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Oxytocin modulates alcohol-cue induced functional connectivity in the nucleus accumbens of social drinkers

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ABSTRACT

The brain oxytocin system is involved in a wide range of addictive behaviors, inhibiting prime- and cue-induced relapse in preclinical models of substance use disorders. Especially the ability of oxytocin to modulate connectivity between the nucleus accumbens (NAc) and cortical regions has been identified as a factor likely to be critical to its effects on relapse. We thus investigated the effect of oxytocin on NAc functional connectivity during an alcohol cue-reactivity task.

Thirteen male social drinkers participated in a randomized double-blind placebo-controlled cross-over functional magnetic resonance imaging (fMRI) alcohol cue-reactivity task with and without prior intranasal application of 24 IU oxytocin. Effects of oxytocin and functional connectivity during presentation of alcohol cues were assessed using ROI-to-ROI generalized psychophysiological interaction analyses.

Oxytocin application significantly reduced NAc connectivity with the cuneus and thalamo-occipital connectivity, while enhancing connectivity between the paracingulate gyrus and precentral gyrus. This effect was specific to the alcohol presentation and was not found during processing of neutral pictures. In addition, the NAc-cuneus connectivity significantly correlated with alcohol cue-induced craving during the scanning session.

For the first time, we could show that oxytocin selectively attenuates NAc connectivity during an alcohol cue-reactivity task which was related to changes in subjective craving for alcohol. This might reflect an attenuation of alcohol-cue saliency by oxytocin, which improves inhibitory control over craving and cue reactivity.

1. Introduction

Multiple lines of evidence support the role of oxytocin in modulating addictive behavior. Intriguingly, application of oxytocin resulted in long lasting (6 weeks) reductions in preference for alcoholic beverages (McGregor and Bowen, 2012) and reduced ethanol consumption in rats (MacFadyen et al., 2016), mice (King et al., 2017) and prairie voles (Stevenson et al., 2017). Further animal studies linked oxytocin's effects on drug ingestion to modulation of neurotransmission in the brain's rewards system, the striatum and nucleus accumbens (NAc) (Kovacs et al., 1990). A recent study investigating the effects of intranasal and intraperitoneal oxytocin application in mice demonstrated that both modes of administration resulted in a block of escalated alcohol drinking in the animals (Tunstall et al., 2019). By comparing the effects of intracerebroventricular administration of oxytocin and the oxytocin receptor agonist PF-06655075 to systemic administration, the authors also provided evidence that specifically the actions of oxytocin within the central nervous system (CNS) seem to underlie its effects on alcohol consumption. Especially the modulation of GABAergic neurotransmission in the central nucleus of the amygdala (CeA) was identified as potential correlate of oxytocin's actions within the CNS (Tunstall et al., 2019). A study in healthy human individuals demonstrated that oxytocin in the central nervous systems (CNS) modulates social behavior, anxiety and stress perception (Tully et al., 2018) via binding to G-protein-coupled oxytocin receptors that are located in the hypothalamus and project to mesolimbic and to cortical brain areas, including the amygdala, hippocampus, prefrontal cortex and nucleus accumbens (Bowen and Neumann, 2016; Neumann and Landgraf, 2012). To date, there is only very limited data on the effects of oxytocin in human individuals with substance use disorder. A preliminary study in patients with alcohol use disorder showed that oxytocin can reduce withdrawal symptoms in the first three days of alcohol withdrawal (Pedersen et al., 2013). A more recent study could not find conclusive results on oxytocin's effects during alcohol withdrawal, i.e. neither

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CIWA scores nor the required oxazepam dose significantly differed between the oxytocin and placebo group (Melby et al., 2019). Another study in opiate-dependent patients demonstrated a significant association between the plasma oxytocin levels and craving scores (Lin et al., 2018) and a further study in opiate-dependent patients could also show a significant reduction of subjective craving and withdrawal symptoms by oxytocin (Moeini et al., 2019). The recent parent study of presented work incorporated a sample of social drinkers in addition to animal data and human post-mortem data (Hansson et al., 2017). This study in non-dependent social drinkers could demonstrate that intranasal application of 24 IU oxytocin reduced alcohol cue-induced brain response in several brain areas, including the insula, hippocampus, parahippocampus, cingulate gyrus, the inferior and the medial frontal gyrus, and in visual and motor regions compared to placebo (Hansson et al., 2017). This study provided initial evidence that oxytocin can be used to modify relevant addiction phenotypes (i.e. neural alcohol cue-reactivity) that are associated with treatment response and relapse risk (Mann et al., 2014). Besides the disruption of local neural activation patterns, attention has been drawn to alcohol's effects on the connectivity of different brain areas under different conditions (e.g. watching drugs vs. watching neutral stimuli). The development of an alcohol use disorder (AUD) is associated with widespread neural adaptations that affect cognitive domains, such as executive control and reward processing that seem to interact in contributing to the maintenance of alcohol seeking and problem drinking. Enhanced reward sensitivity and the motivational drive that promote alcohol-seeking behavior may in part result from changes in functional interconnection of distinct brain regions that can be assessed using psychophysiological interaction analyses. Studies in patients with substance use disorder (SUD) demonstrated altered connectivity between midbrain areas and parts of the amygdala and orbitofrontal cortex in AUD patients with a stronger connectivity in patients that abstained from alcohol versus those that relapsed subsequently (Beck et al., 2012). Another study in AUD patients indicated that the effect of psychopharmacological treatment (baclofen) was related to changes in alcohol cue-modulated functional connectivity between midbrain regions (i.e. the VTA) and the anterior cingulate cortex (ACC) as well as left medial prefrontal cortex (MPFC), rendering alcohol-cue modulated functional brain connectivity a relevant endo-phenotype to investigating potential new treatment options. To date, there is no data on the potential effects of oxytocin on alcohol cue-modulated functional brain connectivity, but existing data on functional brain connectivity during different tasks that assessed emotion processing during the presentation of emotional faces (Kirsch et al., 2005; Wittfoth-Schardt et al., 2012) or infant laughter (Riem et al., 2012) and decision making during a prisoner dilemma paradigm (Rilling et al., 2012) demonstrated significant modulation of functional connectivity by oxytocin. Even though preclinical and preliminary clinical data point towards a beneficial effect of oxytocin on behavior related to SUD, no study to date has investigated the effect of oxytocin on functional brain connectivity during processing of alcohol stimuli. As, alcohol cue-induced changes in functional connectivity between the midbrain and other brain regions were related to increased relapse risk and treatment response in previous studies, we set out to investigate whether oxytocin can modulate alcohol cue-induced functional connectivity between the brain reward system and cortical regions in the framework of a preliminary functional magnetic resonance imaging study. We hypothesized that oxytocin would attenuate functional connectivity between meso-cortico-limbic brain regions during the presentation of alcohol pictures.

2. Materials and methods

2.1. Study design and assessment

Effects of intranasal oxytocin (24 international units, IU, dissolved in 600microliters fluid) on functional brain connectivity were investigated in a randomized, double-blind, placebo-controlled, cross-over study (German clinical trial number DRKS00009253). Current analyses are secondary analyses based on the datasets that were collected and published in the parent study by Hansson et al. (2017). The current study was a preliminary study. Due to the fact that oxytocin is not approved for treatment of alcohol use in Germany yet and adverse effects of oxytocin in females during an (unknown) pregnancy were reported, we opted to conduct this pilot study in a sample of only male social drinkers, as recommended by the local ethics committee. Participants were recruited via advertisements in local newspapers, bulletins at public institutions as well as via social networks. Eighteen social male drinkers were included in the study. All participants were required to be aged 18 to 65 years and meet the definition of "social drinking" (i.e. alcohol consumption $\geq$ 1 standard drink, defined as 12 g alcohol, on at least 2 days per week), as well as being right-handed, and having normal vision (see supplementary Figure S1 for a consort flow chart). Subjects that (i) experienced severe withdrawal symptoms in the past, or (ii) underwent inpatient treatment, due to alcohol intoxication in the past, or (iii) met the criteria for any other axis-I disorder, except from nicotine addiction in the last 12 months (according to DSM-IV), or (iv) took any psychoactive substances, anti-craving or anticonvulsive medication within the last months, or (v) had any comorbid severe internal or neurological condition, or (vi) had a positive drug-screening, or (vii) had any contraindications for receiving a MRI-scan (e.g. tattoos, metal implants, pacemakers), or (viii) had contraindications to the administration of oxytocin, or (ix) had positive breath alcohol levels (> 0), were excluded from the study. Substance use patterns during the last three months were assessed using a short semi-structured interview (Form 90; Sobell et al., 1996). To further characterize the extent of alcohol-use and associated physical and psychological consequences, we administered the Alcohol Dependence Scale (ADS; Kivlahan et al., 1989) and the Obsessive Compulsive Drinking Scale for Alcohol Dependence (OCDS; Anton et al., 1995) and collected blood samples to determine the levels of gamma-glutamyl transpeptidase (gGT), glutamate oxaloacetate transaminase (GOT) and glutamic-pyruvate transaminase (GPT).

Unheralded drug urine screenings were conducted on both experimental days and no participant had a positive screening for any substance. Basic medical check-up, including an electrocardiogram, was performed to rule out contraindications for oxytocin application. Additionally, all participants underwent drug urine screening and breath alcohol level measurement. All subjects underwent two separate experimental sessions at an interval of two weeks, comprising the assessment of clinical measures and two fMRI scans. Participants randomly received oxytocin or placebo either before the first or before the second session. Randomization was conducted based on software-generated codes (RITA, version 1.30, StatSol, Lübeck, Germany). The ethics committee of the University of Heidelberg approved all experimental procedures. Prior to the scanning session and oxytocin vs. placebo application, participants completed a series of clinical scales and questionnaires, such as the Beck Depression Inventory (BDI; Hautzinger et al., 2009), the perceived stress scale (PSS; Cohen et al., 1983) and the Fagerstroem Test for Nicotine Dependence (FTND; Heatherton et al., 1991), as well as the State Trait Anxiety Inventory (STAI; Spielberger, 1983). 45 min prior to the imaging session, a single dose of 24 IU oxytocin (or placebo) was administered as 12 spray puffs. The functional MRI tasks were performed about 60 min after oxytocin application. This timeframe seems suitable as recent studies indicated regional cerebral blood flow changes between 25 and 78 min after oxytocin application (Tully et al., 2018). Participants underwent an fMRI measurement comprising an alcohol cue-reactivity task (Vollstadt-Klein et al., 2012).

2.2. fMRI alcohol cue-reactivity task

Individual neural response to alcohol cues was assessed using a

validated alcohol cue-reactivity paradigm (Vollstadt-Klein et al., 2012). During this task, participants watch series of alcohol and neutral pictures in pseudo-randomized order using goggles that are suitable for MRI (MRI Audio/Video Systems, Resonance Technology Inc., Los Angeles, CA, USA). All pictures were presented using a block-design. Each block consisted of a series of 5 pictures of the same category (alcohol vs. neutral) that were presented for 4 s each. In-between each block, participants were asked to rate their subjective craving (i.e. rating phase) on a visual analogue scale, ranging from 0 (= no craving at all) to 100 (= highest conceivable craving intensity) and a fixation-cross was presented in between-separate trials (i.e. rest condition) which served as baseline condition. The whole task incorporated 12 alcohol and 9 neutral picture blocks with 5 pictures of each category in every block (i.e. 21 blocks in total) and took approximately 12 min. Image presentation and data recording was controlled using the Presentation® software (Version 16.0, Neurobehavioral Systems Inc., Albany, CA, USA).

2.3. fMRI acquisition and pre-processing

The fMRI measurement was conducted using a three Tesla whole-body-tomograph (MAGNETOM Trio, TIM technology, Siemens, Erlangen, Germany). While participants performed the alcohol cue-reactivity task a total of 305 T2*-weighted echo-planar images (EPI) were acquired using a TR (repetition time) of 2 s, a TE (echo time) of 30 ms, a flip angle of 80°, 28 slices, slice thickness = 4 mm, 1-mm gap, voxel dimensions $3 \times 3 \times 5\,mm^3$, FOV = $192 \times 192\ mm^2$, 64×64 in-plane resolution). In order to reduce artefacts due to magnetic saturation effects, the first five scans were excluded from analyses. All MRI data were pre-processed using the statistical parametric mapping software for Matlab (SPM, Wellcome Department of Cognitive Neurology, London, UK) version 12 (v6906). Functional images underwent realignment, slice-timing correction, normalisation to Montreal Neuroloigical Institute (MNI) space using a standardized EPI template image in SPM and spatial smoothing (8 mm full width half maximum Gaussian kernel filter). Motion parameters from the realignment procedure were evaluated and datasets were excluded if translation or rotation exceeded > 3 mm or > 1 ° respectively. For every participant, first level statistics were computed, modeling the different experimental conditions (alcohol, neutral, rest) in a generalized linear model and adding movement parameters and rating phase as covariates of no interest.

2.4. ROI-to-ROI functional connectivity analysis

The Functional Connectivity Toolbox (CONN-Toolbox v18.4 (Whitfield-Gabrieli and Nieto-Castanon, 2012)) for Matlab and SPM12 (v6906) was used to perform ROI-to-ROI functional connectivity analyses using the implemented generalized Psychophysiological Interaction procedure (gPPI). Different task conditions (alcohol, neutral, rest) and realignment parameters from preprocessing were imported into the CONN-Toolbox using the import function for SPM files. In addition, functional images and anatomical volumes for every participant and both assessment sessions were imported, following the standard procedure, detailed in the CONN-toolbox manual. In distinction to resting state connectivity analyses, task main condition effects and sequence of oxytocin application (i.e. before first vs. second scan) were included in the list of confounds, in order to reflect the cross-over design and control for period effects and low-pass filtering during preprocessing was removed. Noise correction was performed using the anatomical component-based noise correction (aCompCor) method (Behzadi et al., 2007) implemented in the CONN Toolbox. This method extracts principal components from white-matter (WM) and cerebrospinal fluid (CSF) time series. WM and CSF voxels are identified via a segmentation of the anatomical images. These components, together with motion parameters derived from SPM preprocessing, were added as confounds in the denoising step of the CONN toolbox. Finally, images were denoised and spatial smoothing using a Gaussian kernel of FWHM 8 mm was conducted. The different task main conditions (alcohol, neutral) were defined as separate blocks for oxytocin and placebo trials. We used that default atlas-based definition of 132 cortical and subcortical regions of interest (ROIs) implemented in the CONN toolbox (v18.a) that uses a combination of the Harvard-Oxford atlas and the AAL atlas.

The parent study that provided the datasets for the current analyses demonstrated significant attenuation of neural responses to alcohol cues in a set of disparate brain areas including mesolimbic and hippocampal regions, as well as parts of the frontal occipital and motor cortices (Hansson et al., 2017). Hence, we expected to detect changes in a variety of cortical and subcortical brain regions and therefore opted to use a ROI-to-ROI whole-brain analytical approach without restriction to a small number of ROIs. We used a standardized parcellation of the whole brain including 91 cortical areas and 15 subcortical areas from the FSL Harvard-Oxford Atlas as well as 26 cerebellar areas from the AAL atlas, totaling in 132 regions, which is included as standard whole-brain parcellation in the recent version of the CONN-toolbox. According to the procedure described in detail in the CONN toolbox manual, differences in ROI-to-ROI connectivity (we used the 132 predefined ROIs from the FSL Harvard-Oxford Atlas and AAL atlas that is included as standard in the CONN toolbox) between oxytocin and placebo trials were investigated comparing oxytocin vs. placebo during the presentation of alcohol pictures using bivariate correlation analyses within the framework of a gPPI analysis model in the CONN-toolbox. A two-sided seed-level false discovery rate (FDR) multiple testing correction of $p_{FDR} < 0.05$ (as implemented in the Conn-toolbox software) was applied to the connectivity analyses in order to guard against false positive findings. In addition, subject level beta-values for ROI-to-ROI connectivity were exported to the Statistical Package for the Social Sciences (SPSS) version 24.0 for following correlation analyses with drinking and craving data.

2.5. Analyses of demographic, clinical and performance data

Data analyses were performed using SPSS version 24.0. Descriptive results are reported as means and standard deviations (SD). Paired t-tests were applied to analyze differences in clinical characteristics (i.e. BDI scores) between scanning sessions. Secondly, correlation analyses were performed to test the associations between ROI-to-ROI connectivity strength (i] NAc – Cuneus; ii] Thalamus – lat. Occipital Cortex; iii] Paracingulate Gyrus – Precentral Gyrus) and 1] alcohol craving during the alcohol cue-reactivity task and 2] OCDS total score. Statistical significance level was set to an alpha of 0.05. All analyses were performed under the assumption of no carryover effect, because investigation days were separated by one week.

3. Results

3.1. Sample characteristics and task performance

Thirteen social drinkers (mean age = 34.5 years, SD = 16.7; mean weight = 83.35 kg, SD = 10.9) provided fMRI data for both investigation days (see Table 1). On average, participants drank 34 g alcohol per day (SD = 21.6 g, range = 10–73 g) that is 2.86 standard drinks of alcohol (à 12 g) per day (SD = 1.8, range = 0.8–6.1 drinks). During the 90 days prior to the experiment, participants reported a high percentage of drinking days (52.5%, SD = 24.4, range = 27–100%) and heavy drinking days (21.6%, SD = 27.9, range 0–100%), defined as days with alcohol consumption > 60 g. According to the previous literature, the drinking patterns indicate that the majority of participants of the current sample are moderate to heavy-social drinkers (Squeglia et al., 2015). Complementary psychometric data show a mean ADS score of 6.6 (SD = 4.3) and a mean OCDS score of 7.2 (SD = 3.8). The majority of participants had normal liver enzyme levels [mean gGT levels 22.8

Table 1
Demographic and psychometric data, expressed as means and standard deviations (SD).

Social drinkers (n = 13)	Mean (SD)	Mean (SD)	Statistics	Significance
Clinical scales	First scan	Second scan		
OCDS (total score)	7.2 (3.8)	7.2 (4.2)	$t_{(12)} = 0.210$	p = 0.837
ADS (total score)	6.6 (4.3)	–	–	–
Smokers (N)	2 (15%)	–	–	–
FTND (total score)	2.0 (2.8)	–	–	–
STAI (trait total score)	32.9 (6.9)	31.0 (6.9)	$t_{(12)} = 1.345$	p = 0.204
PSS (total score)	6.1 (4.5)	6.9 (3.6)	$t_{(12)} = 1.046$	p = 0.316
BDI (total score)	5.4 (2.7)	4.2 (4.1)	$t_{(12)} = 2.004$	p = 0.068

ADS = Alcohol Dependence Severity Scale; BDI = Beck Depression Inventory; FTND = Fagerstroem Test for Nicotine Dependence; OCDS = Obsessive-Compulsive Drinking Scale; PSS = perceived stress scale; STAI = State-Trait-Anxiety Inventory; SD = standard deviation; * = significant differences $p < 0.05$.

U/l (SD = 8.9, range 9–42); mean GOT levels 28.3 U/l (SD = 5.3, range 19–35); mean GPT levels were 25.2 U/l (SD = 9.2, range 15–40)].

3.2. ROI-to-ROI whole-brain connectivity

3.2.1. Placebo

During placebo trials, increased ROI-to-ROI connectivity was observed for the alcohol picture presentation blocks between several brain regions (see Table 2a). The right precentral gyrus seed ROI showed increased connectivity with the left inferior temporal gyrus and left fusiform gyrus. The left planum polare seed ROI showed increased connectivity with the right middle frontal gyrus and the left superior frontal gyrus. In addition, the left anterior supramarginal gyrus seed showed a positive connectivity with the left inferior temporal gyrus. The left supplementary motor area (SMA) showed a positive connectivity with the left lateral occipital cortex (lOC) and vice versa (i.e. for seed SMA and seed lOC). Further, the right parahippocampal cortex seed showed a positive connectivity with the precuneus. In contrast, only the left posterior supramarginal gyrus seed ROI showed a negative connectivity with the precuneus.

3.2.2. Oxytocin

After oxytocin application, decreased ROI-to-ROI connectivity was observed for the alcohol picture presentation blocks between the left thalamus seed and right lOC, as well as between the right angular gyrus seed and precuneus (see Table 2b). The right frontal pole ROI showed a positive connectivity with the right lOC.

3.2.3. Oxytocin vs. Placebo

Contrasting oxytocin to placebo (contrast: oxytocin > placebo) trials showed significantly reduced connectivity between the right NAc seed ROI and the right cuneus, as well as between the left thalamus seed and the right lOC after oxytocin application (see Table 2c). In addition, oxytocin application resulted in an increased connectivity between the right paracingulate gyrus seed and the right precentral gyrus. Subsequent analyses on extracted connectivity weights from oxytocin and placebo blocks indicated that oxytocin significantly reduces the positive connectivity that is found during placebo trials between the NAc and the right cuneus, as well as between the left thalamus and the right lOC (see Fig. 1 and 2). On the other hand, oxytocin application seems to increase the negative connectivity during placebo trials between the right paracingulate gyrus and the right precentral gyrus.

3.2.4. Neutral picture blocks

No significant functional connectivity between the seed regions (see above) that showed significant connectivity with other brain areas (increased or decreased) during the presentation of alcohol pictures could be determined during the neutral picture blocks. Comparing oxytocin to placebo trials however showed lower functional coupling between the precuneus and the right fusiform cortex and increased connectivity between the right posterior inferior temporal gyrus and the right lOC (see Table 3).

3.2.5. Associations between functional connectivity and alcohol craving

Pearson correlation analysis showed a significant association between the oxytocin-modulated connectivity between the NAc and Cuneus (contrast: oxytocin > placebo) with mean subjective alcohol cue-induced craving during the scanning session (r = 0.538, $p = 0.024$, see Fig. 3). No significant association between NAc – Cuneus connectivity and OCDS scores could be determined (r = 0.361, p = 0.102). In addition, neither oxytocin-modulated connectivity between the thalamus and lateral occipital cortex, nor oxytocin-modulated connectivity between paracingulate gyrus and precentral gyrus correlated significantly with subjective craving during the alcohol cue-reactivity task or OCDS total scores ($r_{max} = -0.361$, $p_{min} = 0.102$).

Table 2
Results of ROI-to-ROI connectivity analyses during alcohol trials (n = 13, two-sided multiple testing correction of $p_{FDR[seed\ level]} < 0.05$).

	Seed	Target	t	pFDR	β
a] Placebo					
	Precentral Gyrus (r)	Inferior Temporal Gyrus (l)	6.92	0.001	0.17
	Planum Polare (l)	Middle Frontal Gyrus (r)	5.83	0.006	0.14
	Anterior Supramarginal Gyrus (l)	Inferior Temporal Gyrus (l)	5.80	0.007	0.11
	Supplementary Motor Area (l)	Lat. Occipital Cortex (l)	5.58	0.009	0.15
	Planum Polare (l)	Middle Frontal Gyrus (l)	4.74	0.020	0.14
	Posterior Supramarginal Gyrus (l)	Precuneus	−5.02	0.025	−0.09
	Lat. Occipital Cortex (l)	Supplementary Motor Area (l)	5.00	0.026	0.14
	Precentral Gyrus (r)	Fusiform Gyrus (l)	4.87	0.032	0.10
	Planum Polare (l)	Superior Frontal Gyrus (l)	4.23	0.034	0.09
	Parahippicampal Cortex (r)	Precuneus (r)	4.68	0.045	0.16
b] Oxytocin					
	Thalamus (l)	Lat. Occipital Cortex (r)	−5.99	0.005	−0.07
	Frontal Pole (r)	Lat. Occipital Cortex (r)	5.26	0.020	0.11
	Angular Gyrus (r)	Precuneus	−4.77	0.048	−0.12
c] Oxytocin > Placebo					
	NAc (r)	Cuneus (r)	−5.76	0.010	−0.14
	Thalamus (l)	Lat. Occipital Cortex (r)	−5.17	0.029	−0.12
	Paracingulate Gyrus (r)	Precentral Gyrus (r)	4.88	0.031	0.16

NAc = Nucleus accumbens, (l) = left brain hemisphere, (r) = right brain hemisphere.

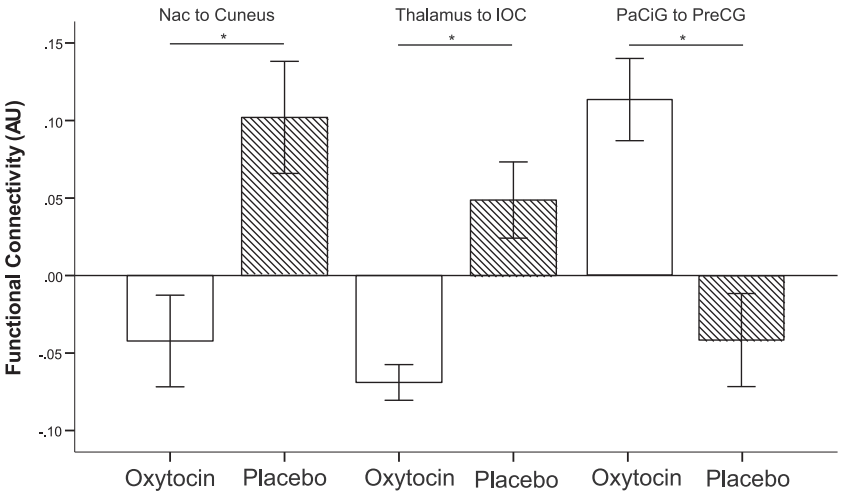

Fig. 1. Bar graphs depicting the comparison of functional connectivity strength during alcohol cue-presentation between (A) right Nucleus accumbens and Cuneus (B) left Thalamus and right lateral Occipital Cortex and (C) left Paracingulate Gyrus and Precentral Gyrus after oxytocin (white bars) vs. placebo (striped bars) application (* = significant difference with $p < 0.001$, Labels: AU = arbitrary unit, lOC = right lateral occipital cortex, PaCiG = left Paracingulate Gyrus, Cuneus = right Cuneus, PreCG = left Precingulate Gyrus, Thalamus = left Thalamus, NAc = right Ncl. Accumbens, two-sided multiple testing correction of $p_{FDR\,[seed\ level]} < 0.05$).

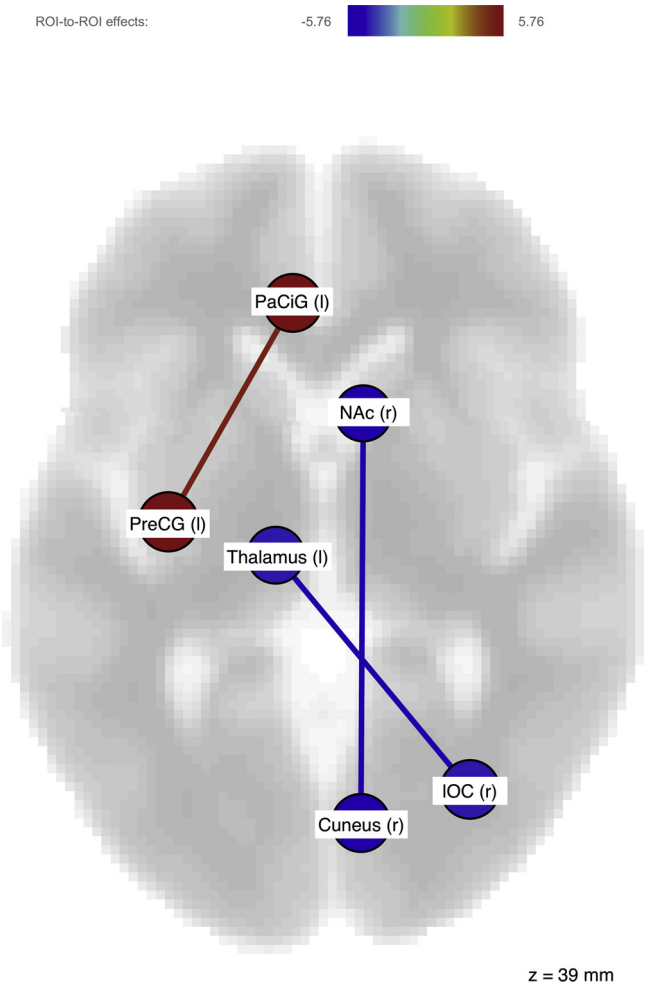

Fig. 2. Two-dimensional rendering of the location of the brain regions that showed significant changes in functional connectivity after oxytocin application vs. placebo during alcohol cue presentation with blue lines representing reduced connectivity after oxytocin application vs. placebo and red lines representing stronger connectivity after oxytocin application vs. placebo (alcohol cue trials, $n = 13$, contrast: oxytocin > placebo, $p_{FDR} < .05$, Labels: lOC (r) = right lateral occipital cortex, PaCiG (l) = Paracingulate Gyrus, PreCG (l) = left Precingulate Gyrus, NAc (r) = right Ncl. accumbens, two-sided multiple testing correction of $p_{FDR\,[seed\ level]} < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Results of ROI-to-ROI connectivity analyses during neutral trials (n = 13, two-sided multiple testing correction of $p_{FDR\,[seed\ level]} < 0.05$).

Seed	Target	t	pFDR	beta
Oxytocin > Placebo				
Post. Inferior Temporal Gyrus (r)	Lateral Occipital Cortex (r)	5.60	0.009	0.09
Precuneus	Fusiform Cortex (r)	−5.21	0.018	−0.12

(l) = left brain hemisphere, (r) = right brain hemisphere.

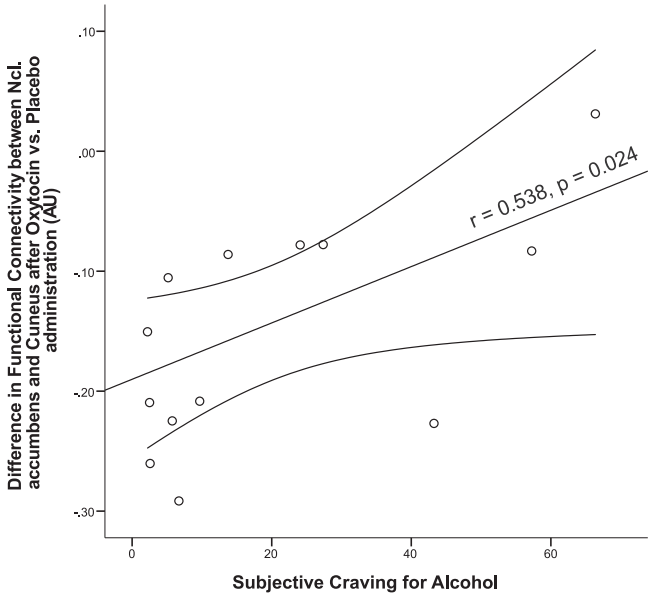

Fig. 3. Scatterplot depicting the positive correlation between Nucleus accumbens – Cuneus connectivity and mean subjective alcohol cue-induced craving during both fMRI sessions (n = 13, r = 0.538, p = 0.024, Labels: AU = arbitrary units).

4. Discussion

The main finding of our study is that oxytocin application attenuates alcohol-cue modulated connectivity between the NAc and cuneus as well as the thalamus and lOC, while it enhances connectivity between precentral gyrus and paracingulate gyrus.

These results harmonize with previous studies that demonstrated the capacity of oxytocin to modulate task-dependent functional brain connectivity. While some studies reported a reduction in functional

coupling after oxytocin administration, e.g. between the amygdala and brainstem during the presentation of emotional faces (Kirsch et al., 2005) or between the pallidum, hippocampus and frontal cortex in response to own vs. familiar faces (Wittfoth-Schardt et al., 2012), other studies reported increased in functional connectivity after oxytocin application, e.g. between the amygdala, orbitofrontal cortex, anterior cingulate cortex, precuneus, hippocampus and supramarginal gyrus in response to infant laughter (Riem et al., 2012) or between the amygdala and insula during a prisoner dilemma paradigm (Rilling et al., 2012). As the authors indicated, oxytocin's effects on functional connectivity might be task-specific. The current study is the first to investigate oxytocin's effects on alcohol cue-modulated brain connectivity on moderate to heavy social drinkers. Current results demonstrate that oxytocin reduces connectivity between the NAc and cuneus during processing of alcohol pictures. Previous studies in cocaine users showed that elevated connectivity between the thalamus, precuneus, medial prefrontal cortex and the limbic reward system (particularly the NAc) were associated with poor outcomes in treatment (for review see (Hanlon et al., 2016)). In addition, a recent study in AUD subjects showed that patients that relapsed to alcohol showed increased connectivity between a striatum seed and the cuneus, as well as the posterior insula, superior temporal gyrus, brainstem and thalamus (Kohno et al., 2017). This study also demonstrated that striato-insula and striato-cortical resting state connectivity was positively correlated with increased alcohol craving in AUD patients. Further, the role of the NAc in alcohol cue-induced craving has been repeatedly demonstrated (Schacht et al., 2013; Yalachkov et al., 2012). The involvement of the cuneus in processing of visual stimuli has been reported. In addition, visual processing in the cuneus seems to be modulated by attention and reward expectation. This suggests that NAc - cuneus connectivity might reflect increased visual saliency of the alcohol-related stimuli that drive craving for alcohol. This idea is supported by the positive association between NAc-cuneal connectivity and alcohol craving in the present study. Hence, attenuation of functional connectivity between the NAc and cuneus following oxytocin application could give hint on an oxytocin-induced decrease of saliency of the alcohol-related stimuli. This is in line with findings that oxytocin levels negatively correlate with drug craving (Lin et al., 2018) and oxytocin application reduces craving for alcohol during the early phase of alcohol withdrawal, i.e. the first three days of alcohol withdrawal (Pedersen et al., 2013) and also other drugs of abuse (e.g. heroin) (Moeini et al., 2019) during withdrawal. Still, this hypothesis has to be validated in future studies.

Increased alcohol cue-induced brain activation in the occipital cortices and the thalamus were reported by multiple cue-reactivity studies in patients with AUD, suggesting that both areas are involved in processing the (visual) properties of alcohol-related stimuli (Yalachkov et al., 2012). In addition, multiple lines of evidence suggest that the thalamus relays information to cortical areas, such as the occipital visual cortices, and controls functional connectivity within and across cortical regions, mediating attentional control (Nakajima and Halassa, 2017; Schmitt et al., 2017). This would be compatible with the idea that oxytocin-induced attenuation of thalamo-occipital connectivity might reflect reduced attention to alcohol-related stimuli after oxytocin-application. A connectivity-based parcellation of the cingulate cortex demonstrated that the precentral gyrus had a high probability of interconnection with the paracingulate gyrus region (Beckmann et al., 2009). Authors further investigated the involvement of the paracingulate cortex region in functional domains using a meta-analytic approach. Results showed that the paracingulate cortex showed overlapping activation during functional imaging tasks that involved conflict, error detection and reward processing, indicating that this region is involved in multiple cognitive processes. Involvement of the precentral gyrus in processing cues that predict reward relative to no-reward was reported by previous studies (Padmala and Pessoa, 2011). Hence, enhanced paracingulate-precentral connectivity after oxytocin activation might reflect enhanced monitoring of alcohol-cue related consequences.

Oxytocin differentially affected connectivity during the presentation of neutral pictures. NAc, thalamus and paracingulate gyrus connectivity were unaffected, suggesting that the effect of oxytocin on the connectivity of reward-related brain regions, such as the NAc, is condition-specific. The data are compatible with the idea that oxytocin selectively attenuates NAc connectivity during the presentation of alcohol cues, which might reflect higher saliency of alcohol-related stimuli, resulting in lower craving for alcohol.

The parent study of current work showed increased brain response in response to alcohol cues under oxytocin and also placebo in parts of the striatum (to whom the NAc belongs), as well in other parts of the putamen and caudate, the thalamus, the amygdala, the cuneus, the middle occipital gyrus, the inferior occipital gyrus and the fusiform gyrus (Hansson et al., 2017). In addition, the results demonstrated an attenuation of neural cue-reactivity by oxytocin in parts of the mesolimbic system (i.e. the insula) and parts of the frontal and occipital cortices as well as the thalamus and amygdala. Current results add to this by demonstrating that oxytocin selectively attenuates NAc connectivity during the presentation of alcohol cues. As increased NAc connectivity has been associated with relapse risk in clinical populations, this could reflect a potentially protective effect of oxytocin alcohol-cue modulated connectivity in reward-associated brain regions. Replication of current findings and further assessment in clinical samples seems warranted. To date, the picture of oxytocin's effects on task-modulated connectivity is far from being understood and future studies are needed to replicate oxytocin's effect on task-modulated connectivity in larger samples of clinical patients.

4.1. Strengths and limitations

The within-subject design of the current study contributed to a better control of potential confounding variables and enhanced power, compared to a between-subject design. In addition, the assessment of a well-characterized that in majority reflect a sample of moderate to heavy social drinkers with no comorbid drug consumption supported internal validity of presented data. Even though current results withheld stringent correction for multiple testing, the small sample limited the power to detect small effects of oxytocin administration and should be regarded as a major limitation of the presented preliminary study. Thus, current results should be regarded as preliminary proof-of-concept that oxytocin could modulate alcohol cue-induced functional brain activation. It should be noted that the correlation analyses are based on a rather small sample and should therefore be interpreted with all due caution and has to be replicated in larger samples. In order to support current results, we conducted leverage and outlier analyses using linear regression analyses in SPSS (with leverage boundary set to 2*[p/n]) that indicated that none of the values is indeed an outlier or has excessive leverage. Hence, we argue that – with all caution due to the small sample size – the results of the correlation analysis are not driven by outliers or values with excessive leverage. Several previous studies indicated that oxytocin's effects – at least on social domains of individual behavior – seem to be partially moderated by contextual and individual (e.g. psychological) factors (Bartz et al., 2011). With regards to addictive behavior, a recent study indicated that effects of oxytocin on alcohol craving might be moderated by anxious attachment style (Mitchell et al., 2016). While the investigation and consideration of such potential moderators might further elucidate the currently sometimes heterogenous findings on oxytocin's effects in substance use disorders, it was beyond the scope of current work to determine potential contextual or psychological moderators of oxytocin effects. Still, controlling for anxiety symptoms (i.e. STAI scores) and depressive symptoms (i.e. BDI scores) in the connectivity analyses did not change the significance or direction of observed results. Current results await further replication and confirmation in following trials that assess clinical populations. While the incorporation of a sample of moderate to heavy

social drinkers does not grant conclusions about processes in alcohol addiction, it might still elucidate preceding stages. The investigation of the effects of oxytocin in patients with alcohol use disorder in the future is important as multiple lines of evidence demonstrated altered neurotransmitter levels and neural responses in those group. Hence, effects of oxytocin might differ from the effects that were found in the current sample of non-dependent social drinkers.

5. Conclusion

For the first time, we show a condition-specific and significant attenuation of NAc connectivity by oxytocin in a sample of moderate to heavy social drinkers that was related to subjective alcohol craving during the fMRI task. Oxytocin-induced attenuation of NAc connectivity was specific to processing alcohol stimuli and might reflect an attenuation of alcohol-cue saliency by oxytocin that could lead to a reduction of the sensitivity towards the appetitive aspects of alcohol cues.

Declaration of competing interest

All authors state that they have no conflicts of interests except for the below-mentioned funding.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.104385.

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