1   # Oxytocin enhancement of emotional empathy: generalization

2   # across cultures and effects on amygdala activity

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27   **Abstract**

28

29   Accumulating evidence suggests that the neuropeptide oxytocin can enhance empathy

30   although it is unclear which specific behavioral and neural aspects are influenced, and

31   whether the effects are modulated by culture, sex and trait autism. Based on previous findings

32   in Caucasian men, we hypothesized that a single intranasal dose of oxytocin would

33   specifically enhance emotional empathy via modulatory effects on the amygdala in an Asian

34   (Chinese) population and explored the modulatory role of sex and trait autism on the effects.

35   We first conducted a double-blind, randomized between-subject design experiment using a

36   modified version of the multifaceted empathy task (MET) to determine whether oxytocin's

37   facilitation of emotional empathy can be replicated in Chinese men (n = 60). To further

38   explore neural mechanisms behind and potential sex differences, functional MRI and skin

39   conductance measures were acquired in an independent experiment incorporating men and

40   women (n = 72). Oxytocin enhanced emotional empathy across experiments and sex, an

41   effect that was accompanied by reduced amygdala activity and increased skin conductance

42   responses. On the network level oxytocin enhanced functional coupling of the right amygdala

43   with the insula and posterior cingulate cortex for positive valence stimuli but attenuated

44   coupling for negative valence stimuli. The effect of oxytocin on amygdala functional

45   connectivity with the insula was modulated by trait autism. Overall, our findings provide

46   further support for the role of oxytocin in facilitating emotional empathy and demonstrate

47   that effects are independent of culture and sex and involve modulatory effects on the

48   amygdala and its interactions with other key empathy regions.

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50   **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

92   have been variable, with OXT effects in the RMET being either restricted to difficult items
93   (Feeser et al., 2015) or unable to be reproduced at all even when taking into account stimulus
94   difficulty and valence (Radke and de Bruijn, 2015). Other studies have also reported that
95   effects were more pronounced in individuals with poor baseline performance (Riem, et al.,
96   2014) or high trait autism (Bartz, et al., 2010). Studies that aimed specifically at determining
97   effects of OXT on EE focused on empathy for pain, an evolutionary conserved primary
98   emotional component (Decety, 2011; Panksepp and Panksepp, 2013), and found no effect on
99   pain empathy towards a partner (Singer, et al., 2008), although an enhanced pain empathic
100   response towards members of an out-group (Shamay-Tsoory, et al., 2013). In contrast,
101   another study in men using the Multifaceted Empathy Test (MET) (Dziobek, et al., 2008),
102   which assesses both CE and EE, observed that OXT specifically enhanced both EED and EEI,
103   but not CE (Hurlemann, et al., 2010). This latter study additionally demonstrated selective EE
104   deficits in amygdala lesion patients and therefore suggested that the amygdala may mediate
105   the EE enhancing effects of OXT. Although several neuroimaging studies have demonstrated
106   modulatory effects of intranasally administered OXT on the core neural components of the
107   empathy network, including the insula, ACC and amygdala and their functional interactions,
108   across different task paradigms (Bakermans-Kranenburg and van IJzendoorn, 2013; Herpertz
109   and Bertsch, 2016; Wigton, et al., 2015), to date only two studies have directly explored the
110   neural mechanisms underlying OXT's empathy enhancing effects. The first reported that
111   OXT increased activation in the superior temporal gyrus and insula during the RMET task
112   (Riem, et al., 2014), whereas the second reported reductions in left insula activity during pain
113   empathic processing (Bos, et al., 2015).

114

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

122

123   Previous studies on the empathy enhancing potential of intranasal OXT are entirely based on
124   observations in Caucasian populations. However, there is accumulating evidence from OXT-
125   administration studies either employing comparable experimental protocols in Caucasian and
126   Chinese subjects (Hurlemann, et al., 2010; Hu, et al., 2015) or examining moderating effects
127   of key cultural orientation differences such as a collectivistic orientation (Pfundmair, et al.,
128   2014; Xu, et al., 2017), suggesting culture-dependent social-cognitive effects of OXT. To this
129   end, the first experiment aimed to replicate findings in a male Caucasian sample showing that
130   OXT enhances EE but not CE (Hurlemann, et al., 2010) in a male Chinese sample. In a
131   second independent sample, male and female Chinese participants performed the same MET
132   paradigm during fMRI to determine the neural substrates involved. Analyses on the neural

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133   level focused on the insula, amygdala and ACC as core empathy regions (Bernhardt and

134   Singer, 2012; Lamm, et al., 2011; Lamm, et al., 2007; Leigh, et al., 2013; Schulte-Ruther, et

135   al., 2007; Singer and Lamm, 2009). Given that the amygdala has been specifically (Cox, et al.,

136   2012; Leigh, et al., 2013) and critically (Hurlemann, et al., 2010) associated with emotional

137   facets of empathy, we expected that OXT's enhancement of EE would be accompanied by

138   altered regional activity and network level connectivity of the amygdala. Previous studies

139   reported increased as well as decreased amygdala activity and connectivity following OXT

140   (Domes, et al., 2007a; Hu, et al., 2015; Striepens, et al., 2012; Tully, et al., 2018; Wigton, et

141   al., 2015) therefore no directed hypothesis with respect to OXT's neural effect was

142   formulated.

143

144   Based on a growing number of findings suggesting sex-dependent effects of OXT on social

145   cognition (Gao, et al., 2016; Chen, et al., 2016; Luo, et al., 2017), the second experiment

146   additionally explored whether OXT differentially affects empathic processing in men and

147   women. In line with a previous study reporting that sex does not affect OXT's modulation of

148   empathy (Shamay-Tsoory, et al., 2013), we hypothesized that OXT facilitation of EE would

149   generalize across sexes. Finally, in the context of increasing interest in the therapeutic

150   application of OXT as a potential treatment to improve social cognitive deficits, including

151   empathy, in autism spectrum disorders (Young and Barrett, 2015), and in line with previous

152   studies in healthy subjects (Bartz, et al., 2010; Scheele, et al., 2014; Xu, et al., 2015), the

153   modulatory role of trait autism (assessed by the Autism Spectrum Quotient questionnaire,

154   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.,

196   China, or placebo nasal spray with identical ingredients except oxytocin) was administered in
197   line with recommendations for the intranasal administration of OXT in humans (Guastella, et
198   al., 2013) and 45min before the start of the experimental paradigm. In Exp 1, three puffs per
199   nostril (at 30s intervals) were administered (24 IU) and in Exp 2, five puffs per nostril (40 IU).
200   Both doses are in the typical range employed by other studies (Guastella, et al., 2013;
201   Striepens, et al., 2011) with the rationale for increasing the dose in Exp 2 being to explore
202   dose-dependent behavioral effects of OXT. In a previous study we found equivalent
203   behavioral and neural effects of 24 and 48 IU OXT doses (Zhao, et al., 2017). However, it
204   should be noted that findings from some other studies investigating dose-dependent effects of
205   intranasal OXT have suggested an inverted-U-shaped dose-response curve (Cardoso, et al.,
206   2013; Quintana, et al., 2017; Quintana, et al., 2016; Spengler, et al., 2017) and thus a stronger
207   enhancement of EE with 24 IU relative to 40 IU is conceivable. In post experiment interviews,
208   participants were unable to guess better than chance whether they had received the OXT
209   nasal spray, confirming successful blinding.
210
211   **2.3 Experimental paradigm**
212   In line with a previous study on male Caucasian participants (Hurlemann, et al., 2010),
213   empathy was assessed using the MET (Dziobek et al., 2008; Domes, et al., 2013; Edele, et al.,
214   2013; Hurlemann, et al., 2010; Wingenfeld, et al., 2014), which assesses both EE and CE
215   components using ecologically valid photo-based stimuli of either negative or positive
216   valence. To account for potential confounding effects of OXT on in-group versus out-group
217   empathy (De Dreu and Kret, 2016) and a cultural empathy bias (Cao, et al., 2015; Luo, et al.,
218   2015a), the original Caucasian MET stimuli were exchanged with corresponding pictures
219   displaying Chinese protagonists. The Chinese stimuli were initially evaluated in an
220   independent sample (supplementary materials) and the final set of Chinese stimuli (30
221   positive, 30 negative valence) closely resembled the Caucasian stimuli depicting daily life
222   scenarios and conveying emotional mental states via facial expression, body posture and
223   contextual cues. To assess CE, participants were instructed to infer the emotional state of the
224   protagonist in each scene and choose the corresponding answer from 4 options listed. The 4
225   options presented similar but distinct emotional states to ensure at least 70% accuracy for
226   each stimulus picture. For EED, participants were required to rate how they felt for the
227   protagonist in the depicted scene (1-9 scale, 1 = not at all, 9 = very strong), for EEI
228   participants were required to rate how much they were aroused by the scene (1-9 scale, 1 =
229   very calm, 9 = very aroused).
230
231   The different components of empathy were presented in a mixed event/block-design.
232   Following a 3 second(s) instruction cue and a jittered inter-trial interval of 3.9s (2.3-5.9s), 10
233   stimuli per block were each presented for 3s followed by either a choice of the emotion
234   depicted for the CE condition (displayed for 4 s) or a rating scale (1-9) for the EED and EEI
235   conditions (displayed for 5 s). Six blocks were presented for each condition, resulting in a
236   total of 18 blocks. The order of blocks was counterbalanced across the experimental

237 conditions, and the fMRI experiment was divided into 6 runs, each containing one block per
238 empathy component. During fMRI (Exp 2) electrodermal activity was simultaneously
239 acquired as an index of autonomic sympathetic activity (Stern, et al., 2001) (technical details
240 on the electrodermal data acquisition are provided in the supplementary materials). To allow
241 baseline recovery of the electrodermal signal a mean inter-trial interval of 5s (4-6s) and a
242 mean interval separating stimulus presentation and behavioral response of 4s (3-5s) was
243 adopted for the fMRI experiment.
244

245 **2.4 fMRI data acquisition**
246 The fMRI data in Exp 2 were collected using a GE (General Electric Medical System,
247 Milwaukee, WI, USA) 3.0T Discovery 750 MRI scanner. fMRI time series were acquired
248 using a T2*-weighted echo planar imaging pulse sequence (repetition time, 2000 millisecond
249 (ms); echo time, 30ms; slices, 39; thickness, 3.4 millimeter (mm); gap, 0.6 mm; field of view,
250 240×240 mm^2; resolution, 64×64; flip angle, 90°). Additionally, a high resolution T1-
251 weighted structural image was acquired using a 3D spoiled gradient recalled
252 (SPGR) sequence (repetition time, 6 ms; echo time, 2ms; flip angle 9°; field of view,
253 256×256 mm^2; acquisition matrix, 256×256; thickness, 1 mm without gap) to exclude
254 participants with apparent brain pathologies and to improve normalization of the fMRI data.
255

256 **2.5 fMRI data processing**
257 fMRI data were analyzed using SPM12 (Wellcome Trust Center of Neuroimaging, University
258 College London, London, United Kingdom). The first five volumes were discarded to allow
259 T1 equilibration and images were realigned to the first image to correct for head motion.
260 Tissue segmentation, bias-correction and skull-stripping were done for the high-resolution
261 structural images. The functional time series were co-registered with the skull-stripped
262 anatomical scan and normalized to MNI space with voxel size of 3 mm^3. Normalized images
263 were then spatially smoothed using a Gaussian kernel with full-width at half-maximum
264 (FWHM) of 8 mm. On the first level, event-related responses were modelled and
265 subsequently convolved with the standard hemodynamic response function (HRF). The first
266 level design matrix included valence- (positive, negative) and empathy type- (CE, EED, EEI)
267 specific regressors for the viewing phases as main experimental conditions. In addition,
268 regressors for the cue presentation, valence- and empathy type-specific regressors for the
269 rating phases, and for viewing and rating phases of incorrect trials as well as the six
270 movement regressors were included. The experimental contrasts were next submitted to a
271 second level random effects analysis.
272

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

278   on the bilateral amygdala, insula and ACC which were structurally defined using 60%
279   probability maps from the Harvard-Oxford (sub)cortical atlas. For the regionally focused
280   analysis approach condition-specific parameter estimates were extracted from these regions
281   of interest (ROI) using the Marsbar toolbox (Brett, et al., 2002) and subjected to empathy
282   type-specific ANOVAs with the between-participant factor treatment (OXT, PLC) and the
283   within-participant factor valence (positive, negative) in SPSS (Statistical Package for the
284   Social Sciences, Version 22). P-values for the post-hoc tests of the ROI analysis were
285   Bonferroni-corrected ($P < 0.05$). An exploratory voxel-wise whole-brain analysis in SPM that
286   served to determine contributions of brain regions outside of the predefined network of
287   interest was thresholded at $P < 0.05$, corrected using the family-wise error (FWE) approach.
288   To investigate the effects of OXT on the network level, a generalized form of
289   psychophysiological interaction analysis (gPPI; http://brainmap.wisc.edu/PPI; McLaren, et al.,
290   2012) was conducted using regions showing significant OXT effects in the BOLD level
291   analysis as seeds and implementing an empathy-type specific voxel-wise whole-brain
292   ANOVA approach including the between-participant factor treatment (OXT, PLC) and the
293   within-participant factor valence (positive, negative) thresholded at $P < 0.05$, FWE-corrected
294   at the cluster level. In line with recent recommendations for the control of false-positives in
295   cluster-based correction approaches an initial cluster forming threshold of $P < 0.001$ was
296   applied to data with a resolution of $3 \times 3 \times 3$ mm (Eklund, et al., 2016; Slotnick, 2017).
297   Parameter estimates were extracted from the significant regions to disentangle the specific
298   effects in post-hoc comparisons. Finally, associations between neural indices and trait autism
299   (ASQ scores) were conducted in SPSS using Pearson correlation analysis.
300

301 ## 3. Results

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

307

308 ### 3.1 Behavioral results
309 Based on previous conceptualizations of empathy, proposing that CE is a prerequisite for EE
310 (Decety and Jackson, 2004; Hillis, 2014), for EED and EEI measures only trials for which
311 subjects successfully recognized the emotions displayed by the protagonist were analyzed
312 (for a similar approach see (Luo, et al., 2015a)). To this end, correctly recognized trials were
313 initially determined based on the CE performance, with only correct trials subsequently
314 entering the analyses for the EED and EEI facets.

315

316 There were no significant differences in CE accuracy between the two treatment groups in
317 both experiments (Exp 1, OXT, $77.53\% \pm 6.02\%$, PLC, $78.94\% \pm 5.78\%$, $T_{57} = 0.92$, $p = 0.36$;
318 Exp 2, OXT, $80.10\% \pm 6.18\%$, PLC, $81.57\% \pm 5.83\%$, $T_{67} = 1.02$, $p = 0.31$). In Exp 1, there
319 was a main effect of treatment ($F(1, 57) = 6.46$, $p = 0.01$, $\eta^2_p = 0.10$) for EED indicating that
320 OXT generally enhanced EED (Fig. 1A). There was no significant treatment $\times$ valence
321 interaction ($F(1, 57) = 0.96$, $p = 0.33$, $\eta^2_p = 0.02$). Analysis of EEI did not reveal a treatment
322 main effect ($F(1, 57) = 2.19$, $p = 0.14$, $\eta^2_p = 0.04$) or valence $\times$ treatment interaction effect (F
323 $(1, 57) = 3.16$, $p = 0.08$, $\eta^2_p = 0.05$).

324

325 Consistent with the findings for EED in Exp 1, Exp 2 also yielded a significant main effect of
326 treatment on EED ($F(1, 67) = 5.81$, $p = 0.02$, $\eta^2_p = 0.08$) with higher ratings following OXT
327 compared to PLC (Fig. 1B). There was also a significant valence $\times$ treatment interaction ($F(1,$
328 $67) = 4.18$, $p = 0.05$, $\eta^2_p = 0.06$) with more pronounced effects of OXT on negative compared
329 to positive valence stimuli (positive: $F(1,67) = 1.68$, $p = 0.2$, $\eta^2_p = 0.02$; negative: $F(1, 67) =$
330 9.96, $p = 0.002$, $\eta^2_p = 0.13$). For EEI there was also a significant main effect of treatment (F
331 $(1, 67) = 4.84$, $p = 0.03$, $\eta^2_p = 0.07$) but no treatment $\times$ valence interaction ($F(1, 67) = 2.02$, p
332 $= 0.16$, $\eta^2_p = 0.03$). For CE, there were neither significant main effects nor interactions (F
333 $(1,65) = 0.80$, $p = 0.37$, $\eta^2_p = 0.01$). In Exp 2, no significant main or interaction effects
334 involving sex were observed (all ps > 0.18) arguing against sex-dependent effects of OXT on
335 empathy.

336

337 ### 3.2 Associations between behavior and trait autism
338 In Exp 1, there was a trend towards a negative correlation between the ASQ score and the
339 total EED and EEI scores in the OXT group (ASQ Total: EED $r = -0.47$, $p = 0.09$; EEI $r = -$
340 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 $(1,47) = 10.19$, $\eta^2_p = 0.18$; EEI: p = 0.02, F $(1,47) = 5.74$, $\eta^2_p = 0.11$, 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 $= 2.19$, $\eta^2_p = 0.05$; Interaction: p = 0.9, F $(1,47) = 0.02$, $\eta^2_p < 0.001$). 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, $\eta^2_p = 0.05$; EEI: F $(1, 66) = 4.50$, p = 0.04, $\eta^2_p = 0.06$), but

374 not during CE trials (F $(1, 66) = 2.14$, p = 0.15, $\eta^2_p = 0.03$). 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**

380   In view of the absence of sex-dependent effects in the behavioral analysis, and to increase the
381   statistical power to determine OXT effects on the neural level, the data from male and female
382   participants were pooled for the fMRI analyses. Four further participants were excluded from
383   the fMRI analysis due to excessive head motion (head motion > 3 mm). The neural
384   mechanisms underlying the behavioral effects of OXT were initially explored in the different
385   priori ROIs (amygdala, insula and ACC) using separate repeated measures ANOVAs for the
386   three empathy (CE, EE and EEI) conditions. Main treatment effects were only observed in
387   the amygdala (Fig. 5A) for EED (left amygdala: $F_{(1,63)} = 6.55$, $p = 0.01$, $\eta^2_p = 0.09$; right
388   amygdala: $F_{(1,63)} = 5.18$, $p = 0.03$, $\eta^2_p = 0.08$). There were no significant main effects for
389   CE or EEI or any treatment $\times$ valence interactions for CE, EED or EEI. An exploratory whole
390   brain analysis revealed no regions that showed significant treatment–dependent changes
391   under CE, EED or EEI (all $P_{FDR\_corrected} > 0.05$) outside of the prior defined ROIs.
392
393   **3.6 Oxytocin effects on functional connectivity**
394   Repeated measures ANOVA models in SPM that included the between-subject factors
395   treatment (OXT, PLC) and sex (male, female) and the within-subject factor valence (positive,
396   negative) revealed a significant Treatment $\times$ Valence interaction effect for EED-associated
397   functional coupling of the right amygdala with the bilateral insula and the bilateral PCC (left
398   insula peak located at x/y/z, -33/6/-15, $P_{FWE} = 0.02$, cluster size = 143 voxels; right insula
399   peak located at 45/18/-12, $P_{FWE} = 0.03$, cluster size = 121 voxels; left PCC peak located at -
400   30/-33/30, $P_{FWE} = 0.003$, cluster size = 213 voxels; right PCC peak located at 21/-36/33, P_{FWE}
401   = 0.01, cluster size = 149 voxels; coordinates given in MNI-space). Extraction of parameter
402   estimates further revealed that OXT increased functional connectivity for positive valence
403   stimuli whereas it decreased connectivity for negative valence ones (left insula: positive, F
404    $(1,61) = 10.52$, $p = 0.002$, $\eta^2_p = 0.15$, negative, $F_{(1,61)} = 3.86$, $p = 0.05$, $\eta^2_p = 0.06$; right
405   insula: positive, $F_{(1,61)} = 3.34$, $p = 0.07$, $\eta^2_p = 0.05$, negative, $F_{(1,61)} = 5.53$, $p = 0.02$, η^2_p
406   = 0.08; left PCC: positive, $F_{(1,61)} = 4.34$, $p = 0.04$, $\eta^2_p = 0.07$, negative, $F_{(1,61)} = 5.67$, $p =$
407   0.02, $\eta^2_p = 0.09$; right PCC: positive, $F_{(1,61)} = 8.00$, $p = 0.006$, $\eta^2_p = 0.12$, negative, $F_{(1,61)}$
408   = 5.48, $p = 0.02$, $\eta^2_p = 0.08$) (Fig. 6).
409
410   **3.7 Associations between neural and SCR effects of OXT and behavioral and autism**
411   **trait scores**
412   There was a significant positive correlation between EED scores and bilateral amygdala
413   responses in the PLC group (left r = 0.49, p = 0.004; right r = 0.35, p = 0.04) which was
414   absent in the OXT group (left r = 0.005, p = 0.98; right r = -0.007, p = 0.97). The correlation
415   difference between the PLC and OXT groups was significant for the left (Fisher's Z = 2.05, p
416   = 0.04) but not the right (Fisher's Z = 1.43, p = 0.15) amygdala (Fig. 5B). There was no
417   correlation between left or right amygdala responses with total ASQ scores.
418   For the functional connections showing OXT effects for EED in terms of a treatment $\times$
419   valence interaction, coupling strength between the right amygdala and left insula during
420   positive valence EED trials was positively correlated with the total ASQ in the PLC group

421   (total ASQ – r = 0.40, p = 0.02,) but not in the OXT group (total ASQ – r = -0.22, p = 0.22,
422   Fisher's Z = 2.50, p = 0.01; Fig. 7A). OXT particularly appears to increase the strength of
423   right amygdala functional connections with the insula in individuals with lower ASQ scores,
424   although only for positive valence EED. The strength of link between the right amygdala and
425   left PCC during negative valence EED trials was positively correlated with the total ASQ
426   score in the PLC group but not the OXT, although the difference between the groups was not
427   significant (total ASQ – PLC r = 0.36, p = 0.04; OXT r = 0.15, p = 0.41; Fisher's Z = 0.85, p
428   = 0.39; Fig. 7B). There were no significant correlations between SCR values and ASQ scores
429   during either EED or EEI trials (all ps > 0.41).
430

4. Discussion

431
432
433   The present study confirmed in two independent samples that intranasal OXT specifically
434   facilitates EE but not CE as assessed by the MET paradigm in Chinese participants, thereby
435   replicating previous findings in Caucasian participants (Hurlemann, et al., 2010). Our
436   findings also demonstrated for the first time that the OXT-induced enhancement of EED is
437   associated with decreased bilateral amygdala reactivity and enhanced functional coupling of
438   the right amygdala with the insula and PCC for positive valence stimuli but attenuated
439   coupling for negative valence stimuli. These behavioral and neural effects were not
440   modulated by subject sex, suggesting a generalization across men and women. Finally, an
441   exploratory analysis of associations with trait autism revealed that both behavioral and neural
442   effects of OXT were modulated to some extent by trait autism scores.
443
444   Although many studies have reported cultural differences between Asian and Caucasian
445   participants in the context of OXT receptor polymorphisms and empathy (Jessica, et al., 2016;
446   Kim, et al., 2010; Luo, et al., 2015b), we did not find any substantive difference with respect
447   to the effects of intranasal OXT on empathy processing as assessed by MET. Thus, in both
448   cultures, OXT enhanced EE but not CE (Hurlemann, et al., 2010) for both valences, although
449   in our second experiment we found stronger effects for negative valence stimuli. Effects of
450   the scanning environment on emotional empathy ratings per se precluded the direct
451   evaluation of dose-response effects between the two experiments, however OXT specifically
452   increased EE in both suggesting that its effects generalize across 24 and 40IU doses, in line
453   with our previous finding (Zhao, et al., 2017). In general, the magnitude of the reported
454   behavioral OXT effect on both EED and EEI reported in Caucasian participants was however
455   somewhat stronger compared to both 24 and 40 IU doses administered in our study, although
456   different MET stimuli were used.
457
458   In agreement with other studies, there were no sex-differences in EE, trait empathy (Wu et al.,
459   2012) or trait autism (Kawamura et al., 2011; Montag et al., 2017) scores in our Chinese
460   study cohort, whereas in Caucasian participants we found that females scored significantly
461   higher than males for both positive and negative valence stimuli (Hurlemann, et al., 2010).
462   Thus, it is conceivable that in Caucasian females the effects of OXT in the MET might not be
463   as pronounced as in males. The absence of an effect of OXT on CE in the MET contrasts
464   with reports using other paradigms, notably the reading the mind in the eyes test (RMET)
465   (Domes, et al., 2007b; Feeser, et al., 2015). However, the robustness of these findings has
466   been questioned by another study which failed to replicate them even when taking into
467   account both item difficulty and valence (Radke and de Bruijn, 2015). Moreover, there are
468   also notable differences between the MET and RMET with the images in the MET including
469   more complex natural scenes and emotions conveyed by multiple cues (face, body posture
470   and context) whereas in the RMET emotions are only interpreted from pictures of eye regions
471   and are also often more subtle. Thus, OXT can facilitate CE in some contexts, particularly

472   with cues restricted to eyes, but not in others where multiple cues are present. Additionally,

473   and in contrast to previous studies, we measured SCR responses during trials involving the

474   three empathy components and OXT only increased the SCR in EE and not CE trials. Thus,

475   OXT enhancement of EE is paralleled by increased physiological arousal not only in EEI

476   trials (where participants are asked to score how aroused they are by the stimulus picture) but

477   also in EED trials (where they are scoring the strength of their feelings towards to protagonist

478   in the picture).

479

480   In line with the specific, and critical contribution of the amygdala to emotional, rather than

481   cognitive aspects of empathy (Hurlemann, et al., 2010), OXT's enhancement of EE was

482   accompanied by a reduction of associated amygdala activity. Exploratory analyses revealed

483   that EED scores were positively associated with the magnitude of amygdala responses during

484   positive valence trials in the PLC group, whereas this association was absent under OXT,

485   possibly reflecting an enhancement of amygdala processing efficiency. While some previous

486   studies found that OXT specifically reduced amygdala responses to negative emotional

487   stimuli (Gamer, et al., 2010; Kirsch, et al., 2005), the suppression of EED-associated

488   amygdala activity was observed irrespective of valence. A similar pattern of OXT-induced

489   valence-independent suppression of amygdala activity has previously been suggested to

490   reflect reduced uncertainty of a social stimulus which in turn motivates approach behavior

491   (Domes, et al., 2007a). In line with this interpretation, the valence-independent EED-

492   associated amygdala suppression may reflect that OXT's approach-facilitating properties

493   (Arakawa, et al., 2010) promote EE regardless of whether the emotions expressed by the

494   protagonist are positive or negative, which is also in line with a rodent study reporting an

495   overall reduction of amygdala EEG power following OXT (Sobota, et al., 2015). Other

496   studies have found that OXT's modulation of amygdala responses dependent upon sex (Gao,

497   et al., 2016, Luo, et al., 2017) and it is generally considered that the salience of cues as well

498   as their context may play an important role in determining OXT's effects (Shamay-Tsoory

499   and Abu-Akel, 2016). In the present study, neither sex nor valence influenced amygdala

500   reactivity. This possibly reflects the fact that both salience and context are broadly similar for

501   EE responses in the two sexes.

502

503   OXT also differentially altered the functional connectivity between the right amygdala and

504   bilateral insula in a valence-dependent manner. In EED trials, the strength of the functional

505   connectivity between the right amygdala and insula following OXT was significantly

506   increased during positive valence stimuli but decreased during negative ones. A few previous

507   studies have also reported OXT effects on functional connectivity between the insula and

508   amygdala (Gao, et al., 2016; Hu, et al., 2015; Rilling, et al., 2012; Striepens, et al., 2012) and

509   these two regions are key hubs of the brain salience network (Uddin, 2015). Thus, in the

510   current context OXT may have acted to increase the salience of both positive and negative

511   valence stimuli during EED trials by differentially altering the functional connectivity

512   between the amygdala and insula. Rilling et al., (2012) have also previously suggested that

513 the stronger the functional coupling between amygdala and insula, the more able the

514 amygdala is to elicit subjective feeling states in response to salient social stimuli.

515 The effect of OXT on increasing functional connectivity between the right amygdala and

516 bilateral PCC for positive valence stimuli and decreasing it for negative ones in EED trials

517 may similarly reflect a modulatory influence on salience processing. A previous study has

518 reported that OXT enhanced functional connectivity between amygdala and PCC during

519 exposure to infant laughter (Riem, et al., 2012), suggesting that it increased the incentive

520 salience of infant laughter. In our current study, the consistent patterns of functional

521 connectivity changes elicited by OXT for positive and negative valence stimuli for amygdala

522 functional connectivity with the insula and PCC may indicate that these three regions

523 comprise an integrated network mediating valence-dependent OXT effects.

524

525 Both the behavioral and neural effects of OXT were modified to some extent by trait autism

526 scores, as measured by the ASQ. In both experiments OXT tended to produce a negative

527 correlation between EE and ASQ scores, whereas this correlation was absent in the PLC

528 group. However, this effect of OXT only achieved significance in Exp 1, which included only

529 male participants, and indicates that increased EE scores were more evident in individuals

530 with lower ASQ scores. For the neural associations functional connectivity between the

531 amygdala and insula was positively associated with total ASQ scores for positive valence EE

532 trials in the PLC group, but this was absent in the OXT group. This indicates that OXT

533 effects on functional connections between the right amygdala and left insula (for positive

534 valence stimuli) were also strongest in individuals with lower ASQ scores. Thus overall,

535 while both behavioral and neural OXT effects on EE were modified by ASQ scores, the

536 extent to which these findings represent support for possible therapeutic use in ASD remains

537 unclear. Indeed, a recent study on OXT enhancement of behavioral and neural responses to

538 affective touch also reported stronger effects in individuals with lower ASQ scores (Scheele,

539 et al., 2014).

540

541 There are several limitations which should be acknowledged in the current study. Firstly, we

542 were unable to directly compare behavioral and neural responses during the MET task in

543 Caucasian as well Chinese participants, so we cannot totally exclude the possibility that some

544 cultural differences in response to OXT during empathic processing may exist. Secondly, we

545 only investigated effects using the MET paradigm and it is possible that OXT effects on CE

546 as well as EE would have been found using other paradigms. Lastly, the absence of sex-

547 differences in OXT effects in the current study might have been contributed to by our

548 Chinese male and female participants exhibiting similar EE scores, in contrast to Caucasian

549 participants (Hurlemann, et al., 2010), and also similar ASQ scores.

550

551 In summary, in the current study we have shown that in the MET paradigm, OXT enhances

552 EE but not CE in Chinese participants, similar to Caucasian ones, and additionally that this

553 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.

559

560

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564

565 **Author Contributions Statement**

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

569

570 **Conflicts of Interest Statement**

571 Authors declare no conflict of interest.

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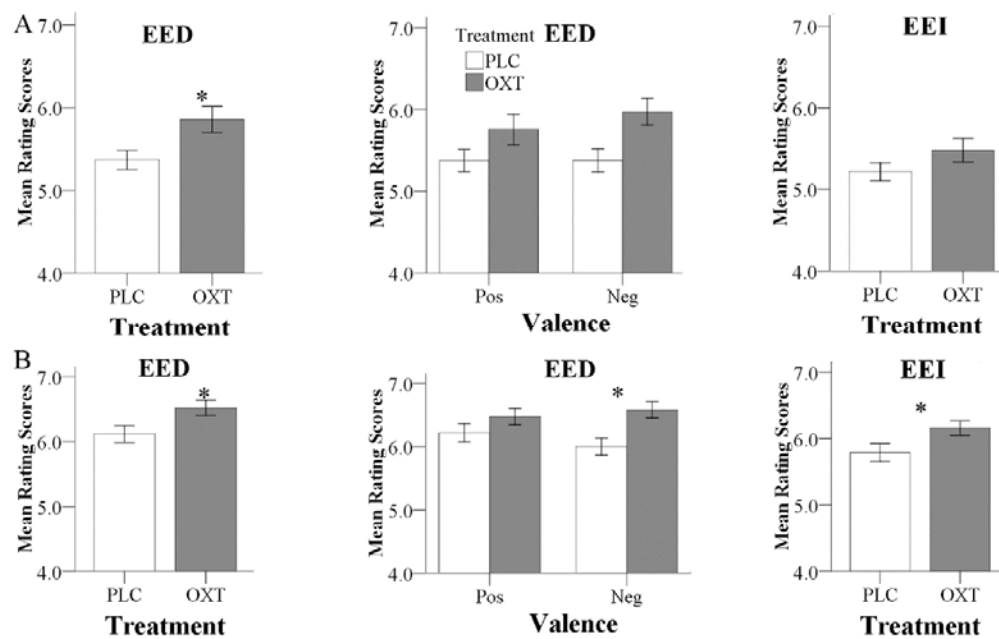
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863 **Fig. 1 Behavioral results for Exp 1 (A) and Exp 2 (B). (A)** In Exp 1, OXT significantly
864 increased EED ratings. To allow for a better comparison with Exp 2, effects of OXT on
865 positive and negative EED trials, as well as on EEI are also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

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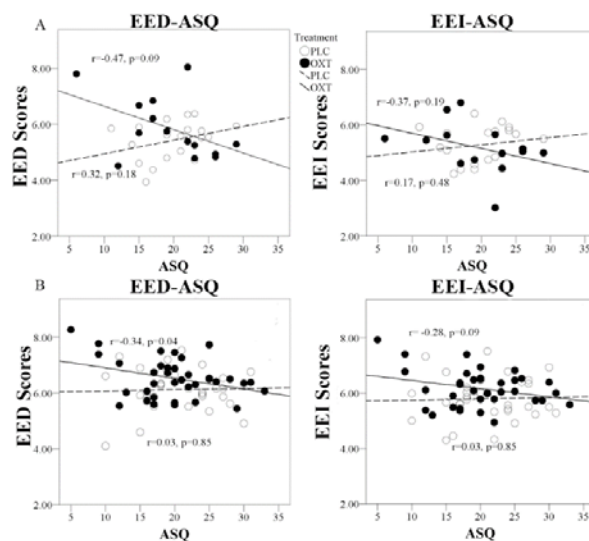
871   **Fig. 2** Regression plots for ASQ score and EED and EEI ratings. Exp 1 (**A**) and Exp 2 (**B**) in
872   OXT and PLC groups.
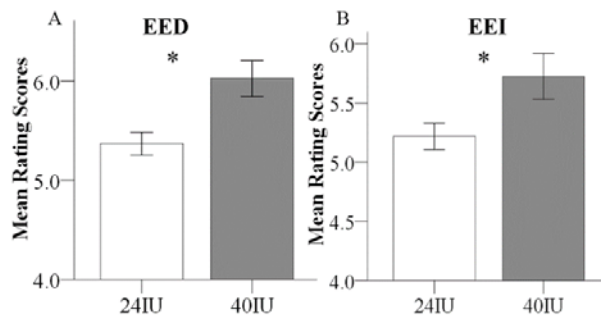
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874   **Fig. 3 EE differences between Exp 1 and Exp 2 in the placebo treated subjects.**
875   Examining the placebo treated male subjects from the two experiments revealed that EE was
876   significantly increased in the MRI environment (Exp 2, 40 IU) compared to the behavioral
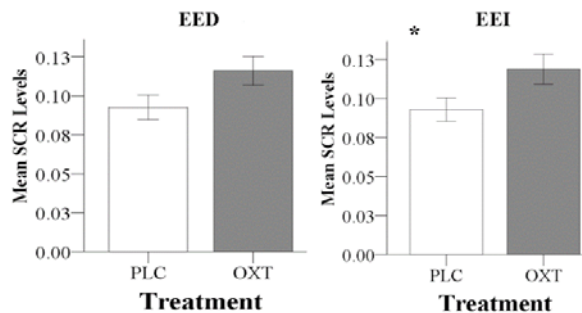877   testing room (Exp 1, 24 IU).
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882   **Fig. 4 OXT effect on SCR in Exp 2.** In Exp 2, OXT increased the SCR during both EED (P
883   = 0.06) and EEI trials. (* P < 0.05)
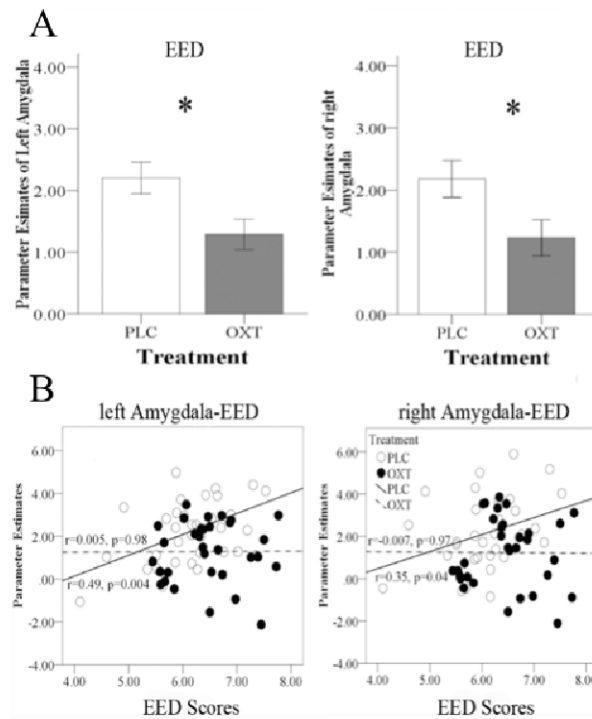884
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893 **Fig. 5** Effects of OXT on bilateral amygdala responses. (**A**) Region of interest analysis results
894 for left and right amygdala responses during EED trials; (**B**) Regression plots show
895 correlations between left and right amygdala responses and EED scores in OXT and PLC
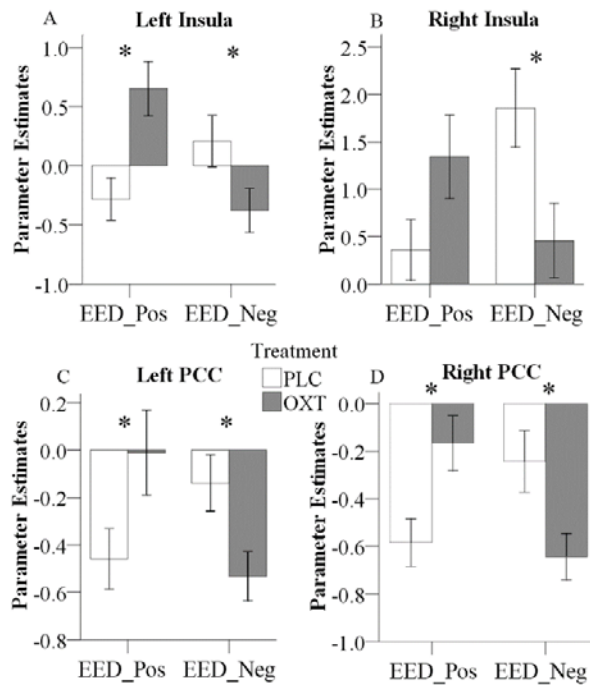896 groups. *P < 0.05
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Fig. 6 Effects of OXT effect on right amygdala functional connectivity. Effects of treatment on functional connectivity of the right amygdala, indicating valence-dependent effects of OXT on the coupling of the right amygdala with the left (**A**) and right (**B**) insula, as well as the left (**C**) and right (**D**) PCC (*$P < 0.05$). (Pos: Positive; Neg: Negative).
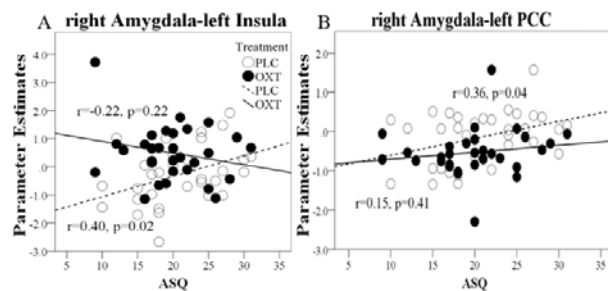

Fig. 7 Regression plots for correlations of ASQ scores with amygdala responses and functional connectivity during EE trials in OXT and PLC groups. (**A**) Correlation between right amygdala-left PCC functional connectivity during negative EED trials and total ASQ score; (**B**) Correlation of right amygdala 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.