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Neural correlates of fear-induced sympathetic response associated with the peripheral temperature change rate

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ABSTRACT

Activation of the sympathetic nervous system is essential for coping with environmental stressors such as fearful stimuli. Recent human imaging studies demonstrated that activity in some cortical regions, such as the anterior cingulate cortex (ACC) and anterior insula cortex (aIC), is related to sympathetic activity. However, little is known about the functional brain connectivity related to sympathetic response to fearful stimuli. The participants were 32 healthy, right-handed volunteers. Functional magnetic resonance imaging (fMRI) was used to examine brain activity when watching horror and control movies. Fingertip temperature was taken during the scanning as a measure of sympathetic response. The movies were watched a second time, and the degree of fear (9-point Likert-type scale) was evaluated every three seconds. The brain activity of the ACC, bilateral aIC, and bilateral anterior prefrontal cortex (aPFC) was correlated with the change rate of fingertip temperature, with or without fearful stimuli. Functional connectivity analysis revealed significantly greater positive functional connectivity between the amygdala and the ACC and between the amygdala and the aIC when watching the horror movie than when watching the control movie. Whole-brain psycho-physiological interaction (PPI) analysis revealed that the functional connectivity between the left amygdala and the ACC was modulated according to the fear rating.
Our results indicate that the increased functional connectivity between the left amygdala and the ACC represents a sympathetic response to fearful stimuli.

1. Introduction

Visceral functions, such as cardiac, digestive, and thermoregulatory functions, are involuntarily controlled by the autonomic nervous system, which is divided into the sympathetic and parasympathetic nervous systems. Activation of the sympathetic nervous system is essential for coping with environmental stressors such as fearful stimuli. Fearful stimuli increase sympathetic nerve activity, which influences our physiological state, such as palpitation, peripheral vasoconstriction, decreased peripheral temperature, increased blood pressure, increased blood flow to the skeletal muscles, and increased trunk temperature (Kistler et al., 1998; Kreibig et al., 2007; Krumhansl, 1997; Rimm-Kaufman and Kagan, 1996; Vianna and Carrive, 2005).

Many reports on the emotional aspects of fear and the brain have observed hyperactivity in the amygdala when the subject was confronted with a frightening situation (reviewed in (Phan et al., 2002; Phan et al., 2004)). Generally, information about the content of a fearful situation has been reported as being conveyed directly from the amygdala to a primary autonomic center, such as the hypothalamus or brainstem, to induce the sympathetic response (reviewed in (Nolte, 2009; Rodrigues et al., 2009)). In contrast, there is some evidence that stimulation of the amygdala, the anterior cingulate cortex (ACC), or the insula cortex induces sympathetic responses (Pool and Ransohoff, 1949; Yasui et al., 1991) and that lesions of the ACC change the autonomic response (reviewed in (Devinsky et al., 1995)). Recent human imaging studies have demonstrated that activity in some cortical regions, such as the ACC and anterior insula cortex (aIC), is related to a sympathetic component of heart rate variability during cognitive processes (Critchley et al., 2000, 2003). In addition, Critchley et al. reported that the ACC regulates autonomic response by demonstrating diminished autonomic response to cognitive effort by three patients with ACC damage, when compared with normal controls (Critchley et al., 2003). Thus, some cortical regions, such as the ACC and aIC, may influence fear-induced sympathetic activity,

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such as by regulating cardiovascular response, which means that the ACC or aIC may be an interface between fear and sympathetic activity. The results of the above-mentioned studies also suggest that information about the content of a fearful situation may be conveyed indirectly from the amygdala to a primary autonomic center via the ACC or aIC.

In neuroimaging studies, functional connectivity analysis based on functional Magnetic Resonance Imaging (fMRI) has been used to examine the correlation between spatially separated brain regions. As for sympathetic-related functional connectivity, to our knowledge only one study has reported that functional connectivity between the amygdala and ACC was related to stress-evoked blood pressure reactivity (Gianaros et al., 2008). However, little is known about connectivity between the amygdala and cortical regions that may be related to sympathetic response to fearful stimuli.

To clarify the neural substrates, we tested the hypothesis that functional connectivity between the amygdala and ACC and between the amygdala and aIC would be enhanced by fearful stimuli and that it would be increased proportionately with the subjective rating of fear. Elucidating the brain activity and its functional connectivity of sympathetic-related cortical regions induced by fearful stimuli will provide a foothold to understanding the neural substrates related to the regulation of visceral functions influenced by emotion through the autonomic nervous system.

This study was done to elucidate the neural correlates of fear-induced sympathetic response. To accomplish this, we used a horror movie as fearful stimuli. Our hypotheses were as follows: 1) there would be significantly greater functional connectivity between both the amygdala and ACC and between the amygdala and aIC while watching a horror movie than while watching a control movie; 2) The functional connectivity between the amygdala and ACC and between the amygdala and aIC would be increased proportionately with the subjective rating of fear while watching the horror movie.

2. Methods and materials

2.1. Participants

Thirty-two healthy, right-handed volunteers (19 men and 13 women, aged 20–39 years) participated in this study. All had normal vision, and none had a history of neurological or psychiatric illness. The exclusion criteria included persons with a cold constitution. We confirmed that all participants had fingertip temperature above 30 °C at the start of the experiment. The participants were asked to avoid all food, caffeine-containing beverages, and smoking for two hours and alcohol and medication for 24 h prior to the experiment. The participants were told that they would be taking part in an experiment that measures brain activity while watching parts of movies. Handedness was evaluated using the Edinburgh Handedness Inventory (Oldfield, 1971). Written informed consent was obtained from all participants. The protocol was approved by the ethics committee of the National Institute for Physiological Sciences. The data of two participants was excluded from the fear rating analysis because they did not comply with the experimental instructions for the self-reported rating of fear, and the data of five was excluded from the fMRI data analysis because of head motion over 3 mm, leaving the data of 30 participants available for the fear rating analysis, 27 for the fMRI data analysis, and 25 for the analysis of both the fear rating and fMRI data.

2.2. Stimuli

A horror movie and a control movie were chosen based on the criteria recommended by Gross and Levenson (Gross and Levenson, 1995). Scenes from the movie "I Know What You Did Last Summer (Directed by Jim Gillespie, USA, dubbed in Japanese, 1997)" were used as the horror movie. It evokes themes of anticipation of immediate bodily injury or impending death by a pursuer and a final confrontation with the source of the threat. A previous report (Kreibig et al., 2007) and our pilot study assured that this movie selectively elicited fear. The control movie was excerpted from the movie "Earth (Directed by Alastair Fothergill & Mark Linfield, UK & Germany, dubbed in Japanese, 2007)". It depicts nature scenery and wild animals. These movies were edited to last 10 min 37 s each (the details of the editing are shown in the supplemental methods). Before watching each movie, the participants read a synopsis and context information (the introductory text is also shown in the supplemental methods).

2.3. Questionnaires

Anxiety was assessed using Spielberger's State–Trait Anxiety Inventory (STAI), which consists of state anxiety and trait anxiety scales (Spielberger, 1983). The state anxiety scale consists of 20 statements that evaluate how the respondent feels "right now, at this moment". The trait anxiety scale consists of 20 statements that evaluate how the respondent feels "generally". The items are answered on a 4-point, Likert-type scale. The total score for each scale ranges from 20 to 80. The higher the total score, the stronger the degree of anxiety. The validity and reliability of the Japanese version of STAI have been confirmed (pages 7–15 of the "Manual for the Japanese version of state-trait anxiety inventory" published by Sankyobo in Kyoto, Japan).

2.4. Procedure

To avoid possible order effects on emotion elicitation, the participants were randomly assigned to one of two movie presentation conditions, the horror movie first and the control second or the reverse. The participants were provided with instructions for the experimental tasks, such as to focus on the video and audio without thinking of other things while watching the video movie and to not sleep. Following an orientation period, the STAI was filled out and physiological sensors were attached, as described later. The physiological indices were continuously monitored during the baseline resting state and the movie presentations.

We examined brain activity with fMRI while resting, watching the control movie, and watching the horror movie (Fig. 1). First, after entering an MRI room in which the temperature was kept at 24 °C, the participant, in a resting state with eyes open, did the first scanning of 10 min 36 s. (The resting fMRI data will be used for analysis in another experiment.) The participant then filled out the state anxiety part of STAI, followed by a 5-min quiet period. The movies, separated by 5-min quiet periods, were then watched. Immediately after each film, the participant filled out the state anxiety part of STAI. After watching both movies, the participant was disconnected from the monitoring devices and left the MRI room. The movies, divided into 212 film clips of three seconds, were again watched with the participant remembering the feeling of fear when they had first watched the movies. The self-reported fear rating for each film clip was input into a computer by

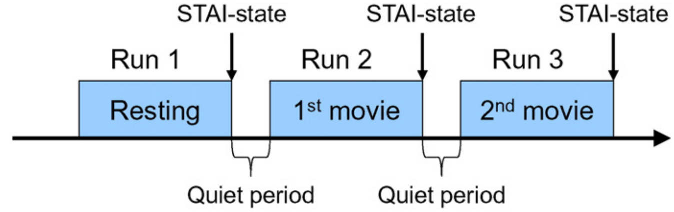

Fig. 1. Schematic diagram of the experiment in the magnetic resonance imaging (MRI) room. For the first scanning (Run 1), the participants were in a resting state with eyes open. They then completed the state anxiety section of Spielberger's State–Trait Anxiety Inventory (STAI), followed by a 5-min quiet period. They were then randomly assigned to scanning while watching one of the two movies (a horror movie or a control movie) (Run 2), with the other watched during the third period of scanning (Run 3). Immediately after each movie, the participant completed the state anxiety section of STAI, followed by a 5-min quiet period.

keyboard using a 9-point Likert-type scale, from 1 (None) to 9 (Most horrible sense of fear in this experiment). The participant was instructed that if there were film clips equally deserving a rating of the most horrible rating of fear, the "9" rating could be used multiple times. Finally, the participant answered the question "How much fear did you feel while watching the first and the second movies?" using a 5-point Likert-type scale (1. None; 2. Very little; 3. A little; 4. Moderate; 5. Strong) to confirm the level at which the film induced a feeling of fear.

2.5. Physiological measurements

Among the possible physiological responses, fingertip temperature has often been utilized as an indicator of sympathetic response to fearful stimuli because fearful stimuli promote a reduction of fingertip temperature via peripheral vasoconstriction (Kistler et al., 1998). Fingertip temperature (in degrees Celsius) was measured by a temperature probe (TSD202A, BIOPAC, Biopac Systems Inc., Goleta, CA, USA) attached to the palmar surface of the distal phalanx of the left fifth finger. The temperature sensor was connected to a Skin Temperature Amplifier Module (SKT100C, BIOPAC, Biopac Systems, Inc., Goleta, CA, USA) and linked to the data acquisition system (Powerlab, ADInstruments, Colorado Springs, CO, USA), which was set to begin recording with a sampling rate of 1 ms when the first MRI scanning started. These data were acquired by the purpose-built software Power Lab Chart Ver.5 (Powerlab, ADInstruments, Colorado Springs, CO, USA).

2.6. Physiological data analysis

The skin temperature data were reduced with a frequency of 1 Hz. We compared the average of these measurements for the first seven scans, when the participant had not yet watched the first fearful scene in the horror movie and for the last seven scans in each condition. Statistical analysis was performed by the paired *t*-test with Bonferroni correction. Change rates of skin temperature (°C/s) were calculated as follows; the value of skin temperature data at 0.5 s after the time point was subtracted from that at 0.5 s before the time point. Because the sympathetic response induced by fearful stimuli was expected to correlate negatively with the fingertip temperature reduction rate, we investigated the correlation between the subjective ratings of fear and the fingertip temperature change rates with a time-lag. Briefly, the temperature change rate data with the time delay (from 0 to 23 s) was down-sampled to match the fear ratings (one-third Hz) for each participant. We then calculated the averages of the temperature change rate data and the averages of subjective rating of fear for each time point. Lastly, we calculated the correlation coefficients between them.

2.7. FMRI data acquisition and basic analysis

The fMRI experiment was conducted using a Siemens 3 Tesla Magnetom Verio MRI scanner (Erlangen, Germany). Functional images were acquired using an echo-planar imaging (EPI) gradient-echo sequence (repetition time [TR] = 3000 ms, echo time [TE] = 30 ms, flip angle = 83°, field of view [FOV] = 192 mm, matrix size = 64 × 64 pixels, 47 slices, voxel size = 3.0 × 3.0 × 3.0 mm). A high-resolution structural image was acquired using T1-weighted magnetization-prepared rapid acquisition in a gradient echo sequence (repetition time [TR] = 1800 ms, echo time [TE] = 2.97 ms, flip angle = 9°, field of view [FOV] = 256 × 256 mm^2, 208 slices, voxel size = 1.0 × 1.0 × 1.0 mm). Presentation software (Neurobehavioral Systems, Albany, CA, USA) was used for the presentation of the audio-visual stimulus and to record the responses of the participants. Visual stimuli were presented on a screen using a liquid crystal display projector, with the participant viewing the screen through a mirror. A total of 215 functional images were collected during each session, and the first three images were discarded from the data analysis to allow for stabilization of the magnetization. SPM8 revision 4010 software (Wellcome Department of Imaging Neuroscience, University College London, http://www.fil.ion.ucl.ac.uk/spm/) was used for image processing and analysis. To reduce head-motion artifacts, the functional images were realigned to the first functional image. The images were smoothed spatially using an anisotropic Gaussian kernel of 4 mm full-width half-maximum to increase the signal-to-noise ratio.

To identify the components of sympathetic-related brain regions, we examined parametrically changed brain activity of the fingertip temperature change rate while watching the control and horror movies. We used the time-lag and delay derived from hemodynamic change to make a regressor. The time-lag was determined based on when the absolute value of the correlation coefficient between the subjective rating of fear and the fingertip temperature change rate was maximal while watching the horror movie, as will be described later. The convolution of the hemodynamic response and the temperature change rate data with the time-lag were used in fMRI model specification in SPM8. The procedure is as follows: Firstly, the 13 second time-lag plus the 9 second delay for the first three images of the scan of the temperature change rate data was down-sampled to match the fear ratings (one-third Hz). We then used the SPM hemodynamic response function (spm_hrf). A vector of SPM hemodynamic response (v), when TR = 3, was calculated from the following formula in MATLAB2010b (The MathWorks, Natick, MA, USA).

$$v = \text{spm\_hrf}(3)$$

A MATLAB convolution function (conv) was used to calculate the convolution of the hemodynamic response and the temperature change rate data (conv_hdr_temp) as in the following formula in MATLAB.

$$\text{conv\_hdr\_temp} = \text{conv}(u, v);$$

$$u : \text{a vector of the temperature change rate data}$$

Lastly, the first 7 convolved down-sampled data (corresponding to the data of the 13 second time-lag plus the 9 second delay) of the temperature change rate were discarded. The convolved down-sampled data of the temperature change rate through the last scan were used in the module "fMRI model specification" of SPM as a regressor in the first-level (individual) analysis.

In the first-level analysis of each contrast definition after the first-level model specification and estimation, the T-contrast weight vector, with (−1) for the regressor of the temperature change rate data with a time-lag and (0) for the other regressor, was entered in the module "Contrast manager" of SPM to identify the regions in which the brain activity was negatively correlated with the fingertip temperature change rate with a time-lag. In the second-level (group) analysis, a "full factorial" design of model specification in which the contrast images resulting from the first-level analysis were used in the module, "Factorial design specification" of SPM, estimation, and contrast definition were conducted to perform the conjunction analysis. In order to evaluate which regions from the maps generated for the two conditions (watching the control and horror movies) had common areas of activation negatively correlated with the fingertip temperature change rate with a time-lag, conjunction analysis was done.

We extracted significant cluster(s) that were positively correlated with the subjective rating of fear when the participant was watching the horror movie using the anatomical region of interest (ROI) of the whole-amygdala in the SPM anatomy toolbox (http://www.fz-juelich.de/inm/inm-1/DE/Forschung/_docs/SPMAnatomyToolbox/SPMAnatomyToolbox_node.html) (Eickhoff et al., 2005). To identify the fear-related amygdala regions activated while watching the horror movies, we examined parametrically changed brain activity by the convolution of the fear ratings and a hemodynamic response function, using SPM8 and MATLAB 2010b, as a regressor in the same way as in the convolution of the fingertip temperature change rate and a hemodynamic response function. A MATLAB convolution function (conv) was used to calculate the convolution of the hemodynamic response and the fear rating data (conv_hdr_rating), as in the following formula in MATLAB.

$$\mathrm{conv\_hdr\_rating} = \mathrm{conv(w, v)};$$

$$\mathrm{w: a\ vector\ of\ (the\ fear\ rating-1)}$$

The convolved data of the fear ratings were used in the module "fMRI model specification" of SPM as a regressor at the first-level analysis. The height threshold was set at $p < 0.001$ (uncorrected for multiple comparisons), and the cluster threshold was set at familywise error (FWE)-corrected $p < 0.05$ (corrected for multiple comparisons).

2.8. Functional connectivity analysis

To investigate the difference of functional connectivity between the fear-related amygdala and a sympathetic-related brain region with and without fearful stimuli, i.e. while watching the horror movie and control movies, the CONN software (http://web.mit.edu/swg/software.htm) of the SPM8 toolbox was used for functional connectivity analysis. Using the default preprocessing parameters, the possible confounding effects of head motion artifacts and the white matter and CSF BOLD signal were defined and addressed (Whitfield-Gabrieli and Nieto-Castanon, 2012). Seed-to-voxel and ROI-to-ROI functional connectivity maps were created for each participant. The mean BOLD time series was computed across all voxels within each ROI. Bivariate-regression analyses were used to determine the linear association of the BOLD time series between each pair of sources. CONN uses a seed-driven functional connectivity analysis strategy in which the Pearson's correlation coefficient is calculated between the seed time course and the time course of all other voxels(Whitfield-Gabrieli and Nieto-Castanon, 2012). The correlation coefficients are then converted to normally distributed scores using Fisher's transformation to allow for second-level General Linear Model analysis (Whitfield-Gabrieli and Nieto-Castanon, 2012). We used the extracted clusters of the ACC, aIC, and anterior prefrontal cortex regions for which brain activity was negatively correlated with the fingertip temperature change rate and those of the amygdala regions for which brain activity was positively correlated with the self-reported fear rating as the seeded voxels (source regions of interest (ROIs)). The threshold of the second-level analysis for data was a false discovery rate (FDR)-corrected $p < 0.05$ in the selected ROI-to-ROI analysis.

2.9. Psychophysiological interaction analysis

To clarify that connectivity between the fear-related amygdala region and the sympathetic-related brain regions was increased in proportion to the degree of fear, the investigation while watching the horror movie was done using psychophysiological interaction (PPI) analysis. The height threshold was set at $p < 0.001$ (uncorrected for multiple comparisons), and the cluster threshold was set at FWE-corrected $p < 0.05$ (corrected for multiple comparisons).

2.10. Statistical analysis

Statistical analyses of the psychophysiological data were performed by use of a statistical software package (PASW Statistics 18, version 18.0.0 for Windows; SPSS Inc., Chicago, IL, USA). The Wilcoxon *t*-test and paired *t*-test were used to compare the psychological and physiological parameters, respectively. Spearman rank correlation analysis was used to test the relation between the time-line of the average fingertip temperature change rate for all participants with the time lag and the time-line of the average of the subjective fear rating, because we found that the subjective fear rating did not come from a normal distribution using the Kolmogorov–Smirnov test (watching horror movie: $p < 0.01$, watching control movie: $p < 0.01$). Pearson's correlation coefficient was used to test the relationship between the signal intensities of the brain region. *P* values < 0.05 were considered to be statistically significant. "Cocor", a free software package for the R programming language, was used for tests for the significance of the difference between correlations (Diedenhofen and Musch, 2015).

3. Results

3.1. Feelings of fear and fingertip temperature

To confirm the feelings of fear elicited when the participant was watching the horror movie, we compared the self-reported fear experience to the control and horror movies using 5-point Likert-type scale (1. None; 2. Very little; 3. A little; 4. Moderate; 5. Strong) and the change from baseline of the state anxiety score of the STAI. The result from the 5-point Likert-type scale for fear from the horror movie ($M = 4.4$, $SE = 0.2$, $p < 0.01$) was significantly higher than that of the control movie ($M = 1.4$, $SE = 0.1$). The increase in the state anxiety score from baseline after watching the horror movie ($M = 17.7$, $SE = 1.7$, $p < 0.01$) was also higher than that after watching the control movie ($M = -5.3$, $SE = 1.3$).

The difference between the fingertip temperature while watching the first and last scenes of the horror movie ($M = -0.55$, $SE = 0.14$, $p < 0.01$) was greater than that between the fingertip temperature while watching the corresponding scenes of the control movie ($M = 0.17$, $SE = 0.09$).

These results show that the horror movie elicited feelings of fear and that this fearful stimuli promoted a reduction of fingertip temperature.

3.2. Influence of the subjective rating of fear on the fingertip temperature change rate

Because the sympathetic response induced by fearful stimuli was expected to correlate negatively with the fingertip temperature reduction rate, we investigated the correlation between the subjective ratings of fear and the fingertip temperature change rates. Fig. 2A and B show the significant negative correlation between the subjective rating of fear and the fingertip temperature change rate while watching the horror movie (Fig. 2B; *rs*: Spearman's rank correlation coefficient $= -0.233$, $p = 0.001$), whereas there was no correlation while watching the control movie (Fig. 2A; $rs = 0.011$, $p = 0.873$). The correlation coefficient while watching the horror movie was significantly greater than that while watching the control movie ($p < 0.05$). The absolute value of the correlation coefficient while watching the horror movie was largest in 13 second shifts (Fig. 2C; $rs = -0.433$, $p < 0.001$).

3.3. Sympathetic-related cortical regions with or without fearful stimuli

To identify the cortical regions associated with sympathetic nerve activity while watching the control and horror movies, we examined parametrically changed BOLD signals using the fingertip temperature change rate with a time-lag of 13 s as a regressor. A conjunction analysis of the control and horror movie conditions revealed that activation of the ACC, bilateral aIC, and bilateral anterior prefrontal cortex (aPFC) was negatively correlated with the fingertip temperature change rate with a time-lag (Fig. 3, Table 1). These cortical regions were associated with sympathetic response with or without fearful stimuli.

3.4. Amygdala region response by fear rating

To identify fear-related amygdala regions, we examined parametrically changed BOLD signals within anatomical ROI of amygdala regions while watching the horror movie using the subjective rating of fear as a regressor. The fear-related amygdala regions (right: 20 voxels, [18, −8, 10], Z = 3.84; left: 40 voxels, [−20, −8, −12], Z = 4.14) were identified by examining the brain activity positively correlated with the

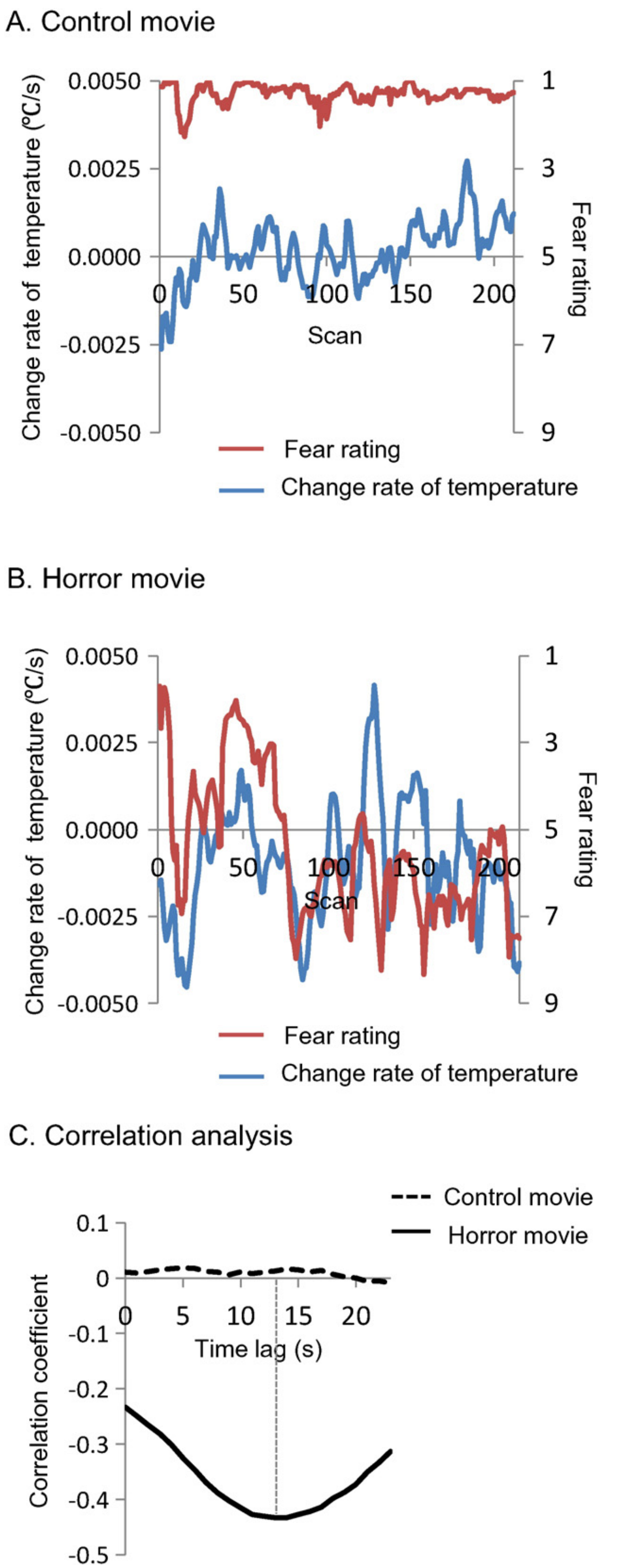

Fig. 2. Subjective ratings of fear and the fingertip temperature change rate, and their correlation. (A, B) The subjective ratings of fear and the fingertip temperature change rates while watching the control (A) and horror (B) movies are shown. (C) The absolute value of the correlation coefficient between the subjective ratings of fear and the fingertip temperature change rate while watching the horror movie was highest in 13 second shifts. Data is expressed as means.

subjective rating of fear while watching the horror movie using the anatomical ROI of the amygdala (corrected $p < 0.05$ at cluster level using small volume correction; Fig. 4).

3.5. Functional connectivity between the fear-related amygdala regions and the sympathetic-related cortical region

The functional connectivity analysis revealed that there was significant bilateral functional connectivity between the amygdala and ACC and between the amygdala and aIC while watching the horror movie, whereas no such connectivity was found while watching the control movie (Fig. 5 upper panel; FDR-corrected $p < 0.05$ in the selected ROI-to-ROI analysis). Additionally, among these ROIs there was significantly stronger positive bilateral functional connectivity between the amygdala and ACC and between amygdala and aIC while watching the horror movie than while watching the control movie (Fig. 5 lower panel; FDR-corrected $p < 0.05$ in the selected ROI-to-ROI analysis).

3.6. Increased functional connectivity between the left amygdala and the ACC proportionate to the subjective rating of fear

PPI analysis revealed that the activation of the ACC (left panel in Fig. 6) and the right associative visual cortex (right panel in Fig. 6) was influenced by the activation of the fear-related left amygdala region and the subjective rating of fear (FEW-corrected $p < 0.05$ at cluster level). An interaction region overlap with the sympathetic-related cortical regions was only seen in the ACC region (orange colored regions in the left panel of Fig. 6; corrected $p < 0.05$ at cluster level using small volume correction). Additionally, Fig. 7 shows the significant positive correlation between the signal intensity of the left amygdala and that of the ACC at a higher subjective rating of fear ($r = 0.296$, $p = 0.002$), whereas there was no such correlation at a lower subjective rating of fear ($r = -0.136$, $p = 0.263$), which shows that the functional connectivity between the fear-related left amygdala region and the ACC was enhanced by the higher fear rating. For the right amygdala, however, PPI analysis found no regions that were significantly influenced by the activation of the right amygdala and the subjective rating of fear.

3.6.1. Connectivity between the ACC and aIC in fear-induced sympathetic response

Because functional connectivity between the sympathetic-related ACC and the aIC is important for sympathetic nervous system response, especially in the context of cognitive and emotional stimuli, we analyzed the functional connectivity between the ACC and the aIC. The results showed significant bilateral functional connectivity between the sympathetic-related ACC and the aIC, not only while watching the horror movie but also while watching control movie (FDR-corrected $p < 0.05$ in the selected ROI-to-ROI analysis).

4. Discussion

We investigated the neural correlates of fear-induced sympathetic response associated with the peripheral temperature change rate. This is the first study to demonstrate both amygdala-ACC and amygdala-aIC connectivity associated with sympathetic nervous system response to fearful stimuli, although previous studies demonstrated that amygdala-ACC connectivity was increased during fearful stimuli (Etkin et al., 2010; Gold et al., 2015; Prater et al., 2013; Robinson et al., 2014). We also firstly demonstrated that connectivity between the fear-related left amygdala region and the sympathetic-related ACC region is increased proportionately with the subjective rating of fear. Our findings provide new insights into the mechanisms of the indirect pathway of fear-induced sympathetic response from the amygdala to the autonomic nervous system.

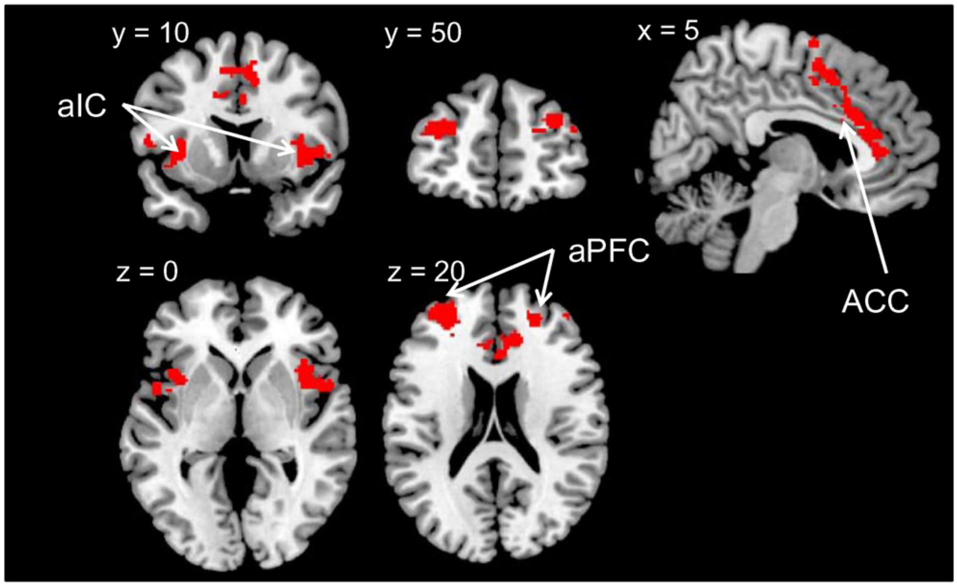

Fig. 3. Activation negatively correlated with the fingertip temperature change rate. Whole-brain conjunction analysis revealed that the activation of the anterior cingulate cortex (ACC), bilateral anterior insula (aIC), and bilateral anterior prefrontal cortex (aPFC) while watching the control and horror movies was negatively correlated with the fingertip temperature change rate. The height threshold was set at $p < 0.001$ (uncorrected for multiple comparisons), and the cluster threshold was set at familywise error (FWE)-corrected $p < 0.05$.

4.1. Influence of the subjective rating of fear on the fingertip temperature change rate

Our results about the influence of the subjective rating of fear on the fingertip temperature change rate demonstrate that the sympathetic response induced by fearful stimuli leads to a reduction of fingertip temperature, with a time-lag of 13 s (Fig. 2). A previous study reported that reduced finger skin blood flow by sympathetic stimuli was reflected by a decrease in fingertip temperature, with a time-lag of 14.6 s (Kistler et al., 1998). The difference between our time-lag measurement and that of Kistler et al. may depend on whether the time-lag is described as being between vasoconstriction and temperature change or between behavior and temperature change. It also may depend on how a sympathetic stimulus is created, such as by watching a fearful movie or by chewing a cotton swab. The sympathetic response to a fearful situation may be faster than the sympathetic response to the stress of chewing. Our results and those of the above mentioned study indicate that the sympathetic response induced by fearful stimuli is negatively correlated with the fingertip temperature reduction rate and that there is a time-lag between sympathetic activity and the reduction of fingertip temperature.

4.2. Sympathetic-related cortical regions with or without fearful stimuli

We found that the ACC, aIC, and aPFC are associated with the fingertip temperature change rate, with or without fearful stimuli (Fig. 3, Table 1). These results demonstrate that the ACC, bilateral aIC, and bilateral aPFC are candidate components of the sympathetic-related cortical region. Previous papers also demonstrated that the ACC, aIC and aPFC are associated with sympathetic activity (Critchley et al., 2000, 2003, 2005; Patterson et al., 2002), consistent with our results.

Table 1
Whole-brain conjunction analysis: regions of brain activation negatively correlated with the fingertip temperature change rate while watching the control and horror movies.

Brain region	BA	Side	Number of voxels	Z-value	MNI coordinates		
Anterior cingulate cortex	32	R	817	4.54	8	10	46
+ Supplementary motor area	6	R		4.32	2	4	54
Insula	13	R	534	5.13	42	8	0
Insula	13	L	358	4.76	−36	10	0
Prefrontal cortex	10	L	218	4.49	−28	50	22
Prefrontal cortex	10	R	129	4.00	30	50	26

The height threshold was set at $p < 0.001$ (uncorrected for multiple comparisons), and the cluster threshold was set at familywise error (FWE)-corrected $p < 0.05$.

Critchley and his colleagues reported that the ACC is involved in the regulation of the autonomic nervous system related to cognitive processes, such as mental and physical effort and error processing (Critchley et al., 2000, 2003, 2005) and that patients with lesions in the ACC had an autonomic reaction disorder (Critchley et al., 2003). Pool and Ransohoff demonstrated that electrical stimulation of the ACC can cause autonomic response (Pool and Ransohoff, 1949). Neuroanatomically, the neurons in the ACC project to the primary autonomic centers, such as the hypothalamus and brainstem (An et al., 1998; Ongur et al., 1998). These previous studies and the anatomical connections support the possibility that the ACC affects sympathetic response. In addition, the ACC has been related not only sympathetic activity but also to cognitive and emotional function (reviewed in (Bush et al., 2000)). Thus, the role of the ACC may be to regulate sympathetic nerve system response to cognitive activity or emotional feelings about fearful stimulation.

The role of the insula in the control and representation of autonomic states has been well established from stimulation and electrophysiological studies of animals (reviewed in (Benarroch, 1993; Cechetto and Saper, 1990)). An animal study showed that the insular region has direct projections to the areas of the hypothalamus and brainstem that are involved in autonomic control (Saper, 1982). A human study showed that electrical stimulation of the right insula elicited tachycardia and hypertension (Oppenheimer et al., 1992). The aIC also plays an important role in monitoring the internal state of the body, which is partly changed by the autonomic nervous system, to maintain human homeostasis (Craig, 2009; Critchley et al., 2004). Taken together, these previous papers and our results suggest that information on the bodily state, which plays an important role in the generation of some emotions, is input to the aIC, then the aIC controls sympathetic response, even to fearful stimuli.

4.3. Functional connectivity between the fear-related amygdala regions and the sympathetic-related cortical region

Our results of functional connectivity revealed that functional connectivity between the fear-related amygdala and the sympathetic-

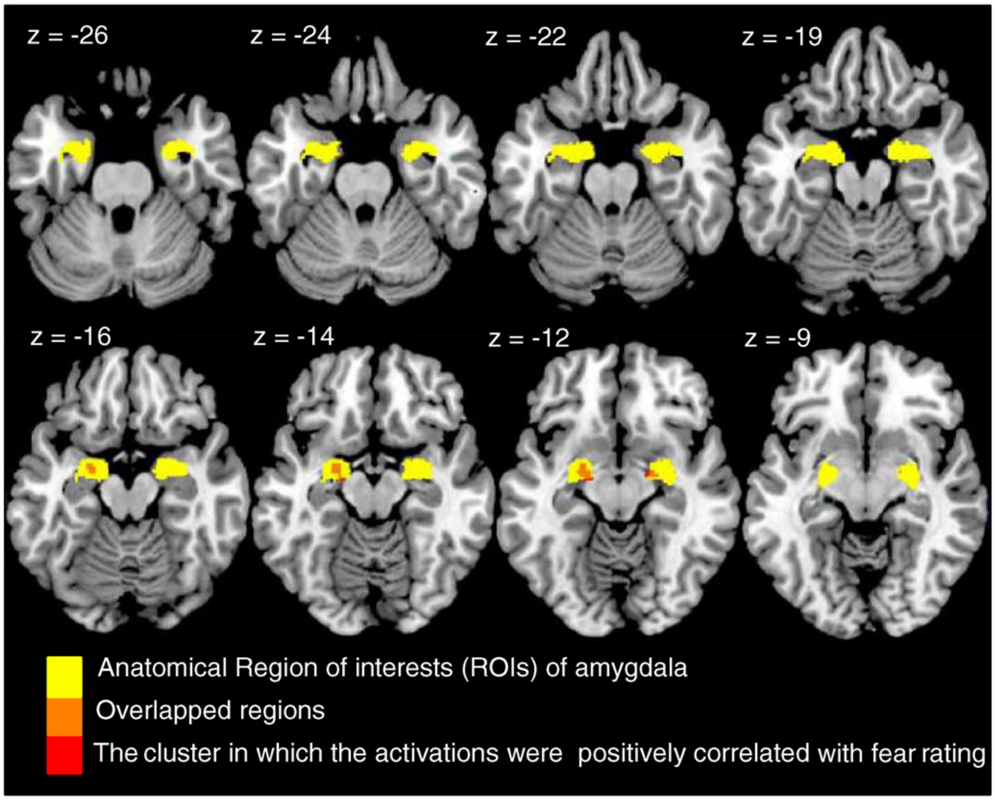

Fig. 4. Anatomical regions of interest (ROIs) of the amygdala and the cluster in which the activation was positively correlated with the self-reported fear rating within the anatomical ROIs. The anatomical ROIs of the amygdala are shown in yellow. The regions in which activation while watching the horror movie was positively correlated with the self-reported fear rating are shown in red. Overlapping regions between the anatomical ROI of the amygdala and the cluster in which the activation was positively correlated with the self-reported fear rating are shown in orange. The height threshold was set at $p < 0.001$ (uncorrected for multiple comparisons), and the cluster threshold was set at familywise error (FWE)-corrected $p < 0.05$ using small volume correction.

related cortical regions, such as ACC and aIC, is enhanced by fearful stimuli. In terms of anatomical and functional connection of the amygdala, reciprocal connection from the amygdala to the ACC and the aIC has been reported (Nolte, 2009; Robinson et al., 2010). These previous papers and our results suggest that the ACC and aIC are candidates for the cortical interface between fear and sympathetic activity.

4.4. Functional connectivity between the fear-related left amygdala region and the sympathetic-related ACC region is proportionate to the subjective rating of fear

Our results of PPI analysis can be interpreted in two ways; that connectivity between the left amygdala and the ACC was altered according to the fear rating (left panel in Fig. 8) or that the response of the ACC, according to the fear rating, is due to the influence of the left amygdala (right panel in Fig. 8). The former is more probable than the latter because our functional connectivity analysis demonstrated that the connectivity between the left amygdala and the ACC was enhanced by fearful stimuli (Fig. 5). Also, we confirmed that functional connectivity between the fear-related left amygdala region and the ACC was enhanced as the fear rating became higher (Fig. 7). These results also support the former interpretation of the PPI analysis as being more appropriate than the latter. Thus, our results of PPI analysis suggest that functional connectivity between the left amygdala and the ACC increases proportionately with the subjective rating of fear. Neuroanatomically, the ACC receives input from the amygdala and sends output to the hypothalamus (Nolte, 2009; Ongur et al., 1998). It was reported that the ACC has functions related to both cognitive and emotional processes (reviewed in (Devinsky et al., 1995)). Our findings and those of these previous reports suggest that the ACC is the cortical interface between fear and sympathetic activity. The ACC may integrate the information regarding the context of the fearful situation from the left amygdala, which influences the autonomic nervous system.

As for the laterality of the amygdala, we found that functional connectivity between the left amygdala and the ACC was increased proportionately with the subjective rating of fear, but not between the right amygdala and the ACC. Phelps et al. demonstrated that activation of the left amygdala was correlated with the expression of fear response as measured by skin conductance which is a sympathetic response (Phelps et al., 2001). This previous study and our results suggest that left amygdala activation and its connectivity are related to both fear and sympathetic response.

4.5. Connectivity between the ACC and aIC in fear-induced sympathetic response

The insula has bidirectional connections with the ACC and previous papers have reported functional connectivity between the ACC and aIC (Craig, 2009; Critchley et al., 2004; Dosenbach et al., 2007; Medford and Critchley, 2010). Moreover, the ACC and aIC may be considered together as input and output regions of the functional system, which is typically engaged across cognitive, affective, and behavioral contexts (Medford and Critchley, 2010). In this study, we found a significant functional connectivity between the ACC and aIC, not only with fearful stimuli but also without fearful stimuli. In

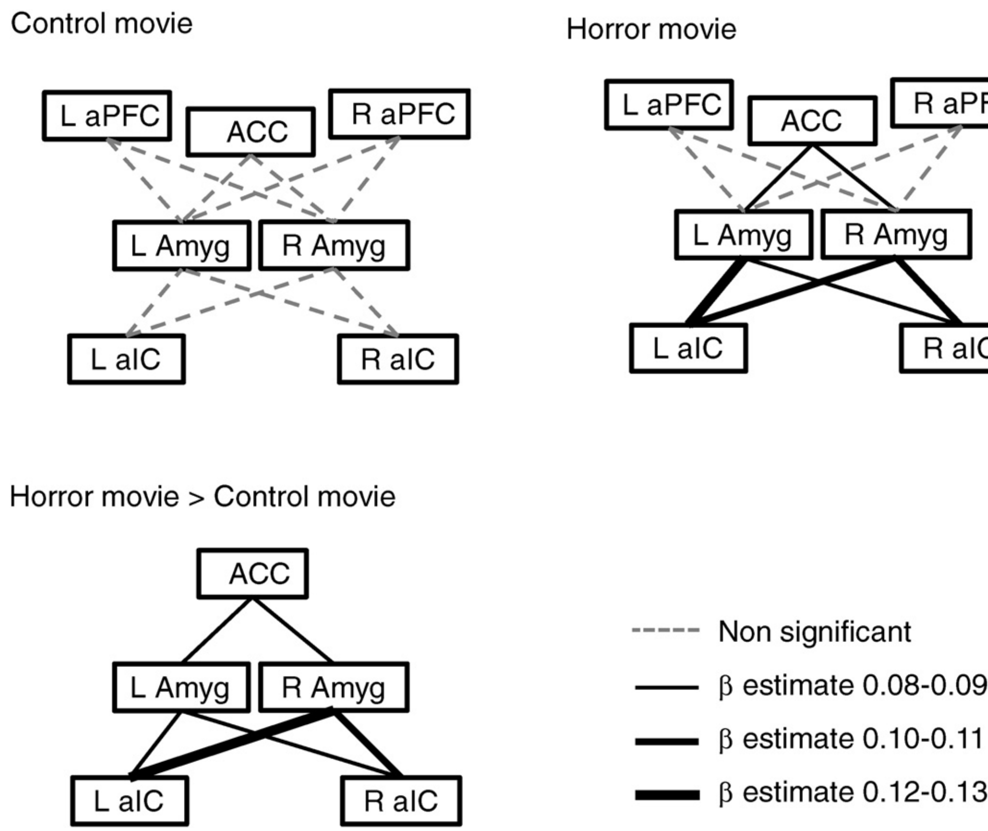

Fig. 5. Functional connectivity between the fear-related bilateral amygdala and sympathetic-related brain regions. Functional connectivity analysis revealed that the activity of the bilateral amygdala was positively correlated with the activity of the anterior cingulate cortex (ACC) and the bilateral anterior insula cortex (aIC) while watching the horror movie, whereas these correlations were not seen while watching the control movie. Additionally, among these regions of interest (ROIs) there was also significantly greater functional connectivity between the bilateral amygdala and the ACC or bilateral aIC while watching the horror movie than while watching the control movie. The statistical threshold is false discovery rate (FDR)-corrected $p < 0.05$ in the selected ROI-ROI analysis. L: left, R: right, aPFC: anterior prefrontal cortex, Amyg: amygdala.

addition, both the ACC and aIC are important regions of emotion and the autonomic nervous system, as previously mentioned. Therefore, functional connection between the ACC and aIC may be important for controlling the sympathetic nervous system, regardless of fearful stimuli.

4.6. Similarities and differences of the role of ACC and aIC in fear-induced sympathetic response

As for the similarities of the roles of the ACC and aIC, our results indicate that both the ACC and aIC are involved in sympathetic nervous

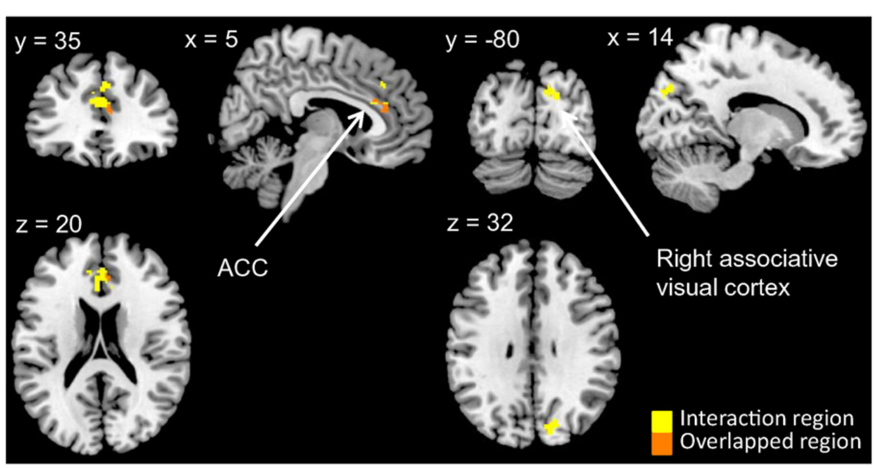

Fig. 6. Psycho-physiological interaction (PPI) analysis of subjective ratings of fear, and the brain activity of the left amygdala. Whole-brain PPI analysis revealed that the activation of the anterior cingulate cortex (ACC: yellow + orange colored regions in left panel) and the right associative visual cortex (yellow colored regions in right panel) was influenced by the activation of the left amygdala and the subjective ratings of fear (The height threshold was set at $p < 0.001$ (uncorrected for multiple comparisons), and the cluster threshold was set at familywise error (FWE)-corrected $p < 0.05$). The orange colored regions are the interaction regions that overlap the sympathetic-related brain regions (The height threshold was set at $p < 0.001$ (uncorrected for multiple comparisons), and the cluster threshold was set at familywise error (FWE)-corrected $p < 0.05$ using small volume correction).

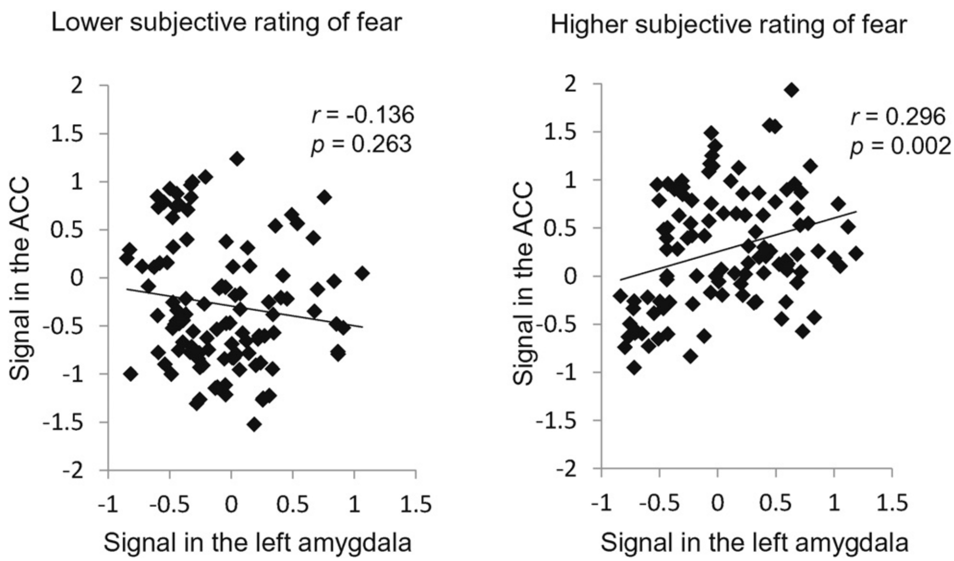

Fig. 7. Signal intensity plots of the left amygdala and anterior cingulate cortex (ACC) for the lower (≤median: 4.968) and higher (>median: 4.968) subjective ratings of fear (average for each time point). Time points of the time-series data (average for each time point) extracted from the ACC region compared with those from the left amygdala. A higher subjective rating of fear was associated with significant connectivity between the left amygdala and the ACC, but a lower subjective rating of fear was not. Each dot represents a pair of corresponding time points. *r*: correlation coefficient.

system activity, regardless of fearful stimuli, and that both the ACC and aIC are functionally connected with the amygdala in fear-inducing situations. As for the differences of the roles of the ACC and aIC, the ACC plays an important role in connectivity to the amygdala according to the degree of fear (Etkin et al., 2010; Gold et al., 2015; Prater et al., 2013; Robinson et al., 2014) and may be deeply involved in the connection between fear and the sympathetic nervous system. On the other hand, we did not find significant connectivity between the amygdala and aIC according to the degree of fear, possibly because the aIC has a more important role in monitoring interoceptive information from the body in fear-inducing situations, as many previous studies have demonstrated (Craig, 2009; Critchley et al., 2004), than the connectivity to amygdala according to the degree of fear.

5. Conclusion

The present findings suggest that fear-induced sympathetic response is influenced by connectivity between the left amygdala and the ACC. Functional connectivity between both the fear-related amygdala and the ACC and the amygdala and the aIC is enhanced by fearful stimuli. Functional connectivity between the left amygdala and the ACC increased proportionately with the subjective rating of fear. These results suggest that the increased functional connectivity between the left amygdala and the ACC represents sympathetic response to fearful stimuli. Our findings provide important insights into the role of the ACC in the connection between fear and the autonomic nervous system.

Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

Acknowledgments and disclosures

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2016.04.040.

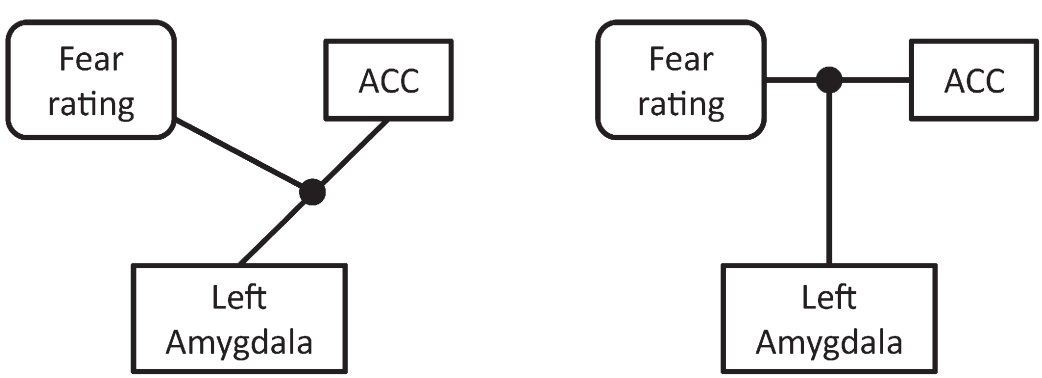

Fig. 8. Possible interpretations of the psycho-physiological interaction (PPI) result. One is that connectivity between the left amygdala and the anterior cingulate cortex (ACC) was altered according to the fear rating (left panel) and another is that the response of the ACC, according to the fear rating, is due to the influence of the left amygdala (right panel).

References

An, X., Bandler, R., Ongur, D., Price, J.L., 1998. Prefrontal cortical projections to longitudinal columns in the midbrain periaqueductal gray in macaque monkeys. J. Comp. Neurol. 401, 455–479.

Benarroch, E.E., 1993. The central autonomic network: functional organization, dysfunction, and perspective. Mayo Clin. Proc. 68, 988–1001.

Bush, G., Luu, P., Posner, M.I., 2000. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn. Sci. 4, 215–222.

Cechetto, D.R., Saper, C.B. (Eds.), 1990. Role of the Cerebral Cortex in Autonomic Function. Oxford University Press, Oxford, UK.

Craig, A.D., 2009. How do you feel—now? The anterior insula and human awareness. Nat. Rev. Neurosci. 10, 59–70.

Critchley, H.D., Corfield, D.R., Chandler, M.P., Mathias, C.J., Dolan, R.J., 2000. Cerebral correlates of autonomic cardiovascular arousal: a functional neuroimaging investigation in humans. J. Physiol. 523 (Pt 1), 259–270.

Critchley, H.D., Mathias, C.J., Josephs, O., O'Doherty, J., Zanini, S., Dewar, B.K., Cipolotti, L., Shallice, T., Dolan, R.J., 2003. Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. Brain 126, 2139–2152.

Critchley, H.D., Wiens, S., Rotshtein, P., Ohman, A., Dolan, R.J., 2004. Neural systems supporting interoceptive awareness. Nat. Neurosci. 7, 189–195.

Critchley, H.D., Tang, J., Glaser, D., Butterworth, B., Dolan, R.J., 2005. Anterior cingulate activity during error and autonomic response. NeuroImage 27, 885–895.

Devinsky, O., Morrell, M.J., Vogt, B.A., 1995. Contributions of anterior cingulate cortex to behaviour. Brain 118 (Pt 1), 279–306.

Diedenhofen, B., Musch, J., 2015. cocor: a comprehensive solution for the statistical comparison of correlations. PLoS One 10, e0121945.

Dosenbach, N.U., Fair, D.A., Miezin, F.M., Cohen, A.L., Wenger, K.K., Dosenbach, R.A., Fox, M.D., Snyder, A.Z., Vincent, J.L., Raichle, M.E., Schlaggar, B.L., Petersen, S.E., 2007. Distinct brain networks for adaptive and stable task control in humans. Proc. Natl. Acad. Sci. U. S. A. 104, 11073–11078.

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.

Etkin, A., Prater, K.E., Hoeft, F., Menon, V., Schatzberg, A.F., 2010. Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. Am. J. Psychiatry 167, 545–554.

Gianaros, P.J., Sheu, L.K., Matthews, K.A., Jennings, J.R., Manuck, S.B., Hariri, A.R., 2008. Individual differences in stressor-evoked blood pressure reactivity vary with activation, volume, and functional connectivity of the amygdala. J. Neurosci. 28, 990–999.

Gold, A.L., Morey, R.A., McCarthy, G., 2015. Amygdala-prefrontal cortex functional connectivity during threat-induced anxiety and goal distraction. Biol. Psychiatry 77, 394–403.

Gross, J.J., Levenson, R.W., 1995. Emotion elicitation using films. Cogn. Emot. 9, 87–108.

Kistler, A., Mariauzouls, C., von Berlepsch, K., 1998. Fingertip temperature as an indicator for sympathetic responses. Int. J. Psychophysiol. 29, 35–41.

Kreibig, S.D., Wilhelm, F.H., Roth, W.T., Gross, J.J., 2007. Cardiovascular, electrodermal, and respiratory response patterns to fear- and sadness-inducing films. Psychophysiology 44, 787–806.

Krumhansl, C.L., 1997. An exploratory study of musical emotions and psychophysiology. Can. J. Exp. Psychol. 51, 336–353.

Medford, N., Critchley, H.D., 2010. Conjoint activity of anterior insular and anterior cingulate cortex: awareness and response. Brain Struct. Funct. 214, 535–549.

Nolte, J., 2009. The Human Brain: An Introduction to its Functional Anatomy. sixth ed. Mosby/Elsevier, Philadelphia, PA.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.

Ongur, D., An, X., Price, J.L., 1998. Prefrontal cortical projections to the hypothalamus in macaque monkeys. J. Comp. Neurol. 401, 480–505.

Oppenheimer, S.M., Gelb, A., Girvin, J.P., Hachinski, V.C., 1992. Cardiovascular effects of human insular cortex stimulation. Neurology 42, 1727–1732.

Patterson 2nd, J.C., Ungerleider, L.G., Bandettini, P.A., 2002. Task-independent functional brain activity correlation with skin conductance changes: an fMRI study. NeuroImage 17, 1797–1806.

Phan, K.L., Wager, T., Taylor, S.F., Liberzon, I., 2002. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. NeuroImage 16, 331–348.

Phan, K.L., Wager, T.D., Taylor, S.F., Liberzon, I., 2004. Functional neuroimaging studies of human emotions. CNS Spectr. 9, 258–266.

Phelps, E.A., O'Connor, K.J., Gatenby, J.C., Gore, J.C., Grillon, C., Davis, M., 2001. Activation of the left amygdala to a cognitive representation of fear. Nat. Neurosci. 4, 437–441.

Pool, J.L., Ransohoff, J., 1949. Autonomic effects on stimulating rostral portion of cingulate gyri in man. J. Neurophysiol. 12, 385–392.

Prater, K.E., Hosanagar, A., Klumpp, H., Angstadt, M., Phan, K.L., 2013. Aberrant amygdala-frontal cortex connectivity during perception of fearful faces and at rest in generalized social anxiety disorder. Depress. Anxiety 30, 234–241.

Rimm-Kaufman, S.E., Kagan, J., 1996. The psychological significance of changes in skin temperature. Motiv. Emot. 20, 63–78.

Robinson, J.L., Laird, A.R., Glahn, D.C., Lovallo, W.R., Fox, P.T., 2010. Metaanalytic connectivity modeling: delineating the functional connectivity of the human amygdala. Hum. Brain Mapp. 31, 173–184.

Robinson, O.J., Krimsky, M., Lieberman, L., Allen, P., Vytal, K., Grillon, C., 2014. Towards a mechanistic understanding of pathological anxiety: the dorsal medial prefrontal-amygdala 'aversive amplification' circuit in unmedicated generalized and social anxiety disorders. Lancet Psychiatry 1, 294–302.

Rodrigues, S.M., LeDoux, J.E., Sapolsky, R.M., 2009. The influence of stress hormones on fear circuitry. Annu. Rev. Neurosci. 32, 289–313.

Saper, C.B., 1982. Convergence of autonomic and limbic connections in the insular cortex of the rat. J. Comp. Neurol. 210, 163–173.

Spielberger, C.D., 1983. Manual for the State–trait Anxiety Inventory (form Y): "Self-evaluation Questionnaire". Consulting Psychologists Press, Palo Alto, CA.

Vianna, D.M., Carrive, P., 2005. Changes in cutaneous and body temperature during and after conditioned fear to context in the rat. Eur. J. Neurosci. 21, 2505–2512.

Whitfield-Gabrieli, S., Nieto-Castanon, A., 2012. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain Connect. 2, 125–141.

Yasui, Y., Breder, C.D., Saper, C.B., Cechetto, D.F., 1991. Autonomic responses and efferent pathways from the insular cortex in the rat. J. Comp. Neurol. 303, 355–374.