Oxytocin improves behavioural and neural deficits in inferring others' social emotions in autism

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Abstract

Recent studies have suggested oxytocin's therapeutic effects on deficits in social communication and interaction in autism spectrum disorder through improvement of emotion recognition with direct emotional cues, such as facial expression and voice prosody. Although difficulty in understanding of others' social emotions and beliefs under conditions without direct emotional cues also plays an important role in autism spectrum disorder, no study has examined the potential effect of oxytocin on this difficulty. Here, we sequentially conducted both a case-control study and a clinical trial to investigate the potential effects of oxytocin on this difficulty at behavioural and neural levels measured using functional magnetic resonance imaging during a psychological task. This task was modified from Sally-Anne task, a well-known first-order false belief task. The task was optimised for investigation of the abilities to infer another person's social emotions and beliefs distinctively, in order to test hypothesis that oxytocin improves deficit in inferring others' social emotions rather than beliefs, under conditions without direct emotional cues. In the case-control study, 17 men with autism spectrum disorder showed significant behavioural deficits in inferring others' social emotions ($P = 0.018$) but not in inferring others' beliefs ($P = 0.064$) compared with 17 typically developing demographically-matched male participants. They also showed significantly less activity in the right anterior insula and posterior superior temporal sulcus during inferring others' social emotions, and in the dorsomedial prefrontal cortex during inferring others' beliefs compared with the typically developing participants ($P < 0.001$ & cluster size > 10 voxels). Then, to investigate potential effects of oxytocin on these behavioural and neural deficits, we conducted a double-blind placebo-controlled crossover within-subject trial for single-dose intranasal administration of 24 IU oxytocin in an independent group of 20 men with autism spectrum disorder. Behaviourally, oxytocin significantly increased the correct rate in inferring others' social emotions ($P = 0.043$,

one-tail). At the neural level, the peptide significantly enhanced the originally-diminished brain activity in the right anterior insula during inferring others' social emotions ($P = 0.004$), but not in the dorsomedial prefrontal cortex during inferring others' beliefs ($P = 0.858$). The present findings suggest that oxytocin enhances the ability to understand others' social emotions that have also required second-order false belief rather than first-order false beliefs under conditions without direct emotional cues in autism spectrum disorder at both the behaviour and neural levels.

(385 words)

Keywords: empathy; mentalising; neuropeptide; perspective taking; theory of mind

Abbreviations:

anterior insula (AI); Autism Diagnostic Interview-Revised (ADI-R); autism spectrum disorder (ASD); blood-oxygen-level dependent (BOLD); dorsomedial prefrontal cortex (dmPFC); functional MRI (fMRI); intelligence quotient (IQ); National Adult Reading Test (NART); regions-of-interest (ROI); Social Responsiveness Scale (SRS); superior temporal sulcus (STS); typically developing (TD)

Introduction

Autism spectrum disorder (ASD) is a developmental disorder that affects approximately 1% of the general population and has no established pharmacological treatment for its core symptoms (Centers for Disease Control and Prevention., 2012). The disorders are characterized by deficits in social communication and interaction, and repetitive and restricted behaviour (American Psychiatric Association, 2013). Among several cognitive components associated with these symptoms, inferring others' emotions and beliefs is suggested to be one of the most important cognitive components whose disturbance is deeply related to deficits in social communication and social interaction of ASD (Frith, 2001).

Inferring others' emotions and beliefs is achieved even without direct emotional cues, such as emotional facial expressions or prosody (Gallagher *et al.*, 2000; Shamay-Tsoory *et al.*, 2007). Individuals with ASD have difficulty in inferring others' emotions and beliefs under conditions without direct emotional cues as well as those with direct emotional cues (Castelli *et al.*, 2002; Kana *et al.*, 2006; Kaland *et al.*, 2008; Masten *et al.*, 2011). It is supposed that individuals with ASD with high intelligence compensate for their difficulty with the ability to infer others' beliefs (Shamay-Tsoory, 2008; Senju *et al.*, 2009), but they still have deficits in the ability to infer others' emotions (Baron-Cohen *et al.*, 1997). As the ability of inferring others' emotions without emotional cues is considered to play an important role in social relationships (Kidd and Castano. 2013), this disability is one potential reason that individuals with ASD show deficits in social communication and social interaction (van Riekel *et al.*, 2010).

Accumulating evidence supports the concept that oxytocin can induce effects on social and affiliative behaviours (Young and Wang, 2004; Jin *et al.*, 2007; Guastella *et al.*, 2008; Kim *et al.*, 2010; Bartz *et al.*, 2011; Guastella and MacLeod, 2012; Striepens *et al.*, 2012). Although oxytocin may also promote aggression and

other antisocial behaviour in some circumstances (Miller, 2013), it is thought to be a potential therapeutic approach for deficits in social communication and interaction in individuals with ASD (Van IJzendoorn and Bakermans-Kranenburg, 2011; Yamasue *et al.*, 2012; Bakermans-Kranenburg and Van IJzendoorn, 2013; Tachibana *et al.*, 2013; Veening and Olivier, 2013). In fact, several previous studies have reported oxytocin-induced improvements of autistic behaviour (Hollander *et al.*, 2003, 2007; Andari *et al.*, 2010; Guastella *et al.*, 2010; Anagnostou *et al.*, 2012), and its neural basis (Domes *et al.*, 2013a, 2013b; Gordon *et al.*, 2013; Watanabe *et al.*, 2014). It should be noted that previous studies have repeatedly investigated and demonstrated oxytocin's effectiveness in reducing difficulty in emotion recognition under conditions with direct emotional cues in individuals with ASD (Hollander *et al.*, 2007; Guastella *et al.*, 2010; Domes *et al.*, 2013b; Gordon *et al.*, 2013; Watanabe *et al.*, 2014).

However, to the best of our knowledge, no studies have reported oxytocin's effect on the abilities to infer others' emotions and beliefs under conditions without direct emotional cues in individuals with ASD. There are many behavioural studies involving typically developing (TD) participants that reported that oxytocin improves recognition of emotion (Guastella and MacLeod, 2012; Shahrestani *et al.*, 2013), and one study with TD participants showed oxytocin's effect on inferring others' emotions but not on inferring others' beliefs (Hurlemann *et al.*, 2010). Thus, we hypothesized that oxytocin may ease the deficit in inferring others' emotions rather than beliefs in individuals with ASD and at least partially mitigate neural deficits of inferring others' emotions rather than those of beliefs.

To test our hypothesis, we conducted two experiments consisting of a case-control study and a clinical trial. For this purpose, we developed a psychological task by optimising the Sally-Anne task, a well-known task for test of first-order false beliefs, to investigate abilities to infer another person's emotions and beliefs distinctively

under conditions without direct emotional cues. We used the task as is, to investigate neural correlates of

difficulty in inferring others' beliefs (Sommer *et al.*, 2007). Additionally, we also investigated neural bases of

deficit in inferring others' emotions by adding a question at the end of the story using the same cartoon. The

emotions in the present study are the social emotions that involve a two-person situation where one's loss

depends on another's gain (Shamay-Tsoory *et al.*, 2007) and include, for example, gloating when one

successfully deceives another (Ortony *et al.*, 1990; van Dijk *et al.*, 2006). To correctly answer the question for

social emotion conditions, an understanding of second-order false belief is also required. First, we conducted a

functional MRI (fMRI) study to distinctively investigate neural correlates of these behavioural characteristics in

individuals with ASD by comparing brain activity between individuals with ASD and TD. In the second

experiment, we performed a double-blind, placebo-controlled, crossover trial for single-dose intranasal

administration of oxytocin to investigate the behavioural and neural effect of the neuropeptide on deficits in

inferring others' social emotions and belief without direct emotional cues in individuals with ASD.

Materials and methods

Procedure

The first experiment (Experiment 1) was a case-control fMRI study with a psychological task designed to study

behavioural and neural deficits in inferring others' social emotions and beliefs distinctively under conditions

without direct emotional cues, which involved individuals with ASD and TD. In Experiment 1, we compared

both behavioural performance and brain activity during the psychological task. To examine neural correlates for

deficit in inferring others' social emotions and beliefs, we explored brain regions where individuals with ASD

show less brain activity during the psychological task compared with TD participants.

The second experiment (Experiment 2) was an fMRI study in a placebo-controlled double-blind crossover clinical trial where participants with ASD were intranasally administered a single-dose of oxytocin or placebo between an interval of 1 week. This experiment was performed with the same psychological task as Experiment 1 in totally different individuals in Experiment 1, to minimise habituation of the participants to the psychological task. In Experiment 2, we tested whether oxytocin improves the behavioural deficit shown in Experiment 1 and enhances the decreased brain activity during the task in brain regions identified in Experiment 1 in individuals with ASD, by comparing behavioural results and brain activity in the oxytocin session with those given placebo.

Participants and diagnosis

None of the individuals with ASD who participated in Experiment 1 were in Experiment 2. Experiment 2 examined the main outcome of the clinical trial registered in the University Hospital Medical Information Network clinical trials registry (UMIN000004393). The participants in Experiment 2 were also participants of our study in a recent publication that reported a different outcome of the present clinical trial (UMIN000004393) in combination with another clinical trial (UMIN000002241) (Watanabe *et al.*, 2014). The recruiting method and inclusion/exclusion criteria were the same as in our previous studies and a recent report of a different outcome of the present trial (Aoki *et al.*, 2012; Watanabe *et al.*, 2012, 2014). Briefly, among 323 people with probable ASD who visited the outpatient services of The University of Tokyo hospital and Showa University Karasuyama Hospital, 40 high-functioning ASD males, who satisfied the eligibility criteria (firm diagnosis, age ≥20 years, full scale intelligence quotient (IQ) >80), were recruited. Seventeen individuals with ASD out of the 40 were assigned to Experiment 1 (29.6 ± 8.0 years old, mean ± SD)(Table 1) and 20 were

assigned to Experiment 2 (30.8 ± 6.0 y.o.)(Table 2), while the other 3 participants did not participate because of

the schedule restriction (Fig. 1). The 17 individuals with ASD participated in Experiment 1 and 3 people who

didn't participate in the study are participants in another clinical trial (UMIN000002241) that was conducted at

least 1 week apart from Experiment 1. Two individuals with ASD who participated in Experiment 1 were on

antidepressants, while one was on antipsychotics in Experiment 2. Seventeen age-, IQ-, and parental

socioeconomic status -matched TD adult males who served as controls in Experiment 1 were college students,

graduate school students, hospital staff and their acquaintances (30.4 ± 5.6 y.o.)(Table 1) (Hollingshead, 1957).

Handedness was determined using the Edinburgh Handedness Inventory (Oldfield, 1971), with a laterality

index of >0.5 used as the cut-off for right-handedness. Participants whose laterality index score ranged from

-0.5 to 0.5 were defined as mixed-handedness. The IQ of the TD group was estimated using a Japanese version

of the National Adult Reading Test (NART) (Nelson, 1982; Matsuoka *et al*., 2006). Although the scores of the

NART are well-correlated with the full scale IQ measured with Wechsler Adult Intelligence Scale Revised in

TD individuals (Matsuoka *et al*., 2006), the application of the estimation of full scale IQ using the NART is

problematic for individuals with ASD because of the well-known imbalances in their intellectual abilities.

Therefore, the IQs of the ASD participants were assessed using the full scale of the Wechsler Adult Intelligence

Scale Revised Japanese version (Wechsler, 1981).

The exclusion criteria for both groups were: current or past neurological comorbidity, traumatic brain injury

with any known cognitive consequences or loss of consciousness for more than 5 min, a history of

electroconvulsive therapy and substance abuse or addiction. An additional exclusion criterion for the control

group was a history of neuropsychiatric disorders in the participants themselves.

The protocol for diagnoses in the current study was the same as our previous studies (Aoki *et al*., 2012;

Watanabe *et al.*, 2012, 2014) that used the Diagnostic and Statistical Manual-Revision IV-Text Revision

(American Psychiatric Association, 2000) in combination with the Japanese version of the Autism Diagnostic

Interview-Revised (ADI-R) (Tsuchiya *et al.*, 2012). The ASD diagnoses of seven individuals who did not meet

the threshold in the ADI-R social domain were confirmed through an evaluation with the Autism Diagnostic

Observation Schedule (Lord *et al.*, 2000) by another certified psychologist (Miho Kuroda). All seven

individuals were diagnosed with autism based on the Autism Diagnostic Observation Schedule social

reciprocity + communication scores, Module 4, (15.2 ± 3.16, mean ±SD, range: 12−20, in which 10 is the

minimum threshold for autism). In addition to the ADI-R that is mainly based on participants' behaviour during

their childhood, we also obtained the maternal-reported Social Responsiveness Scale (SRS) to measure

participants' behaviour at the time of experiments (Constantino, 2002).

Interventions, randomisation, and masking in Experiment 2

Description of interventions, randomisation, and masking is available elsewhere (Watanabe *et al.*, 2014).

Briefly, 40 min before scanning, the individuals with ASD received a single-dose oxytocin (24 IU; Syntocinon

Spray, Novartis) intranasally (Kosfeld *et al.*, 2005) or placebo, in a randomised fashion. Participants abstained

from food and drink for 2 hr before the experiment, and from exercise, caffeine, and alcohol during the 24 hr

before the session. No adverse side effects were observed.

The randomisation and masking manager assigned each participant to an oxytocin-initially-administered or

placebo-initially-administered group with a computer-generated randomised order. Individuals in an

oxytocin-initially-administered group received placebo in the following scan 1 week later, and vice versa (Fig.

1). Concretely, the individuals with ASD who had initially received placebo received oxytocin the following

week, while the other 10 individuals with ASD who had received oxytocin in the first session were administered placebo the following week. Thus, on the whole, the 20 individuals with ASD experienced both placebo and oxytocin sessions. The manager completely covered the label of the sprays to maintain the blindness of the participants and experimenters.

MRI scanning

In both experiments, participants were scanned in the same MR scanner with the same setting. A 3T MRI scanner (GE Signa HDxt,Waukesha, WI, USA) in The University of Tokyo Hospital was used. The anatomical scanning sequence was a three-dimensional Fourier-transform spoiled-gradient-recalled acquisition during steady state (TR = 6.8 s, slice thickness = 1 mm, in-plane resolution = 1 × 1 mm). A trained neuroradiologist (H.T./H.S./W.G.) found no gross abnormalities in any of the participants. Gradient-echo echo-planar sequences were used for functional imaging (TR = 3s, TE = 35 ms, FA = 80°, $4 \times 4 \times 4$ mm^3, 42 slices, ventral to dorsal interleaved acquisition). The first five functional images in each run were discarded to allow for equilibrium of longitudinal magnetisation.

fMRI experimental procedure and paradigm

This event-related fMRI experiment was performed using E-prime 2.1 (Pittsburgh, PA, USA). During a scan, participants underwent two runs consecutively. During one run, 10 different stories were presented to the participants (Supplementary Material). Each story was presented for three times consecutively and participants were required to answer one yes-no question at the end of each presentation. Thus, participants were demanded to answer 30 questions during one run. The same set of 10 stories was presented in both 1st and 2nd runs. But

questions whose answers are "yes" in the 1st run were modified to be questions whose answers are "no" in the

2nd run, vice versa. The order of questions with "yes" and "no" was determined in a pseudo-random fashion.

The scan time for each run was approximately 8 min. Each story consisted of four black and white comic

frames based on Sally-Anne story and other stories that also require understanding of first-order false beliefs

developed by Vollm *et al.,* (Völlm *et al.,* 2006). Before the scanning procedure, all the participants were shown

the cartoon stories demonstrated in the scanner and required to answer to questions modified from the

questions in the "Control" condition in the psychological task. By doing so, the participants were trained to

understand the cartoon stories in order to complete the task appropriately without experiencing the questions of

"Belief" and "Social emotion" condition in the task.

Task conditions and trial timing are summarized in Figure 2A. Each trial consists of 1) 3.5 sec story

presentation with four frames, 2) 2 sec of a frame with a question written in white on a black screen, 3) 2.5–3.5

sec fixation cross, 4) 3 sec of a frame with comic vignette that illustrates an arrow and a star to help to answer

the question, and 5) 3 sec fixation cross. Participants were required to answer this question during the last frame

with comic and fixation cross (i.e. 4) & 5)), by pressing a button to answer yes or no. We measured the reaction

time and correct rate during this psychological task.

As summarised in Figure 2B, the four frames show a short story with two characters with neutral faces. In

the story, one character (Sally) places a ball in a left box (Frame 1) and leaves the room (Frame 2). In her

absence, another character (Anne) moves the ball to a right box (Frame 3) and then Sally returns (Frame 4)

(Baron-Cohen *et al.,* 1985; Senju *et al.,* 2009). At the end of each presentation of the story, participants are

asked the following three types of questions in a pseudo-random order: "Is actually the ball in this box?

(indicating a left box with a star)" for control, "Does she look for her ball in this box? (indicating Sally with an

arrow and a left box by a star)" for belief inference, and "Does she feel playful, seeing the box opened?" for social emotion inference (Fig. 2C).

To answer the first question, participants are required to understand the actual location of the ball. This condition was used as "Control".

For the second question, they are required to understand that Sally's actions will be based on what she believes to be true, rather than the actual location. To answer the question, understanding of first-order false beliefs and knowing the actual location is necessary. This condition that requires one to infer others' false beliefs was named as "Belief".

With regard to the third question, participants were required to answer the question about Anne's emotional status after she completed the practical joke. To rationally imagine the emotional status of the character, it is required to understand the situation correctly, i.e. that Anne believes that Sally falsely believes the ball is in the left box, but actually the ball is in the right box. Thus, to answer the question correctly, understanding of the second-order false belief (Shamay-Tsoory *et al.*, 2009), the first-order false belief, and the actual location of the ball itself, are necessary and the ability to infer Anne's emotion. This condition was named "Social emotion" (Völlm *et al.*, 2006) (Fig. 2C).

In this context, as we recognised that the "Belief" condition requires the ability to infer others' beliefs and understanding of the actual location, which is required to answer "Control" condition, the difference between "Belief" and "Control" conditions represents the ability to infer others' false beliefs; thus we named the brain activity of the contrast as "belief minus control activity". Similarly, we also recognised that the "Social emotion" condition requires the ability to infer others' social emotions and false beliefs without direct emotional cues in addition to understanding the actual location. In other words, "Social emotion" condition comprises both

cognitive components to infer others' emotion without direct emotional cues and to answer "Belief" condition.

Thus, we took difference between "Social emotion" and "Belief" conditions to represent inferring others' social

emotions without direct emotional cues and named the brain activity of the contrast as "social minus belief

activity".

Behavioural analysis

For Experiment 1, to investigate whether individuals with ASD have a behavioural deficit in inferring others'

social emotions and beliefs under conditions without direct emotional cues, we performed independent t-tests

to compare reaction time and correct rate of the psychological task between individuals with ASD and TD. As

we predicted that individuals with ASD have a deficit in inferring others' social emotions, thresholds for

statistical significance of reaction time and correct rate of Social emotion condition were set at one-tailed $P <$

0.025 (=0.05/2, number of behavioural variables in Social emotion condition). On the other hand, as we have

not assumed a behavioural deficit in high-functioning individuals with ASD in inferring others' first-order false

beliefs and understanding the story (Shamay-Tsoory, 2008; Senju *et al.*, 2009), we set the threshold of statistical

significance of reaction time and correct rate in Belief and Control conditions set at $P < 0.0125$ (=0.05/4,

number of behavioural variables of two conditions).

For Experiment 2, a paired t-test was conducted to test whether oxytocin, compared with placebo, mitigates

behavioural performance where individuals with ASD showed significant deficit in Experiment 1 compared

with TD participants (i.e. low correct rate for Social emotion, see Results). Statistical significance was set at

one-tailed $P < 0.05$.

fMRI analysis

The fMRI data were analysed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). Functional images were realigned, slice timing corrected, normalised to the default template with interpolation to a $2 \times 2 \times 2$ mm^3 space, and smoothed (full width half maximum = 8 mm, Gaussian filter). To remove low-frequency drift from the data, high-pass temporal filtering with a cut-off of 128 s was applied. Independent t-tests have showed there was no significant difference in the extent of motion (x/y/z/pitch/yaw/roll) between individuals with ASD and TD in Experiment 1. Repeated measure ANOVA has demonstrated that there was also no significant difference in it between the oxytocin and placebo sessions in individuals with ASD in Experiment 2.

At a single-subject level, we used a general linear model with three regressors (Social emotion, Belief, and Control). Each event-related regressor had an onset at the time of presentation of question and a duration that corresponded to each response time. Two contrast images of interest were estimated for each participant: (i) social minus belief activity and (ii) belief minus control activity. Only brain activities during the event with correctly answered questions were analysed.

In the second-level analysis for Experiment 1, the resulting contrast images were entered into separate group-level analyses for each contrast of interest, in which one-sample t-tests were performed. For the interaction analysis between social minus belief and belief minus control activities by group (TD vs. ASD), a statistical threshold was set at $P < 0.001$, whole brain analysis, uncorrected for multiple comparisons with minimum cluster extent 10 voxels, based on recent fMRI studies with similar sample sizes (Hsu *et al.*, 2008; Harding *et al.*, 2012; Wymbs *et al.*, 2012; Politis *et al.*, 2013). The analyses identified brain regions where individuals with ASD showed significantly less brain activity compared with TD participants. The detected

brain regions were used to determine regions-of-interest (ROI) in Experiment 2.

Then, for Experiment 2, to assess the enhancement effect of oxytocin on originally-less-than-TD brain activity in Experiment 1, we conducted an ROI analysis by performing paired t-tests of two contrasts (social minus belief activity and belief minus control activity) separately in individuals with ASD between oxytocin and placebo sessions. The ROIs were defined as spheres with radii of 4 mm around the peak coordinate, identified in Experiment 1 as the centres (Table 3). Averaged BOLD signal in these ROIs in oxytocin and placebo sessions were extracted, and paired t-tests were conducted to assess the effect of oxytocin on brain activity. As we have *a priori* hypothesis that oxytocin administration may increase originally-diminished social minus belief activity, statistical significance was set at one-tailed $P < 0.025$ (= 0.05/2, Bonferroni-corrected for two ROIs, see Results) for social minus belief activity. With regard to belief minus control activity, we adopted a statistical threshold at $P < 0.05$.

To additionally explore the global trend of oxytocin's effect on brain activity, we also conducted a voxel-wise whole-brain search for brain regions that showed significant increases induced by oxytocin in social minus belief activity compared with placebo, adopting a relatively liberal threshold (whole brain $P < 0.002$, uncorrected, minimum cluster extent 10 (Rossion *et al.*, 2003)).

Correlation between behavioural result and clinical indices

In Experiment 1, we calculated Pearson's correlation coefficients between the task performance impaired in ASD and the SRS in individuals with ASD. A statistical threshold was set at $P < 0.05$. On the other hand, to explore the influence of potential confounds, we also performed Pearson's correlation analyses between task performance, where individuals with ASD showed significant deficit, and age, IQ and parental socioeconomic

status in individuals with ASD. We also adopted a threshold for statistical significance with correction of multiple comparisons at $P < 0.017$ (=0.05/3, number of potential confounds).

Likewise for the behavioural results, we assessed a potential association between averaged BOLD signals in the ROIs where individuals with ASD showed significantly decreased social minus belief-/belief minus control activity compared with TD participants, and clinical information of interest, such as the SRS score. A statistical threshold was set at $P < 0.05$. Additionally, potential associations between averaged BOLD signals in the ROIs identified in Experiment 1 and potential confounds, such as age, IQ, and parental socioeconomic status were also assessed. A statistical threshold was set at $P < 0.017$ (=0.05/3, number of potential confounds).

Correlation between oxytocin-induced changes in brain activities and those in behaviour during the task

In Experiment 2, to test potential association between oxytocin induced brain activity enhancement and oxytocin-induced change of behaviour, we calculated relation between oxytocin induced averaged BOLD signal changes in ROIs and oxytocin-induced behavioural changes of the corresponding condition (e.g. social minus belief activity and correct rate for Social emotion condition). As there was significant effect of oxytocin only on the correct rate of Social emotion condition, the significance level was set at $P < 0.05$ (see Results section).

Evaluation of effect of recruiting different groups of individuals with ASD in identifying the ROIs in Experiment 1 and examining the effect of oxytocin in Experiment 2

We recognise it beneficial to recruit independent groups between the case-control and clinical trials. Because

participants in Experiment 2 did not experience the psychological task before, the effect of habituation to the

task is supposed to be minimised. However, one potential drawback of this method is that ROIs identified by

different groups of individuals with ASD in the Experiment 1 may not be the brain region where individuals

with ASD in Experiment 2 to whom oxytocin was actually administered would show less-than-TD brain

activity. Thus, there may not be the effect of oxytocin on the brain regions identified with individuals with ASD

in Experiment 2. To test whether the statistical conclusion is preserved after controlling the effect of differences

in groups of individuals with ASD between Experiment 1 and Experiment 2, we compared contrast images of

social minus belief activity of 20 individuals with ASD in the placebo session of Experiment 2 with those in 17

TD participants in Experiment 1. To identify the ROI where individuals with ASD in the placebo session of

Experiment 2 showed less brain activity, a statistical threshold was set at $P < 0.001$, whole brain analysis,

uncorrected for multiple comparisons with minimum cluster extent 10. Then, paired t-tests were conducted to

test the effect of oxytocin on brain activity in the ROI identified in this comparison. As this is a confirmatory

analysis, we set the same threshold for statistical significance at $P < 0.025$, as the threshold for the right anterior

insula (AI) in Experiment 2.

Excluding the effect of psychotropic medication on identifying ROIs and the effect of oxytocin

Furthermore, to examine the potential effect of psychotropic medication, we repeated the series of analyses by

excluding individuals with ASD on psychotropic medication, i.e. two individuals with ASD from Experiment 1

and one from Experiment 2.

Comparison of the behavioural results of Experiments 1 and 2

To characterise the effect of oxytocin on behaviour as an exploratory step, we have compared results of

Experiments 1 and 2. First, we conducted an independent t-test to compare the correct rate of Social emotion in

individuals with ASD after oxytocin administration in Experiment 2 with those in TD participants as well as in

individuals with ASD in Experiment 1. Then, the correct rate of Social emotion in individuals with ASD on

placebo session in Experiment 2 was compared with those in ASD participants in Experiment 1. Statistical

thresholds for these analyses were set at $P < 0.05$.

Results

Experiment 1 (ASD vs. TD)

Behavioural data

Independent t-tests revealed that the correct rate among individuals with ASD was significantly lower than that

among TD participants in the Social emotion condition ($t(32) = 2.50$, *Cohen's d* $= 0.80$, $P = 0.009$, one-tailed)

(Fig. 3A), but there was no significant difference in the correct rate in Belief ($t(32) = 1.92$, $d = 0.63$, $P = 0.064$)

and Control conditions ($t(32) = 1.04$, $d = 0.36$, $P = 0.307$) (Fig. 3A). With regard to the reaction time, there was

no significant difference between individuals with ASD and TD in Social emotion ($t(32) = 1.28$, $d = 0.44$, $P =$

0.104, one-tailed), Belief ($t(32) = 2.03$, $d = 0.67$, $P = 0.051$) and Control conditions ($t(32) = 1.04$, $d = 0.54$, $P =$

0.307) (Fig. 3B).

fMRI analysis

Social minus belief activity

We identified brain regions where individuals with ASD showed significantly less social minus belief brain

activity compared with TD participants. As illustrated in Figure 3, the whole brain analysis demonstrated less

social minus belief activity in the right AI ([*x, y, z*]=[48, 20, −14] (Montreal Neurological Institute coordinates),

k = 17; *t*(32) = 3.91; *P* < 0.001 uncorrected) (Fig. 3A, Table 3) and the right posterior superior temporal sulcus

(STS) ([56, −64, 36], *k* = 19; *t*(32) = 3.78; *P* < 0.001 uncorrected) (Fig. 3B, Table 3).

Belief minus control activity

We further examined brain regions where individuals with ASD showed significantly less brain activity

compared with TD participants during "Belief" relative to "Control" conditions. As illustrated in Figure 3,

whole brain analysis demonstrated significantly less belief minus control activity in the dorsomedial prefrontal

cortex (dmPFC) ([0,18,20], *k* = 21; *t*(32) = 3.78; *P* < 0.001 uncorrected) (Fig. 3C).

Correlation between the behavioural result and clinical indices

In Experiment 1, Pearson's correlation analyses demonstrated that the SRS was negatively correlated with the

correct rate of Social emotion (*r* = -0.675, *P* = 0.004) across individuals with ASD, whereas the ADI-R scores

were not correlated with the correct rate in Social emotion (*P* > 0.405). On the other hand, there was no

significant association between the correct rate of Social emotion and potential confounds, such as age, parental

SES and full scale IQ (*P* > 0.439).

With regard to belief minus control activity, although averaged BOLD signals in the dmPFC showed

correlation with the SRS (P = 0.029) and ADI-R reciprocal social interaction scores (*P* = 0.036), the

significances disappeared after correcting multiple comparison (*P* > 0.062). There was no significant

correlation between averaged BOLD signals in three brain regions where individuals with ASD showed

significantly lower-than-TD activity and potential confounds ($P > 0.158$).

Experiment 2 (Oxytocin vs. Placebo in individuals with ASD)

Oxytocin's effects on behaviour

As Experiment 1 showed a significantly lower correct rate in Social emotion condition among individuals with

ASD compared with TD participants, the purpose in Experiment 2 was to test whether oxytocin increases the

basically low correct rate in individuals with ASD. Paired t-test demonstrated that oxytocin significantly

increased the correct rate of Social emotion in individuals with ASD compared with placebo ($t(19) = 1.81$, $r^2 =$

0.15, $P = 0.043$, one-tailed). As the analysis has shown a trend level difference in the correct rate of Belief

condition, we have additionally investigated the potential effect of oxytocin on the correct rate. Paired t-test has

shown that there was no significant difference in the correct rate of Belief between oxytocin and placebo

sessions ($t(19) = 0.948$, $r^2 = 0.045$, $P = 0.355$).

Oxytocin's effects on social minus belief activity in the right AI

We then investigated whether oxytocin increased the originally-less-than-TD brain activity in individuals with

ASD in Experiment 1, by conducting paired t-tests to compare brain activities at the oxytocin session with

those at the placebo session. For the social minus belief activity contrast, the analysis demonstrated that

oxytocin significantly increased brain activity in the right AI ($t(19) = 3.00$, $r^2 = 0.32$, $P = 0.004$, one-tailed) (Fig.

4B, Table 4). On the other hand, there were no significant effects of oxytocin on brain activity in the right

posterior STS ($t(19) = 1.50$, $P = 0.075$, one-tailed) (Fig. 4B, Table 4). With regard to ROIs where individuals

with ASD showed significantly less belief minus control activity in Experiment 1, there were no significant

effects of oxytocin on brain activity in the dmPFC ($t(19) = 0.18$, $r^2 < 0.01$, $P = 0.858$) (Fig. 4C, Table 4).

Results of voxel-wise whole-brain analysis

To ensure global specificity of the effects of oxytocin, we also conducted a whole-brain analysis. Significant activities were found in the cuneus ([4,-86,40], $k = 47$; $t(19) = 4.36$; $P < 0.001$ uncorrected), the right anterior middle temporal gyrus ([60,0,-22], $k = 19$; $t(19) = 3.85$; $P = 0.001$ uncorrected), the right inferior frontal gyrus ([60,18,-2], $k = 39$; $t(19) = 3.60$; $P = 0.001$ uncorrected) and the right AI ([46,24,-18], $k = 17$; $t(19) = 3.57$; $P = 0.001$ uncorrected) in the oxytocin session compared with the placebo session in social minus belief activity. Analysis showed that oxytocin induced an enhancement of social minus belief activity in the brain region that covers the right AI, which partially overlapped with the right AI ROI defined from the results of Experiment 1 (Fig. 4D).

Relationship between oxytocin-induced increase of the right AI brain activity and behavioural change

There was no significant relationship between oxytocin-induced changes in social minus belief activity in the right AI and those in the correct rate of Social emotion ($r = 0.138$, $P = 0.561$).

Evaluation of effect of recruiting different groups of individuals with ASD in identifying the ROIs and examining the effect of oxytocin

The individuals with ASD in the placebo session of Experiment 2 showed significantly less social minus belief brain activity in the right AI compared TD participants in Experiment 1 ([50,18,-12], $k = 54$; $t(35) = 4.13$; $P < 0.001$ uncorrected, whole brain analysis), which includes the peak coordinate identified in Experiment 1

([48,20,-14]), in social minus belief activity contrast in this comparison (Supplementary Fig. 1A). Furthermore,

a paired t-test that compared brain activity of 4 mm radii of the peak coordinate identified by the comparison

between individuals with ASD at the placebo session and TD participants showed a significant increase of the

originally-diminished brain activity in the oxytocin session compared with placebo ($t(19) = 2.716$, $r^2 = 0.28$, P

$= 0.007$, one-tailed) (Supplementary Fig. 1A).

Excluding the effect of psychotropic medication on identifying ROIs and effect of oxytocin

The series of analyses excluding 3 out of the 37 individuals with ASD on psychotropic medication

demonstrated significantly less social minus belief activity in the right AI ([48,20,-14], $k = 11$; $t(30) = 3.34$; $P <$

0.001 uncorrected, whole brain analysis) in individuals with ASD compared with TD (Supplementary Fig. 1C),

and oxytocin induced statistically significant enhancement of social minus belief activity in the brain region

($t(18) = 3.11$, $r^2 = 0.35$, $P = 0.003$, one-tailed), indicating that the statistical conclusions are free from the effect

of psychotropic medication (Supplementary Fig. 1D).

Comparison of the behavioural results of Experiments 1 and 2

The analysis showed that there was no significant difference between the correct rate of Social emotion in

individuals with ASD after oxytocin administration in Experiment 2 ($83.5 \pm 17.0\%$) and those found in TD

participants in Experiment 1 ($84.1 \pm 13.0\%$) ($t(35) = 0.12$, $P = 0.90$). On the other hand, the correct rate of

Social emotion in individuals with ASD after oxytocin administration was significantly higher than the correct

rate of Social emotion among individuals with ASD in Experiment 1 ($70.0 \pm 19.7\%$) ($t(35) = 2.26$, $P = 0.03$).

Furthermore, it is shown that there was no significant difference in the correct rate of Social emotion between

individuals with ASD in Experiment 1 and those in the placebo session in Experiment 2 ($t(35) = 1.28$, $P = 0.21$).

Discussion

The crucial finding of the present study is that intranasal administration of oxytocin enabled high-functioning individuals with ASD to recover their behavioural and neural deficits in their ability to infer others' social emotions under conditions without direct emotional cues. We first demonstrated that individuals with ASD have behavioural difficulties, such as low accuracy in inferring others' social emotions without direct emotional cues compared with matched TD participants in Experiment 1. Experiment 1 also demonstrated that the individuals with ASD showed lower activities in the right AI and STS while inferring others' social emotions, and in the dmPFC while inferring beliefs, compared with TD participants. Then, in Experiment 2, we found that oxytocin behaviourally improved accuracy in inferring others' social emotions without direct emotional cues. At the neural level, Experiment 2 further showed that oxytocin enhanced the originally-diminished social minus belief activity in the right AI in individuals with ASD, but such effect was not observed in the belief minus control activity.

Although individuals with ASD showed significantly lower correct rate in the Social emotion condition than individuals with TD, their correct rate was much higher than 50%, a random choice, suggesting that it is unlikely that individuals with ASD did not understand the psychological task accurately. In addition, the participants stated that they understood that they were expected to imagine the emotion of the protagonist in the cartoon stories, based on a situation in which one deceived the other. It should be noted that the current findings cannot be directly extended to female individuals with ASD, or ASD individuals with intellectual disability.

Furthermore, we did not administer oxytocin in TD individuals. Because the ability to infer others' social emotions is not impaired in individuals with TD, the administration of oxytocin to individuals with TD is outside the aims of the present study, which examines the potential of oxytocin in treating this deficit. Thus, it remains unknown whether oxytocin's effects are specific to individuals with ASD.

The present result, suggesting that the AI is a neural correlate of the deficits in inferring others' social emotions without direct emotional cues, is concordant with the results from previous studies that demonstrated involvement of the AI in disturbed processing of others' social emotions, such as empathy, and also inferring others' social emotions in individuals with ASD (Bird *et al.*, 2010; Schulte-Rüther *et al.*, 2013).

The present study showed less-than-TD social minus belief activity in the STS, whose peak coordinate is [56, -65, 36], in individuals with ASD. The peak coordinate is close to the posterior STS [59, -44, 17] reported by Pelphrey *et al.*, which is also known as a neural basis of the deficit in inferring others' beliefs in individuals with ASD (Pelphrey *et al.*, 2007, 2011; Pelphrey and Carter, 2008). On the other hand, the peak coordinate is also close to the temporoparietal junction [57, -57, 18] reported by Saxe *et al.*, which is supposed to be involved in the false belief task (Saxe *et al.*, 2006). In the present study, the Social emotion condition also demands understanding of a higher order of false belief than the Belief condition (second-order vs. first-order). Thus, the less brain activity in this brain region in the social minus belief activity contrast may represent a stronger burden of understanding of false belief (Happe, 1994; Singer, 2006).

It has been repeatedly reported that the dmPFC is one of the potential neural bases in individuals with ASD for disturbed social cognition, such as inferring others' beliefs (Redcay *et al.*, 2013; Bernhardt *et al.*, 2013). Thus, it is expected that the present study has successfully detected a neural basis of deficit in inferring others' belief in individuals with ASD.

It should be noted that the task in the present study does not provide direct emotional cues, in contrast to previous studies with individuals with ASD that demonstrated oxytocin's effect on recognition of emotion under conditions with direct emotional cues, such as facial or prosodic expression (Hollander *et al.*, 2007; Guastella *et al.*, 2010; Domes *et al.*, 2013b). Additionally, the emotion in the present study is gloating when one successfully deceives another, which is a social rather than basic emotion (Ortony *et al.*, 1990; van Dijk *et al.*, 2006) and involves a two-person situation in which one's loss depends on another's gain (Shamay-Tsoory *et al.*, 2007). The present study extends oxytocin's effects on recognition of emotions to a deficit in inferring social emotions of others under conditions without direct emotional cues in individuals with ASD.

The present study showed that oxytocin enhances originally-diminished social minus belief activity in the right AI of ASD participants. The limbic/paralimbic structures are supposed be one of the brain regions where oxytocin has an influence (Ferguson *et al.*, 2001; Inoue *et al.*, 2010; Insel, 2010; Meyer-Lindenberg *et al.*, 2011; Tost *et al.*, 2011; Yamasue *et al.*, 2011). In line with this notion, previous studies with TD participants have repeatedly demonstrated that oxytocin administration enhances AI activity and its functional connectivity with other limbic/paralimbic structures underlying enhancement of social behaviour (Baumgartner *et al.*, 2008; Singer *et al.*, 2008; Riem *et al.*, 2011; Rilling *et al.*, 2012; Striepens *et al.*, 2012; Wittfoth-Schardt *et al.*, 2012). One study with TD individuals reported that the peripheral level of oxytocin has also been reported to be associated with AI activity (Strathearn *et al.*, 2009). Additionally, our recent study reported that individuals having smaller right AI volume show low prosociality and higher frequency in a common allele in the oxytocin receptor gene rs2254298A (Saito *et al.*, 2013), a risk allele for ASD in the Asian population (Yamasue, 2013). Together with these studies, the current results further support the finding that the AI is one of several potential target regions where oxytocin acts.

The results of the present study suggest that less-than-TD brain activity in the right AI is a potential neural basis of deficit in inferring others' social emotions without direct emotional cues in individuals with ASD. A single-dose of intranasal administration of oxytocin may improve the behavioural deficit in inferring others' social emotions under conditions without direct emotional cues, and enhance the originally-diminished right AI activity in individuals with ASD. These results potentially extend the potential usage of oxytocin to autistic deficits in inferring others' social emotions without direct emotional cues both at the behavioural and neural levels.

Figure legends

Figure 1
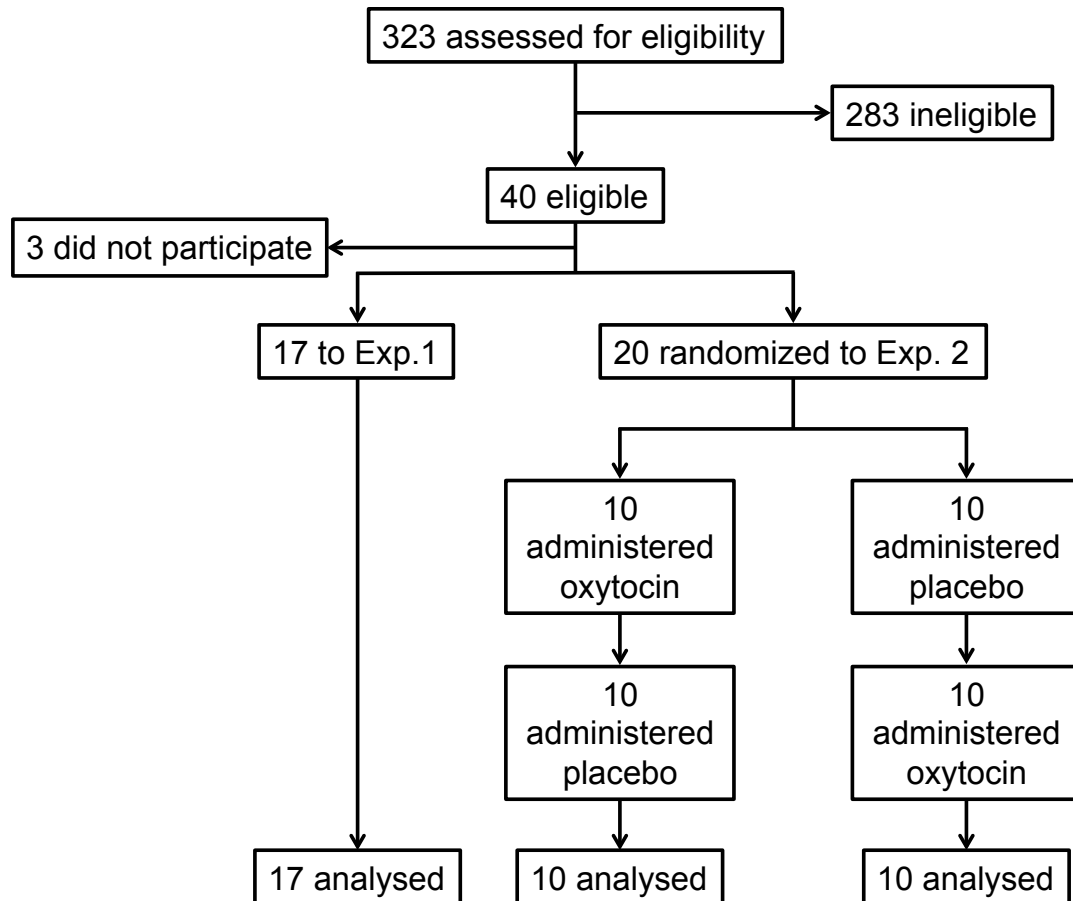
Participant recruitment. Among 323 people with probable ASD, 40 individuals with ASD were recruited.

Among them, 17 individuals with ASD were assigned to Experiment 1 and another 20 were assigned to

Experiment 2. Three individuals with ASD did not participate because of a schedule conflict. Among 20

individuals with ASD enrolled in Experiment 2, 10 individuals with ASD were assigned to an

oxytocin-initially-administered group, whereas the other 10 individuals with ASD were assigned to a
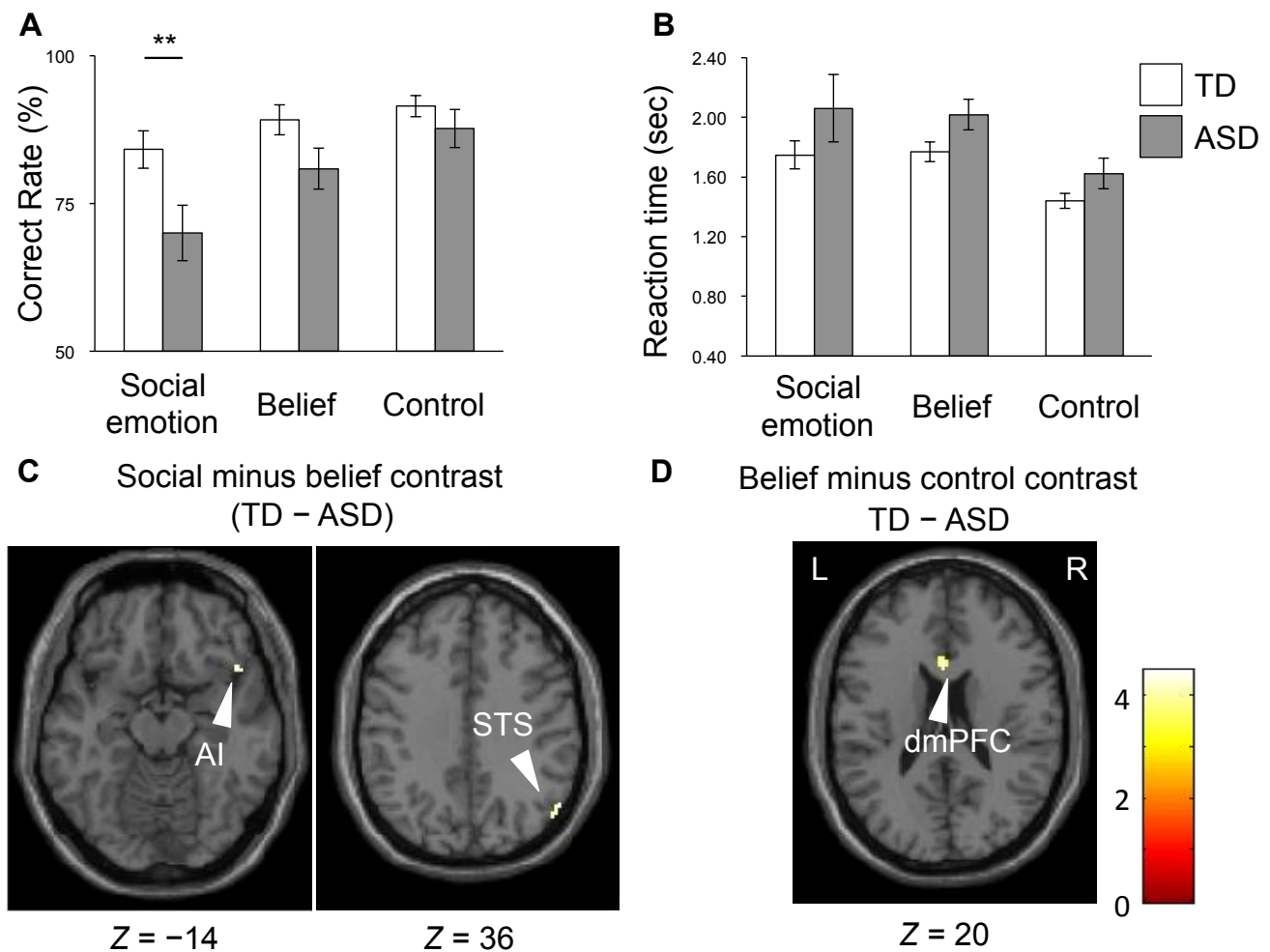
placebo-initially-administered group. Individuals in both groups received both oxytocin and placebo with an

interval of 1 week.

Figure 2
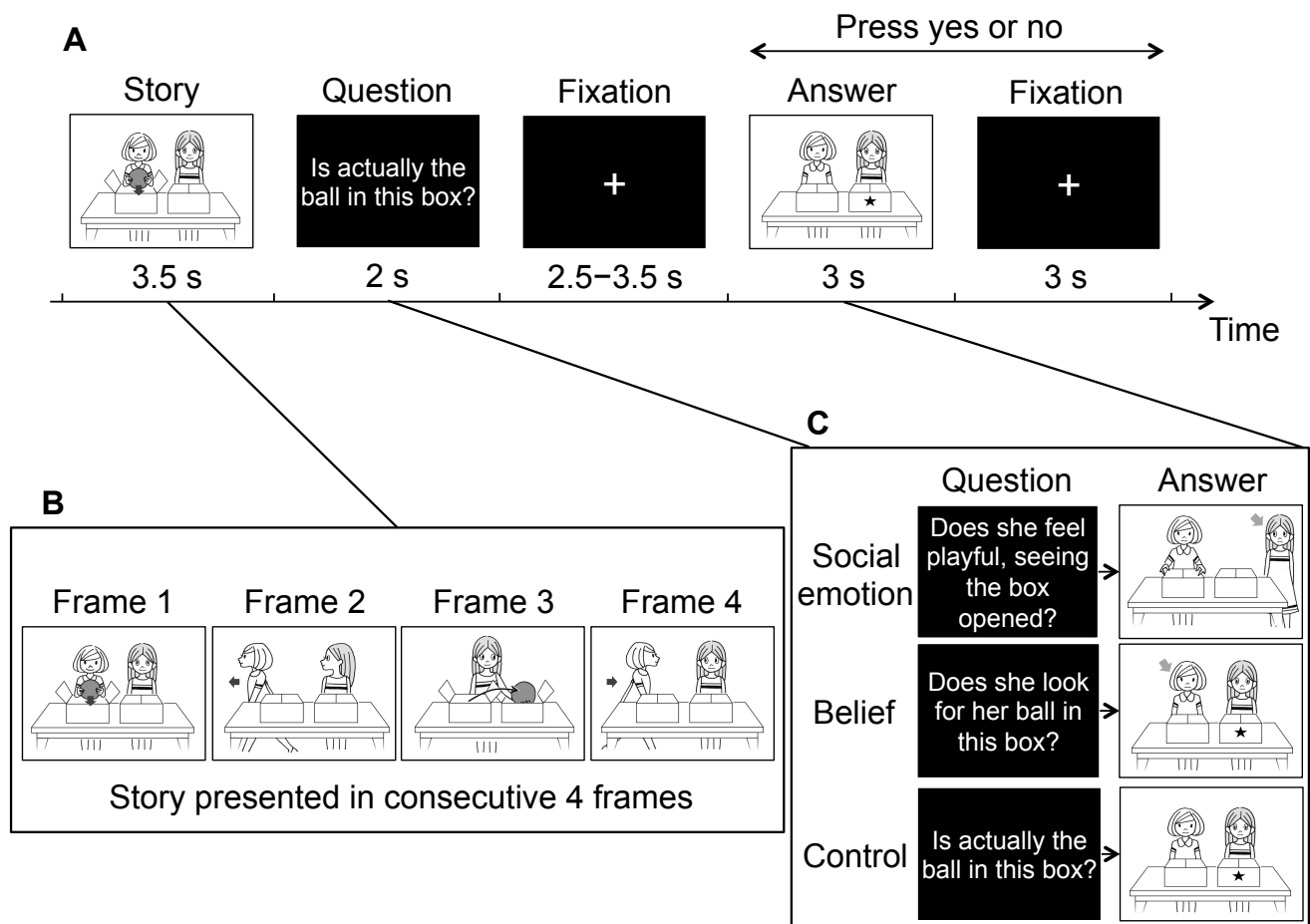
Task design. A) The figure summarises the task design with one of ten stories. 1) Each story includes four

frames of a black and white comic (3.5 sec). 2) Then, a frame with a question written in white letters on the

black screen was shown (2 sec). 3) After 2.5–3.5 sec white fixation cross on the black screen, 4) another frame

was presented to illustrate an arrow and a star to help to answer the question (3 sec). At the end of one story, 5)

a 3 sec fixation cross was shown. During the last frame and fixation cross, participants were instructed to

answer the question with yes or no. B) Each story consisted of four frames with two people. There are ten

different types of stories that require understanding of first-order false beliefs. C) Each story was presented

three times consecutively per each run. At the end of the story presentation, participants were asked to answer

the following three types of questions that asked emotion of the character, belief of the character, and fact.

These three questions appear in a pseudo-random order. Each time, an appropriate comic frame follows after

the fixation cross.

Figure 3

A

Correct Rate (%)

B

Reaction time (sec)

□ TD
■ ASD

Social emotion   Belief   Control

C Social minus belief contrast (TD − ASD)

AI

STS

Z = −14   Z = 36

D Belief minus control contrast TD − ASD

L   R

dmPFC

Z = 20

Behaviour and fMRI results in Experiment 1. (ASD vs TD). A) Effect of ASD diagnosis on correct rate for Social emotion, Belief, and Control conditions. ** TD participants demonstrated significantly higher correct rate of Social emotion condition compared with individuals with ASD ($P = 0.009$); bar: s.e.. B) Effect of diagnosis on reaction time for Social emotion, Belief, and Control conditions. C) Two panels show the brain regions where individuals with ASD showed less brain activity compared with TD participants in social minus belief brain contrast ($P < 0.001$ uncorrected) (AI: anterior insula, STS: superior temporal sulcus). Brain regions used in analyses reported in the subpanels B and C were determined from the results of Experiment 1. D) Panel shows the brain regions where individuals with ASD showed less brain activity compared with TD participants in belief minus control brain contrast ($P < 0.001$ uncorrected) (dmPFC: dorsomedial prefrontal cortex).
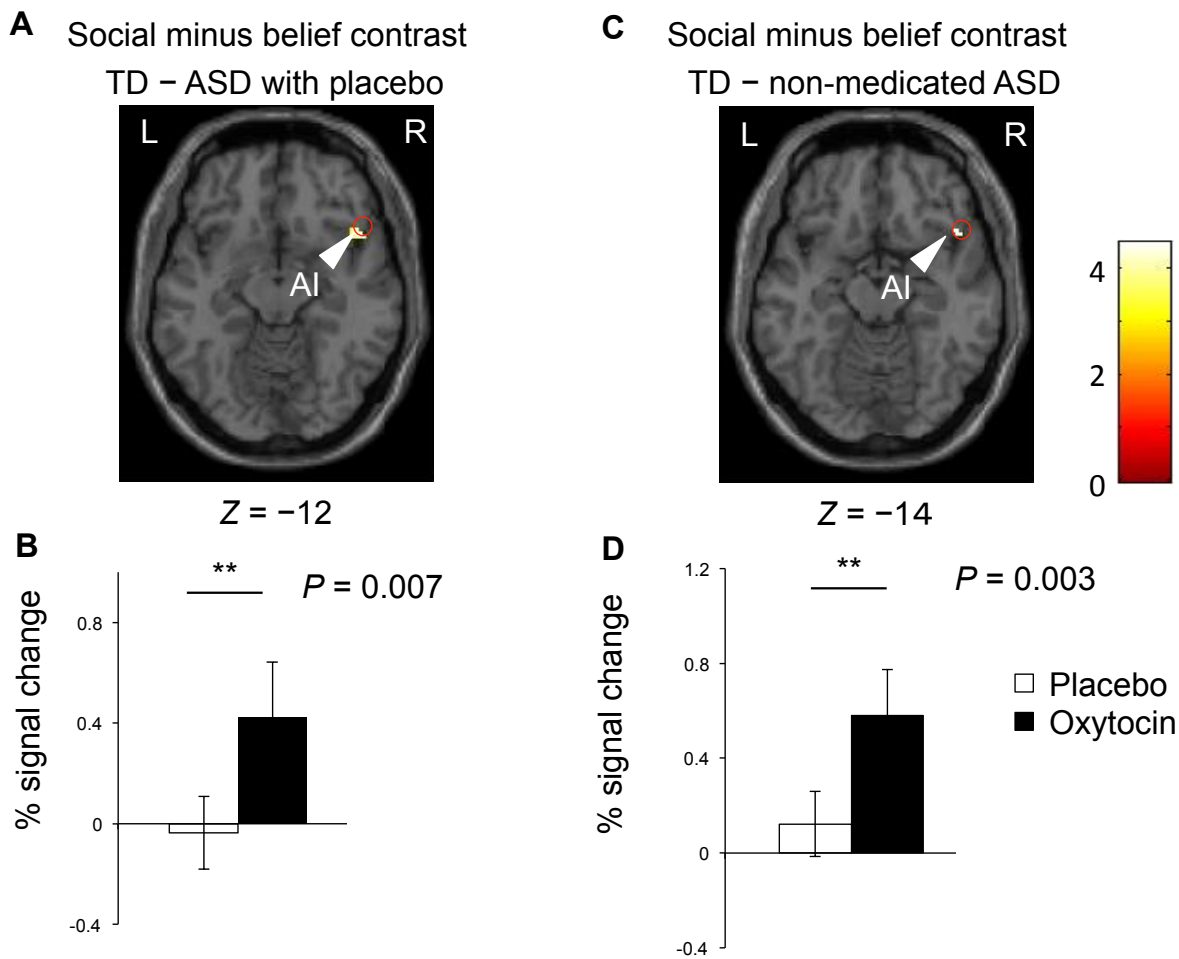
Figure 4
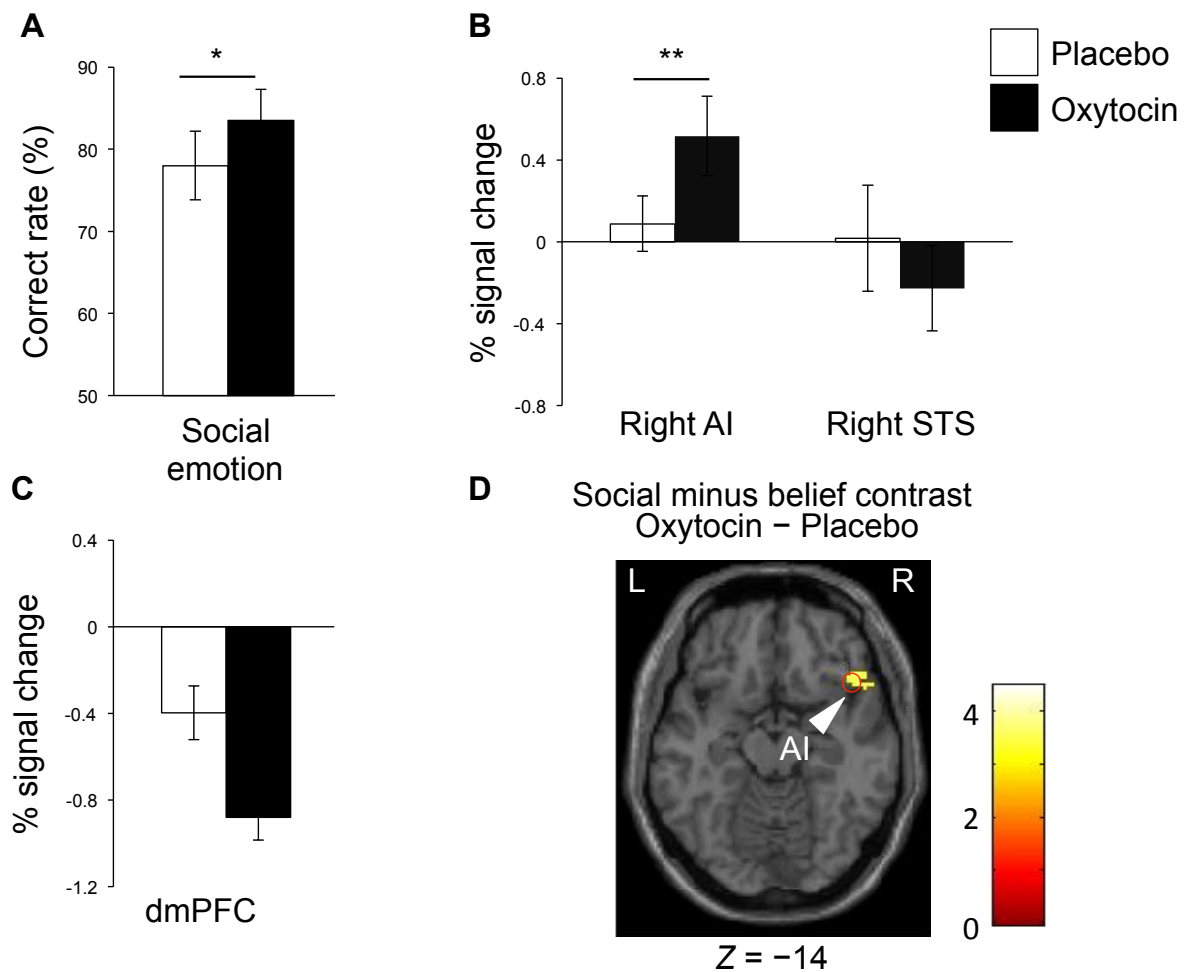
Behavioural and fMRI results of Experiment 2. randomised trial. A) *Individuals with ASD showed

significantly higher correct rate in Social emotion condition in the oxytocin session compared with the placebo

session ($P = 0.043$); bar: s.e.. B) Percent signal changes in oxytocin and placebo sessions in brain regions where

individuals with ASD showed basically less brain activity in social minus belief contrast. C) Percent signal

changes in oxytocin and placebo sessions in brain regions where individuals with ASD showed less brain

activity in belief minus control contrast. **Oxytocin significantly improved brain activity of the right AI in

individuals with ASD ($P = 0.004$, one-tailed). D) Result of a voxel-wise whole-brain search for brain regions

that showed an increase in social minus belief activity in the oxytocin session compared with the placebo

session in the right anterior insula ($P < 0.002$, whole brain uncorrected). The location where oxytocin activated

partially overlapped with the region of interest defined based on ASD-TD comparison in Experiment 1 (red

circle).

Supplementary Figure 1

A Social minus belief contrast
TD − ASD with placebo

$Z = -12$

C Social minus belief contrast
TD − non-medicated ASD

$Z = -14$

B $P = 0.007$ **

D $P = 0.003$ **

□ Placebo
■ Oxytocin

fMRI results of supplementary analyses to explore replicability of social minus belief activity in the right anterior insula and oxytocin's effect on it. A) This panel shows the brain region where individuals with ASD in the placebo session of Experiment 2 showed less brain activity compared with TD participants in Experiment 1 in social minus belief brain contrast ($P < 0.001$ uncorrected) (AI: anterior insula). B) Percent signal changes of the sphere of 4 mm radii from the peak coordinate shown in panel A) in oxytocin and placebo sessions in brain regions where individuals with ASD in the placebo session of Experiment 2 showed less brain activity in social minus belief contrast. **Oxytocin significantly improved brain activity of the right AI in individuals with ASD ($P = 0.007$); bar: s.e.. C) This panel shows the brain region where individuals with ASD without psychotropic medication in Experiment 1 showed less brain activity compared with TD participants in Experiment 1 in

social minus belief brain contrast ($P < 0.001$ uncorrected) (AI: anterior insula). D) Percent signal changes in the sphere of 4 mm radii from the peak coordinate shown in panel C) in oxytocin and placebo sessions in brain regions where individuals with ASD without medication showed less brain activity in social minus belief contrast. **Oxytocin significantly improved brain activity of the right AI in individuals with ASD ($P = 0.003$); bar: s.e..

Supplementary Material Supplementary material shows ten different types of story with four black and white comic frames presented during the scan. They are based on Sally-Anne story and modified from stories that also require understanding of first-order false beliefs developed by Vollm et al., (Völlm et al., 2006). Four frames were presented for the same rate and interval. Ten stories appeared in a pseudo-random fashion. "S"s and "E"s stand for "start" and "end" of the story in this supplementary material.

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