Sadness enhances the experience of pain via neural activation in the anterior cingulate cortex and amygdala: An fMRI study

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A B S T R A C T
Pain is a multidimensional experience. Human pain perception can be modulated by subjective emotional responses. We examined this association within the context of a neuroimaging study, using functional MRI to examine neural responses to electrical pain-inducing stimuli in 15 healthy subjects (6 females; age range = 20–30 years). Pain-inducing stimuli were presented during different emotional contexts, which were induced via the continuous presentation (5 s) of sad, happy, or neutral pictures of faces. We found that subjective pain ratings were higher in the sad emotional context than in the happy and neutral contexts, and that pain-related activation in the ACC was more pronounced in the sad context relative to the happy and neutral contexts. Psychophysiological interaction (PPI) and dynamic causal modeling (DCM) analyses demonstrated amygdala to ACC connections during the experience of pain in the sad context. These findings serve to highlight the neural mechanisms that may be relevant to understanding the broader relationship between somatic complaints and negative emotion.

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Introduction

Pain is a multidimensional phenomenon that is characterized by clear physiological and psychological elements. Various studies have examined the relationship between negative emotion and pain perception (Bueno et al., 1997; Campbell et al., 2003; Katon et al., 1982).

Human neuroimaging studies have examined the relationships between cognition and sensory perception. For example, previous studies have demonstrated that cognitive contexts such as distraction (Frankenstein et al., 2001; Petrovic et al., 2000; Valet et al., 2004), anticipation (Keltner et al., 2006; Porro et al., 2003; Sawamoto et al., 2000; Wager et al., 2004), empathy (Jackson et al., 2006; Ogino et al., 2007; Singer et al., 2004), and attention (Bantick et al., 2002; Buffington et al., 2005; Davis et al., 1997) can modulate the neural activities that underlie sensory perception, including pain perception. It has also been reported that emotional context can affect both subjective pain sensitivity and the neural activities that appear to underlie pain perception. Ploghaus et al. (2001) reported that perceived pain intensity is enhanced by pain-relevant anxiety, via neural activation in certain brain regions. Ochsner et al. (2006) examined the relationship between individual differences in fear or anxiety sensitivity and the patterns of neural activation thought to underlie pain responses and demonstrated that this relationship is itself associated with activity in particular brain regions. These imaging studies suggest that the anterior cingulate cortex (ACC) acts as a central component in the modulation of pain perception. The ACC is a pivotal area that receives various inputs that are associated with the processing of painful stimuli, and activation here is closely associated with the perceived degree of pain unpleasantness (Peyron et al., 2000; Phillips et al., 2003a; Rainville et al., 2002).

Sadness is one of the basic human emotions, and it is generally accepted that sadness occurs in response to an aversive experience (Ellsworth and Smith, 1988). Previous neuroimaging studies have investigated neural responses to sadness-inducing emotional stimuli (Habel et al., 2005; Posse et al., 2003) and suggested that amygdala has been implicated. The amygdala is directly involved in autonomic nervous system activation, escape, arousal, and the experience of fear associated with painful stimuli (Bernard et al., 1989; Bernard and Besson, 1990). Importantly, there are the behavioral and subjective relationships between sad or depressed mood states and somatic complaints, including pain (Breslau et al., 2003; Carroll and Besson, 1990).
neural responses to painful stimuli and to reveal a possible association between pain perception and the contexts evoked by emotional stimuli. Furthermore, we explored the relationship between those brain regions that are directly associated with affective aspects of pain stimuli and other regions and examined the mechanism by which pain pathways are modulated by emotion.

Methods

Subjects

Fifteen healthy adults (6 female and 9 males, mean age = 25.8 years, range = 20–30 years, all right-handed) participated in the present study. All subjects gave their written informed consent before participation, according to a protocol approved by the ethics committee of Hiroshima University.

Experimental paradigm and stimuli

An intraepidermal stimulation method (Inui et al., 2002a,b) was used to induce minor pain at the superficial skin level. The original method was slightly modified, to provide a higher selectivity for the activation of nociceptors (Inui et al., 2006). We used a stainless steel concentric bipolar needle electrode (Nihon Kohden, Tokyo, Japan) for intraepidermal stimulation. The anode was an outer ring 1.2 mm in diameter, and the cathode was an inner needle that protruded 0.1 mm from the outer ring. This needle electrode permitted the selective stimulation of cutaneous A-delta fibers. The electrical stimuli used were 50 Hz current constant double pulses of 0.5 ms in duration. The electrical stimuli were intended to evoke the feeling of receiving an injection. The needle electrode was exchanged for each subject. The constant current stimulator (SEN-2201; Nihon Kohden, Tokyo, Japan) was located outside the MRI room, and the electrode was connected to the stimulator via a magnet-compatible extension cable.

Each of the subjects rated pain stimulus intensity on a verbal scale from 0 (no pain) to 10 (extremely intense pain) outside the MRI room before imaging was conducted, and a current intensity that corresponded to a rating of 4 (moderate pain) was used during the later imaging phase. Current intensity was determined before recordings were made. We stimulated the left forearm of each subject. Mean stimulus intensity in the imaging study was 0.49±0.15 mA (mean ± SD). The insertion of the needle electrode caused no bleeding or visible damage to the skin of any subject.

We used pictures of faces as emotional stimuli, given that such stimuli have been employed in many previous functional neuroimaging studies that examined neural responses to emotional stimuli (Killgore and Yurgelun-Todd, 2004; Morris et al., 1996; Phillips et al., 1997). We used sad, happy, and neutral facial expressions to induce different emotional contexts, while the subjects were exposed to the pain-inducing stimuli. Eight sad, eight happy, or eight neutral facial expressions displayed by eight different identities (4 females and 4 males) were taken from a standardized series of stimuli (Kamachi et al., 2001) and were presented for 5 s each, with a 1-s interstimulus interval. Each face was randomly used across 60 trials, with a total of 180 trials being conducted. The total experimental duration was 18 min.

For half of the facial stimulus trials (randomly selected), pain stimuli were randomly delivered in 1, 2, or 3 s from the presentation onset of the facial stimulus. For the other half of the face stimuli, no corresponding pain stimuli were delivered. This approach permitted us to avoid producing anticipatory effects. The appearances of the pain stimuli were randomized, with the pain stimuli being delivered on a total of 30 trials in each emotional context. A schematic representation of the experimental design is shown in Fig. 1.

Before fMRI recording were made, all subjects completed the Beck Depression Inventory (BDI) (Beck et al., 1961) (2.1±2.1) and the State–Trait Anxiety Inventory (STAI) (Spielberger, 1983) (STAI-S = 38.7±7.5; STAI-T = 36.3±6.9) to rate levels of depression and anxiety, respectively. During fMRI recording, subjects were instructed to imagine how the person depicted in each image felt, at the point when the image appeared on the screen inside the MRI room. An MR-compatible back projection screen (Silent Vision SV-6011; Avotec, USA) was used to present the facial stimuli.

After the fMRI session, the whole study protocol was replicated outside of the scanner as a behavioral experiment. Each of the subjects rated pain intensity and affective intensity of the facial images on 7-point scales (ranging from +3 to −3). The intensity of pain used here remained the same (a rating of +4 (moderate pain)) as that established initially for each of the subjects during the fMRI session. This was confirmed using subject ratings (i.e., if subjects felt the same intensity of pain as that they initially confirmed, they provided a rating of “0,” and if they felt a much stronger intensity of pain than that they did initially, they provided a rating of “+3”). After the pain ratings were obtained, subjects then provided ratings to confirm the affective intensity of the facial images. The affective intensity of each facial image was rated on a scale that included the following anchor points: “+3” = completely happy, “0” = neutral, and “−3” = completely sad.

fMRI acquisition

The fMRI procedure was performed using a Magnex Eclipse 1.5 T Power Drive 250 (Siemens, Munich, Germany). A time course series of 366 scans was acquired using T2⁎-weighted, gradient echo, echo planar imaging (EPI) sequences. Each volume consisted of 28 slices, with a slice thickness of 4 mm with no gap, and covered the entire cerebral and cerebellar cortices. The time interval between two successive acquisitions of the same image (TR) was 3000 ms, the echo time (TE) was 46 ms, and the flip angle was 90°. The field of view (FOV) was 256 mm, and the matrix size was 64×64, giving voxel

![Fig. 1. Schematic representation of the experimental design. Each sad, happy, or neutral facial expression was presented for 5 s, with an interstimulus interval of 1 s. Each face was randomly used across 60 trials, with a total of 180 trials being performed. Pain stimuli were randomly delivered 1, 2, or 3 s upon onset of half of the facial stimuli (determined at random). Thirty pain trials were performed for each emotional condition.](image-url)
dimensions of 4 mm × 4 mm × 4 mm. Scan acquisition was synchronized to the onset of each trial. After functional scanning, structural scans were acquired using a T1-weighted gradient echo pulse sequence (TR = 2160 ms; TE = 3.93 ms; flip angle = 15°; FOV = 256 mm; voxel dimensions of 1 mm × 1 mm × 1 mm) to facilitate localization.

fMRI analysis

Image processing and statistical analyses were carried out using Statistical Parametric Mapping (SPM5) software (Wellcom Department of Cognitive Neurology, London, UK). The first three volumes of each fMRI run were discarded because the MRI signal was unsteady. Each set of functional volumes was realigned to the first volume. A slice timing correction was performed on the model slice to correct for the sequential sampling of the brain in the slice direction. Volumes were spatially normalized to a standard template based upon the Montreal Neurological Institute (MNI) reference brain, and finally smoothed using an 8-mm FWHM Gaussian kernel. For each subject, task-related activity was identified by convolving a vector of the stimulus onset times with a synthetic hemodynamic response. The general linear model (GLM) was used to examine effects of interest. Six regressors were modeled: Pain during Sad Faces, Pain during Happy Faces, Pain during Neutral Faces, Sad Faces, Happy Faces, and Neutral Faces. We established the duration of only the faces’ regressors as 5 s.

Initially, regions of pain activation common to all subjects were determined using group analysis, according to a random effect model that permitted inferences to the general population. The degree of activation was calculated by averaging across all three emotional conditions. Secondly, a whole-brain ANOVA with face type as a factor (sad, happy, and neutral) was performed to identify differential activity between brain regions. Mask images were identified via an examination of the voxels activated during presentation of all pain stimuli (uncorrected p < 0.001) to confirm whether the ANOVA results were pertinent to brain activity associated with pain stimuli in our study. For voxels exhibiting the maximum main effect, multiple comparisons between faces were made using Bonferroni corrections.

Psychophysiological interaction (PPI) analysis captures the interaction between brain regions in relation to an experimental paradigm (Friston et al., 1997). For example, this approach can capture the way in which activity in one brain region modulates activity in another region by specifically assessing responses to the active task relative to an informative baseline. To undertake PPI analysis, a design matrix was established, which typically contains three columns of variables as follows: (1) A psychophysiological variable that reflects the experimental paradigm. (2) A time series variable representing the time course of the source region. The source region was a 6-mm sphere with a center defined by the peak coordinate of the foregoing analysis. (3) A variable that represents the interaction between (1) and (2). The regression coefficient for the interaction term provides a measure of PPI. In the present context, a significant effect for PPI means that the correlation (or covariance) between the source and the sink region during an emotional pain condition is significantly different from that during another emotional condition. In this regard, PPI analysis assesses differences in functional connectivity between the regions of interest. To perform PPI analyses, the first eigenvariate time series of the 6-mm sphere activated from the previous analyses was extracted. The effect of the interaction term was then studied using the contrast [1 0 0], where the first column represents the interaction term. The extracted individual images were then taken to the second level to perform a random effects analysis, using a one-sample t-test. Regions of interest were determined using the data obtained from voxels activated during presentation of all pain stimuli (uncorrected p < 0.05): the brain map in each case was used as a mask image. The whole-brain ANOVA with face type as a factor (sad, happy, and neutral) revealed that BOLD responses for the ACC during the presentation of pain stimuli were larger for the sad condition than for the happy and neutral conditions (see Results). Initially, we analyzed the functional connections for the ACC, comparing the pain for sad facial images with the happy (A: pain for sad facial image > pain for happy facial image) and neutral images (B: pain for sad facial image > pain for neutral facial image). We then assessed the difference between (A) and (B) ((A) − (B) and (B) − (A)). Finally, we assessed a sadness-specific pain component by comparing the functional connections noted during the sad facial condition with those connection that were averaged across the happy and neutral facial images (pain for sad facial image − 1/2 ((pain for happy facial image) + (pain for neutral facial image))). The statistical threshold for the imaging connections noted during the sad facial condition with those connection that were averaged across the happy and neutral facial images (pain for sad facial image − 1/2 ((pain for happy facial image) + (pain for neutral facial image))). The statistical threshold for the imaging analysis described above was set at an uncorrected p value of 0.001 and at a minimum cluster size of 20 voxels, based on previous pain-related fMRI studies (Bornhövd et al., 2002; Jackson et al., 2006; Ochsnner et al., 2006).

We used dynamic causal modeling (DCM) to examine the directional influence between the brain regions that were detected using the PPI analysis (Friston et al., 2003). DCM is a nonlinear systems identification procedure that uses Bayesian estimates of parameters to make inferences about effective connectivity between brain regions, as well as how this connectivity is affected by experimental conditions. BOLD signal time courses were extracted from a 6-mm sphere centered on subject-specific maxima located within 10 mm for each area detected by the PPI analysis. Subjects who did not demonstrate activation in these regions were excluded from the DCM analysis. DCM connection parameters (“connection strengths”) were obtained for the following effects, for each subject: (1) the direct influence of stimuli on regional activity, (2) the intrinsic connections between regions, and (3) the changes in the intrinsic connectivity between regions induced by the experimental design (or modulatory effects) (Friston et al., 2003). One-sample t-tests were performed on the connection parameters.

Finally, we examined the correlations between the brain regions involved in modulating pain within the context of sadness and the sadness-specific emotional and pain rating scores. We subtracted the

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Sad (mean ± SD)</th>
<th>Neutral (mean ± SD)</th>
<th>Happy (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postscan emotional</td>
<td>−1.66 ± 0.29</td>
<td>−0.08 ± 0.11</td>
<td>1.94 ± 0.30</td>
</tr>
<tr>
<td>rating (ranging from 3 to −3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postscan pain</td>
<td>0.19 ± 0.70</td>
<td>−0.09 ± 0.67</td>
<td>−0.22 ± 0.52</td>
</tr>
<tr>
<td>rating (ranging from 3 to −3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Pain-related regions</th>
<th>Cluster extent</th>
<th>L/R</th>
<th>MNI coordinates</th>
<th>z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate cortex</td>
<td>142 R</td>
<td>−2</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>Anterior insula</td>
<td>139 R</td>
<td>40</td>
<td>−12</td>
<td>8</td>
</tr>
<tr>
<td>Anterior insula</td>
<td>226 L</td>
<td>−38</td>
<td>−12</td>
<td>6</td>
</tr>
<tr>
<td>Thalamus</td>
<td>119 R</td>
<td>10</td>
<td>−16</td>
<td>10</td>
</tr>
<tr>
<td>Thalamus</td>
<td>312 L</td>
<td>−8</td>
<td>−20</td>
<td>0</td>
</tr>
<tr>
<td>Amygdala</td>
<td>38 R</td>
<td>30</td>
<td>−4</td>
<td>14</td>
</tr>
<tr>
<td>Second somatosensory area</td>
<td>302 R</td>
<td>60</td>
<td>−20</td>
<td>20</td>
</tr>
<tr>
<td>Second somatosensory area</td>
<td>266 L</td>
<td>−60</td>
<td>−20</td>
<td>12</td>
</tr>
<tr>
<td>Dorsolateral prefrontal cortex</td>
<td>21 R</td>
<td>34</td>
<td>52</td>
<td>24</td>
</tr>
</tbody>
</table>
rating scores in the happy condition from those in the sad emotional condition to derive sadness-specific factors as emotional valence differences. Brain regions of interest (for sadness-specific brain activity) were determined using data obtained from a 10-mm sphere centered on the brain area for which a significant main effect of emotional condition was noted (ACC \( x = -4, y = 12, z = 26 \)). In addition, the right amygdala \( x = 24, y = -2, z = -12 \) was functionally connected with the ACC during pain experienced in the sad emotional context (see results of the PPI analysis), and regions of interest were also drawn on a 10-mm sphere centered on the right amygdala. These brain activities were extracted as the contrast of sad with pain vs. happy with pain to maintain consistency between variables. We then conducted a simple regression analysis (uncorrected \( p < 0.05 \)) to examine whether the sadness-specific emotional and pain rating scores were related to activity patterns associated with the sadness-specific brain regions. Parameter estimates for these regions were extracted from the peak activation coordinates. Pearson correlation coefficients were calculated using SPSS (Chicago, IL) for Windows (release 12.0 for PC). The statistical significance level was set at \( p < 0.05 \).

Fig. 2. (A) Brain areas activated by pain stimuli for all 3 emotional conditions. (B) Effects of emotional faces on pain processing. The ACC \( x = -4, y = 12, z = 26 \) showed significant activation (uncorrected \( p < 0.001 \)). Brain regions elicited by the pain stimuli are marked in yellow. The ACC showed a significant main effect of condition (marked in green). (C) Activation of the statistical peak voxel (ANOVA) at the ACC. The bars represent the activation of each condition, and error bars show the standard error of the mean. (D) A psychophysiological interaction analysis (PPI) was conducted to test the association of other brain areas with the ACC. (E) The graph showing parameter estimate in peak coordinate as the difference of connectivity strength in 3 emotions. ACC activity covaried with that of the right amygdala. ACC: anterior cingulate cortex, Ins: insula, SII: second somatosensory area, Tha: thalamus, Amy: amygdala.
Results

Behavioral results

An examination of postscanning emotional intensity ratings of the facial images shows that subjects reported comparably intense affective estimates for the images that evoked sadness and happiness (Table 1). An ANOVA revealed a significant main effect of emotion $F(2, 28) = 849.5$, $p < 0.0001$, and a post hoc Bonferroni multiple comparison test revealed that the sad facial image received significantly lower ratings than the neutral facial image ($p < 0.05$) and that the happy facial image was also rated as significantly more intense than the neutral facial image ($p < 0.05$).

Importantly, subjects reported different pain intensities across the emotional context conditions (see Table 1). An ANOVA revealed a significant main effect of emotion, $F(2, 28) = 8.42$, $p < 0.005$, and a post hoc Bonferroni test revealed that pain intensities in the sad emotional context condition were significantly higher than in both the neutral ($p < 0.05$) and happy conditions ($p < 0.05$). There was no significant difference in pain intensity between the happy and neutral conditions ($p = 0.33$).

Brain regions involved in pain perception

The BOLD signal elicited by the pain stimuli was first assessed by contrasting the hemodynamic response to the pain stimuli accompanied by facial images with that elicited by facial images alone for all of the facial images combined. Significant changes in signal intensity were detected in a number of brain regions related to pain perception (Table 2 and Fig. 2A), including the ACC, insula, thalamus, amygdala, second somatosensory area (SII), and the dorsolateral prefrontal cortex.

<table>
<thead>
<tr>
<th>Path</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAD → Amy</td>
<td>0.052*</td>
</tr>
<tr>
<td>Amy → ACC</td>
<td>0.039*</td>
</tr>
<tr>
<td>ACC → Amy</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

One-sample $t$-test. Depicted are path and means.

SAD, sad-specific pain; Amy, amygdala; ACC, anterior cingulate cortex. * $p < 0.05$.

Pain perception across three different emotional contexts

A one-way ANOVA was performed across the three emotional context conditions to examine possible differences in pain processing across the conditions. The regions that showed significant differences across the conditions are depicted in Fig. 2B. A significant main effect of condition was observed for the ACC ($x = -4, y = 12, z = 26$, size $= 28$, z-score $= 3.95$) (uncorrected $p < 0.001$). As shown in Fig. 2C, the parameter estimates for the ACC were larger for the sad condition than for the happy and neutral conditions (post hoc $p$ values $< 0.05$).

Psychophysiological interaction (PPI) analysis

PPI analyses were performed to assess the functional connections of the ACC [6-mm sphere centered $x = -4, y = 12, z = 26$] with other areas focusing primarily on the pain with sadness emotional context condition. No regions showed the difference between functional connection comparing the pain for sad facial images with the happy (A) and that comparing the pain for sad facial images with the neutral (B) (A, B: see PPI analysis, Methods), and we found essentially the same functional connectivities in the happy and neutral conditions. The PPI analysis for the sad-specific pain component (pain with sad facial image $- 1/2 (($pain with happy facial image $) + ($pain with neutral facial image $)))$ revealed that the activity observed during the sad emotional context condition was accompanied by increased functional interaction with the right amygdala ($x = 24, y = -2, z = -12$, size $= 20$, z-score $= 3.63$) (Fig. 2D). ACC activity in the sadness-specific pain was associated with amygdala activity.

DCM results

We identified two regions [ACC [$x = -4, y = 12, z = 26$], right amygdala [$x = 24, y = -2, z = -12$]] via PPI analysis and further analyzed the effective connectivity between these brain regions using DCM. Four subjects who did not show activation in these two regions were excluded from the DCM analysis. The DCM model built here is illustrated in Fig. 3, given that previous studies have reported that the amygdala has an important direct influence on the emotional dimension of pain (Gauriau and Bernard, 2004; Ikeda et al., 2007; Neugebauer et al., 2004; Rhudy and Meagher, 2001) and that the amygdala and ACC are closely connected (Anand et al., 2005; Gao et al., 2004; Mobascher et al., 2009) during the processing of pain stimuli.

The direct influence of pain in the sad context on the amygdala and intrinsic connectivity parameters are displayed in Table 3. The direct input to the amygdala was significantly greater than zero, and intrinsic connections for all modeled directions were significantly greater than zero. We confirmed that pain in the sad emotional context is associated with activation in the amygdala and that the amygdala and ACC are closely connected during the experience of pain under such conditions. There was no relationship between increased functional connectivity and enhanced pain intensity ratings in the sad emotional context.

Fig. 3. Dynamic causal modeling (DCM) neural network. Pain during the sad condition was entered as a driving input directly into the amygdala (Amy). The intrinsic connectivity between Amy and ACC was modeled as bidirectional. ACC: anterior cingulate cortex.
Another DCM model featured the ACC as the brain region exerting a direct influence on pain within the sad context. However, we did not find a significant connection in this model.

**Correlations between emotional/pain intensity rating scores and pain stimuli with specific brain regions**

No regions showed positive and negative correlations with emotional rating scores.

Sadness-specific pain rating scores (sad–happy) were positively correlated with sadness-specific activation (the contrast of sad with pain vs. happy with pain) in the ACC \((x = 2, y = 6, z = 22, \text{size} = 6, r = 0.52)\). No regions showed negative correlations with pain rating scores. Furthermore, there were no correlations between the sadness-specific pain rating scores and brain activation during the presentation of painful stimuli in the happy and neutral emotional contexts.

**Discussion**

We demonstrated that emotional context differences affect subjective pain intensities and neural activation associated with pain stimuli: Subjective pain intensities in the sad context condition were greater than those in the happy and neutral conditions, and ACC activation was greater in the sad condition than in the happy and neutral conditions. We found effective connections between the amygdala and ACC during the experience of pain in the sad condition and direct input from the amygdala when subjects experienced pain in this context. Pain intensity ratings were positively correlated with activation of the ACC in the sad condition. To our knowledge, this is the first fMRI study that has reported a relationship between pain perception and a sad emotional context.

The ACC is an important region for pain perception, at both the cognitive and emotional levels. For example, cognitive distraction (Frankenstein et al., 2001; Petrovic et al., 2000; Valet et al., 2004) may attenuate pain-evoked ACC activity, and Wager et al. (2004) showed that pain-related ACC activity can be reduced via placebo, such that the mere belief that one is receiving an effective analgesic treatment can reduce pain. It has also been reported that the ACC is activated during the anticipation of painful stimuli (Hsieh et al., 1999; Porro et al., 2003). In humans, surgical lesions of the ACC decrease the physical perception of pain (Wilkinson et al., 1999). Thus, the ACC plays a key role in the cognitive aspects of pain perception. The ACC is more directly involved in the evaluation of pain intensity and in the affective evaluation of pain than in the sensory component (Buchel et al., 2002; Rainville et al., 1997). Rainville et al. (1997) and Tolle et al. (1999) found a positive relationship between ACC activity and ratings of pain unpleasantness. Phillips et al. (2003b) have researched neural responses to esophageal sensation during presentation of fearful and neutral faces, and they showed that negative emotional states enhance pain-evoked activity in various limbic regions, including the ACC and insula. It appears that negative emotional states can make individuals more sensitive to pain. We also found greater pain-related ACC activity in the sad condition, and we suggest that the ACC activation we observed reflected the subjective pain ratings that we obtained. In the present study, one might assume that the stronger ACC activity during the processing of painful stimuli in the sad condition merely reflects the effects of facial emotion. However, we used a one-way ANOVA to analyze facial stimuli with face type as a factor (sad, happy, and neutral), using GLM modeling data from the four conditions (Sad Faces, Happy Faces, Neutral Faces, and Pain). Data were analyzed separately for the three emotions and pain context to remove any possible effects of factors other than facial emotion, and no main effect of condition was observed for ACC activation. Furthermore, analysis of the pain stimuli themselves revealed a significant main effect at the ACC. We therefore conclude that the ACC activity during the processing of painful stimuli was indeed modulated by the sad emotional context.

We hypothesized that pain in the sad context would directly influence the amygdala, given that previous studies reported that the amygdala plays an important role in the emotional–affecive dimension of pain, from the viewpoint of anatomical, neurochemical, electrophysiological, and behavioral studies (Gauriau and Bernard, 2004; Ikeda et al., 2007; Ji and Neugebauer, 2007; Neugebauer et al., 2004; Rhudy and Meagher, 2001) as well as in the processing of sad expressions (Habel et al., 2005; Posse et al., 2003). The amygdala contributes to convergence and integration in the sensitivity of pain perception (Maren, 2005; Pare et al., 2004; Shi and Davis, 1999). The intrinsic connections between regions were modeled as bidirectional, in accordance with anatomical and functional evidence demonstrating that the ACC projects to the amygdala (Anand et al., 2005; Gao et al., 2004; Mobascher et al., 2009). Our functional connectivity analysis revealed that the amygdala was directly connected with the ACC during the experience of pain in the sad condition and that afferent nociceptive processing in the amygdala was associated with pain in the sad condition. These data suggest an important role for the amygdala in the modulation of pain perception as a function of emotional contexts, and the ACC is thought to be activated by the amygdala, subsequently strengthening the representations of pain perception. Previous studies have demonstrated effective connectivity between the amygdala and middle ACC (Dhond et al., 2008; Keightley et al., 2003; Mériaux et al., 2006; Mitchell et al., 2008). The amygdala is also known to be interconnected with the ACC during pain processing (Bingel et al., 2006; Tracey and Mantyh, 2007), and both have been implicated in the emotional–affecive dimensions of pain perception rather than the sensory-discriminative dimensions (Wager et al., 2004). Furthermore, some evidence suggests that the amygdala and ACC are involved in the anticipation in pain (Hutchison et al., 1999; Ploghaus et al., 1999; Singer et al., 2004). It has been reported that amygdala activation modulates pain processing by amplifying signals to those brain areas that are directly involved in pain processing (Poremba and Gabriel, 1997). In the auditory system, the amygdala is thought to cause stronger auditory cortex responses associated with emotional stimuli (Morris et al., 2001; Phelps and LeDoux, 2005). This could suggest that the amygdala–ACC interaction observed in our study may be important for the emotional processing of painful stimuli and that the amygdala may uniquely enhance activation of the ACC during pain processing under sad conditions. On the contrary, there was a weaker connectivity from the ACC to the amygdala than vice versa, at least in the present study. We suggest that this connection represents the top-down modulation of pain by the ACC. However, the modulatory effects of the ACC that we observed might have been quite weak because our experiment was an instant emotional paradigm rather than cognitive one caused the top-down modulation such as distraction and attention. We did not find a statistically significant correlation between increases in functional connectivities between the amygdala and the ACC and enhanced pain intensity ratings in the sad emotional context. It is possible that some of the operative factors (e.g., modulation of the ACC or amygdala by other brain regions) are latent, although we could detect functional connectivities during the processing of pain in the sad condition, using our DCM model.

Our behavioral results revealed that the intensity of pain in the sad context was rated as significantly higher than in the neutral and happy contexts, although pain stimulus intensities were set to similar levels for each subject. Our data strongly suggest that sad emotional contexts modulate pain perception. One previous study reported a relationship between experimentally induced sadness and pain sensitivity in healthy subjects, demonstrating enhanced pain perception in response to sustained noxious stimuli (Zelman et al., 1991). It has also been reported that enhanced perception of experimentally evoked pain is found during major depressive episodes (Adler and Gattaz, 1993; Merskey, 1965; Suarez-Roca et al., 2003), suggesting that there might be an overall facilitation in the perception of unpleasant sensations during depressed mood states.
The present study has several limitations. First, although our paradigm was appropriate for detecting neuronal processing of pain stimuli, some uncertainties remain: (1) The display duration for the facial images (5 sec) was longer than that used in previous studies (Killgore and Yurgelun-Todd, 2004; Morris et al., 1996; Phillips et al., 1997), and we must therefore concede the possibility that any context-induced emotional effects might have been attenuated via habituation. (2) Each facial image was presented with a 1-s interstimulus interval, and the emotional context of the last facial image in a sequence might have affected the next one. (3) Although the facial stimuli appear to effectively induce corresponding emotional contexts, their limited relevance to pain perception may lead to fairly small effects on the latter phenomenon. It must be noted that the differences in pain ratings across the three conditions are very small, although they did reach statistical significance. It might therefore be difficult to accept any broad conclusion based on such small behavioral observations. The subjects rated pain intensities outside of the scanner. The behavioral results were retrospective ratings and therefore might have been subject to response biases and memory distortion.

Conclusions

Our neuroimaging results provide evidence that people tend to show higher pain sensitivities when they are feeling sad and that the amygdala and ACC are the brain regions that are particularly active under such conditions. These results provide some insight into negative emotion-induced changes in subjective pain sensitivity and neural activity and the potential importance of emotional context in understanding somatic complaints such as pain.

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References