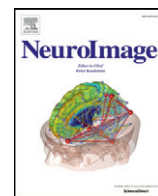


Contents lists available at [ScienceDirect](http://ScienceDirect.com)

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Neural substrates of shared attention as social memory: A hyperscanning functional magnetic resonance imaging study



Takahiko Koike ^{a,1}, Hiroki C. Tanabe ^{a,b,1}, Shuntaro Okazaki ^a, Eri Nakagawa ^{c,a}, Akihiro T. Sasaki ^{a,d,e}, Koji Shimada ^{a,f,g}, Sho K. Sugawara ^a, Haruka K. Takahashi ^{a,h}, Kazufumi Yoshihara ^{a,i}, Jorge Bosch-Bayard ^{J,a}, Norihiro Sadato ^{a,h,g,*}

^a Division of Cerebral Integration, Department of Cerebral Research, National Institute for Physiological Sciences (NIPS), Aichi 4448585, Japan

^b Division of Psychology, Department of Social and Human Environment, Graduate School of Environmental Studies, Nagoya University, Nagoya 4648601, Japan

^c Graduate School of Intercultural Studies, Kobe University, Kobe 6578501, Japan

^d Pathophysiological and Health Science Team, RIKEN Center for Life Science Technologies, Kobe 6500047, Japan

^e Department of Physiology, Osaka City University Graduate School of Medicine, Osaka 4678603, Japan

^f Research Center for Child Mental Development, University of Fukui, Fukui 9101193, Japan

^g Biomedical Imaging Research Center (BIRC), University of Fukui, Fukui 9101193, Japan

^h Department of Physiological Sciences, School of Life Sciences, SOKENDAI (The Graduate University for Advanced Studies), Kanagawa 2400193, Japan

ⁱ Department of Psychosomatic Medicine, Kyushu University, Fukuoka 8128582, Japan

^J Institute for Neurobiology, National Autonomous University of Mexico, Santiago de Queretaro, Mexico

ARTICLE INFO

Article history:

Received 29 May 2015

Accepted 26 September 2015

Available online 26 October 2015

Keywords:

Hyperscanning

Shared attention

Eye-blink synchronization

Inter-individual neural synchronization

Joint attention

Mutual gaze

ABSTRACT

During a dyadic social interaction, two individuals can share visual attention through gaze, directed to each other (mutual gaze) or to a third person or an object (joint attention). Shared attention is fundamental to dyadic face-to-face interaction, but how attention is shared, retained, and neutrally represented in a pair-specific manner has not been well studied. Here, we conducted a two-day hyperscanning functional magnetic resonance imaging study in which pairs of participants performed a real-time mutual gaze task followed by a joint attention task on the first day, and mutual gaze tasks several days later. The joint attention task enhanced eye-blink synchronization, which is believed to be a behavioral index of shared attention. When the same participant pairs underwent mutual gaze without joint attention on the second day, enhanced eye-blink synchronization persisted, and this was positively correlated with inter-individual neural synchronization within the right inferior frontal gyrus. Neural synchronization was also positively correlated with enhanced eye-blink synchronization during the previous joint attention task session. Consistent with the Hebbian association hypothesis, the right inferior frontal gyrus had been activated both by initiating and responding to joint attention. These results indicate that shared attention is represented and retained by pair-specific neural synchronization that cannot be reduced to the individual level.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Social interactions enable us to evaluate what the mental states and intentions of others might be. Importantly, the type of social experience is fundamentally different when we directly interact with others (second-person view) rather than merely observing them (spectator view; [Schilbach et al., 2013](#)). Social interactions have been postulated to have three prominent characteristics ([Schilbach et al., 2013](#)). First, there are different roles for the interacting individuals (e.g., initiator

and responder at the simplest level). Second, sharing of attention, intention, and motivation are created de novo within an interaction, which are critical for the progress and continuation of the interaction itself. Finally, there is a context for the interaction based on past events and experience. Shared attention, or coordinated visual attention during face-to-face interaction, such as joint attention and mutual gaze ([Emery, 2000](#)), is a typical and fundamental process that fulfils the above three characteristics.

Humans use eye gaze to detect another individual's focus of attention, orient their own attention to the same locus, and draw inferences regarding the other individual's goals ([Allison et al., 2000](#); [Calder et al., 2007](#); [Nummenmaa and Calder, 2009](#)). Mutual gaze provides a communicative link between humans by sharing the message of "I am attending to you" ([Farroni et al., 2002](#); [Schilbach, 2015](#)). Joint attention (JA)

* Corresponding author at: National Institute for Physiological Sciences (NIPS), Myodaiji, Okazaki 444-8585, Japan. Fax: +81 564 55 7843.

E-mail address: sadato@nips.ac.jp (N. Sadato).

¹ T.K. and H.C.T. equally contributed to this work.

coordinates attention between partners to share an awareness of objects or events (Mundy et al., 1986). There are two types of JA: Initiating JA (IJA) is the ability to create spontaneously a shared point of reference using mutual gaze, and by alternating gaze between objects and other individuals; and responding JA (RJA) is the ability to follow the direction of the initiator's gaze in order to share attention towards the object (Mundy et al., 2009). Thus IJA, RJA, and mutual gaze are tightly linked (Emery, 2000; Perrett and Emery, 1994) and function to share attention within a dyad or to a third object. The importance of mutual gaze and JA in the development of social cognition has been stressed (Mundy and Newell, 2007). However, it is unknown if the attention shared between interactants is retained as social memory (Oullier et al., 2008), nor its neural substrates. As shared attention is an interactively constituted phenomenon which cannot be reduced to responses at the individual level, hyperscanning is really needed to depict its neural mechanisms and the hypothesized memory trace (Konvalinka and Roepstorff, 2012; Schilbach, 2015).

A previous hyperscanning functional magnetic resonance imaging (fMRI) study showed inter-individual neural synchronization within the right inferior frontal gyrus (IFG) during JA after the removal of common effects of task (Saito et al., 2010). JA is regarded as a two-way behavioral stimulus-to-brain coupling phenomenon, such that the behavior of one person is coupled to the brain activation of the other, and vice versa (Hari et al., 2009). Thus neural synchronization in the right IFG may represent inter-individual shared attention as a 'readiness potential' for subsequent gaze based interaction (Schilbach, 2015).

Inter-individual neural synchronization can be understood based on the premise that the perceptual system of one brain can become coupled to the motor system of another (Dumas, 2011; Jacob, 2009; Schippers and Keysers, 2011) through Hebbian association. This Hebbian account was previously invoked to explain automatic mimicry (Keysers and Perrett, 2004; Del Giudice et al., 2009; Sasaki et al., 2012). That is, the basis of automatic mimicry is motor and perception action representations becoming tightly linked in such a way that perceiving another person's action activates the same representations as performing the action. It was argued that the action representation, or motor-perceptual common representation, could be formed as an internal model through Hebbian associations trained during motor execution (Keysers and Perrett, 2004; Del Giudice et al., 2009). Given that we continuously monitor our own actions, their sensory consequences are systematically and synchronously paired with motor commands. This predicts the emergence of Hebbian connections that link motor programs to sensory consequences (forward internal models), and vice versa (inverse internal models), even during social interaction (Wolpert et al., 2003): In social Hebbian connections, one's own motor programs are linked to the sensory consequences provided by another's actions. We applied this motor-perceptual common representation account to attention control. Our hypothesis was that the training of joint attention, JA causes a social Hebbian association between initiating and responding joint attention, IJA and RJA. This is because the control of directing attention toward a third object for initiating JA is temporally linked to sensory consequences of the partner's response of directing attention to the same object, that is, RJA. Thus, social Hebbian association could link the neural activities induced by IJA to those by induced by RJA of the partner, resulting in neural synchronization. If this is true, then both IJA and RJA should activate the right IFG, and the synchronization should be retained as social memory after the JA experience.

To quantify interpersonal aspects of the social interaction such as shared attention, finding adequate and useful behavioral markers is critical (Schilbach, 2014). Attentional coordination during shared attention is in the spatial domain. Less explicitly included in the shared attention is the common "time window" of the attention directed to each other during mutual gaze, that precedes the JA. To perform a JA task, the initiator is required to confirm that the partner is attending to the initiator during a preceding eye contact condition, and the responder is required

to attend to the initiator's eye movements. Thus, they are to share an attentional temporal window.

Eye-blinks are known to define the attentional temporal window. Demands for attentional resources modulate the rate of eye-blinks (Bentivoglio et al., 1997; Shultz et al., 2011), and the timing of eye-blinks is associated with implicit (Herrmann, 2010) and explicit (Orchard and Stern, 1991) attentional pauses in task content. Eye-blinks of participants are synchronized while viewing the same video stories (Nakano et al., 2009), and between listener and speaker in face-to-face conversation (Nakano and Kitazawa, 2010). Considering that blinks define the attentional "window", synchronization of eye-blinks between face-to-face interactants can be taken as an index of shared attention. Once a Hebbian association is established, the initiation of eye-contact between the previously trained pair will induce the control–response linkage in the attentional domain that can be measured via eye-blink synchronization.

Accordingly, our hypothesis was that shared attention during a JA task would be represented by blink synchronization and retained as the social memory, and that this social memory would be represented by enhanced inter-individual neural synchronization in the right IFG. We also expected the right IFG to be activated by both RJA and IJA. To test these hypotheses, we conducted hyperscanning fMRI during a JA task, and during mutual eye gaze both before and after the JA task (Fig. 1A). Three fMRI experiments were carried out. In Experiment 1, participants performed real-time mutual gaze (MG1 condition, Fig. 1A) followed by the JA tasks (Figs. 1B to D) on Day 1; on Day 2 of Experiment 1, participants again underwent the real-time mutual gaze condition (MG2 condition, Fig. 2A). There was a control condition in which participants believed that they were performing real-time interaction using eye contact, but in actuality they watched a video recorded on Day 1 (VIDEO condition, Fig. 1A). Experiment 2 was a 2-day hyperscanning fMRI study consisting of the real-time mutual gaze task without JA on Day 1. In Experiment 3, participants completed the MG1 and JA tasks as in Experiment 1 on Day 1, but on Day 2 they performed the real-time mutual gaze task with a new partner different from the partner they had on Day 1.

Material and methods

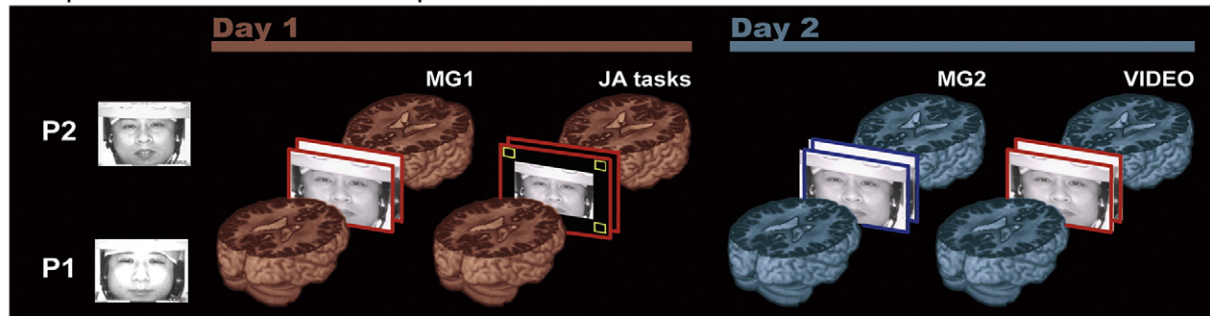
Participants

A total of 96 volunteers participated. Prior to the experiment, we assigned participants of the same gender to pairs. Participants were not mutually acquainted prior to the start of the experiment. All participants except one were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). None of the participants had a history of neurological or psychiatric illness. The protocol was approved by the ethical committee of the National Institute for Physiological Sciences (Okazaki, Japan), and the experiments were undertaken in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (the Declaration of Helsinki). All participants gave their written informed consent to participate in the study.

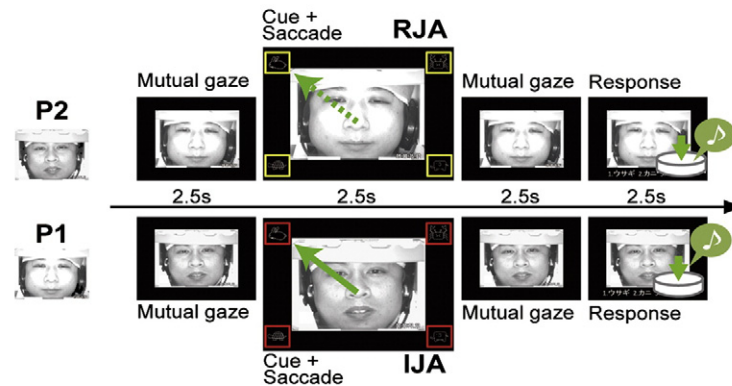
Experimental setup

To measure neural activation during the online exchange of eye signals between pairs of participants, we used a hyperscanning paradigm with two MR scanners (Magnetom Verio 3 T, Siemens, Erlangen, Germany), installed side-by-side in parallel to minimize interference between magnetic fields. The two MR scanners shared one control room, and the onset of scanning was synchronized by an external trigger that was generated by in-house MS-DOS software. To enable reciprocal face-to-face interaction, the two MRI scanners were used alongside online video cameras and infrared eye-tracking systems (NAC Image Technology Inc., Tokyo, Japan). The infrared camera

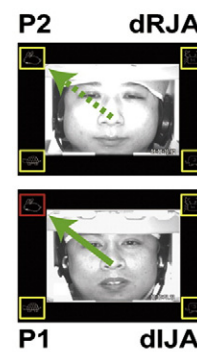
A Experimental conditions in Experiment 1



B Time course of JA tasks



C dIJA/dRJA



D CTRL

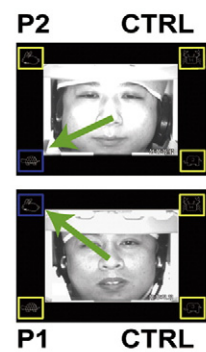


Fig. 1. Procedures of Experiment 1. A, Brain images indicate fMRI data obtained on Day 1 (orange) during real-time mutual gaze via the video system (orange frame, MG1), and during the JA task (red frame). On Day 2, fMRI data (blue brains) were obtained during real-time mutual gaze (blue frame, MG2), and while watching the video of the partner recorded on Day 1 (orange frame, VIDEO). B, Time course of the IJA/RJA tasks. Following 2.5 s of mutual gaze, the “all four red” cue prompted participant 1 (P1) to freely select one of the objects and shift his/her gaze to it (green solid arrow). The same objects with yellow frames were simultaneously presented to the counterpart (P2). The “all four yellow” cue prompted P2 to shift his/her gaze to the object that P1 attended to (green dashed arrow). Once the objects disappeared, participants were required to return their gaze back to the mutual gaze. The names of four objects were then presented. Participants were requested to select the name of the object that they had attended to using a button press, with aural feedback indicating successful JA. C, In the designated-choice IJA/RJA (dIJA/dRJA) condition, P1 was instructed to shift their gaze to the designated target indicated by a red frame. D, In the Control (CTRL) condition, both participants were instructed to shift their gaze to the blue target without reference to the partner's eye movement.

captured images of each participant's face including the eyes and eyebrows, which were transferred to a personal computer (Dimension 9200, Dell Computer Co., Round Rock, TX). Video data of participants' faces were recorded to analyze eye-blink synchronization. The current study used a 32-channel phased array coil modified to consist of 24 channels. The Siemens Verio standard 32-channel phased array coil consists of a bottom component of 20 channels and a top component of 12 channels. Since the top component covers the eye region, it is unsuitable for the present study of joint attention. To visualize the eye region fully, the top component of the standard 32-channel coil was replaced with a four-channel small flex coil (Siemens) that was attached with a special holding fixture (Takashima Seisakusho Co., Tokyo, Japan). This modification causes a difference in the spatial distribution of temporal signal to noise ratio (SNR, Triantafyllou et al., 2005). Specifically, because of the relative paucity of the coils, the temporal SNR of 24-channel was lower on the frontal region than 32-channel, and vice versa in the central and posterior brain region. However, considering far better temporal SNR of multi-channel phased array coil compared with a single circular polarized (CP) coil (Wiggins et al., 2006), the 24 channel setting in the present study is acceptable.

The visual stimuli for the JA tasks were generated using Presentation software (Neurobehavioral Systems, Albany, CA). Video images of participants' faces were captured using an on-line video camera system and combined using a Picture-in-Picture system (NAC Image Technology and Panasonic System Solutions Japan Co. Ltd., Tokyo, Japan). The combined visual stimuli were projected using a liquid crystal display (LCD) projector (CP-SX12000J, Hitachi Ltd., Tokyo, Japan) onto a half-transparent screen that sat on the scanner bed approximately

190.8 cm from participants' eyes, and were presented at a visual angle of $13.06^\circ \times 10.45^\circ$.

Experiment 1

Thirty-four participants took part in Experiment 1, which consisted of a mutual gaze task and a JA task on Day 1, followed by a mutual gaze task and other control tasks on Day 2 (Fig. 1A). One pair could not complete the Day 2 experiments due to technical difficulties. Thus, we acquired JA task data from 34 participants (18 men and 16 women; mean \pm standard deviation [SD] age, 21.8 ± 4.63 years), and mutual gaze data from 32 participants (16 men and 16 women; mean \pm SD age, 22.0 ± 4.73 years). The mean \pm SD interval between days 1 and 2 was 7.25 ± 7.58 days.

Mutual gaze task (Day 1, MG1). On the first day, participants performed a real-time mutual gaze task for 9 min, during which they watched their partner's face through a video system in real time (Fig. 1A). Participants were instructed to gaze at their partner's eyes projected on the screen, and to imagine what their partner was thinking at that time. We did not try to control for the frequency or timing of eye-blinks or natural eye movements around the partner's eyes. Only one pair showed a sudden and clear emergence of emotion (i.e., laughing) shortly after the start of fMRI recording. At that time, the recording was stopped. We restarted the mutual gaze experiment, and the re-recorded fMRI data were used in subsequent analysis. Participants' faces were recorded, and the video data were used on Day 2. fMRI data were acquired simultaneously from the two participants. After MG1, participants rated how well they were able to focus

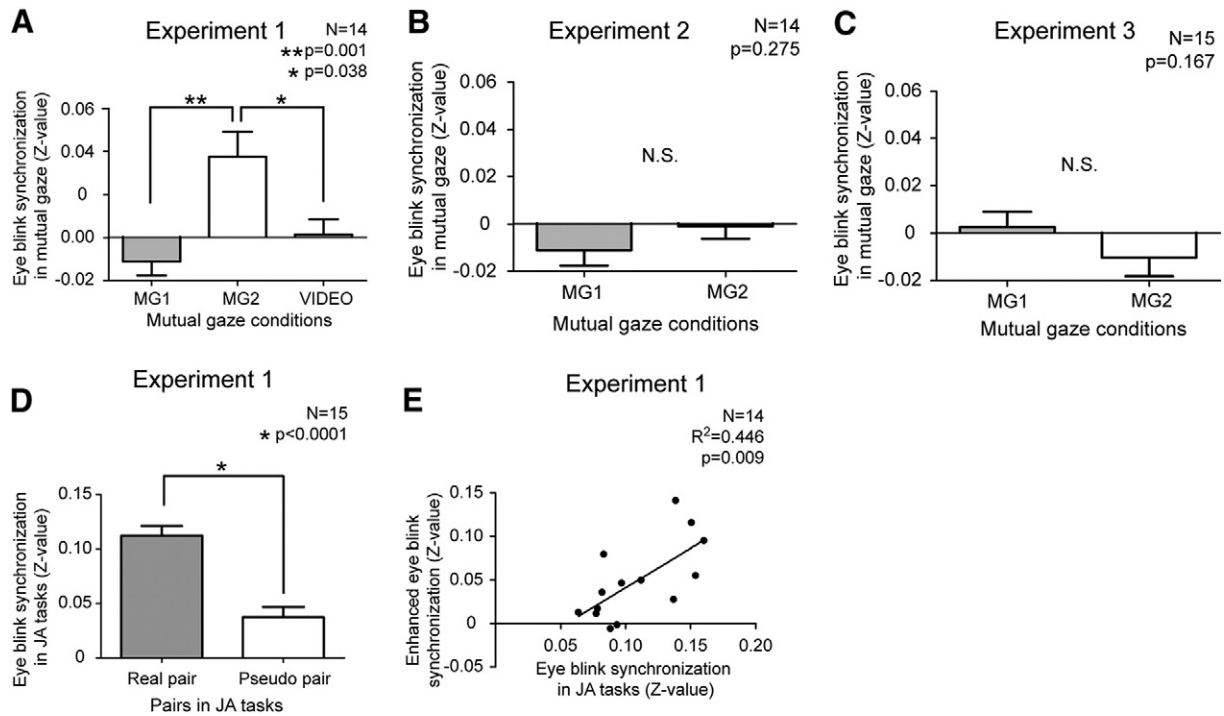


Fig. 2. Inter-individual eye-movement synchronization in three experiments. A, The eye-movement synchronization between paired participants during the MG1, MG2, and VIDEO conditions in Experiment 1. B, The synchronization in Experiment 2 in which participants did not perform JA task in Day 1. C, The synchronization in Experiment 3 in which participants were paired with a different partner in Day 2. D, Eye-movements synchronization in the JA task between paired participants (real pair) and two participants who were not paired but performing JA tasks along with the same time schedule (pseudo pair). E, Correlation between the eye-blink synchronization during JA tasks and enhancement of eye-blink synchronization from MG1 to MG2.

on their partner's mind during the task, using a visual analogue scale (VAS).

JA tasks (Day 1). Participants performed the JA task by exchanging eye gaze information in real time through a video system. During MRI scanning, the other participant's face was presented at the center of the screen, and four target objects were displayed in the corners of the screen (Fig. 1B). Thus, the target objects and the partner's face were presented to participants in both scanners at the same time (Fig. 1B). The target objects were standardized line drawings of five animals (rabbit, crab, turtle, elephant, and cat) and five objects (chair, fan, clock, bus, and ribbon). In each trial, four of the five images were randomly selected within each category (i.e., animal or object).

Free choice IJA/RJA tasks. The JA tasks involved the initiation of JA by looking at one of the target objects spontaneously (IJA) and also looking at the object indicated by the partner's eye movement (RJA), and are shown in Fig. 1B. The "all four red" cue indicates that the participant has to initiate JA, and the "all four yellow" cue indicates that the participant has to respond to their partner's eye gaze (Fig. 1B). The roles of initiator and responder were switched randomly between trials within a run. The duration of each trial was 10 s, and the total time of the IJA/RJA run was approximately 7 min. The run was repeated twice.

Designated-choice IJA/RJA tasks. A designated-choice IJA/RJA (dIJA/dRJA) task was identical to the free-choice IJA/RJA tasks except that one object had a red frame and the other three objects had yellow frames when presented to the participant who had to initiate JA (P1). This "one red" cue prompted participant 1 to shift his/her gaze to the designated red-framed object (Fig. 1C). The responder (P2) did the same as in the IJA/RJA task: participant 2 followed the initiator's eye movement. The dIJA/dRJA run was also repeated twice.

Control task. The control task (CTRL) was identical to the designated IJA/RJA tasks except that one blue-framed object and three yellow-framed objects were presented to both participants (Fig. 1D). This "one blue" cue prompted both participants to shift their gaze to the

designated, blue-framed object. We required participants to perform this task without reference to their partner, even though the live image of the partner was displayed on the screen. One CTRL run was performed.

Mutual gaze task (Day 2, MG2). On Day 2, participants were paired with same partner as on Day 1, and did the real-time mutual gaze task again. The instructions, setting, and fMRI parameters were identical to the MG1 condition, except for the duration of the condition (7 min) (Fig. 1A). No pair showed a sudden and clear emergence of emotion.

Control gaze task (Day 2, VIDEO). This task was identical to that in the MG2 condition, except that participants actually watched the video recorded during the MG1 condition instead of viewing their partner's face in real time. This condition is named VIDEO (Fig. 1A). The order of conditions (MG2 and VIDEO) was randomized across pairs of participants. After the experiment, a VAS score was used to assess whether participants were able to focus on their partner's thoughts. VAS scores showed no differences in focusing on partners' thoughts between VIDEO, MG2, and MG1 conditions ($p = 0.08$, $F[2, 31] = 2.635$, one-way repeated measures ANOVA followed by post hoc tests with Bonferroni correction for multiple comparisons). In addition, we confirmed via a post-experiment interview that no participants thought that they were viewing a recorded video image.

Experiment 2

Thirty participants (16 men and 14 women; mean \pm SD age, 20.6 ± 2.92 years) took part in Experiment 2, which was identical to Experiment 1 except that participants did not perform the JA task on Day 1 or the VIDEO condition on Day 2. No pair showed a sudden and clear emergence of emotion. The mean \pm SD interval between days 1 and 2 in Experiment 2 was 9.40 ± 9.23 days. There was no significant difference between Day 1 and 2 intervals in Experiment 1 compared to Experiment 2.

Experiment 3

Thirty-two participants took part in Experiment 3, which was identical to Experiment 1 except that participants did not complete the VIDEO condition on Day 2, and had a different partner during the MG2 (mutual gaze in Day 2) task than that on Day 1. The new partner had also performed the MG1 and JA tasks on Day 1. No pair showed a sudden and clear emergence of emotion. Due to technical difficulties, fMRI data were not acquired from one participant for the JA task. Therefore, data from 31 participants (11 men and 20 women; mean \pm SD age, 23.1 ± 5.45 years) were analyzed for the JA tasks, and data from 32 participants (12 men and 20 women; mean \pm SD age, 23.0 ± 5.39 years) were analyzed for the mutual gaze conditions. The mean \pm SD interval between days 1 and 2 in Experiment 3 was 5.81 ± 6.67 days, which did not significantly differ from the intervals in Experiments 1 and 2.

MRI data acquisition

MRI time-series data were acquired using ascending-order T2*-weighted, gradient echo, echo planar imaging (EPI) sequences. Each volume consisted of 36 slices, each 3.0 mm thick with a 0.5-mm gap, to cover the entire cerebral cortex and cerebellum. The acquisition time was 2300 ms and the delay-in-repetition time (TR) was 200 ms. Thus, the time interval between two volumes was 2500 ms with a flip angle (FA) of 80° and a 30-ms echo time (TE). The field of view (FOV) was 192 mm and the in-plane matrix size was 64×64 pixels. For the JA experiments, we acquired 168 volumes (7 min) per run. For the mutual gaze condition, we acquired 216 volumes (9 min) in the MG1 conditions, and 168 volumes (7 min) in the MG2 and VIDEO conditions. For anatomical reference, T1-weighted high-resolution images were obtained with three-dimensional (3D) magnetization-prepared rapid-acquisition gradient echo sequencing (TR = 1800 ms; TE = 2.97 ms; FA = 9° ; FOV = 256 mm; and voxel dimensions = $1 \times 1 \times 1$ mm) using the full 32-channel phased array coil.

Behavioral data analysis

Because of technical problems with the video-recording system, eye-blink synchronization analysis was carried out with only 14 of the 16 participant pairs in the mutual gaze conditions in Experiment 1, 15 of the 16 pairs in the JA tasks in Experiment 1, 14 of the 15 pairs in Experiment 2, and 15 of the 16 pairs in Experiment 3. To obtain the time course of eye-blink responses, we measured pupil size (i.e., the vertical diameter of the pupil) using the Audio Visual Interleave (AVI) video data (640×480 pixels, 30 frame/s, and 8-bit gray scale) that were recorded during the fMRI experiments. Using in-house MATLAB R2010a (MathWorks, Natick, MA, USA) scripts, the center of the pupil was detected in each video frame of individual data. Then, the vertical pupil size in each video frame was calculated with binarized AVI frames. The continuous time course of the eye-blink during each experiment condition was linearly de-trended and normalized to make the average and standard deviation equal to 0 and 1, respectively. Using the normalized time-courses of the first 7 min of the recorded data, we calculated Pearson's correlation coefficients (r) of eye-blink between paired participants. The correlation coefficient r was transformed to a z-score using the Fisher's r-to-z transformation. Finally, the z-score was used in the group-level statistical analyses. To test whether the JA-related eye-blink synchronization was pair specific, synchronization was also evaluated between randomly generated pseudo pairs in each of the experimental conditions.

fMRI image preprocessing

Image preprocessing was performed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) implemented in MATLAB 2010b. After all of the volumes were realigned, differences in slice-timing within each image volume were corrected. The reference

image was on the center of volume. The whole-head 3D MPRAGE volume was co-registered with the EPI volumes, and the whole-head 3D MPRAGE volume was normalized to the Montréal Neurological Institute (MNI) T1 image template (ICBM152) using a non-linear basis function. Subsequently, normalization parameters were applied to all of the EPI volumes. The normalized EPI images were then spatially smoothed in three dimensions using an 8-mm full-width at half-maximum Gaussian kernel. For the inter-individual synchronization analysis, we additionally applied a low-frequency band-pass filter (0.01–0.08 Hz) to the blood oxygen level dependent (BOLD) time-series data to eliminate physiological noise components (Cordes et al., 2001). Motion-related artifacts were removed using a general linear model with six regressors of no interest.

Inter-individual neural synchronization between participants

As in previous studies (Saito et al., 2010; Tanabe et al., 2012), inter-individual synchronization was evaluated by calculating the correlation coefficients between the BOLD time-series data at homologous MNI coordinate positions (x, y, z). In the evaluation of inter-individual neural synchronization, regardless of differences in conditions, the first 7 min of the BOLD time-series was analyzed.

The calculation was performed with in-house scripts using MATLAB 2010b. To minimize computational processing time, the analysis was performed only on grey-matter voxels, defined using the probabilistic map of gray matter (grey.nii) in SPM8. The calculated correlation coefficient was then transformed to a z-score using Fisher's r-to-z transformation. Using the z-score images and SPM8, inter-individual neural synchronization in the MG1 and MG2 was compared in a random effects model (Friston et al., 1999).

Regions of interest definition

Based on the regions showing inter-individual neural synchronization, two regions of interest (ROIs) were defined. The right middle temporal gyrus (MTG) ROI was defined by the cluster that had significant inter-individual neural synchronization in MG1 in Experiment 1 (Fig. 2D and Table 2). The right IFG ROI was defined as the cluster showing enhanced synchronization during MG2 compared with MG1 (Fig. 3C and Table 2).

As the extrastriate body area (EBA) is known to receive both sensory inputs of others' body information (Downing et al., 2001) and efference copies from one's own body information (Astafiev et al., 2004; Orlov et al., 2010), we hypothesized that it might receive both self and other eye-blink information; hence, EBA activity might be synchronized between participants during mutual gaze. To define the location of the EBA in the middle temporal gyrus, a subset of participants in Experiment 1 (8 men and 9 women; mean \pm SD age, 22.8 ± 5.19 years) completed an EBA localizer task with a conventional block design (Downing et al., 2001) as described in a previous study (Okamoto et al., 2014). Imaging parameters for image acquisition and preprocessing procedures were identical to those used in JA experiments except for the number of volumes. We performed a random-effects analysis using SPM8 (Friston et al., 1999).

JA activation

In total, we collected fMRI data from 65 participants in the JA task (34 in Experiment 1, and 31 in Experiment 3). We adopted a summary statistics approach in order to depict the neural substrates of the tasks. In the individual analyses, we fitted a general linear model to the fMRI data from each participant. Neural activity was modeled using delta functions convolved with a canonical hemodynamic response function. The design matrix included five regressors (IJA, RJA, dIJA, dRJA, and CTRL) that were modeled at the onsets of each event, and the duration was 0 s. The data were high-pass filtered with a cut-off period of 128 s

to remove low-frequency signal drifts. Serial autocorrelation assuming a first-order autoregressive model was estimated from the pooled active voxels using the restricted maximum likelihood procedure, and was used to whiten the data (Friston, 2002). No global scaling was performed. The estimated parameters were calculated by performing least-squares estimation on the high-pass filtered and whitened data and design matrix. The weighted sum of the parameter estimates in the individual analyses constituted contrast images that were used for the second-level analysis. The resulting set of voxel values for each contrast constituted a statistical parametric map of the t statistic (SPM $\{t\}$). Our hypothesis was that enhanced synchronization, caused by social Hebbian association learning, would occur during JA tasks, expecting that the neural areas of enhanced synchronization would be activated by both IJA and RJA. Therefore, using MarsBaR toolbox (<http://sourceforge.net/projects/marsbar/>) with the “mean” function, we calculated the mean Z-value of voxels included within the right IFG ROI that was obtained as the area of enhanced inter-individual neural synchronization during mutual gaze (MG2–MG1 contrast). Using analysis of variance (ANOVA), we tested whether the ROI was commonly activated by both IJA and RJA.

Full description of the JA related activation will be presented elsewhere (Koike et al., in preparation).

Eye-blink-related activation

As in a previous study (Nakano et al., 2013), we evaluated eye-blink-related activation during the MG2 condition in Experiment 1 by modeling the onset of eye-blinks within the framework of the general linear model of SPM12 which is equivalent to SPM8. Through individual data analysis we obtained contrast images of the estimated parameter, which were used for a random-effects analysis (Friston et al., 1999). We also acquired the residual BOLD time-course by modeling out eye-blink-related activation. Using MarsBaR toolbox, we calculated the mean Z-value of voxels included within the cluster for preparing bar graphs. An ANOVA was done to test for a difference in activation between clusters.

Inter-individual neural synchronization with residual BOLD signal

To test whether the enhancement of inter-individual neural synchronization in the right IFG was merely caused by eye-blink-related activation, inter-individual synchronization was recalculated using the residual BOLD time-course resulting from the process of evaluating eye-blink-related activation. Except for the use of the residual BOLD image, the procedure of calculating inter-individual neural synchronization was identical to that described above. The MarsBaR toolbox was used to calculate the mean Z-value of voxels included within the right MTG and IFG ROIs.

Statistical thresholding of imaging results and anatomical labeling

The threshold for significance of the SPM $\{t\}$ was set at $p < 0.05$ with a family-wise error (FWE) correction at the cluster level for the entire brain with an uncorrected height threshold of $p < 0.001$ (Friston et al., 1996).

Anatomical labeling was based on Automated Anatomical Labeling (Tzourio-Mazoyer et al., 2002) and the Anatomy toolbox v1.8 (Eickhoff et al., 2007, 2005).

Correlation between behavioral synchronization, neural synchronization, and JA-related neural synchronization

As there was a significant increment in blink synchronization and neural synchronization in the right IFG during mutual gaze following the JA task (MG2 as compared with MG1, Figs. 2A and 3C), correlation analysis was performed. Using MarsBaR, we calculated the mean Z-value of voxels included within the right IFG ROI. To test the relationship

between JA-related synchronization and enhanced behavioral and neural synchronization, the mean contrast estimate of voxels included within the right IFG ROI was calculated. The correlation coefficient was calculated.

Results

Eye-blink synchronization

By visual inspection of the video data of participants' face during mutual gaze condition, we confirmed that there was no aversion of the gaze from the partner or continuous closing of eyes. In Experiment 1, the mutual gaze condition on Day 1 (MG1) with an unknown partner did not elicit significant eye-blink synchronization (Fig. 2A, $p = 0.108$, $t[13] = -1.724$, one-sample t -test). On Day 2, during the mutual gaze condition (MG2), eye-blink synchronization was significant (Fig. 2A, $p = 0.002$, $t[13] = 3.804$, one-sample t -test), and eye-blink synchronization in MG2 was significantly more prominent than during MG1 (Fig. 2A, $p = 0.001$, $F[1.256, 16.586] = 12.923$, one-way ANOVA with Greenhouse–Geisser correction followed by post hoc tests with Bonferroni correction for multiple comparisons). Without online interaction between participants (VIDEO condition), eye-blink synchronization was not significant (Fig. 2A, $p = 0.828$, $t[13] = 0.221$, one-sample t -test). The difference in eye-blink synchronization between the MG2 and VIDEO conditions was also statistically significant (Fig. 2A, $p = 0.038$, $F[1.256, 16.586] = 12.923$, one-way ANOVA with Greenhouse–Geisser correction followed by post hoc tests with Bonferroni correction for multiple comparisons).

Without JA experience (Experiment 2), no enhancement of behavioral synchronization ($p = 0.275$, $t[13] = 1.140$, paired t -test) was observed (Fig. 2B). Even following JA (Experiment 3), synchronization was not enhanced when the partner was swapped (Fig. 2C).

There was significant inter-individual eye-blink synchronization even during JA tasks between paired partners in Experiment 1 (real pair, Fig. 2D, $p < 0.0001$, $t[14] = 12.3$, one-sample t -test). While eye-blink synchronization between randomly selected participants was significant (pseudo pair, Fig. 2D, $p = 0.001$, $t[14] = 3.957$, one-sample t -test), real pair eye-blinks showed more prominent synchronization (Fig. 2D, $p < 0.0001$, $t[28] = -5.700$, paired t -test). The strength of eye-blink synchronization during JA was positively correlated with enhanced eye-blink synchronization during MG2 compared with MG1 (Fig. 2E, $R^2 = 0.446$, $p = 0.009$). There was no correlation of t1–t2 intervals (i.e., time interval between MG1 and MG2) with enhanced eye blink synchronization ($R^2 = 0.038$, $p = 0.5053$).

Neural synchronization

During the mutual gaze condition on Day 1 (MG1) in Experiment 1, inter-individual neural synchronization was found in the middle occipital gyrus and MTG (Fig. 3A, Table 2) adjacent to the right EBA (white outline in Figs. 3A to G, Table 1). During the mutual gaze condition on Day 2 (MG2), inter-individual synchronization extended anteriorly to the right posterior superior temporal sulcus, bilateral IFG, and ventral premotor cortex (Fig. 3B, Table 2). The enhancement in inter-individual synchronization during MG2 compared with MG1 was statistically significant only in the right IFG (Fig. 3C, Table 2). There was no correlation of t1–t2 intervals with enhancement of the neural synchronization in the right IFG ($R^2 = 0.0092$, $p = 0.7443$). Consistent with eye-blink synchronization, no significant inter-individual neural synchronization was found during the VIDEO condition (data not shown). Consistent with behavioral synchronization, without JA experience (Experiment 2), no enhancement of neural synchronization was observed (Figs. 3D and E, Table 3). Even following JA (Experiment 3), neural synchronization was not enhanced when the partner was swapped (Figs. 3F and G, Table 4).

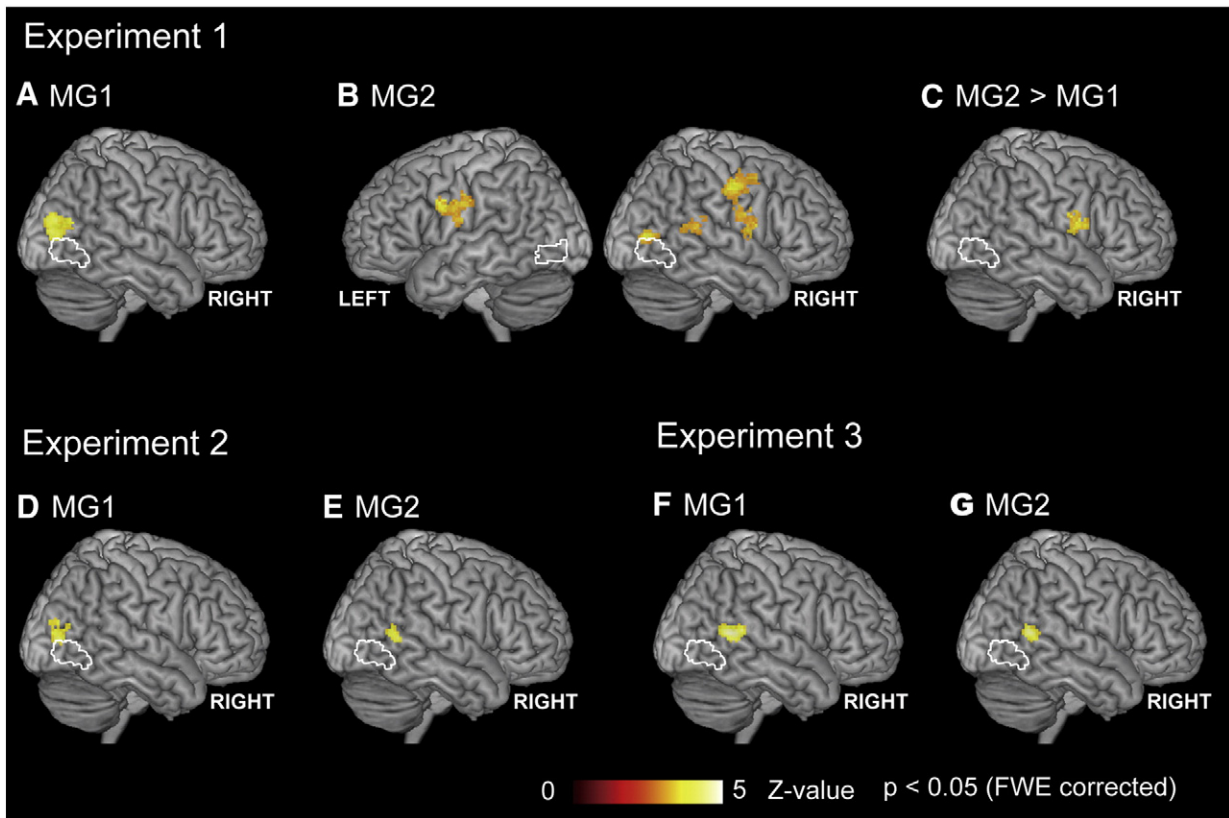


Fig. 3. Inter-individual neural synchronization in three experiments. Significant inter-individual neural synchronization in A, MG1, B, MG2 condition, and C, their increment in Experiment 1 (Table 2) was superimposed on the 3D surface of a template brain. D and E, Significant inter-individual neural synchronization during MG1 and MG2 in Experiment 2. F and G, Significant inter-individual neural synchronization during MG1 and MG2 in Experiment 3. The same imaging format as Experiment 1 (A and B) was utilized for D to G. White contour indicates functionally defined EBA (Table 1).

Relationship between neural and behavioral synchronization

The enhancement of inter-individual neural synchronization in the right IFG in Experiment 1 was significantly correlated with eye-blink synchronization during JA tasks (Fig. 4A, $R^2 = 0.379$, $p = 0.014$), and with the enhancement of eye-blink synchronization (Fig. 4B, $R^2 = 0.307$, $p = 0.040$). Consistent with the social Hebbian learning hypothesis, the right IFG was activated by both IJA and RJA, while no activation was found during the control condition (Fig. 4C).

Neural substrates of eye-blink per se

Eye-blinks per se activated extended cortical areas including visual and parietal areas (Figs. 5A and B, Table 5). In particular, significant eye-blink-related activation was observed in the right parietal operculum (SII) extending to the insula cortex, consistent with a previous study (Nakano et al., 2013). There was no overlap between the right

SII ROI and IFG that showed enhanced inter-individual neural synchronization (Fig. 5A). Eye-blink-related activation in the right IFG was significantly weaker than that in SII (Fig. 5B, $p < 0.001$, $F[1.314, 35.486] = 12.729$, one-way ANOVA with Greenhouse–Geisser correction followed by post hoc tests with Bonferroni corrections for multiple comparisons) and the regions of primary visual cortex (V1) that showed a significant eye-blink effect (Fig. 5B, $p < 0.001$, $F[1.314, 35.486] = 12.729$, one-way ANOVA with Greenhouse–Geisser correction followed by post hoc tests with Bonferroni corrections for multiple comparisons).

To test whether neural synchronization in the right MTG and IFG in Experiment 1 (see, Fig. 3C) is caused by eye-blinks per se, we calculated the inter-individual neural synchronization in the right MTG and IFG ROIs with the residual time series obtained by removing eye-blink-related activation. As shown in Fig. 6A, in Experiment 1, even after removing the eye-blink-related activation, right MTG showed significant inter-individual neural synchronization both in MG1 ($p = 0.020$, $t[13] = 2.656$, one-sample t-test) and MG2 ($p = 0.037$, $t[13] = 2.324$,

Table 1
Brain regions showing significant activation associated with seeing body parts in the EBA localizer task.

Cluster		Peak		MNI coordinates				Location
p-Value (FWE corr)	Cluster size	p-Value (FWE corr)	T value	x	y	z	Side	
0.014	283	0.492	5.71	46	-78	-2	R	IOG
		0.816	5.04	56	-68	0	R	MTG
		0.880	4.87	48	-60	-8	R	ITG, hOC5 (V5) (10%)
0.014	283	0.532	5.63	-36	-82	-6	L	IOG, hOC4v (V4) (10%)
		0.603	5.48	-44	-70	-10	L	IOG

IOG, inferior occipital gyrus; ITG, inferior temporal gyrus; MTG, middle temporal gyrus. L, left; R, right; MNI, Montreal Neurological Institute (MNI) space; FWE, family-wise error correction; x, y, z = location (in mm) with the three axes. The locations of local maxima are defined by the SPM Anatomy Toolbox v1.8 (Eickhoff et al., 2005, 2007). Reported results are $p < 0.05$ FWE for the whole brain.

Table 2
Inter-individual neural synchronization in Experiment 1.

Cluster		Peak		MNI coordinates				Location	Probability
p-Value (FWE corr)	Cluster size	p-Value (FWE corr)	T value	x	y	z	Side		
0.000	260	0.7956	4.79	40	-82	10	R	MOG	PGp (20%), V5 (20%)
		0.8365	4.73	38	-78	18	R	MOG	
		0.9780	4.39	46	-76	8	R	MOG	
MG1									
0.025	109	0.1657	5.75	48	-74	6	R	MOG	V5 (10%)
0.000	433	0.2162	5.62	56	-12	40	R	PoG	Area 1 (60%), Area 3b (50%), Area 4a (20%)
		0.9547	4.49	68	-8	22	R	PoG	OP4 (30%), Area 3b (30%), Area 1 (20%)
		0.9899	4.31	48	-10	38	R	PoG	
0.001	187	0.6048	5.03	-62	4	30	L	PrG	Area 6 (50%), Area 44 (20%)
		0.6903	4.93	-50	-14	30	L	PoG	Area 3b (40%), Area 3a (20%)
		0.8674	4.68	-64	-14	28	L	PoG	OP4 (50%), Area 1 (30%)
0.027	107	0.6164	5.02	34	4	12	R	INS	
		0.9792	4.39	44	0	14	R	Rop	
		0.9999	3.99	36	-4	18	R	Rop	OP3 (20%)
0.043	96	0.9985	4.14	54	-38	12	R	STG	PF (10%)
		0.9999	3.99	64	-48	14	R	MTG	PGa (40%)
		0.9999	3.98	48	-38	18	R	STG	PFcm (30%), PFm (20%)
MG2-MG1									
0.005	151	0.3102	5.43	46	2	16	R	Rop	
		0.8498	4.71	66	2	16	R	PoG	Area 1 (10%), OP4 (10%), Area 44 (10%)
		0.9953	4.23	60	8	14	R	IFG	Area 44 (50%)

INS, insular cortex; MOG, middle occipital gyrus; MTG, middle temporal gyrus; PoG, postcentral gyrus; PrG, precentral gyrus; Rop, rolandic operculum; STG, superior temporal gyrus. L, left; R, right; MNI, Montreal Neurological Institute (MNI) space; FWE, family-wise error correction; x, y, z = location (in mm) with the three axes. The locations of local maxima are defined by the SPM Anatomy Toolbox v1.8 (Eickhoff et al., 2005, 2007). Reported results are $p < 0.05$ FWE for the whole brain.

one-sample t-test) without a significant condition effect ($p = 0.639$, $t[13] = 0.480$, paired t-test). Fig. 6B shows that within the right IFG, after removing eye-blink-related activation, synchronization was significant in the MG2 condition ($p < 0.0001$, $t[13] = 5.961$, one-sample t-test), but not in the MG1 condition ($p = 0.987$, $t[13] = 0.017$, one-sample t-test). Enhanced inter-individual neural synchronization within the right IFG was statistically significant even without eye-blink-related activation ($p = 0.008$, $t[13] = 3.154$, paired t-test).

Discussion

Eye-blink synchronization

The JA task caused blink synchronization as expected. To successfully conduct the task, participants had to coordinate the timing of opening and closing their window of attention with their partner's, resulting in eye-blink synchronization (task effect). Consistent with the task effect, significant eye-blink synchronization was also observed in the pseudo pair (Fig. 2D). As the task design was identical across the pairs, this indicates that the JA task aligned the attentional window within the dyad. Therefore, any difference in blink synchronization between real and pseudo pairs (Fig. 2D) constitutes a pair-specific effect.

There was no eye-blink synchronization during first mutual gaze (MG1), reflecting no commonly shared task that can provide cues for eliciting similar behavior. Thus eye-blink synchronization during mutual gaze which emerged after the JA task (Fig. 2A) does not reflect a task effect. The lack of eye-blink synchronization in the VIDEO condition confirms the importance of on-line mutual interaction for the emergence of eye-blink synchronization during MG2. Furthermore, the strength of eye-blink synchronization during JA was positively correlated with enhanced eye-blink synchronization during MG2 (Fig. 2E). Given constant task effects in synchronization during JA, this correlation indicates that blink synchronization during MG2 is affected by the pair specific effect of blink synchronization during JA. In other words, the shared attention induced by JA was retained as a pair-specific "social" memory and represented by enhanced synchronization during mutual gaze.

Neural synchronization

Enhanced synchronization following JA

Inter-individual neural synchronization in the bilateral PMv, extending to M1/S1, was also evident during the MG2 condition (Fig. 3B). It is possible that this corresponds to the premotor face area (Hanakawa

Table 3
Inter-individual neural synchronization in Experiment 2.

Cluster		Peak		MNI coordinates				Location	Probability
p-Value (FWE corr)	Cluster size	p-Value (FWE corr)	T value	x	y	z	Side		
0.029	103	0.5288	5.26	42	-78	6	R	MOG	V5 (10%)
		1.0000	3.89	36	-78	16	R	MOG	
		1.0000	3.80	44	-72	14	R	MTG	PGp (40%)
MG2									
0.013	121	0.3210	5.56	46	-54	8	R	MTG	PGp (10%)

MOG, middle occipital gyrus; MTG, middle temporal gyrus. L, left; R, right; MNI, Montreal Neurological Institute (MNI) space; FWE, family-wise error correction; x, y, z = location (in mm) with the three axes. The locations of local maxima are defined by the SPM Anatomy Toolbox v1.8 (Eickhoff et al., 2005, 2007). Reported results are $p < 0.05$ FWE for the whole brain.

Table 4
Inter-individual neural synchronization in Experiment 3.

Cluster		Peak		MNI coordinates				Location	Probability
p-Value (FWE corr)	Cluster size	p-Value (FWE corr)	T value	x	y	z	Side		
0.000	243	0.1815	5.81	64	−48	10	R	MTG	PGa (20%)
		0.6820	5.05	54	−42	12	R	STG	PFm (20%), PGa (10%)
		1.0000	3.68	50	−58	10	R	MTG	PGp (10%)
MG1									
0.005	120	0.3585	5.46	56	−54	10	R	MTG	PGa (40%)
MG2									

MTG, middle temporal gyrus; STG, superior temporal gyrus. L, left; R, right; MNI, Montreal Neurological Institute (MNI) space; FWE, family-wise error correction; x, y, z = location (in mm) with the three axes. The locations of local maxima are defined by the SPM Anatomy Toolbox v1.8 (Eickhoff et al., 2005, 2007). Reported results are $p < 0.05$ FWE for the whole brain.

et al., 2005). In the present study, full-face visual stimuli were used for the JA task and the eye contact condition, while only the eye regions were presented in previous studies that did not show inter-individual synchronization of the bilateral PMv (Saito et al., 2010). Furthermore, recent studies indicate that S1 is involved in social cognition (Keysers et al., 2010). In particular, somatosensory cortex is known to receive an efference copy (forward model) of motor plans (Cui et al., 2014). Thus, inter-individual synchronization in the bilateral PMv extending to M1/S1 may be related to the learned internal model of the JA-related facial movements.

Neural synchronization in IFG

Across the whole brain, only the right IFG showed enhanced neural synchronization following JA (MG2–MG1, Fig. 3C), whereas no synchronization was observed during VIDEO. Enhanced synchronization in the right IFG was positively correlated with eye-blink synchronization during JA tasks, and with the enhancement of eye-blink synchronization. Finally, the right IFG was activated by both IJA and RJA. These findings indicate that the right IFG is related to the generation of shared attention through social Hebbian association during JA, and to its retention that is evoked by mutual gaze.

Previous studies have suggested that the right IFG is an interface between self and other, especially during social situations. The right IFG is involved in unconscious incorporation of facial information of one's partner (Leslie et al., 2004), and in distinguishing self-related facial information from that of others (Sugiura et al., 2005). Furthermore, the right IFG is involved in the release of attention that is linked to spontaneous eye-blinks (Nakano et al., 2013). The release of attention activates the default mode network that is associated with internal processing, while suppressing the dorsal attentional network (Nakano et al., 2013). As the right IFG switches between central-executive and default-mode networks (Sridharan et al., 2008), neural synchronization in the right IFG may represent synchronized shifting of attention towards self and others (Pfeiffer et al., 2013).

The right IFG was activated by both responding and initiating JA (Fig. 4C), consistent with previous studies (Redcay et al., 2012; Saito et al., 2010; Williams et al., 2005). Furthermore, neural synchronization of the right IFG occurred spontaneously during MG2. These findings are in line with the notion that mirror neuron properties of the right IFG and ventral premotor cortex (Gallese et al., 1996; Rizzolatti et al., 1996; Keysers et al., 2010) are caused by social Hebbian learning (Keysers and Perrett, 2004; Wolpert et al., 2003) which binds self-derived behavior to that of others through on-line interaction (Mundy and Newell, 2007; Treur, 2011). The present study suggests that the right IFG was affected by social Hebbian association which binds self-derived directed attention (Tomasello and Carpenter, 2007) to that of others.

Synchronized neural activity within right IFG during mutual gaze is unlikely caused by the blink per se, because right IFG did not show blink-related activation: Instead, spontaneous eye-blinks activated the right SII/insula (Figs. 4A and B), consistent with a previous study (Nakano et al., 2013). Enhanced synchronization of right IFG was significant even after removal of eye-blink related activation (Fig. 6B). Thus the neural synchronization of the right IFG represents learned shared attention. Considering that shared attention is to be understood as a complementary action due to its social salience, relevance in initiating communication, and joint action (Pfeiffer et al., 2013), the present finding is consistent with a previous study by Newman-Norlund et al. (2007) who showed that the right IFG is more active during complimentary as compared to imitative actions.

Neural synchronization in right MTG

Right MTG showed significant and consistent inter-individual synchronization during MGs. Unlike the right IFG, there was no learning effect. As no inter-individual neural synchronization occurred during the VIDEO condition, MTG synchronization should have emerged as a result of on-line mutual interaction during mutual gaze. The EBA are known to receive both sensory inputs of others' body information

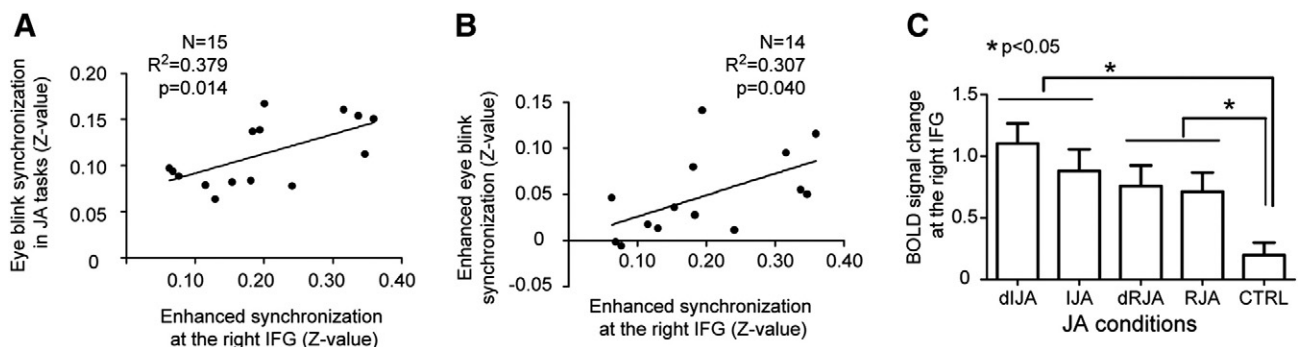


Fig. 4. Enhanced neural synchronization of the right IFG after JA experience in Experiment 1. The enhancement of neural synchronization at the right IFG cluster defined by MG2–MG1 (see, Fig. 3C) was correlated with A, eye-blink synchronization during JA tasks, and with B, enhanced eye-blink synchronization. C, Task-related activation during JA task. Error bars, standard error of the mean (s.e.m.).

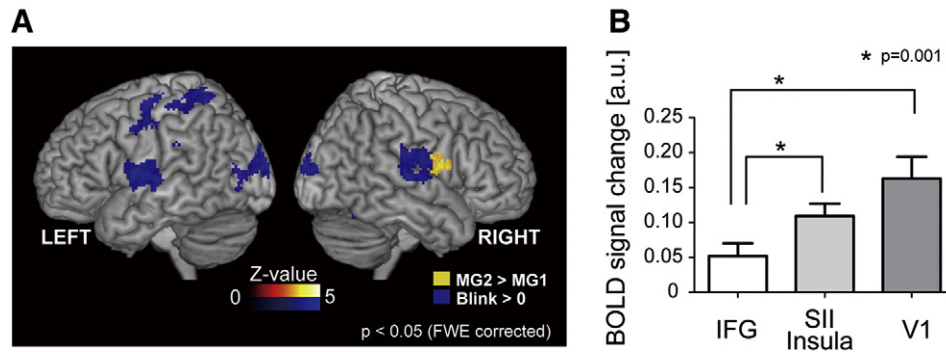


Fig. 5. Eye-blink related activation in MG2 of Experiment 1. A, Brain regions that are significantly activated by eye-blink (blue) and right IFG cluster showing significant enhancement of synchronization during MG2 compared with MG1 in Experiment 1 (yellow). See Table 5 for detailed information. B, BOLD signal changes in the right IFG, SII/insula, and V1. Error bars, standard error of the mean (s.e.m.).

(Downing et al., 2001) and efference copies (Astafiev et al., 2004; Orlov et al., 2010), thus the adjacent MTG may conceivably receive information about self and other's eye-blinks. Consistent with this notion, MTG has a role in detecting contingency between own and partner's behavior (Redcay et al., 2010). Given that the summation of inputs to the MTG region is identical between the two participants, even pairs of new partners synchronize their visual area activation.

Neural synchronization in the 'social default mode'

Previously, there have been several studies that investigated the flow of information between the brains of two partner by scanning participants one after another during offline interactions (pseudo hyperscanning, Anders et al., 2011; Konvalinka and Roepstorff, 2012; Schippers et al., 2010; Stephens et al., 2010, for review). However, this technique cannot capture mutual influence during the interaction.

Inter-individual neural synchronization during social interaction tasks has been reported repeatedly (Astolfi et al., 2010; Cui et al., 2012; De Vico Fallani et al., 2010; Dumas et al., 2010; Jiang et al., 2012; Müller et al., 2013; Osaka et al., 2014; Saito et al., 2010; Sängner et al., 2012; Tanabe et al., 2012; Yun et al., 2012). These studies fail to exclude the possibility that the observed neural synchronization reflects similarity in their behavior (Konvalinka and Roepstorff, 2012). Most recently, Stolk et al. (2014) showed enhanced neural synchronization at the right superior temporal gyrus during participation in a cooperative

game, in parallel with establishing mutual understanding of novel signals, independent of the occurrence of each signal (Stolk et al., 2014). They attributed the observed synchronization to the generation of a shared conceptualization of the social signals that were used to successfully conduct the cooperative game.

In contrast, the present study showed enhanced synchronization of eye-blinks within a dyad that could not be attributable to similarity in their behavior, but was instead due to the pair-specific relation (Konvalinka and Roepstorff, 2012). Regarding the inter-individual functional connectivity by means of neural correlation, we treated the two brains as spontaneous "two-in-one" system during the mutual gaze condition that can be regarded as 'social default mode', as the activity of an individual brain consists of spontaneously organized networks during the resting state (Fox et al., 2005, 2006). Inter-individual connectivity became more conspicuous after partners became familiar with one another, i.e., after the JA training (Figs. 3A to C), and the connectivity profiles showed pair-specificity (Figs. 3E and G). Thus, the property of the two-in-one system during the social default mode reflects the relationship between two participants, as the property of an intra-brain network reflects the mental state during a no-task condition or default mode (Yan et al., 2009). Mutual eye contact underlies almost all face-to-face social interactions. Therefore, the effect of eye contact should be carefully considered to explore inter-individual networks involved in face-to-face communication. Further investigation

Table 5
Brain regions showing significant activation associated with eye-blink in MG2 in Experiment 1.

Cluster p-Value (FWE corr)	Cluster size	Peak		MNI coordinates				Location	Probability
		p-Value (FWE corr)	T value	x	y	z	Side		
0.000	8469	0.159	5.42	-26	-74	12	L	LG	
		0.172	5.38	-14	-44	-4	L	LG	
		0.198	5.31	-10	-68	-4	L	LG	Area 18 (40%)
0.000	1037	0.271	5.15	-38	-16	18	L	Rop	OP3 (80%), OP2 (40%)
		0.445	4.86	-60	-4	8	L	Rop	OP4 (40%)
		0.517	4.77	-50	-20	-2	L	Rop	OP4 (50%), OP1 (20%)
0.002	350	0.275	5.14	-18	-42	52	L	SPL	Area 3b (30%), Area 3a (20%)
		0.600	4.66	-14	-44	62	L	Precuneus	Area 3b (40%), SPL (5 L) (30%)
		0.897	4.23	-16	-32	50	L	Precuneus	SPL (5 L) (70%), Area 1 (20%)
0.000	815	0.320	5.06	36	-22	14	R	INS	OP2 (80%)
		0.431	4.88	60	-22	8	R	STG	OP1 (10%)
		0.657	4.59	68	-14	20	R	PoG	OP4 (50%)
0.015	229	0.703	4.53	-44	-10	60	L	PrG	Area 6 (90%)
		0.959	4.07	-54	-10	50	L	PoG	PoG (50%), Area 1 (40%)
		0.993	3.84	-42	-18	40	L	PoG	Area 6 (60%), Area 1 (40%)
0.026	202	0.877	4.27	-30	-40	64	L	PoG	Area 1 (60%), Area 2 (50%)
		0.892	4.24	-32	-54	68	L	SPL	SPL (7A) (30%), SPL (7PC) (30%)
		0.991	3.87	-50	-28	60	L	Precuneus	SPL (7PC) (50%), SPL (7A) (40%)

INS, insular; LG, lingual gyrus; PoG, postcentral gyrus; PrG, precentral gyrus; Rop, rolandic operculum; SPL, superior parietal lobule; STG, superior temporal gyrus. L, left; R, right; MNI, Montreal Neurological Institute (MNI) space; FWE, family-wise error correction; x, y, z = location (in mm) with the three axes. The locations of local maxima are defined by the SPM Anatomy Toolbox v1.8 (Eickhoff et al., 2005, 2007). Reported results are $p < 0.05$ FWE for the whole brain.

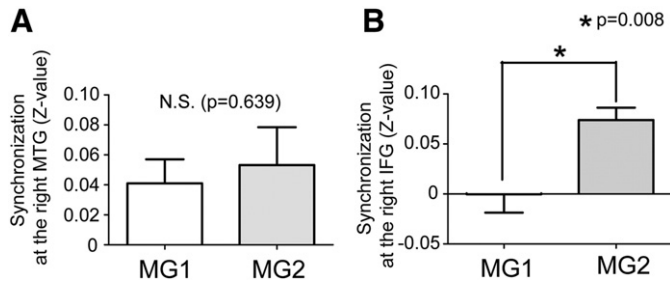


Fig. 6. Inter-individual neural synchronization after removing eye-blink effect in Experiment 1. A, Synchronization within the right MTG cluster shows no enhancement of inter-individual synchronization between MG1 and MG2. B, By contrast, in the right IFG cluster, the synchronization was significantly enhanced in MG2. Error bars, standard error of the mean (s.e.m.).

of this two-in-one system, during minimum task constraints, i.e., eye contact, might help to reveal the functional roles of inter-individual neural synchronization, as default mode network studies in the resting state have shed light on task-related brain networks (Fox et al., 2005, 2006).

Conclusion

The enhancement of behavioral and neural synchronization during mutual gaze after a JA task represents a pair-specific construct of shared attention that cannot be reduced to the individual. As default mode network studies on the resting state have shed light on state-related brain activities (Fox et al., 2005), further investigation of inter-individual neural interaction will help to reveal the neural underpinnings of the state of interacting persons (Konvalinka and Roepstorff, 2012).

Acknowledgment

This work was supported, in part, by Grant-in-Aid for Scientific Research (#21220005 and #15H01846 to N.S.) and Challenging Exploratory Research grant (#23650224 to H.C.T.) from the Japan Society for the Promotion of Science, and Scientific Research on Innovative Areas grant (#22101007 to H.C.T.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT). Part of this study is the result of “Development of biomarker candidates for social behavior” carried out under the strategic research program for Brain Sciences MEXT.

Conflict of interest

The authors declare no competing financial interests.

References

- Allison, T., Puce, a, McCarthy, G., 2000. Social perception from visual cues: role of the STS region. *Trends Cogn. Sci.* 4, 267–278.
- Anders, S., Heinzle, J., Weiskopf, N., Ethofer, T., Haynes, J., 2011. Flow of affective information between communicating brains. *Neuroimage* 54, 439–446. <http://dx.doi.org/10.1016/j.neuroimage.2010.07.004>.
- Astafiev, S.V., Stanley, C.M., Shulman, G.L., Corbetta, M., 2004. Extrastriate body area in human occipital cortex responds to the performance of motor actions. *Nat. Neurosci.* 7, 542–548.
- Astolfi, L., Toppi, J., De Vico Fallani, F., Vecchiato, G., Salinari, S., Mattia, D., Cincotti, F., Babiloni, F., 2010. Neuroelectrical hyperscanning measures simultaneous brain activity in humans. *Brain Topogr.* 23, 243–256. <http://dx.doi.org/10.1007/s10548-010-0147-9>.
- Bentivoglio, A.R., Bressman, S.B., Cassetta, E., Carretta, D., Tonali, P., Albanese, A., 1997. Analysis of blink rate patterns in normal subjects. *Mov. Disord.* 12, 1028–1034. <http://dx.doi.org/10.1002/mds.870120629>.
- Calder, A.J., Beaver, J.D., Winston, J.S., Dolan, R.J., Jenkins, R., Eger, E., Henson, R.N.A., 2007. Separate coding of different gaze directions in the superior temporal sulcus and inferior parietal lobule. *Curr. Biol.* 17, 20–25.

- Cordes, D., Haughton, V.M., Arfanakis, K., Carew, J.D., Turski, P. a, Moritz, C.H., Quigley, M. a, Meyerand, M.E., 2001. Frequencies contributing to functional connectivity in the cerebral cortex in “resting-state” data. *AJNR Am. J. Neuroradiol.* 22, 1326–1333.
- Cui, X., Bryant, D.M., Reiss, A.L., 2012. NIRS-based hyperscanning reveals increased inter-personal coherence in superior frontal cortex during cooperation. *Neuroimage* 59, 2430–2437. <http://dx.doi.org/10.1016/j.neuroimage.2011.09.003>.
- Cui, F., Arnstein, D., Thomas, R.M., Maurits, N.M., Keyzers, C., Gazzola, V., 2014. Functional magnetic resonance imaging connectivity analyses reveal efference-copy to primary somatosensory area, BA2. *PLoS One* 9, e84367. <http://dx.doi.org/10.1371/journal.pone.0084367>.
- De Vico Fallani, F., Nicosia, V., Sinatra, R., Astolfi, L., Cincotti, F., Mattia, D., Wilke, C., Doud, A., Latora, V., He, B., Babiloni, F., 2010. Defecting or not defecting: how to “read” human behavior during cooperative games by EEG measurements. *PLoS One* 5, e14187. <http://dx.doi.org/10.1371/journal.pone.0014187>.
- Del Giudice, M., Manera, V., Keyzers, C., 2009. Programmed to learn? The ontogeny of mirror neurons. *Dev. Sci.* 12, 350–363. <http://dx.doi.org/10.1111/j.1467-7687.2008.00783.x>.
- Downing, P.E., Jiang, Y., Shuman, M., Kanwisher, N., 2001. A cortical area selective for visual processing of the human body. *Science* 293, 2470–2473. <http://dx.doi.org/10.1126/science.1063414>.
- Dumas, G., 2011. Towards a two-body neuroscience. *Commun. Integr. Biol.* 4, 349–352. <http://dx.doi.org/10.4161/cib.4.3.15110>.
- Dumas, G., Nadel, J., Soussignan, R., Martinerie, J., Garnero, L., 2010. Inter-brain synchronization during social interaction. *PLoS One* 5, e12166. <http://dx.doi.org/10.1371/journal.pone.0012166>.
- Eickhoff, S.B., Stephan, K.E., Mohlberg, H., Grefkes, C., Fink, G.R., Amunts, K., Zilles, K., 2005. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* 25, 1325–1335.
- Eickhoff, S.B., Paus, T., Caspers, S., Grosbras, M.-H., Evans, A.C., Zilles, K., Amunts, K., 2007. Assignment of functional activations to probabilistic cytoarchitectonic areas revisited. *Neuroimage* 36, 511–521. <http://dx.doi.org/10.1016/j.neuroimage.2007.03.060>.
- Emery, N.J., 2000. The eyes have it: the neuroethology, function and evolution of social gaze. *Neurosci. Biobehav. Rev.* 24, 581–604.
- Farroni, T., Csibra, G., Simion, F., Johnson, M.H., 2002. Eye contact detection in humans from birth. *Proc. Natl. Acad. Sci. U. S. A.* 99, 9602–9605. <http://dx.doi.org/10.1073/pnas.152159999>.
- Fox, M.D., Snyder, A.Z., Vincent, J.L., Corbetta, M., Van Essen, D.C., Raichle, M.E., 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Natl. Acad. Sci. U. S. A.* 102, 9673–9678. <http://dx.doi.org/10.1073/pnas.0504136102>.
- Fox, M.D., Corbetta, M., Snyder, A.Z., Vincent, J.L., Raichle, M.E., 2006. Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems. *Proc. Natl. Acad. Sci. U. S. A.* 103, 10046–10051. <http://dx.doi.org/10.1073/pnas.0604187103>.
- Friston, K.J., 2002. Bayesian estimation of dynamical systems: an application to fMRI. *Neuroimage* 16, 513–530.
- Friston, K.J., Holmes, a, Poline, J.B., Price, C.J., Frith, C.D., 1996. Detecting activations in PET and fMRI: levels of inference and power. *Neuroimage* 4, 223–235. <http://dx.doi.org/10.1006/nimg.1996.0074>.
- Friston, K.J., Holmes, A.P., Worsley, K.J., 1999. How many subjects constitute a study? *Neuroimage* 10, 1–5.
- Gallese, V., Fadiga, L., Fogassi, L., Rizzolatti, G., 1996. Action recognition in the premotor cortex. *Brain* 119, 593–609.
- Hanakawa, T., Parikh, S., Bruno, M.K., Hallett, M., 2005. Finger and face representations in the ipsilateral precentral motor areas in humans. *J. Neurophysiol.* 93, 2950–2958.
- Hari, R.R., Kujala, M.V.M., 2009. Brain basis of human social interaction: from concepts to brain imaging. *Physiol. Rev.* 89, 453–479. <http://dx.doi.org/10.1152/physrev.00041.2007>.
- Herrmann, A., 2010. The interaction of eye blinks and other prosodic cues in German sign language. *Sign Lang. Linguist.* 13, 3–39.
- Jacob, P., 2009. The tuning-fork model of human social cognition: a critique. *Conscious. Cogn.* 18, 229–243.
- Jiang, J., Dai, B., Peng, D., Zhu, C., Liu, L., Lu, C., 2012. Neural synchronization during face-to-face communication. *J. Neurosci.* 32, 16064–16069. <http://dx.doi.org/10.1523/JNEUROSCI.2926-12.2012>.
- Keyzers, C., Perrett, D.I., 2004. Demystifying social cognition: a Hebbian perspective. *Trends Cogn. Sci.* 8, 501–507. <http://dx.doi.org/10.1016/j.tics.2004.09.005>.
- Keyzers, C., Kaas, J.H., Gazzola, V., 2010. Somatosensation in social perception. *Nat. Rev. Neurosci.* 11, 417–428. <http://dx.doi.org/10.1038/nrn2833>.
- Konvalinka, I., Roepstorff, A., 2012. The two-brain approach: how can mutually interacting brains teach us something about social interaction? *Front. Hum. Neurosci.* 6, 215. <http://dx.doi.org/10.3389/fnhum.2012.00215>.
- Leslie, K.R., Johnson-Frey, S.H., Grafton, S.T., 2004. Functional imaging of face and hand imitation: towards a motor theory of empathy. *Neuroimage* 21, 601–607.
- Müller, V., Sänger, J., Lindenberger, U., 2013. Intra- and inter-brain synchronization during musical improvisation on the guitar. *PLoS One* 8, e73852. <http://dx.doi.org/10.1371/journal.pone.0073852>.
- Mundy, P., Newell, L., 2007. Attention, joint attention, and social cognition. *Curr. Dir. Psychol. Sci.* 16, 269–274. <http://dx.doi.org/10.1111/j.1467-8721.2007.00518.x>. Attention.
- Mundy, P., Sigman, M., Ungerer, J., Sherman, T., 1986. Defining the social deficits of autism: the contribution of non-verbal communication measures. *J. Child Psychol. Psychiatry* 27, 657–669.
- Mundy, P., Sullivan, L., Mastergeorge, A.M., 2009. A parallel and distributed processing model of joint attention, social-cognition and autism. *Autism* 2, 2–21. <http://dx.doi.org/10.1002/aur.61.A>.
- Nakano, T., Kitazawa, S., 2010. Eyeblink entrainment at breakpoints of speech. *Exp. Brain Res.* 205, 577–581.

- Nakano, T., Yamamoto, Y., Kitajo, K., Takahashi, T., Kitazawa, S., 2009. Synchronization of spontaneous eyeblinks while viewing video stories. *Proc. Biol. Sci.* 276, 3635–3644. <http://dx.doi.org/10.1098/rspb.2009.0828>.
- Nakano, T., Kato, M., Morito, Y., Itoi, S., Kitazawa, S., 2013. Blink-related momentary activation of the default mode network while viewing videos. *Proc. Natl. Acad. Sci. U. S. A.* 110, 702–706. <http://dx.doi.org/10.1073/pnas.1214804110>.
- Newman-Norlund, R.D., van Schie, H.T., van Zuijlen, A.M.J., Bekkering, H., 2007. The mirror neuron system is more active during complementary compared with imitative action. *Nat. Neurosci.* 10, 817–818. <http://dx.doi.org/10.1038/nn1911>.
- Nummenmaa, L., Calder, A.J., 2009. Neural mechanisms of social attention. *Trends Cogn. Sci.* 13, 135–143.
- Okamoto, Y., Kitada, R., Tanabe, H.C., Hayashi, M.J., Kochiyama, T., Munesue, T., Ishitobi, M., Saito, D.N., Yanaka, H.T., Omori, M., Wada, Y., Okazawa, H., Sasaki, A.T., Morita, T., Itakura, S., Kosaka, H., Sadato, N., 2014. Attenuation of the contingency detection effect in the extrastriate body area in autism spectrum disorder. *Neurosci. Res.* 87, 66–76. <http://dx.doi.org/10.1016/j.neures.2014.06.012>.
- Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9, 97–113.
- Orchard, L.N., Stern, J.A., 1991. Blinks as an index of cognitive activity during reading. *Integr. Physiol. Behav. Sci.* 26, 108–116.
- Orlov, T., Makin, T.R., Zohary, E., 2010. Topographic representation of the human body in the occipitotemporal cortex. *Neuron* 68, 586–600. <http://dx.doi.org/10.1016/j.neuron.2010.09.032>.
- Osaka, N., Minamoto, T., Yaoi, K., Azuma, M., Osaka, M., 2014. Neural synchronization during cooperated humming: a hyperscanning study using fNIRS. *Procedia-Soc. Behav. Sci.* 126, 241–243. <http://dx.doi.org/10.1016/j.sbspro.2014.02.395>.
- Oullier, O., de Guzman, G.C., Jantzen, K.J., Lagarde, J., Kelso, J. a S., 2008. Social coordination dynamics: measuring human bonding. *Soc. Neurosci.* 3, 178–192. <http://dx.doi.org/10.1080/17470910701563392>.
- Perrett, D.I., Emery, N.J., 1994. Understanding the intentions of others from visual signals: neurophysiological evidence. *Curr. Psychol. Cogn.* 13, 683–694.
- Pfeiffer, U.J., Vogeley, K., Schilbach, L., 2013. From gaze cueing to dual eye-tracking: novel approaches to investigate the neural correlates of gaze in social interaction. *Neurosci. Biobehav. Rev.* 37, 2516–2528. <http://dx.doi.org/10.1016/j.neubiorev.2013.07.017>.
- Redcay, E., Dodell-Feder, D., Pearrow, M.J., Mavros, P.L., Kleiner, M., Gabrieli, J.D.E., Saxe, R., 2010. Live face-to-face interaction during fMRI: a new tool for social cognitive neuroscience. *Neuroimage* 50, 1639–1647. <http://dx.doi.org/10.1016/j.neuroimage.2010.01.052>.
- Redcay, E., Kleiner, M., Saxe, R., 2012. Look at this: the neural correlates of initiating and responding to bids for joint attention. *Front. Hum. Neurosci.* 6, 169. <http://dx.doi.org/10.3389/fnhum.2012.00169>.
- Rizzolatti, G., Fadiga, L., Matelli, M., Bettinardi, V., Paulesu, E., Perani, D., Fazio, F., 1996. Localization of grasp representations in humans by PET: 1. Observation versus execution. *Exp. Brain Res.* 111, 246–252.
- Saito, D.N., Tanabe, H.C., Izuma, K., Hayashi, M.J., Morito, Y., Komeda, H., Uchiyama, H., Kosaka, H., Okazawa, H., Fujibayashi, Y., Sadato, N., 2010. “Stay tuned”: inter-individual neural synchronization during mutual gaze and joint attention. *Front. Integr. Neurosci.* 4, 127. <http://dx.doi.org/10.3389/fnint.2010.00127>.
- Sänger, J., Müller, V., Lindenberger, U., 2012. Intra- and interbrain synchronization and network properties when playing guitar in duets. *Front. Hum. Neurosci.* 6, 312. <http://dx.doi.org/10.3389/fnhum.2012.00312>.
- Sasaki, T.A., Kochiyama, T., Sugiura, M., Tanabe, H.C., Sadato, N., 2012. Neural networks for action representation: a functional magnetic-resonance imaging and dynamic causal modeling study. *Front. Hum. Neurosci.* 6.
- Schilbach, L., 2014. On the relationship of online and offline social cognition. *Front. Hum. Neurosci.* 8, 278. <http://dx.doi.org/10.3389/fnhum.2014.00278>.
- Schilbach, L., 2015. Eye to eye, face to face and brain to brain: novel approaches to study the behavioral dynamics and neural mechanisms of social interactions. *Curr. Opin. Behav. Sci.* 3, 130–135. <http://dx.doi.org/10.1016/j.cobeha.2015.03.006>.
- Schilbach, L., Timmermans, B., Reddy, V., Costall, A., Bente, G., Schlicht, T., Vogeley, K., 2013. Toward a second-person neuroscience. *Behav. Brain Sci.* 36, 393–414.
- Schippers, M.B., Keysers, C., 2011. Mapping the flow of information within the putative mirror neuron system during gesture observation. *Neuroimage* 57, 37–44. <http://dx.doi.org/10.1016/j.neuroimage.2011.02.018>.
- Schippers, M.B., Roebroek, A., Renken, R., Nanetti, L., Keysers, C., 2010. Mapping the information flow from one brain to another during gestural communication. *Proc. Natl. Acad. Sci. U. S. A.* 107, 9388–9393. <http://dx.doi.org/10.1073/pnas.1001791107> / DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1001791107.
- Shultz, S., Klin, A., Jones, W., 2011. Inhibition of eye blinking reveals subjective perceptions of stimulus salience. *Proc. Natl. Acad. Sci. U. S. A.* 108, 21270–21275. <http://dx.doi.org/10.1073/pnas.1109304108>.
- Sridharan, D., Levitin, D.J., Menon, V., 2008. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc. Natl. Acad. Sci. U. S. A.* 105, 12569–12574. <http://dx.doi.org/10.1073/pnas.0800005105>.
- Stephens, G.J., Silbert, L.J., Hasson, U., 2010. Speaker-listener neural coupling underlies successful communication. *Proc. Natl. Acad. Sci. U. S. A.* 107, 14425–14430. <http://dx.doi.org/10.1073/pnas.1008662107>.
- Stolk, A., Noordzij, M.L., Verhagen, L., Volman, I., Schoffelen, J., Oostenveld, R., Hagoort, P., Toni, I., 2014. Cerebral coherence between communicators marks the emergence of meaning. *Proc. Natl. Acad. Sci. U. S. A.* 111, 18183–18188. <http://dx.doi.org/10.1073/pnas.1414886111>.
- Sugiura, M., Watanabe, J., Maeda, Y., Matsue, Y., Fukuda, H., Kawashima, R., 2005. Cortical mechanisms of visual self-recognition. *Neuroimage* 24, 143–149.
- Tanabe, H.C., Kosaka, H., Saito, D.N., Koike, T., Hayashi, M.J., Izuma, K., Komeda, H., Ishitobi, M., Omori, M., Munesue, T., Okazawa, H., Wada, Y., Sadato, N., Sciences, S.W., 2012. Hard to “tune in”: neural mechanisms of eye contact and joint attention in high-functioning autistic spectrum disorder. *Front. Hum. Neurosci.* 6. <http://dx.doi.org/10.3389/fnhum.2012.00268>.
- Tomasello, M., Carpenter, M., 2007. Shared intentionality. *Dev. Sci.* 10, 121–125. <http://dx.doi.org/10.1111/j.1467-7687.2007.00573.x>.
- Treuer, J., 2011. A computational agent model for Hebbian learning of social interaction. *Lect. Notes Comput. Sci.* 7062, 9–19.
- Triantafyllou, C., Hoge, R.D., Krueger, G., Wiggins, C.J., Potthast, a., Wiggins, G.C., Wald, L.L., 2005. Comparison of physiological noise at 1.5 T, 3 T and 7 T and optimization of fMRI acquisition parameters. *Neuroimage* 26, 243–250. <http://dx.doi.org/10.1016/j.neuroimage.2005.01.007>.
- Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15, 273–289.
- Wiggins, G.C., Triantafyllou, C., Potthast, a., Reykowski, a., Nittka, M., Wald, L.L., 2006. 32-Channel 3 Tesla receive-only phased-array head coil with soccer-ball element geometry. *Magn. Reson. Med.* 56, 216–223. <http://dx.doi.org/10.1002/mrm.20925>.
- Williams, J.H.G., Waiter, G.D., Perra, O., Perrett, D.I., Whiten, A., 2005. An fMRI study of joint attention experience. *Neuroimage* 25, 133–140. <http://dx.doi.org/10.1016/j.neuroimage.2004.10.047>.
- Wolpert, D.M., Doya, K., Kawato, M., 2003. A unifying computational framework for motor control and social interaction. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358, 593–602. <http://dx.doi.org/10.1098/rstb.2002.1238>.
- Yan, C., Liu, D., He, Y., Zou, Q., Zhu, C., Zuo, X., Long, X., Zang, Y., 2009. Spontaneous brain activity in the default mode network is sensitive to different resting-state conditions with limited cognitive load. *PLoS One* 4, e5743. <http://dx.doi.org/10.1371/journal.pone.0005743>.
- Yun, K., Watanabe, K., Shimojo, S., 2012. Interpersonal body and neural synchronization as a marker of implicit social interaction. *Sci. Rep.* 2, 959. <http://dx.doi.org/10.1038/srep00959>.