Oxytocin effects on brain and behavior are influenced by dose-frequency and receptor genotype: therapeutic implications

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Abstract

There is considerable interest in therapeutic application of intranasal oxytocin in psychiatric disorders but while clinical trials typically use daily dosing protocols it has not been established whether this is optimal or if there are moderating influences of oxytocin receptor genotype. In a randomized, placebo-controlled pre-registered trial on 138 adult male subjects we investigated effects of single and repeated (24IU daily versus alternate days for 5 days) doses of oxytocin on two neural biomarkers (attenuated amygdala fear reactivity and increased intrinsic amygdala-prefrontal functional connectivity) and modulating effects of oxytocin receptor polymorphisms rs53576 and rs2254298 strongly associated with autism. Results confirmed that after a single dose, amygdala responses to fear faces were reduced and its resting state connectivity with medial frontal cortex increased. Suppression of amygdala responses to fear faces was restricted to AA homozygotes of rs53576 and A+ carriers of rs2254298. Importantly, amygdala responses to fear faces were absent after daily oxytocin treatment but maintained after treatment every other day and included reduced ratings of emotional intensity. In contrast, effects on intrinsic amygdala-prefrontal coupling remained constant. Overall, results suggest infrequent rather than daily doses of oxytocin may be more effective therapeutically and that its actions on amygdala responses are strongly genotype-dependent.

Key words: amygdala; dose-frequency; fearful faces; oxytocin; oxytocin receptor gene; resting-state; task-related

50 **Significance statement**

51 Despite increasing interest in therapeutic use intranasal oxytocin no studies have
52 established whether the twice daily treatment protocol used is optimal, or if effects are
53 influenced by receptor genotype. Here we report that giving intranasal oxytocin daily
54 to healthy subjects blunts its ability to reduce amygdala responses to fearful faces
55 after only 5 days, whereas administering it every other day does not and additionally
56 reduces emotional intensity ratings. The amygdala effects of oxytocin are also only
57 present in specific receptor genotypes. While resting-state effects of oxytocin were
58 not influenced by repeated doses or genotype there was no advantage of daily
59 administration. Overall, our results demonstrate that infrequent doses of oxytocin
60 given to specific genotypes may be most efficacious therapeutically.

61

62 Intranasal oxytocin (OT) has been proposed as a potential therapy for social
63 dysfunction in psychiatric disorders particularly in relation to autism spectrum
64 disorder, schizophrenia and social anxiety (1-3). To date the functional effects of
65 intranasal OT administration have been based primarily on a single dose and there is
66 evidence that a 24IU dose may be optimal (4). However, initial clinical trials
67 reporting some therapeutic efficacy have typically used twice-daily treatment
68 protocols lasting a number of weeks (1, 5-8), even though no studies have
69 investigated if this is an optimal strategy. This is an important issue since clinical
70 trials to date have reported relatively modest or inconsistent effects of chronic daily
71 intranasal OT treatment and there is evidence from animal studies for rapid
72 desensitization of OT receptors in some brain regions (9), most notably in the
73 amygdala which mediates many of OTs functional effects on social cognition and
74 anxiety (1). Rodent models have reported that in contrast to single doses, chronic
75 administration of OT can actually produce social impairment (10-12) and reduced
76 receptor expression in the amygdala and nucleus accumbens (12). Furthermore,
77 chronic versus single administration of OT can produce differential neurochemical
78 changes in both the rodent and human frontal cortex (13).

79 Different OT receptor (OXTR) genotypes have been associated with social
80 behaviors and may also influence sensitivity of behavioral and neural responses to
81 intranasal OT. In particular, OXTR polymorphisms rs53576 and rs2254298 have been
82 associated with autism (14), deficits in social and emotional processing including
83 empathy (15-17) and individual variations in behavioral and neural responses to

84 intranasal OT (18, 19). Variants of rs2254298 may also be a more general biomarker
85 for social dysfunction across a number of psychiatric disorders (20).

86 While single doses of OT have been reported to produce numerous functional
87 effects two have emerged as promising candidate neural biomarkers: reduced
88 amygdala responses to fear faces and increased resting state functional connectivity
89 between the amygdala and medial prefrontal cortex (1, 4). Both biomarkers may be
90 particularly relevant in the context of anxiety in terms of reduced responses to social
91 threat and enhanced top-down control of emotion, although OT effects on the
92 amygdala are important for a range of social cognition functions (1-4). In the current
93 pre-registered neuroimaging study we therefore conducted a double-blind,
94 randomized between subject placebo (PLC)-controlled design experiment including
95 138 adult male subjects to investigate the effects of acute (day 1, single dose) and
96 repeated (5 days – group 1: once daily (OT_5), group 2 every other day with PLC
97 treatment on the intervening days (OT_3) versus group 3: PLC every day) 24IU
98 intranasal dose regimes on these two biomarkers and valence, arousal and intensity
99 ratings of fear faces. Additionally, we assessed the influence of OTR rs53576 and
100 rs2254298 polymorphisms on the acute and repeated dose effects of intranasal OT and
101 associations with trait autism as assessed by the Autism Spectrum Quotient (AQ) (21)
102 (see **SI Fig. S1** for protocol).

103 **Acute effects of OT on neural responses to emotional faces**

During fMRI subjects were shown emotional faces (fear, angry, happy, neutral; balanced male-female stimuli) in a randomized event-related design. To ensure attentive processing subjects were required to identify the gender of each face after it was presented (see **SI Fig. S2**). In accordance with the primary outcome measure of the trial analyses focused on the neural responses to fearful faces, however no significant effects of OT were found for happy or angry faces (for a similar strategy see also (4)). Comparison of single-dose OT- and PLC-treated subjects (1st day) revealed significantly decreased right amygdala reactivity towards fearful faces on the whole brain level (k = 78, $p_{FWE} = 0.043$, x=27, y=-4, z=-13) (**Fig.1A**). Cytoarchitectonic probabilistic localization (Anatomy toolbox V1.8 (22)) mapped the peak coordinate with >80% probability to the basolateral sub-region of the amygdala. In addition, OT also decreased fear-reactivity in the right superior frontal gyrus and bilateral primary visual cortex (see **SI Table S1**). Importantly, control analyses did not reveal significant differences between the OT_3 and OT_5 groups on the 1st day.

Primary outcome measures: effects of repeated doses on OT-evoked changes

Mixed ANOVAs with treatment (PLC, OT_3, OT_5) and time point (1st day, acute effects; 5th day, chronic effects) as factors and right amygdala fear reactivity as dependent variable revealed a significant main effect of treatment ($F_{2, 135} = 7.854$, p = 0.001, $\eta^2_p = 0.104$) and a treatment x time point interaction ($F_{2, 135} = 5.737$, p = 0.004, $\eta^2_p = 0.078$). Post-hoc Bonferroni corrected comparisons between groups on the 5th day revealed that the OT_3 group exhibited a suppression of right amygdala fear reactivity relative to both the PLC (p = 0.001, Cohen's $d = 0.70$) and OT_5 groups (p =

126 0.018, $d = 0.52$) but the OT_5 group did not ($p = 0.266$, $d = 0.24$, relative to the PLC
127 group). Within group comparisons revealed that whereas amygdala reactivity did not
128 change on day 1 relative to day 5 in the PLC group ($p = 0.090$, $d = 0.29$), its
129 suppression was significantly enhanced in the OT_3 group ($p = 0.019$, $d = 0.40$) and
130 attenuated in the OT_5 one ($p = 0.044$, $d = 0.38$) (**Fig. 1B**). Thus, OT administration
131 every other day actually resulted in an enhanced suppression of amygdala responses
132 after 5 days compared to day 1 whereas the opposite occurred with daily treatment.

133 Ratings of the valence, arousal and intensity of all emotional faces were made by
134 subjects immediately after their scan was completed. Mixed ANOVAs including
135 treatment (PLC, OT_3, OT_5), time point (1st day, acute effects; 5th day, chronic effects)
136 and emotional expression (fear, angry, neutral or happy) as within-subject factors
137 revealed a significant main effect of emotional expression ($F_{1, 135} = 214.83$, $p < 0.001$,
138 $\eta^2_p = 0.61$) and a three-way interaction ($F_{1, 135} = 2.98$ $p = 0.007$, $\eta^2_p = 0.042$) for
139 emotional intensity ratings. Post-hoc comparisons indicated no significant between-
140 group differences on the 1st day, but the OT_3 group showed a significant decrease in
141 emotional intensity ratings for fearful faces on the 5th day as compared to the PLC
142 group ($p = 0.05$, $d = 0.42$) (**Fig.2**). No significant treatment x emotional expression
143 interactions on valence or arousal ratings were found ($ps > 0.05$).

144 Right-amygdala seed-to-whole brain fMRI resting-state analysis by mixed-effect
145 ANOVA revealed a main effect of treatment (OT_3, OT_5 and PLC) on amygdala
146 functional coupling with the ventromedial prefrontal cortex (vmPFC, peak MNI x = -
147 3, y =53, z = -19, $F_{2, 135} = 16.55$, $p_{FWE} = 0.001$, k = 88) before subjects underwent the

face paradigm, whereas no brain regions showed a significant time point x treatment interaction. Post-hoc analyses demonstrated that right amygdala intrinsic connectivity with the vmPFC was stronger in both the OT_3 ($k = 74$, $p_{FWE} = 0.014$, $x = -3$, $y = 53$, $z = -19$) and OT_5 groups ($k = 127$, $p_{FWE} = 0.001$, $x = -3$, $y = 53$, $z = -19$) relative to PLC. Subsequent confirmatory analyses of resting state functional connectivity in this pathway acquired after the face task paradigm confirmed a significant main effect of treatment ($F_{2, 135} = 7.081$, $p = 0.001$, $\eta^2_p = 0.095$). Post hoc comparisons showed both groups exhibited increased connectivity in this pathway after a single OT dose on the 1st day (OT_3, $p = 0.001$, $d = 0.71$; OT_5, $p = 0.063$, $d = 0.40$; relative to PLC) as well as after repeated doses on the 5th day (OT_3 $p = 0.047$, $d = 0.46$; OT_5, $p = 0.001$, $d = 0.69$; relative to PLC). No significant differences between the OT_3 and OT_5 groups were observed on the 1st ($p = 0.140$) or 5th ($p = 0.208$) day (see **Fig. 3B**). Thus, unlike the amygdala responses to fearful faces, for resting state functional connectivity between amygdala and vmPFC there was no reduction in OT-efficacy following daily repeated doses, although after 5 days, doses given every other day equally effective.

Although not included in our primary outcome measures we additionally analyzed the effects of single and repeated effects of OT on the right superior frontal gyrus and the bilateral primary (calcarine) visual cortex responses to fear faces. The visual cortex also showed the same reduced responsivity to daily OT as the amygdala, although the superior frontal gyrus did not (see **SI**). An examination of brain structural data using voxel based morphometry revealed no evidence for acute or

169 repeated dose effects of OT on gray matter volume and therefore observed functional
170 effects are unlikely to have been contributed to by structural changes (see **SI**).

171 **Secondary outcome measures: associations with OXTR genotype and trait**
172 **autism**

173 From the genotyping performed on the study subjects the number of G-carriers and G-
174 non-carriers of rs53576 as well as A-carriers and A-non-carriers of rs2254298 did not
175 differ between the three groups and both SNPs satisfied the Hardy Weinberg
176 Equilibrium (see **SI Tables S2 and S3**). With Bonferroni-correction for the multiple
177 SNPs and alleles (i.e. 2 x 2 = 4), $p < 0.0125$ was considered significant. For amygdala
178 responses there was a significant treatment x genotype interaction for rs53576 ($F_{2, 105}$
179 $= 6.01$, $p = 0.003$, $\eta^2_p = 0.10$) although for rs2254298 it did not reach significance ($F_{2,}$
180 $_{105} = 1.48$, $p = 0.232$, $\eta^2_p = 0.03$). For rs53576, OT-induced amygdala suppression on
181 day 1 was only significant in G-non-carriers (AA - $p < 0.001$, Cohen's $d = 1.00$) and
182 also on the 5th day in the OT_3 group (AA - $p < 0.001$, $d = 1.41$). An exploratory post
183 hoc analysis for rs2254298 revealed that OT-induced suppression on the 1st day across
184 treatment groups was only significant in A-carriers (AA and AG - $p = 0.001$, $d = 0.91$)
185 and also on the 5th day in the OT_3 group (AA and AG - $p = 0.004$, $d = 0.81$) (see **Fig.**
186 **4**). For emotional intensity ratings there was a significant treatment x genotype
187 interaction for rs2254298 ($F_{2, 105} = 6.25$, $p = 0.003$, $\eta^2_p = 0.11$). Post-hoc analyses
188 revealed no group differences on the 1st day, however on the 5th day the reduction in
189 intensity ratings in the OT_3 group was only significant in A-carriers (AA and AG - p
190 < 0.001, $d = 1.19$) (**Fig. 4**). Thus, OT-modulation of amygdala and behavioral

responses to fearful faces are strongly influence by receptor genotype. Associations with trait autism scores (AQ) did not achieve significance. Similar patterns of genotype association were found for the visual cortex but not superior frontal gyrus responses to fear faces (see **SI**).

Since comparable patterns of OT effects were observed on pre- and post-task resting state data right amygdala-vmPFC functional connectivity values were pooled to increase the power to determine associations with secondary outcome measures (**Fig. 3B**). No significant main or interaction effects of genotype for either SNP (rs 2254298 all ps > 0.262; rs53576 all ps > 0.396) were found although a negative association between total AQ scores and coupling strengths was observed in the PLC group on the 1st day (r = -0.32, p = 0.029) which was significantly reduced by OT in both groups on the 1st day (OT_3, r = 0.21 p = 0.170; Fisher's z = 2.51, p = 0.012; OT_5, r = 0.10, p = 0.500; z = 2.07, p = 0.038). The same effect was also seen on the 5th day (PLC: r = -0.33 p = 0.025; OT_3, r = -0.03 p = 0.837; z = 1.41, p = 0.16; OT_5 group, r = 0.10 p = 0.475; z = 2.11, p = 0.035) (**Figs. 3C-3D**). Thus, OT was particularly effective in increasing resting-state functional connectivity in individuals with higher trait autism scores and effects were independent of genotype.

Implications of findings for potential therapeutic use of oxytocin

Overall, our findings firstly validate the use of OT-reductions in amygdala responses to fear-faces and associated emotional intensity ratings together with increased resting state functional connectivity between the amygdala and vmPFC as robust markers for

212 its effects. Intriguingly, these task-dependent and resting-state effects of OT show a
213 markedly different sensitivity to repeated intranasal doses and OXTR genotype,
214 although our results indicate that to achieve both maximal task and resting-state
215 changes an optimal protocol using the standard 24IU dose is to administer it every
216 other day rather than daily. Both amygdala and behavioral responses to fearful faces
217 were actually enhanced after 5 days when OT was administered every other day and
218 even with the resting-state and superior frontal gyrus changes there was no advantage
219 of giving OT daily as opposed to every other day.

220 Our findings are therefore consistent with those in animal models demonstrating
221 OXTR desensitization following repeated doses of OT in some brain regions (9) and
222 that chronic administration can reduce brain OXTR expression (12) and altered
223 patterns of neurochemical release (13). While we did not use the twice daily
224 administration protocol employed to date in clinical trials we anticipate that this
225 would likely produce even stronger receptor-desensitization effects. However, at this
226 point we have only demonstrated repeated-dose effects on amygdala and visual cortex
227 fear reactivity in this respect and those on other behavioral and neural domains remain
228 to be established. The amygdala is however one of the main targets for the functional
229 effects of OT and is dysfunctional in many psychiatric disorders including social
230 impairments and anxiety (23, 24). Interestingly, chronic doses of OT in rodents failed
231 to produced anxiolytic effects normally seen with single doses (12) and thus daily
232 repeated doses in humans might also reduce its efficacy in decreasing social anxiety.

233 Our second major finding is that the effects of intranasal OT on reducing
234 amygdala and other neural responses to fear faces are highly dependent on OXTR
235 genotype. Thus, for the amygdala only G-non-carriers of rs53576 (i.e. AA) and A-
236 carriers of rs2254298 (i.e. AA and AG) showed significant responses and AA
237 homozygotes for rs53576 and A+ carriers of rs2254298 were the most sensitive to
238 OT. A+ carriers of these two genotypes have frequently, although not universally,
239 been associated with social dysfunction in autism as well as empathy (14, 15). A
240 recent haplotype-based analysis of OXTR SNPs including rs53576 and rs2254298
241 also indicated that individually they have some association with sensitivity to OT-
242 effects on face recognition (19). Again, while our current study clearly indicates that
243 OT modulation of amygdala and visual cortex responses to fear are strongly
244 associated with the AA genotype and with large effect sizes (Cohen's *ds* from 0.81-
245 1.41), it is entirely possible that effects of OT on other regions and behaviors might
246 show a different association. It should also be emphasized that subject numbers are
247 relatively low for establishing robust genetic associations. The AA allele of rs53576
248 occurs more frequently in Asian compared with Caucasian populations (25) amd so
249 there may be some cultural differences. Also rs53576 exhibits some sex-dependent
250 differences in social cooperation effects of OT (18) and our current study focused
251 only on males to avoid potential menstrual cycle effect issues.

252 Interestingly, task-related and intrinsic network changes produced by OT showed
253 a strikingly different sensitivity to repeated doses and OXTR genotype and only the
254 resting state changes were actually significantly associated with trait autism.

However, importantly there was no advantage of giving OT daily as opposed to every other day. This might potentially suggest that intrinsic networks are less influenced by repeated doses of OT or OXTR genotype than those activated by tasks, although this remains to be more fully established. Clearly however it will be important in future studies to measure OT effects on both task-related and intrinsic networks.

In conclusion, the current study provides strong evidence for an important influence of dose frequency and receptor genotype on the neural and behavioral actions of intranasal OT in healthy human subjects. These factors should therefore be taken into consideration when designing future clinical trials using repeated doses of intranasal OT as an intervention in psychiatric disorders.

Methods

Participants. A total of n = 147 healthy, right-handed healthy adult male subjects were enrolled according to common inclusion and exclusion criteria for human OT-administration studies (details see **SI**). Subject numbers were chosen on the basis of achieving >90% power for a medium effect size of 0.45. A total of 9 subjects had to be excluded due to failure to complete the study, head movement (see Consort Flow Diagram for details **SI Fig S3**). Subjects were randomly assigned to repeated intranasal treatment (single daily dose on five consecutive days) of (1) placebo (PLC; n = 46, M ± SD, 22.46± 2.3 years), (2) oxytocin (OT_5; n = 49, M ± SD, 21.78 ± 2.3 years), or interleaved OT and PLC (OT on days 1, 3, 5, PLC on days 2, 4; OT_3; n =

276 43, M ± SD, 21.02 ± 2.0 years). To ensure compliance all subjects were required to
277 come to the experimental laboratory every day and supervised during self-
278 administration of the nasal sprays. To control for between-group differences in
279 potential confounders for behavioral and neural effects of OT pre-treatment levels of
280 anxiety, depression, and empathy were assessed using validated scales. Additionally,
281 measures of trait autism were assessed using the AQ to compute associations with
282 neural and behavioral effects of OT. There were no significant differences between
283 the OT group and PLC groups (all ps > 0.05, see **SI and Table S4**). The study had
284 full approval by the local ethics committee (Institutional Review Board, University of
285 Electronic Science and Technology of China) and subjects provided written informed
286 consent and the study procedures were in accordance with the latest revision of the
287 Declaration of Helsinki. The study was pre-registered at Clinical Trials.gov
288 (NCT03610919). Subjects received monetary compensation for participation.

289 **Experimental procedures.** The study employed a double-blind, randomized,
290 placebo-controlled, between-subject design with repeated intranasal single daily dose
291 administration on five consecutive days. Participants were randomly assigned to 3
292 groups: group 1 OT (24 IU, single daily dose) for 5 days (OT_5 group); group 2 OT
293 every other day (24 IU, single dose on days 1, 3, 5) and PLC (days 2, 4) (OT_3 group);
294 group 3, PLC for 5 days (PLC group) (see **SI Fig S1**). The OT and PLC sprays were
295 both supplied by Sichuan Meike Pharmaceutical Co. Ltd, Sichuan, China in identical
296 dispenser bottles containing identical ingredients (e.g. glycerine and sodium chloride)
297 other than OT. In line with recommended guidelines experiments started 45 minutes

298 after intranasal administration (26). In post-treatment interviews collected on days 1
299 and 5 subjects were unable to identify better than chance whether they had received
300 OT or PLC ($\chi^2 < 0.1$, ps > 0.2, see **SI Table S5**) confirming successful blinding over
301 the entire study period. To explore the potential contribution of individual differences
302 in OXTR genotypes to treatment effects subjects provided buccal swaps on the 1st day
303 for analysis of OXTR rs2254298, rs53576 SNPs (see **SI** and (27)).

304 **Stimuli and face emotion task.** 208 grayscale facial stimuli displaying happy,
305 neutral, angry or fearful facial expressions (n = 26 per category, 50% female) were
306 used in the implicit emotional face processing task. Stimuli were initially rated with
307 respect to arousal and valence by an independent group of subjects (n = 24, 10
308 females, age, M ± SD, 21.3 ± 1.9 years). Based on these valence and arousal ratings
309 two matched independent sets of stimuli were produced for use on the 1st and 5th days
310 (all ps > 0.3, see **SI Table S6**) and the order of the two sets was counter balanced
311 across individuals in OT and PLC groups. Subjects were instructed to view the facial
312 stimuli attentively and were required to indicate by a button press the gender of the
313 face after each presentation to ensure attentive processing. Stimuli were presented for
314 3s followed by a jittered fixation (1.5-2.1s) followed by the gender discrimination task
315 (2s) (subjects achieved >80% accuracy), and finally a jittered inter-trial interval (4.5-
316 6s) that served as low level baseline. Order of gender and emotion was pseudo-
317 randomized (see **SI Fig. S2**). After each scanning session participants rated emotional
318 valence, arousal and intensity of the faces presented during fMRI. Stimuli were

presented for 10s and subjects provided ratings by 1-9 Self-Assessment-Manikin scales (SAM) for the three different domains.

Primary outcomes and analysis plan. Face emotion-related amygdala responses were assessed using the event-related implicit face processing fMRI paradigm on treatment days 1 and 5. Intrinsic amygdala connectivity was assessed by means of two resting state fMRI assessments (before and after the task-paradigm). Valence, arousal and intensity ratings (scale: 1-9) for the facial stimuli were collected immediately after MRI acquisition as additional behavioral outcomes (further details see **SI**). In line with the main aim of the study changes in amygdala fear reactivity and amygdala intrinsic connectivity between the 1st and 5th day served as primary outcome measures. Changes in valence, arousal and intensity ratings for the fear faces represented an associated behavioral outcome measure.

Imaging acquisition and analysis. MRI data was acquired using standard sequences on a 3T GE MR750 system. In addition to the functional time series high resolution T1-weighted structural MRI data was acquired at both time points to improve normalization and control for acute and chronic effects of treatment on brain structure. MRI data was preprocessed using validated procedures in SPM12 (28) (Statistical Parametric Mapping; http://www.fil.ion.ucl.ac.uk/spm) and Data Processing Assistant or Resting-State fMRI (29) (DPARSFA; http://rfmri.org/DPARSF) (for details see **SI**). First level General Linear Models (GLM) for the task-related fMRI data included separate regressors for the four

340 emotional conditions, gender identity rating period and 6 movement parameters and
341 appropriate contrasts were subjected to a second level random effects analyses.

342 **Analysis strategy.** To identify OT-sensitive regions and confirm previous findings on
343 suppression of amygdala reactivity following single-dose OT-administration (2) a
344 voxel-wise whole-brain two-sample t-test was conducted in SPM comparing neural
345 activity in OT- and PLC-treated subjects on the1st day. In line with previous studies
346 and our a priori hypothesis single-dose OT suppressed amygdala fear reactivity (see
347 Results). For all subsequent analyses a 6-mm sphere centered at the maximum t-
348 values of the acute OT effects in the amygdala served as target region to determine
349 different trajectories of amygdala functioning following acute versus repeated
350 treatment administration (for a similar approach see (4)).

351 **Primary outcome: amygdala fear-reactivity.** Differences in stimulus-induced
352 amygdala fear reactivity were examined by means of mixed ANOVAs with treatment
353 (PLC, OT_3, OT_5) as between-subject factor, time point (1st day, acute effects; 5th day,
354 chronic effects) as within-subject factor and extracted target region amygdala-
355 reactivity towards fearful faces as dependent variable (parameter estimates were
356 extracted using Marsbar, http://marsbar.sourceforge.net). Effects were further
357 disentangled using appropriate Bonferroni-corrected post-hoc comparisons.

358 **Primary outcome: intrinsic amygdala connectivity.** Differences in amygdala
359 connectivity were determined using a voxel-wise seed-to-whole-brain ANOVA as
360 implemented in the SPM flexible factorial design with treatment (PLC, OT_3, OT_5) as

361 between-subject factor, time point (1st day, acute effects; 5th day, chronic effects) as
362 within-subject factor and amygdala connectivity maps as dependent variable. To
363 account for potential effects of task-engagement on intrinsic amygdala connectivity
364 the primary analysis focused on pre-task resting state maps. Based on findings from
365 the pre-task data, a subsequent ROI analyses on the post-task data (focusing on
366 extracted estimates from a 6mm sphere centered at vmPFC determined by the pre-task
367 data) served to replicate and further validate them.

368 **Secondary outcome measures: contributions of OXTR genetics and associations**
369 **with trait autism.** Subjects were genotyped (OXTR SNPs, rs2254298 and rs53576)
370 from buccal cells as described previously (27) (see **SI**). Only 120 of the study subjects
371 were successfully genotyped for both SNPs. For rs2254298 subjects were divided into
372 A+ carriers (AA and AG) and A- non-carriers (GG) and rs53576 into G+ carriers (GG
373 and GA) and G- non-carriers (AA). To explore effects of genotype as a secondary
374 outcome measure, OXTR group was included as additional between-subject factor in
375 the corresponding ANOVAs and amygdala fear reactivity, amygdala-vmPFC intrinsic
376 functional connectivity and behavior rating response served as dependent variables.
377 Associations between neural and behavioral effects of OT and trait autism were
378 carried out by analyzing correlations with AQ scores.

379 **Control analysis: treatment effects on brain structure.** To further control for
380 potential confounding effects of single- and repeated OT-administration brain
381 structure, a voxel-based morphometry (VBM) analysis was conducted on the T1-
382 weighted images acquired on both testing days using SPM12 standard procedures (30,

383 31). Effects of single- and repeated dose administration were explored using analyses
384 concordant with the fMRI analyses (see **SI**).

385 **Thresholding and statistical implementation.** Voxel-wise whole brain analyses in
386 SPM were thresholded at a cluster-based Family-wise error (FWE) corrected $p < .05$
387 with an initial cluster forming threshold of $p < .001$ (in line with recent
388 recommendations for the control of false positives in cluster-based control of multiple
389 comparisons (32, 33)). Behavioral and neural (extracted parameter estimates) indices
390 were analyzed using SPSS 22.0 and appropriate ANOVA models. Partial eta squared
391 (η^2_p) and Cohen's *d* were computed as measures of effect size. Sphericity was
392 assessed with Mauchly's test, Greenhouse-Geisser correction for non-sphericity when
393 applicable. Group differences in parameter estimates extracted from significant main
394 effect in SPM were further evaluated using two-sample t tests. Associations between
395 neural and behavioral changes and AQ scores were computed using Pearson
396 correlation. All reported p values were Bonferroni-corrected, two-tailed, and $p \leq 0.05$
397 considered significant.
398

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Figures

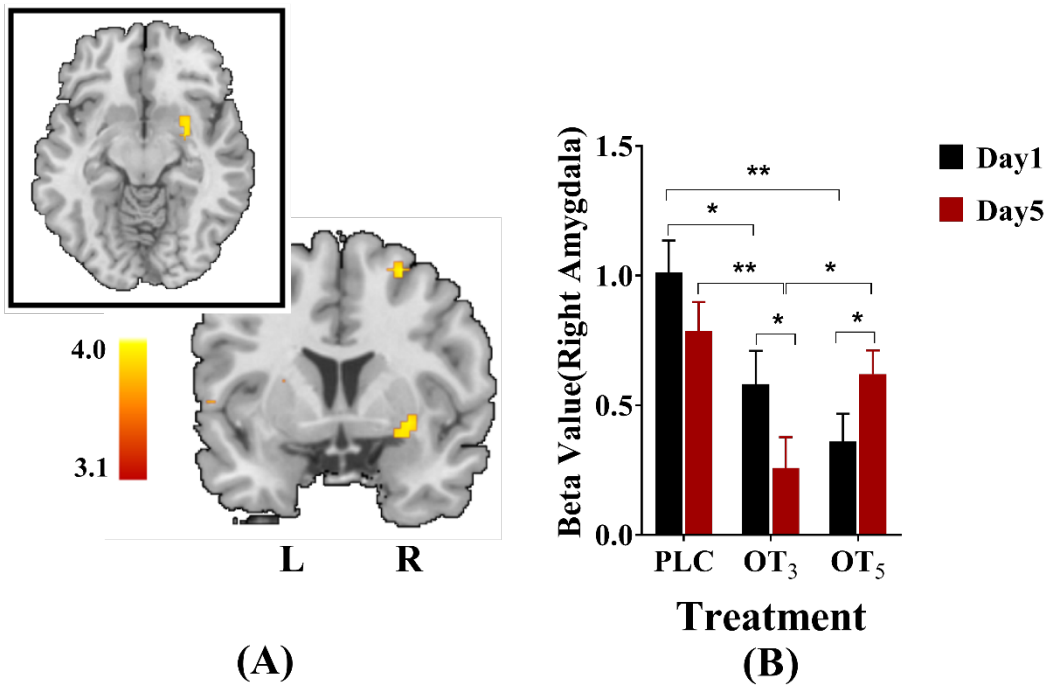
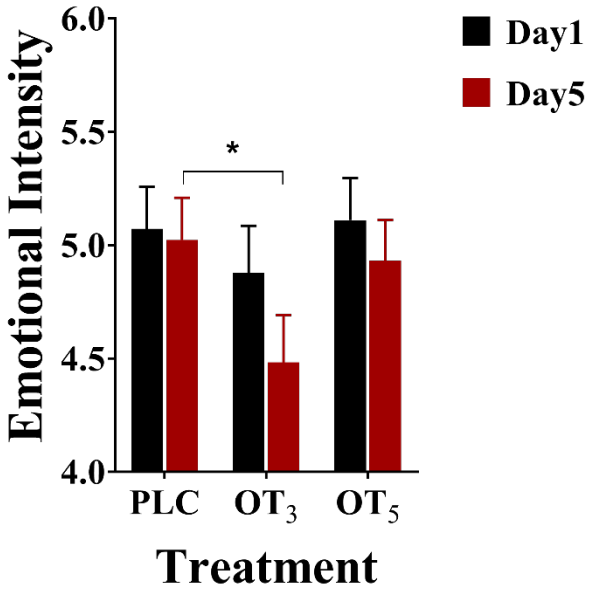


Fig.1 The effect of intranasal oxytocin (OT) treatment on neural responses to fearful faces on the 1st day. (A) The t-map of the treatment effect (FWE c = 78, t = 3.93, p = 0.043) showed an activated cluster peaking at the right amygdala (x = 27, y = −4, z = −13). (B) Parameter estimates extracted using a 6-mm radius sphere centered at the peak MNI coordinates at the right amygdala on the 1st and 5th day separately revealed that OT treatment on alternate days (OT_3) decreased amygdala responses on both the 1st and 5th days whereas for the daily OT treatment group (OT_5) group the decrease only occurred on the 1st day. * $p < 0.05$, ** $p < 0.01$, two-tailed t-test. Bars indicate M ± SE.



507

508 **Fig.2** Oxytocin decreased intensity ratings of fearful faces only on the 5th day of

509 treatment in the group receiving OT on alternate days (OT_3) compared with placebo

510 (PLC). *p = 0.05. Bars depict M ± SE.

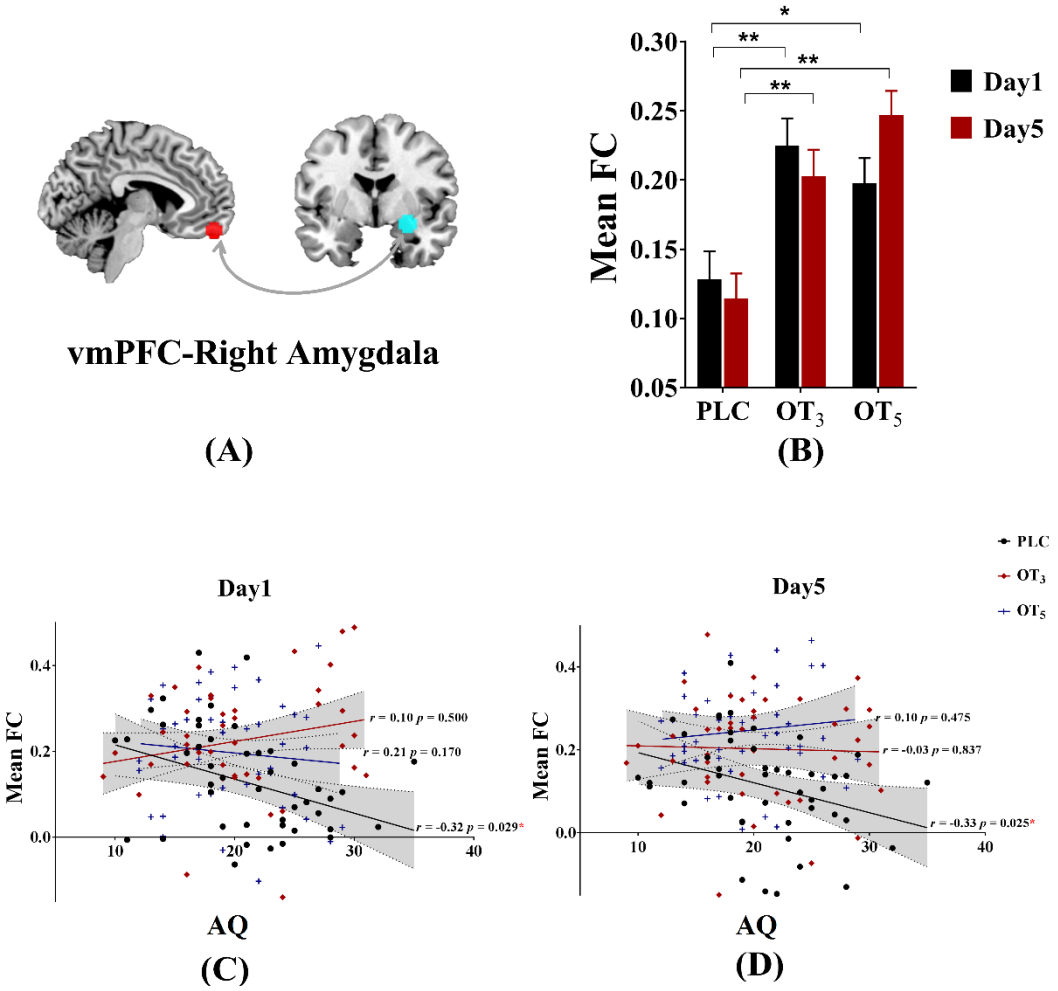
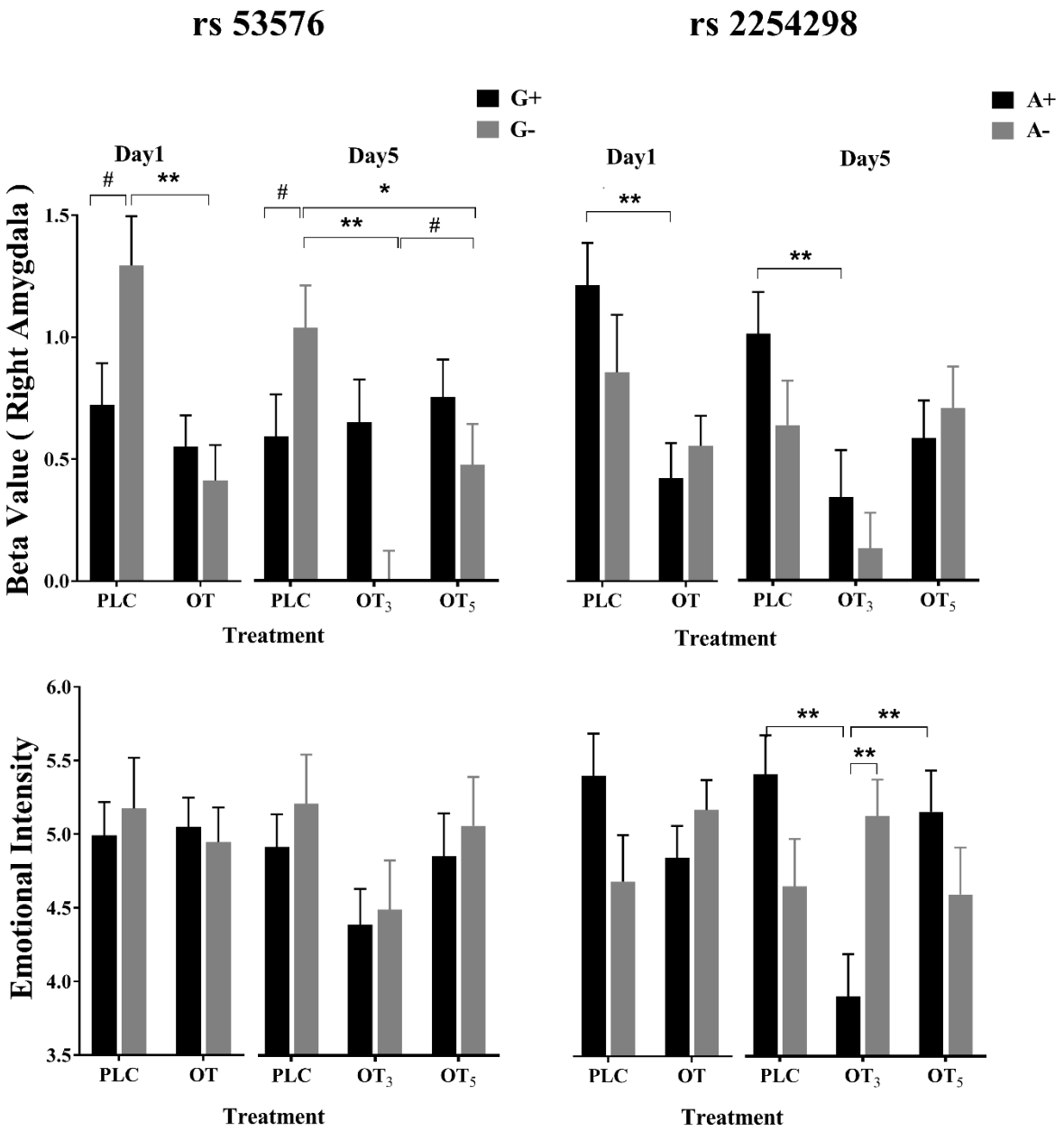


Fig.3 The effects of oxytocin (OT) on resting state functional connectivity (A) schematic showing functional connection between the right amygdala and ventromedial medial prefrontal cortex (vmPFC). Whole brain functional connectivity used the right amygdala as a region of interest (6mm sphere, x=27, y=-4, z=-13) and (B) revealed significant increased functional connectivity between vmPFC and right amygdala (p_{FWE} <0.05, x=-3, y=53, z=-19) in both OT treatment groups (every day – OT and every other day - OT on both days 1 and 5. Data for the two resting state periods before and after the face emotion task were similar and are therefore showed here combined. (C) Regression graphs showing correlations between resting-state functional connectivity and autism spectrum quotient scores (AQ) in the three treatment groups on day 1 and (D) day 5. Bar graph illustrates the extraction of parameter estimates from right amygdala connectivity with vmPFC (M ± SE). * $p < 0.05$, ** $p < 0.01$, two-tailed t-test. Bars indicate M ± SE.



524

525 **Fig.4.** Influence of oxytocin receptor genotype on right amygdala responses to fearful faces (n
526 = 111 subjects). Top: for rs 53576 only G-non carriers (i.e. AA) showed reduced amygdala
527 response to fearful faces and for rs2254298 only A-carriers (i.e. AA and AG) in the groups
528 with intranasal oxytocin treatment either daily (OT_5) or every other day (OT_3). Bottom: A
529 similar pattern was found for rs2254298, but not rs53576 for intensity ratings of fearful faces
530 although only on day 5 in the OT_3) group. ** $p < 0.001$, * $p < 0.01$, # $p < 0.05$, two-tailed
531 t-test (Bonferroni corrected significance threshold of $p = 0.0125$). Bars indicate M ± SE.

532