased connectivity with frontal regions were mediated by effects of OT on the right SF and left BLA subregions. Enhanced coupling with the right inferior parietal lobule following IN-OT appeared to be specifically mediated by the left BLA. In contrast to the analysis on the level of the total amygdala, the subregion-specific analysis additionally revealed reduced connectivity following IN-OT, with the bilateral CM showing decreased coupling with a network spanning occipital, temporal and parietal regions.

OT-effects on the level of the total amygdala

Largely converging with previous studies that investigated connectivity effects of IN-OT on the level of the total amygdala (Sripada et al., 2013; Kovács and Kéri, 2015; Ebner et al., 2016), OT increased the functional coupling of the total amygdala with medial prefrontal regions. The medial PFC shares extensive projections with the amygdala (Ghashghaei et al., 2007) and together these regions represent core nodes in the cortico-limbic emotion processing and regulation circuitry. In this circuitry the amygdala plays a pivotal role in emotional processing, particularly automatic detection of potential threat and acquisition of fear (LeDoux, 2007, Calder et al., 2001), whereas prefrontal regions are thought to implement regulatory control through top-down inhibition of amygdala activity promoting flexible and adaptive behavior. The dorso-medial PFC has been involved in both volitional emotion regulation processes such as cognitive reappraisal (Etkin et al., 2011; Ochsner et al., 2012) as well as automatic control processes including inhibition of the stress response (Dedovic et al., 2009) and extinction of previously acquired fear responses (Phillips et al., 2008).

In addition to the previously reported IN-OT effect on amygdala-prefrontal connectivity, the present study revealed initial evidence that OT also enhances coupling of the amygdala with cerebellar regions. The use of cytoarchitectonic high-probability maps in the present investigation probably allowed a more precise mapping of the amygdala and thus enabled the initial characterization of IN-OT effects on down-stream cerebellar networks. In addition to the well-validated role of the cerebellum in motor control, accumulating evidence suggests an important role of the cerebellum in emotion and cognition (Stoodley and Schmahmann; 2010). Together with the amygdala and the mPFC, cerebellar regions critically contribute to the acquisition and extinction of fear responses (Farley et al., 2016, Magal and Mintz, 2014), suggesting that modulatory IN-OT effects on these pathways reflect the essential contribution of the endogenous OT system in fear learning (Neumann and Slattery, 2016).

In line with previous animal research reporting subregion-specific effects of OT on amygdala-associated functions (Calcagnoli et al., 2015; Campbell-Smith et al., 2015) and a previous task-based fMRI study reporting subregion-specific activity changes following IN-OT in humans (Gamer et al., 2010), the present study revealed a differential impact of OT on amygdala-subregion functional coupling with distinct up-stream cortical nodes, particularly prefrontal and parietal, and cerebellar down-stream regions.

OT-effects on basolateral connectivity

OT increased the functional coupling of the BLA subregions with a widespread bilateral cerebellar network, and additionally increased

functional coupling of the left BLA with the dorsolateral PFC. Axonal tracing models in animals (Stefanacci and Amaral, 2002) and probabilistic tractography studies in humans (Bach et al., 2011) suggest that the BLA is a core hub for integrating highly processed multisensory information and for connecting this information with emotional contents. This integrative function of perceptual input and emotional valence might underlie the critical role of the BLA in fear conditioning and extinction (LeDoux, 2007; Pessoa, 2008). Both, prefrontal as well as cerebellar regions strongly interact with the BLA during fear extinction (Farley et al., 2016, Magal and Mintz, 2014; Sehlmeyer et al., 2009). OT-administration facilitates fear extinction across species (Eckstein *et al.*, 2014a; Campbell-Smith et al., 2015), with animal data indicating that specifically increased OT transmission in the BLA subregion facilitates fear extinction (Campbell-Smith et al., 2015). In line with these observations, the present findings might reflect a specific involvement of BLA OT-transmission in fear extinction in humans.

The left BLA additionally exhibited increased coupling with contralateral regions in the dorsolateral PFC and inferior parietal lobule following IN-OT. Both regions have been previously associated with top-down control of the amygdala, particularly the implementation of volitional emotion regulation strategies in response to negative stimuli (Ochsner and Gross, 2005; Buhle et al., 2014; Seo et al., 2014). Increased connectivity of the BLA with cerebellar as well as frontoparietal regions following IN-OT therefore might reflect the complex and party overlapping engagement of the OT-system in different emotion regulation processes.

OT-effects on centromedial connectivity

OT increased the connectivity of the CM subregions with an extensive bilateral cerebellar network. Previous studies demonstrated a particularly strong coupling of the CM subregion with cerebellar regions (Roy et al., 2009) and suggest an important role of these connections in mediating the emotional functions of the cerebellum (Etkin et al., 2009). Animal data additionally indicates important contributions of CM-cerebellar interactions during fear extinction (Magal and Mintz, 2014) and specific contributions of CM oxytocinergic signaling in fear extinction and fear expression (Campbell-Smith et al., 2015). In accordance with the common and distinct functions of BLA and CM oxytocin-signaling in fear acquisition and distinction (Campbell-Smith et al., 2015), OT enhanced the connectivity of both subregions with the cerebellum.

In contrast to the analyses on the level of the whole amygdala, examining subregion-specific connectivity additionally revealed that IN-OT reduced amygdala functional coupling. Reduced functional connectivity following OT was specifically observed for the CM subregions, and engaged a network incorporating occipital, temporal and parietal regions. More specifically, OT decreased coupling of the CM subregions with a network spanning core regions of the emotional face processing network, including the fusiform gyrus, lingual gyrus, middle occipital gyrus and the middle temporal gyrus (Fusar-Poli et al., 2009). Recent meta-analytic reports covering findings from OT administration experiments have consistently revealed that OT enhances the recognition of emotional faces (Van Ijzendoorn and Bakermans-Kranenburg, 2012; Shahrestani et al., 2013), with functional imaging studies suggesting that the amygdala plays a pivotal role in facilitated facial emotion recognition following OT (Domes *et al.*, 2009; Domes et al., 2014).

Whereas the role of the CM in fear and anxiety is well established, recent research suggests that the CM is also implicated in the modulation of social behavior (Varlinskaya et al., 2013). OT administration studies in rodents furthermore emphasize the specific involvement of the CM subregion in social aggression and social interest (Calcagnoli et al., 2015; Dumais et al., 2016). Previous neuroimaging studies in humans suggest strong modulatory influence of the amygdala on the face processing networks, even during rest (e.g. Kruschwitz et al., 2015) and a specific sensitivity of the CM in processing the emotional significance of social stimuli including facial expressions (Hrybouski et al., 2016; Boll et al., 2011). Although negative correlations in rsFC depend on analysis strategies and their interpretation remains a matter of debate (Murphy et al., 2009; Weissenbacher et al., 2009), the present findings might suggest that the modulatory effects of OT on the face processing networks are mediated by the CM subregion.

OT-effects on superficial connectivity

Administration of intranasal-OT increased functional coupling of the right SF amygdala with a ventromedial prefrontal network. Previous neuroimaging research in humans suggests that the SF subregion is specifically sensitive to social information (Goossens et al., 2009, Bzdok et al., 2013) and shows negative coupling with medial prefrontal regions during rest (Roy et al., 2009). Task-based fMRI research indicates an important role of the prefrontal-amygdala circuitry in regulatory control processes (Etkin et al., 2011; Ochsner et al., 2012), with the amygdala-ventromedial prefrontal pathway being specifically involved in implicit regulation of negative emotions (Etkin et al., 2015) and emotional conflict resolution (Egner et al., 2008). Further support for a specific involvement of the SF-mPFC pathway in negative emotion processing and regulation comes from a study reporting stronger connectivity in the right SF amygdala-mPFC pathway after sleep deprivation which was directly associated with mood disturbances during sleep deprivation (Lei et al., 2015). In line with the proposed anxiolytic effects of OT (MacDonald and Feifel, 2014) and animal research suggesting that the mPFC mediates the anxiolytic effects of OT (Sabihi et al., 2014), the present findings might indicate a specific association between OT's effects on the SF subregion and facilitated automatic control of negative emotions.

Associations with depression

An exploratory analysis revealed preliminary evidence that subregion-specific effects of OT, but not its effects at the level of the entire amygdala, might be related to subclinical levels of depression. Specifically, coupling of the right SF subregion with the mPFC inversely correlated with depressive symptom load following OT, however not PL, suggesting that elevated levels of depression interfere with responsivity of this pathway to IN-OT. The inverse association within the OT group in the present study might suggest that the SF-mPFC pathway showed the strongest responsivity to OT in participants with the lowest level of subclinical depression, possibly indicating a blunted response to IN-OT in individuals with higher depressive symptom load. These findings are in line with growing evidence suggesting that contextual and inter-individual factors moderate the effects of IN-OT (Bartz et al., 2011), and corroborate previous reports on detrimental effects of IN-OT on emotional processing in populations with elevated depressive symptoms (Ellenbogen et al., 2013; Pincus et al., 2010). Together these findings suggest that higher depression levels might be associated with a higher risk for negative effects of IN-OT. Given the important role of reduced amygdala-prefrontal coupling during rest and emotional processing in major depression (Etkin and Schatzberg, 2011; Greening et al., 2013; Phillips et al., 2015) and of reinstated amygdala-prefrontal coupling for anti-depressive treatment response (Dichter et al., 2015) future studies exploring the therapeutic potential of IN-OT in patients with depressive disorders should consider to monitor effects on amygdala functioning on the subregion-level.

Concordant and unique OT-effects on left and right amygdala regions

In line with previous studies examining rsFC of the amygdala subregions and effects of IN-OT on amygdala networks (Sripada et al., 2013; Frijling et al., 2016; Roy et al., 2009; however, see e.g. Ebner

et al., 2016) the present study separately examined left and right amygdala regions. Consistent with previous studies (e.g. Sripada et al., 2013) OT produced concordant as well as lateralized effects on left and right amygdala connectivity. In the present study OT-produced concordant effects on cerebellar coupling of the left and right regions, while unique enhancement was observed on left total/BLA – right prefrontal/inferior parietal connectivity as well as right SF – left ventromedial PFC connectivity. Although lateralized effects of OT on amygdala functioning have not been systematically examined, it might be hypothesized that differences might reflect hemisphere-specific functional specialization of the amygdala. OT increased left total/BLA coupling with core regions of the emotion regulation and fear extinction networks. Previous meta-analytic data suggests that volitional regulation and fear extinction recruit a right-lateralized prefrontal-parietal network and predominately reduce activation of the left amygdala (Dörfel et al., 2014; Diekhof et al., 2011). The right SF demonstrated hemisphere-specific OT-enhanced coupling with prefrontal regions engaged in rapid implicit regulation (Egner et al., 2008) possibly reflect the stronger contribution of the left amygdala in the rapid detection of facial threat cues (Hardee et al., 2008) and OT's suppression of early left amygdala reactivity in this context (Kanat et al., 2015).

Limitations

Findings from the present study need to be considered in the context of some limitations. A number of recent studies have demonstrated gender-specific effects of IN-OT (Ditzen et al., 2012; Gao et al., 2016), thus the present results observed in a male sample cannot be directly extrapolated to women. The present analysis approach used functional and not effective connectivity methods and thus does not allow drawing of direct conclusions on the directionality of the observed functional coupling effects. Although prefrontal regions have been proposed to exert regulatory control over the amygdala, reciprocal connections and interactions have also been documented (Etkin et al., 2011). Future work might combine IN-OT administration with statistical modeling approaches to connectivity data in order to elucidate the directionality of hemodynamic interactions in the context of OT administration. Given that the BDI has been developed to assess depression severity in clinical samples, the BDI scores acquired in the present study might not reflect a comprehensive assessment of depressive traits in a sample of healthy individuals. Conclusions regarding associations with BDI scores therefore need to be interpreted with caution. Finally, although we were able to replicate previous findings on increased amygdala-prefrontal connectivity following OT, the present analysis included state anxiety as covariate to account for potential effects of between-group differences in this variable. However, given previous findings on associations between amygdala resting state connectivity and state anxiety (Baur et al., 2013), the inclusion of state anxiety as covariate might limit the comparability of the present findings with previous OT-resting state connectivity studies.

Conclusions

In conclusion, the findings indicate subregional specific effects of OT on amygdala connectivity with functionally distinct networks. Moreover, the present findings indicate that examining the effects of OT on the sub-regional level of the amygdala might provide a more comprehensive approach to delineate the complex behavioral effects of OT on the neural level and that examining effects on the level of the entire amygdala might not account for the complex effects of OT. Finally, preliminary evidence suggests that effects of OT on the SF-prefrontal pathway might be associated with preclinical measures of depression.

Funding

This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) BE 5465/2-1 (B.B.), and HU1302/4-1 (R.H.) and the National Natural Science Foundation of China (NSFC) 31530032 (K.M.K.).

Acknowledgements

All authors approved the final version of the manuscript. The authors declare no conflict of interest. The authors wish to thank Katrin Preckel, Claudia Scholz and Annika Walter for their help with data collection.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.neuroimage.2017.01.078.

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