Regional Intensive and Temporal Patterns of Functional MRI Activation Distinguishing Noxious and Innocuous Contact Heat

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Submitted 28 September 2004; accepted in final form 9 November 2004

Moulton, E. A., M. L. Keaser, R. P. Gullapalli, and J. D. Greenspan. Regional intensive and temporal patterns of functional MRI activation distinguishing noxious and innocuous contact heat. J Neurophysiol 93: 2183–2193, 2005; doi:10.1152/jn.01025.2004. Cortical responses to painful and nonpainful heat were measured using functional magnetic resonance imaging (fMRI) region of interest analysis (ROI) of primary somatosensory cortex (S1), secondary somatosensory cortex (S2), anterior cingulate (ACC), supplementary motor area (SMA), insula, and inferior frontal gyrus (IFG). Previous studies indicated that innocuous and noxious stimuli of different modalities produce responses with different time courses in S1 and S2. The aim of this study was to determine whether temporally distinct nociceptive blood oxygen level–dependent (BOLD) responses are evoked in multiple somatosensory processing cortical areas and whether these responses discriminate small noxious stimulus intensity differences. Thirty-three subjects underwent fMRI scanning while receiving three intensities of thermal stimuli, ranging from innocuous warm (41°C) to 1°C below tolerance, applied to the dorsum of the left foot. Innocuous and noxious responses were distinguishable in contralateral S1, the mid-ACC, and SMA. The peak of the nociceptive response was temporally delayed from the innocuous response peak by 6–8 s. Responses to noxious but not to innocuous stimuli were observed in contralateral posterior insula. Responses to innocuous and noxious stimuli were not statistically different in contralateral S2. In contralateral S1 only, the nociceptive response could differentiate heat stimuli separated by 1°C. These results show that multiple cortical areas have temporally distinguishable innocuous and noxious responses evoked by a painfully hot thermode, 2) the nociceptive processing properties vary across cortical regions, and 3) nociceptive responses in S1 discriminate between painful temperatures at a level unmatched in other cortical areas.

Introduction

Many somesthetic cortical areas receive both innocuous and noxious neural signals. Electrophysiological recordings from primate primary and secondary somatosensory (S1 and S2) cortices have revealed that, although most neurons respond exclusively to innocuous stimuli, a subset responded selectively or differentially to noxious stimuli (Dong et al. 1989; Kenshalo and Isensee 1983; Kenshalo et al. 1988, 2000). Functional neuroimaging studies extend these observations to humans, where both types of stimuli activate the cortical areas S1 and S2 (Bornhovd et al. 2002; Coghill et al. 1999; Davis et al. 1998; Peyron et al. 2000). Given this overlap, it may be difficult to distinguish between innocuous and nociceptive processing with functional neuroimaging data (Davis 2003). Temporally distinguishable responses to innocuous versus noxious stimuli have been reported in primate S1 (Chen et al. 2004; Tommerdahl et al. 1996, 1998) and in human S1 and S2 (Chen et al. 2002a). To our knowledge, no studies have looked beyond these areas for both intensity and temporal differentiation of innocuous and nociceptive responses. Frequently used in pain imaging studies, a noxious thermode can activate warm fibers and mechanoreceptors with contact, as well as heat-sensitive nociceptors (Raja et al. 1999). Regardless of the stimulus modality used, differentiation of innocuous and nociceptive responses is essential for the interpretation of cerebral processing related to pain.

Identifying nociceptive responses permits the explicit measurement of their capacity for encoding nociceptive intensity. Previous studies have relied on the entire response to painful stimuli to identify areas where blood flow or blood oxygen level–dependent (BOLD) signals were graded with stimulus intensity in the painful range (Bornhovd et al. 2002; Buchel et al. 2002; Coghill et al. 1999; Derbyshire et al. 1997; Porro et al. 1998; Ringler et al. 2003). The areas that were significantly related to nociceptive intensity in this manner included S1, S2, anterior cingulate cortex (ACC), and insula, among others. Both the ACC and insula are routinely divided into anterior and middle/posterior portions, each with putatively different functional properties (Bingel et al. 2003; Buchel et al. 2002; Craig et al. 2000; Davis et al. 1997; Derbyshire et al. 1998; Peyron et al. 1999; Vogt et al. 1995). The encoding properties of these areas may be more closely related to nociception by considering only the response selective to nociception.

The current study used innocuous stimuli, stimuli 2°C below pain tolerance (Pain1), and stimuli 1°C below pain tolerance (Pain2) applied to the dorsum of the left foot during functional magnetic resonance imaging (fMRI) scanning. The BOLD signal was analyzed for its ability to distinguish innocuous versus noxious thermally evoked cerebral activation in regions of interest (ROIs) within the pain neuromatrix, including S1, S2, anterior insula (aINS), posterior insula (pINS), mid-ACC, rostral ACC (rACC), SMA, and inferior frontal gyrus (IFG). Additionally, the BOLD responses were tested for nociceptive intensity discriminative properties within a narrow stimulus range (1.0°C).

Methods

Subjects

Sixty healthy subjects were recruited using advertising flyers posted throughout the University of Maryland, Baltimore campus. Subjects

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provided written informed consent to participate in the study and were paid $25/h. Thirty-three subjects (14 male: 19 female) aged 29 ± 7 yr old completed the study. Subjects were excluded on the basis of psychophysical screening (n = 20; criteria listed below), scheduling problems (n = 2), and technical problems with fMRI scanning (n = 5). The University of Maryland Institutional Review Board for the Protection of Human Subjects approved the procedures and protocols for this study.

Thermal stimulation

An MR-compatible Peltier thermal probe with a 2.6-cm² contact surface (TSA-II, medoc Advanced Medical Systems, Ramat Yishai, Israel) was used to apply heat stimuli. During pretesting, a 41°C search stimulus was applied to the dorsum of the subject’s foot to identify areas sensitive to innocuous heat. Six probe-sized (2.6 cm²) warmth-sensitive areas were identified and used as guides for placement of the thermal stimuli, to avoid warmth-insensitive regions during the experiment (Green and Cruz 1998).

The probe was preheated to a target temperature before being placed onto the subject’s foot for 16 s. A pneumatic handle attached to the thermode allowed the experimenter to apply the probe with 300–400 g of consistent pressure. Three target temperatures were selected: one innocuous (41°C) and 2 painfully hot. The painfully hot temperatures were determined separately for each subject based on pain tolerance. Pain tolerance was assessed during a separate test session, using an ascending series of stimuli presented in 1°C steps. Tolerance was defined as the highest temperature a subject accepted without withdrawing from the stimulus. The hottest temperature used in the fMRI sessions was identified as 1°C below pain tolerance (Pain2), and the other as 2°C below tolerance (Pain1). Subjects who described either of these temperatures as nonpainful were excluded (n = 12), as were subjects who rated the thermal intensity of the 2 temperatures within 5 points of each other on a 100-point Visual Analogue Scale (VAS) (n = 1). Subjects that had no sensation of heat with the 41°C stimulus were also excluded (n = 2). Subjects were also excluded if their pain tolerance was below 45°C (n = 3) or above our range of testing (50°C) (n = 2).

Experimental protocol

During the fMRI session, subjects were scanned while each of the 16-s target temperatures were applied 3 times each in random sequence (Fig. 1). Each thermode application period was separated by 38 s. To avoid primary afferent habituation or sensitization, each thermode placement was rotated sequentially according to the warmth maps, effectively lengthening the interstimulus interval for any single stimulated area to 5 min and 8 s.

After each stimulus, subjects were presented with a computerized VAS (DAPSYS, Brian Turnquist, Johns Hopkins University, http://www.dapsys.net) by MR Vision 2000 goggles (Resonance Technologies, Van Nuys, CA) at which time they rated their peak sensation of thermal intensity using an MR-compatible trackball (Fellowes, www.fellowes.com), which controlled a cursor moving along the vertical VAS. The extremes of the VAS were labeled “no warmth” and “most intense pain imaginable,” with a marker for “just painful” located at the lower quarter of the scale. Subjects were instructed to use the range from “no warmth” to “just painful” to rate nonpainful heat, and to use the range from “just painful” to “most intense pain imaginable” to rate painful heat. Ratings were stored as numbers from 0 to 100, with 25 corresponding to “just painful.”

The temperatures used for the Pain1 and Pain2 stimulus levels in the scanner environment were determined on an individual basis: 89% of the ratings of the 41°C stimulus were nonpainful warmth, whereas the prescribed Pain1 and Pain2 temperatures were both consistently painful and rated significantly different from one another [2-tailed paired t-test, t(32) = 6.416, P < 0.001]. The mean Pain1 temperature applied was 46.4°C (±1.1 SD), whereas the mean Pain2 temperature was 47.4°C (±1.1 SD).

Image acquisition

Functional MR scans were carried out using the rectilinear echo planar imaging (EPI) method with a 1.5-T Philips Eclipse scanner (formerly Marconi Medical Systems, Cleveland, OH). A gradient echo single-shot EPI sequence was used to provide 3.2 × 3.2 mm acquired resolution over a 24-cm field-of-view (FOV). These images were zero padded to 128 × 128 pixels to provide a resolution of 1.875 × 1.875 mm. T2*-weighting from this sequence was accomplished with a gradient echo time (TE) of 35 ms. The repetition time (TR) was 2.000 ms, which allowed the cerebrum to be covered using 23 slices, with a slice thickness of 6 mm and no gaps between slices. High-resolution anatomical volumetric scans (4.5 ms TE, 29 ms TR, 92 slices, with a slice thickness of 1.5 mm and a 0.938 × 0.938 mm in-plane resolution over a 24-cm FOV) were acquired for anatomical reference of the functional slices.

Image processing and analysis

Image processing and statistical analysis were performed using AFNI (Cox 1996). The first 4 volumes were removed from the functional scans to allow for signal equilibration. For motion correction, functional time series images were spatially registered to the first of the remaining volumes using the AFNI script 3dvolreg. The mean correction for displacement across individuals was 0.38 (x), 0.63 (y), and 0.28 mm (z). Inspection of the volumes revealed no visible errors in motion correction. Spiking artifacts in any time series that exceeded 2.5 SDs of the overall signal were reduced using the AFNI routine 3dDespike. Time series were temporally smoothed using a moving 3-point weighted (0.15–0.70–0.15) average. To increase the signal to noise ratio and to accommodate interindividual differences in brain morphology, spatial blurring was applied to the images for all time points using a 5-mm full width half-maximum Gaussian blur. Linear, second-order, and third-order trends within the time series were subtracted using the AFNI routines 3dDeconvolve and 3dDeconvolve2.
removed, and voxelwise normalization was achieved by dividing the signal intensity at each time point by the voxel’s mean intensity. If required, the high-resolution anatomical images were manually registered to the functional images to achieve maximum alignment using the AFNI software routine Nudger.

A general linear model (GLM) was used to identify regions in individuals whose signal variation was significantly related to the experimental protocol. Through voxelwise regression of fMR signal time courses, the GLM was used to model temporally discrete responses, or regressors, to each of 4 conditions: each of the 3 levels of thermal stimulation and the rating task. The timing of the experimental paradigm dictated which intervals of the signal corresponded to each condition. To allow for variability and uncertainty of the precise timing of the hemodynamic response, responses were modeled using a box-car that allowed for 4 different lags, from 4 to 10 s. Statistical parametric maps were based on t-statistics of the full GLM. Voxels outside of the brain and those subject to susceptibility artifact were eliminated from further analysis. Monte Carlo simulations were run to estimate the likelihood of detecting false positives over multiple comparisons (3dAlphaSim in AFNI). An individual voxel threshold of \( P < 0.05 \) and a cluster threshold of 7 voxels (in original coordinate space) provided a corrected overall alpha of \( P < 0.05 \). The thresholded activation maps and the anatomical scans were then spatially normalized to fit the human brain atlas of Talairach–Tournoux (Talairach and Tournoux 1988), and the voxels resampled to 1 × 1 × 1 mm.

A group activation map was constructed by performing a conjunction analysis (Friston et al. 1999) to measure the spatial overlap of activation across subjects. Voxels in this group map were accepted if 20 or more of the 33 subjects showed activation per voxel. In addition to this criterion, only clusters of 20 or more contiguous voxels (in Talairach space) were considered further. The locations of active voxel clusters were categorized into different regions of interest (ROIs) based on structural landmarks. If more than one cluster was identified within an ROI, the largest cluster was selected for further analyses.

A priori ROIs included S1 (foot representation), S2, pINS, aINS, rACC, mid-ACC, IFG, and SMA. The foot area of S1 was defined as the paracentral lobule and the portion of the postcentral gyrus extending from the midline of the brain laterally to the beginning of the curve of the incus. S2 was defined as part of the parietal operculum located on the upper bank of the Sylvian fissure and caudal to the postcentral gyrus. The pINS was identified as the part of the insula at the same rostrocaudal level as S2. The aINS consisted of the rest of the insula, rostral to the pINS. The ACC as a whole was identified as the area between the corpus callosum and cingulate sulcus, extending rostrally from the marginal branch of the cingulate sulcus to the cingulate sulcus anterior to the genu. Additionally, the rostral ACC (rACC) was identified as the rostral third of the cingulate gyrus, and the midcingulate ACC (mid-ACC) as the middle third. SMA was marked as the area immediately superior to the cingulate sulcus, and extending from the posterior extent of the superior frontal gyrus to the precentral gyrus. The IFG was marked as the triangular and opercular parts of the inferior frontal gyrus, which included Brodmann Areas (BAs) 44/45/46. A nonsomatosenory ROI was drawn that sampled a portion of right hemisphere V1, consisting of a sphere with a 4-mm radius in the center of BA 17.

The active clusters in the ROIs were applied as masks to the spatially normalized individual subject data sets, such that a corresponding set of voxels could be compared across subjects. For the voxel clusters identified within each ROI, single-trial averages were calculated for each of the 3 different stimulus temperatures for each individual. The BOLD signal time course was averaged over all significantly activated voxels within each ROI cluster, so as to generate a mean ROI signal time course. The ROI single-trial averages were then averaged across all the subjects to create ROI group signal time courses.

For signal analysis of the ROI single-trial averages, the time courses were divided into 4 phases: baseline, early stimulus response, late stimulus response, and the rating-task response. Signal intensities were averaged over 4 time points corresponding to each phase. The baseline phase value was based on the average of the 4 prestimulus baseline points (0–6 s into the cycle). The early and late phase responses were identified by equally dividing the time of stimulus presentation into halves and adjusting for a minimal hemodynamic lag of 4 s (12–18 and 20–26 s). The rating phase response captured the end of the rating period (32–38 s). Note that stimulus level is a separate factor from phase, and each stimulus level has an associated baseline, early, late, and rating phase.

To characterize the responses of each ROI, a 2-way repeated-measures ANOVA with factors of stimulus intensity and cycle phase was performed. The dependent variable was average signal intensity. Innocuous responses, Pain1 responses, nociceptive responses, nociceptive intensity-dependent responses, and rating-related responses were determined as significant through post hoc tests using the following criteria: For an ROI to be classified as responsive to innocuous stimulation, the response must be greater than the baseline for the innocuous stimulus trials \( (P < 0.05) \). An ROI was identified as responsive to the Pain1 temperature if the response was greater than the baseline for the Pain1 trials \( (P < 0.05) \). For an ROI response to be classified as nociceptive, the response during the Pain1 trials must be significantly greater than the response during the innocuous trials \( (P < 0.05) \). ROIs were determined to be involved in nociceptive intensity coding if the higher of the 2 painful temperatures had a significantly greater response \( (P < 0.05) \). An ROI was rating-related if the signal during the rating phase was significantly greater than the baseline phase \( (P < 0.05) \). All post hoc tests were 1-tailed because the hypotheses were unidirectional.

**RESULTS**

Conjunction analysis on fMRI data identified widespread paradigm-related activation across subjects (Fig. 2). Many of the hypothesized pain-related ROIs showed substantial intersubject spatial overlap of activation (Table 1). A control ROI, primary visual cortex (V1), did not show a significant stimulus-related response to any of the temperatures presented, although a significant response to the rating task was observed (Fig. 3).

Innocuous stimuli activates several ROIs of the pain neuromatrix.

ROI-based single-trial average analysis revealed responses to innocuous heat with differing temporal characteristics across brain regions. For contralateral S1, mid-ACC, and SMA, significant transient early phase responses to the innocuous temperature were observed, without a significant late period response (Table 2). These transient innocuous responses peaked 6 s after the onset of the stimulus, and then returned to baseline levels before the stimulus was removed (Fig. 2). Other areas showed significant responses to innocuous stimuli during both the early and late phase, including contralateral S2, contralateral and ipsilateral IFG, and rACC (Table 2). These persistent responses to warmth/contact reached a plateau 6–12 s after stimulus onset (Fig. 2). The innocuous response in contralateral S2, although statistically “persistent,” was visibly similar to the transient early phase responses in contralateral S1, mid-ACC, and SMA (Fig. 2). The persistent warmth/contact response in rACC, although statistically significant, was weak relative to the other ROIs.
Noxious stimuli evoke complex persistent responses across the pain neuromatrix

Significant responses to Pain1 thermal stimuli were observed in contralateral S1, mid-ACC, contralateral S2, contralateral pINS, SMA, rACC, as well as contralateral and ipsilateral IFG (Table 3). For the ROIs with early transient innocuous responses (contralateral S1, mid-ACC, and SMA), the single-trial averages for both the painful temperatures showed rise times similar to the innocuous responses (6–8 s), but the responses were more persistent and complex. Although the Pain1 response in contralateral S2 was not statistically different from the innocuous response, a trend toward a more persistent response was observed (Fig. 2).

Nociceptive and innocuous components extracted from a noxious response

Nociceptive-selective responses were extracted from Pain1 and Pain2 responses. By considering each complex painful response as a compound response to both innocuous somatosensory and nociceptive stimuli, subtraction of the innocuous response from both the Pain1 and the Pain2 responses were taken to represent a selective nociceptive response (Fig. 4).

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**FIG. 2.** Conjunction activation maps and group single-trial averages of region of interest (ROI) activation. Average of a subset of Talairach high-resolution anatomical scans serve as the anatomical underlay. Yellow lines in each brain image denote the a priori boundaries of each ROI. ROIs are characterized as those with late-phase nociceptive responses (A–C), nociceptive responses (D–F), response to the Pain1 stimuli (G, H), and without a heat response (I, J). Dashed lines in the graphs represent the average of the 4 prestimulus baseline points. Values are means + SE.
By comparing innocuous versus Pain1 responses, nociceptive responses were found in contralateral S1, SMA, contralateral IFG, contralateral pINS, and rACC (Table 4). Nociceptive responses in contralateral S1, SMA, and mid-ACC were found only in the late phase (Fig. 5, Table 4). The latency to peak of the nociceptive late phase responses averaged 12.7 s, with a range from 12 to 14 s. In contrast, cIFG, contralateral pINS, and rACC showed significant nociceptive responses in both early and late phases, although the signal was greater during the late phase (Fig. 5). V1 did not show significant responses at any time, when considering these same types of subtracted responses (Fig. 3).

**Contralateral S1 distinguishes small differences in noxious heat**

Of all the ROIs, only contralateral S1 showed a significant difference in response between the 2 noxious stimulus levels. The nociceptive late phase response in S1 showed a clear graded response to the 2 levels of nociceptive stimuli (Fig. 6, Table 4).

**Rating task–related activation**

Regardless of stimulus intensity, most of the ROIs showed significant signal increases during the rating task phase (Table 5). The V1 control ROI also showed a significant signal increase during this phase (Fig. 3A).

**DISCUSSION**

Separately identifiable innocuous and nociceptive responses were observed in contralateral S1, mid-ACC, and SMA. The nociceptive response was distinguishable from the innocuous response in terms of signal amplitude and temporal features.
The contralateral pINS also showed a nociceptive response, but with no significant innocuous response. The rACC and contralateral IFG had both innocuous and nociceptive responses, which were distinguished by amplitude, but not by temporal differences. Contralateral S2 and ipsilateral IFG responses to the Pain1 stimuli were not significantly different from the innocuous responses, thus failing to exhibit a selective nociceptive response. For comparison, the V1 ROI showed a significant response only during the rating task, which was attributed to the presentation of the VAS. Furthermore, this response did not vary according to thermal stimulus intensity.

This study is the first to temporally separate innocuous from noxious fMRI responses arising from the same stimulus, although temporally distinguishable responses to different modalities of innocuous and noxious stimuli have been reported previously (Chen et al. 2002a). In Chen et al., innocuous brushing evoked early responses in S1 and S2 that were longer lasting than the responses observed in the present study. The longer duration was likely a result of the continuous brushing, compared with the warming and the weak pressure of the thermode used here, which would activate warm fibers and slowly adapting mechanoreceptors, as well as transiently activate rapidly adapting mechanoreceptors. The response to the pain-evoking thermode in Chen et al. occurred about 5 s after onset of the innocuous response, consistent with our calcu-

<table>
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Only activation in a priori ROIs is displayed. Talairach coordinates reported for the center of maximum subject overlap. Number of voxels indicates the cluster size common to at least 20 of the 33 subjects in Talairach space. Maximum subject overlap is the greatest number of subjects showing overlapping significant activation for at least one voxel of the cluster.

FIG. 3. Right hemisphere primary visual cortex (V1) ROI responses. A: conjunction map and single-trial averages for each temperature level. Blue circle highlights the portion of V1 sampled for this control ROI. B: subtraction of the innocuous response from the Pain1 and Pain2 responses.
the same oxygen-dependent signal, so this correspondence as was the amplitude of fMRI-measured S1 late nociceptive correlated with human pain intensity (Tommerdahl et al. 1996), tionally, the long latency noxious response in S1 was positively time separation between responses is strikingly similar. Addi-

monkey is not expected to be identical to humans, although the exact timing of the peak responses in the smaller squirrel noxious heat peaked at 6.3 s (Tommerdahl et al. 1998). The peak response 2.5–3.5 s after onset, whereas the response to peak complement the data presented here: flutter elicited a innocuous and noxious responses in S1 (Tommerdahl et al. 1996, 1998). Although flutter (tactile) and noxious heat were innocuous and noxious responses in S1 (Tommerdahl et al. 1996, 1998). Although flutter (tactile) and noxious heat were

innocuous and noxious responses in S1 (Tommerdahl et al. 1996). Using cortical structures found SMA equally driven by pain- and

was positively correlated with human pain intensity (Tommerdahl et al. 1996), as was the amplitude of fMRI-measured S1 late nociceptive component observed here. Optical imaging and fMRI measure the same oxygen-dependent signal, so this correspondence reinforces the evidence for temporal separation of innocuous and noxious responses.

Optical imaging in squirrel monkeys has also distinguished innocuous and noxious responses in S1 (Tommerdahl et al. 1996, 1998). Although flutter (tactile) and noxious heat were presented separately, the interval between the responses’ time to peak complement the data presented here: flutter elicited a peak response 2.5–3.5 s after onset, whereas the response to noxious heat peaked at 6.3 s (Tommerdahl et al. 1998). The exact timing of the peak responses in the smaller squirrel monkey is not expected to be identical to humans, although the time separation between responses is strikingly similar. Additionally, the long latency noxious response in S1 was positively correlated with human pain intensity (Tommerdahl et al. 1996), as was the amplitude of fMRI-measured S1 late nociceptive component observed here. Optical imaging and fMRI measure the same oxygen-dependent signal, so this correspondence reinforces the evidence for temporal separation of innocuous and noxious responses.

Temporally separable innocuous and noxious responses were also observed in mid-ACC. Recordings from microelectrodes and the cortical surface of human ACC have identified neuronal and cortical responses to painful stimuli (Hutchinson 1999; Lenz et al. 1998), whereas EEG recordings have identified a pain-related negative difference potential overlying ACC (Dowman and Schell 1999). Innocuous heat stimuli do not typically activate ACC areas (Becerra et al. 1999; Buchel et al. 2002), although such stimuli can activate ACC when paired with the expectation of pain (Sawamoto et al. 2000) and during the anticipation of pain (Ploghaus et al. 1999; Porro et al. 2003). This early response observed in the present study may also be related to selective attention provoked by a stimulus (Peyron et al. 1999). Previous attention- and pain-related responses have been separately localized in anterior and posterior parts of mid-ACC, respectively (Davis et al. 1997). Our data did not reveal such a spatial distinction in mid-ACC. However, a nociceptive response with only a weak innocuous response was discerned in rACC, clearly distinguishing it from the mid-ACC response.

Although SMA is routinely activated in pain imaging studies, and its responses are similar to those in mid-ACC, it is not typically identified as part of the pain neuromatrix (Becerra et al. 1999; Bornhovd et al. 2002; Coghill et al. 1999; Gelnar et al. 1999; Peyron et al. 2000). Previous studies tend to identify activation on the border of mid-ACC and SMA as mid-ACC activation (Peyron et al. 2000). An fMRI study on midline cortical structures found SMA equally driven by pain- and motor-related conditions (Kwan et al. 2000). Furthermore, an EEG study identified a heat-evoked potential localized to SMA that correlated with pain intensity (Chen et al. 2002b). It should be noted that in the present study, the SMA response had distinctly separate peaks, allowing for temporal separation of activation related to stimulus presentation and the subject’s response.

A previous fMRI study also identified early and late activation in response to thermal stimuli (Becerra et al. 2001). Using

### Table 2. ROIs showing a significant innocuous response

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</tbody>
</table>

Determined using a 2-way repeated-measures ANOVA with factors of temperature (3 levels) and phase (4 levels). *Huynh–Feldt to correct for sphericity violation (Mauchley’s Test, P < 0.05).

### Table 3. ROIs showing a significant response to Pain 1 stimuli

<table>
<thead>
<tr>
<th>Area</th>
<th>Factor</th>
<th>F-Statistic</th>
<th>P</th>
<th>Post hoc</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>cS1</td>
<td>phase</td>
<td>8.278</td>
<td>&lt;0.01*</td>
<td>early&gt;bsl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>mid-ACC</td>
<td>phase</td>
<td>49.643</td>
<td>&lt;0.001*</td>
<td>late&gt;bsl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cS2</td>
<td>phase</td>
<td>9.959</td>
<td>&lt;0.001*</td>
<td>early&gt;bsl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>c-pINS</td>
<td>phase</td>
<td>7.005</td>
<td>&lt;0.01*</td>
<td>early&gt;bsl</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SMA</td>
<td>phase</td>
<td>46.995</td>
<td>&lt;0.001*</td>
<td>late&gt;bsl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>rACC</td>
<td>phase</td>
<td>10.896</td>
<td>&lt;0.001</td>
<td>early&gt;bsl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cIFG</td>
<td>phase</td>
<td>25.113</td>
<td>&lt;0.001*</td>
<td>early&gt;bsl</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>iIFG</td>
<td>phase</td>
<td>31.736</td>
<td>&lt;0.001*</td>
<td>early&gt;bsl</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Determined using a 2-way repeated-measures ANOVA with factors of temperature (3 levels) and phase (4 levels). *Huynh–Feldt to correct for sphericity violation (Mauchley’s Test, P < 0.05).
a 25-s stimulus, early activation was identified using a 12.5-s modeling window, whereas late activation captured the last 12.5 s. Becerra et al. suggested that the late phase might relate more specifically to pain because of the number of classic pain structures with late activation independent of the early phase activation. Although the duration of the stimuli in that study was longer than that used here, the early and late activation of that study may correspond to the innocuous and noxious responses reported here. Becerra et al. identified late activation in S1, insula, and ACC, all of which had nociceptive-selective responses in this study. Early activation was less prominent in pain-related structures than was found in the current study, perhaps the result of the thermode being strapped to the application site, which would reduce the dynamic tactile component of the stimulus.

Despite evidence that pINS is linked to discriminative innocuous thermal sensation (Craig et al. 2000), no response to the warm stimulus was observed in this study. This may be a consequence of the low sensitivity of our analysis to detect a warm response in pINS, as others have found that warm stimuli only weakly activate pINS (Bornhovd et al. 2002). The nociceptive responsiveness found here and in other studies (Bingel et al. 2003; Brooks et al. 2002) is consistent with the idea that this region of insula may correspond to the region in monkeys that receives nociceptive input from the VMpo thalamic nucleus (Craig 2004; Craig et al. 2000).

Responses to innocuous and Pain1 stimuli were not found to be significantly different in contralateral S2. Most imaging studies find S2 nociceptive (Bornhovd et al. 2002; Coghill et al. 1999; Ringler et al. 2003), although it also responds to innocuous stimuli (Krubitser et al. 1995; Peyron et al. 2000). Without an explicit comparison to a matching innocuous response, one cannot distinguish a nociceptive-related response per se in most testing conditions. S2 is often grouped together with pINS, particularly in positron emission tomography studies using large spatial blurs. Including the selectively nociceptive pINS as part of S2 could make the combined region appear to have a distinct nociceptive intensity differentiation (late phase only)

cS1 temp*phase 2.582 <0.05* Pain2-early<Pain1-early NS Pain2-early>Pain1-early NS Pain2-early>Pain1-early NS Pain2-early>Pain1-early NS

TABLE 4. ROIs showing a significant nociceptive response

<table>
<thead>
<tr>
<th>Area</th>
<th>Factor</th>
<th>F-Statistic</th>
<th>P</th>
<th>Post hoc</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMA</td>
<td>temp</td>
<td>4.712</td>
<td>&lt;0.05</td>
<td>Pain1&gt;innoc</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>cIFG</td>
<td>temp</td>
<td>5.482</td>
<td>&lt;0.05*</td>
<td>Pain1&gt;innoc</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>c-pINS</td>
<td>temp</td>
<td>5.117</td>
<td>&lt;0.05*</td>
<td>Pain1&gt;innoc</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>rACC</td>
<td>temp</td>
<td>3.393</td>
<td>&lt;0.05</td>
<td>Pain1&gt;innoc</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>cS1</td>
<td>temp*phase</td>
<td>2.582</td>
<td>&lt;0.05*</td>
<td>Pain1-early=Pain2-early NS Pain1-early&gt;Pain2-early NS Pain1-early&gt;Pain2-early NS Pain1-early&gt;Pain2-early NS</td>
<td></td>
</tr>
<tr>
<td>SMA</td>
<td>temp*phase</td>
<td>2.714</td>
<td>&lt;0.05*</td>
<td>Pain1-early=Pain2-early NS Pain1-early&gt;Pain2-early NS Pain1-early&gt;Pain2-early NS Pain1-early&gt;Pain2-early NS</td>
<td></td>
</tr>
<tr>
<td>mid-ACC</td>
<td>temp*phase</td>
<td>3.060</td>
<td>&lt;0.05*</td>
<td>Pain1-early=Pain2-early NS Pain1-early&gt;Pain2-early NS Pain1-early&gt;Pain2-early NS Pain1-early&gt;Pain2-early NS</td>
<td></td>
</tr>
</tbody>
</table>

Determined using a 2-way repeated measures ANOVA with factors of temperature (3 levels) and phase (4 levels). *Huynh–Feldt to correct for sphericity violation (Mauchley’s Test, P < 0.05).
ceptive response. Differentiation of these adjacent, yet spatially distinct, regions reveals separate functional properties. However, inspection of the S2 single-trial average suggests that it may have nociceptive-selective responses that just missed statistical detection, but are qualitatively similar to those found in S1, mid-ACC, and SMA.

Nociceptive responses were found in IFG, whereas the nearby aINS failed to show a significant nociceptive response. The aINS, as defined here, was limited to the depths of the Sylvian fissure, excluding areas within the adjacent IFG. The rostrolateral border of the aINS of previous studies might have overlapped with the posterior margins of the IFG in the current study, thus blurring their functional characteristics. In the current study, the IFG produced a nociceptive-selective response, with a gradually ascending signal that peaked near the end of the stimulus. The IFG has been suggested to play a role in spatial attention and pain evaluation (Becerra et al. 2001; Coghill et al. 1999; Peyron et al. 1999). Thus gradual activation could reflect either increasing attention to noxious stimuli or transitioning to the impending rating task.

FIG. 5. Subtracted signals of nociceptive encoding ROIs. Traces are splines of the average difference between the innocuous response and the perceptually painful temperatures. Differences were derived for each subject, then averaged across subjects.

FIG. 6. Nociceptive components extracted from contralateral primary somatosensory cortex (S1) responses. Pain2 nociceptive component is significantly larger than the Pain1 nociceptive component in the late phase. Values are means ± SE.
When using a subtractive approach to identify nociceptive responses (as exemplified in Fig. 4), the danger exists that some portion of the nociceptive responses may be subtracted as well (Davis 2003). This potential complication could lead to missing a nociceptive response overshadowed by a much stronger innocuous response—a possibility for the S2 results reported here. Regardless, even with the potential for false negative results with the subtractive method used here, nociceptive responses were detected across many of the expected ROIs. Nearly all ROIs showed significant activation associated with the poststimulus rating period of the trial. The late activation described by Becerra et al. (2001) occurs too soon after stimulus onset to match this rating-related activation. Rating-related activation, when compared with the nociceptive responses, had similar or larger amplitudes in many ROIs. Although we refer to this as a “rating response,” this activation may reflect a stimulus change/offset response, in that stimulus offset is nearly simultaneously with VAS presentation—cuing the response period. The rating-related activation may alternatively be attributable to the subjects’ perceptual perseveration, so as to “maintain representation” of the sensory event while evaluating it to make a report. Tactile imagery can activate somatosensory networks nearly as well as the physical application of the imagined stimulus (Yoo et al. 2003). Our results suggest that these nociceptive-related areas are engaged in some level of sensorimotor integration, self-assessment, and/or retained representation of perception. The gradual progression of the IFG response seems to peak at the beginning of the rating task, suggesting that it may be an intermediary of sensory processing and the motor response. Subsequent studies will be necessary to differentiate among activation arising from stimulus change, poststimulus imagery, subsequent cognitive processing, or motor-related components of VAS manipulation. Significant pain intensity coding response properties were observed only in contralateral S1. The finding that S1 activation varied with nociceptive intensity are consistent with prior studies, although other regions, such as S2, mid-ACC, aINS, and IFG, have also been reported to show intensity-dependent responses (Bornhovd et al. 2002; Buchel et al. 2002; Coghill et al. 1999; Derbyshire et al. 1997; Porro et al. 1998; Ringler et al. 2003; Timmerman et al. 2001). Unlike the other studies, which used a wider stimulus/pain intensity range, this study evaluated response differences between stimuli separated by only 1°C. Thus our study evaluates nociceptive intensity discrimination at a more limited, but higher resolution than that used in previous imaging studies. Our data suggest that the contralateral S1 is capable of finer heat nociceptive intensity discrimination than that of other ROIs. In a related manner, nociceptive neurons in primate contralateral S1 have been shown to increase their firing rate with similarly minute changes in noxious heat (Kenshalo et al. 1988). However, the failure to identify significant differences in other ROIs, like negative results in any fMRI study, must be interpreted with caution.

In conclusion, distinctly different innocuous and noxious heat responses to the same thermode application have been identified in contralateral S1, mid-ACC, and SMA. Nociceptive responses were also found in contralateral IFG, contralateral pINS, and rACC. Of these regions, only contralateral S1 activation varied significantly with nociceptive intensity, supporting a pain intensity coding function for S1 distinct from its innocuous somesthetic response properties. These findings do not rule out a pain intensity coding function for other ROIs, but suggests that pain intensity coding may be of greater resolution in S1 than other nociceptive ROIs.

**ACKNOWLEDGMENTS**

The authors thank C. Cordes, R. Arya, and S. Roys for technical assistance.

**GRANTS**

This work was supported by National Institute of Neurological Disorders and Stroke Grant R01-NS-38493.

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