Original Investigation

Mitigation of Sociocommunicational Deficits of Autism Through Oxytocin-Induced Recovery of Medial Prefrontal Activity
A Randomized Trial

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IMPORTANCE Sociocommunicational deficits make it difficult for individuals with autism spectrum disorders (ASD) to understand communication content with conflicting verbal and nonverbal information. Despite growing prospects for oxytocin as a therapeutic agent for ASD, no direct neurobiological evidence exists for oxytocin's beneficial effects on this core symptom of ASD. This is slowing clinical application of the neuropeptide.

OBJECTIVE To directly examine whether oxytocin has beneficial effects on the sociocommunicational deficits of ASD using both behavioral and neural measures.

DESIGN, SETTING, AND PARTICIPANTS At the University of Tokyo Hospital, we conducted a randomized, double-blind, placebo-controlled, within-subject-crossover, single-site experimental trial in which intranasal oxytocin and placebo were administered. A total of 40 highly functioning men with ASD participated and were randomized in the trial.

INTERVENTIONS Single-dose intranasal administration of oxytocin (24 IU) and placebo.

MAIN OUTCOMES AND MEASURES Using functional magnetic resonance imaging, we examined effects of oxytocin on behavioral neural responses of the participants to a social psychological task. In our previous case-control study using the same psychological task, when making decisions about social information with conflicting verbal and nonverbal contents, participants with ASD made judgments based on nonverbal contents less frequently with longer time and could not induce enough activation in the medial prefrontal cortex. Therefore, our main outcomes and measures were the frequency of the nonverbal information–based judgments (NVJs), the response time for NVJs, and brain activity of the medial prefrontal cortex during NVJs.

RESULTS Intranasal oxytocin enabled the participants to make NVJs more frequently ($P = .03$) with shorter response time ($P = .02$). During the mitigated behavior, oxytocin increased the originally diminished brain activity in the medial prefrontal cortex ($P < .001$). Moreover, oxytocin enhanced functional coordination in the area ($P < .001$), and the magnitude of these neural effects was predictive of the behavioral effects ($P \leq .01$).

CONCLUSIONS AND RELEVANCE These findings provide the first neurobiological evidence for oxytocin's beneficial effects on sociocommunicational deficits of ASD and give us the initial account for neurobiological mechanisms underlying any beneficial effects of the neuropeptide.

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Autism spectrum disorders (ASD) affect approximately 1% of the general population,[1] and currently there is no established pharmacological treatment for their core symptoms. The disorders have 3 core symptoms: impaired social interaction, impairments in communication, and repetitive and restricted behavior.[2,3] As indicated through revision of the definition of the disorder (*DSM-5*, http://www.dsm5.org/), sociocommunicational deficits derived from the integration of the former 2 core symptoms make it difficult for individuals with ASD to understand the content of complex social communications, such as irony and humor, that have conflicting nonverbal and verbal information.[4-11] Because of this difficulty, individuals with ASD, even those with high intelligence, often fail to properly understand others' intentions in their daily communications.

Recently, a series of studies raised the possibility that some ASD symptoms may be relieved by administration of oxytocin,[3,12,13] a neuropeptide known to play a key role in social and affiliative behavior.[12-21] Prior studies with typically developed (TD) individuals have shown that oxytocin modulates their neural responses to social stimuli, enhances social perception, and promotes social interactions with modulation of functional connectivity.[14,15,18,22,23] Although there is no neurobiological evidence for the effects of oxytocin on individuals with ASD and few studies have included oxytocin's effect as a main effect in their analyses, previous preliminary behavioral studies in individuals with ASD in small sample sizes ($N < 20$) have supported oxytocin's potential as a therapy for some of the core symptoms of ASD. Restricted and repetitive behaviors were significantly reduced by oxytocin infusion.[24] Social interactions and social recognition were significantly enhanced by intranasal administration of oxytocin to individuals with ASD when the social interactions mostly consisted of nonverbal information, such as facial expressions and eye gazes.[25-28]

However, to our knowledge, no previous study has directly demonstrated that oxytocin improves sociocommunicational deficits of ASD in simultaneous processing of both verbal and nonverbal information. This lack of study is likely partly attributable to the difficulty in designing a psychological task that is sensitive to the deficits, which are occasionally masked by the preserved intelligence of highly functioning individuals with ASD.[4,25-27,29] We devised a psychological task that requires participants to make judgments regarding the intentions of others based on sociocommunicational content in which the verbal and nonverbal information conflicts. This task is thought to be appropriate for examining oxytocin's effects on sociocommunicational deficits of ASD because, in our previous case-control study using functional magnetic resonance imaging (fMRI),[8] the same task enabled us to detect ASD-specific neural responses whose magnitudes significantly correlated with the severity of sociocommunicational deficits. Moreover, previous studies have also suggested that psychological tasks focusing on understanding others' intention, based on a process of verbal and nonverbal conflicts, can effectively detect sociocommunicational deficits in individuals with ASD.[4,6,7,9-11] Furthermore, previous studies have also demonstrated oxytocin's effects on perception and interpretation of nonverbal social information including facial expression and emotional prosody.[25-28,30,31] Considering these previous findings, it is reasonable to assume that observation of behavioral and neural activity during the judgment of others' intention, based on the verbal and nonverbal conflicting information, may allow detection of oxytocin's effects on sociocommunicational deficits in individuals with ASD.

In the present task, participants are presented with short movies in which professional actors speak an emotional word (verbal information) with an emotional facial expression and expressive voice prosody (nonverbal information) (**Figure 1**A). The movies consist of 2 types of emotionally congruent movies with negative (NV–V–) or positive (NV+V+) nonverbal and verbal information and 2 types of incongruent movies with negative nonverbal and positive verbal (NV–V+) or positive nonverbal and negative verbal (NV+V–) information (eFigure 1 in Supplement). On the basis of the integration of the verbal and nonverbal information, participants are instructed to make a "friend or foe" judgment of the actor in each movie (Figure 1B). According to the type of information with the strongest effects on the judgments, the responses to the incongruent stimuli were classified into nonverbal information-based judgments (NVJs) and verbal information-based judgments (VJs). For example, a judgment of foe in response to an NV–V+ stimulus was regarded as an NVJ, and a judgment of foe in response to an NV+V– stimulus was regarded as a VJ (Figure 1B). It should be noted that this psychological task does not focus on the difference in cognitive processes between a friend judgment and a foe judgment but instead focuses on the difference between NVJs and VJs.

In our previous case-control fMRI study that compared behavioral and neural responses during the task between TD individuals and those with ASD,[8] the individuals with ASD showed significantly fewer NVJs with significantly longer response times (eFigure 2 in Supplement). During the judgments, they also showed significantly diminished brain activity in several brain regions, including medial prefrontal regions (eTable 2 in Supplement).

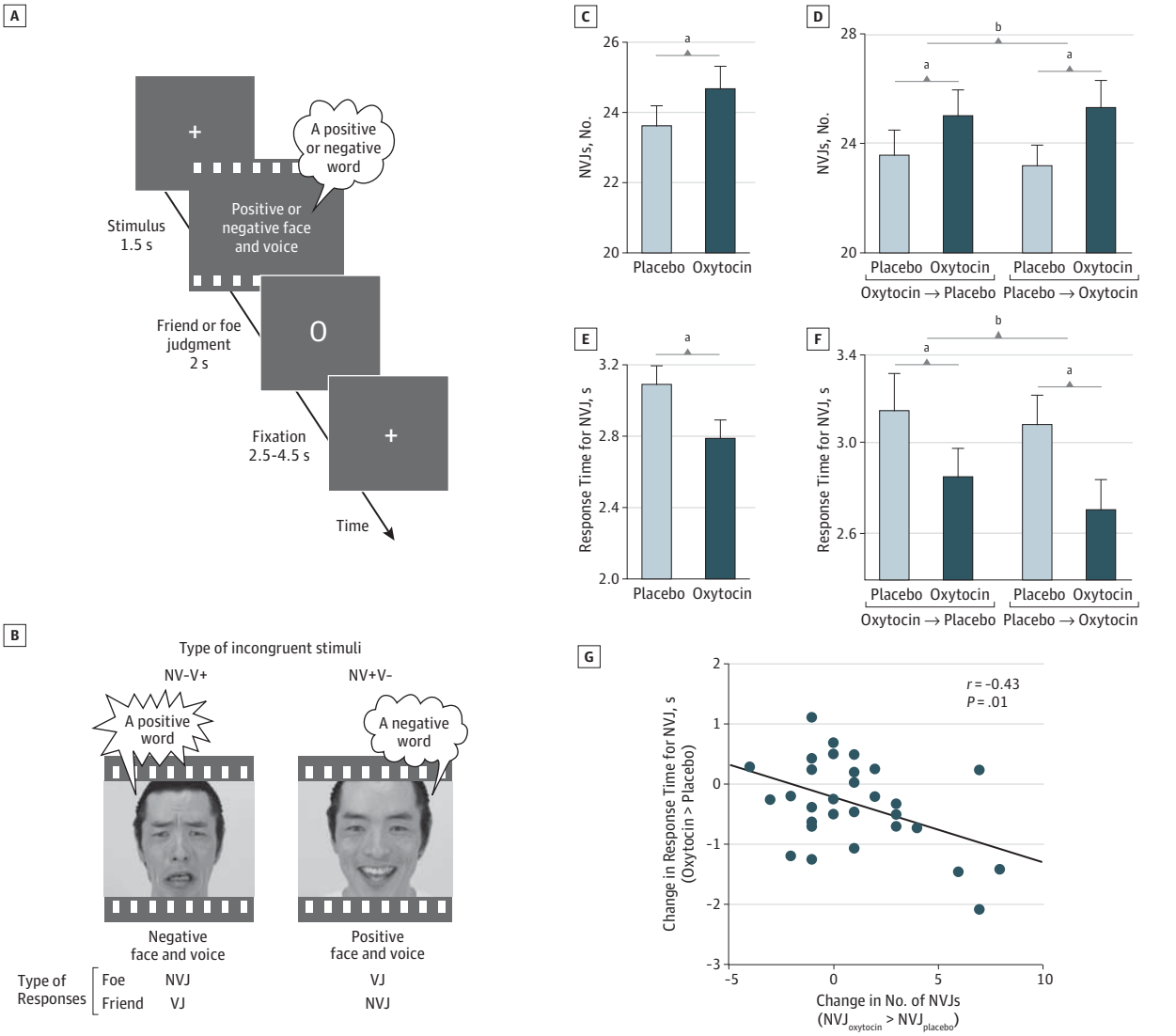
In this double-blind, placebo-controlled, within-subject-crossover trial for single-dose intranasal administration of oxytocin, we examined the behavioral and neural effects of the neuropeptide on the 3 impaired outcomes, ie, the number of NVJs, the response time for NVJs, and the medial prefrontal activity during NVJs.

Methods

Participants

Forty highly functioning men with ASD participated in this study (**Figure 2**) because of their firm diagnosis, age (≥20 years), sex (male), full-scale IQ (>80), and written consent. The 40 participants with ASD included all 15 participants with ASD in our previous case-control fMRI study.[8] Seven of them were excluded because of technical problems (2 participants), current use of a psychotropic medication (2 participants), or frequent atypical responses to congruent stimuli (3 participants). Participants showing frequent atypical responses were de-

Figure 1. Task Design and Effects on Behavior



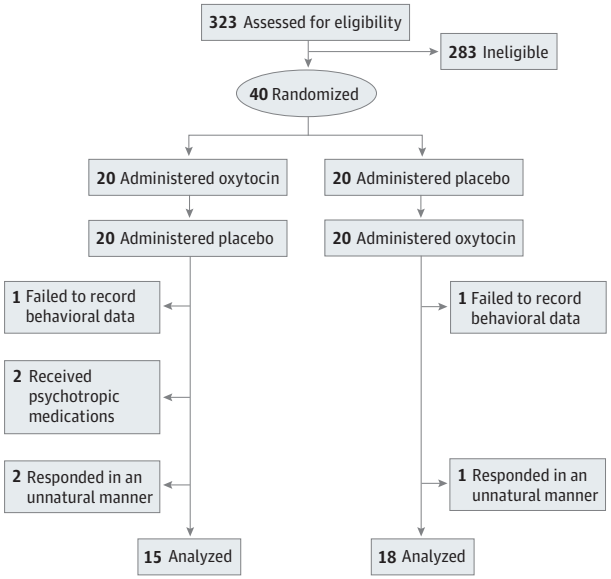
A, Participants were instructed to make friend or foe judgments for each movie in a series of short movies in which a professional actor spoke an emotionally positive or negative word (verbal information) while exhibiting emotionally positive or negative facial and vocal expressions (nonverbal information). B, Responses to incongruent stimuli were classified as nonverbal information–based judgments (NVJs) or verbal information–based judgments (VJs). NV–V+ indicates stimuli with negative nonverbal information and positive verbal information; NV+V–, stimuli with positive nonverbal information and negative verbal information. C, Intranasal administration of oxytocin significantly increased the number of NVJs, which was lower in untreated individuals with autism spectrum disorders compared with typically developing individuals in our previous study.[8] Error bars indicate standard error of the mean. D, This oxytocin-induced increase in the number of NVJs was observed regardless of the order of drug administration. E, Intranasal oxytocin significantly decreased response times for the NVJs, which were longer in untreated individuals with autism spectrum disorders than in typically developing individuals (eFigure 2 in Supplement). F, This oxytocin-induced shortening of response times for NVJs was observed regardless of the order of drug administration. G, The oxytocin-induced increase in the number of NVJs significantly correlated with oxytocin-induced shortening of response times to incongruent stimuli, which suggests a common neural effect of oxytocin.
[a] $P < .05$.
[b] $P > .05$.

fined as those who judged congruent stimuli in counterintuitive directions in more than half of the trials (ie, friend and foe judgments of NV–V– and NV+V+, respectively) (Figure 2). Consequently, we analyzed fMRI data collected from the remaining 33 nonmedicated participants (mean [SD] age, 28.5 [5.9] years) (eTable 1 in Supplement). Written informed consent was obtained from all the participants. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the institutional review board of the University of Tokyo Hospital.

Diagnosis

Experienced psychiatrists (H.Y. and N.K.) carefully diagnosed the participants as having ASD on the basis of the strict criteria of *DSM-IV-TR*[2] after more than 2 months of follow-up examinations. Diagnoses were validated by 1 psychiatrist or psy-

Figure 2. Enrollment and Randomization of Participants



Among the 40 individuals with autism spectrum disorders enrolled in this trial, 7 were excluded from the analysis because of technical problems in recording behavioral data (2 participants), current use of psychotropic medications (2 participants), or frequent unnatural responses to the congruent stimuli (3 participants). As their unnatural responses, 1 of the 3 excluded participants judged actors with positive nonverbal and positive verbal information as foe in 19 of 20 stimuli in the oxytocin session and 11 of 20 stimuli in the placebo session, 1 participant judged actors with positive nonverbal and positive verbal information as foe in 20 of 20 stimuli in both the oxytocin and placebo sessions, and the other judged actors with negative nonverbal and negative verbal information as friend in 11 of 20 stimuli in both the oxytocin and placebo sessions. In the remaining 33 participants, the number of atypical responses ranged from 0 to 4. We observed essentially the same statistical conclusions when the 2 medicated participants were not excluded (eAppendix 2 in Supplement).

chologist (H.K. or Miho Kuroda, PhD) who confirmed the diagnoses based on the Japanese version of the Autism Diagnostic Interview-Revised[32] and/or the Autism Diagnostic Observation Schedule.[33] All participants with ASD exhibited IQs ranging from average to above average in the full scale of the Wechsler Adult Intelligence Scale-Revised, Japanese version[34] (eAppendix 1 in Supplement).

Randomization and Masking of Drug Administration

The randomization and masking manager randomly assigned participants to initially administered oxytocin or a placebo group. The manager completely covered the label of sprays for blinding from the participants and the other research members (eAppendix 1 in Supplement).

Interventions

The participants with ASD intranasally[35] received a single dose of oxytocin (24 IU; Syntocinon Nasal Spray)[14,30,36] or placebo at a 1-week interval in a pseudorandom order 40 minutes before the scanning. To avoid any subjective effects of the substances other than those caused by oxytocin, the placebo contained all inactive ingredients.[14] No adverse effects of oxytocin were observed in this study (eAppendix 1 in Supplement).

Task and Stimuli

The task and stimuli in this study were the same as those used in our previous case-control[8] and healthy participant[37] studies. The stimuli consisted of 80 original monochrome movies with a length of 1.5 seconds (**Video**). After sufficient training using different movie stimuli, the participants underwent the friend or foe judgment task using the 80 stimuli (Figure 1A; for details of the task paradigm, see eAppendix 1 in Supplement).

MRI Scanning

A 3-T MRI scanner (GE Healthcare) at the University of Tokyo Hospital was used. Axial T2-weighted images were recorded for the anatomical coregistration. Gradient-echo echo-planar sequences were used for functional imaging (repetition time, 3 seconds; echo time, 35 milliseconds; flip angle, 80°; matrix, 4 × 4 × 4 mm^3; 22 slices; ventral to dorsal interleaved acquisition). The first 5 functional images in each run were discarded to allow for equilibrium of longitudinal magnetization (eAppendix 1 in Supplement).

Statistical Analysis

Behavioral Analysis

After classifying the participants' responses to the incongruent stimuli into NVJs and VJs (Figure 1B), we examined the effects of oxytocin on the number of NVJs by conducting a repeated-measures 3-way analysis of variance (ANOVA) of the number of judgments for the incongruent stimuli (type of drug [oxytocin or placebo] × type of response [friend or foe] × type of stimuli [NV−V+ or NV+V−]). Using a 3-way ANOVA with the same structure, we also examined the effects of oxytocin on response times to incongruent stimuli. Response time was calculated as time spent from the start of each movie to each button press (ie, each response time includes time for viewing each 1.5-second movie) (eAppendix 1 in Supplement).

fMRI Analysis

The fMRI data were analyzed using SPM8 software (http://www.fil.ion.ucl.ac.uk/spm/). The data were preprocessed through realignment, correction of slice timing, normalization to the default template with interpolation to a 2 × 2 × 2-mm space (eAppendix 1 in Supplement),[38] and spatial smoothing (full-width half-maximum, 8 mm; Gaussian filter), and high-pass temporal filtering (128 seconds). For our event-related fMRI design at the single-participant level, we used a general linear model with regressors for the 4 types of stimuli × the 2 types of responses. Using these single participant-level data, we evaluated oxytocin's NVJ-specific effects on brain activity by estimating the effects of the interaction in a repeated-measures 3-way ANOVA of brain activity (type of drug × type of response × type of stimuli). This analysis was conducted as a region-of-interest (ROI) analysis for 7 brain regions (eTable 2 in Supplement) that were a priori selected on the basis of our previous case-control study.[8] The ROIs were defined as 4-mm-radius spheres. To validate the spatial specificity of oxytocin's neural effects, we also conducted a whole-brain analysis using a random-effects model ($P < .05$ corrected based on the family-wise error [FWE] rate). By comparing brain activ-

ity between NVJs and VJs, we reduced the effects of cognitive components related to general judgments on brain activity.

Because we found significant effects of oxytocin only on the 2 medial prefrontal ROIs (the anterior cingulate cortex [ACC] and dorsal medial prefrontal cortex [dmPFC]) among the 7 ROIs, we then estimated functional connectivity between them by calculating the psychophysiological interaction (PPI)[39,40] using the method implemented in SPM8. The PPI analyses were first conducted between the ACC and dmPFC. To confirm the spatial specificity of the ROI-based PPI analyses, we then conducted voxelwise whole-brain PPI analyses with the dmPFC as a seed (eAppendix 1 in Supplement).

As a control, we also evaluated oxytocin's effects on brain activity during congruent stimuli trials at a whole-brain level.

Results

Oxytocin Increases the Number of NVJs

A repeated-measures 3-way ANOVA on the number of judgments of the incongruent stimuli detected a significant 3-way interaction ($F_{1,32}$ = 5.1, P = .03) (eTable 4 in Supplement). A post hoc paired t test showed that the number of NVJs was significantly greater during the oxytocin session than during the placebo session in the individuals with ASD (t_{32} = 2.2, P = .03, Cohen d = 0.55) (Figure 1C). This effect was independent of the order in which drugs were administered (Figure 1D) because in a mixed-design repeated-measures 2-way ANOVA on the number of NVJs (the order of drug × the type of drug), we did not detect a significant main effect of the order of drug administration or any significant interaction (P = .74 and P = .86, respectively) but detected a significant main effect of drug type ($F_{1,32}$ = 4.9, P = .03). These findings suggest that intranasal oxytocin significantly increases the number of NVJs, which was originally below the levels of TD individuals.[8]

Oxytocin Shortens Response Times in the Processing of Incongruent Stimuli

A repeated-measures 3-way ANOVA on the response times for the incongruent stimuli detected a significant 3-way interaction ($F_{1,32}$ = 5.9, P = .02). A post hoc paired t test revealed that oxytocin administration significantly shortened the response times for NVJs as compared with placebo administration (t_{32} = 2.5, P = .02, d = 0.63) (Figure 1E). Conducting a mixed-design repeated-measures 2-way ANOVA on the response times for NVJs (the order of drug × the type of drug), we confirmed that this behavioral effect was independent of the order in which drugs were administered (no significant main effect of the order of drug administration [P = .67] or any type of significant interaction [P = .71]; significant main effect of types of drug [$F_{1,32}$ = 4.8, P = .04]) (Figure 1F). In addition, 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 = .04) (eFigure 4 in Supplement).

Together, these behavioral results suggest that intranasal oxytocin enables individuals with ASD to process verbal and nonverbal incongruent social information in a more typical and smoother manner. Furthermore, the magnitudes of the 2 behavioral effects were significantly correlated (r = −0.43, P = .01) (Figure 1G), which suggests the existence of common neural mechanisms by which oxytocin affects these behavioral processes.

Oxytocin Recovers Impaired Medial Prefrontal Activity

We next investigated the neural mechanisms underlying the behavioral effects of oxytocin. A repeated-measures 3-way ANOVA on brain activity of the 7 ROIs (eTable 2 in Supplement) revealed significant 3-way interactions in the ACC and dmPFC ($F_{1,32}$ > 15.5, P < .05, Bonferroni corrected for the 7 ROIs; neither main effects nor 2-way interactions, P > .40) (**Figure 3**A). A post hoc paired t test showed that oxytocin significantly increased NVJ-specific brain activity (NVJs minus VJs) in these 2 medial prefrontal cortex regions (mPFCs) (ACC: t_{32} = 4.1, P < .001, d = 1.1; dmPFC: t_{32} = 3.9, P < .001, d = 1.1).

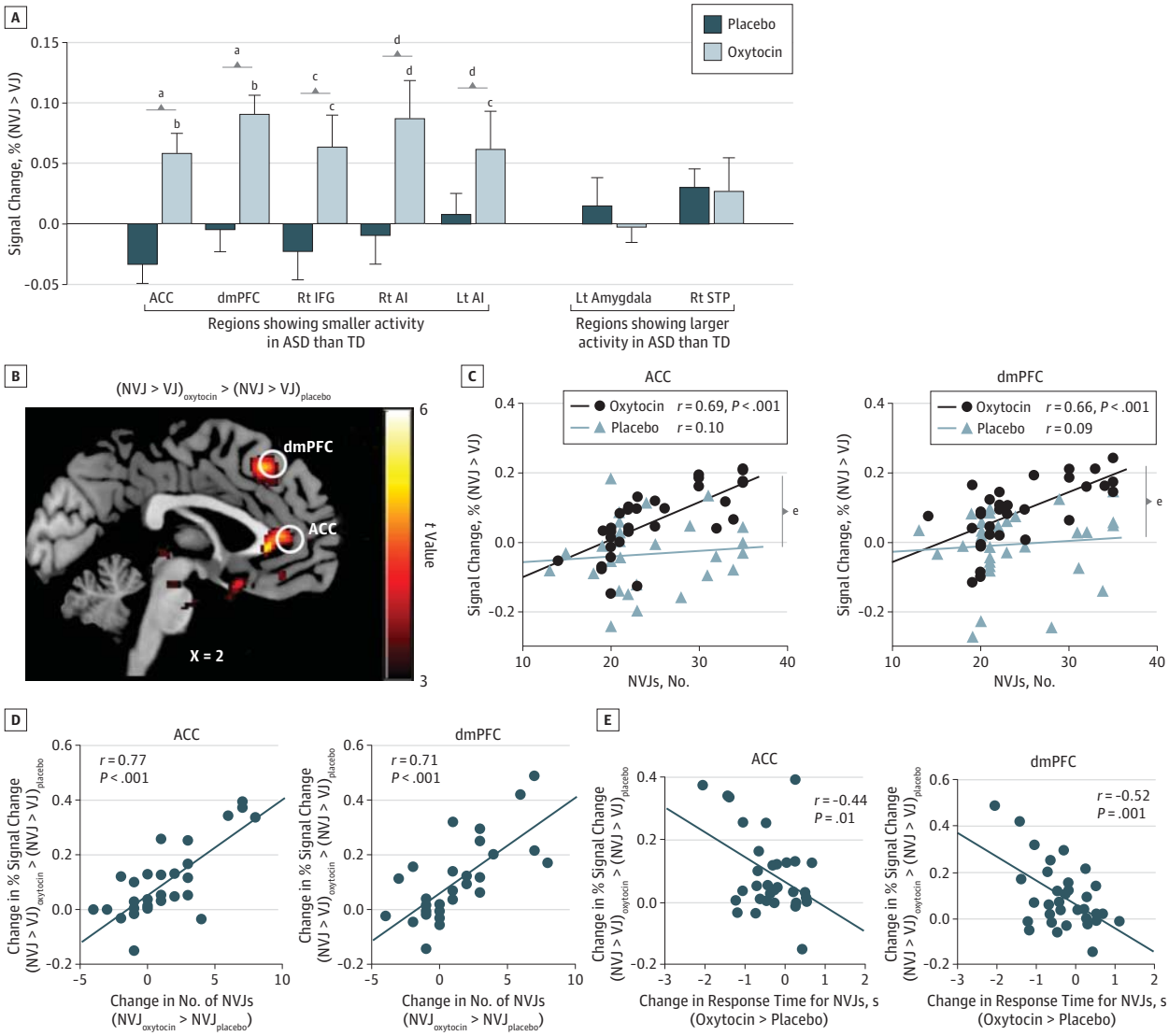
The spatial specificity of our ROI analysis was confirmed by a stricter voxelwise whole-brain search, which found that the 2 mPFCs showed significant oxytocin-induced increases in NVJ-specific brain activity (ie, $[\text{NVJs} > \text{VJs}]_{\text{oxytocin}} > [\text{NVJs} > \text{VJs}]_{\text{placebo}}$; t_{32} > 5.1, P_{FWE} < .05, d > 1.5) (Figure 3B). Although other brain regions also showed significant increases in activity (eFigure 6 in Supplement; **Table**), the locations of the medial prefrontal activations in this whole-brain analysis were close to and partially overlapped with those of our predefined ACC and dmPFC ROIs (Figure 3B), suggesting that oxytocin does not increase overall brain activity but specifically recovers the originally diminished activities of the ACC and dmPFC.

Oxytocin Makes Medial Prefrontal Activity Patterns of ASD Resemble Those of TD

We found that intranasal oxytocin not only restores activity but also makes the activity patterns in the mPFCs more similar to those we observed in TD individuals in our previous study.[8] First, only in the oxytocin session, brain activity in the ACC and dmPFC was significantly greater during NVJs than VJs (ACC: t_{32} = 3.6, P < .001; dmPFC: t_{32} = 5.8, P < .001) (Figure 3A). In our previous study,[8] this activity pattern was observed only in TD individuals but not in individuals with ASD without any drug administration.

Moreover, we found another similarity in the relationship between behavioral and neural responses between TD individuals and individuals with ASD who received oxytocin. In our previous case-control study,[8] we observed significantly positive correlations across participants between the number of NVJs and NVJ-specific brain activity in the ACC and dmPFC in TD individuals but not in individuals with ASD. Here, we found significant positive correlations between the number of NVJs and NVJ-specific brain activity in both the ACC and dmPFC only after oxytocin administration (ACC: r = 0.69, P < .001; dmPFC: r = 0.66, P < .001; P < .05 Bonferroni corrected for the 7 ROIs) (Figure 3C). The differences between the correlation coefficients under oxytocin and placebo sessions

Figure 3. Effects on Brain Activity



A, Oxytocin significantly increased nonverbal information–based judgment (NVJ)–specific activity (NVJ > verbal information–based judgment [VJ]) only in the anterior cingulate cortex (ACC) and dorsal medial prefrontal cortex (dmPFC), whose activity was lower in untreated individuals with autism spectrum disorders (ASD) than in typically developing (TD) individuals in our previous study.[8] Even in the oxytocin session alone, activity in the ACC and dmPFC was significantly greater during NVJs than VJs, which had previously been observed only in TD individuals and not in individuals with ASD.[8] To show the interaction of brain activity (type of drug × type of response × type of incongruent stimuli), we previously calculated the interaction between the latter 2 factors as the difference between NVJ and VJ. AI indicates anterior insula; IFG: inferior prefrontal gyrus; Lt, left; Rt, right; STP: superior temporal pole; and error bars, standard error of the mean. B, A voxelwise whole-brain search found significant activations in the medial prefrontal cortex (Table; eFigure 6 in Supplement), an area related to various introspective and social cognitions. Its location partially overlapped with our predefined ACC and dmPFC regions of interest (circles), which supports the regional specificity of oxytocin's neural effects. $P < .001$, uncorrected for presentation purposes. C, The NVJ-specific activity in the ACC and dmPFC showed significant positive correlations with the number of NVJs only after oxytocin administration. In our previous case-control study,[8] these correlations were also observed in TD individuals but not in ASD individuals. Oxytocin-induced increases in NVJ-specific brain activity in the ACC and dmPFC were significantly correlated with oxytocin-induced increases in the number of NVJs (D) and oxytocin-induced shortening of response times for NVJs (E).

[a] $P < .05$, Bonferroni corrected.
[b] $P < .001$.
[c] $P < .05$.
[d] $P < .01$.
[e] $P < .01$ in a test of the differences between 2 Pearson correlation coefficients.

were statistically significant in both regions (ACC: $Z = 2.9$, $P = .002$; dmPFC: $Z = 2.7$, $P = .003$). These results suggest that oxytocin enabled the participants with ASD to recruit their ACCs and dmPFCs for processing of social communication content in a manner that is similar to TD individuals.

Oxytocin's Neural Effects Are Predictive of Its Behavioral Effects

Furthermore, we examined whether our observed neural effects are the basis for the observed behavioral effects and found significant correlations between oxytocin-engendered in-

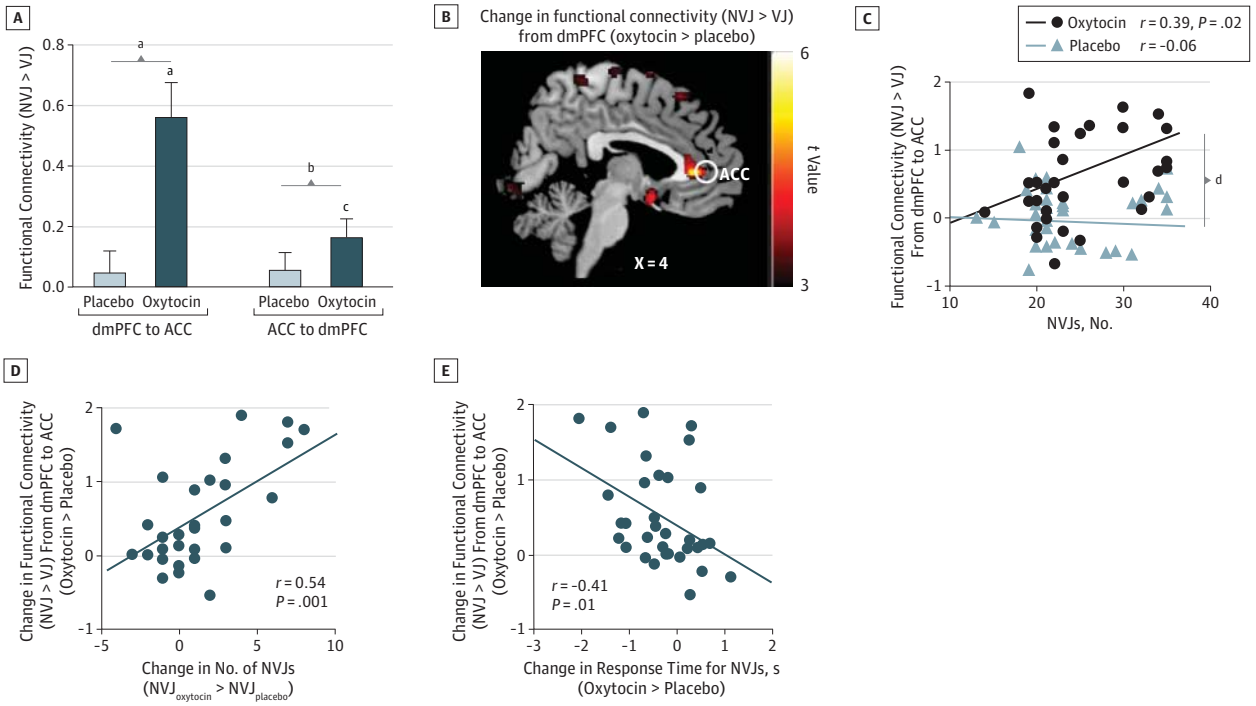
Table. Results of a Voxelwise Whole-Brain Analysis[a]

Right or Left	Anatomical Label	MNI Coordinate			Cluster Size, No. of Voxels	*t* Value
		x	y	z		
Right	ACC	0	36	4	316	5.1
Right	dmPFC	2	26	48	412	5.5
Left	SFG	−34	50	−2	348	5.9
Left	STS	−58	−16	4	582	5.3

Abbreviations: ACC, anterior cingulate cortex; dmPFC, dorsal medial prefrontal cortex; MNI, Montreal Neurological Institute; SFG, superior frontal gyrus; STS, superior temporal sulcus.

[a] (Nonverbal information–based judgment > verbal information–based judgment)$_{oxytocin}$ > (nonverbal information–based judgment > verbal information–based judgment)$_{placebo}$; $P < .05$, family-wise error corrected.

Figure 4. Effects of Oxytocin on Functional Connectivity



A, Intranasal oxytocin significantly enhanced nonverbal information–based judgment (NVJ)–specific functional connectivity (psychophysiological interaction) from the dorsal medial prefrontal cortex (dmPFC) to the anterior cingulate cortex (ACC), whereas it did not enhance the opposite connectivity. Significance is shown for greater functional connectivity during NVJs than verbal information–based judgments (VJs) in the oxytocin session alone. Error bars indicate standard error of the mean. B, Our predefined ACC region of interest partially overlapped with the region that showed increased connectivity from the dmPFC in a voxelwise whole-brain connectivity analysis with the dmPFC seed (eTable 5 in Supplement). This result reveals the spatial specificity of oxytocin-induced enhancements of functional connectivity from the dmPFC. $P < .001$, uncorrected for presentation purposes. C, The NVJ-specific connectivity from the dmPFC to the ACC significantly correlated with the number of NVJs only after oxytocin administration, which suggests that this connectivity is associated with NVJs. Oxytocin-induced enhancement of functional connectivity from the dmPFC to the ACC significantly correlated with oxytocin-induced increases in the number of NVJs (D) and shortening of response times for NVJs (E).

[a] $P < .001$.
[b] $P > .05$.
[c] $P < .01$ in 1-sample *t* test.
[d] $P < .05$.

creases in the number of NVJs and the increases in NVJ-specific brain activity in both the ACC and dmPFC across participants (ACC: $r = 0.77$, $P < .001$; dmPFC: $r = 0.71$, $P < .001$; $P < .05$ Bonferroni corrected for the 2 regions) (Figure 3D). We also detected significant correlations between oxytocin-engendered decreases in response times for NVJs and increases in NVJ-specific activity in both the ACC and dmPFC (ACC: $r = -0.44$, $P = .01$; dmPFC: $r = -0.52$, $P = .001$; $P < .05$ Bonferroni corrected for the 2 regions) (Figure 3E).

Oxytocin Enhances Functional Coordination Among mPFCs

These findings imply that coordinated activity between the ACC and dmPFC mediates the beneficial effects of oxytocin on behavioral difficulties in individuals with ASD. Indeed, functional connectivity analyses revealed that oxytocin significantly increased the NVJ-specific connectivity from the dmPFC to the ACC ($t_{32} = 4.4$, $P < .001$, paired *t* test) (**Figure 4**A). The spatial specificity of this effect was confirmed by a voxelwise whole-brain connectivity analysis, which detected a sig-

nificant oxytocin-induced increase in connectivity from the dmPFC to a medial prefrontal region that was near our predefined ACC ROI (t_{32} = 4.8, P_{FWE} < .05) (Figure 4B; eFigure 7 and eTable 5 in Supplement). Moreover, the magnitude of this NVJ-specific functional connectivity exhibited a significantly positive correlation with the number of NVJs only after oxytocin administration (r = 0.39, P = .02) (Figure 4C); the difference in the correlations between the oxytocin and placebo sessions was statistically significant (Z = 1.8, P = .03). Furthermore, the oxytocin-induced enhancement of functional connectivity from the dmPFC to the ACC was significantly positively correlated with both oxytocin-induced increases in the number of NVJs (r = 0.54, P = .001) (Figure 4D) and oxytocin-induced shortening of NVJ response times (r = −0.41, P = .01) (Figure 4E). These results suggest that oxytocin might mitigate the sociocommunicational deficits of ASD through the coordinated enhancement of activity in the dmPFC and ACC.

Oxytocin's Effects on Amygdalar Activity in Congruent Control Stimuli Trials

As a control, we examined oxytocin's suppressive effect on amygdalar responses to emotional stimuli, which has been reported in a series of previous studies.[15,17,26,36,41-45] A repeated-measures 2-way ANOVA of amygdalar activity during congruent stimuli trials (type of drug [oxytocin or placebo] × type of congruent stimuli) detected a significant decrease in the amygdala, whose location was predefined based on our previous case-control study[8] ($F_{1,32}$ = 13.5, P < .001; no other significant main effect or interaction) (eFigure 9A in Supplement). A voxelwise whole-brain analysis confirmed the effect on the amygdala as well as on other brain regions (P_{FWE} < .05) (eFigure 9B and eFigure 10 in Supplement).

Additional results such as oxytocin's effects on eye gaze (eFigure 3 in Supplement), relationship of oxytocin-induced shortening of response times to social responsiveness scale (eFigure 5 in Supplement), functional connectivity from the ACC to the dmPFC (eFigure 8 in Supplement), robustness of results to participant selection (eFigure 11 in Supplement), and behavior to control stimuli (eTable 3 in Supplement) are also reported.

Discussion

This study demonstrates that intranasal administration of oxytocin enables highly functioning individuals with ASD to exhibit more typical and smoother behavioral responses to social communication for which verbal and nonverbal information is conflicting. Moreover, we showed that the behavioral effects of oxytocin could be explained by oxytocin-induced restoration of deficits in brain activity in the ACC and dmPFC and oxytocin-induced enhancement of functional connectivity from the dmPFC to the ACC. These findings elucidate the neural mechanisms underlying oxytocin's beneficial effects for sociocommunicational deficits in ASD, and they provide, to our knowledge, the initial evidence regarding the neurobiological basis for any useful effect of oxytocin for the core symptoms of ASD. Furthermore, our results suggest that medial prefrontal activity during processing of incongruent verbal and nonverbal stimuli may be a promising neural biomarker for the evaluation of beneficial effects on sociocommunicational deficits in highly functioning individuals with ASD.

It is not clear whether the administration of oxytocin can fully recover the sociocommunicational deficits in highly functioning individuals with ASD. Indeed, in this study, the main behavioral and neurological outcomes of individuals with ASD who received oxytocin were not significantly different from those observed in TD individuals in our previous study (P > .30) (eAppendix 2 in Supplement). However, we cannot assume therapeutic effects of oxytocin based on these results. This study examined only some of the sociocommunicational deficits in ASD and did not investigate whether these beneficial effects can be maintained after long-term administration. To validate the effects found in this study, future studies need to examine both the effects of oxytocin on other aspects of sociocommunicational deficits and its effects after long-term administration.

In addition, caution should be used concerning oxytocin's potential adverse effects in future long-term clinical trials. Some previous studies with TD participants reported that oxytocin increased defensive responses to negative emotional stimuli[46] and induced avoidance of positive stimuli in a certain condition.[47] These studies imply that repeated administration of oxytocin might induce highly functioning individuals with ASD to hold overdefensive attitudes to emotionally negative information. Future long-term administration of the neuropeptide will need to take into account these potential adverse effects.

The 33 participants examined in this study include all 15 highly functioning individuals with ASD who participated in our previous case-control study.[8] Although this overlap of participants might increase the possibility of false-positives in the present findings, at the very least, the effects on the ACC and dmPFC are likely free from this potential error. Similar decreases of activity in a region close to the dmPFC have been reported in other previous studies that examined brain activity in individuals with ASD during the comprehension of irony.[20,29] Based on the correspondence between the results of this study and previous studies, it is reasonable to interpret our findings as evidence that intranasal oxytocin recovers deficits in brain activity in highly functioning individuals with ASD that are linked to the clinical severity of their sociocommunicational impairments.

Our results regarding the sensitivity of the mPFCs to exogenous oxytocin are consistent with previous studies showing the existence of a large number of oxytocin receptors in the mPFC of prairie voles[48,49] and the associations between oxytocin receptor alleles and functions and structures in the human mPFC.[19,50-52] In addition, previous fMRI studies of TD participants have suggested that oxytocin's functional effects are observed particularly in regional activity and functional connectivity among the brain regions related to social cognition.[23] Considering these microscopic and mesoscopic findings, changes in activity and connectivity related to the mPFC may be an endophenotype that links oxytocin's behavioral effects with its influences on cellular and molecular systems.

Conclusions

Our findings provide both behavioral and neural evidence for oxytocin's therapeutic effects on sociocommunicational deficits of ASD. The study also suggests an important role of mPFC activity as an endophenotype linking oxytocin's behavioral effects with its influences on cellular and molecular systems, which is translatable into future animal models.

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