Extended cortical activations during evaluating successive pain stimuli

Jörn Lötsch, Carmen Walter, Lisa Felden, Christine Preibisch, Ulrike Nöth, Till Martin, Sandra Anti, Ralf Deichmann, and Bruno G. Oertel

1Pharmazentrum Frankfurt/ZAFES, Institute of Clinical Pharmacology, Goethe-University, Frankfurt am Main, Germany and
2Brain Imaging Center, Goethe-University, Frankfurt am Main, Germany

Comparing pain is done in daily life and involves short-term memorizing and attention focusing. This event-related functional magnetic resonance imaging study investigated the short-term brain activations associated with the comparison of pain stimuli using a delayed discrimination paradigm. Fourteen healthy young volunteers compared two successive pain stimuli administered at a 10 s interval to the same location at the nasal mucosa. Fourteen age- and sex-matched subjects received similar pain stimuli without performing the comparison task. With the comparison task, the activations associated with the second pain stimulus were significantly greater than with the first stimulus in the anterior insular cortex and the primary somatosensory area. This was observed on the background of a generally increased stimulus-associated brain activation in the presence of the comparison task that included regions of the pain matrix (insular cortex, primary and secondary somatosensory area, midcingulate cortex, supplemental motor area) and regions associated with attention, decision making, working memory and body recognition (frontal and temporal gyri, inferior parietal lobule, precuneus, lingual cortices). This data provides a cerebral correlate for the role of pain as a biological alerting system that gains the subject's attention and then dominates most other perceptions and activities involving pain-specific and non-pain-specific brain regions.

Keywords: pain; brain

INTRODUCTION

Comparing sensations of pain is an inherent task of any pain experience, readily performed in daily life and frequently included in experimental assessments of pain. When pain is successively perceived, this discriminative task involves short-term memorizing and attention focusing (Rainville et al., 2004). The pain-relevant brain structures contributing to this memory and attention task are incompletely known. Presumably, they include parts of the ‘pain matrix’ comprising cerebral projection areas of pain (e.g. insular, anterior cingulate, primary and secondary somatosensory, premotor and supplementary motor cortices, thalamus (Peyron et al., 2000; Apkarian et al., 2005)).

Studies employing positron emission tomography and functional magnetic resonance imaging (fMRI) (Casey et al., 1996; Derbyshire et al., 1997; Coghill et al., 1999; Tölle TR et al., 1999; Bornhövd et al., 2002; Büchel et al., 2002) provided information about distinct functions and interplay of components of the pain matrix. It is known that the subjective experience of pain is processed by distinct brain regions (Coghill et al., 1999). However, brain representation specifically related to pain comparison tasks was only rarely addressed (Albanese et al., 2007).

We hypothesized that a pain comparison task involves brain areas that exceed those activated following the mere administration of pain stimuli. The memorizing, attention focussing and comparative quantitative evaluation of the stimuli are likely to be reflected in an activity of additional brain areas as compared with the simpler mere perception of pain. This hypothesis was tested by analysing fMRI recordings of brain activations evoked by pairs of subsequent pain stimuli in two groups, only one of which performed a pain comparison task.

EXPERIMENTAL PROCEDURES

Subjects and study design

The difference between brain activations in response to pain stimuli that are not further rated and those in response to pain stimuli that have to be compared with each other was investigated in two separate groups (‘comparing group’ and ‘non-comparing group’), each comprising 14 healthy volunteers matched for age and sex (seven men and seven women per group, age 22–36.6 years, mean age ± standard deviation: comparing group: 29.7 ± 3.3 years, non-comparing group: 25.3 ± 2.6 years). This case number is sufficient (Desmond and Glover, 2002; Thirion et al., 2007) for the intended group comparisons. It should be noted that the number
of subjects would not be sufficient for additional sex comparisons, which, however, were not in the focus. The independent-groups design was preferred to a randomized cross-over design because in preliminary studies during the study set-up we had found it very difficult to suppress the deliberate comparison of pain stimuli once this task had been done in the experiment.

Using a delayed discrimination paradigm (Rainville et al., 2004), 50 pairs of painful stimuli of different intensities were administered at an interval within pairs of 10 s and between pairs of 20 s, i.e. the stimulation design was [stimulus #1, 10 s interval, stimulus #2, 20 s interval], _i_ = 1, ..., 50. Four seconds after the second stimulus (Bornhövd et al., 2002; Büchel et al., 2002) subjects were visually prompted to press one of three buttons. While the first group (comparing group) was instructed to compare the painfulness of the second stimulus of each pair to that of the first one by pressing one of three different buttons (left button = stimulus 1 > stimulus 2, middle: 1 < 2, right: 1 = 2), the second group (non-comparing group) was just prompted to press a particular button without requiring any judgment of the preceding pain stimuli.

The number of left, middle and right button presses in the comparing group were 19 ± 4, 19 ± 6 and 12 ± 5 (mean ± standard deviation), respectively. The subjects in the non-comparing group were instructed to press a particular button as often as this button had been pressed on average in the comparing group to avoid that an uneven distribution of button presses might contribute to the differences between both groups. The time between the visual request and the actual button press was 0.91 ± 0.44 s.

The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty of the Goethe-University. All participants had given informed written consent and their state of health was assessed by anamnesis and a short medical examination. Medications were prohibited for one week and alcohol for 24 h before the actual experiments, which started between 9 a.m. and 2 p.m. In a separate training session, subjects received 30 pain stimuli.

### Pain stimulation

Short stinging pain was evoked at the nasal mucosa by local administration of pulses of gaseous CO₂ into the subject’s right nostril (Kobal, 1981, 1985). They excite nociceptors probably via TRPV1 (Reeh and Kress, 2001), TRPA1 (Wang et al., 2010). It is likely that in addition other proton sensitive ion channels (e.g. acid sensing ion channels (Ugawa et al., 1998) or proton receptors (e.g. proton sensing G-protein coupled receptors (Huang et al., 2007)) are involved, which are expressed at nociceptive nerve endings and play a key role in pain perception (Schaible et al., 2006). The CO₂ stimuli are known to activate trigeminal A<sub>δ</sub>-fibres, with co-activation of C-fibres (Steen et al., 1992). Their nociceptive specificity is supported by showing in a magnetoencephalography experiment that they evoke cortical potentials generated in the pain relevant secondary somatosensory area (Huttunen et al., 1986). The pain model used is well established (Kobal et al., 1990; Lötsch J et al., 1998; Lötsch J et al., 2006) including fMRI assessments (Small et al., 2005; Oertel et al., 2008).

Concomitant alteration of mechanical or thermal conditions at the mucosa was avoided by embedding the CO₂ pulses (duration 300 ms) in a constantly flowing air stream (8 l/min) with controlled temperature and humidity (36.5 °C, 80% relative humidity). This stream was led to the right nasal cavity via a Teflon tube (outer diameter 1.5 mm). A special device (olfactometer OM/2, Burghart Instruments, Wedel, Germany) precisely controlled the concentration and duration of the rectangular (rise time ~50 ms) CO₂ stimuli (Kobal, 1985).

During the actual experiments, subjects received 50 pairs of stimuli. To keep habituation and adaptation low (Hummel et al., 1994), long intervals (interstimulus interval of 10 s within the stimulus pair and of 20 s between pairs) were applied. One stimulus (either the first or the second) was always at a CO₂ concentration of 65% v/v, the other stimulus had a CO₂ concentration ranging from 55% v/v CO₂ to 75% v/v CO₂, in 0.5% v/v CO₂ steps. The difference was randomly below and above the just noticeable difference (Hummel et al., 2003) making comparison difficult. However, pain stimuli were always well above the pain threshold (Hummel et al., 2003). On average, the first stimulus had a CO₂ concentration of 65.4 ± 3.8% v/v and the second stimulus a concentration of 64.6 ± 4.1% v/v (t-test of stimuli 1 versus 2 with respect to the CO₂ concentrations: P = 0.29).

### Image recording

With an event-related design (Friston et al., 1998), the blood-oxygenation level dependent (BOLD) (Ogawa et al., 1990) response to the pain stimuli was recorded on a 3 T magnetic resonance (Bloch, 1946; Purcell et al., 1946) head scanner (Siemens Magnetom Allegra, Siemens Medical Solutions, Erlangen, Germany) equipped with a combined single channel transmit and 4-channel receive head coil. The subject’s head was immobilized with foam pads. For the fMRI study, a T₁*-weighted gradient echo (GE) echo planar imaging (EPI) (Mansfeld, 1977) sequence was used in combination with parallel imaging (GRAPPA) (Griswold et al., 2002), acceleration factor of 2. Imaging parameters were: repetition time (TR) 1530 ms, echo time (TE) 30 ms, flip angle 90°, matrix size 64 × 64, in-plane resolution 3 × 3 mm<sup>2</sup>, 1090 imaging volumes, 29 slices (axial with 30° tilt towards coronal orientation, slice thickness 3 mm, inter-slice gap 1.2 mm). In addition, magnetic field mapping was done for subsequent offline correction of distortions in the EPI images resulting from inhomogeneities of the static magnetic field B₀ (Andersson et al., 2001; Hutton et al., 2002). High resolution (voxel size 1 × 1 × 1 mm<sup>3</sup>) T₁-weighted
anatomical images were acquired for each subject using a three-dimensional magnetization-prepared rapid GE (MP-RAGE) sequence.

Data analysis
The individual EPI volumes were corrected for slice timing, realigned to the first volume (Friston et al., 1995) and unwarped (statistical parametric mapping software SPM8, Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). The high-resolution $T_1$-weighted anatomical image was co-registered to the mean EPI volume, segmented and normalized using 4th-degree B-spline interpolation. The resulting spatial normalization parameters were applied to the EPI volumes, which were subsequently smoothed by convolution with an isotropic 8 mm full-width-at-half-maximum Gaussian kernel.

The fMRI data was analysed by applying a general linear model (Friston et al., 1995). The pain stimuli were modelled as separate delta functions convolved with the canonical hemodynamic response function as implemented in SPM8. The delta functions representing the stimuli were additionally modulated by the CO$_2$ concentration of the respective stimuli. The visual requests for stimulus rating and the subsequent button-press, recorded by the ‘Presentation’ software (Neurobehavioral Systems, Albany, CA, USA), were modelled within the design matrix but omitted from second level analysis. Furthermore, the six rotational and translational parameters from the rigid body transformation, obtained during image realignment and unwarping, were modelled as covariates of no interest. The serial autocorrelation of the BOLD time series was modelled using a first-order autoregressive model. Low-frequency fluctuations of the MR signal were removed with a high-pass filter at 128 Hz. Voxelwise regression coefficients for all regressors were estimated using least squares fitting. Effects were tested with linear contrasts of the parameter estimates, resulting in $t$-statistics for every voxel. The contrast images of the pain stimuli (#1 and #2) were then included in a second level group analysis.

Second-level processing used a $2 \times 2$ flexible factorial design and subsequent contrast analysis (Gläser and Gitelman, 2008). The factor ‘subject’ was additionally included in the factor matrix but is not reported. The factor matrix further included the factors ‘group’ and ‘pain stimulus number’. The design was applied to analyse (i) whether the CO$_2$ stimulus-specific activation in the comparing group differed from the non-comparing group due to the attention focus and memorizing task (main effect ‘group’), (ii) whether activation levels differed among the first and second stimuli (main effect ‘pain stimulus’) and (iii) whether the differences were group specific (interaction ‘group’ by ‘pain stimulus’) because comparison was only requested in the comparing task group. In a separate analysis (iv), the equality of the activations after the first stimuli between the comparing and the non-comparing group was verified by means of two-sample $t$-tests, using contrasts $1 - 1$ and $-1$ $1$ for greater activations in the comparing or non-comparing group, respectively.

The resulting parametrical maps of $T$ statistics were interpreted regarding the probabilistic behaviour of Gaussian random fields (Worsley, 1994). Only results that were significant at the Family Wise Error (FWE) (Loring et al., 2002) corrected level at $P < 0.01$ are reported. The localization of brain activation was aided by the Anatomical Automatic Labelling toolbox (Tzourio-Mazoyer et al., 2002). Significant peak activations are reported as Montreal Neurological Institute (MNI) coordinates specifying the distance from the anterior commissure in $x$ (right to left), $y$ (anterior to posterior), and $z$ (top to bottom) directions.

RESULTS
As reported in a previous study (Albanese et al., 2007), subjects described the noxious stimuli as painful but tolerable. The subjects of the comparing group correctly rated stimulus 1 > stimulus 2, 1 < 2 or 1 = 2 in 46 ± 1.6% of the stimulus pairs [95% confidence interval (CI) 42.6–49.3%, one-sample $t$-test against the chance of 33.3%: $P < 0.001$]. The first stimulus was correctly identified as more intense in 45.1 ± 2.1% of the pairs, the second stimulus was correctly identified as more intense in 49.4 ± 3.2% ($P < 0.001$). However, comparative judgement of the stimulus was false in more than half the pairs, which shows the intended difficulty of the comparison task. Subjects rated the second stimulus as weaker than the first one, more intense or similar in 37.4 ± 1.7%, 38.7 ± 2.2% and 23.9 ± 2.1% of the stimulus pairs, respectively.

The first stimulus yielded similar activations in the comparing and the non-comparing groups as indicated by the absence of a significant difference in activation between groups (two-sample $t$-test). The second stimulus evoked stronger activations than the first, especially when a comparison task was involved. The factorial analysis of the $2 \times 2$ matrix (‘group’ × ‘stimulus number’) revealed several differences between the comparing group and the non-comparing group suggesting marked activations related to the comparison task. Brain activation levels during the experiments were significantly higher in the comparing group (Figure 1; SPM contrast 1 1 $-1$ $-1$ for the analysis applied to stimulus 1 and 2 in the comparing group and stimulus 1 and 2 in the non-comparing group, respectively, denoting the main effect of ‘group’; the opposite contrast $-1$ $-1$ 1 1 revealed no significant differences). This was observed in several regions (Table 1) across the brain including those of the pain matrix (left and right anterior insular cortices, left primary and secondary somatosensory area, the midcingulate cortex, the left and right supplemental motor area, and the right middle frontal gyrus). Pronounced activation differences were additionally found outside the pain matrix (the inferior and superior frontal gyrus, the inferior parietal lobule, the
precentral gyrus, the middle- and superior temporal gyrus, the precuneus and the lingual cortices).

Activations associated with the second stimulus were generally higher than those associated with the first (Figure 2 and Table 2; contrast \(-1 \times 1 -1\) denoting the main effect of ‘pain stimulus’; the opposite contrast \(1 -1 \times 1\) revealed no significant differences). This effect was clearly related to the stimulus comparison task in a subset of brain regions (Figure 3; interaction ‘pain stimulus’ by ‘group’, contrast \(-1 \times 1\)). Specifically, higher brain activation following the second stimulus in the comparing group (contrast \(-1 \times 1\); Table 3) was observed mainly in the left and right anterior insular cortex (peak MNI coordinates: \(x = -36, y = 17, z = -2\) mm and \(x = 39, y = 23, z = -5\) mm, respectively). In addition, similar significant activation differences were seen contra-laterally to the pain stimulation side in the left postcentral gyrus corresponding to the primary somatosensory area (peak MNI coordinates: \(x = -39, y = -34, z = 43\) mm).

DISCUSSION

Subjects who compared pain stimuli had a generally increased responsiveness in the major part of the so-called ‘pain matrix’ (Peyron et al., 2000; Bingel et al., 2004; Apkarian et al., 2005) including the insular cortex, primary and secondary somatosensory area, midcingulate cortex and supplemental motor area. Outside of this ‘pain matrix’, activations were increased in brain regions that can be characterized as being associated with attention, decision-making, working memory or body recognition.

By exceeding the ‘pain matrix’, the extension of these activations already suggests that they are not pain-specific. A similar observation was reported for subjects receiving non-painful and painful mechanical stimuli (Schoedel et al., 2008). Moreover, increasing evidence casts doubt on the pain-specificity of the ‘pain matrix’. It has been proposed to represent a sensory matrix (Mouraux and Iannetti, 2009) or a salience matrix (Iannetti and Mouraux, 2010) that comprises brain activations evoked by sensory stimulation in general, and not exclusively painful. The ‘pain matrix’ is also activated by non-painful stimuli (Mouraux and Iannetti, 2009). Using the same type of stimuli, we recently showed that the operculo-insular cortex, especially SII and the posterior insular cortex, are more pain specific (Oertel et al., 2011), which agrees with results using other noxious stimuli or assessments in patients suffering particular central pain syndromes (Peyron et al., 2002; Baumgartner et al., 2010; Garcia-Larrea et al., 2010). The present findings further support observations that pain stimuli are associated with complex and not only pain specific activations in the brain.

Fig. 1 Main effect of ‘group’, i.e. the presence or absence of a comparison task of two pain stimuli, on the brain activations associated with two successive pain stimuli (contrast \(1 \times 1 -1\) in the succession of comparing group, stimulus 1 and 2 and non-comparing group, stimulus 1 and 2, respectively). The localizations of differences in brain activations (left side, brain surface) due to the comparison task are superimposed upon axial slices of the canonical MR template implemented in SPM8 (right side). The significance at voxel level is colour coded from dark red to yellow with increasing t-values (Table 1). Voxels are shown at a threshold of \(P < 0.01\) (FWE-corrected; \(t > 6.67\)).
Table 1 Clusters of brain regions, which were activated more when pain stimuli were connected with an attention/memory task than just administered without requiring any action from the subject (main effect of ‘group’, i.e. presence or absence of the comparison task, contrast 1 1 −1 −1 in the succession of comparing group, stimulus 1 and 2 and non-comparing group, stimulus 1 and 2, respectively)

<table>
<thead>
<tr>
<th>Brain regions within the cluster</th>
<th>Number of voxel in cluster</th>
<th>Peak coordinates x y z</th>
<th>t-value of peak coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left postcentral/middle temporal/inferior</td>
<td>354</td>
<td>−39 −34 43</td>
<td>13.56</td>
</tr>
<tr>
<td>parietal gyrus</td>
<td></td>
<td>−51 −46 7</td>
<td>7.51</td>
</tr>
<tr>
<td>Left supplementary motor area, right supplementary motor area/midcingulate cortex</td>
<td>177</td>
<td>−5 5 58</td>
<td>11.66</td>
</tr>
<tr>
<td>Right insular cortex/inferior frontal gyrus</td>
<td>169</td>
<td>36 20 −2</td>
<td>10.21</td>
</tr>
<tr>
<td>Right lingual gyrus</td>
<td>133</td>
<td>−12 −82 −5</td>
<td>14.66</td>
</tr>
<tr>
<td>Left precentral/inferior frontal gyrus</td>
<td>118</td>
<td>−45 2 37</td>
<td>11.45</td>
</tr>
<tr>
<td>Right inferior parietal lobule</td>
<td>104</td>
<td>51 −34 49</td>
<td>9.32</td>
</tr>
<tr>
<td>Right inferior frontal/precentral gyrus</td>
<td>87</td>
<td>54 20 19</td>
<td>8.08</td>
</tr>
<tr>
<td>Right inferior parietal lobule</td>
<td>87</td>
<td>54 11 40</td>
<td>8.05</td>
</tr>
<tr>
<td>Left lingual gyrus</td>
<td>60</td>
<td>−18 −82 −8</td>
<td>12.40</td>
</tr>
<tr>
<td>Right middle temporal/superior temporal gyrus</td>
<td>42</td>
<td>57 −49 7</td>
<td>8.21</td>
</tr>
<tr>
<td>Left postcentral gyrus</td>
<td>28</td>
<td>−54 −19 22</td>
<td>11.03</td>
</tr>
<tr>
<td>Right superior parietal lobule</td>
<td>22</td>
<td>−51 −46 52</td>
<td>10.24</td>
</tr>
<tr>
<td>Right superior frontal gyrus</td>
<td>17</td>
<td>−21 −10 58</td>
<td>8.46</td>
</tr>
<tr>
<td>Right middle frontal gyrus</td>
<td>8</td>
<td>36 17 37</td>
<td>7.60</td>
</tr>
<tr>
<td>Precuneus</td>
<td>6</td>
<td>−27 −46 7</td>
<td>7.11</td>
</tr>
<tr>
<td>Right inferior frontal gyrus</td>
<td>5</td>
<td>45 35 16</td>
<td>7.25</td>
</tr>
<tr>
<td>Right superior frontal gyrus</td>
<td>5</td>
<td>66 −37 22</td>
<td>7.23</td>
</tr>
<tr>
<td>Right inferior frontal/middle frontal gyrus</td>
<td>5</td>
<td>42 32 28</td>
<td>6.92</td>
</tr>
</tbody>
</table>

Results reflect a 14/14-subject group comparison analysis. Voxels are given at a threshold of P < 0.01 (FWE-corrected; t > 6.67, Cluster size threshold 5 voxel, coordinates in MNI-space). L and R denote the left and right hemispheres, respectively. Coordinates are reported in the MNI space [mm].

Due to technical restrictions imposed by the equipment for automated stimulus administration, the study design was based on a fixed interval between stimuli. This may impair the independence between the second and first stimuli and advises for caution when interpreting the results. Due to this design, differences between first and second pain stimuli may be less meaningful considering the order-effect on the BOLD signal with two delta functions at a 10 s interval. There are two main effects that should be taken into account:

First, it has been shown that a design based on randomized interstimulus delays may be more efficient than a fixed delay design, and that the accuracy with which the event-related hemodynamic response to different stimuli can be estimated, improves monotonically with decreasing mean interstimulus interval for the randomized delay approach (Dale, 1999). However, at long intervals of ≥20 s, variable and fixed interval designs have a very similar efficiency. Only for shorter mean intervals, the efficiency of the variable interval design increases dramatically while that of fixed interval designs decreases. Still, at an interval of 10 s, the variable design is not substantially more effective than the fixed design (Figure 1 in (Dale, 1999)) and a major improvement becomes evident at intervals <4 s, which were excluded in the present experiment to avoid habituation and adaptation to the pain stimuli (Hummel et al., 1994).

Furthermore, it should be noted that parts of the presented results are similar to results observed with a comparable design where the technical limitation of a fixed interstimulus interval did not apply (Albanese et al., 2007).

Secondly, a bias may arise from the fact that the second stimulus was administered always 10 s after the first one, so the maximum of its hemodynamic response function coincided with the minimum (undershoot) of the hemodynamic response triggered by the first stimulus. Therefore, the response to the second stimulus should actually be weakened by this design. In contrast, there were delays of 20 s between pairs of stimuli, so the maximum of the hemodynamic response triggered by the first of a pair of stimuli always occurred when hemodynamic responses triggered by previous stimuli had already relaxed. Therefore, the design should rather weaken the response to the second stimulus in relation to the response to the first stimulus. In contrast, the opposed effect was observed, thus strengthening the present findings.

The intention was to choose amplitude differences for each pair of stimuli that were below or slightly above the just noticeable level as described in the literature (Hummel et al., 2003). In fact, the subjects perceived the stimuli often as equally intense and were not able to identify the stronger one in more than half of the pairs despite a one third chance. However, although the second stimulus was equally often rated as being stronger or weaker than the first one, it evoked on average stronger brain activations. It is unlikely that this effect is a false positive induced by the experimental design, because there were distinct group differences, in spite of the fact that all experimental conditions (apart from the comparing task) were identical for both groups, including the number of button presses. Nevertheless, more details about pain specific versus comparison task-specific brain activation differences could be analysed in follow-up studies employing broader ranges of pain stimulus intensities.

Among regions displaying task-related activation differences, the right inferior frontal gyrus is known to respond to items that are most relevant for a currently intended task (Hampshire et al., 2009) and has been associated with increased attention towards a painful stimulus (Peyron et al., 1999; Coghill et al., 2001; Brooks et al., 2002; Kurata et al., 2002). The middle/superior frontal gyri have been
suggested to be part of an attention system, additionally comprising the anterior cingulate cortex, the inferior frontal gyrus and the inferior parietal lobule, which helps in alerting the organism to behaviourally relevant stimuli (Symonds et al., 2006) such as pain. With the inferior parietal lobule, the middle frontal gyrus was previously reported as activated during painful stimulations (Peyron et al., 1999; Coghill et al., 2001; Bornhövd et al., 2002). Activations in the inferior parietal lobule were previously found when subjects were paying attention to a thermal stimulus (Peyron et al., 1999). The superior frontal gyrus, also presently more activated in the comparing group, was activated when painful heat stimulation was unexpected (Ploghaus et al., 2000).

Since the middle frontal gyrus is a brain region associated with working memory and attention-related processing (Petrides et al., 1993; Coghill et al., 2001), its present pain-related activations probably reflect working memory and attention-related processing of the stimuli triggered by the required comparison task. Similarly, the observed activation in the precuneus may reflect the suggested role of this region in integrated tasks including memory retrieval (Schmidt et al., 2002) and recognition memory judgments (Henson et al., 1999). The superior temporal gyrus is a region suggested to play an important role in integrating previous actions and successful outcomes into decision-making strategy (Paulus et al., 2005). Decision making under uncertainty has also been assigned to the inferior parietal lobule (Vickery and Jiang, 2009). With respect to pain, activation of the middle temporal gyrus was related to the amount of tactile allodynia in postherpetic neuralgia (Geha et al., 2008).

The effect of stronger activations being evoked by the second stimulus was more pronounced when subjects were required to compare stimulus intensities. This effect was mainly found in the anterior insular cortex and to a lesser degree in the postcentral cortex. Based on previous knowledge, both regions are reasonable localizations for a comparison task of pain requiring pain perception and evaluative judgment. The anterior insula is involved in the cognitive evaluation of sensory pain intensity (Kalisch et al., 2006) and in circuits processing the affective dimension of pain (Saper, 1982; Fulbright et al., 2001). These are components likely to be addressed in pain comparisons. Although this seems to support a pain-specificity of the anterior insular cortex, due to the study design and analysis the pain component of the study was subtracted out of the linear contrast, leaving the functional interpretation of the data
activations after the second stimulus were observed in the basal ganglia (nucleus caudatus, putamen, pallidum). These regions process non-noxious and noxious somatosensory information (Chudler and Dong, 1995) apart from their importance in motor functions. They may be involved in processing the sensory-discriminative, affective and cognitive dimensions of pain and in the modulation and sensory gating of nociceptive information to motor areas (Chudler and Dong, 1995). In addition to this interpretation linked to pain processing, the effect of stronger activation of basal ganglia being induced by the second rather than the first stimulus might simply reflect their function in coordinating the imminent motor activity required to press a button 4 s after the second stimulus, which the subjects of both groups knew to be requested after the second stimulus.

In the study of (Albanese et al., 2007) more diverse stimuli than those employed in this study were applied (47.5–53°C with differences in intensity of the two stimulus presentations ranging from 0.1°C to 1.5°C). The activations were separated into those related to the administration of the stimuli, the memory tasks or the discrimination task. The activations associated with the stimuli were observed in SI, SII, the anterior insular cortex, the posterior parietal cortex and the anterior cingulate cortex. These are key regions of the pain matrix, especially the operculo-insular cortex (Peyron et al., 2002) has been repeatedly shown to specifically respond to pain while other parts of the pain matrix may be activated by non-painful sensory input and have therefore been suggested to represent a sensory brain matrix that includes pain but is not dedicated to it (Mouraux and Iannetti, 2009). The presently observed activations included these regions, i.e. the insular and cingulate cortex, postcentral gyrus and secondary somatosensory area. However, activations in the anterior insular cortex, as observed with the present design and analysis, pointed at an involvement in the salience network rather than specifically in pain. Further activations are likely to be related more to the memory or discrimination tasks than to sensory perception of the stimuli. In the cited study (Albanese et al., 2007), memory-associated regions were reported to be SI, inferior and posterior parietal cortex. Presently, the inferior parietal lobule was also found, however, the main differences related to the comparing task were seen in SI and in the insular cortex but not in SII (Albanese et al., 2007).

The comparison task leads to increased pain-associated activations in brain regions known as cortical representations of attention, memory, judgment and body recognition, which significantly exceeds the pain matrix. These extended activations served as a background for activations in the insula and primary somatosensory cortex (Albanese et al., 2007). The present results further support that pain is associated with complex cerebral processing involving pain specific and non-pain-specific brain areas.

### Table 2

<table>
<thead>
<tr>
<th>Brain regions within the cluster</th>
<th>Number of voxel in cluster</th>
<th>Peak coordinates</th>
<th>t-value of peak coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right insular cortex/inferior frontal gyrus</td>
<td>266</td>
<td>36 23 7</td>
<td>13.05</td>
</tr>
<tr>
<td>Right insular cortex/inferior frontal gyrus</td>
<td>140</td>
<td>-36 20 1</td>
<td>11.76</td>
</tr>
<tr>
<td>Left pallidum</td>
<td>17</td>
<td>-15 5 1</td>
<td>7.66</td>
</tr>
<tr>
<td>Right inferior frontal gyrus</td>
<td>16</td>
<td>45 44 -5</td>
<td>7.46</td>
</tr>
<tr>
<td>Left postcentral gyrus/inferior parietal lobe</td>
<td>13</td>
<td>-39 -34 43</td>
<td>7.68</td>
</tr>
<tr>
<td>Left thalamus</td>
<td>12</td>
<td>-15 -19 -5</td>
<td>7.72</td>
</tr>
<tr>
<td>Right pallidum</td>
<td>11</td>
<td>15 11 2</td>
<td>7.26</td>
</tr>
<tr>
<td>Left postcentral gyrus</td>
<td>9</td>
<td>-54 -19 22</td>
<td>7.16</td>
</tr>
<tr>
<td>Right superior frontal gyrus</td>
<td>8</td>
<td>6 29 40</td>
<td>7.54</td>
</tr>
<tr>
<td>Right thalamus</td>
<td>6</td>
<td>5 13 7</td>
<td>7.48</td>
</tr>
<tr>
<td>Left putamen</td>
<td>6</td>
<td>-24 -4 7</td>
<td>7.35</td>
</tr>
<tr>
<td>Left inferior occipital gyrus</td>
<td>6</td>
<td>-21 -91 -11</td>
<td>7.11</td>
</tr>
<tr>
<td>Right lingual gyrus</td>
<td>5</td>
<td>24 -91 -5</td>
<td>7.78</td>
</tr>
</tbody>
</table>

Results reflect a 14/14-subject group comparison analysis. Voxels are given at a threshold of $P<0.01$ (FWE-corrected; $t>6.67$, Cluster size threshold 5 voxel, coordinates in MNI-space). L and R denote the left and right hemispheres, respectively. Coordinates are reported in the MNI space [mm].

with the influence of pain removed. This is more consistent with either a non-specific rating and would support a role of the anterior insula in a salience or comparative evaluation network. Moreover, the stimulus pair paradigm of the present study induced an anticipation of pain that agrees with the role of the anterior insula in learning (Koyama et al., 2005), even without sensory stimulation (Porro et al., 2002; Kong et al., 2006; Helmchen et al., 2008), and may thus reflect cortical processes of anticipation of sensory input including pain (Ploghaus et al., 1999; Porro et al., 2002).

An additional region required for comparing a pain stimulus with a preceding one is the postcentral gyrus. This region represents the primary somatosensory area (S1) reflecting the somatic localization of the sensory experience, and its localization corresponds to the reported cortical representation of the CO2 trigeminal stimulus (Bensafi et al., 2008). Exclusive identification of the anterior insular and the post-central cortical areas as regions related to comparing a pain stimulus with a preceding one agrees with findings associating short-term retention of spatial and intensity aspects of noxious stimuli (Albanese et al., 2007).

Not specifically related to a pain comparison task was the observed effect of stronger activations following the second of a pair of successive pain stimuli.
Brain activation when comparing successive pain stimuli

Fig. 3 Brain regions, in which the comparison task was specifically associated with larger activations following the second as compared to the first pain stimulus (interaction ‘group’, i.e. presence or absence of comparison task, by ‘pain stimulus number’ in a 2 × 2, group × stimulus number, flexible factorial design, contrast −1 1 −1). The localization of differences in brain activations (left side, brain surface) due to the stimulus number in the presence of a comparison task are superimposed upon axial slices of the canonical MR template implemented in SPM8 (middle). The significance at voxel level is colour coded from dark red to yellow with increasing intensity, and stimulus awareness within the anterior cingulate cortex: a single-trial fMRI study. Cerebral Cortex, 14, 1340–5.


Table 3 Clusters of brain regions in which the comparison task was specifically associated with larger activations following the second as compared to the first of two pain stimuli successively administered to the right nasal mucosa at an interval of 10 s (interaction ‘group’, i.e. presence or absence of comparison task, by ‘pain stimulus number’ in a 2 × 2, group × stimulus number, flexible factorial design, contrast −1 1 −1). The localizations of differences in brain activations (left side, brain surface) due to the stimulus number in the presence of a comparison task are superimposed upon axial slices of the canonical MR template implemented in SPM8 (middle). The significance at voxel level is colour coded from dark red to yellow with increasing intensity, and stimulus awareness within the anterior cingulate cortex: a single-trial fMRI study. Cerebral Cortex, 14, 1340–5.


REFERENCES


