Temporal and Spatial Dynamics of Human Forebrain Activity During Heat Pain: Analysis by Positron Emission Tomography

KENNETH L. CASEY, THOMAS J. MORROW, JÜRGEN LORENZ, AND SATOSHI MINOSHIMA

Department of Neurology, Department of Physiology, and Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan; and Neurology Research Laboratories, Veteran’s Affairs Medical Center, Ann Arbor, Michigan 48105

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Casey, Kenneth L., Thomas J. Morrow, Jürgen Lorenz, and Satoshi Minoshima. Temporal and spatial dynamics of human forebrain activity during heat pain: analysis by positron emission tomography. J Neurophysiol 85: 951–959, 2001. To learn about the sequence of brain activation patterns during heat pain, we acquired positron emission tomographic (PET) brain scans at different times during repetitive heat stimulation (40 or 50°C, 5-s contact) of each subject’s left forearm. Early scans began at the onset of 60 s of stimulation; late scans began after 40 s of stimulation, which continued throughout the 60-s scan period (total stimulus duration 100 s). Each subject (14 normal, right-handed subjects; 10 male, 4 female; ages 18–42) used a visual analog scale to rate the perceived stimulus intensity (0 = no heat, 7 = pain threshold, 10 = barely tolerable pain) after each scan. The 40°C stimulation received an average intensity rating of 2.19 ± 1.22 (mean ± SD) and the 50°C an average rating of 8.93 ± 1.33. During the scan sessions, subjects did not report a difference between early and late scans. To examine the effect of the duration of stimulation specifically, 8 of these subjects rated the perceived intensity of each of 20 sequential 5-s duration contact heat stimuli (40 or 50°C; 100 s of stimulation). We used a graphical method to detect changes in perceived unpleasantness. There was no difference in perceived intensity or unpleasantness during the 40°C stimulation. However, during 50°C stimulation, perceived unpleasantness increased and subjects perceived the last five, but not the second five, stimuli as more intense than the first five stimuli. These psychophysical changes could be mediated by brain structures with increasing activity from early to late PET scans or that are active only during late scans. These structures include the contralateral M1/S1 cortex, bilateral S2 and mid-insular cortex, contralateral VP thalamus, medial ipsilateral thalamus, and the vermis and paravermis of the cerebellum. Structures that are equally active throughout stimulation (contralateral mid-anterior cingulate and premotor cortex) are less likely to mediate these psychophysical changes. Some cortical, but not subcortical, structures showed significant or borderline activation only during the early scans (ipsilateral premotor cortex, contralateral perigenual anterior cingulate, lateral prefrontal, and anterior insular cortex); they may mediate pain-related attentive or anticipatory functions. Overall, the results reveal that 1) the pattern of brain activation and the perception of heat pain both change during repetitive noxious heat stimulation, 2) cortical activity can be detected before subcortical responses appear, and 3) timing the stimulation with respect to the scan period can, together with psychophysical measurements, identify brain structures that are likely to participate in the perception of pain.

INTRODUCTION

A major purpose of functional brain imaging in humans is to identify the neural structures that mediate different aspects of neurologic function. In functional magnetic resonance imaging (fMRI) and in positron emission tomography (PET) studies, one strategy is to relate changes in the intensity of synthaptically induced regional cerebral blood flow (rCBF) to changes in sensory, motor, or higher cognitive function. This strategy is useful in investigations of the cerebral mechanisms mediating the perception of pain. For example, Rainville et al. used hypnosis to dissociate the perception of pain intensity from the unpleasantness of pain and showed that unpleasantness correlated positively with rCBF increase just posterior to the perigenual cingulate, but not primary somatosensory, cortex (Rainville et al. 1997). In related studies, Derbyshire and colleagues, and more recently Coghill and colleagues, demonstrated that changes in perceived pain intensity show a significant positive correlation with rCBF responses in several cerebral structures in both hemispheres (Coghill et al. 1999; Derbyshire et al. 1997). Abnormal pain states and other pain-related sensations have also been used to identify brain mechanisms mediating different aspects of the pain experience (Baron et al. 1999; Casey et al. 1996; Hsieh et al. 1994–1996; Jadad et al. 1998; Svensson et al. 1997).

In the example studies cited above, subjects were aware of a change that they expected to alter their perception of pain. In the Rainville study, hypnotic suggestion was used. In the other investigations, stimulus parameters or other conditions were altered in an obvious way, and each subject was asked to report on the sensation. It is possible that heightened vigilance and directed attention toward stimulus differences recruits brain mechanisms that are more specifically related to these alerting and cognitive functions than to differences in the perception of pain. Here we present evidence that 1) both the perception of heat pain and the activation of specific brain regions change during the course of repetitive contact heat stimulation, and 2) activity in some cortical areas is detected before subcortical activation. We use this information as evidence about the functional role of specific brain structures in heat pain.

Address for reprint requests: K. L. Casey, VA Medical Center, 2215 Fuller Rd., Ann Arbor, MI 48105 (E-mail: kencasey@umich.edu).
**Methods**

Fourteen healthy, right-handed subjects (10 male, 4 female; ages 18–42) participated in this investigation. None were taking analgesics or other drugs that alter CNS function. All subjects gave written informed consent to participate and refrained from smoking and consuming alcohol or caffeine for 24 h before the study. The Human Studies Committee of the Ann Arbor Veteran’s Affairs Medical Center and the Institutional Review Board for Human Studies at the University of Michigan Medical Center approved the consent form and the study protocol.

**PET protocol and data analysis**

The scanner used in this study was a Siemens/CTI 931/08-12 with 15 tomographic slices covering an axial field of view of 10 cm. We used a transmission scout view to position each subject in the scanner approximately parallel to the canthomeatal line. For each of eight scans, each subject received a 50-mCi intravenous bolus injection of H$_2$O. At least 15 min elapsed between each scan. Data acquisition began 5 s after the estimated arrival of radioactivity in the brain and continued for approximately 60 s. After normalizing each image set to whole brain counts (Fox and Raichle 1984), mean radioactivity concentration images estimating rCBF were created for each experimental condition across all subjects by stereotactic anatomical standardization techniques (Minoshima et al. 1992, 1994). We made subtraction images for each subject by subtracting the images acquired during the lower intensity stimulation from those acquired during the highest intensity stimulation. We analyzed only those voxels with normalized CBF values larger than 60% of the global value in this study. A voxel-by-voxel statistical subtraction analysis (Z-score) with adjustment for multiple comparisons was performed by estimating the smoothness of subtraction images (Friston et al. 1991) following three-dimensional Gaussian filtering (FWHM = 9 mm) to enhance signal-to-noise ratio and compensate for anatomical variance. Voxels with a significantly increased CBF compared with the average noise variance computed across all voxels (pooled variance) were identified (Worsley et al. 1992). The critical level of significance was determined by adjusting $P = 0.05$ using this information (Adler and Hasofer 1976).

To detect intraregional rCBF increases between the early and late phases of stimulation, we selected volumes of interest (VOI) developed from peak activations ($Z > 3.5$) in a previous heat pain study (Casey et al. 1994) and applied these to structures with late heat pain peak activations. The average volume of the VOI template was 7.22 ml (mean ± SD; approximate spherical diameter of 1.4 mm). We found eight sites for which the peak activation in the present study was within 5 mm of that in the previous study in one or more stereotactic planes (Table 3). For statistical analysis, we computed a paired $t$ statistic for each VOI from the average percentage change in rCBF within the early and late phases (50–40°C) and between the early and late phases [(50–40°C late stimulation) − (50–40°C early stimulation)] across all subjects. A chance probability level of $P < 0.05$, uncorrected for multiple comparisons, was accepted as significant.

**Procedure for PET scans**

We told subjects that the purpose of the study was to relate their brain activity, as measured by PET, to their ability to estimate the intensity of stimulation delivered to the left arm during the scan. We emphasized the importance of remaining motionless and concentrating on the stimulation during the scan.

Two intensities of heat (40 and 50°C) were applied repetitively on alternate scans for a total of eight scans. Heat stimuli were delivered sequentially to six separate sites on the left volar forearm with a digitally controlled feedback contact thermode (Cygnus, Paterson, NJ) with a 254-mm$^2$ gold-plated copper contact surface that was heated by DC. Each stimulus was 5 s in duration. The temperature at the skin-thermode interface was estimated from a thermocouple embedded 0.83 mm below the contact plate. The temperature of the thermode was set and held at the stimulus temperature before applying the probe to the skin. Probe temperature was constant throughout each scan.

On four of the scans, the onset of stimulation coincided with the earliest detection of radioactive counts in the subject’s head and continued repetitively throughout the 60-s duration of the scan. Because these scans began during the early phase of stimulation, we refer to them as early scans. The other four scans began approximately 40 s after the onset of stimulation, which then continued for the 60-s duration of the scan for a total of approximately 100 s (Fig. 1); these we call late scans. The four early scans were obtained first in seven subjects and last in the remaining seven subjects.

**Psychophysical studies**

After being positioned in the scanner, each subject was instructed in magnitude estimation based on the scale used previously (Casey et al. 1994, 1996) for which 0 indicated “no heat sensation,” 7 indicated “just barely painful,” and 10 indicated “just barely tolerable.” This scale accounts for the larger range of detectable innocuous, compared with noxious, heat stimuli below 50°C. Several practice trials were given with four to five stimulus intensities ranging from 36 to 50°C at least three trials at each end of the range showed that 36°C was perceived as painless and warm and that 50°C was definitely painful. We told all subjects that the intensity of each stimulus would be constant during each of the scans, that stimulus intensity may or may not change between scans, and that some, none, or all of the stimuli might be in the noxious range. We instructed them to average mentally the intensity of the total number of repetitive stimuli delivered during each scan. We specifically did not mention any differences in the timing of the scans or any expected differences associated with scan timing or duration. Because we wished each subject to concentrate on estimating average stimulus intensity, we did not request a separate estimation of stimulus unpleasantness during the scan sessions in this study. All subjects remained silent and immobile during each scan and, only after each scan was completed, did they verbalize...
their rating of stimulus intensity and give their own personal narrative description of the stimulus.

During the scan sessions, subjects gave mentally averaged ratings over the total number of stimuli after each scan. We analyzed data by a two-way repeated measures ANOVA with temperature (2 levels: 40 and 50°C) and scanning time (2 levels: early and late) as within subject variables. Because the durations of the stimulation delivery periods were different for the early and late scans (approximately 60 compared with 100 s, respectively), we wished to determine whether psychophysical effects occurred during the temporal progression of the repetitive heat stimulation. It is not possible to do this during the scanning procedure because the subject cannot perform and express an evaluation of each stimulus during the scanning period. Therefore, we performed separate psychophysical testing of eight normal subjects (4 males and 4 females, ages 18–26 yr), who also participated in the PET study. The remaining six subjects were not available for these separate studies for various personal reasons. We conducted these separate psychophysical studies within 1 mo after the PET scans. We chose this sequence of data acquisition so that the subjects would not introduce cognitive activities developed from this psychophysical investigation into the PET scanning sessions.

We measured perceived intensity by the same magnitude estimation scale used in the PET studies (0 = no heat, 7 = just barely painful, and 10 = barely tolerable). We told each subject that the purpose of the study was to determine how accurately and reliably they could rate the intensity of different heat stimuli applied repetitively to the left (nondominant) forearm. They were given instructions in the use of the same rating scale described above for the PET studies and were given several stimuli at 40, 43, 45, 47, and 50°C as examples of the range of intensities to be used. We told all subjects that we might apply several different stimulus intensities during each of four separate stimulation sessions. Unknown to the subjects, however, stimulus intensity was constant throughout each of these stimulation sessions. Each session was separated by a 10-min rest period and consisted of 20 5-s stimuli (2 repetitions of either 40 or 50°C counterbalanced across subjects) delivered to randomly spaced alternating sites on the volar surface of the left forearm at 2-s intervals. Subjects rated the intensity of each stimulus during each inter-stimulus interval. To determine whether changes in perceived stimulus intensity during 60 s of stimulation (approximating the early scan condition) differed from that during 100 s of stimulation (approximating the late scan condition), we computed the subjects’ mean verbal numerical rating for the first, second, and fourth sets of five stimuli in each session. We analyzed the data by a three-way repeated-measures ANOVA with set (3 levels: 1st, 2nd, and 4th set), temperature (2 levels: 40 and 50°C), and repetition (1st and 2nd block) as within-subject variables. We corrected P values by adjusting the degrees of freedom with Greenhouse-Geisser epsilon values for all effects and interactions with more than two levels. Significant main and interaction effects (P < 0.05) were analyzed by post hoc t-test comparisons. This experiment allowed us to determine whether perceived changes in pain intensity took place during the course of repetitive stimulation, and, if so, whether such changes were different for stimulus sequences of 60 s (as in the early scans) compared with sequences of 100 s (as in the late scans).

Because it is difficult to rate both perceived stimulus intensity and unpleasantness within each interstimulus interval, we instructed each subject to represent graphically the unpleasantness of the series of stimuli immediately after each presentation. We provided subjects with a sheet containing 4 squares, 60 mm on each side, with the left side labeled “very unpleasant” at the top and “not unpleasant” at the bottom. We used olfactory and auditory examples to inform each subject that the degree of perceived unpleasantness could be different from the degree of perceived stimulus intensity. Subjects were then instructed to draw a line from the left to the right sides of each blank square, indicating the level of unpleasantness, if any, they experienced at the beginning of the stimulation series on the left and at the end of stimulation on the right. Two squares were used for each of the 40°C set of stimuli and 2 for the 50°C set of stimuli. For analysis, the graphs were normalized across subjects by placing the estimate of beginning unpleasantness at the left vertical midpoint of the standard graph and each subject’s estimate of ending unpleasantness at a corresponding relative point on the right vertical axis. We averaged the distance (in mm) each right-hand point was above (+) or below (−) the horizontal meridian across subjects, and the significance of the deviation from 0 was determined with a Mann-Whitney rank sum test.

RESULTS

Psychophysical studies

During the scanning sessions, subjects described the 40°C stimulation as warm and gave it an average intensity rating of 2.19 ± 1.22 (mean ± SD). They described the 50°C stimulation as painfully hot and gave it an average rating of 8.93 ± 1.33. As expected, a two-way ANOVA showed a highly significant main effect of temperature (F = 575.15; P < 0.0001). Scanning time effects were not significant (F = 0.22; P = 0.65), for either nonpainful or painful stimulus intensities as indicated by the absence of a significant interaction between temperature and scanning period (F = 0.25; P = 0.63).

To determine whether the perception of stimulus characteristics changed specifically during the 100-s repetitive application of heat (late scan) compared with the 60-s stimulus duration during the early scan, we performed the separate psychophysical experiment as described above (METHODS). Comparing the second set of five 5-s stimuli with the first would estimate the perceptual changes during early scans (10 5-s stimuli). Comparing the fourth set of five stimuli with the first would estimate the perceptual changes during late scans (20 5-s stimuli). We had no hypothesis relevant to the third set of five stimuli because none of the scans were 45 s in duration, so this period was not analyzed. The three-way repeated measures ANOVA yielded a significant main effect of temperature (F = 102.3; P < 0.001). Temperature significantly interacted with the set of five stimuli (1st, 2nd, or 4th set; F = 6.94; P = 0.013 corrected). Reppetition did not influence the ratings (F = 1.76; P = 0.23). We therefore averaged both repeated blocks at a given temperature and set. Figure 2 illustrates the temperature by set interaction as resulting from enhanced ratings in the fourth set compared with the second (t = 2.95; P = 0.02) as well as compared with the first set (t = 2.9; P = 0.02) of five 5-s stimuli only for stimulus intensities of painful 50°C, but not of nonpainful 40°C.

Subjects reported no unpleasantness at all for 40°C stimuli throughout the duration of stimulation. In contrast, some degree of time dependency of the subjects’ estimates for unpleasantness did occur for 50°C stimulus intensities. Figure 3 displays each individual’s values of perceived unpleasantness at the end of stimulation as changes from a normalized beginning of zero. There is a significant median increase of unpleasantness (2.75 mm) at the end of 50°C stimulation compared with the beginning (Mann-Whitney rank sum test; P = 0.04).

Early phase of heat pain

During the first 60 s of noxious repetitive cutaneous heat stimulation, voxel-by-voxel analysis reveals significant activation of the contralateral mid-anterior cingulate cortex by heat pain. The volume of the activated region includes some of the
A total of 20 5-s duration stimuli was applied every 2 s in 4 separate sessions at constant intensities of either 40 or 50°C. A 10-min rest period separated each session. Subjects (n = 8), all of whom participated in the PET study and were uninformed about the intensity of each stimulus applied during each session, rated the perceived intensity of each stimulus during the interstimulus using values from 0 to 10, with 7 indicating pain threshold. The average (±SD) rating of the 1st, 2nd, and 4th sets of 5 stimuli are plotted for stimulus intensities of 40 and 50°C. A series of 10 stimuli approximates the number of stimuli applied during “early” scans, and a series of 20 stimuli approximates the number of stimuli applied during the “late” scans. A 3-way repeated-measures ANOVA reveals a significant interaction of temperature with stimulus set (F = 6.94, P = 0.013 corrected). Post hoc t-test comparisons show no significant change in the perceived intensity throughout the 40°C stimulation series. However, the last 5 stimuli of the 20 stimulus series applied at 50°C is perceived significantly more intense than the 1st 5 stimuli (P = 0.02, 2-tailed paired t-test) and than the 2nd 5 stimuli (P = 0.02, 2-tailed paired t-test).

supplementary motor cortex. Different regions of the premotor cortex (B6) are activated bilaterally just below the level of significance selected for this study. The contralateral perigenual anterior cingulate, lateral prefrontal, and anterior insular cortices show trends of activation also (Figs. 4 and 5, Table 1). The VOI analysis reveals significant activation of the ipsilateral mid-insula and the anterior cingulate cortex bilaterally. A trend of activation appears in the contralateral sensorimotor cortex and mid-insula (Table 3). No subcortical structures are activated significantly during these early scans.

**Late phase of heat pain**

A more extensive pattern of activation occurs when noxious cutaneous heat is applied for 40 s before, as well as during, the scan. Ten regions show significant responses in the voxel-by-voxel analysis (Figs. 4 and 5, Table 2). There is bilateral activation of the mid-insular cortex, the lenticular nuclei, and the cerebellum. Contralaterally activated structures include the lenticular nucleus and the sensorimotor (M1/S1), S2, and mid-anterior cingulate cortices; a trend of activation appears in the contralateral premotor cortex and ventral posterior thalamus. There is strong activation of the ipsilateral ventral lateral and medial thalamus. The ipsilateral S2 (B2/40) and mid-anterior cingulate cortices show a trend of activation. A similar pattern of activation appears in the VOI analysis (Table 3). The thalamus and insular cortex respond bilaterally while the sensorimotor and mid-anterior cingulate cortex show only contralateral activation. The cerebellar vermis responds also.

**Intraregional increases: a comparison of early and late phases of stimulation**

Analysis of responses within the VOI shows that rCBF increases within specific structures as the painful heat stimulation continues (Figs. 4 and 5, Table 3). Activity increases significantly within five of the eight structures analyzed: the contralateral sensorimotor and insular cortices, the ipsilateral insular cortex and thalamus, and the cerebellar vermis. Of the remaining structures, the contralateral mid-anterior cingulate cortex is active throughout the stimulation while the corresponding ipsilateral anterior cingulate cortex becomes less active. The contralateral ventral posterior thalamus becomes significantly active during the late phase of stimulation, but the increased activity is not significantly different from the previous period.

**Discussion**

**Temporal and spatial pattern of pain-related activation**

In our previous heat pain studies, stimulation began approximately 10 s before the slow bolus injection of H$_2$O (about

![FIG. 2. Temporal changes of perceived intensity of repetitive contact heat stimuli applied at pseudo-randomly alternating sites to the volar forearm. A total of 20 5-s duration stimuli was applied every 2 s in 4 separate sessions at constant intensities of either 40 or 50°C. A 10-min rest period separated each session. Subjects (n = 8), all of whom participated in the PET study and were uninformed about the intensity of each stimulus applied during each session, rated the perceived intensity of each stimulus during the interstimulus using values from 0 to 10, with 7 indicating pain threshold. The average (±SD) rating of the 1st, 2nd, and 4th sets of 5 stimuli are plotted for stimulus intensities of 40 and 50°C. A series of 10 stimuli approximates the number of stimuli applied during “early” scans, and a series of 20 stimuli approximates the number of stimuli applied during the “late” scans. A 3-way repeated-measures ANOVA reveals a significant interaction of temperature with stimulus set (F = 6.94, P = 0.013 corrected). Post hoc t-test comparisons show no significant change in the perceived intensity throughout the 40°C stimulation series. However, the last 5 stimuli of the 20 stimulus series applied at 50°C is perceived significantly more intense than the 1st 5 stimuli (P = 0.02, 2-tailed paired t-test) and than the 2nd 5 stimuli (P = 0.02, 2-tailed paired t-test).

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**Discussion**

**Temporal and spatial pattern of pain-related activation**

In our previous heat pain studies, stimulation began approximately 10 s before the slow bolus injection of H$_2$O (about
30 s preceding scan data acquisition). Stimulation then continued throughout the 60 s duration of the scan for a total of approximately 90–100 s. Our results show that, by appropriate timing of the stimulation, it is possible to reveal changes in the intensity and pattern of the rCBF response during the course of the repetitive heat stimulation. For example, if scan data acquisition begins with the onset of stimulation, as in the early scans of this study, activation is detected in relatively few structures during the first 60 s of noxious heat stimulation. Combining the voxel-by-voxel and VOI analyses of the early stimulation phase, we find highly significant activation limited to the bilateral anterior cingulate and ipsilateral insular cortices. We also observe strong trends of activation bilaterally in the premotor cortex and in the contralateral insular and lateral prefrontal cortices. Only if the scan begins after approximately 40 s of stimulation is additional significant activity detected bilaterally in the thalamus, lenticular nucleus, and cerebellum and contralaterally in the S2 cortex, and M1/S1 sensorimotor cortex. Strong trends of activation are observed also in the ipsilateral mid-anterior cingulate and S2 cortex.

The VOI analysis suggests that some of the later activations occur within regions that are marginally active in the early phase of stimulation. For example, the bilateral insular cortex, the contralateral sensorimotor cortex, and the cerebellum show either significant or marginally significant activity in the early phase before subsequently becoming significantly more active (Table 3).

Overall, the pattern of activation in the late phase of stimulation is consistent with our previous results and with the results of other PET studies using similar stimulation paradigms. In direct comparison with our previous results, it appears that the additional period of noxious heat stimulation is responsible for the additional cortical and subcortical activations observed in the previous studies compared with the shorter early stimulation phase in this investigation. Specifically, the difference between the early and late activations suggests that significant components of the cerebral response to noxious heat may not appear until after nearly 40 s of noxious heat stimulation. This is consistent with the increasing responses we observe in some VOI and with earlier cognitive studies showing progressively increasing activations with PET acquisition times beyond 60 s (Silbersweig et al. 1993).

When the bolus injection method is used, H215O PET scan sensitivity to cerebral blood flow is maximal and heavily concentrated within the first 20 s of scan acquisition (Beason-Held et al. 1999). Therefore it is likely that some late activations began during the last 30–40 s of stimulation during the early scans but were not detected because of reduced signal-to-noise ratio during the last 40 s of acquisition (Beason-Held et al. 1999; Hurtig et al. 1994; Koepppe et al. 1987).
Possible gender-related responses

In a recent report (Paulson et al. 1998), we showed that a sample of right-handed women rate the intensity of noxious, but not innocuous, heat stimuli higher than a sample of right-handed men of similar age. A PET activation study of these subjects showed that the same structures were activated by noxious heat in both genders except for the lateral prefrontal cortex (Broadmann’s areas 9/46), which showed a significantly greater contralateral response in females. In this study of 10 males and 4 females, the gender distribution in a sample of this size was insufficient to detect a gender-related difference in heat pain perception. Nonetheless, we cannot rule out the possibility that the females in our sample made a disproportionate contribution to the response in an adjacent area of the brain.

TABLE 1. Heat pain: early phase (voxel-by-voxel analysis)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Coordinates (x, y, z)</th>
<th>% Increase rCBF</th>
<th>Z-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cingulate cortex, mid-anterior (B24)</td>
<td>−3, −4, 45</td>
<td>3.4</td>
<td>4.3</td>
</tr>
<tr>
<td>Premotor cortex (B6)</td>
<td>−17, −10, 58</td>
<td>3.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Cingulate cortex, perigenual</td>
<td>−1, 23, 25</td>
<td>2.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Lateral prefrontal cortex, (B10)</td>
<td>−46, 48, 4</td>
<td>2.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Anterior insula</td>
<td>−33, 23, 7</td>
<td>2.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premotor cortex (B6)</td>
<td>48, −1, 11</td>
<td>3.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Voxel-by-voxel analysis. Structures with significant and borderline peak activations during repetitive noxious heat compared to warm stimulation. These “early” scans began at the onset of stimulation and continued for 60 s. Stereotactic coordinates are according to the atlas of Talairach and Tournoux (1988), right (contralateral) negative. rCBF, regional cerebral blood flow.

TABLE 2. Heat pain: late phase (voxel-by-voxel analysis)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Coordinates (x, y, z)</th>
<th>% Increase rCBF</th>
<th>Z-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid-insula</td>
<td>−39, 1, 11</td>
<td>5.2</td>
<td>6.6</td>
</tr>
<tr>
<td>N. lenticularis/posterior insula</td>
<td>−28/−33, −13/−15, 7/9</td>
<td>3.8</td>
<td>4.9</td>
</tr>
<tr>
<td>M1/S1 cortex</td>
<td>−24, −17, 58</td>
<td>4.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Cingulate cortex (mid-anterior B24)</td>
<td>−6, −1, 38</td>
<td>3.4</td>
<td>4.3</td>
</tr>
<tr>
<td>S2 cortex (B40)</td>
<td>−37, −19, 20</td>
<td>3.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Cerebellar vermis</td>
<td>−81/−60/−51, −11/−16</td>
<td>3.1/3.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Premotor (B6)</td>
<td>−51, −4, 38</td>
<td>2.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Thalamus, ventral posterior</td>
<td>−15, −24, 9</td>
<td>2.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Ipsilateral

| Thalamus, ventral lateral/n. lenticularis | 21, −15, 9 | 4.2 | 5.3 |
| Medial thalamus | 8, −19, 9 | 3.9 | 4.9 |
| Cerebellum, paravermal | 21, −60, −20 | 4.2 | 4.8 |
| Mid-insula | 35, 3, 4 | 3.4 | 4.4 |
| S2 cortex (B2/40) | 48, −19, 25 | 2.8 | 3.6 |
| Cingulate cortex, mid-anterior B24 | 10, 3, 29 | 2.7 | 3.4 |

Same as Table 1 except that these “late” scans began approximately 40 s after the onset of stimulation, which continued throughout the 60-s duration of the scan. rCBF, regional cerebral blood flow.
Volume of interest (VOI) analysis. We developed VOI around the peak coordinates of structures with heat pain related activations in a previous study (Casey et al. 1994). We applied these VOI to the analysis of heat pain activations obtained during the “early” and “late” scans. The peak coordinates of the VOI we selected overlapped those obtained in the voxel-by-voxel analysis of the “late” scans. Bold designates those structures showing a significant regional cerebral blood flow rCBF increase to noxious, compared to innocuous, heat stimulation during early and/or late scans as determined by single tailed paired t-test (p values shown in left column). Five of the 8 VOI showed significant activity during the early scan and significantly increased rCBF responses during the late scan (contralateral M1/S1 cortex, bilateral insulae, ipsilateral thalamus, and cerebellar vermis). The mid-anterior cingulate cortex was active during both early and late scans and did not show an additional increased response during the late scan. The contralateral ventral posterior thalamus became significantly active during the late scan, but this activity was not significantly different from that obtained in the early scan.* One-tailed test. ** Means ± 5D.

Table 3. Heat pain: early and late phases (VOI analysis)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Peak Coordinates (Late Phase)</th>
<th>Peak Coordinates (1994 Heat Study)</th>
<th>%Increase rCBF** (Early/Late)</th>
<th>%Increase rCBF (Late-Early)</th>
<th>P (Early, Late, Late-Early)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior cingulate (B24)</td>
<td>−6, −1, 38</td>
<td>−10, 3, 34</td>
<td>2.1 ± 3.1/2.5 ± 3.0</td>
<td>0.34 ± 3.4</td>
<td>0.01, 0.004, 0.35</td>
</tr>
<tr>
<td>M1/S1 cortex</td>
<td>−24, −17, 58</td>
<td>−26, −17, 52</td>
<td>1.4 ± 3.3/3.2 ± 2.6</td>
<td>1.8 ± 3.4</td>
<td>0.06, 0.0003, 0.035</td>
</tr>
<tr>
<td>Insula (mid/posterior)</td>
<td>−39, 1, 11</td>
<td>−37, −19, 14</td>
<td>0.7 ± 1.9/2.7 ± 2.2</td>
<td>1.9 ± 2.6</td>
<td>0.08, 0.0003, 0.007</td>
</tr>
<tr>
<td>Thalamus (VP)</td>
<td>−15, −24, 9</td>
<td>−8, −15, 4</td>
<td>0.5 ± 2.6/1.2 ± 2.0</td>
<td>0.7 ± 3.9</td>
<td>0.24, 0.02, 0.25</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior cingulate (B24)</td>
<td>10, 3, 29</td>
<td>8, 14, 29</td>
<td>1.7 ± 3.0/0.7 ± 1.9</td>
<td>−0.9 ± 4.1</td>
<td>0.03, 0.08, 0.43</td>
</tr>
<tr>
<td>Mid-insula</td>
<td>35, 3, 4</td>
<td>33, 1, 4</td>
<td>0.9 ± 1.9/3.1 ± 2.8</td>
<td>2.1 ± 2.9</td>
<td>0.04, 0.0005, 0.009</td>
</tr>
<tr>
<td>Thalamus (medial VP)</td>
<td>8, −19, 9</td>
<td>6, −15, 11</td>
<td>0.3 ± 1.5/2.6 ± 2.2</td>
<td>2.4 ± 2.5</td>
<td>0.25, 0.0004, 0.002</td>
</tr>
<tr>
<td>Midline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum (vermis)</td>
<td>−8, −60, −11</td>
<td>8, −53, −9</td>
<td>0.6 ± 1.6/2.2 ± 2.4</td>
<td>1.6 ± 2.7</td>
<td>0.08, 0.002, 0.019</td>
</tr>
</tbody>
</table>

Relationship to changes in the perception of heat pain

The psychophysical studies show that the perception of heat pain, but not warmth, changes as the repetitive stimulation continues over the presentation of 20, but not 10, stimuli. Subjects perceived the intensity and unpleasantness of the longer 50°C, but not the 40°C, repetitive stimulation to increase during the stimulation. The longer stimulus duration approximates the stimulation period of each late scan in this study. It is therefore reasonable to expect that brain activation patterns and intensities would change accordingly.

The early activation of some structures and the delayed response of others may be related to the temporal evolution of perceptual changes. For example, the voxel-by-voxel and VOI analyses reveal contralateral mid-anterior cingulate activity during both the early and late phases of stimulation. The VOI analysis shows further that the intraregional intensity of this early activation does not change during the late stimulation phase (Table 3). This suggests that at least this mid-anterior portion of the contralateral anterior cingulate cortex may not participate in mediating the perceptual increases we observed in the psychophysical studies. A similar conclusion is suggested for the ipsilateral mid-anterior cingulate cortex because, although it shows borderline significant activation during the late, but not early, stimulation (Tables 1 and 2), VOI analysis reveals no increase in the rCBF response; indeed, rCBF tends to decrease in this structure. It is possible that any perceived differences between early and late scans were insufficient to change rCBF in the anterior cingulate cortex. Nonetheless, the lack of relationship to increases in perceived stimulation characteristics is in accord with recent investigations showing that synaptic activity in specific subsections of the mid-anterior portion of the cingulate cortex may be more closely related to attention, response preparation, and selection than to the perceptual phenomena of pain specifically (Davis et al. 1997; Derbyshire et al. 1998; Devinsky et al. 1995; Vogt et al. 1992). This interpretation gains additional support from the recent study of a patient with a focal mid-anterior cingulate lesion and a specific deficit in manual motor, but not vocal, responses in executive decision functions (Turken and Swick 1999). The premotor cortex, which is active during pain (Casey et al. 1999; Casey et al. 1996; Coghill et al. 1999; Derbyshire et al. 1997; Paulson et al. 1998; Svensson et al. 1997), itch (Hsieh et al. 1994), and cognitive functions unrelated to pain (Jonides et al. 1993; Pardo et al. 1990), also shows strong trends of activation during both early and late scans (Tables 1 and 2). However, the stereotactic coordinates of peak activation are quite different, so it is possible that this shift of activity is somehow related to changes in the perception of heat pain.

Structures that are active only during the early scans are also unlikely to mediate the changes in heat pain perception associated with the late scans. For example, we observed borderline activation of the mid- and perigenual portion of the anterior...
cingulate during the early, but not late, phase of stimulation (Tables 1 and 2). The region just posterior to the perigenual cingulate has been shown to participate in mediating the hedonic features of nociceptive processing (Rainville et al. 1997). However, the peak coordinates of the exclusively early activations in both the perigenual anterior cingulate cortex and the anterior insular cortex (Table 1) are each within the territory of the activations that Ploghaus et al. (1999) found were related to the anticipation of pain instead of the perception of pain intensity (Ploghaus et al. 1999). Activity in the lateral prefrontal cortex (Table 1) has been associated with mechanisms of attention, orientation, and higher cognitive functions that may be more prominent during the early stages of pain perception (Baron et al. 1999; Derbyshire et al. 1997; Iadarola et al. 1998; Pardo et al. 1991).

Our approach to the neurophysiological analysis of pain-related perception complements the correlation analysis employed by Derbyshire et al. (1997) or by Coghill et al. (1999). In these correlation studies, subjects made judgments about a series of repetitive stimuli delivered at the same intensity during each scan. Therefore they ignored differences between the early and late phases of the stimulation or at least averaged these differences into the final report at the end of each scan. In our study, subjects also made an “averaged” report of stimulus intensity after each scan, which may have obscured any perceived differences between early and late scans during the scan acquisition sessions. We cannot determine whether, during the scan sessions, our subjects actually perceived a difference between noxious early and late scans because we did not include this distinction as part of the task. Our separate psychophysical analysis shows that, when specifically examined, the perceived intensity and unpleasantness of repetitive contact noxious heat does increase over time and could be mediated by the corresponding changes in forebrain activation. Nonetheless, it is quite possible that the changing pattern of brain activation is not related to increasing pain, but mediates cerebral functions other than those with which we measured.

Neurophysiological implications

Several neurophysiological factors may determine the temporal sequence of the pain-related activation pattern revealed by PET. One determinant is the amount of rCBF increase generated by similar intensities of neuronal activity in different brain areas. Another factor is the inter-regional variability in the timing of the rCBF response relative to neuronal activity. It is possible that these local variables are sufficiently great to produce rCBF responses that are out of sequence with the underlying neuronal activity. Although this problem has not been thoroughly and specifically investigated, several functional magnetic resonance (fMR) (Apkarian et al. 1999; Davis et al. 1995, 1997, 1998) and optical imaging studies (Malonek and Grinvald 1996; Tommerdahl et al. 1996; Vanzetta and Grinvald 1999) suggest that these variables are not likely to produce the degree of temporal dispersion of activation that we observed. It is more likely that the sequence of neuronal activities determines the temporal sequence of activation revealed by PET. This neurophysiological variable should be considered in future PET studies of pain and other sensory functions; it may account for some of the differences among PET studies in the pattern and intensity of activation.

Given the above argument, it is notable that cortical activation precedes thalamic activation in this study. One obvious possibility is that an amount of thalamic neuronal activity that is insufficient to generate a detectable rCBF response nonetheless generates cortical activation. Assuming a small inter-regional variance in the relationship between neuronal activity and rCBF response, this explanation suggests that a relatively small amount of thalamic activity could produce a large cortical response, consistent with the extensive intracortical divergence of thalamocortical fibers (Castro-Alamancos and Connors 1997; Jones 1998; Rausell et al. 1998). Another possibility is that the early cortical activation reflects activity that is independent of sensory stimulation and the perception task. It is possible that anticipation, anxiety, fear, and other unidentified factors within the environment of the experiment contribute to early cortical activity early in the course of stimulus-evoked subcortical activation. This early cortical activity, in turn, may strongly influence subcortical nociceptive transmission at several levels of the CNS (Ghosh et al. 1994; Liu et al. 1995; Nudo and Masterton 1990; Ralston and Ralston 1985). By appropriately timing PET acquisition with respect to changes in various experimental conditions, it may be possible to investigate these and related dynamic aspects of cerebral mechanisms mediating pain.

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REFERENCES


