Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

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eAppendix 1. Methods

Participants

Among 323 patients with probable ASD who visited the University of Tokyo Hospital or Showa University Karasuyama Hospital between Nov/1/2009 and April/30/2011. Forty participants participated in the present study. None of these participants had histories of current or past neurological illness, traumatic brain injuries with any known cognitive consequences or loss of consciousness for more than five minutes, or histories of substance abuse or addiction.

Because no previous study examined oxytocin's effects on fMRI signals in ASD subjects, we estimated the required size of sample based on a previous fMRI study[1] examining oxytocin's effects on TD adults reported findings with an effect size ranging between approximately 0.5 and 0.75 at the two-sided $P<0.001$. A sample size of 36 was required to detect an effect size of 0.75 at the two-sided $P<0.001$ in a whole-brain-level fMRI analysis with a power of 80%. Forty participants were enrolled to accommodate approximately 10% dropout.

Based on the diagnosis protocol in the following section, 31 of the 33 participants were clinically diagnosed with high-functioning autism, one of the remaining subjects was diagnosed with Asperger's syndrome, and the other remaining subject was diagnosed with pervasive developmental disorder-not otherwise specified.

The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the institutional review board of the University of Tokyo Hospital. This study was a combined examination of two registered clinical trials in the University Hospital Medical Information Network (UMIN) clinical trials registry – UMIN000004393 and its preliminary version with the same protocol UMIN000002241. After a complete explanation of the study, written informed consent was obtained from all participants. Using longitudinal clinical assessments, a trained psychiatrist (H.Y.) confirmed that all of these adult participants had no intellectual disabilities and were capable of providing informed consent.

Diagnostic protocol and clinical scores

The protocol for diagnoses in the present study was the same as that used in our previous case-control study[2].

Another trained psychiatrist confirmed the diagnoses using the Japanese version of the Autism Diagnostic Interview-Revised (ADI-R)[3] (H.K.). The ASD diagnoses of eight participants who did not meet the threshold in the ADI-R social domain were confirmed by evaluating social scores in Autism Diagnostic Observation Schedule (ADOS)[4] by another trained psychologist (M.K.). All the eight participants were diagnosed with autism on the basis of ADOS social scores (Range: 11-20, in which 10 is the minimum threshold for autism). The Social Responsiveness Scale (SRS)[5], a quantitative measure of autistic traits, was assessed by mothers of the participants on the day of the first MR scan

eTable 1 shows the clinical scores of the participants. The IQs of the ASD participants

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were assessed using the full scale of the Wechsler Adult Intelligence Scale-Revised, Japanese version[6], and all the ASD participants exhibited IQs ranging from average to above average. Handedness was determined using the Edinburgh Handedness Inventory[7], and participants with a laterality index of more than 0.5 were regarded as right-handed. Participants whose laterality index score ranged from -0.5 to 0.5 were defined as ambidextrous. The SES of the participants and their parents were assessed using the Hollingshead scale[8]. Sleepiness during the MR scans was assessed using the Stanford Sleepiness Scale[9], and no participants fell asleep during the fMRI experiment. To monitor for potential side effects of oxytocin administration, heart rate and blood pressure were measured immediately after MR scan completion.

Randomized intranasal administration of oxytocin and placebo

This study adopted a randomized, placebo-controlled, double-blind, within-subjects, crossover experimental design (Figure 2). ASD participants received a single dose of oxytocin (24 IU; Syntocinon-Spray, Novartis)[10-12] or placebo at a one-week interval in a pseudo-random order 40 minutes before the MR scanning. A randomization and masking manager randomly assigned each participant to an oxytocin-initially-administered or placebo-initially-administered group in a computer-generated randomized order. The manager completely covered the label of the nasal sprays to maintain the blindness of the participants and other research members. Thus, both experimenters and participants were blind to identity of the drug administered.

To avoid any subjective effects of the substances other than those caused by oxytocin (e.g., olfactory effects), the placebo contained all inactive ingredients. Participants abstained from food and drink (other than water) for 2 hours before the experiment and from exercise, caffeine, and alcohol for 24 hours before the session. As in previous studies, no adverse side effects of oxytocin were observed in this study.

Exclusion of medicated individuals with ASD

When we planned the present clinical trials, we assumed that it would be difficult to recruit enough medication-free ASD participants. Therefore, although we recruited non-medicated ASD participants with priority, ASD participants taking psychotropic medication were also recruited. However, unexpectedly, we were able to recruit a fairly large number of medication-free ASD participants. To minimize the potential confounding effects, we mainly analyzed data obtained from drug-free participants. In the final section of this supplementary information, we provide results with data from the medicated subjects included.

Task and stimuli

The task and stimuli in the present study were the same as those used in our previous case-control study[2].

The stimuli consisted of 80 original monochrome movies with a length of 1,500 ms (Video). In each movie, one of 20 professional actors (10 male and 10 female) spoke a

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different emotional word accompanied by an emotional facial expression and expressive prosody (Figure 1A, eFigure 1). Healthy Japanese adults (T.W., N.Y., Y.K., and H.Y.) confirmed that the movies are natural and realistic. As verbal information, words with high emotional valence and arousal were selected from the list of Affective Norms for English Words (40 words had a positive valence, and the other 40 words had a negative valence)[13]. Overly aggressive words were excluded for ethical considerations. As nonverbal information, the professional actors were instructed to display positive or negative facial expressions and prosody while speaking each word. The emotional directionality of the facial expressions was always the same as that of the accompanying voice prosody. The emotional directionality of the verbal and nonverbal information was separately validated in our previous study[2]. In total, there were four types of stimuli (eFigure 1): a negative facial expression and prosody paired with a negative word (i.e., NV-V-, e.g., Video part 1), a negative facial expression and prosody paired with a positive word (NV-V+, e.g., Video part 2), a positive facial expression and prosody paired with a negative word (NV+V-, e.g., Video part 3), and a positive facial expression and prosody stimuli with a positive word (NV+V+, e.g., Video part 4). There were 20 videos of each type.

Validation of stimuli

Although the sample size in the test was small (N=12), an independent behavioral test in our previous study[2] validated the emotional valence of the verbal/nonverbal information with a large effect size (Cohen's d>1.45). Furthermore, in another recent fMRI study conducted by us in TD participants[14], the present task and stimuli also enabled us to find hierarchal brain network structure underlying the resolution of incongruent social information. Although the test and studies provide some validation of the present stimuli, we note that the stimuli were evaluated in a scale with two extremes and that they may need to be evaluated in a free-choice paradigm test.

Task paradigm

During fMRI scanning, the participants were presented with the 80 movies sequentially and instructed to intuitively judge whether they believed the actor in the movie was a friend or foe by pressing a button (Figure 1A). Each movie stimulus was presented immediately before a 2 s response period that was followed by a waiting period whose duration was jittered between 2.5 s and 4.5 s (Figure 1B). To optimize the event-related design, we also added ten 7 s dummy trials in each run. The fMRI scanning consisted of two runs of approximately 6 min, each of which involved 10 NV-V-, 10 NV-V+, 10 NV+V-, and 10 NV+V+ stimuli, pseudo-randomly ordered. Before fMRI scanning, all participants underwent sufficient training to allow them to acclimate to and complete the judgment task in a limited time. Movie stimuli used in the training session were totally different from those used in the fMRI scanning.

Influence of training before scanning

The individuals with ASD were required to undergo a sufficient length of training of the preset

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psychological task. All the movie stimuli used in the training were different from those used during the following fMRI scanning. The training was repeated until the individuals with ASD could respond to the stimuli within the allotted time period. Because some of the individuals with ASD had difficulty in the smooth and integrative operation of the response pad, the participants also underwent training with pressing a button. These training sessions are considered to make the ASD participants habituated against the psychological task. Therefore, the present experimental paradigm might not be able to detect the processes of habituation to novel psychological tasks.

MRI scanning

A sagittal localizer scan was obtained first, followed by the axial T2 weighted images (echo time (TE)=82.32 ms, repetition time (TR)=4400 ms, field of view (FOV)=240×240 mm, matrix=256×256, slice thickness=2.5 mm, number of axial slices=62) for the anatomical screening. Because the stimuli included human speech, we used an MRI-compatible headphone system (Hitachi, Corp. Tokyo, Japan), and confirmed before and after the fMRI experiment that the speech content and prosody in the stimuli could be heard clearly. A trained neuroradiologist (H.T. or O.A.) evaluated the MRI scans and found no gross abnormalities in any participant. Magnetic field inhomogeneities in our scanner were monitored with basic quality control that was conducted daily, and they were stable over the course of this study.

Gaze tracking and analysis

For 16 of the 33 participants, eye movements, fixations, and pupil diameters were recorded during fMRI scanning using a remote infrared-light camera. The acquired data were analyzed in accordance with previous studies[11,15-17]. After smoothing of the raw data with a Gaussian filter, blink and artifact detection was performed[18]. Over the whole trial, we calculated the average percent fixation duration on the eyes or nose/mouth area relative to the whole screen. Fixations were coded when the gaze remained within a target area for at least 100ms. The eye or nose/mouth areas were defined as a common area for all the movies in a relative liberal manner.

We recorded from only a subset of the participants because we did not have equipment for eye-gaze tracking in the first half of the present clinical trial, which is registered as a preliminary clinical trial (UMIN 000002241). Based on results of this preliminary trial with 20 ASD participants, we were allowed to prepare a device to track eye gaze during MRI scanning. Therefore, we could record eye gaze in the following trial (UMIN 000004393) in which other 20 individuals with ASD participated. Among the 20 participants, four participants were excluded because of their history of medication (one participant) and atypical responses to congruent stimuli (three). As a result, we analyzed eye-gaze-tracking data recorded from 16 ASD participants.

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Behavioral analysis

Based on this classification, we examined oxytocin's effects on the number of NVJs by conducting a repeated measures three-way ANOVA of the number of judgments on incongruent stimuli (type of drug: oxytocin/placebo × type of response: friend/foe × type of stimuli: NV-V+/NV+V-); This analysis was chosen because our previous study showed a significant difference in the number of NVJs between TD and ASD individuals[2]. Using a three-way ANOVA with the same structure, we also examined oxytocin's effects on response times to incongruent stimuli because the data in the case-control study[2] also showed a significant difference in the response times for NVJs (eFigure 2). Note that, to keep consistency with the present study, this re-analysis does not include TD and ASD participants who showed too many unnatural responses to the congruent stimuli.

Difference in motion during scanning between oxytocin and placebo sessions

The fMRI data were analyzed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). There was no significant difference in the extent of motion during scanning between the oxytocin sessions and placebo sessions, as a repeated-measures ANOVA (type of drug: oxytocin/placebo × absolute values of motion parameters: x/y/z/pitch/yaw/roll) did not find a significant main effect of the type of drug or interaction ($P > 0.8$).

Normalization of fMRI images

In the present study, fMRI images were not directly normalized to an EPI template in SPM. For spatial normalization, we first estimated warping parameters through fitting each obtained high-resolution T2 image to a T2 template in SPM. We then applied the parameters to functional images (i.e., EPI image) and obtained the normalized functional images.

fMRI analysis

Correlations between the behavioral and neural effects of oxytocin were estimated by calculating Pearson's correlation coefficients between the brain activity in the ROIs and several behavioral scores. Brain activity was taken as the NVJ-specific percent signal in each of the ROIs (i.e., NVJ > VJ). The behavioral scores were the number of NVJs or the response times to the incongruent stimuli. The statistical significance of the correlation was corrected for the seven ROIs using the Bonferroni correction.

In the ROI and behavior correlation analyses, we set $P < 0.05$ as the statistical threshold and employed Bonferroni corrections for multiple comparisons. In the whole brain analysis, we used a statistical threshold of $P < 0.05$ corrected based on the family-wise error (FWE) rate.

Validation of ROI selection

On the basis of the findings of our previous case-control study, we focused on oxytocin's effects on the brain activity of seven ROIs (eTable 2). Findings of other previous studies also allowed us

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to estimate the sensitivity of ROIs to oxytocin as follows. Deviated activity in the ACC and dmPFC during the social cognition task in individuals with ASD has been repeatedly reported[19]. The ASD-specific brain activity during processing social stimuli was also found in the right IFG[20-23], and amygdala[19,20,24,25]. The bilateral AI are also known to show ASD-specific regional activity and functional connectivity during social cognition tasks[19,26-29]. These previous findings provide support for our selection of ROIs.

Functional connectivity analysis (PPI analysis)

Functional connectivity was estimated by calculating the psycho-physiological interaction (PPI)[2,30] between the two medial prefrontal ROIs (i.e., the ACC and dmPFC). At the single subject level, PPIs specific to the NVJ trials were calculated using a contrast between NVJ and VJ. In this analysis, we used three regressors consisting of a regressor representing fMRI signals of the seed region, one representing a psychological factor (i.e., NVJ and VJ), and a psycho-physiological interaction factor. PPI was separately calculated for the oxytocin and placebo sessions. At the group level, we evaluated the significance of PPI using a random effects model at a whole-brain level. Because the ROI analysis described above demonstrated the importance of the ACC and dmPFC, we first focused on these two medial prefrontal regions and estimated the PPI from the ACC to the dmPFC and from the dmPFC to the ACC. As a validation of the spatial specificity, we also present the results of the whole-brain level analysis.

In the ROI analysis and analysis of the correlation with behavioral scores, we adopted a Bonferroni corrected $P < 0.05$ as the statistical threshold. In the whole brain connectivity analysis, we used an FWE-corrected $P < 0.05$ as the statistical threshold.

Correction for multiple comparisons

The present clinical trial was essentially hypothesis-based research. Therefore, it is necessary to correct for multiple comparisons that are based on the hypothesis.

On the basis of our previous case-control study, in the present trial, we hypothesize that oxytocin increases the number of NVJs, shortens the response time for NVJs, and normalizes the deviated activity during NVJ in seven brain regions. Considering this hypothesis, we do not have to correct for multiple comparisons when we examine oxytocin's effects on the number of NVJs and the response time for NVJs. In the case of brain activity specific to NVJs, it is necessary to correct for multiple comparisons. Thus, in the present study, we applied a Bonferroni correction for the seven regions, and each effect of oxytocin was evaluated with a significance level of $P < 0.0071$ (=0.05/7).

In the voxelwise whole-brain analyses of activation and PPI, we evaluated the statistical significance under a threshold of FWE-corrected $P < 0.05$.

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eAppendix 2. Results

Oxytocin's effects on blood pressure, pulse rate, and sleepiness score

We confirmed oxytocin's effects on the autonomic nervous system (eTable 1). Both systolic and diastolic blood pressures were significantly higher after administration of oxytocin than after administration of placebo (systolic blood pressure: $t(32) = 2.4$, $P = 0.02$; diastolic blood pressure: $t(32) = 2.1$, $P = 0.04$, in paired *t*-tests). However, neither the pulse rate nor Stanford sleepiness score showed significant differences after administration of oxytocin or placebo (pulse rate: $t(32) = 1.9$, $P > 0.07$ in paired *t*-test; sleepiness score: $z = 0.58$, $P > 0.56$ in Wilcoxon rank test).

When we accounted for the covariance of these autonomic responses to the drugs in the following analyses, all of the statistically significant results described in the following sections were unchanged.

Oxytocin's effects on eye-gaze

Although the sample size of the eye-gaze tracking was limited to 16 of the 33 participants, we observed a significant effect of oxytocin on fixation time in the eye area (eFigure 3). After intranasal oxytocin, the averaged percentage of time spent fixating on the eye area was significantly greater than after administration of placebo ($t(15) = 2.2$, $P = 0.02$); fixation time on the nose/mouth area was unaltered ($t(15) = 0.2$, $P > 0.4$). This effect of oxytocin on fixation time on the eye area is consistent with previous studies involving TD or ASD subjects[31,32].

This result suggests that oxytocin improved the ability to perceive nonverbal information. However, even after oxytocin administration, there was neither a significant correlation between the percentage of fixation time on the eye area and number of NVJs ($r = 0.07$) nor between the percentage of fixation time on the eye area and response time to incongruent stimuli ($r = 0.11$). Similarly, none of these correlations were significant after placebo administration. These results suggest that the effects of oxytocin on the number of NVJs and response times to incongruent stimuli cannot be explained only by the oxytocin-induced alterations of eye-gaze.

Details of the behavioral effects of oxytocin

eTable 3 shows the numbers of friend/foe responses to the congruent stimuli. After the exclusion of the participants who showed unnatural responses to the congruent control stimuli, all the participants responded to the stimuli in natural ways (i.e. Friend to NV+V+ and Foe to NV-V-).

eTable 4 shows the numbers of friend/foe responses to the verbal-nonverbal incongruent stimuli. In a repeated-measures three-way ANOVA (type of drug: oxytocin/placebo × type of response: friend/foe × type of incongruent stimuli: NV-V+/NV+V-) detected a significant three-way interaction ($F(1, 32) = 5.1$, $P = 0.03$), and a post-hoc paired t-test showed that the number of NVJs was significantly greater in oxytocin session than in placebo session (Figure 1C; $t(32) = 2.2$, $P = 0.03$). However, there was no significant effect of friend/foe types.

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Relationship between oxytocin's effect on the response time and that on the number of NVJs.
Oxytocin's effect on response time was independent of that on the number of NVJs, as oxytocin's shortening effect on response time was present even when we compared response time for stimuli to which the participants showed the same judgments in both oxytocin and placebo sessions (t_{32}=2.2, P=0.035, eFigure 4).

Details of oxytocin-induced shortening of response times for NVJs.
A repeated-measures two-way ANOVA of the response times to the incongruent stimuli (type of drug: oxytocin/placebo × type of judgments: NVJ/VJ) found a significant three-way interaction ($F(1,32) = 5.9$, $P = 0.02$). The response times for NVJs were significantly correlated with SRS scores only after placebo administration ($rho = 0.38$, $P = 0.03$ in Spearman's correlation coefficient test; no significant difference between the two correlation coefficients, $P > 0.1$; eFigure 5). These results suggest that the length of the response times for NVJs might capture part of the symptoms of individuals with ASD.

Results of voxelwise whole-brain analysis
We conducted a voxelwise whole-brain search for brain regions that showed significant increases in NVJ-specific activity between oxytocin and placebo administrations. We detected significant activation in the ACC, dmPFC (Figure 3A), left superior frontal gyrus (SFG), and left superior temporal sulcus (STS) ($P < 0.05$, FWE-corrected, eFigure 6, Table 1). The locations of the ACC and dmPFC found in this whole-brain search were close to and partially overlapped those of our pre-defined ACC and dmPFC ROIs.

In contrast, we could not find oxytocin-induced significant supersession of NVJ-specific brain activity.

Other main effects of three-way ANOVAs
In the three-way ANOVA of the number of judgments for the incongruent stimuli (type of drug: oxytocin/placebo × type of response: friend/foe × type of stimuli: NV-V+/NV+V-), there were no significant main effects ($P > 0.3$). In the three-way ANOVA of the response time to incongruent stimuli (type of drug: oxytocin/placebo × type of response: friend/foe × type of stimuli: NV-V+/NV+V-), there were also no significant main effects ($P > 0.6$). In the three-way ANOVA of brain activity (type of drug × type of response × type of stimuli), there were no significant main effects ($P > 0.4$).

Although not a main effect of the three-way ANOVA, we observed drug-independent brain activity patterns that were related to VJ and NVJ, respectively. In our recent fMRI study in TD individuals[14], we reported that a hierarchal brain network underlies the resolution of verbal-nonverbal incongruent social information. The network contains two sub-modules each of which is specifically involved in VJ and NVJ. Moreover, the two sub-modules are bridged by a judgment-common anterior dmPFC. Another fMRI study in individuals with ASD without

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oxytocin and placebo has also reported that similar brain regions are related to VJ and NVJ, though some of the brain activity was diminished compared with TD[2].

Results of the voxelwise whole-brain analysis of functional connectivity.

We also conducted a voxelwise whole-brain analysis of NVJ-specific functional connectivity from the dmPFC and found a significant increase in connectivity in the ACC and left anterior insula (AI) (eFigure 7, eTable 5). The location of the ACC found in this analysis was close to that of our pre-defined ACC ROI.

Relationship between the number of NVJs and functional connectivity from the ACC to the dmPFC.

Although change in the functional connectivity from the dmPFC to the ACC was closely associated with the number of NVJs (Figure 4D) and change in responses time for NVJs (Figure 4E), the connectivity from the ACC to the dmPFC did not show this association (eFigure 8C).

Effects on amygdalar activity.

In addition to the suppressive effects on amygdalar brain activity during congruent stimuli trials, the NVJ-specific functional connectivity from the amygdala to the ACC after oxytocin administration was significantly smaller than that after placebo administration (t_{32}=3.8, P<0.001 in a paired t-test, eFigure 9C); however, this reduction was not observed in the NVJ-specific connectivity from the amygdala to the dmPFC (P>0.1).

Oxytocin's effects on brain activity during congruent stimuli trials

Oxytocin-induced suppression was observed in the right SFG and bilateral amygdala, whereas activity in the orbitomedial frontal cortex and STS was increased by oxytocin (eFigure 10).

Robustness of the present results to participant selection.

We confirmed the robustness of these results to participant selection, as shown in eFigure1. We observed essentially the same statistical conclusions displayed in Figures 1, 3, and 4, eFigures 3 to 9, and Tables 1 and eTable 5 when the two medicated participants were not excluded (i.e., number of participants = 35). Essentially the same observations were also confirmed when we included data from the three different participants who showed non-straight behavioral responses (i.e., number of participants = 36) and when we used all available data (i.e., number of participants = 38, eFigure 11).

Comparison with TD individuals

The main behavioral and neurological outcomes of oxytocin-administered individuals with ASD were not significantly different from those observed in TD individuals from our previous study (*P*>0.3). The number of NVJ: ASD and oxytocin: 24.7±1.0, TD: 26.4±1.1; the response time for

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NVJ: ASD and oxytocin: 2.8±0.13, TD: 2.9±0.15; NVJ-specific brain activity in the ACC: ASD and oxytocin: 0.051±0.02, TD: 0.053±0.01; NVJ-specific brain activity in the dmPFC: ASD and oxytocin: 0.09±0.02, TD: 0.082±0.02

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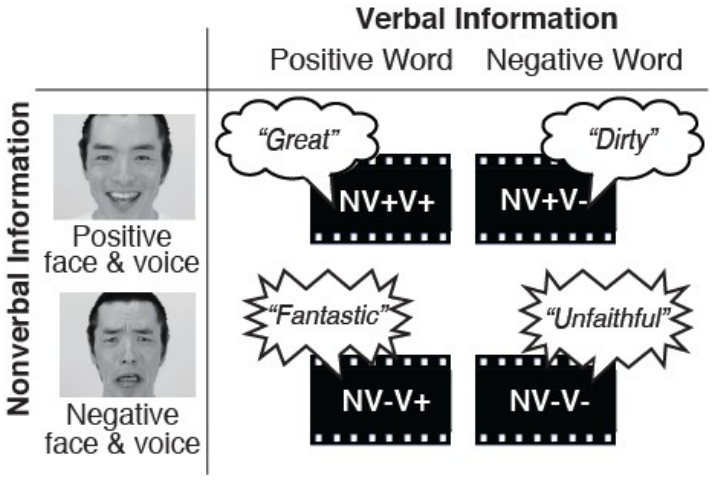
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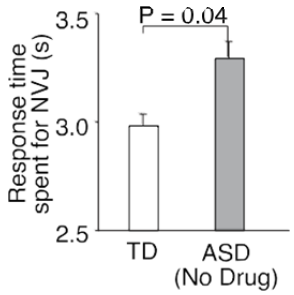
eFigure 1. Types of Movie Stimuli

The movie stimuli consisted of four types: positive/negative nonverbal information × positive/negative verbal information. NV: nonverbal information, V: verbal information. These stimuli were used in our previous case-control study[1].



eFigure 2. Difference in Response Time for NVJ Between TD and Individuals With ASD

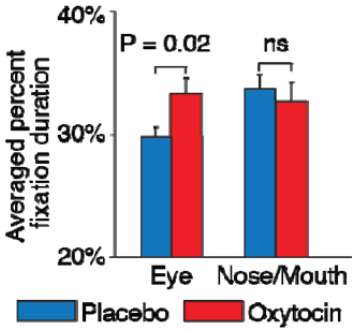
Behavioral data obtained in our previous case-control study [1] showed a significant difference in the response time for NVJs (t = 2.4, P = 0.04, in a two-sample t-test). Note that, to keep consistency with the present study, this re-analysis does not include TD and ASD participants who showed too many unnatural responses to the congruent stimuli.



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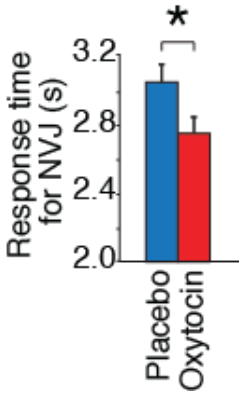
eFigure 3. Oxytocin's Effects on Eye Gaze

Intranasal oxytocin significantly increased the fixation time on the eye area but did not affect fixation time on the nose/mouth area.



eFigure 4. Oxytocin's Effect on Response Time for NVJs

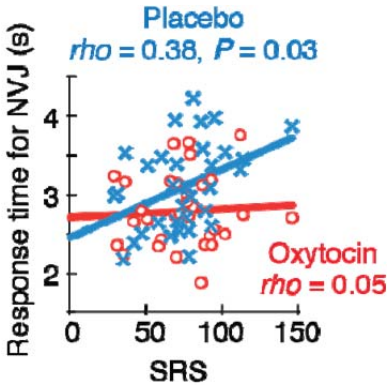
Oxytocin shortened the response time for NVJs, independently of oxytocin's effects on the number of NJVs.



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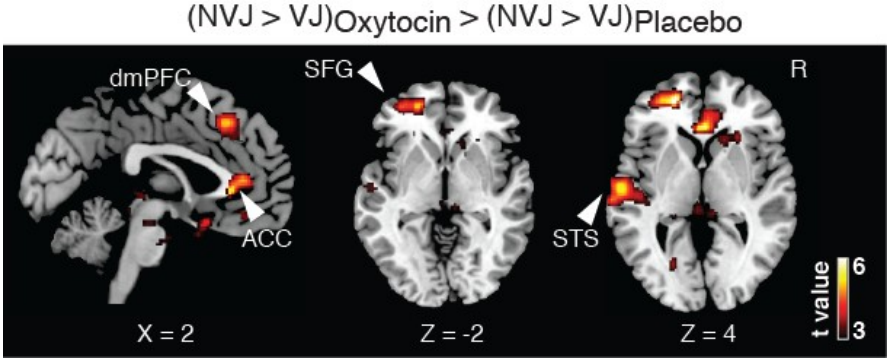
eFigure 5. Oxytocin-Induced Shortening of Response Times to Incongruent Stimuli

The response times for NVJs showed a significantly positive correlation with SRS only after administration of placebo.



eFigure 6. Results of Whole-Brain Analysis of Oxytocin-Induced Activity Changes

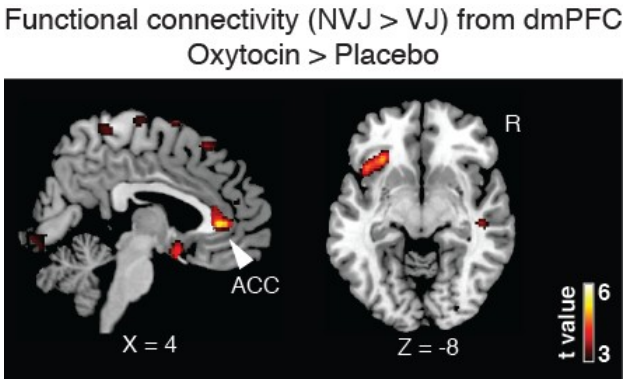
Activity in the ACC, dmPFC, left SFG, and left STS showed significant increases with oxytocin. The threshold of this map was set at $P < 0.001$, uncorrected for presentation purposes.



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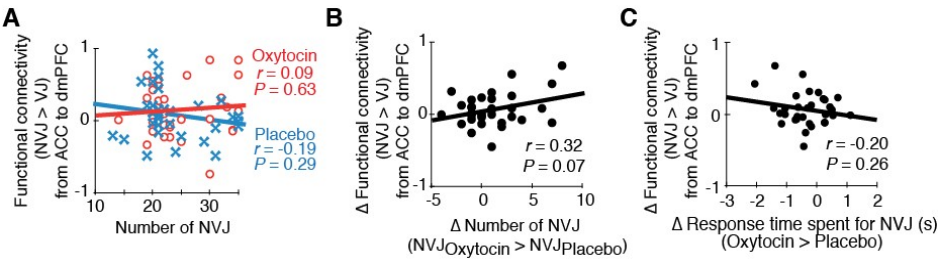
eFigure 7. Results of the Whole-Brain Analysis of Functional Connectivity

Oxytocin significantly increased the NVJ-specific functional connectivity from the dmPFC to the ACC and left AI.



eFigure 8. Functional Connectivity From the ACC to the dmPFC

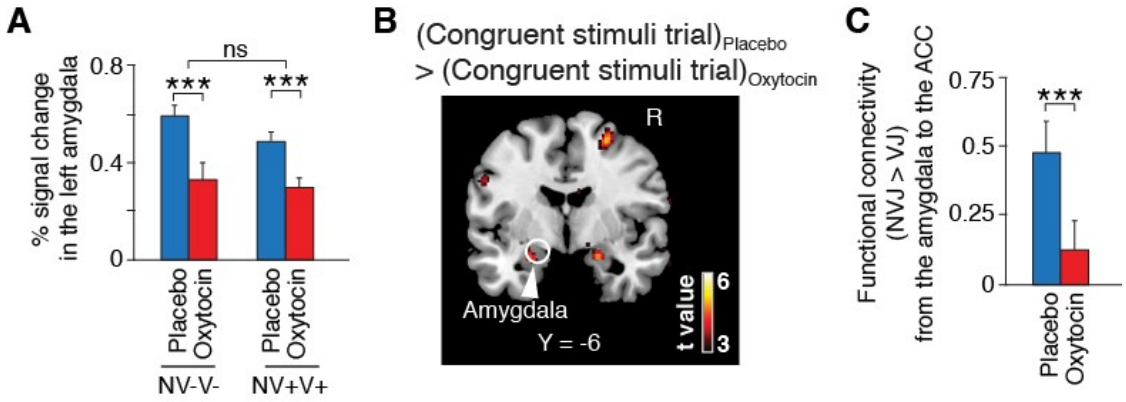
(A) Unlike the connectivity from dmPFC to the ACC, the functional connectivity from the ACC to the dmPFC did not show a significant correlation with the number of NVJs. **(B)** The oxytocin-induced change in the connectivity was not significantly correlated with the oxytocin-induced change in the number of NVJs. **(C)** There was not a significant correlation between the oxytocin-induced change in connectivity and oxytocin-induced change in response times for NVJs.



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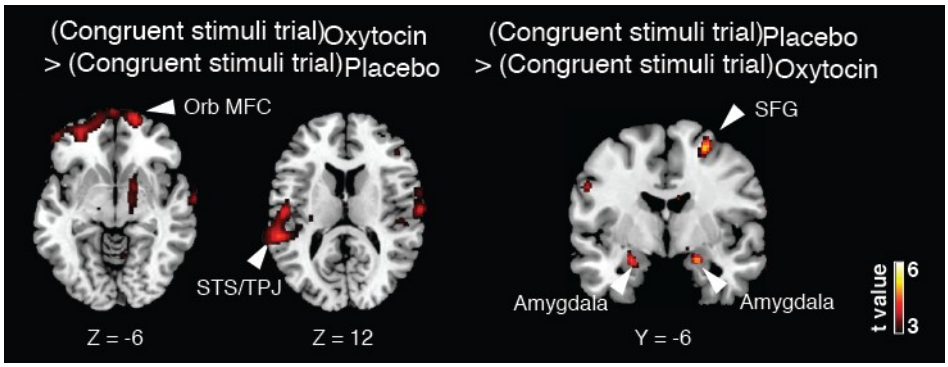
eFigure 9. Oxytocin's Effects on Amygdalar Activity

(A) Oxytocin decreased brain activity in the pre-defined left amygdala (eTable 2). This effect was independent of the emotional direction of the congruent stimuli. ***: $P < 0.001$ in post-hoc paired *t*-tests. **(B)** The effect of oxytocin on amygdalar activity was confirmed in the whole brain analysis. **(C)** Oxytocin also decreased the NVJ-specific functional connectivity from the amygdala to the ACC.



eFigure 10. The Effects of Oxytocin on Processing of Congruent Stimuli

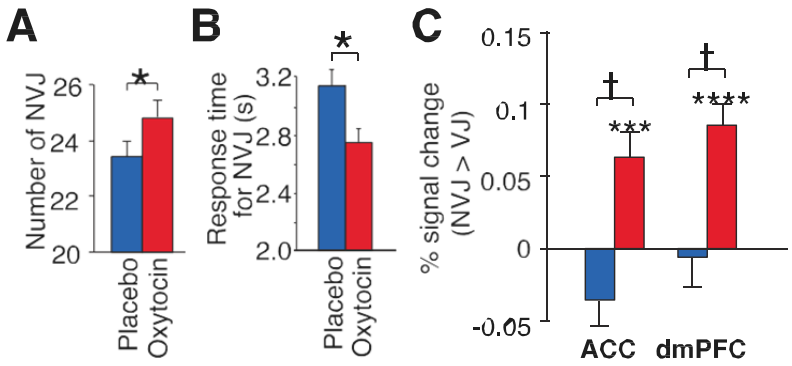
Oxytocin increased brain activity during congruent stimuli trials in the right orbitomedial frontal cortex (Orb MFC) and left STS/TPJ. In contrast, activity in the bilateral amygdalae and right SFG was decreased by oxytocin.



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eFigure 11. Robustness of Results to Participant Selection

Even when we included all available data (i.e., $N = 38$), we obtained essentially the same results on the main outcome measures. *: P < 0.05, ***: P < 0.001, ****: P < 0.0005, †: P < 0.05, Bonferroni-corrected.



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eTable 1. Demographic Characteristics of Participants Whose Data Were Analyzed

The effects of oxytocin on blood pressure and pulse rate were evaluated with paired *t*-tests, and oxytocin's effects on sleepiness scores were evaluated with the Wilcoxon rank sum test. SD: standard deviation, IQ: intelligence quotient, FIQ: full IQ, VIQ: verbal IQ, PIQ: Performance IQ, SES: socio-economic status, which was assessed based on the Hollingshead index. Higher scores indicate lower status. HFA: high-functioning autism. PDD-NOS: pervasive developmental disorder-not otherwise specified.

	Subjects with ASDs (N=33)
Variables	Mean ± SD
Age (range)	28.5 (20-41) ± 5.9
Height, cm	170.4 ± 4.8
Body wegiht, kg	66.9 ± 12.9
SES*	2.9 ± 1.2
Parental SES*	2.3 ± 0.7
Handedness: Right / Mixed / Left	27 / 3 / 3
IQ**	
FIQ	106.4 ±11.2
VIQ	111.8 ± 13.4
PIQ	95.2 ± 16.6
Autism spectrum disorder subtype	32 HFA / 1 Asperger
Autism Diagnostic Interview-Revised	
Social	14.5 ± 6.5
Communication	11.7 ± 4.0
Repetitive	4.3 ± 2.1
Social Responsiveness Scale	74.5 ± 25.5

	Oxytocin	Placebo	Statistical value	P value
Systolic blood pressure	123 ± 14.9	115.5 ± 12.4	t(32) = 2.4	0.02
Diastolic blood pressure	81.7 ± 8.3	76.8 ± 10.3	t(32) = 2.1	0.04
Pulse	70.5 ± 10.1	67.5 ± 9.5	t(32) = 1.9	0.07
Stanford sleepiness score	3.2 ± 1.7	3.4 ± 1.8	z = 0.58	0.56

*Socio-economic status, assessed using the Hollingshead. Higher scores indicate lower status.

**HFA: High-functioning autism

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eTable 2. Regions of Interest Whose Activity Showed Significant Difference Between ASD and TD Individuals in Our Previous Study

Brain activity in the seven regions of interest showed significant increases or decreases in our previous case-control study [1] in which we employed the same psychological task and analyzed the fMRI data in the same manner, i.e., by comparing NVJ-specific brain activity between ASD and TD individuals. MNI: Montreal Neurological Institute; ACC: anterior cingulate cortex; dmPFC: dorsal medial prefrontal cortex; IFG: inferior frontal gyrus; AI: anterior insula; STP: superior temporal pole.

		MNI coordinate		
Right/Left	**Anatomical label**	**x**	**y**	**z**
Regions whose activity was less in ASD individuals than in TD individuals.				
Left	ACC	2	34	8
Left	dmPFC	0	30	52
Right	IFG	54	28	22
Right	AI	38	42	-4
Left	AI	-38	16	-8
Regions whose activity was larger in ASD individuals than in TD individuals.				
Left	Amygdala	-26	-6	-20
Right	STP	56	2	0

ACC: Anterior cingulate cortex; vmPFC: ventral medial prefrontal cortex; dmPFC: dorsal medial prefrontal cortex; IFG: inferior frontal gyrus; AI: anterior insula; STP: superior temporal pole.

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eTable 3. Behavioral Responses to Congruent Stimuli (Control Stimuli)

Each cell shows the number of each type of the judgments of the congruent stimuli. In our analysis, we used data obtained during only natural responses (i.e. Friend to NV+V+ and Foe to NV-V-).

	Placebo		Oxytocin	
Type of judgment	NV-V-	NV+V+	NV-V-	NV+V+
Friend	1.4±1.8	18.3±2.1	1.4±1.9	18.6±1.7
Foe	18.6±1.8	1.7±2.1	18.6±1.9	1.4±1.7

mean ± s.d.

eTable 4. Behavioral Responses to Incongruent Stimuli

Each cell shows the number of each type of the judgments of the incongruent stimuli. Grey cells are NVJ, whereas white ones VJ. Although there was no significant main effect of friend/foe judgments, there was significant difference in the number of NVJ between oxytocin and placebo conditions (Figure 1C).

	Placebo		Oxytocin	
Type of judgment	NV-V+	NV+V-	NV-V+	NV+V-
Friend	8.0±4.1	11.6±4.8	7.6±4.3	12.3±4.9
Foe	12.0±4.1	8.4±4.8	12.4±4.3	7.7±4.9

NVJ	VJ

mean ± s.d.

eTable 5. Results of Voxelwise Whole-Brain Analysis of Functional Connectivity

P < 0.05, FWE-corrected. Abbreviations are described in eTable 2.

		MNI coordinate				
Right/Left	**Anatomical label**	**x**	**y**	**z**	**cluster size**	**t value**
(NVJ > VJ) x (Oxytocin > Placebo)						
Right	ACC	4	40	4	276	4.8
Left	AI	-36	20	-8	359	5.1

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