Oxytocin enhancement of emotional empathy: generalization across cultures and effects on amygdala activity

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Abstract

Accumulating evidence suggests that the neuropeptide oxytocin can enhance empathy although it is unclear which specific behavioral and neural aspects are influenced, and whether the effects are modulated by culture, sex and trait autism. Based on previous findings in Caucasian men, we hypothesized that a single intranasal dose of oxytocin would specifically enhance emotional empathy via modulatory effects on the amygdala in an Asian (Chinese) population and explored the modulatory role of sex and trait autism on the effects. We first conducted a double-blind, randomized between-subject design experiment using a modified version of the multifaceted empathy task (MET) to determine whether oxytocin's facilitation of emotional empathy can be replicated in Chinese men (n = 60). To further explore neural mechanisms behind and potential sex differences, functional MRI and skin conductance measures were acquired in an independent experiment incorporating men and women (n = 72). Oxytocin enhanced emotional empathy across experiments and sex, an effect that was accompanied by reduced amygdala activity and increased skin conductance responses. On the network level oxytocin enhanced functional coupling of the right amygdala with the insula and posterior cingulate cortex for positive valence stimuli but attenuated coupling for negative valence stimuli. The effect of oxytocin on amygdala functional connectivity with the insula was modulated by trait autism. Overall, our findings provide further support for the role of oxytocin in facilitating emotional empathy and demonstrate that effects are independent of culture and sex and involve modulatory effects on the amygdala and its interactions with other key empathy regions.

Key words: amygdala; autism; cognitive empathy; culture; emotional empathy; oxytocin**.**

1. Introduction

Empathy is a key social-cognitive capacity that facilitates interpersonal functioning by allowing us to recognize, understand and respond appropriately to mental and affective states experienced by others (Decety and Jackson, 2004; Dziobek, et al., 2008; Reniers, et al., 2010). Impaired empathy is a core deficit in psychiatric disorders characterized by interpersonal dysfunctions, including autism (Dziobek, et al., 2008), schizophrenia (Lee, et al., 2011; Rosenfeld, et al., 2011; Shamay-Tsoory, et al., 2007), and personality disorders (Herpertz and Bertsch, 2014).

Empathy is a multidimensional construct, entailing cognitive processes of perspective-taking, to make inferences about others' mental states (cognitive empathy, CE), as well as emotional processes reflecting a direct affective reaction involving understanding, sharing and responding appropriately to others' feelings (emotional empathy, EE) (Bernhardt and Singer, 2012; Shamay-Tsoory, 2011; Shamay-Tsoory, et al., 2009). EE has been further divided into a direct component (direct emotional empathy, EED), referring to explicit emotional evaluation and empathic concern, and an indirect component (indirect emotional empathy, EEI), referring to a more general physiological arousal response to both person and context (Dziobek, et al., 2008). Although the cognitive and emotional components of empathy represent partly dissociable systems (Shamay-Tsoory, et al., 2009), integrative approaches propose that the experience of empathy evolves as a dynamic interplay between them requiring an explicit representation of the specific affective state of the other person, thereby making CE a prerequisite for EE (Decety and Jackson, 2004; Hillis, 2014). On the neural level the functional organization of empathy is partially mirrored in shared and separable anatomical representations (Bernhardt and Singer, 2012; Lamm, et al., 2011; Lamm, et al., 2007; Leigh, et al., 2013; Schulte-Ruther, et al., 2007; Singer and Lamm, 2009), with the bilateral insula, posterior cingulate cortex (PCC) and anterior cingulate cortex (ACC) contributing to both (Fan, et al., 2011), and the amygdala contributing to the emotional component of empathy (Cox, et al., 2012; Leigh, et al., 2013).

Converging evidence suggests that the hypothalamic neuropeptide oxytocin (OXT) facilitates empathy (Riem, 2012; Rosenfeld, et al., 2011; Striepens, et al., 2011). Genetic approaches have consistently revealed associations between individual variations in the OXT receptor gene and levels of trait empathy in Caucasian (Rodrigues, et al., 2009; Smith, et al., 2014) and Chinese populations (Wu, et al., 2012), with more recent studies suggesting that the specific associations evolve in interaction with other factors, particularly culture (Luo, et al., 2015b; Montag, et al., 2017) and sex (Weisman, et al., 2015). Studies investigating the behavioral effects of intranasal OXT administration on CE have reported enhanced accuracy in the reading the mind in the eyes test (RMET) (Domes, et al., 2007b) and a paradigm requiring participants to infer the intensity of positive or negative emotions expressed by subjects portrayed in videos (Bartz, et al., 2010). However, findings in the domain of CE

have been variable, with OXT effects in the RMET being either restricted to difficult items (Feeser et al., 2015) or unable to be reproduced at all even when taking into account stimulus difficulty and valence (Radke and de Bruijn, 2015). Other studies have also reported that effects were more pronounced in individuals with poor baseline performance (Riem, et al., 2014) or high trait autism (Bartz, et al., 2010). Studies that aimed specifically at determining effects of OXT on EE focused on empathy for pain, an evolutionary conserved primary emotional component (Decety, 2011; Panksepp and Panksepp, 2013), and found no effect on pain empathy towards a partner (Singer, et al., 2008), although an enhanced pain empathic response towards members of an out-group (Shamay-Tsoory, et al., 2013). In contrast, another study in men using the Multifaceted Empathy Test (MET) (Dziobek, et al., 2008), which assesses both CE and EE, observed that OXT specifically enhanced both EED and EEI, but not CE (Hurlemann, et al., 2010). This latter study additionally demonstrated selective EE deficits in amygdala lesion patients and therefore suggested that the amygdala may mediate the EE enhancing effects of OXT. Although several neuroimaging studies have demonstrated modulatory effects of intranasally administered OXT on the core neural components of the empathy network, including the insula, ACC and amygdala and their functional interactions, across different task paradigms (Bakermans-Kranenburg and van IJzendoorn, 2013; Herpertz and Bertsch, 2016; Wigton, et al., 2015), to date only two studies have directly explored the neural mechanisms underlying OXT's empathy enhancing effects. The first reported that OXT increased activation in the superior temporal gyrus and insula during the RMET task (Riem, et al., 2014), whereas the second reported reductions in left insula activity during pain empathic processing (Bos, et al., 2015).

In summary, although the empathy enhancing effects of OXT are central to its proposed social-cognitive and therapeutic properties, it remains unclear whether it selectively enhances CE or EE, and which specific neural substrates are involved. To systematically address these questions, we employed two independent pharmacological between-subject placebo (PLC) controlled experiments in healthy Chinese individuals investigating the effects of intranasal OXT on CE and EE and the underlying neural basis of this effects during the MET (Dziobek, et al., 2008).

Previous studies on the empathy enhancing potential of intranasal OXT are entirely based on observations in Caucasian populations. However, there is accumulating evidence from OXT-administration studies either employing comparable experimental protocols in Caucasian and Chinese subjects (Hurlemann, et al., 2010; Hu, et al., 2015) or examining moderating effects of key cultural orientation differences such as a collectivistic orientation (Pfundmair, et al., 2014; Xu, et al., 2017), suggesting culture-dependent social-cognitive effects of OXT. To this end, the first experiment aimed to replicate findings in a male Caucasian sample showing that OXT enhances EE but not CE (Hurlemann, et al., 2010) in a male Chinese sample. In a second independent sample, male and female Chinese participants performed the same MET paradigm during fMRI to determine the neural substrates involved. Analyses on the neural

level focused on the insula, amygdala and ACC as core empathy regions (Bernhardt and Singer, 2012; Lamm, et al., 2011; Lamm, et al., 2007; Leigh, et al., 2013; Schulte-Ruther, et al., 2007; Singer and Lamm, 2009). Given that the amygdala has been specifically (Cox, et al., 2012; Leigh, et al., 2013) and critically (Hurlemann, et al., 2010) associated with emotional facets of empathy, we expected that OXT's enhancement of EE would be accompanied by altered regional activity and network level connectivity of the amygdala. Previous studies reported increased as well as decreased amygdala activity and connectivity following OXT (Domes, et al., 2007a; Hu, et al., 2015; Striepens, et al., 2012; Tully, et al., 2018; Wigton, et al., 2015) therefore no directed hypothesis with respect to OXT's neural effect was formulated.

Based on a growing number of findings suggesting sex-dependent effects of OXT on social cognition (Gao, et al., 2016; Chen, et al., 2016; Luo, et al., 2017), the second experiment additionally explored whether OXT differentially affects empathic processing in men and women. In line with a previous study reporting that sex does not affect OXT's modulation of empathy (Shamay-Tsoory, et al., 2013), we hypothesized that OXT facilitation of EE would generalize across sexes. Finally, in the context of increasing interest in the therapeutic application of OXT as a potential treatment to improve social cognitive deficits, including empathy, in autism spectrum disorders (Young and Barrett, 2015), and in line with previous studies in healthy subjects (Bartz, et al., 2010; Scheele, et al., 2014; Xu, et al., 2015), the modulatory role of trait autism (assessed by the Autism Spectrum Quotient questionnaire, ASQ, Baron-Cohen, et al., 2001) was explored.

Materials and Methods

2.1 Participants

To fully replicate the previous study on Caucasian participants (Hurlemann, et al., 2010), only males were recruited in the first experiment but both males and females were enrolled in the second experiment to explore potential sex-dependent effects of OXT on empathy. Experiment 1 (Exp 1) included 60 participants (M ± SD, mean age = 22.42 ± 2.23, all male) and Experiment 2 (Exp 2) included an independent sample of 72 participants (34 females, mean age = 21.18 ± 1.95, 38 males, mean age = 22.61 ± 2.01). Both experiments incorporated a double-blind, between-participant design, with participants being randomly assigned to receive either OXT or PLC nasal-spray, resulting in n = 30 (Exp 1) and n = 36 (Exp 2, female = 17) participants treated with OXT. The experimental groups in both experiments were of comparable age (Exp 1, $p = 0.53$, $T_{58} = 0.63$; Exp 2, $p = 0.66$, $T_{70} = -0.44$), education (Exp 1, $p = 0.66$, $T_{58} = 0.44$; Exp 2, $p = 0.63$, $T_{70} = -0.49$) and, in Exp 2, of equivalent sex distribution (chi-square < 0.001, df = 1, p = 1). Exclusion criteria for all participants were past or current physical, neurological or psychiatric disorders, regular or current use of medication or tobacco.

Participants were required to abstain from alcohol, caffeine or nicotine for at least 12 hours before the experiment. None of the females in Exp 2 were taking oral contraceptives or were tested during their menstrual period. Menstrual cycle phase was determined using validated procedures as described in (Penton-Voak, et al., 1999). The proportion of females estimated to be in their follicular or luteal phases did not differ significantly between the treatment groups (chi-square = 0.12, df = 1, p = 0.73). In Exp 1, one participant (in the OXT group) and in Exp 2, three participants (in the PLC group) failed to understand task instructions and were consequently excluded from all further analysis, leading to a total of n = 59 participants in Exp 1 and n = 69 participants in Exp 2.

Before the experiment, written informed consent was obtained from all participants. The study was approved by the local ethics committee of the University of Electronic Science and Technology of China and all procedures were in accordance with the latest revision of the declaration of Helsinki.

2.2 Experimental Protocol

To control for potential confounding variables, all participants initially completed the following questionnaires: Becks Depression Inventory (BDI; Beck, et al., 1961), WLEIS-C Emotional Intelligence Scale (Wleis-C; Wong and Law, 2002), State Trait Anxiety Inventory (STAI; Spielberger, et al., 1970), Empathy Quotient (EQ; Baron-Cohen and Wheelwright, 2004), and Positive and Negative Affect Scale (PANAS; Watson, et al., 1988). To examine associations with trait autism, the ASQ questionnaire (Baron-Cohen, et al., 2001) was administered. Intranasal treatment (oxytocin nasal spray, Sichuan Meike Pharmacy Co., Ltd.,

China, or placebo nasal spray with identical ingredients except oxytocin) was administered in line with recommendations for the intranasal administration of OXT in humans (Guastella, et al., 2013) and 45min before the start of the experimental paradigm. In Exp 1, three puffs per nostril (at 30s intervals) were administered (24 IU) and in Exp 2, five puffs per nostril (40 IU). Both doses are in the typical range employed by other studies (Guastella, et al., 2013; Striepens, et al., 2011) with the rationale for increasing the dose in Exp 2 being to explore dose-dependent behavioral effects of OXT. In a previous study we found equivalent behavioral and neural effects of 24 and 48 IU OXT doses (Zhao, et al., 2017). However, it should be noted that findings from some other studies investigating dose-dependent effects of intranasal OXT have suggested an inverted-U-shaped dose-response curve (Cardoso, et al., 2013; Quintana, et al., 2017; Quintana, et al., 2016; Spengler, et al., 2017) and thus a stronger enhancement of EE with 24 IU relative to 40 IU is conceivable. In post experiment interviews, participants were unable to guess better than chance whether they had received the OXT nasal spray, confirming successful blinding.

2.3 Experimental paradigm

In line with a previous study on male Caucasian participants (Hurlemann, et al., 2010), empathy was assessed using the MET (Dziobek et al., 2008; Domes, et al., 2013; Edele, et al., 2013; Hurlemann, et al., 2010; Wingenfeld, et al., 2014), which assesses both EE and CE components using ecologically valid photo-based stimuli of either negative or positive valence. To account for potential confounding effects of OXT on in-group versus out-group empathy (De Dreu and Kret, 2016) and a cultural empathy bias (Cao, et al., 2015; Luo, et al., 2015a), the original Caucasian MET stimuli were exchanged with corresponding pictures displaying Chinese protagonists. The Chinese stimuli were initially evaluated in an independent sample (supplementary materials) and the final set of Chinese stimuli (30 positive, 30 negative valence) closely resembled the Caucasian stimuli depicting daily life scenarios and conveying emotional mental states via facial expression, body posture and contextual cues. To assess CE, participants were instructed to infer the emotional state of the protagonist in each scene and choose the corresponding answer from 4 options listed. The 4 options presented similar but distinct emotional states to ensure at least 70% accuracy for each stimulus picture. For EED, participants were required to rate how they felt for the protagonist in the depicted scene (1-9 scale, 1 = not at all, 9 = very strong), for EEI participants were required to rate how much they were aroused by the scene (1-9 scale, 1 = very calm, 9 = very aroused).

The different components of empathy were presented in a mixed event/block-design. Following a 3 second(s) instruction cue and a jittered inter-trial interval of 3.9s (2.3-5.9s), 10 stimuli per block were each presented for 3s followed by either a choice of the emotion depicted for the CE condition (displayed for 4 s) or a rating scale (1-9) for the EED and EEI conditions (displayed for 5 s). Six blocks were presented for each condition, resulting in a total of 18 blocks. The order of blocks was counterbalanced across the experimental

conditions, and the fMRI experiment was divided into 6 runs, each containing one block per empathy component. During fMRI (Exp 2) electrodermal activity was simultaneously acquired as an index of autonomic sympathetic activity (Stern, et al., 2001) (technical details on the electrodermal data acquisition are provided in the supplementary materials). To allow baseline recovery of the electrodermal signal a mean inter-trial interval of 5s (4-6s) and a mean interval separating stimulus presentation and behavioral response of 4s (3-5s) was adopted for the fMRI experiment.

2.4 fMRI data acquisition

The fMRI data in Exp 2 were collected using a GE (General Electric Medical System, Milwaukee, WI, USA) 3.0T Discovery 750 MRI scanner. fMRI time series were acquired using a T2*-weighted echo planar imaging pulse sequence (repetition time, 2000 millisecond (ms); echo time, 30ms; slices, 39; thickness, 3.4 millimeter (mm); gap, 0.6 mm; field of view, 240 × 240 mm^2; resolution, 64×64; flip angle, 90°). Additionally, a high resolution T1-weighted structural image was acquired using a 3D spoiled gradient recalled (SPGR) sequence (repetition time, 6 ms; echo time, 2ms; flip angle 9°; field of view, 256 × 256 mm^2; acquisition matrix, 256 × 256; thickness, 1 mm without gap) to exclude participants with apparent brain pathologies and to improve normalization of the fMRI data.

2.5 fMRI data processing

fMRI data were analyzed using SPM12 (Wellcome Trust Center of Neuroimaging, University College London, London, United Kingdom). The first five volumes were discarded to allow T1 equilibration and images were realigned to the first image to correct for head motion. Tissue segmentation, bias-correction and skull-stripping were done for the high-resolution structural images. The functional time series were co-registered with the skull-stripped anatomical scan and normalized to MNI space with voxel size of 3 mm^3. Normalized images were then spatially smoothed using a Gaussian kernel with full-width at half-maximum (FWHM) of 8 mm. On the first level, event-related responses were modelled and subsequently convolved with the standard hemodynamic response function (HRF). The first level design matrix included valence- (positive, negative) and empathy type- (CE, EED, EEI) specific regressors for the viewing phases as main experimental conditions. In addition, regressors for the cue presentation, valence- and empathy type-specific regressors for the rating phases, and for viewing and rating phases of incorrect trials as well as the six movement regressors were included. The experimental contrasts were next submitted to a second level random effects analysis.

To evaluate empathy-type specific main and interaction effects of treatment and valence, repeated-measured ANOVAs were employed in a flexible-factorial design. Based on our regional hypothesis and the core empathy network (Bakermans-Kranenburg and van IJzendoorn, 2013; Bernhardt and Singer, 2012; Hillis, 2014; Hurlemann, et al., 2010; Leigh, et al., 2013; Shamay-Tsoory and Abu-Akel, 2016; Wigton, et al., 2015), the analyses focused

on the bilateral amygdala, insula and ACC which were structurally defined using 60% probability maps from the Harvard-Oxford (sub)cortical atlas. For the regionally focused analysis approach condition-specific parameter estimates were extracted from these regions of interest (ROI) using the Marsbar toolbox (Brett, et al., 2002) and subjected to empathy type-specific ANOVAs with the between-participant factor treatment (OXT, PLC) and the within-participant factor valence (positive, negative) in SPSS (Statistical Package for the Social Sciences, Version 22). P-values for the post-hoc tests of the ROI analysis were Bonferroni-corrected ($P < 0.05$). An exploratory voxel-wise whole-brain analysis in SPM that served to determine contributions of brain regions outside of the predefined network of interest was thresholded at $P < 0.05$, corrected using the family-wise error (FWE) approach. To investigate the effects of OXT on the network level, a generalized form of psychophysiological interaction analysis (gPPI; http://brainmap.wisc.edu/PPI; McLaren, et al., 2012) was conducted using regions showing significant OXT effects in the BOLD level analysis as seeds and implementing an empathy-type specific voxel-wise whole-brain ANOVA approach including the between-participant factor treatment (OXT, PLC) and the within-participant factor valence (positive, negative) thresholded at $P < 0.05$, FWE-corrected at the cluster level. In line with recent recommendations for the control of false-positives in cluster-based correction approaches an initial cluster forming threshold of $P < 0.001$ was applied to data with a resolution of $3 \times 3 \times 3$ mm (Eklund, et al., 2016; Slotnick, 2017). Parameter estimates were extracted from the significant regions to disentangle the specific effects in post-hoc comparisons. Finally, associations between neural indices and trait autism (ASQ scores) were conducted in SPSS using Pearson correlation analysis.

3. Results

In both experiments, there were no significant differences in trait and mood questionnaire scores between OXT and PLC treatment groups (supplementary Table S1 for Exp 1, Table S2 for Exp 2). In line with previous studies in Chinese populations (Melchers et al., 2015; Montag et al., 2017), no significant sex differences in ASQ and EQ scores were observed in Exp 2 (Supplementary Table S3).

3.1 Behavioral results

Based on previous conceptualizations of empathy, proposing that CE is a prerequisite for EE (Decety and Jackson, 2004; Hillis, 2014), for EED and EEI measures only trials for which subjects successfully recognized the emotions displayed by the protagonist were analyzed (for a similar approach see (Luo, et al., 2015a)). To this end, correctly recognized trials were initially determined based on the CE performance, with only correct trials subsequently entering the analyses for the EED and EEI facets.

There were no significant differences in CE accuracy between the two treatment groups in both experiments (Exp 1, OXT, 77.53% ± 6.02%, PLC, 78.94% ± 5.78%, $T_{57} = 0.92$, $p = 0.36$; Exp 2, OXT, 80.10% ± 6.18%, PLC, 81.57% ± 5.83%, $T_{67} = 1.02$, $p = 0.31$). In Exp 1, there was a main effect of treatment ($F(1, 57) = 6.46$, $p = 0.01$, $\eta^2_p = 0.10$) for EED indicating that OXT generally enhanced EED (Fig. 1A). There was no significant treatment × valence interaction ($F(1, 57) = 0.96$, $p = 0.33$, $\eta^2_p = 0.02$). Analysis of EEI did not reveal a treatment main effect ($F(1, 57) = 2.19$, $p = 0.14$, $\eta^2_p = 0.04$) or valence × treatment interaction effect ($F(1, 57) = 3.16$, $p = 0.08$, $\eta^2_p = 0.05$).

Consistent with the findings for EED in Exp 1, Exp 2 also yielded a significant main effect of treatment on EED ($F(1, 67) = 5.81$, $p = 0.02$, $\eta^2_p = 0.08$) with higher ratings following OXT compared to PLC (Fig. 1B). There was also a significant valence × treatment interaction ($F(1, 67) = 4.18$, $p = 0.05$, $\eta^2_p = 0.06$) with more pronounced effects of OXT on negative compared to positive valence stimuli (positive: $F(1,67) = 1.68$, $p = 0.2$, $\eta^2_p = 0.02$; negative: $F(1, 67) = 9.96$, $p = 0.002$, $\eta^2_p = 0.13$). For EEI there was also a significant main effect of treatment ($F(1, 67) = 4.84$, $p = 0.03$, $\eta^2_p = 0.07$) but no treatment × valence interaction ($F(1, 67) = 2.02$, $p = 0.16$, $\eta^2_p = 0.03$). For CE, there were neither significant main effects nor interactions ($F(1,65) = 0.80$, $p = 0.37$, $\eta^2_p = 0.01$). In Exp 2, no significant main or interaction effects involving sex were observed (all ps > 0.18) arguing against sex-dependent effects of OXT on empathy.

3.2 Associations between behavior and trait autism

In Exp 1, there was a trend towards a negative correlation between the ASQ score and the total EED and EEI scores in the OXT group (ASQ Total: EED $r = -0.47$, $p = 0.09$; EEI $r = -0.37$, $p = 0.19$) but not the PLC group (EED $r = 0.319$, $p = 0.18$; EEI $r = 0.17$, $p = 0.48$). The

341 correlation significantly differed between the PLC and OXT groups for EED (Fisher's z = -
342 2.13, p = 0.03) although not for EEI (Fisher's z = -1.43, p = 0.15). In Exp 2, there was a
343 similar pattern of correlation differences between EED and EEI scores and total ASQ scores,
344 although these associations did not reach statistical significance other than for EED under
345 OXT (EED – PLC r = 0.03, p = 0.85, OXT r = -0.34, p = 0.04, Fisher's z = 1.53, p = 0.13;
346 EEI – PLC r = 0.03, p = 0.85; OXT r = -0.28, p = 0.09, Fisher's z =1.29, p = 0.20).
347 Regression plots are shown in Fig. 2 and suggest that OXT is producing its main behavioral
348 effects in participants with lower autism traits.
349

350 **3.3 Dose-dependent effects between Experiments 1 and 2 (24 IU vs 40 IU)**

351 Dose effects were explored by combining the data from male participants in Exp 1 (24 IU)
352 and Exp 2 (40 IU). To initially explore potential effects of the different experimental
353 environments (Exp 1, 24 IU, behavioral testing room; Exp 2, 40 IU, inside the MRI-scanner)
354 on empathy per se, a first analysis focused on the placebo-treated subjects. A repeated
355 ANOVA with environment (behavioral vs MRI room) as a between-subject factor and
356 valence as a within-subject factor revealed a significant environment main effect for both EE
357 facets, indicating elevated EE ratings in the MRI room (Main effects: EED: p = 0.003, F
358 (1,47) = 10.19, η^2_p = 0.18; EEI: p = 0.02, F (1,47) = 5.74, η^2_p = 0.11, both interactions with
359 valence > 0.38, non-significant, Fig. 3), but no effects on CE (Main effect: p = 0.15, F (1,47)
360 = 2.19, η^2_p = 0.05; Interaction: p = 0.9, F (1,47) = 0.02, η^2_p < 0.001). These findings suggest
361 that the MRI-environment per se increased EE, an effect possibly related to elevated levels of
362 stress during the MRI assessments, which would be in line with a previous study reporting
363 that stress-induction specifically increased EE, but not CE in the MET (Wolf, et al., 2015).
364 The environmental differences and potential interactions with OXT preclude the
365 interpretation of dose-related differences between the experiments.
366

367 **3.4 Oxytocin effects on SCR**

368 One participant was excluded from SCR analysis due to low skin impedance and thus a total
369 of 68 participants from Exp 2 were included. Analyses of the SCR data paralleled the
370 analyses of the empathy ratings, using ANOVAs with the between-subject factors treatment
371 (OXT, PLC) and sex (male, female), and the within-subject factor valence. There was a
372 marginal main effect of treatment on SCR during EED trials and significant during EEI trials
373 (EED: F (1, 66) = 3.77, p = 0.06, η^2_p = 0.05; EEI: F (1, 66) = 4.50, p = 0.04, η^2_p = 0.06), but
374 not during CE trials (F (1, 66) = 2.14, p = 0.15, η^2_p = 0.03). This was due to SCR responses
375 being increased in the OXT group during EE and EEI trials (Fig. 4). There were no
376 significant main effects of valence or treatment × valence or treatment × sex interactions for
377 CE, EE or EEI trials (all ps>0.2).
378

379 **3.5 Oxytocin effects on neural activity**

In view of the absence of sex-dependent effects in the behavioral analysis, and to increase the statistical power to determine OXT effects on the neural level, the data from male and female participants were pooled for the fMRI analyses. Four further participants were excluded from the fMRI analysis due to excessive head motion (head motion > 3 mm). The neural mechanisms underlying the behavioral effects of OXT were initially explored in the different priori ROIs (amygdala, insula and ACC) using separate repeated measures ANOVAs for the three empathy (CE, EE and EEI) conditions. Main treatment effects were only observed in the amygdala (Fig. 5A) for EED (left amygdala: $F(1,63) = 6.55$, $p = 0.01$, $\eta^2_p = 0.09$; right amygdala: $F(1,63) = 5.18$, $p = 0.03$, $\eta^2_p = 0.08$). There were no significant main effects for CE or EEI or any treatment × valence interactions for CE, EED or EEI. An exploratory whole brain analysis revealed no regions that showed significant treatment–dependent changes under CE, EED or EEI (all $P_{FDR\_corrected} > 0.05$) outside of the prior defined ROIs.

3.6 Oxytocin effects on functional connectivity

Repeated measures ANOVA models in SPM that included the between-subject factors treatment (OXT, PLC) and sex (male, female) and the within-subject factor valence (positive, negative) revealed a significant Treatment × Valence interaction effect for EED-associated functional coupling of the right amygdala with the bilateral insula and the bilateral PCC (left insula peak located at x/y/z, -33/6/-15, $P_{FWE} = 0.02$, cluster size = 143 voxels; right insula peak located at 45/18/-12, $P_{FWE} = 0.03$, cluster size = 121 voxels; left PCC peak located at -30/-33/30, $P_{FWE} = 0.003$, cluster size = 213 voxels; right PCC peak located at 21/-36/33, $P_{FWE} = 0.01$, cluster size = 149 voxels; coordinates given in MNI-space). Extraction of parameter estimates further revealed that OXT increased functional connectivity for positive valence stimuli whereas it decreased connectivity for negative valence ones (left insula: positive, $F(1,61) = 10.52$, $p = 0.002$, $\eta^2_p = 0.15$, negative, $F(1,61) = 3.86$, $p = 0.05$, $\eta^2_p = 0.06$; right insula: positive, $F(1,61) = 3.34$, $p = 0.07$, $\eta^2_p = 0.05$, negative, $F(1,61) = 5.53$, $p = 0.02$, $\eta^2_p = 0.08$; left PCC: positive, $F(1,61) = 4.34$, $p = 0.04$, $\eta^2_p = 0.07$, negative, $F(1,61) = 5.67$, $p = 0.02$, $\eta^2_p = 0.09$; right PCC: positive, $F(1,61) = 8.00$, $p = 0.006$, $\eta^2_p = 0.12$, negative, $F(1,61) = 5.48$, $p = 0.02$, $\eta^2_p = 0.08$) (Fig. 6).

3.7 Associations between neural and SCR effects of OXT and behavioral and autism trait scores

There was a significant positive correlation between EED scores and bilateral amygdala responses in the PLC group (left $r = 0.49$, $p = 0.004$; right $r = 0.35$, $p = 0.04$) which was absent in the OXT group (left $r = 0.005$, $p = 0.98$; right $r = -0.007$, $p = 0.97$). The correlation difference between the PLC and OXT groups was significant for the left (Fisher's $Z = 2.05$, $p = 0.04$) but not the right (Fisher's $Z = 1.43$, $p = 0.15$) amygdala (Fig. 5B). There was no correlation between left or right amygdala responses with total ASQ scores.

For the functional connections showing OXT effects for EED in terms of a treatment × valence interaction, coupling strength between the right amygdala and left insula during positive valence EED trials was positively correlated with the total ASQ in the PLC group

(total ASQ – r = 0.40, p = 0.02,) but not in the OXT group (total ASQ – r = -0.22, p = 0.22, Fisher's Z = 2.50, p = 0.01; Fig. 7A). OXT particularly appears to increase the strength of right amygdala functional connections with the insula in individuals with lower ASQ scores, although only for positive valence EED. The strength of link between the right amygdala and left PCC during negative valence EED trials was positively correlated with the total ASQ score in the PLC group but not the OXT, although the difference between the groups was not significant (total ASQ – PLC r = 0.36, p = 0.04; OXT r = 0.15, p = 0.41; Fisher's Z = 0.85, p = 0.39; Fig. 7B). There were no significant correlations between SCR values and ASQ scores during either EED or EEI trials (all ps > 0.41).

4. Discussion

The present study confirmed in two independent samples that intranasal OXT specifically facilitates EE but not CE as assessed by the MET paradigm in Chinese participants, thereby replicating previous findings in Caucasian participants (Hurlemann, et al., 2010). Our findings also demonstrated for the first time that the OXT-induced enhancement of EED is associated with decreased bilateral amygdala reactivity and enhanced functional coupling of the right amygdala with the insula and PCC for positive valence stimuli but attenuated coupling for negative valence stimuli. These behavioral and neural effects were not modulated by subject sex, suggesting a generalization across men and women. Finally, an exploratory analysis of associations with trait autism revealed that both behavioral and neural effects of OXT were modulated to some extent by trait autism scores.

Although many studies have reported cultural differences between Asian and Caucasian participants in the context of OXT receptor polymorphisms and empathy (Jessica, et al., 2016; Kim, et al., 2010; Luo, et al., 2015b), we did not find any substantive difference with respect to the effects of intranasal OXT on empathy processing as assessed by MET. Thus, in both cultures, OXT enhanced EE but not CE (Hurlemann, et al., 2010) for both valences, although in our second experiment we found stronger effects for negative valence stimuli. Effects of the scanning environment on emotional empathy ratings per se precluded the direct evaluation of dose-response effects between the two experiments, however OXT specifically increased EE in both suggesting that its effects generalize across 24 and 40IU doses, in line with our previous finding (Zhao, et al., 2017). In general, the magnitude of the reported behavioral OXT effect on both EED and EEI reported in Caucasian participants was however somewhat stronger compared to both 24 and 40 IU doses administered in our study, although different MET stimuli were used.

In agreement with other studies, there were no sex-differences in EE, trait empathy (Wu et al., 2012) or trait autism (Kawamura et al., 2011; Montag et al., 2017) scores in our Chinese study cohort, whereas in Caucasian participants we found that females scored significantly higher than males for both positive and negative valence stimuli (Hurlemann, et al., 2010). Thus, it is conceivable that in Caucasian females the effects of OXT in the MET might not be as pronounced as in males. The absence of an effect of OXT on CE in the MET contrasts with reports using other paradigms, notably the reading the mind in the eyes test (RMET) (Domes, et al., 2007b; Feeser, et al., 2015). However, the robustness of these findings has been questioned by another study which failed to replicate them even when taking into account both item difficulty and valence (Radke and de Bruijn, 2015). Moreover, there are also notable differences between the MET and RMET with the images in the MET including more complex natural scenes and emotions conveyed by multiple cues (face, body posture and context) whereas in the RMET emotions are only interpreted from pictures of eye regions and are also often more subtle. Thus, OXT can facilitate CE in some contexts, particularly

with cues restricted to eyes, but not in others where multiple cues are present. Additionally, and in contrast to previous studies, we measured SCR responses during trials involving the three empathy components and OXT only increased the SCR in EE and not CE trials. Thus, OXT enhancement of EE is paralleled by increased physiological arousal not only in EEI trials (where participants are asked to score how aroused they are by the stimulus picture) but also in EED trials (where they are scoring the strength of their feelings towards to protagonist in the picture).

In line with the specific, and critical contribution of the amygdala to emotional, rather than cognitive aspects of empathy (Hurlemann, et al., 2010), OXT's enhancement of EE was accompanied by a reduction of associated amygdala activity. Exploratory analyses revealed that EED scores were positively associated with the magnitude of amygdala responses during positive valence trials in the PLC group, whereas this association was absent under OXT, possibly reflecting an enhancement of amygdala processing efficiency. While some previous studies found that OXT specifically reduced amygdala responses to negative emotional stimuli (Gamer, et al., 2010; Kirsch, et al., 2005), the suppression of EED-associated amygdala activity was observed irrespective of valence. A similar pattern of OXT-induced valence-independent suppression of amygdala activity has previously been suggested to reflect reduced uncertainty of a social stimulus which in turn motivates approach behavior (Domes, et al., 2007a). In line with this interpretation, the valence-independent EED-associated amygdala suppression may reflect that OXT's approach-facilitating properties (Arakawa, et al., 2010) promote EE regardless of whether the emotions expressed by the protagonist are positive or negative, which is also in line with a rodent study reporting an overall reduction of amygdala EEG power following OXT (Sobota, et al., 2015). Other studies have found that OXT's modulation of amygdala responses dependent upon sex (Gao, et al., 2016, Luo, et al., 2017) and it is generally considered that the salience of cues as well as their context may play an important role in determining OXT's effects (Shamay-Tsoory and Abu-Akel, 2016). In the present study, neither sex nor valence influenced amygdala reactivity. This possibly reflects the fact that both salience and context are broadly similar for EE responses in the two sexes.

OXT also differentially altered the functional connectivity between the right amygdala and bilateral insula in a valence-dependent manner. In EED trials, the strength of the functional connectivity between the right amygdala and insula following OXT was significantly increased during positive valence stimuli but decreased during negative ones. A few previous studies have also reported OXT effects on functional connectivity between the insula and amygdala (Gao, et al., 2016; Hu, et al., 2015; Rilling, et al., 2012; Striepens, et al., 2012) and these two regions are key hubs of the brain salience network (Uddin, 2015). Thus, in the current context OXT may have acted to increase the salience of both positive and negative valence stimuli during EED trials by differentially altering the functional connectivity between the amygdala and insula. Rilling et al., (2012) have also previously suggested that

the stronger the functional coupling between amygdala and insula, the more able the amygdala is to elicit subjective feeling states in response to salient social stimuli. The effect of OXT on increasing functional connectivity between the right amygdala and bilateral PCC for positive valence stimuli and decreasing it for negative ones in EED trials may similarly reflect a modulatory influence on salience processing. A previous study has reported that OXT enhanced functional connectivity between amygdala and PCC during exposure to infant laughter (Riem, et al., 2012), suggesting that it increased the incentive salience of infant laughter. In our current study, the consistent patterns of functional connectivity changes elicited by OXT for positive and negative valence stimuli for amygdala functional connectivity with the insula and PCC may indicate that these three regions comprise an integrated network mediating valence-dependent OXT effects.

Both the behavioral and neural effects of OXT were modified to some extent by trait autism scores, as measured by the ASQ. In both experiments OXT tended to produce a negative correlation between EE and ASQ scores, whereas this correlation was absent in the PLC group. However, this effect of OXT only achieved significance in Exp 1, which included only male participants, and indicates that increased EE scores were more evident in individuals with lower ASQ scores. For the neural associations functional connectivity between the amygdala and insula was positively associated with total ASQ scores for positive valence EE trials in the PLC group, but this was absent in the OXT group. This indicates that OXT effects on functional connections between the right amygdala and left insula (for positive valence stimuli) were also strongest in individuals with lower ASQ scores. Thus overall, while both behavioral and neural OXT effects on EE were modified by ASQ scores, the extent to which these findings represent support for possible therapeutic use in ASD remains unclear. Indeed, a recent study on OXT enhancement of behavioral and neural responses to affective touch also reported stronger effects in individuals with lower ASQ scores (Scheele, et al., 2014).

There are several limitations which should be acknowledged in the current study. Firstly, we were unable to directly compare behavioral and neural responses during the MET task in Caucasian as well Chinese participants, so we cannot totally exclude the possibility that some cultural differences in response to OXT during empathic processing may exist. Secondly, we only investigated effects using the MET paradigm and it is possible that OXT effects on CE as well as EE would have been found using other paradigms. Lastly, the absence of sex-differences in OXT effects in the current study might have been contributed to by our Chinese male and female participants exhibiting similar EE scores, in contrast to Caucasian participants (Hurlemann, et al., 2010), and also similar ASQ scores.

In summary, in the current study we have shown that in the MET paradigm, OXT enhances EE but not CE in Chinese participants, similar to Caucasian ones, and additionally that this occurs in female as well as male participants. Furthermore, we have shown for the first time

554 that this EE effect of OXT is associated with decreased amygdala responses and differentially
555 altered functional connectivity between the amygdala and insula and PCC for positive and
556 negative valence stimuli. Finally, we have shown that both behavioral and neural effects of
557 OXT are modified to some extent by trait autism scores, although behavioral and functional
558 connectivity effects were strongest in individuals with lower scores.
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Author Contributions Statement

Geng, Hurlemann and Kendrick designed this experiment, Geng collected the data, Geng, Zhao, Zhou, Kendrick and Becker analysed the data, Geng, Zhao, Ma, Yao, Becker and Kendrick interpreted the results. Geng, Becker and Kendrick wrote the paper.

Conflicts of Interest Statement

Authors declare no conflict of interest.

References

Arakawa, H., Arakawa, K., and Deak, T. (2010). Oxytocin and vasopressin in the medial amygdala differentially modulate approach and avoidance behavior toward illness-related social odor. *Neuroscience* 171(4), 1141-1151. doi: 10.1016/j.neuroscience.2010.10.013.

Bakermans-Kranenburg, M.J., and van IJzendoorn, M.H. (2013). Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. *Translational Psychiatry* 3.

Baron-Cohen, S., and Wheelwright, S. (2004). The Empathy Quotient: An Investigation of Adults with Asperger Syndrome or High Functioning Autism, and Normal Sex Differences. *Journal of Autism and Developmental Disorders* 34(2), 163-175. doi: 10.1023/b:jadd.0000022607.19833.00.

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., and Clubley, E. (2001). The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. *J Autism Dev Disord* 31(1), 5-17.

Bartz, J.A., Zaki, J., Bolger, N., Hollander, E., Ludwig, N.N., Kolevzon, A., et al. (2010). Oxytocin Selectively Improves Empathic Accuracy. *Psychological Science* 21(10), 1426-1428. doi: 10.1177/0956797610383439.

Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., and Erbaugh, J. (1961). An inventory for measuring depression. *Arch Gen Psychiatry* 4, 561-571.

Bernhardt, B.C., and Singer, T. (2012). The Neural Basis of Empathy. *Annual Review of Neuroscience, Vol 35* 35, 1-23.

Bethlehem, R.A.I., van Honk, J., Auyeung, B., and Baron-Cohen, S. (2013). Oxytocin, brain physiology, and functional connectivity: A review of intranasal oxytocin fMRI studies. *Psychoneuroendocrinology* 38(7), 962-974.

Bos, P.A., Montoya, E.R., Hermans, E.J., Keysers, C., and van Honk, J. (2015). Oxytocin reduces neural activity in the pain circuitry when seeing pain in others. *Neuroimage* 113, 217-224.

Brett, M., Anton, J.-L., Valabregue, R., and Poline, J.-B. (2002). Region of interest analysis using the MarsBar toolbox for SPM 99. *Neuroimage* 16(2), S497.

Cao, Y., Contreras-Huerta, L.S., McFadyen, J., and Cunnington, R. (2015). Racial bias in neural response to others' pain is reduced with other-race contact. *Cortex* 70, 68-78.

Cardoso, C., Ellenbogen, M.A., Orlando, M.A., Bacon, S.L., and Joober, R. (2013). Intranasal oxytocin attenuates the cortisol response to physical stress: a dose-response study. *Psychoneuroendocrinology* 38(3), 399-407. doi: 10.1016/j.psyneuen.2012.07.013.

Chen, X., Hackett, P.D., DeMarco, A.C., Feng, C., Stair, S., Haroon, E., et al. (2016). Effects of oxytocin and vasopressin on the neural response to unreciprocated cooperation within brain regions involved in stress and anxiety in men and women. *Brain Imaging Behav* 10(2), 581-593. doi: 10.1007/s11682-015-9411-7.

613 Cox, C.L., Uddin, L.Q., Di Martino, A., Castellanos, F.X., Milham, M.P., and Kelly, C.
614 (2012). The balance between feeling and knowing: affective and cognitive empathy
615 are reflected in the brain's intrinsic functional dynamics. *Social Cognitive and*
616 *Affective Neuroscience* 7(6)**,** 727-737. doi: 10.1093/scan/nsr051.
617 De Dreu, C.K., and Kret, M.E. (2016). Oxytocin Conditions Intergroup Relations Through
618 Upregulated In-Group Empathy, Cooperation, Conformity, and Defense. *Biol*
619 *Psychiatry* 79(3)**,** 165-173. doi: 10.1016/j.biopsych.2015.03.020.
620 Decety, J. (2011). The neuroevolution of empathy. *Ann N Y Acad Sci* 1231**,** 35-45. doi:
621 10.1111/j.1749-6632.2011.06027.x.
622 Decety, J., and Jackson, P.L. (2004). The functional architecture of human empathy. *Behav*
623 *Cogn Neurosci Rev* 3(2)**,** 71-100. doi: 10.1177/1534582304267187.
624 Domes, G., Heinrichs, M., Glascher, J., Buchel, C., Braus, D.F., and Herpertz, S.C. (2007a).
625 Oxytocin attenuates amygdala responses to emotional faces regardless of valence.
626 *Biological Psychiatry* 62(10)**,** 1187-1190. doi: 10.1016/j.biopsych.2007.03.025.
627 Domes, G., Heinrichs, M., Michel, A., Berger, C., and Herpertz, S.C. (2007b). Oxytocin
628 improves "mind-reading" in humans. *Biological Psychiatry* 61(6)**,** 731-733.
629 Domes, G., Hollerbach, P., Vohs, K., Mokros, A., and Habermeyer, E. (2013). Emotional
630 Empathy and Psychopathy in Offenders: An Experimental Study. *Journal of*
631 *Personality Disorders* 27(1)**,** 67-84.
632 Dziobek, I., Rogers, K., Fleck, S., Bahnemann, M., Heekeren, H.R., Wolf, O.T., et al. (2008).
633 Dissociation of cognitive and emotional empathy in adults with asperger syndrome
634 using the multifaceted empathy test (MET). *Journal of Autism and Developmental*
635 *Disorders* 38(3)**,** 464-473. doi: 10.1007/s10803-007-0486-x.
636 Eklund, A., Nichols, T.E., and Knutsson, H. (2016). Cluster failure: Why fMRI inferences for
637 spatial extent have inflated false-positive rates. *Proc Natl Acad Sci U S A* 113(28)**,**
638 7900-7905. doi: 10.1073/pnas.1602413113.
639 Edele, A., Dziobek, I., and Keller, M. (2013). Explaining altruistic sharing in the dictator
640 game: The role of affective empathy, cognitive empathy, and justice sensitivity.
641 *Learning and Individual Differences* 24**,** 96-102. doi: 10.1016/j.lindif.2012.12.020.
642 Fan, Y., Duncan, N.W., de Greck, M., and Northoff, G. (2011). Is there a core neural network
643 in empathy? An fMRI based quantitative meta-analysis. *Neuroscience and*
644 *Biobehavioral Reviews* 35(3)**,** 903-911. doi: 10.1016/j.neubiorev.2010.10.009.
645 Feeser, M., Fan, Y., Weigand, A., Hahn, A., Gärtner, M., Böker, H., et al. (2015). Oxytocin
646 improves mentalizing–pronounced effects for individuals with attenuated ability to
647 empathize. *Psychoneuroendocrinology* 53**,** 223-232.
648 Gamer, M., Zurowski, B., and Buchel, C. (2010). Different amygdala subregions mediate
649 valence-related and attentional effects of oxytocin in humans. *Proceedings of the*
650 *National Academy of Sciences of the United States of America* 107(20)**,** 9400-9405.
651 doi: 10.1073/pnas.1000985107.
652 Gao, S., Becker, B., Luo, L., Geng, Y., Zhao, W., Yin, Y., et al. (2016). Oxytocin, the peptide
653 that bonds the sexes also divides them. *Proc Natl Acad Sci U S A* 113(27)**,** 7650-7654.

654 doi: 10.1073/pnas.1602620113.
655 Guastella, A.J., Hickie, I.B., McGuinness, M.M., Otis, M., Woods, E.A., Disinger, H.M., et al.
656 (2013). Recommendations for the standardisation of oxytocin nasal administration
657 and guidelines for its reporting in human research. *Psychoneuroendocrinology* 38(5)**,**
658 612-625. doi: 10.1016/j.psyneuen.2012.11.019.
659 Herpertz, S.C., and Bertsch, K. (2014). The social-cognitive basis of personality disorders.
660 *Curr Opin Psychiatry* 27(1)**,** 73-77. doi: 10.1097/YCO.0000000000000026.
661 Herpertz, S.C., and Bertsch, K. (2016). Oxytocin Effects on Brain Functioning in Humans.
662 *Biological Psychiatry* 79(8)**,** 631-632.
663 Hillis, A.E. (2014). Inability to empathize: brain lesions that disrupt sharing and
664 understanding another's emotions. *Brain* 137**,** 981-997.
665 Hu, J.H., Qi, S., Becker, B., Luo, L.Z., Gao, S., Gong, Q.Y., et al. (2015). Oxytocin
666 Selectively Facilitates Learning with Social Feedback and Increases Activity and
667 Functional Connectivity in Emotional Memory and Reward Processing Regions.
668 *Human Brain Mapping* 36(6)**,** 2132-2146.
669 Hurlemann, R., Patin, A., Onur, O.A., Cohen, M.X., Baumgartner, T., Metzler, S., et al.
670 (2010). Oxytocin Enhances Amygdala-Dependent, Socially Reinforced Learning and
671 Emotional Empathy in Humans. *Journal of Neuroscience* 30(14)**,** 4999-5007.
672 Jessica, L.J., Y. Sasaki; Keiko, Ishii; Mizuho, Shinada; Heejung, S. Kim (2016). gene-culture
673 interation influence of culture and oxytocin receptor gene polymorphism on loneliness.
674 *Culture and Brain*. doi: 10.1007/s40167-
675 Kawamura, Y., Liu, X., Shimada, T., Otowa, T., Kakiuchi, C., Akiyama, T., et al. (2011).
676 Association between oxytocin receptor gene polymorphisms and autistic traits as
677 measured by the Autism□Spectrum Quotient in a non□clinical Japanese population.
678 *Asia□Pacific Psychiatry* 3(3)**,** 128-136.
679 Kim, H.S., Sherman, D.K., Sasaki, J.Y., Xu, J., Chu, T.Q., Ryu, C., et al. (2010). Culture,
680 distress, and oxytocin receptor polymorphism (OXTR) interact to influence emotional
681 support seeking. *Proceedings of the National Academy of Sciences of the United*
682 *States of America* 107(36)**,** 15717-15721. doi: 10.1073/pnas.1010830107.
683 Kirsch, P., Esslinger, C., Chen, Q., Mier, D., Lis, S., Siddhanti, S., et al. (2005). Oxytocin
684 modulates neural circuitry for social cognition and fear in humans. *J Neurosci* 25(49)**,**
685 11489-11493. doi: 10.1523/JNEUROSCI.3984-05.2005.
686 Lamm, C., Decety, J., and Singer, T. (2011). Meta-analytic evidence for common and distinct
687 neural networks associated with directly experienced pain and empathy for pain.
688 *Neuroimage* 54(3)**,** 2492-2502. doi: 10.1016/j.neuroimage.2010.10.014.
689 Lamm, C., Nusbaum, H.C., Meltzoff, A.N., and Decety, J. (2007). What Are You Feeling?
690 Using Functional Magnetic Resonance Imaging to Assess the Modulation of Sensory
691 and Affective Responses during Empathy for Pain. *PLOS ONE* 2(12).
692 Lee, J., Zaki, J., Harvey, P.O., Ochsner, K., and Green, M.F. (2011). Schizophrenia patients
693 are impaired in empathic accuracy. *Psychological Medicine* 41(11)**,** 2297-2304.
694 Leigh, R., Oishi, K., Hsu, J., Lindquist, M., Gottesman, R.F., Jarso, S., et al. (2013). Acute

695 lesions that impair affective empathy. *Brain* 136**,** 2539-2549.
696 Luo, L., Becker, B., Geng, Y., Zhao, Z., Gao, S., Zhao, W., et al. (2017). Sex-dependent
697 neural effect of oxytocin during subliminal processing of negative emotion faces.
698 *Neuroimage* 162**,** 127-137. doi: 10.1016/j.neuroimage.2017.08.079.
699 Luo, S.Y., Li, B.F., Ma, Y., Zhang, W.X., Rao, Y., and Han, S.H. (2015a). Oxytocin receptor
700 gene and racial ingroup bias in empathy-related brain activity. *Neuroimage* 110**,** 22-31.
701 Luo, S.Y., Ma, Y.N., Liu, Y., Li, B.F., Wang, C.B., Shi, Z.H., et al. (2015b). Interaction
702 between oxytocin receptor polymorphism and interdependent culture values on
703 human empathy. *Social Cognitive and Affective Neuroscience* 10(9)**,** 1273-1281. doi:
704 10.1093/scan/nsv019.
705 McLaren, D.G., Ries, M.L., Xu, G., and Johnson, S.C. (2012). A generalized form of context-
706 dependent psychophysiological interactions (gPPI): a comparison to standard
707 approaches. *Neuroimage* 61(4)**,** 1277-1286. doi: 10.1016/j.neuroimage.2012.03.068.
708 Melchers, M., Li, M., Chen, Y., Zhang, W., and Montag, C. (2015). Low empathy is
709 associated with problematic use of the Internet: Empirical evidence from China and
710 Germany. *Asian journal of psychiatry* 17**,** 56-60.
711 Montag, C., Sindermann, C., Melchers, M., Jung, S., Luo, R., Becker, B., et al. (2017). A
712 functional polymorphism of the OXTR gene is associated with autistic traits in
713 Caucasian and Asian populations. *Am J Med Genet B Neuropsychiatr Genet* 174(8)**,**
714 808-816. doi: 10.1002/ajmg.b.32596.
715 Panksepp, J., and Panksepp, J.B. (2013). Toward a cross-species understanding of empathy.
716 *Trends Neurosci* 36(8)**,** 489-496. doi: 10.1016/j.tins.2013.04.009.
717 Penton-Voak, I.S., Perrett, D.I., Castles, D.L., Kobayashi, T., Burt, D.M., Murray, L.K., et al.
718 (1999). Menstrual cycle alters face preference. *Nature* 399(6738)**,** 741-742. doi:
719 10.1038/21557.
720 Pfundmair, M., Aydin, N., Frey, D., and Echterhoff, G. (2014). The interplay of oxytocin and
721 collectivistic orientation shields against negative effects of ostracism. *Journal of*
722 *Experimental Social Psychology* 55**,** 246-251.
723 Quintana, D.S., Westlye, L.T., Alnaes, D., Rustan, O.G., Kaufmann, T., Smerud, K.T., et al.
724 (2016). Low dose intranasal oxytocin delivered with Breath Powered device dampens
725 amygdala response to emotional stimuli: A peripheral effect-controlled within-
726 subjects randomized dose-response fMRI trial. *Psychoneuroendocrinology* 69**,** 180-
727 188. doi: 10.1016/j.psyneuen.2016.04.010.
728 Quintana, D.S., Westlye, L.T., Hope, S., Naerland, T., Elvsashagen, T., Dorum, E., et al.
729 (2017). Dose-dependent social-cognitive effects of intranasal oxytocin delivered with
730 novel Breath Powered device in adults with autism spectrum disorder: a randomized
731 placebo-controlled double-blind crossover trial. *Transl Psychiatry* 7(5)**,** e1136. doi:
732 10.1038/tp.2017.103.
733 Radke, S., and de Bruijn, E.R. (2015). Does oxytocin affect mind-reading? A replication
734 study. *Psychoneuroendocrinology* 60**,** 75-81. doi: 10.1016/j.psyneuen.2015.06.006.
735 Reniers, R.L.E.P., Corcoran, R., Drake, R., Shryane, N., and Vollm, B. (2010). The QCAEP:

736 A questionnaire of cognitive and affective empathy. *Journal of Personality*
737 *Assessment* 93(1)**,** 84-95.
738 Riem, M.M.E. (2012). Oxytocin Modulates Amygdala, Insula, and Inferior Frontal Gyrus
739 Responses to Infant Crying: A Randomized Controlled Trial (vol 70, pg 291, 2011).
740 *Biological Psychiatry* 71(7)**,** 660-660. doi: 10.1016/j.biopsych.2012.02.010.
741 Riem, M.M.E., Bakermans-Kranenburg, M.J., Voorthuis, A., and van IJzendoorn, M.H.
742 (2014). Oxytocin effects on mind-reading are moderated by experiences of maternal
743 love withdrawal: An fMRI study. *Progress in Neuro-Psychopharmacology &*
744 *Biological Psychiatry* 51**,** 105-112.
745 Riem, M.M.E., van IJzendoorn, M.H., Tops, M., Boksem, M.A.S., Rombouts, S.A.R.B., and
746 Bakermans-Kranenburg, M.J. (2012). No Laughing Matter: Intranasal Oxytocin
747 Administration Changes Functional Brain Connectivity during Exposure to Infant
748 Laughter. *Neuropsychopharmacology* 37(5)**,** 1257-1266. doi: 10.1038/npp.2011.313.
749 Rilling, J.K., DeMarco, A.C., Hackett, P.D., Thompson, R., Ditzen, B., Patel, R., et al. (2012).
750 Effects of intranasal oxytocin and vasopressin on cooperative behavior and associated
751 brain activity in men. *Psychoneuroendocrinology* 37(4)**,** 447-461. doi:
752 10.1016/j.psyneuen.2011.07.013.
753 Rodrigues, S.M., Saslow, L.R., Garcia, N., John, O.P., and Keltner, D. (2009). Oxytocin
754 receptor genetic variation relates to empathy and stress reactivity in humans.
755 *Proceedings of the National Academy of Sciences of the United States of America*
756 106(50)**,** 21437-21441. doi: 10.1073/pnas.0909579106.
757 Rosenfeld, A.J., Lieberman, J.A., and Jarskog, L.F. (2011). Oxytocin, Dopamine, and the
758 Amygdala: A Neurofunctional Model of Social Cognitive Deficits in Schizophrenia.
759 *Schizophrenia Bulletin* 37(5)**,** 1077-1087. doi: 10.1093/schbul/sbq015.
760 Scheele, D., Kendrick, K.M., Khouri, C., Kretzer, E., Schlapfer, T.E., Stoffel-Wagner, B., et al.
761 (2014). An oxytocin-induced facilitation of neural and emotional responses to social
762 touch correlates inversely with autism traits. *Neuropsychopharmacology* 39(9)**,** 2078-
763 2085. doi: 10.1038/npp.2014.78.
764 Schulte-Ruther, M., Markowitsch, H.J., Fink, G.R., and Piefke, M. (2007). Mirror neuron and
765 theory of mind mechanisms involved in face-to-face interactions: A functional
766 magnetic resonance imaging approach to empathy. *Journal of Cognitive Neuroscience*
767 19(8)**,** 1354-1372. doi: DOI 10.1162/jocn.2007.19.8.1354.
768 Shamay-Tsoory, S.G. (2011). The neural bases for empathy. *Neuroscientist* 17(1)**,** 18-24. doi:
769 10.1177/1073858410379268.
770 Shamay-Tsoory, S.G., and Abu-Akel, A. (2016). The Social Salience Hypothesis of Oxytocin.
771 *Biological Psychiatry* 79(3)**,** 194-202.
772 Shamay-Tsoory, S.G., Abu-Akel, A., Palgi, S., Sulieman, R., Fischer-Shofty, M., Levkovitz,
773 Y., et al. (2013). Giving peace a chance: Oxytocin increases empathy to pain in the
774 context of the Israeli-Palestinian conflict. *Psychoneuroendocrinology* 38(12)**,** 3139-
775 3144. doi: 10.1016/j.psyneuen.2013.09.015.
776 Shamay-Tsoory, S.G., Aharon-Peretz, J., and Levkovitz, Y. (2007). The neuroanatomical

basis of affective mentalizing in schizophrenia: Comparison of patients with schizophrenia and patients with localized prefrontal lesions. *Schizophrenia Research* 90(1-3)**,** 274-283. doi: 10.1016/j.schres.2006.09.020.

Shamay-Tsoory, S.G., Aharon-Peretz, J., and Perry, D. (2009). Two systems for empathy: a double dissociation between emotional and cognitive empathy in inferior frontal gyrus versus ventromedial prefrontal lesions. *Brain* 132(Pt 3)**,** 617-627. doi: 10.1093/brain/awn279.

Singer, T., and Lamm, C. (2009). The Social Neuroscience of Empathy. *Year in Cognitive Neuroscience 2009* 1156**,** 81-96.

Singer, T., Snozzi, R., Bird, G., Petrovic, P., Silani, G., Heinrichs, M., et al. (2008). Effects of Oxytocin and Prosocial Behavior on Brain Responses to Direct and Vicariously Experienced Pain. *Emotion* 8(6)**,** 781-791. doi: 10.1037/a0014195.

Slotnick, S.D. (2017). Cluster success: fMRI inferences for spatial extent have acceptable false-positive rates. *Cogn Neurosci* 8(3)**,** 150-155. doi: 10.1080/17588928.2017.1319350.

Smith, K.E., Porges, E.C., Norman, G.J., Connelly, J.J., and Decety, J. (2014). Oxytocin receptor gene variation predicts empathic concern and autonomic arousal while perceiving harm to others. *Social Neuroscience* 9(1)**,** 1-9. doi: 10.1080/17470919.2013.863223.

Sobota, R., Mihara, T., Forrest, A., Featherstone, R.E., and Siegel, S.J. (2015). Oxytocin reduces amygdala activity, increases social interactions, and reduces anxiety-like behavior irrespective of NMDAR antagonism. *Behav Neurosci* 129(4)**,** 389-398. doi: 10.1037/bne0000074.

Spengler, F.B., Schultz, J., Scheele, D., Essel, M., Maier, W., Heinrichs, M., et al. (2017). Kinetics and Dose Dependency of Intranasal Oxytocin Effects on Amygdala Reactivity. *Biol Psychiatry* 82(12)**,** 885-894. doi: 10.1016/j.biopsych.2017.04.015.

Spielberger, C.D., Gorsuch, R.L., and Lushene, R.E. (1970). Manual for the state-trait anxiety inventory.

Stern, R.M., Ray, W.J., and Quigley, K.S. (2001). *Psychophysiological recording*. Oxford University Press, USA.

Striepens, N., Kendrick, K.M., Maier, W., and Hurlemann, R. (2011). Prosocial effects of oxytocin and clinical evidence for its therapeutic potential. *Frontiers in Neuroendocrinology* 32(4)**,** 426-450. doi: 10.1016/j.yfrne.2011.07.001.

Striepens, N., Scheele, D., Kendrick, K.M., Becker, B., Schafer, L., Schwalba, K., et al. (2012). Oxytocin facilitates protective responses to aversive social stimuli in males. *Proc Natl Acad Sci U S A* 109(44)**,** 18144-18149. doi: 10.1073/pnas.1208852109.

Tully, J., Gabay, A.S., Brown, D., Murphy, D.G.M., and Blackwood, N. (2018). The effect of intranasal oxytocin on neural response to facial emotions in healthy adults as measured by functional MRI: A systematic review. *Psychiatry Res* 272**,** 17-29. doi: 10.1016/j.pscychresns.2017.11.017.

Uddin, L.Q. (2015). Salience processing and insular cortical function and dysfunction. *Nat*

Rev Neurosci 16(1)**,** 55-61. doi: 10.1038/nrn3857.

Watson, D., Clark, L.A., and Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol* 54(6)**,** 1063-1070.

Weisman, O., Pelphrey, K.A., Leckman, J.F., Feldman, R., Lu, Y., Chong, A., et al. (2015). The association between 2D:4D ratio and cognitive empathy is contingent on a common polymorphism in the oxytocin receptor gene (OXTR rs53576). *Psychoneuroendocrinology* 58**,** 23-32. doi: 10.1016/j.psyneuen.2015.04.007.

Wigton, R., Radua, J., Allen, P., Averbeck, B., Meyer-Lindenberg, A., McGuire, P., et al. (2015). Neurophysiological effects of acute oxytocin administration: systematic review and meta-analysis of placebo-controlled imaging studies. *Journal of Psychiatry & Neuroscience* 40(1)**,** E1-E22.

Wingenfeld, K., Kuehl, L.K., Janke, K., Hinkelmann, K., Dziobek, I., Fleischer, J., et al. (2014). Enhanced Emotional Empathy after Mineralocorticoid Receptor Stimulation in Women with Borderline Personality Disorder and Healthy Women. *Neuropsychopharmacology* 39(8)**,** 1799-1804. doi: 10.1038/npp.2014.36.

Wong, C.-S., and Law, K.S. (2002). The effects of leader and follower emotional intelligence on performance and attitude: An exploratory study. *The leadership quarterly* 13(3)**,** 243-274.

Wolf, O.T., Schulte, J.M., Drimalla, H., Hamacher-Dang, T.C., Knoch, D., and Dziobek, I. (2015). Enhanced emotional empathy after psychosocial stress in young healthy men. *Stress* 18(6)**,** 631-637. doi: 10.3109/10253890.2015.1078787.

Wu, N., Li, Z., and Su, Y.J. (2012). The association between oxytocin receptor gene polymorphism (OXTR) and trait empathy. *Journal of Affective Disorders* 138(3)**,** 468-472. doi: 10.1016/j.jad.2012.01.009.

Xu, L., Ma, X., Zhao, W., Luo, L., Yao, S., and Kendrick, K.M. (2015). Oxytocin enhances attentional bias for neutral and positive expression faces in individuals with higher autistic traits. *Psychoneuroendocrinology* 62**,** 352-358. doi: 10.1016/j.psyneuen.2015.09.002.

Xu, X., Yao, S., Xu, L., Geng, Y., Zhao, W., Ma, X., et al. (2017). Oxytocin biases men but not women to restore social connections with individuals who socially exclude them. *Scientific reports* 7**,** 40589.

Young, L.J., and Barrett, C.E. (2015). Neuroscience. Can oxytocin treat autism? *Science* 347(6224)**,** 825-826. doi: 10.1126/science.aaa8120.

Zhao, W., Geng, Y., Luo, L., Zhao, Z., Ma, X., Xu, L., et al. (2017). Oxytocin Increases the Perceived Value of Both Self- and Other-Owned Items and Alters Medial Prefrontal Cortex Activity in an Endowment Task. *Front Hum Neurosci* 11**,** 272. doi: 10.3389/fnhum.2017.00272.

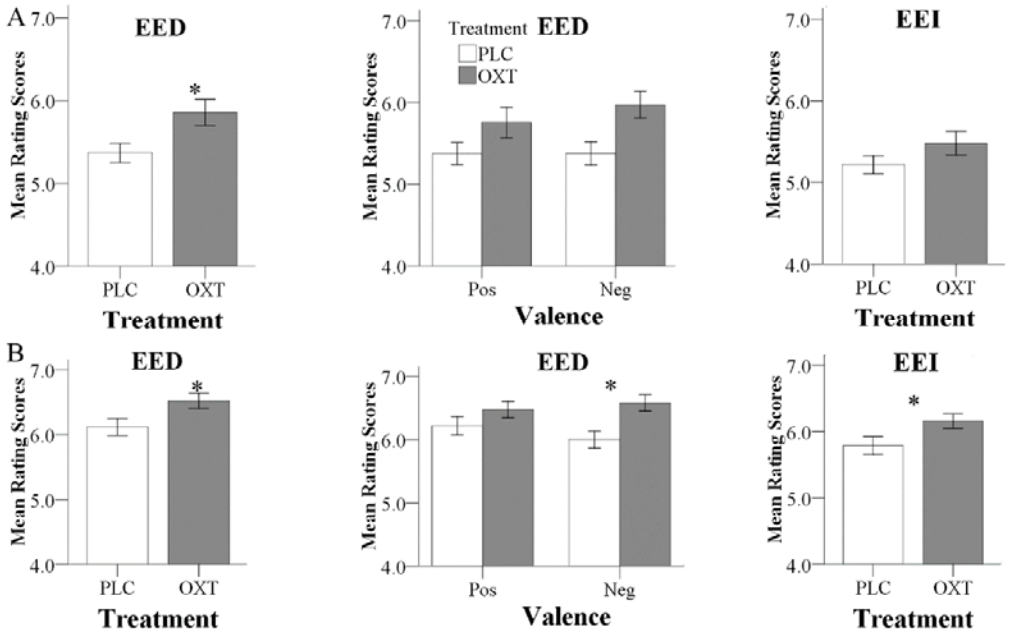
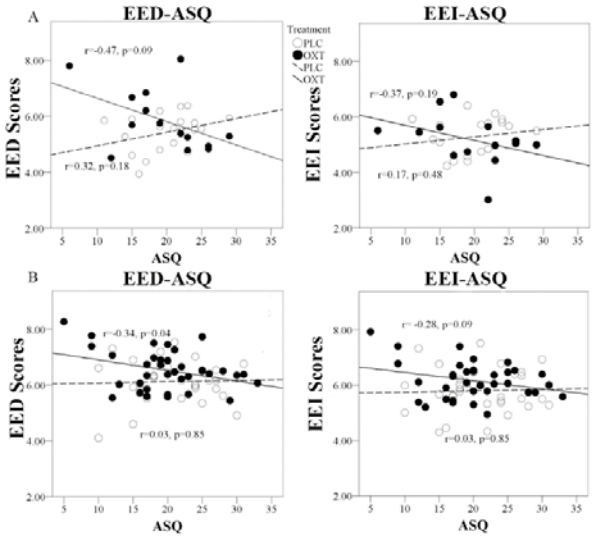
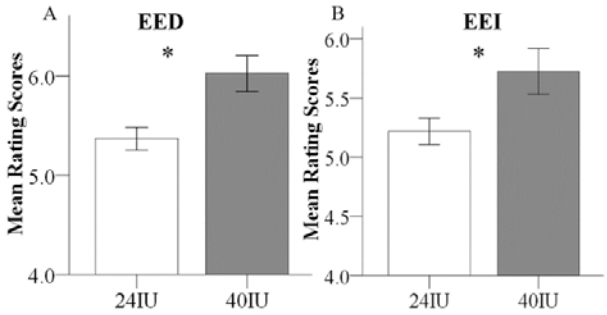


Fig. 1 Behavioral results for Exp 1 (A) and Exp 2 (B). (**A**) In Exp 1, OXT significantly increased EED ratings. To allow for a better comparison with Exp 2, effects of OXT on positive and negative EED trials, as well as on EEI are also shown; (**B**) In Exp 2, OXT increased both EED and EEI ratings; an effect of oxytocin on negative EED drove the significant treatment by valence interaction. (*P < 0.05)



871 **Fig. 2** Regression plots for ASQ score and EED and EEI ratings. Exp 1 (**A**) and Exp 2 (**B**) in
872 OXT and PLC groups.



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874 **Fig. 3 EE differences between Exp 1 and Exp 2 in the placebo treated subjects.**
875 Examining the placebo treated male subjects from the two experiments revealed that EE was
876 significantly increased in the MRI environment (Exp 2, 40 IU) compared to the behavioral
877 testing room (Exp 1, 24 IU).
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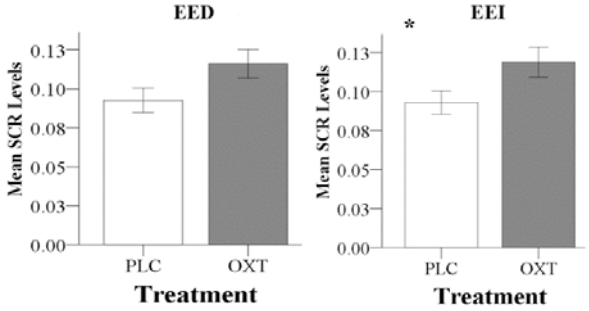

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882 **Fig. 4 OXT effect on SCR in Exp 2.** In Exp 2, OXT increased the SCR during both EED ($P = 0.06$)
883 and EEI trials. (* $P < 0.05$)
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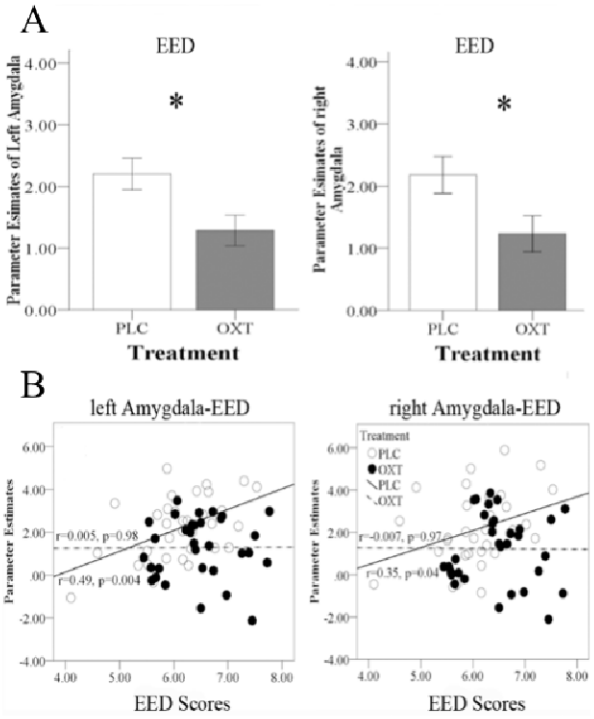

Fig. 5 Effects of OXT on bilateral amygdala responses. (**A**) Region of interest analysis results for left and right amygdala responses during EED trials; (**B**) Regression plots show correlations between left and right amygdala responses and EED scores in OXT and PLC groups. *P < 0.05

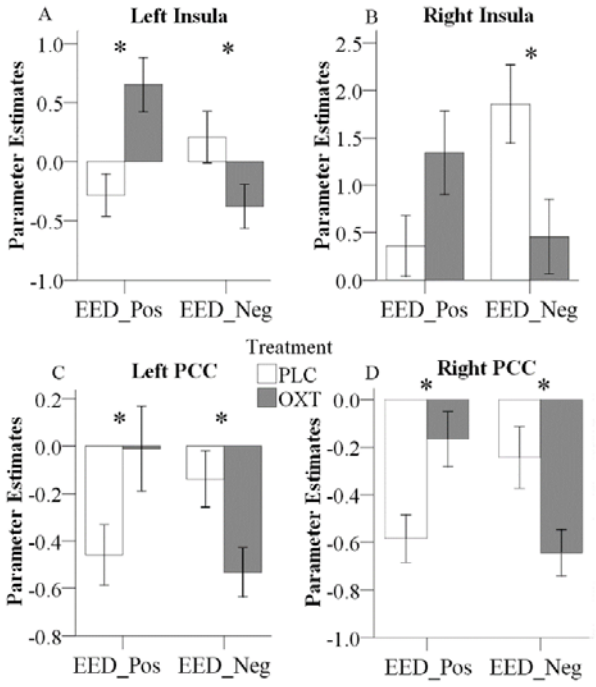

Fig. 6 Effects of OXT effect on right amygdala functional connectivity. Effects of treatment on functional connectivity of the right amygdala, indicating valence-dependent effects of OXT on the coupling of the right amygdala with the left (**A**) and right (**B**) insula, as well as the left (**C**) and right (**D**) PCC (*P < 0.05). (Pos: Positive; Neg: Negative).

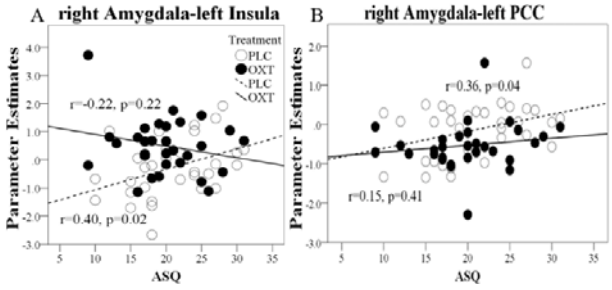

Fig. 7 Regression plots for correlations of ASQ scores with amygdala responses and functional connectivity during EE trials in OXT and PLC groups. (**A**) Correlation between right amygdala-left PCC functional connectivity during negative EED trials and total ASQ score; (**B**) Correlation of right amygdala functional connectivity with left insula cortex during positive EE trials and total ASQ score. In all cases, significant positive correlations during PLC administration are absent in the OXT group.