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Painful stimuli evoke different stimulus–response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study

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Summary

Only recently have neuroimaging studies moved away from describing regions activated by noxious stimuli and started to disentangle subprocesses within the nociceptive system. One approach to characterizing the role of individual regions is to record brain responses evoked by different stimulus intensities. We used such a parametric single-trial functional MRI design in combination with a thulium:yttrium–aluminium–granate infrared laser and investigated pain, stimulus intensity and stimulus awareness (i.e. pain-unrelated) responses in nine healthy volunteers. Four stimulus intensities, ranging from warm to painful (300–600 mJ), were applied in a randomized order and rated by the subjects on a five-point scale (P0–4). Regions in the dorsolateral prefrontal cortex and the intraparietal sulcus differentiated between P0 (not perceived) and P1 but exhibited no further signal increase with P2, and were related to stimulus perception and subsequent cognitive processing. Signal changes in the primary somatosensory cortex discriminated between non-painful trials (P0 and P1), linking this region to basic sensory processing. Pain-related regions in the secondary somatosensory cortex and insular cortex showed a response that did not distinguish between innocuous trials (P0 and P1) but showed a positive linear relationship with signal changes for painful trials (P2–4). This was also true for the amygdala, with the exception that, in P0 trials in which the stimulus was not perceived (i.e. 'uncertain' trials), the evoked signal changes were as great as in P3 trials, indicating that the amygdala is involved in coding 'uncertainty', as has been suggested previously in relation to classical conditioning.

Keywords: pain; single-trial fMRI; laser; neuroimaging; parametric

Abbreviations: ACC = anterior cingulate cortex; BOLD = blood oxygen level-dependent; DLPFC = dorsolateral prefrontal cortex; fMRI = functional MRI; MEG = magnetoencephalography(-ic); SI = primary somatosensory cortex; SII = secondary somatosensory cortex; SPM = statistical parametric map; SRF = stimulus–response function; Tm:YAG = thulium:yttrium–aluminium–granate

Introduction

The pain processing system is essential for the analysis of potentially life-threatening conditions and has to fulfil several tasks. It should (i) receive and analyse nociceptive sensory input, (ii) be able to shift the focus of attention towards pain processing, (iii) hold pain-related information in working memory, (iv) have access to the motor system to prepare a defence and (v) memory-encode the situation to avoid future damage.

The nociceptive system comprises the anterior cingulate cortex (ACC), primary (SI) and secondary (SII) somatosensory cortexes, insular cortex, dorsolateral prefrontal cortex (DLPFC) and parietal cortex (Talbot *et al*., 1991; Casey *et al*., 1996; May *et al*., 1998; Apkarian *et al*., 1999; Kwan *et al*., 2000; Peyron *et al*., 2000). Each of these regions plays a different role within this system. In the primate SI cortex, neurones encode the intensity of tactile and nociceptive stimuli, implicating SI in sensory-discriminative aspects of pain processing (Kenshalo *et al*., 1988). Neurones in SII show complex response patterns (Robinson and Burton, 1980), suggesting a role of SII in coding pain intensity, as has been shown in many neuroimaging studies (Coghill *et al*., 1999; Sawamoto *et al*., 2000). The specific role of the parietal cortex in pain processing seems to be directing attention towards

the painful stimulus (Peyron *et al*., 1999), and this is comparable with shifts of visuospatial attention to unexpected visual targets (Corbetta *et al*., 2000). The DLPFC and its link to working memory processing (Buckner and Petersen, 1996; Courtney *et al*., 1996; Frith and Dolan, 1996) is ideally suited to keeping information about painful stimuli on-line for further processing. Medial temporal lobe areas, including the amygdala, are involved in learning the association between aversive and neutral stimuli in classical conditioning (LaBar *et al*., 1998; Ploghaus *et al*., 1999; Büchel and Dolan, 2000), thus providing a framework for the avoidance of future encounters with similar stimuli.

Only recently have neuroimaging studies moved away from describing regions activated by noxious stimuli and started to disentangle subprocesses within the nociceptive system by direct experimental manipulation of the pain affect (Rainville *et al*., 1997), pain intensity (Hofbauer *et al*., 2001) or attention to pain (Peyron *et al*., 1999), or by the use of different paradigms in the same group of subjects (Davis *et al*., 1997; Kwan *et al*., 2000). Yet another approach utilizes different stimulus intensities from warm to painful to characterize individual regions by their stimulus–response function (SRF) (Coghill *et al*., 1999; Tölle *et al*., 1999). The results that can be achieved with this very elegant approach depend greatly on the spatial and temporal resolution of the neuroimaging technique and might thus be limited when using PET as opposed to functional MRI (fMRI).

We used single-trial fMRI in combination with a thulium:yttrium–aluminium–granate (Tm:YAG) infrared laser, which delivers very brief (1 ms) painful heat stimuli. A parametric design employing four stimulus intensities ranging from warm to painful allowed us to assess individual SRFs, i.e. BOLD (blood oxygenation level-dependent) contrast responses as a function of stimulus and pain intensity (Büchel *et al*., 1998*a*; Coghill *et al*., 1999; Tölle *et al*., 1999). These SRFs can reveal details about the response pattern of different regions and hence about their particular role within the nociceptive system.

On the basis of recent magnetoencephalographic (MEG) (Timmermann *et al*., 2001) and PET (Coghill *et al*., 1999) data, we hypothesized linear dependency between stimulus intensity and BOLD signal in SI, whereas in SII and the anterior insula we expected the response profile to be related to pain intensity, i.e. no BOLD signal differences for non-painful stimuli but an increase for painful stimuli. On the basis of previous PET studies, we hypothesized that responses would be related to stimulus perception, i.e. a signal difference between P0 (pain rated by the subject as not noticed) and all other ratings of pain irrespective of perceived pain or stimulus intensity in the prefrontal (Coghill *et al*., 1999) and parietal cortex (Peyron *et al*., 1999).

The complex response patterns observed in the cingulate cortex are beyond the scope of this report and will be reported elsewhere (Büchel *et al*., 2002).

Methods

Subjects

A total of 10 healthy subjects recruited from the University of Hamburg gave their written informed consent to participation in the study, which was approved by the ethics committee of the Hamburg Chamber of Physicians. There were seven males and three females and their mean age was 28.1 years (range 24–42 years). One subject had to be excluded from the study because of an exceedingly high pain threshold and poor pain discrimination ability. The remaining nine subjects (six male, three female; one left-handed) were free to withdraw from the study at any time.

During scanning, two investigators stayed with the subject in the scanner room. One investigator applied the laser stimulus to the dorsum of the left hand and the other investigator documented the rating of each stimulus.

Laser stimulation

A Tm:YAG laser (Baasel Lasertech, Starnberg, Germany) was used to apply computer-controlled brief radiant pulses to the skin of the subjects. The thulium laser emits near-infrared radiation (wavelength 1.96 μm, spot diameter 5 mm, pulse duration 1 ms) with a penetration depth of 360 μm into the human skin. The laser stimulus allows precise restriction of the emitted heat energy to the termination area of primary nociceptive afferents without damaging the epidermis or affecting the subcutaneous tissue (Spiegel *et al*., 2000). Additionally, the temperature rise in the superficial skin following laser stimulation is fast enough to elicit activation of thinly myelinated Aδ- and unmyelinated C-nociceptors.

Experimental protocol and pain rating

In a single fMRI session, 100 nociceptive stimuli were delivered to the dorsum of the left hand. Interstimulus intervals were randomized within the range between 8 and 12 s to decrease pain anticipation. The stimulation site was changed slightly after each stimulus to avoid sensitization, habituation and tissue damage. Stimulation intensity was fully randomized between 300 and 600 mJ (300, 400, 500 and 600 mJ). Thus it was impossible for the volunteer to predict the upcoming stimulus intensity. Four seconds after the laser stimulus, a tone (1 kHz sine wave, 500 ms) signalled the subject to rate the perceived stimulus intensity. The lowest painful stimulus (P2) was defined as the feeling when pulling a small hair on the dorsum of the hand. P4 was defined as the maximum pain in the experiment and was associated with an energy level of 600 mJ. P3 was defined as pain intermediate between P2 and P4. P0 was defined as pain not noticed, and P1 as a sensation that felt warm but not painful. The subjects indicated their rating by a finger sign. Showing the closed right hand indicated a stimulus intensity rating of zero (P0), one finger (thumb) a stimulus intensity of one (P1) and two, three and four fingers pain intensities P2, P3 and P4,

respectively. Volunteers were exposed to all pain intensities and trained with this rating scale inside the magnet for 20 min before scanning. In addition, the individual pain threshold was derived psychophysically in each subject before scanning by the use of three series of stimuli ascending in steps of 30 mJ, from below sensation threshold to 90 mJ above pain threshold, and back again to below sensation threshold. Data from this prescanning pain threshold estimation were lost for two subjects because of computer failure. Due to the low intersubject variability of the pain threshold in previous behavioural studies performed outside the MRI magnet, we decided to use fixed energy levels for all subjects to simplify the paradigm and the data analysis (Bromm and Lorenz, 1998).

Image acquisition

MRI scanning was performed on a 1.5 T scanner (Siemens Vision; Siemens, Erlangen, Germany). In a single session, 375 volumes (25 contiguous axial slices, each 3 mm thick, 1 mm gap) were acquired using a gradient echo echo-planar (EPI) T_2*-sensitive sequence [repetition time (TR) 2800 ms, echo time (TE) 60 ms, flip angle (FA) 90°, matrix 64 × 64, field of view 210 × 210 mm]. A standard head coil was used and packed with foam pads. Subjects were blindfolded during the experiment. For display purposes, a high-resolution (1 × 1 × 1 mm voxel size) T_1-weighted structural MRI was acquired for each volunteer using a 3D FLASH (fast low angle shot) sequence.

Image processing and statistical analysis

Image processing and statistical analysis were carried out using SPM99 (http://www.fil.ion.ucl.ac.uk/spm) (Friston *et al*., 1995*b*; Worsley and Friston, 1995). The first five fMRI volumes were removed to allow for signal equilibration. All volumes were realigned to the first volume (Friston *et al*., 1995*c*), spatially normalized (Friston *et al*., 1995*a*) to a standard EPI template (Evans *et al*., 1993) and finally smoothed using a 6 mm full width at half maximum isotropic Gaussian kernel. Data analysis was performed by modelling the different trials (perceived pain intensity P0, P1, P2, P3, P4) as delta functions convolved with a set of two basis functions, modelling an early response peaking 5 s after application of the painful stimulus and a second basis function peaking at 9 s (expected peak for the motor component). The basis function was the canonical haemodynamic response function as implemented in SPM99. Voxelwise regression coefficients for all regressors were estimated using least-squares analysis within SPM99 (Friston *et al*., 1995*c*).

Specific effects were tested with appropriate linear contrasts of the regression coefficients (parameter estimates), resulting in a *t* statistic for each voxel. These *t* statistics constitute a statistical parametric map (SPM). SPMs are interpreted by referring to the probabilistic behaviour of Gaussian random fields (Worsley, 1994). Because of strong *a priori* hypotheses of pain-related responses in the SI (Bushnell *et al*., 1999), SII, insular cortex (Coghill *et al*., 1999) and amygdala (Schneider *et al*., 2001), the threshold was set to $P < 0.001$ uncorrected in these regions. The T_1-weighted structural volume was coregistered to the functional scans by normalizing it to a T_1-weighted template in the same space as the T_2* EPI template used to normalize the functional data set.

Results

Behavioural data

The correlation coefficient between perceived intensity and applied stimulus intensity averaged over nine subjects was 0.74 ± 0.02 (range 0.67–0.82). Figure 1A shows the average relationship between applied stimulus intensity and perceived pain. On average, the ratings were associated with the following mean stimulus intensities: P0, 333.5 ± 5.7 mJ; P1, 375.6 ± 11.3 mJ; P2, 446.0 ± 9.5 mJ; P3, 527.2 ± 7.5 mJ; and P4, 577.4 ± 5.3 mJ. The mean pain rating was linearly related to the laser energy applied: average pain rating = laser energy × 0.84–0.15, where laser energy is 300, 400, 500 or 600 mJ (Fig. 1A). On average, subjects gave a rating of P0 in 17.4 trials, P1 in 20.6, P2 in 24.7, P3 in 23.3 and P4 in 14 trials (Fig. 1B). The pain threshold during MRI scanning (average of mean intensity associated with P1 and P2) was 410 ± 28 mJ.

fMRI data

SFRs

The main aim of the study was the characterization of areas activated by painful stimuli by their relationship to stimulus intensity and pain (Büchel *et al*., 1998*a*). Treating differently rated trials as different conditions allowed us to analyse the relationship between the stimulus and the BOLD signal. Although the perceived intensity depended linearly on the applied laser intensity over the whole range of the rating scale used (Fig. 1), only the subrange from P2 to P4 describes increasing pain; P0 and P1 refer to the absence of pain, rendering the scale non-linear with respect to pain.

Essentially, we found three different SRFs, as follows.

(i) Some areas showed a significantly higher BOLD signal for P1 compared with P0 but no further signal increase with P2–4. Given that P0 was used to code stimuli that were not perceived, this simple step function discriminates between perceived and non-perceived stimuli, without any further pain or intensity discrimination, and is related to stimulus perception (Fig. 2A).

(ii) Some areas showed a linear relationship beginning at P0, i.e. they distinguished well among P0, P1 and P2. This SRF differentiates between different stimulus intensities, even though some (P0 and P1) were perceived as not painful.

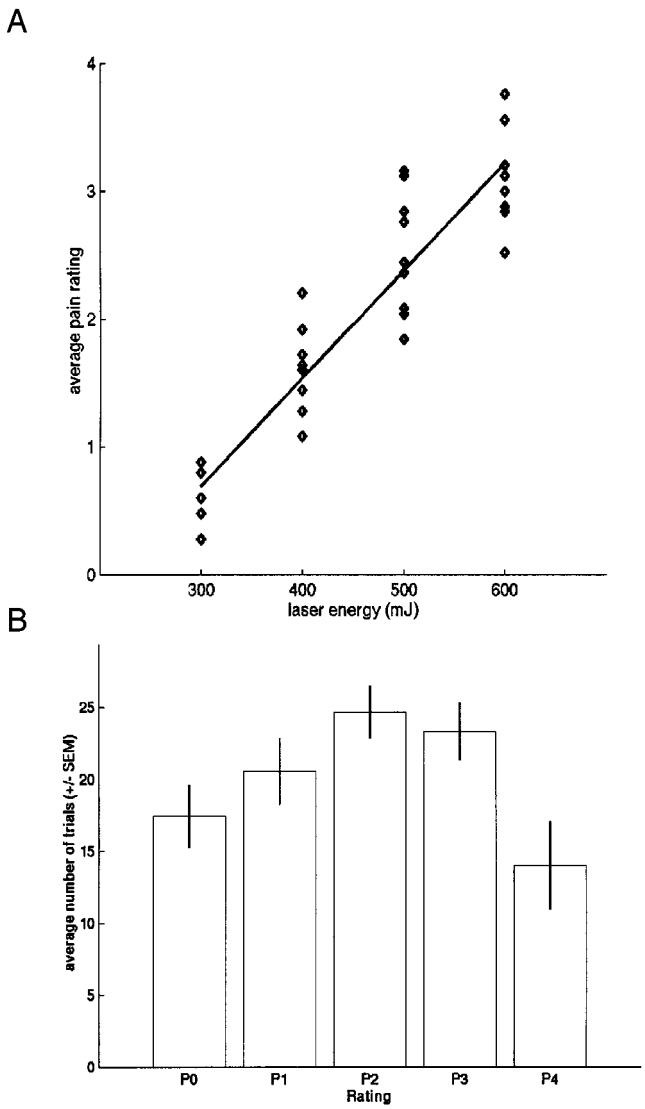


Fig. 1 (**A**) Relationship between applied stimulus energy (*x* axis) and average rating (P0–4, *y* axis). Data points show the average rating for each energy level and each subject. Average pain rating was linearly related to laser energy (average pain rating = laser energy × 0.84 – 0.15), where laser energy is 300, 400, 500 and 600 mJ. (**B**) Average frequency of trials with different ratings (P0–4). On average, subjects rated P0 in 17.4 trials, P1 in 20.6 trials, P2 in 24.7 trials, P3 in 23.3 trials and P4 in 14 trials.

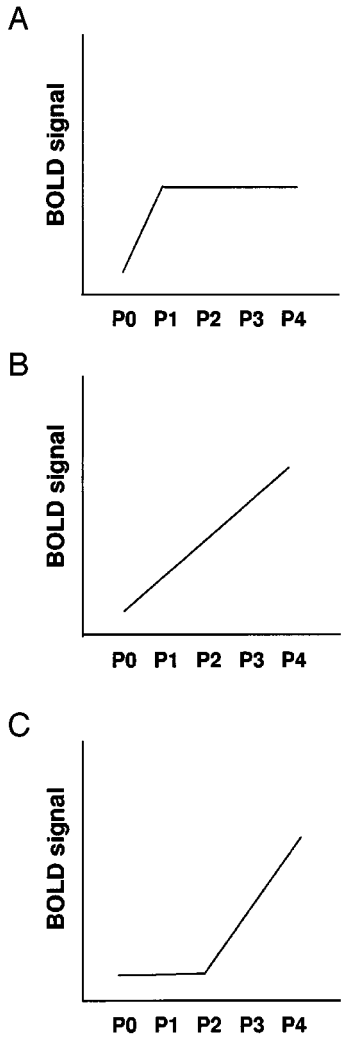


Fig. 2 Different SRFs. (**A**) The SRF shows a higher BOLD signal for P1 (stimulus perceived but not painful) compared with P0 (stimulus not perceived), but no further signal increase with P2–4 (low, middle and high pain levels). This step function discriminates between perceived and unperceived stimuli without any further pain or intensity discrimination and seems to be linked to stimulus awareness and possibly to cognitive processing (e.g. working memory or attention). (**B**) The SRF shows a linear relationship beginning at P0 and differentiates well between stimulus intensities, even though some (P0 and P1) were perceived as not painful. This SRF is related to stimulus intensity. (**C**) The SRF shows an initial plateau, i.e. does not differentiate between P0 and P1 but shows a linear relationship from P2 to P4. According to our pain rating scale, in which P2 was defined as clearly painful but P0 and P1 were not painful, this SRF is related to pain intensity.

Signal changes in these regions are therefore related to stimulus intensity (Fig. 2B).

(iii) Other SRFs showed an initial plateau, i.e. they did not differentiate between P0, P1 and sometimes P2, but showed a linear relationship for P2–4. Given the definition of our pain rating scale, in which P2 was defined as clearly painful and P0 and P1 as not painful, this type of SRF is indicative of areas coding pain intensity (Fig. 2C).

Areas showing a stimulus perception-related SRF

Regions with such a SRF were identified by a contrast comparing P1 with P0. To further exclude regions that also showed differences between P2 and P1, this contrast was exclusively masked with the contrast P2 > P1 at $P < 0.001$. Simply, this masked contrast reveals areas that showed a significant difference between P1 and P0, but not between P1 and P2. Areas showing such an SRF were found in the parietal lobe posterior to the postcentral sulcus, following the intraparietal sulcus. Two distinct regions of the DLPFC bilaterally, which were 2 cm apart in the anterior–posterior

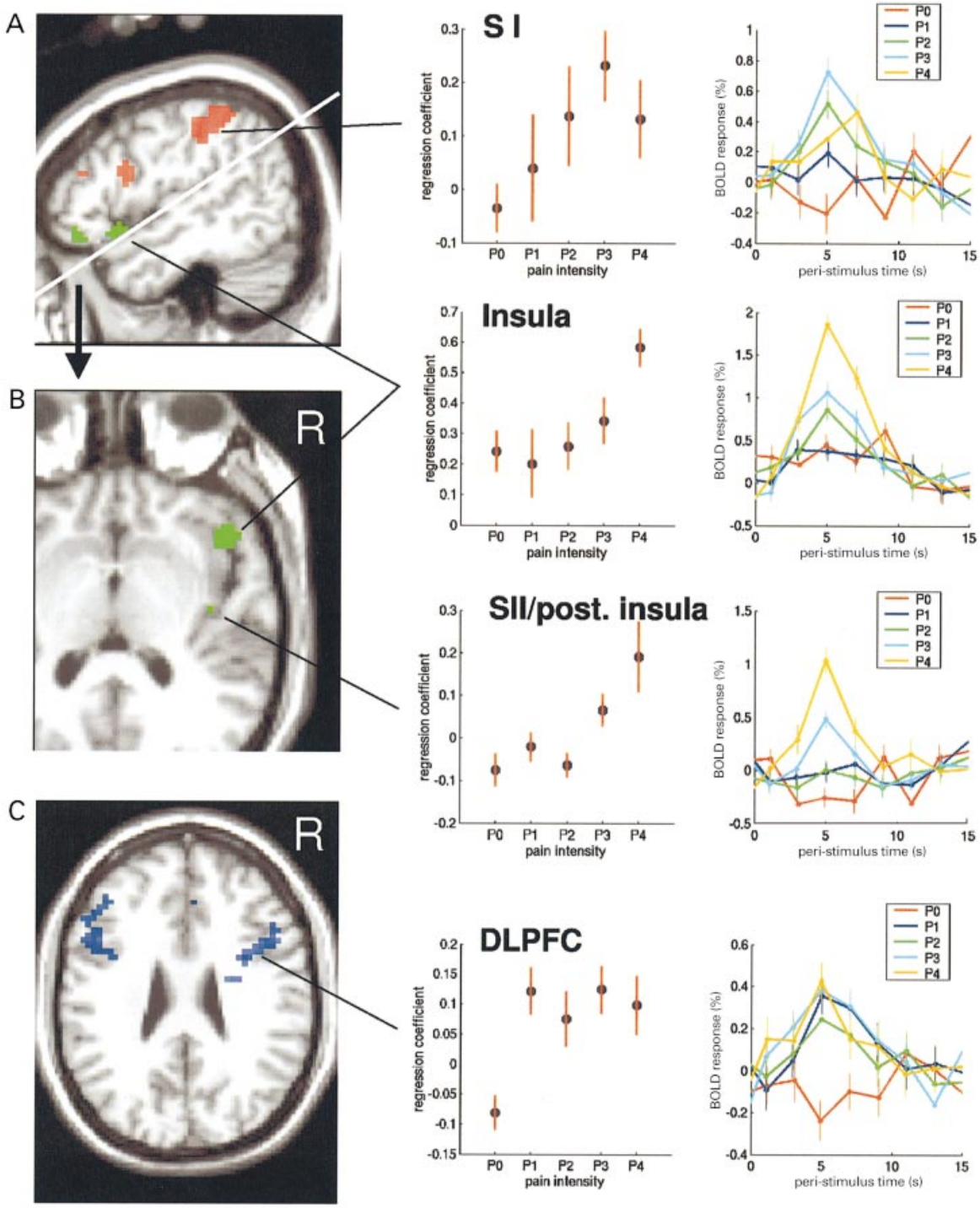


Fig. 3 Activations in the SI, SII/posterior insula, anterior insula ($P < 0.05$, corrected) and DLPFC ($P < 0.001$) overlaid on a structural T_1-weighted MRI, used for spatial normalization. Regions showing different SRFs are colour-coded. Stimulus intensity-related areas are shown in red, pain intensity-related areas in green and cognitive processing-related areas in blue. (**A**) Sagittal slice ($x = 50$ mm) with activations in the SI and anterior insula. (**B**) Axial slice through activations in the SII/posterior insula and anterior insula. The white line in **A** shows the precise location of the slice. To show the spatial relationship between the two areas, this slice is rotated 34° anticlockwise (nose left). (**C**) Slice through the dorsolateral prefrontal cortex. The middle column shows the individual SRF for each region. Fitting canonical haemodynamic response functions (for details see Methods) to the data yields a regression coefficient indicating the magnitude of the response for each trial type (P0–4). This magnitude (± standard error of the mean), plotted as a function of rating, is the SRF. In the right column, the amount of activation (percentage of the whole brain signal change in each region) is plotted in bins of 2 s as a function of peristimulus time separately for P0–4. The insula and SII/posterior insula show a pain-related SRF, whereas the SRF of the SI shows discrimination between P0 and PI, indicative of stimulus-intensity processing. The DLPFC shows a step function related to stimulus perception but not to pain or intensity processing.

Table 1 *Areas showing a significant SRF*

Contrast	Region	*x*, *y*, *z* (mm)	*Z* score
Stimulus perception-related			
Contrast: P1 > P0	DLPFC anterior R	54, 30, 21	4.2*
(not P2 > P1)	L	–48, 27, 21	4.5*
–1 1 0 0 0 not 0 –1 1 0 0	DLPFC posterior R	33, 6, 27	4.7*
	L	–48, 9, 21	5.7†
	Postparietal R	45, –51, 54	5.3†
	L	–36, –60, 57	6.6†
Stimulus intensity-related			
Contrast: P3 > P2 > P1 > P0	SI R	51, –27, 45	7.5†
–1.5 –0.5 0.5 1.5 0	L	–42, –21, 54	6.9†
Pain-related			
Contrast: P4 > P3 > P2 > P1	Perigenual ACC	–6, 54, 12	5.1†
(not P1 > P0)		–6, 36, –12	4.3*
0 –1.5 –0.5 0.5 1.5 not –1 1 0 0 0	SII/posterior insula	42, –12, 6	5.4†
		–45, –18, 12	3.8*
	IC	51, 15, –9	7.0†
		–45, 12, –9	4.5*
		36, 9, 0	4.8*
		–42, 9, –6	3.8*
	Amygdala	24, 0, –24	3.5*
		–27, 0, –27	5.1†

NOT indicates that the first contrast was exclusively masked (see text) with the second one at $P < 0.001$. *Z* scores refer to the first contrast. The contrast weights refer to the regression coefficients for P0, P1, P2, P3 and P4. DLPFC = dorsolateral prefrontal cortex; SI = primary somatosensory cortex; SII = secondary somatosensory cortex; IC = insular cortex; ACC = anterior cingulate cortex. *$P < 0.001$; †$P < 0.05$, corrected.

direction, showed a similar SRF (areas coded blue in Fig. 3). Table 1 summarizes the significance and location of these results.

Areas showing a stimulus intensity-related SRF

To identify regions showing intensity-related changes in the BOLD signal, we employed a contrast modelling a linear signal increase for P0–3. The most prominent linear relationship of the BOLD signal with stimulus intensity in the low stimulus range (P0–2) was observed in the postcentral sulcus bilaterally, anterior to the activations in the intraparietal sulcus revealed by the previous contrast (red area in Fig. 3). In accordance with previous fMRI studies on pain and vibrotactile stimulation, this activation is probably located in the SI (Andersson *et al*., 1997; Gelnar *et al*., 1999). Apart from a linear relationship, this area showed a ceiling effect, i.e. a slight decrease rather than an increase in BOLD signal when moving from P3 to P4 (Fig. 3).

Areas showing a pain-related SRF

To identify regions showing a pain-related BOLD signal, we employed a contrast that modelled a linear signal increase for P1–4 without a significant difference between P0 and P1. Most areas showing a linear relationship between BOLD signal and pain were found in the ventral part of the right hemisphere, buried in the depth of the Sylvian fissure. An anterior activation was located in the anterior insular cortex contralateral to the stimulated hand, whereas a posterior activation was located at the posterior insular cortex approaching the parietal operculum, presumably the location of the human SII (Gelnar *et al*., 1999; Treede *et al*., 2000) (Fig. 4). Although the peak location was closer to a reported activation in the SII than to a reported activation in the insula in a recent PET study (Coghill *et al*., 2001), we will refer to this activation as the SII/posterior insula. At a lower threshold, homologous areas in the left hemisphere were also activated (Table 1).

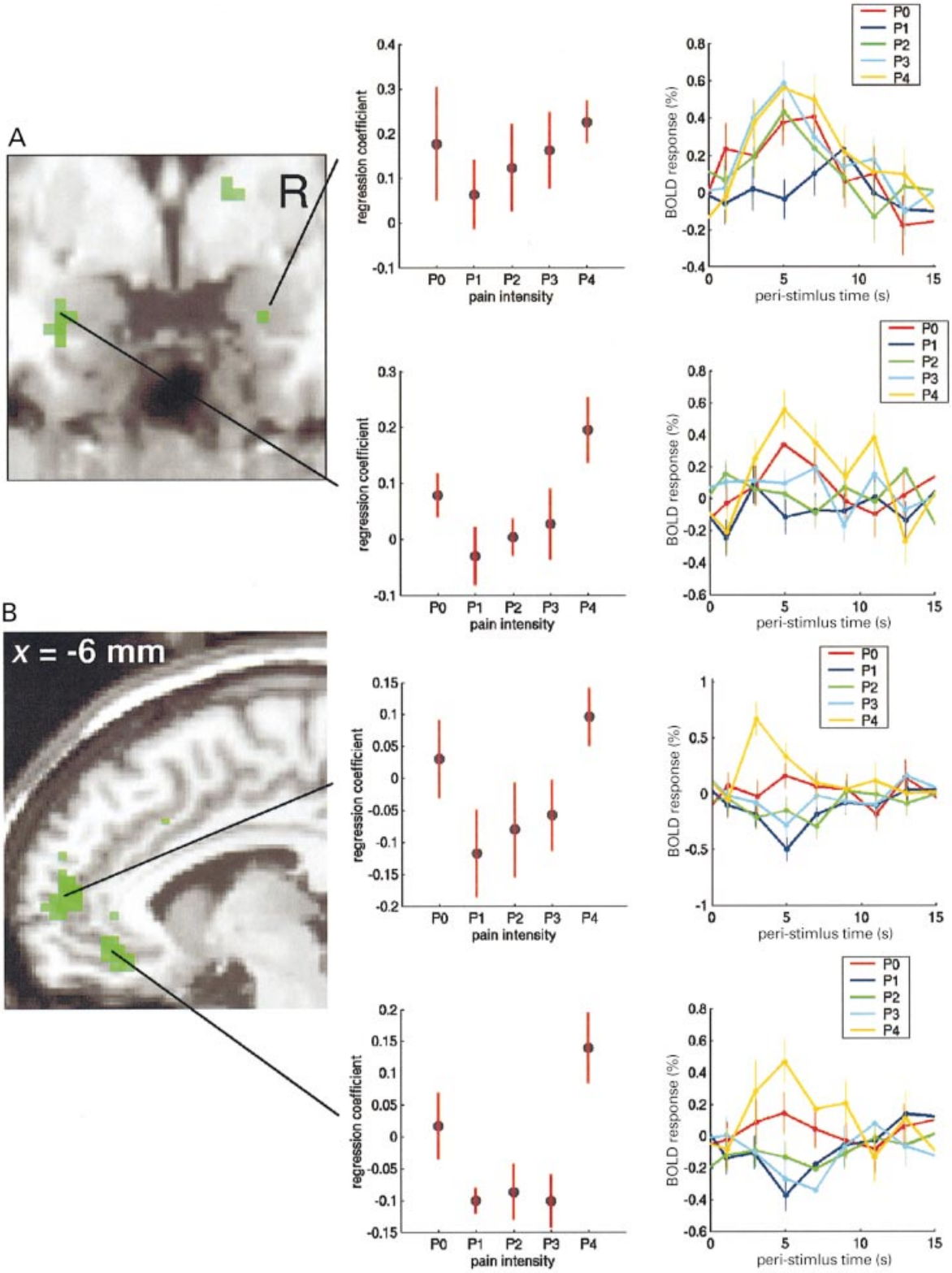
To test for differences in shape between the response in the SI and SII/posterior insula, a second-order polynomial ($y = b_1x^2 + b_2x + b_3$) was fitted to the SRF and the second-order coefficients (b_1) were compared using a paired *t*-test. The stimulus-related SRF in the SI ($x = 51$, $y = –27$, $z = 45$) was significantly different from the pain-intensity-related response in the SII/posterior insula [($x = 42$, $y = –12$, $z = 6$); $t(8) = 2.5$, $P < 0.05$] and the pain-intensity-related SRF in the anterior insula [($x = 51$, $y = 15$, $z = –9$); $t(8) = 2.9$, $P < 0.05$].

A slightly modified SRF with respect to BOLD responses evoked by P0 was seen in the perigenual ACC and the anterior medial temporal lobe, probably the bilateral amygdalae. These SRFs showed a linear relationship with pain intensity (P2–4). However, the BOLD signal evoked by trials rated as not perceived (P0) yielded a BOLD response comparable to that evoked by P3 (Fig. 4).

Discussion

Using a precise laser pain stimulus in combination with a parametric event-related design and fMRI, we characterized

regions of the nociceptive system according to their SRF. In the SI we found a close relationship between BOLD responses and low stimulus intensity; conversely, in the SII/posterior insula and the anterior insula the BOLD responses were related to pain rather than stimulus intensity. Non-specific activations that were probably related to attention and working memory were found in the DLPFC and the intraparietal sulcus. The amygdala and the perigenual ACC



showed a U-shaped, pain-related SRF, with activation levels for P0 comparable to those for P3.

Pain stimulus

The Tm:YAG laser is the ideal stimulation device for event-related fMRI studies of pain. It delivers brief (1 ms) stimuli with defined energy levels and activates only nociceptors, not the vibrotactile sensory system. In recent MEG studies, it was shown that vibrotactile stimuli evoke very short latency responses in the SI, whereas with the laser the latencies of evoked responses in the SI (and SII) were almost three times longer, without an initial short-latency component, highlighting the laser's property of stimulating nociceptors exclusively (Ploner *et al*., 2000; Timmermann *et al*., 2001).

Pain intensity-related responses

We applied four different pain intensities (300, 400, 500 and 600 mJ), which were rated for intensity on a five-point rating scale ranging from 0 to 4. We correlated the BOLD responses with individual ratings rather than the applied intensities, because this allowed us to dissociate painful ($\geqslant$P2) from non-painful (<P1) events accurately.

Primary somatosensory cortex

Our data confirm previous reports of the involvement of the SI in pain processing. The locations of stimulus-related responses found in the postcentral gyrus/sulcus were in good agreement with previous neuroimaging data showing activation of the SI (Andersson *et al*., 1997; Gelnar *et al*., 1999). Our use of a laser pain stimulus that has been shown not to activate the vibrotactile system further supports the view that the SI is implicated in pain processing irrespective of concomitant tactile stimulation, which is unavoidable when using thermodes or electrodes (Bushnell *et al*., 1999).

Furthermore, our data highlight the sensory-discriminative properties of the SI as revealed in its SRF. Responses in the SI contralateral to the pain stimulation were linearly related to stimulus intensity for P0–3. The observation of an SRF showing a linear relationship with stimulus intensity in the SI is in accordance with primate data (Kenshalo *et al*., 1988) and functional neuroimaging studies using PET (Coghill *et al*., 1999) and MEG (Timmermann *et al*., 2001). Primate studies have identified populations of neurones (wide dynamic range neurones) in the SI showing firing rates that are highly correlated with the physical stimulus intensity applied (Kenshalo *et al*., 1988). Surprisingly, our data show a linear increase for trials rated P0–3 but a ceiling effect, or even a decrease, between P3 and P4. We speculate that, at higher pain intensities, additional, antinociceptive mechanisms are initiated that lead to negative modulation of SI activation.

Secondary somatosensory cortex/posterior insula and anterior insula

Contrary to the stimulus intensity-related SRF found in the SI, a pain-related SRF was observed in the SII/posterior insula and anterior insula. This non-linearity is expected in areas coding pain rather than stimulus intensity (i.e. no response for the non-painful ratings P0 and P1, but a linear relationship with increasing pain intensity from P2 to P4). This finding is in accordance with previous functional neuroimaging studies showing a relationship between pain intensity and rCBF in PET (Coghill *et al*., 1999) and MEG (Timmermann *et al*., 2001) in the SII/posterior insula. The latter study found almost no difference in evoked responses for low (non-painful) intensities but a marked response increase when moving to painful stimulation intensities, showing a linear relationship between the magnetic response and pain intensity. Interestingly, the initial plateau was observed with intensities between 150 and 300 mJ, whereas in our study the plateau included P2 with an average intensity of 446 mJ. In accordance with this finding is the increased pain threshold observed in our study during fMRI (mean between P1 and P2 = 410 $\pm$ 28 mJ) as opposed to the pain threshold estimated before scanning (mean between P1 and P2 = 325 $\pm$ 18 mJ). This is probably related to the higher arousal during fMRI caused by the restricted space in combination with the hammering sound of the gradient system. Several studies have documented that attention or arousal can alter pain threshold significantly (Petrovic *et al*., 2000).

Prefrontal and parietal cortex

Areas in the prefrontal cortex and areas along the intraparietal sulcus showed a significant BOLD signal difference between P0 and P1 but no further discrimination between P1, P2, P3 and P4. Similar SRFs were observed in the ACC, within the

Fig. 4 Activations in the bilateral amygdalae and perigenual ACC ($P < 0.001$) overlaid on a structural T_1-weighted MRI, used for spatial normalization. (**A**) Activation in the bilateral amygdalae on a coronal slice ($y = 0$ mm). (**B**) Sagittal slice ($x = -6$ mm) with activations in two locations of the perigenual ACC. The middle column shows the individual SRF for each region. Fitting canonical haemodynamic response functions (for details see Methods) to the data yields a regression coefficient indicating the magnitude of the response for each trial type (P0–4). This magnitude ($\pm$ standard error of the mean), plotted as a function of rating, is the SRF. In the right column, the amount of activation (percentage of whole brain signal change in each region) is plotted in bins of 2 s as a function of peristimulus time separately for P0–4. The SRFs in all regions show an increase with painful stimuli (P2–4), similar to the SII/posterior insula and the anterior insula in Fig. 3. In contrast to the SII/posterior insula responses, the BOLD signal evoked by P0 is much higher than that evoked by P1.

cingulate sulcus. This 'on–off' response profile was related to whether a stimulus was perceived, i.e. whether the rating was greater than P0. Whenever a stimulus is consciously perceived, many cognitive processes are initiated: (i) an exogenous shift of spatial attention to the stimulated site; (ii) rating of intensity; and (iii) keeping this rating in working memory, as required in our task. Therefore, regions showing this response pattern might be related to these cognitive components.

This is in accordance with functional neuroimaging data implicating the association of the DLPFC with working memory (Petrides *et al*., 1993; Courtney *et al*., 1996) and of regions within the intraparietal sulcus with shifts of spatial attention (Driver and Spence, 1998; Corbetta *et al*., 2000). Furthermore, the intraparietal sulcus contains multimodal neurones (tactile, visual, acoustic) and is therefore ideally equipped to participate in 'orienting responses' when a painful stimulus is perceived (Bremmer *et al*., 2001).

A previous PET study employed different noxious and innocuous stimulus temperatures and also found binary on–off responses in the DLPFC. The authors interpreted this pattern as 'pain independent responses' and further speculated that it might reflect memory- and attention-related processing (Coghill *et al*., 1999). A recent study investigated directly the effect of attention in the context of pain processing using a full factorial design. In agreement with our data, attention-related activation was found in the DLPFC and inferior parietal lobule irrespective of whether the stimulus was noxious or innocuous (Peyron *et al*., 1999). It should be noted that the above interpretation assumes that the attentional and working memory loads induced by the very brief (1 ms) laser stimulus do not differ between P1, P2, P3 and P4. This might not be the case when using a long-lasting pain stimulus (e.g. a thermode), to which increasing attentional resources can be allocated over time. Alternatively, a binary on–off SRF could be related to the coding of warmth, indicating that primary afferent warm fibres code the intensity of warmth up to pain threshold and then plateau.

Amygdala and perigenual ACC

Responses in the amygdala to painful stimuli have been reported previously (Schneider *et al*., 2001). However, the response pattern in the amygdala and the perigenual ACC showed a pattern different from that found in the SII/posterior insula or the anterior insula. Stimuli that were not perceived (P0) evoked BOLD responses almost as high as those evoked by P3. Aversive classical conditioning studies revealed that the amygdala is activated only at the initial stages of acquisition (Quirk *et al*., 1997; Büchel *et al*., 1998*b*, 1999) or extinction (LaBar *et al*., 1998), when there is considerable uncertainty about the contiguity of neutral and aversive stimuli. The U shape of the SRF observed in the amygdala could be related to uncertainty or pain expectancy (Ploghaus *et al*., 1999): a clear, warm, but not painful sensation (P1) is interpreted as a 'relief' signal (i.e. no painful stimuli will follow in this trial), whereas in the case of P0, where no stimulus is perceived, the uncertainty or expectancy of receiving a (painful) stimulus increases until the rating tone occurs. Unfortunately, our experimental set-up did not have the temporal resolution to detect a difference in peak latency for P0 and P3, which would be expected if the observed phenomenon were related to an increase in uncertainty for P0. Interestingly, the perigenual ACC showed an almost identical U-shaped SRF. In accordance with this SRF, this subregion of the ACC has been associated with the emotional content of the stimulus (Blair *et al*., 1999) and is connected to the amygdala (Vogt and Pandya, 1987; Devinsky *et al*., 1995; Stefanacci and Amaral, 2000).

ACC

In a companion paper (Büchel *et al*., 2002) we report pain-related activations in the ventral posterior ACC ($x = 3$, $y = 6$, $z = 48$ mm), confirming previous findings (Porro *et al*., 1998; Kwan *et al*., 2000). This pain-related response is almost identical to that found in the SII/posterior insula and the anterior insula. Distinct from these pain-related regions, activations in the dorsal anterior ACC ($x = –3$, $y = 21$, $z = 45$ mm) were found to code stimulus perception without further discrimination of pain or stimulus intensity. This response profile is identical to that found in the DLPFC and parietal cortex and is related to working memory or attention to pain (Peyron *et al*., 1999). In analogy to the basic sensory processing response found in the SI, we discovered an area in the dorsal posterior ACC ($x = –3$, $y = 3$, $z = 51$ mm) that showed a similar pattern.

Conclusion

Using event-related fMRI together with brief laser pain stimuli of different intensities, we were able to characterize precisely regions within the nociceptive system according to their individual SRF. With respect to pain-related processing, we confirmed previous studies showing pain-related SRFs in the SII/posterior insula and the anterior insula. In contrast to these ventral pain-related regions, the SI showed BOLD signal changes correlated with stimulus intensity, indicative of basic sensory processing. Distinct from these pain and stimulus-related regions, activations in the dorsolateral prefrontal cortex and the intraparietal sulcus were found to code stimulus perception without further discrimination of pain or stimulus intensities, suggesting cognitive processes (e.g. working memory or attention to pain). SRFs in the perigenual ACC and the amygdala showed a pain-related SRF, with the exception that unperceived stimuli evoked very high BOLD responses. This might be related to the implicit uncertainty of whether a painful stimulus might follow and is in accordance with the putative role of these areas in the emotional processing of aversive events.

The different response patterns found in the SI (stimulus-related), SII/posterior insula, insula (pain-related), DLPFC and PPC (attention/working memory-related) were all mirrored in individual subregions of the ACC, suggesting that the ACC integrates information on painful stimuli, probably to generate an adequate response through its projections to associated motor areas, such as the supplementary and cingulate motor area (Dum and Strick, 1996).

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