Neural correlates of emotion processing during observed self-face recognition in individuals with autism spectrum disorders

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1. Introduction

In human society, the ability to interact with others in a socially appropriate manner is essential. In addition to basic emotions, such as happiness, fear, and anger, humans experience higher-order self-conscious emotions (e.g., coyness, shyness, pride, embarrassment, shame, and guilt). Unlike basic emotions, self-conscious emotions tend to arise through relationships with others and serve important interpersonal functions (Miller & Leary, 1992; Tangney, 1999; Lewis, 2000). For instance, when individuals are exposed to self-images via mirrors, photographs, or videos, they sometimes experience early forms of self-conscious emotions (e.g., coyness or shyness). This type of emotion emerges at around 18 to 24 months of age, and only after self-recognition appears (Lewis, 1989). Furthermore, when individuals are exposed to visual self-images that deviate substantially from the individual's ideals or standards, they can experience more complex negative self-conscious emotions (e.g., embarrassment) (Duval & Wicklund, 1972; Carver & Scheier, 1981; 1998). Such negative emotions emerge at around 3–4 years of age, when the child has internalized rules or standards for self-evaluation. Therefore, this class of self-conscious emotions are called "self-evaluative emotions", and they can be viewed as part of an alarm system that detects deviations of behaviors and attitudes relative to social standards. This system could play an important role in guiding appropriate social conduct.

Previously, we demonstrated that the anterior insula (AI) and the anterior cingulate cortex (ACC) are involved in the experience of embarrassment (Morita et al., 2008; 2012). These areas were more active when participants viewed self-face images, including those that would be expected to elicit feelings of embarrassment, than when they viewed images of others' faces. These brain regions are co-activated when subjects experience a range of basic emotions, including disgust and fear, as well as social emotions including romantic love, injustice, and social exclusion (Blood & Zatorre, 2001; Eisenberger et al., 2003; Wicker et al., 2003; Bartels & Zeki, 2004; Takahashi et al., 2008; Onoda et al., 2010; Moor et al., 2012). In addition, these regions are also co-activated in response to salient stimuli or events that do not necessarily elicit a specific emotional feeling (Craig, 2002). Therefore, the ACC and AI are thought to be components of a "salience network" that functions to identify the most relevant among several internal and extra-personal stimuli in order to guide appropriate behavior. In this framework, the AI serves as an integral hub in mediating dynamic interactions between other large-scale brain networks: the central executive and default mode networks (Seeley et al., 2007; Menon & Uddin, 2010).

Recently, we also obtained evidence that social situations in which participants are observed by others modulate activation patterns in the AI and ACC in distinct manners (Morita et al., 2014). In that study, we showed that individuals view self-face images while being observed by others, they experience a stronger feeling of embarrassment than when viewing the same images in the absence of an observer. Individual increases in the subjective feeling of embarrassment are positively correlated with individual increases in self-related activity (self vs. others) in the right AI, but not in the caudal part of the ACC. According to the Craig's model of integration across the insula cortex, diverse information, including homeostatic, environmental, hedonic, motivational, social, and cognitive activity, is integrated in a posterior-to-anterior direction to produce subjective experiences representing the sentient self at a particular moment in time (Craig, 2009). Considering this view together, it is suggested that the right AI plays a crucial role in creating the subjective experience of embarrassment.

On the other hand, we also found that being observed increased functional connectivity between the caudal ACC and medial prefrontal cortex (MPFC) when viewing self-face images. The MPFC is consistently activated by self-reflective processing, in which participants are required to think about their own mental or inner states (e.g., emotions or personality traits) (Northoff et al., 2006; Murray et al., 2012). In addition, the MPFC is also activated by tasks that require participants to infer others' mental states or take a third-person perspective (i.e., mentalizing) (D'Argembeau et al., 2007; Gallagher and Frith, 2003; Frith and Frith, 2006). Several recent studies suggested that the MPFC was involved in inference of the more complex mental states of others. For example, the MPFC is recruited when thinking about how another person would appraise us (Ochsner et al., 2005; Amodio and Frith, 2006; D'Argembeau et al., 2007; Frith and Frith, 2008; Izuma et al., 2008, 2010a; Sugiura et al., 2012). All these evidence considered, in our previous study, we proposed that being observed during self-face recognition would increase accessing information about the self that is reflected in the eyes or minds of others. This would lead to an increase in functional connectivity between the caudal ACC and MPFC. That is, unlike the functional role of the AI, the caudal ACC seems to serve as a hub, integrating information about the reflective self that is used for self-evaluative processing.

Autism spectrum disorder (ASD) is characterized by persistent deficits in social communication and social interaction across multiple contexts, in conjunction with restricted, repetitive patterns of behavior, interests, or activities (Diagnostic and Statistical Manual of Mental Disorders, DSM-5, American Psychiatric Association [2013]). A great deal of research has demonstrated that individuals with ASD exhibit deficits in face perception (eye gaze or facial expression) at both the behavioral and neural levels (Dalton et al., 2005; Dawson et al., 2005; Pierce et al., 2001). In contrast to the abundance of evidence regarding atypical face-processing, however, few studies have suggested that self-face processing is atypical in ASD. Indeed, the ability of autistic children to discriminate between their own faces and the faces of others appears to be intact (Akagi et al., 2003; Dawson & McKissick, 1984; Reddy et al., 2010).

However, autistic children do differ from control children in their responses to self-images reflected in a mirror. Typically, developing children aged around 2 years exhibit self-conscious emotions or self-conscious behaviors (e.g., a coy smile) in response to a self-reflection. This early form of self-conscious is thought to occur when one is the object of others' attention (Lewis, 1989). By contrast, autistic children exhibited relatively neutral emotions in this context (Akagi et al., 2003; Dawson & McKissick, 1984; Reddy et al., 2010). Consistent with these findings, we have obtained evidence that adults with ASD respond in an emotionally atypical manner to self-face images. In that study, we found that cognitive evaluation (self-evaluation) of self-face images and emotional responses (i.e., self-conscious emotions) were less coupled in adults with ASD, in parallel with reduced activity of the right insula (Morita et al., 2012). Given that self-conscious emotions arise through social relationships between self and others, atypical self-conscious emotions in ASD patients may be related to their reduced responsiveness to being observed. Indeed, another group has reported that autistic adults exhibit weaker reactions to being observed during social behaviours. When asked to make real charitable donations in the presence or absence of observers, neurotypical controls donated significantly more in the presence of the observers than when alone, whereas the amount of donation by adults with high-functioning autism was not influenced by the presence of observers (Izuma et al., 2011). Therefore, we hypothesized that being observed would have little impact on the emotional response associated with self-face recognition in individuals with ASD.

To test this hypothesis, we employed a modified functional magnetic resonance imaging (fMRI) paradigm in which participants rated the extent of embarrassment elicited by self-face images and those of others in the presence or absence of observers (Morita et al., 2014). Our previous study employed a dual MRI system with an interaction system that allowed two participants to be observed mutually and equally by each other in real time. By contrast, this study employed a single MRI system with one participant inside and an observer outside, similar to the system used in another previous study (Izuma et al., 2010b). For half of the sessions, participants were told that they would be able to see the face of their observer via a live video link, indicating that the observer was watching the face images that the participant was viewing. On the other hand, for the other half of the sessions, participants were told that they would see an empty chair, indicating that the observer was not watching the face images.
Using this experimental setting, we initially confirmed that neurotypical individuals experienced increased embarrassment when viewing self-face images in the presence of an observer. Next, we tested whether the extent of subjective embarrassment and the neural substrates for embarrassment would be modulated by observation in individuals with ASD. For standard analyses and psychophysiological interaction (PPI) analyses, we defined regions of interest

(ROIs) based on peak coordinates in emotion-related regions (caudal ACC and AI), where activation patterns during self-face processing were shown to be modulated by observation in an independent study conducted on neurotypical individuals (Morita et al., 2014).

2. Material and Methods

2.1 Participants

Fourteen males with high-functioning ASD (mean age ± standard deviation [SD] = 24.5 ± 6.7 years) were recruited at the Department of Neuropsychiatry of the University of Fukui Hospital or the Department of Psychiatry and Neurobiology of the Kanazawa University Hospital in Japan (Table 1). The authors (Hirotaka K. and Toshio M.) diagnosed the participants based on the classifications described in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) (American Psychiatric Association, 2000) and standardized criteria taken from the Diagnostic Interview for Social and Communication Disorders (DISCO) (Wing et al., 2002). The DISCO has reliable psychometric properties (Nygren et al., 2009), and also contains items that address early development and a section focused on activities of daily living, which provides data on the individual's level of functioning in several areas beyond the social and communication domains (Wing et al., 2002). The ASD group consisted of 13 participants with autistic disorder and one participant with Asperger syndrome. We also recruited 18 age- and intelligence quotient (IQ)-matched male controls (mean age ± SD = 23.3 ± 3.3 years) from the local community (Table 1). Participants were excluded if they had a history of major medical or neurological illness, including epilepsy, significant head trauma, or a lifetime history of alcohol or drug dependence. They were also screened to exclude individuals who had a first-degree relative with an axis I disorder based on DSM-IV criteria. IQ assessments were performed using the Japanese version of the third edition of the Wechsler Adult Intelligence Scale-III (WAIS-III, Wechsler, 1997; JWAIS-III, Fujita et al., 2006) to check for differences in general ability. All participants scored above the IQ threshold of 80. Autistic traits were quantified using the Autism spectrum quotient (AQ) (Baron-Cohen et al., 2001), which consists of the following five subscales: social skills, attention switching, attention to detail, communication, and imagination (Table 1). One additional participant in the control group was excluded from the analyses due to a high AQ score (cut-off score: 32). The AQ scores of the ASD group were significantly higher than those of the control group ($t(30) = 9.32$, $p < 0.001$). Participants were all right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971), with the exception of two left-handed participants in the ASD group. The protocol was approved by the ethics committee of the University of Fukui. All participants gave written informed consent prior to participation.

2.2 Materials

The experiment took place over 2 days. On the first day, participants made short speeches in front of a video camera. Rather than being told the true aim of the study, participants were informed that the purpose of the recording was to investigate eye movements that occur when a person speaks. Recordings of each participant's face were made throughout the speeches. Twenty-one black-and-white images of each participant's face, with ratings ranging from good (attractive) to bad (unattractive), were selected from the recorded videos. Each face image was rated by an experimenter as to whether each of the following four items was attractive or unattractive: eyes, mouth, chin, and overall. Each item was scored on a 5-point scale, and a total score was calculated for each image. The averages of the 21 scores were matched across participants. Twenty-one images per participant were used as the stimuli for the SELF trials. By contrast, for the OTHERS trials, 21 face images were selected from three gender-matched unfamiliar individuals (seven images per person). The average of the 21 scores for others' face images was matched with that for self-face images.

2.3 fMRI experimental procedure and design

2.3.1 Cover-story setup

A few weeks after the video-recording session, participants underwent fMRI scanning. When participants arrived at the scanner control room, they were introduced to their partner for the experiment (who was actually a male actor), and informed that he would join in the experiment as an observer. For all of the participants, the partner was an unfamiliar individual whom the subject had not previously met.

Outside the scanning room, the participants and their partner were shown the experimental setup, which consisted of a desk, a chair, a display, and a small video camera. The participants and their partner were told that the participants would view their own facial images or those of other men (different from the partner) inside the MRI scanner, and that those face images would be also simultaneously shown on the display. They were also told that in two of four sessions the partner would sit in the chair to rate the photogenicity of the face images, whereas in the other two sessions, the partner would go to another room to be engaged in an unrelated task. We explained that the small video camera placed on the top of the display would capture the inside of the room, and that the captured live images would be presented on the upper left portion of the screen inside the MRI scanner.

The cover story led the participants to believe that the face stimuli shown on the screen inside the MRI scanner were shared with the partner, and that they would be seeing a live video image showing their partner's face as they sat in the chair and rated the photogenicity scores of their own faces or others' (observation condition). An interview at the end of the experiment confirmed that all participants believed the cover story. However, the videos presented in the upper left portion of the screen were actually pre-recorded.

2.3.2 Setup for MRI scanning

After a practice run, participants were asked to lie in the MRI scanner, wearing earplugs and with their heads immobilized by sponge cushions. Inside the scanner, the participants performed the face task under two conditions: non-observation (NOB) and observation (OB) (Figure 1). In both conditions, the participants were asked to rate the extent of embarrassment that they felt upon viewing each face stimulus displayed on the right half of the screen. In each session, 21 images of the participant's own face (SELF) and 21 images of the faces of unfamiliar individuals (OTHERS) were presented in a pseudorandom order. The session also included seven "null events" in which no stimulus was shown. In each trial, a face stimulus appeared for 3 s. After the face stimulus disappeared, a visual analogue scale appeared for 4.5 s; the endpoints of the scale were labelled "None at all" (no embarrassment) and "Strong" (most embarrassed). During the rating period, participants were required to rate the extent of their embarrassment by moving a pointer along the scale, using the index and middle fingers to operate a two-button response box held with their right hand. In order to discourage response preparation during the stimulus-viewing period, the starting position of the pointer was randomly determined for each trial. The visual analogue scales were subsequently divided into 100 equal intervals for analyses.

In the NOB condition, a pre-recorded video showing the upper portion of the chair was presented in the upper left portion of the screen throughout the session. In this condition, the participants were instructed to perform the task alone and informed that the face stimuli were not being shared with their partner. By contrast, in the OB condition, a pre-recorded video showing the partner's face was presented in the upper left portion of the screen throughout the session. When recording the video used for this condition, the partner (an actor) maintained a neutral expression and kept his gaze fixed constantly on the screen, in order to minimize confounding factors elicited by changes in the actor's facial expressions or eye movements. In this condition, the participants were instructed to perform the task and informed that the face stimuli were being shared with their partner.

This experimental design was based on a rapid event-related paradigm, in which efficiency is highly dependent upon the temporal pattern of stimulus presentation (Dale, 1999; Friston et al., 1999). Detailed requirements for a highly efficient experimental design are described elsewhere (Morita et al., 2012). Participants completed four sessions including 21 SELF and 21 OTHERS trials, with each session lasting for 6 min 2.5 s. Each face stimulus was presented once in each session and four times during the entire experiment. Half of the sessions were conducted under the NOB condition, and the others were conducted under the OB condition. The order of conditions was counterbalanced across participants (NOB-OB-NOB-OB or OB-NOB-OB-NOB) to avoid order effects. Sessions were separated by intervals of a few minutes in order to make the participants believe that their partner had moved to a different room and prepared for the next task during the interval.

Task images (generated by Presentation software, Neurobehavioral Systems, Albany, CA) and recordings of the observer or chair, created using a digital camera (HDR-XR520V; Sony, Tokyo, Japan), were combined using a screen splitter (MV-40F; FOR-A, Tokyo, Japan). Throughout the sessions, visual stimuli were projected on a screen at the top end of the scanner bore and viewed by the participants through a mirror.

2.4 Psychological measurements

Immediately following scanning, participants undertook a self-paced rating task using the stimuli from the fMRI session. Participants were asked to rate the images in terms of how photogenic they appeared on a visual analogue scale, the extremes of which were labelled “Good” and “Bad”. The visual analogue scales were subsequently divided into 100 equal intervals for analyses. Following the rating task, participants were asked to complete a self-report questionnaire based on the Japanese version of the self-consciousness scale (Fenigstein et al., 1975; Sugawara et al., 1984), which provides indices for two specific types of self-consciousness: public and private.

2.5 MRI scanning procedure

Functional images were acquired using T2*-weighted, gradient echo, echo-planar imaging (EPI) sequences with a 3-T MR imager (Discovery MR750; GE Healthcare, Milwaukee, WI) and a 32-channel array coil. There were four fMRI sessions, during each of which 145 volumes were acquired. Each volume consisted of 40 slices, acquired in ascending order, with a thickness of 3.5 mm and a 0.5-mm gap, in order to cover the entire brain. The time interval between each two successive acquisitions of the same slice (TR) was 2,500 ms, with an echo time (TE) of 30 ms and a flip angle (FA) of 83°. The field of view (FOV) was 192 × 192 mm and the matrix size was 64 × 64, giving voxel dimensions of 3 × 3 mm. For anatomical reference, three-dimensional (3D), inversion recovery–prepared spoiled gradient echo (IR-SPGR) images (TR = 6.4 ms; TE = 2 ms; FA=11°; matrix size = 256 × 256; slice thickness = 1 mm; total of 176 transaxial images) were also obtained.

2.6 Behavioural data analysis

Behavioural data analysis was carried out using SPSS version 16.0J (SPSS Japan, Tokyo, Japan). To compare the embarrassment ratings measured during MRI scanning for each condition and within each group, we performed three-way analysis of variance (ANOVA), face type (SELF, OTHERS) × observation (NOB, OB) × group (Control, ASD), on the average ratings. We also performed two-way ANOVA, face type (SELF, OTHERS) × group (Control, ASD), on the photogenicity ratings measured outside the scanner. To investigate the relationship between photogenicity ratings and embarrassment ratings for self-faces, we calculated the correlation coefficient (r) between the two scores and transformed it into a Fisher's z coefficient. We entered these z scores into a two-way ANOVA with observation (NOB, OB) and group (Control, ASD) as factors. Questionnaire data were analyzed using t-tests to compare the control and ASD groups. Results were considered statistically significant at $p < 0.05$.

2.7 Imaging data analysis

2.7.1 Pre-processing

The first five volumes of each fMRI session were discarded because of unsteady magnetization, and the remaining 140 volumes per session were used for analysis. Image and statistical analyses were performed using Statistical Parametric Mapping (SPM8; The Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 8.1.0 (Mathworks, Sherborn, MA). Images were motion corrected (with six parameters), corrected for slice acquisition timing, normalized to the Montreal Neurological Institute (MNI) space (Evans et al., 1994), and smoothed with a full-width at half-maximum (FWHM) of 8 mm in the x, y, and z dimensions. According to the record of head motions within the fMRI sessions, all participants had < 3 mm (cut-off) maximum displacement in the x, y, and z dimensions. Mean displacement was 0.42 ± 0.45 mm (range: 0.05–2.09 mm) in the control group and 0.52 ± 0.50 mm (range: 0.05–2.00 mm) in the ASD group.

2.7.2 Activation analyses

After pre-processing, we used a general linear model (GLM) to analyze the fMRI data for each participant (Friston et al., 1995; Worsley and Friston, 1995). We included five regressors of interest in each session: two task-related regressors (SELF and OTHERS conditions), two regressors for parametric modulation (the embarrassment scores for each face type), and one regressor for motor responses related to rating. One irrelevant constant term (session effect) was also included for each session. Of the four sessions, two were under the OB condition and two were under the NOB condition. To eliminate the artifactual low-frequency trend, we used a high-pass filter comprising the discrete cosine basis function with a cut-off period of 128 s. Serial autocorrelation assuming a first-order autoregressive model was estimated from the pooled active voxels using the restricted maximum likelihood (ReML) procedure, and then used to whiten the data and the design matrix (Friston et al., 2002). To calculate the estimated parameters, least-squares estimation was performed on the high-pass filtered and pre-whitened data and design matrix. The weighted sum of parameter estimates in the individual analyses constituted contrast images that were used for the second-level analysis. For each subject, we used the contrast images to identify brain areas exhibiting differential effects of face type (SELF vs. OTHERS, OTHERS vs. SELF), observation conditions (OB vs. NOB, NOB vs. OB), and the interaction between face type and observation (face type × observation).

In the second-level analysis, we searched for brain regions exhibiting differential effects of face type, observation conditions, and face type × observation in each group. We also searched for brain regions that exhibited a significant difference between groups with respect to any of these parameters. For these whole-brain analyses, we applied a statistical height threshold of $p < 0.005$ and an extent threshold of $p < 0.05$, corrected for multiple comparisons.

To investigate whether activation patterns during self-face processing were modulated by being observed in control and ASD participants, we focused on regions related to emotional processing associated with self-face recognition. We identified ROIs as spheres with 6-mm radii centered on the MNI coordinates for coordinates within the right AI (x = 40, y = 14, z = −10) and caudal ACC (x = −4, y = 4, z = 28), in which self-related activities were shown to be enhanced under observation by others in an independent study conducted with neurotypical individuals (Morita et al., 2014). First, we extracted the individual parameter estimates for self-related activity in each observation condition for each ROI. In each group, we tested whether self-related activity was modulated by being observed using a two-way ANOVA, observation (NOB, OB) × group (Control, ASD). We also tested whether the increment in neural activity upon observation depended on the increment in embarrassment, as well as whether the dependency differed between groups. Second, we extracted the individual parameter estimates for the parametric modulation according to embarrassment ratings in each condition for each ROI, and tested whether it was affected by being observed in each group using a two-way ANOVA, observation (NOB, OB) × group (Control, ASD).

2.7.3 Connectivity analyses (PPI)

Finally, we used psycho-physiological interaction (PPI) analysis to search for brain regions in which the effect of being observed on functional connectivity with a seed region (caudal ACC) when viewing self-face images exhibited differences between groups (Friston et al., 1997; Gitelman et al., 2003). To identify the caudal ACC seed for each participant, we defined brain areas exhibiting differential effects of face type (SELF vs. OTHERS) at a lenient threshold of $p < 0.1$ (uncorrected). Then, we detected the nearest peak within 8 mm of the peak coordinate (x = 0, y = 6, z = 30), in which the functional connectivity with frontal regions was enhanced by observation by others in our independent study of neurotypical individuals (Morita et al., 2014). We extracted the time series from 6-mm sphere around the detected peak. The time series from the PPI was calculated as the element-by-element product of each time series and a psychological vector of interest ($OB_{SELF} > NOB_{SELF} = 1$ for viewing one's own face while being observed, −1 for viewing one's own face without being observed). This product was subsequently re-convolved with the hemodynamic response function (HRF). The interaction term was then entered as a regressor in a first-level model, together with the time series of the caudal ACC seed region and the psychological vector of interest ($OB_{SELF} - NOB_{SELF}$). The model parameters were estimated and contrasts were generated to test the effect of PPIs that were used for the second-level analysis. In the second-level random-effects analysis using a two-sample *t*-test, we identified brain regions in which the effect of observation on connectivity with the seed region when viewing self-face images differed between groups. To look for subtle observation × group interactions with small sample sizes, we employed a more lenient threshold of $p < 0.005$ (uncorrected), extent k > 10, for the PPI analysis. In the event of differences between groups, we tested whether the enhanced functional connectivity with the caudal ACC seed associated with being observed was related to the change in psychological ratings associated with being observed.

3. RESULTS

3.1 Behavioural data

The average public self-consciousness scale scores were 41.4 ± 7.0 in the control group and 40.4 ± 9.0 in the ASD group. The average private self-consciousness scale scores were 33.4 ± 5.5 in the control group and 36.0 ± 6.1 in the ASD group. There were no significant differences between groups in either of the subscales (public, t (30) = 0.34, p = 0.75; private, t (30) = −1.24, p = 0.22). Figure 2 shows the range of embarrassment ratings measured during the fMRI session. A three-way ANOVA, face type (SELF, OTHERS) × observation (NOB, OB) × group (Control, ASD), performed on the average ratings revealed a significant main effect of face type (F (1, 30) = 41.3, $p < 10^{-6}$), observation (F (1, 30) = 7.44, $p < 0.05$), and group (F (1, 30) = 4.92, $p < 0.05$), and a marginally significant face type × observation interaction (F (1, 30) = 4.04, $p < 0.1$), whereas no other 2-way (all ps > 0.3) or 3-way interaction (p = 0.67) was significant. The significant main effect of group indicates that individuals with ASD reported less embarrassment relative to neurotypical individuals in response to face images.

However, similar to neurotypical individuals, individuals with ASD reported stronger embarrassment in response to self-face images than face images of others, especially when they were being observed (Figure 2B).

The average photogenicity ratings measured outside the MRI scanner were 35.1 ± 2.3 for SELF images and 44.4 ± 2.6 for OTHERS images in the control group, versus 30.7 ± 3.5 for SELF images and 47.4 ± 2.9 for OTHERS images in the ASD group. A two-way ANOVA, face type (SELF, OTHERS) × group (Control, ASD), revealed a significant main effect of face type (F (1, 30) = 27.4, $p < 10^{-4}$), but no significant main effect of group ($p = 0.82$) or face type × group ($p = 0.15$). These results indicate that the participants reported lower scores for images of their own faces than those of unfamiliar people, regardless of ASD diagnosis.

We then investigated the relationship between embarrassment ratings measured inside the scanner and photogenicity ratings of self-face images measured outside the scanner by calculating the Fisher's z-transformed correlation coefficients between the two ratings for each individual (Figure 3). A two-way ANOVA, observation (NOB, OB) × group (Control, ASD), revealed a significant main effect of observation (F (1, 30) = 8.53, $p < 0.01$) and group (F (1, 30) = 5.45, $p < 0.05$). There was also a significant observation × group interaction (F (1, 30) = 5.00, $p < 0.05$). Post-hoc tests revealed that control individuals exhibited significantly stronger correlations between these two ratings for self-face images when they were observed (OB condition) than when they were not observed (NOB condition) ($p < 0.001$), whereas individuals with ASD exhibited no modulation in response to being observed ($p = 0.68$). These results indicate that the coupling between the two psychological ratings of self-face images was enhanced by observation in the control group, but not in the ASD group.

3.2 fMRI data

3.2.1 Whole-brain analysis

In each group, we identified brain regions in which activity differed between face types (Table 2, Supplementary Figure 1). The control group exhibited significant self-related activation (SELF vs. OTHERS) in bilateral lateral frontal regions including ventral premotor cortex (PMv), inferior frontal gyrus (IFG), and anterior insula (AI); bilateral medial prefrontal regions including supplementary motor area (SMA) and anterior cingulate cortex (ACC); and bilateral subcortical regions including midbrain and thalamus. In addition to these areas, we observed significant activation in the right occipital cortex including inferior occipital gyrus (IOG), inferior temporal gyrus (ITG), and middle occipital gyrus (MOG). Most of these activation peaks were consistent with our previous results (Morita et al., 2008; 2012; 2014). By contrast, the ASD group exhibited significant activation only in the left lateral frontal regions including PMv, IFG, and AI. A direct comparison between groups revealed that the self-related activity in the right occipital cortex including IOG was stronger in the control group than in the ASD group (Table 2). No brain regions exhibited a difference between observation conditions or a face type × observation interaction in either group. In addition, no brain regions exhibited a significant difference between groups in regard to either observation conditions or face type × observation interaction.

3.2.3 PPI analysis

We used PPI analysis with a caudate ACC seed region to evaluate differences in functional connectivity between the control and ASD groups when viewing self-face images. The caudal ACC (seed) exhibited greater enhancement of functional connectivity with medial prefrontal cortex in response to observation in the control group than in the ASD group (Figure 5A, Table 3). Connectivity between caudal ACC and MPFC was significantly higher in the control group in observed vs. non-observed viewing of self-face images (t (17) = 3.03, $p < 0.01$), but was lower in the ASD group (t (13) = −2.58, $p < 0.05$) (Figure 5B). Further, when data were pooled across the two groups, the enhancement of functional connectivity between the caudal ACC and MPFC was significantly correlated with the enhancement of coupling strength between photogenicity and embarrassment ratings for self-face images ($r = -0.355$, $p < 0.05$) (Figure 5C), but not with enhancement of embarrassment ratings ($r = -0.029$, $p = 0.87$) (Figure 5D). A similar group difference was observed in the modulation of functional connectivity between the caudal ACC and the right caudate nucleus. However, because the connectivity between the caudal ACC and the right caudate nucleus was not increased by observation in the control group (t (17) = 0.66, $p = 0.52$), it will not be discussed further here.

3.2.2 ROI analysis

ROI analysis was used to characterize the patterns of activity according to observation condition and group (Figure 4). We investigated activation patterns in emotion-related regions (right AI and caudal ACC) in which self-related activity was enhanced by observation by others in our independent study of neurotypical individuals (Morita et al., 2014).

Right AI: Regarding self-related activity, two-way ANOVA [observation (NOB, OB) × group (Control, ASD)] revealed no significant main effect or interaction (all ps > 0.1) (Figure 4A middle). We also investigated the relationship between the individual increase in the self-related activity of each ROI and the individual increase in embarrassment ratings of self-face images. In the control group, we observed a significant positive across-subjects correlation ($r = 0.489$, $p < 0.05$) (Figure 4A right). Such significant correlation was not observed in the ASD group ($r = -0.420$, $p = 0.13$). Analysis of covariance (ANCOVA) revealed that the slopes of the regression lines between groups were significantly different (F (1, 31) = 7.49, $p < 0.05$). Regarding within-subject modulation of neural activity by embarrassment ratings for self-faces, two-way ANOVA [observation (NOB, OB) × group (Control, ASD)] revealed no significant main effect or interaction.

Caudal ACC: Self-related activity within the caudal ACC ROI exhibited no significant main effect or interaction (all *p*s > 0.1) (Figure 4B middle). The ASD group exhibited a significant positive across-subjects correlation between the individual increase in self-related activity and the individual increase in embarrassment ratings (r = 0.530, p = 0.05), while the control group did not (r = 0.206, p = 0.41) (Figure 4B right). ANCOVA revealed no significant difference in the slopes of the regression lines between groups. Self-related activity in caudal ACC did not exhibit any significant within-subject modulation by embarrassment ratings for self-faces in either the Control or the ASD group.

4. DISCUSSION

4.1 Effect of being observed on emotional response to self-face images

First, we confirmed that the presence of an observer led to an increase in the subjective feeling of embarrassment provoked by self-face images in neurotypical individuals, which is consistent with our previous findings (Morita et al., 2014). In this study, in addition to the embarrassment ratings, the coupling between embarrassment and photogenicity ratings for self-face images was also modulated by the presence of an observer. In the absence of an observer, neurotypical individuals experienced a feeling of embarrassment that was weakly linked with the results of self-face evaluations. This linkage between the two psychological ratings was enhanced by the presence of an observer. These data suggest that being observed can affect the process of shaping self-conscious emotions in normal individuals, and thus provide evidence for the idea that self-conscious emotions arise throughout interactions with others.

We replicated our previous findings with regard to effect of being watched on self-conscious emotions, even though we used a different procedure to induce a sense of being watched from that used in our previous study. In our previous study, a dual interacting MRI system allowed participants to see each other in real time, simulating a social situation in which two people are viewing each other's face images simultaneously during daily life. By contrast, this study used a single MRI system, with one participant inside and one partner (=observer) outside. In this situation, we used a cover story to let the participants believe that the face stimuli shown on the screen inside the MRI scanner were shared with the observer, and that they would be seeing a live video image showing the observer's face as they was in the next room (in the OB condition). Although the participant viewed pre-recorded (rather than live) video images of their observer's face, we confirmed that all participants believed the cover story and presence of a real observer on the debriefing after the fMRI experiment. In this manner, we successfully induced in participants a sense of being watched by a partner while they were inside the MRI scanner.

Recent social psychological experiments showed that when individuals are observed by others, they are concerned about how they are viewed or evaluated and wish to earn a good reputation, which can result in prosocial behaviors (Haley and Fessler, 2005; Bateson et al., 2006; Be´nabouu and Tirole, 2006). Similarly, when viewing self-face images while being observed, individuals might be primarily concerned with how their images are perceived or judged by the observers, and might therefore want to earn a good reputation based on their physical attributes. This could raise the internal standard for the self, leading to an increase in the discrepancy between the actual self and mentally represented ideals or standards, thus enhancing the embarrassment experienced when viewing self-face images. Thus, being observed can modulate emotional processing associated with self-face recognition.

4.2 Atypical effect of being observed in ASD

After confirming our previous findings in neurotypical individuals, we investigated to what extent the emotional response to self-face images is modulated by the presence of an observer in individuals with ASD. We observed a group difference in the effect of being observed on the coupling between embarrassment and photogenicity ratings for self-face images. As described above, in the control group, the coupling between embarrassment and photogenicity ratings was enhanced by the presence of an observer. By contrast, in the ASD group, the presence of an observer had little effect on the linkage between cognitive evaluation of the self-face images and the resultant emotional response.

One possible explanation for this finding in the ASD group is that these individuals are less concerned about how an observer might view or evaluate their face images when being observed, potentially due to their difficulty in representing the mental states of others (Castelli et al., 2002; Frith et al., 1991; Happé et al., 1996). Individuals with ASD do not automatically pay attention to socially relevant information or represent others' mental states, although they can sometimes perform these tasks when explicitly instructed to do so (Frith, 2004; Senju, 2013; Callenmark et al., 2014). However, in this study, the task involved rating the embarrassment felt when viewing each face image, and participants were not explicitly instructed to infer the mental states or perspective of the observer. As described above, none of the participants with ASD reported that they did not believe the cover story. This indicates that none of them was aware that the video images of the observer they saw were a pre-recorded video in the observation condition. Even though the participants in the ASD group believed the presence of a real observer, this would not trigger concern about how the observer might view or evaluate their face images. This could in turn lead to the observed lack of changes across observation conditions in regard to the coupling strength between emotional responses and cognitive evaluation of self-face images.

In a previous study, we observed similar ASD-specific weak coupling between these two psychological ratings (Morita et al. 2012). In that study, individuals with and without ASD rated the photogenicity of self-face images in the MRI scanner and also rated the extent of embarrassment associated with each image outside the scanner. When they were engaged in rating the embarrassment scores, an experimenter sat on a chair several meters behind them without checking the rating scores. This situation was more similar to the observation (OB) condition than the non-observation (NOB) condition in this study. Therefore, ASD-specific weak coupling between the two psychological ratings observed in the previous study may have been caused by ASD participants' lower level of concern about how the experimenter evaluated their own face images. Taken together, these results imply that a participant's concern about how their own face images are viewed or judged by someone not only increases the extent of embarrassment they experience, but also strengthen the linkage between the emotional response and the cognitive evaluation of self-face images, regardless of whether they are actually being observed.

4.3 Activation patterns of right AI in controls

We investigated the effects of being observed on processing in emotion-related brain regions. We replicated our previous finding that in neurotypical individuals, there was a positive correlation between individual increases in activity in the right AI and the extent of the perceived embarrassment for self-face images, even though these participants were recruited from different populations. These results indicate that the right AI is important for the experience of subjective feelings of embarrassment evoked by self-face images.

The AI is active during a wide variety of tasks in addition to emotional processing, including self-related processing without a specific emotional feeling, such as self-face recognition (Kircher et al., 2000; 2001; Platek et al., 2006; Devue et al., 2007), self-agency (Farrer and Frith, 2002), autobiographical memory retrieval (Fink et al., 1996), evaluation of traits concerning the self (Fossati et al., 2003; Modinos et al., 2009), and time perception (Coull et al., 2004; Deary et al., 2004; Livesey et al., 2007). To explain this wide range of functions within a unified framework, Craig proposed a model for the insular cortex in which diverse types of information including homeostatic, environmental, hedonic, motivational, social, and cognitive data are integrated in a posterior-to-anterior direction to produce subjective experience (Craig, 2009). In line with Craig's view, our results strongly suggest that the right AI plays an important role in creating the subjective feeling of embarrassment, as well as other types of experiences.

4.4 Atypical activation pattern of right AI in ASD

In contrast to the control group, the ASD group exhibited atypical activation of the right AI, such that individual increases in the extent of embarrassment were not accompanied by individual increases in right AI activity. That is, in individuals with ASD, the degree of activity in the right AI did not reflect subjective ratings of embarrassment.

Several neuroimaging studies have reported ASD-related structural (Kosaka et al., 2010; Yamasaki et al., 2010) and functional (Di Martino et al., 2009; Silani et al., 2008; Uddin et al., 2015) abnormalities of the AI, mostly in the right hemisphere. Previously, we reported that the gray matter volume of the right AI and right IFG is smaller in individuals with ASD (Kosaka et al., 2010); this effect was negatively correlated with AQ score, which is used to assess individual autistic traits (Baron-Cohen et al., 2001). Regarding functional abnormality, a recent meta-analysis revealed that the right AI is less likely to be activated during social tasks in individuals with ASD than in neurotypical controls (Di Martino et al., 2009). In addition, several studies reported reduced functional connectivity of the insula in adults with ASD (Di Martino et al., 2014; Ebisch et al., 2011; von dem Hagen et al. 2013). In line with these findings, Uddin and Menon (2009) proposed that the function of the AI in integration of inputs from multiple sources necessary for emotional awareness of self and others may be impaired in individuals with ASD. Taken together with our results, these findings suggest that emotional or neural states labeled by individuals with ASD as “embarrassment” might differ from those labeled as such by control individuals, due to the impairment of the integrative function of the AI involved in creating subjective feelings.

4.5 Atypical effects of being observed on functional connectivity in ASD

We also confirmed that being observed enhanced functional connectivity between the caudal subdivision of the ACC and the dorsal part of the MPFC in healthy individuals, consistent with our previous findings (Morita et al., 2014). In our previous study, we proposed that the caudal ACC could serve as a hub to integrate information about the self, which is required for self-evaluative processing. In addition, we suggested that the enhancement of functional connectivity between the caudal ACC and the MPFC in the presence of an observer would reflect increased access to information about the self that is reflected in the eyes or minds of others (Ochsner et al., 2005; Amodio and Frith, 2006; D’Argembeau et al., 2007; Frith and Frith, 2008; Izuma et al., 2008, 2010a; Sugiura et al., 2012). In this way, in healthy individuals, social situations in which participants are observed by others modulate activation patterns in the caudal ACC in a different manner from the right AI, suggesting a functional dissociation between the ACC and the AI in emotional processing associated with self-face recognition.

In contrast to healthy individuals, individuals with ASD exhibited a smaller observer-induced effect on functional connectivity. Furthermore, the reduced effect of being observed in ASD was specifically related to a reduction in the effect of being observed on the coupling strength between photogenicity and embarrassment ratings for self-face images. These results indicate that being observed has little effect on the emotional processing associated with self-face recognition in individuals with ASD, potentially due to their lower level of concern about how an observer might view or evaluate their own face images. This implies that individuals with ASD lack the ability to integrate information from others about self-evaluative processing, even when they are explicitly aware of the presence of observers. Thus, social deficits present in individuals with ASD may be partly caused by impairments in the linkage between information about self and others. By conducting these analyses, which combined psychological and imaging data, our results shed light on the atypical effects of being observed on emotional processing associated with self-face recognition in individuals with ASD, which cannot be detected by self-reported ratings of embarrassment alone.

4.6 Limitations

This study had several limitations. First, the sample size was relatively small. Consequently, some caution must be exercised in interpreting the results of these analyses. Future studies seeking to confirm these findings should include more participants.

Another potential limitation involves the differences between our findings in this study and those of previous studies. The first difference was in the self-related activity of the right AI in the neurotypical control group. In our previous study, we found that activity in this region was significantly enhanced by the presence of an observer (Morita et al., 2014), but we did not see this effect in the present study. One possible reason for this discrepancy relates to gender difference in social sensitivities. Several researchers have reported that women have greater social sensitivity and are more strongly emotionally affected by social situations than men (Miller, 1995; Pettijohn et al., 2010). In our previous study, half of the participants were women and half were men, whereas all of the participants in the present study were men, in order to match the gender composition of the ASD group. Therefore, the lesser degree of right AI sensitivity to observation in this study might be related to the lower sensitivity of men to the effects of being observed. Despite the lack of a statistically significant increase in self-related activity in the right AI, we were able to replicate our previous finding that individual increases in self-related activity of the right AI were significantly correlated with individual increases in the extent of reported embarrassment in response to self-face images.

The second difference involves the extent of embarrassment in response to faces in individuals with ASD. In our previous study, individuals with ASD reported the same level of embarrassment in response to face images as neurotypical individuals did, regardless of whether the faces were their own or others' (Morita et al., 2012). In this study, however, compared with control individuals, individuals with ASD reported less embarrassment in response to faces. As shown in Figure 2, there were large individual differences in average embarrassment ratings for face images, especially in the ASD group. Because of this large individual variation in embarrassment ratings, comparison of these ratings between groups could lead to contradictory results.

Last, we did not assess individual state anxiety or trait social anxiety. Our own face images should be emotionally salient stimuli for us, and this might be related to the anxiety of being observed by others. Elucidation of this issue would require additional psychological assessments, namely, evaluation of the changes in individual state anxiety before and after the short speeches on the first day, as well as the individual trait of social anxiety, which refers to the tendency to experience fear in social situations. These assessments of anxiety might provide insights into the mechanisms underlying atypical self-conscious emotions in individuals with ASD.

Conclusions

In neurotypical individuals, being observed by others had an impact on emotional processing associated with self-face recognition. First, the presence of an observer enhanced subjective feeling of embarrassment, and also enhanced the coupling strength between emotional response (embarrassment) and cognitive evaluations (photogenicity) of the self-images. In addition, the extent of embarrassment was closely correlated with activity in the right AI. This suggests that the right AI is involved in creating the subjective experience of embarrassment. Second, the presence of an observer increased functional connectivity between the caudal ACC and MPFC, reflecting enhanced concern about how self-face images were viewed by an observer.

Individuals with ASD also reported stronger embarrassment when viewing self-face images than the face images of others, especially when being observed. However, activity in the right AI did not correspond to the reported extent of embarrassment. In addition, individuals with ASD did not exhibit enhanced functional connectivity between the caudal ACC and MPFC in association with being observed. The absence of this relationship corresponded to reduced modulation of the coupling between the cognitive evaluation of and emotional responses to self-face images.

These results suggest that the reduction in the impact of being observed on embarrassment induced by self-face images in ASD individuals is related to their lack of ability to integrate information from others regarding self-evaluative processing mediated by the ACC and MPFC, and impaired creation of subjective feelings in the right AI.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TM (first author) was involved in conducting the experiment, analyzing and interpreting the data, and drafting the article. HK and MI were involved in recruiting the participants, diagnosing the participants with ASD, and conducting the experiment. DNS and TF were involved in recruiting the participants and conducting the experiment. TM (sixth author) was involved in recruiting the participants and diagnosing the participants with ASD. KI was involved in conducting the experiment. HO and RK were involved in interpreting the data. NS was involved in interpreting the data and drafting the article. All authors have read and approved the final manuscript.

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Figure legends

Figure 1. Sequence of events during sessions in the NOB and OB conditions. In both conditions, self-faces (SELF) and faces of unfamiliar others (OTHERS) were presented in a pseudorandom order on the right-hand side of the screen. At the beginning of each trial, a face stimulus was presented (3 sec). Immediately after the face stimulus disappeared, participants were required to rate how embarrassed they felt upon viewing each face by using a visual analogue scale with their index and middle fingers within 4.5 sec. All words displayed during the rating phase were in Japanese. In the NOB condition (A), a video showing an empty chair was presented in the upper left portion of the screen throughout the session. In the OB condition (B), a video showing the partner's face was presented in the upper left portion of the screen. Although participants were informed that these videos were live, they were actually pre-recorded. Written informed consent for publication of their individual face images was obtained from the three individuals displayed in this figure.

Figure 2. Results of embarrassment ratings. Embarrassment ratings for SELF and OTHERS' images, rank-ordered according to ratings measured during the fMRI session in the control (A) and ASD (B) groups. Right bar graphs show mean embarrassment ratings for each condition. Data represent means ± standard error (SE).

Figure 3. Correlation coefficients between embarrassment and photogenicity ratings for self-face images in each condition (NOB or OB) and group (Control or ASD). Data were normalized by Fisher's z transformation. Asterisks indicate statistical significance (**, $p < 0.01$).

Figure 4.
Activation patterns in each ROI (A, right AI; B, caudal ACC). The location of each ROI is shown on a single transverse slice of a standard brain template provided in the MRIcron software (http://www.cabiatl.com/mricro/mricro/). The bar graphs show averaged parameter estimates for the mean self-related activity for each condition and group. The scatter plots show the relationship between the observer-induced increases in self-related activity and the individual's self-reported embarrassment for self-face images.

Figure 5.
Results of PPI analysis. (A) MPFC exhibited a group difference (Control vs. ASD) in enhanced connectivity with the caudal ACC (seed) when viewing self-face images as a result of being observed. (B) Averaged parameter estimates for the increase in connectivity between the caudal

ACC seed and MPFC. Individual parameter estimates for the increase in connectivity between the caudal ACC seed and MPFC are plotted against the individual increase in coupling strength between the photogenicity and embarrassment ratings for self-faces (C), and the individual increase in embarrassment ratings for self-faces (D).

Supplementary Figure 1.
Brain areas exhibiting significant activation caused by the SELF vs. OTHERS contrast in each group (control group, red [low] to white [high]; ASD group, blue [low] to green [high]). Each activation was superimposed on a high-resolution anatomical MR image in ten contiguous transaxial slices separated by 6-mm intervals, extending from MNI coordinates Z = −18 to Z = +36. The height threshold was set at $T > 2.75$ ($p < 0.005$), and $p < 0.05$, corrected for multiple comparisons at the cluster level.

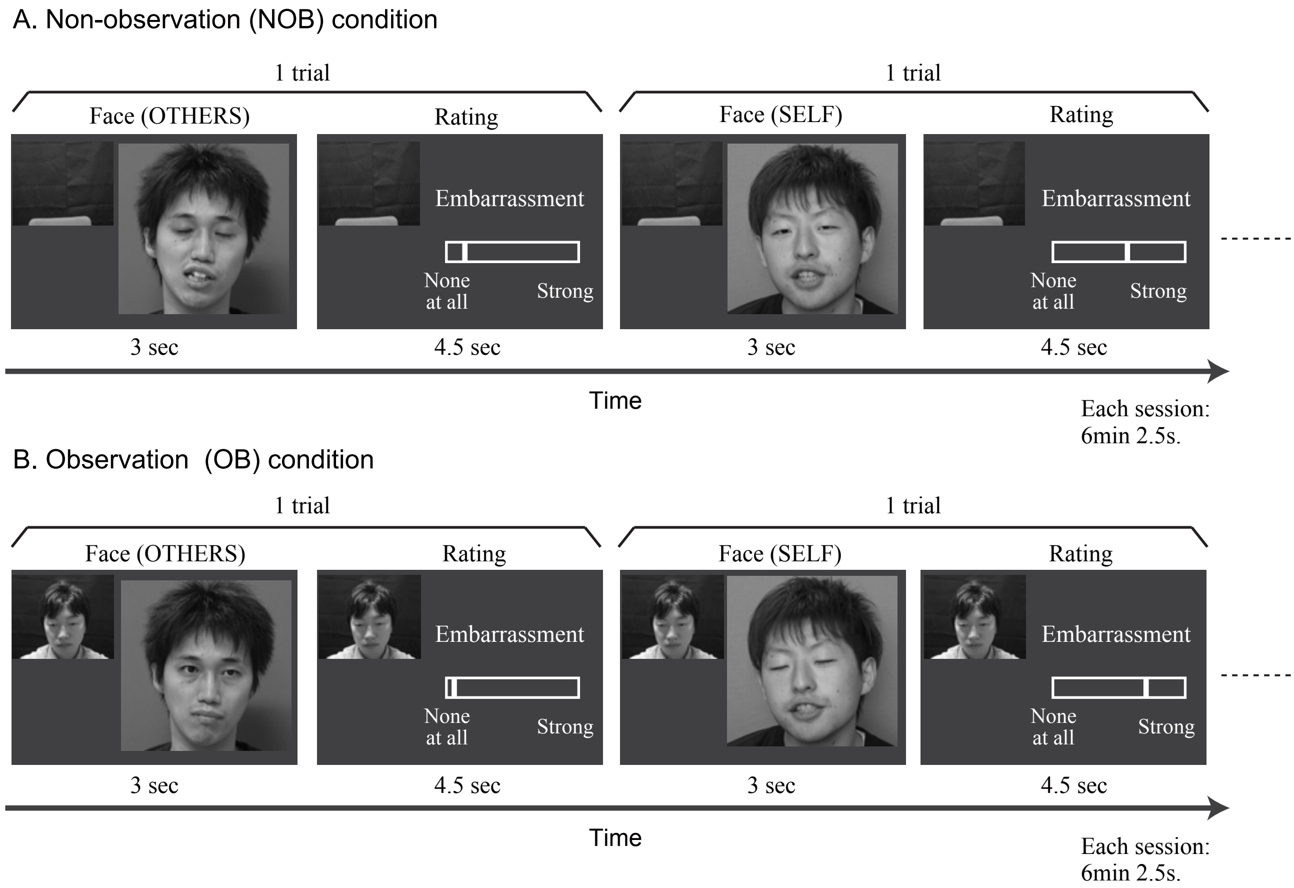

A. Non-observation (NOB) condition
1 trial
1 trial
Face (OTHERS)
Rating
Face (SELF)
Rating
Embarrassment
None at all
Strong
Embarrassment
None at all
Strong
3 sec
4.5 sec
3 sec
4.5 sec
Time
Each session: 6min 2.5s.
B. Observation (OB) condition
1 trial
1 trial
Face (OTHERS)
Rating
Face (SELF)
Rating
Embarrassment
None at all
Strong
Embarrassment
None at all
Strong
3 sec
4.5 sec
3 sec
4.5 sec
Time
Each session: 6min 2.5s.

Figure 1

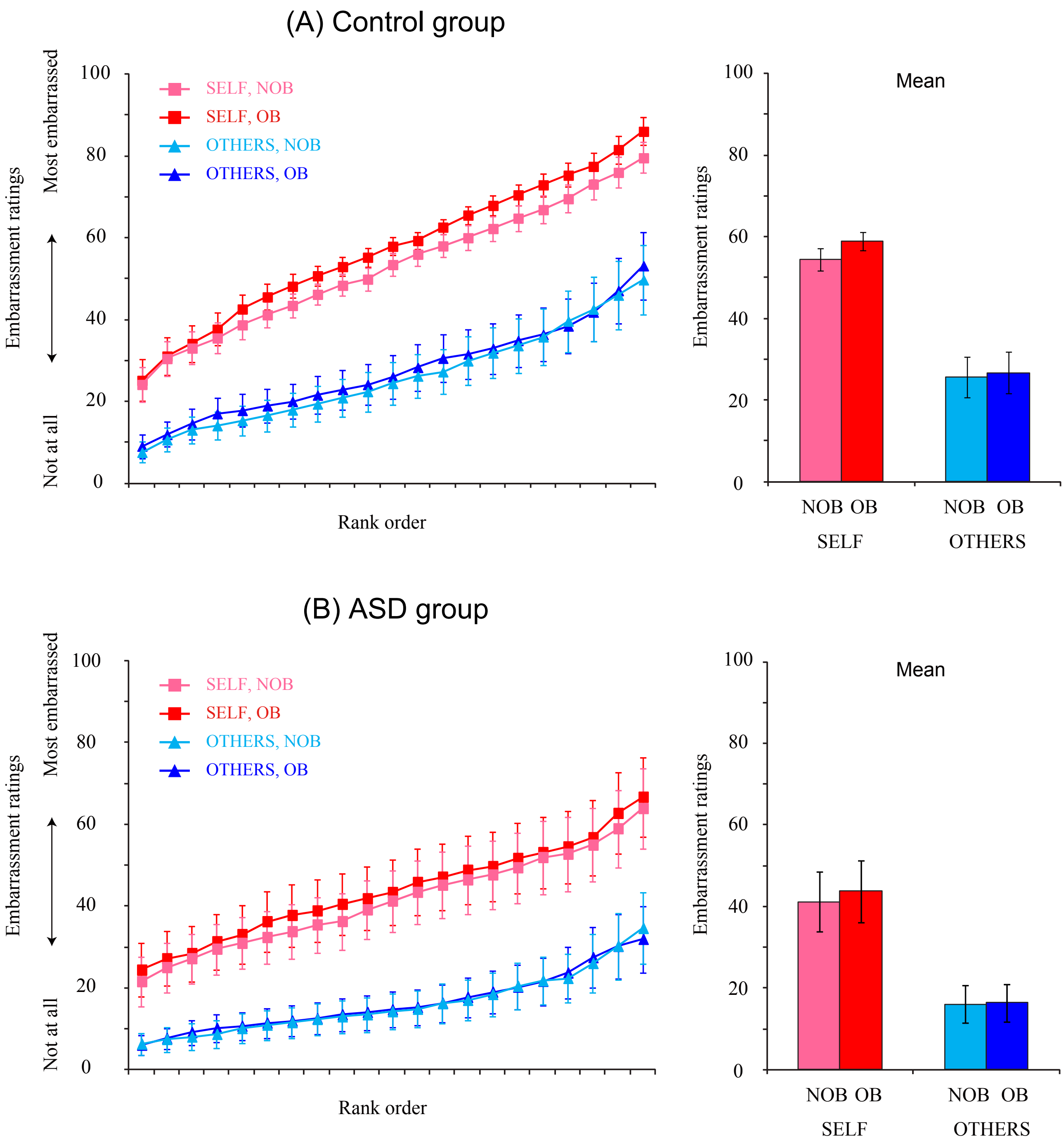
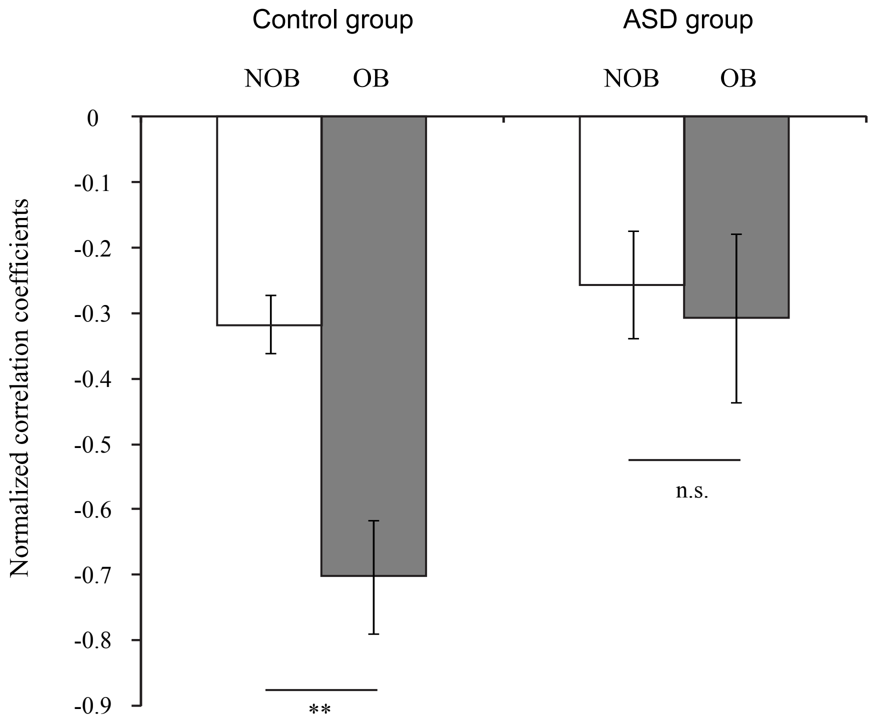

(A) Control group
Most embarrassed
Embarrassment ratings
Not at all
100
80
60
40
20
0
SELF, NOB
SELF, OB
OTHERS, NOB
OTHERS, OB
Rank order
Mean
NOB OB
SELF
NOB OB
OTHERS
(B) ASD group
Rank order
Mean
NOB OB
SELF
NOB OB
OTHERS

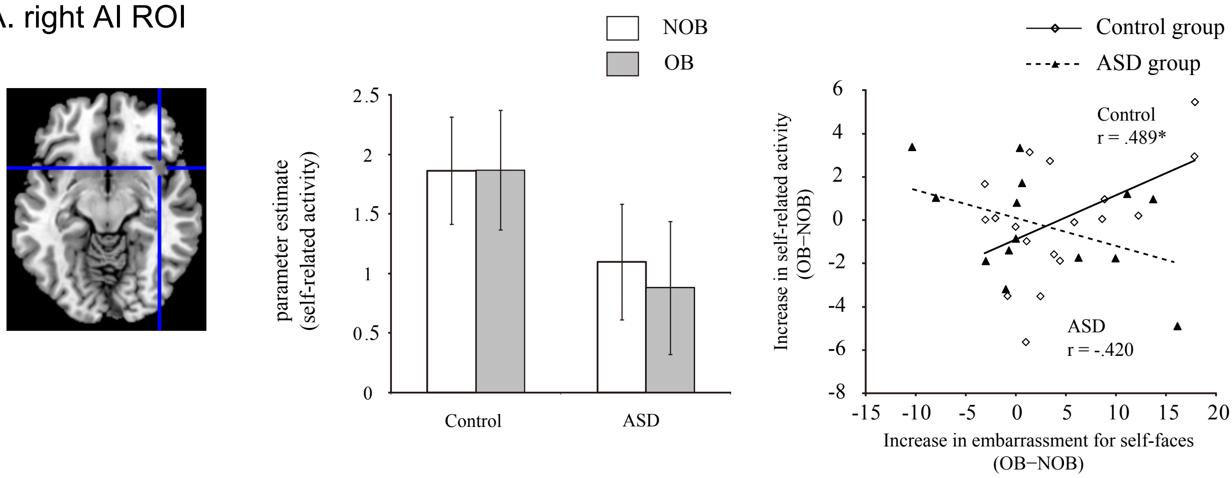
Figure 2



Control group
ASD group
NOB
OB
NOB
OB
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
Normalized correlation coefficients
n.s.
**

Figure 3

A. right AI ROI



B. caudal ACC ROI

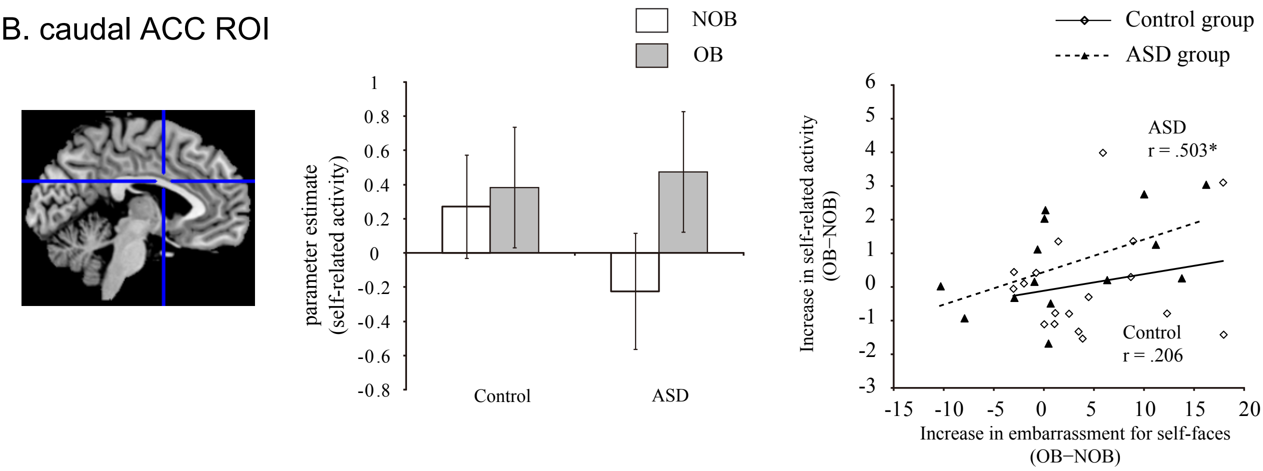


Figure 4

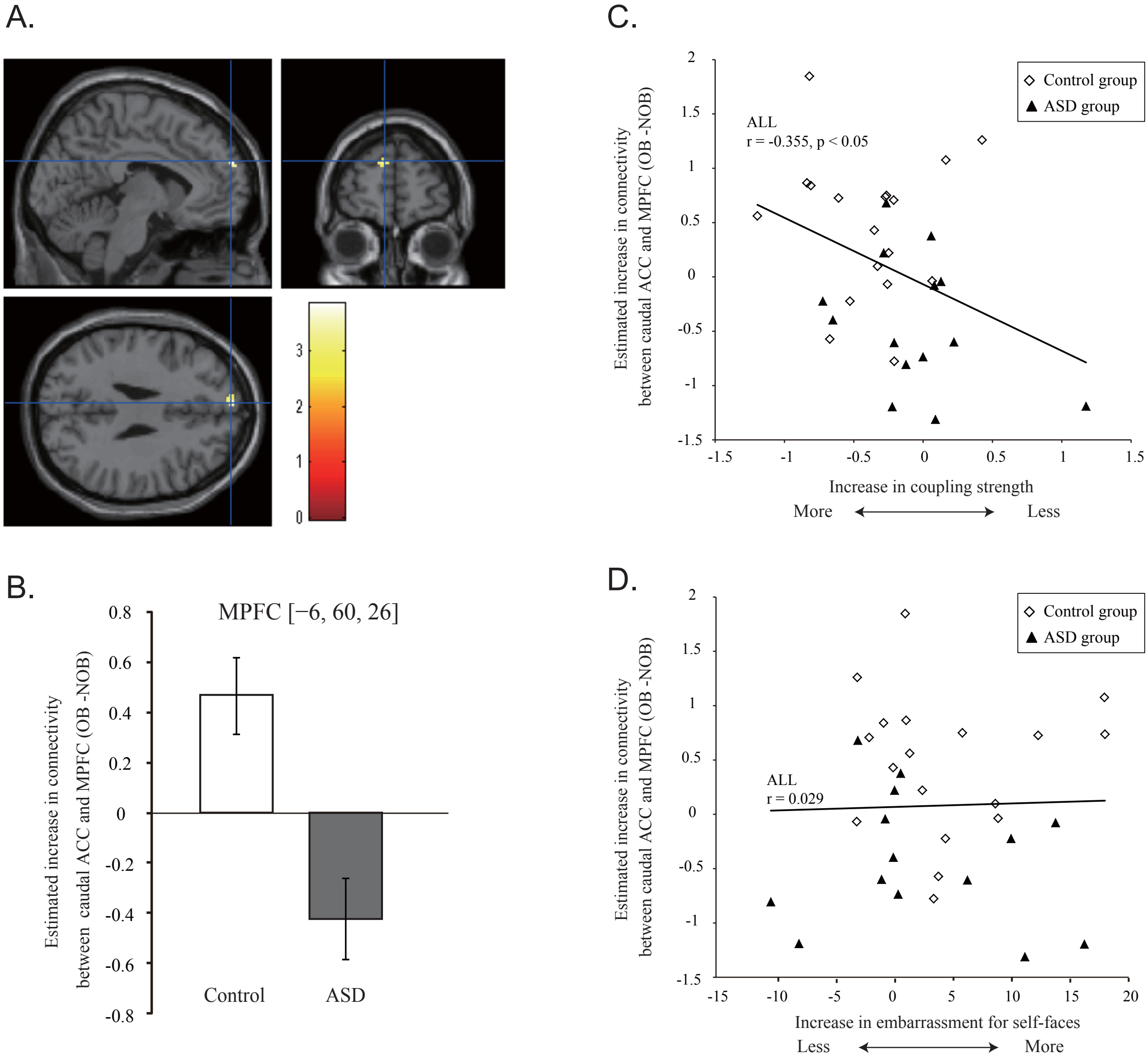

A.
B.
MPFC [−6, 60, 26]
Estimated increase in connectivity between caudal ACC and MPFC (OB -NOB)
Control
ASD
C.
Control group
ASD group
ALL
r = -0.355, p < 0.05
Increase in coupling strength
More
Less
D.
ALL
r = 0.029
Increase in embarrassment for self-faces
Less
More

Figure 5

Supplementary figure 1