

Functional magnetic resonance imaging of capsaicin induced thermal hyperalgesia

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Most clinical pain is associated with increased sensitivity to external stimuli. In both inflammatory and neuropathic conditions, pain may be evoked by touch, pressure, movement, heat or cold. Essentially, these phenomena represent reduced thresholds for eliciting pain, and are termed allodynia (when pain is caused by innocuous stimuli) and hyperalgesia (when an exaggerated response follows application of a normally painful stimulus). To investigate these pain syndromes, several animal models of neuropathic pain have been developed (Devor and Seltzer 1999). More recently, human pain models have been described (Liu et al. 1998; Petersen and Rowbotham 1999), which share some characteristics of neuropathic pain, and thus provide an opportunity to study “clinical” pain under controlled conditions. Our previous functional magnetic resonance imaging (fMRI) studies used intrinsically painful stimuli (thermal pain applied via a contact thermode), and healthy subjects, and thus primarily relate to the study of physiological pain (Brooks et al. 2002). Results from these studies do not necessarily tell us much about clinical pain, in which there are multiple functional alterations in central and peripheral pain signalling pathways.

The need for a human pain model has been addressed by Simone et al (Simone et al. 1989), who injected capsaicin solution intradermally to produce regions of primary and secondary hyperalgesia. By using this approach areas of mechanical allodynia and hyperalgesia to punctate stimuli may be observed (Liu et al. 1998). Three previous neuroimaging studies (Iadarola et al. 1998; Baron et al. 1999; Witting et al. 2001) have used this technique to elicit mechanical allodynia, however, results obtained were inconsistent. The source of these discrepancies may be related to differences in the pain levels evoked by stimulation, prior exposure to the capsaicin stimulus, type

of pain investigated (e.g. dynamic versus punctuate allodynia), or variability in the delay between capsaicin injection and recording functional data. By using the capsaicin model of Petersen et al, we have investigated whether patterns of pain-related brain activity are modified by the presence of heat hyperalgesia, and sought to address some of the technical difficulties of previous studies. In particular, we have compared brain activity, as measured by blood oxygenation level dependent (BOLD) fMRI, following physiological pain evoked using a thermode to heat hyperalgesia elicited after skin sensitisation with capsaicin cream. The relationship between the obtained data and possible mechanisms for central sensitisation are discussed.

METHODS

Sixteen healthy subjects (six female) were recruited, screened for the presence of neurological disease and given a general health check. All subjects were right handed and aged between 20-37 years. Local ethics committee approval was obtained for the study, and subjects consented to thermal stimulation and capsaicin application to the thenar eminence of the right hand.

Experiment 1: physiological heat pain

Offline psychophysics evaluation of heat pain thresholds was performed prior to functional MRI investigation. Thermal stimuli were applied using a 3x3 cm² Peltier thermode (Medoc, Haifa, Israel) adapted for use in the MRI scanner. Before scanning, for each subject, the temperature giving rise to a VAS rating of between 5 and 7 (out of 10) was recorded, corresponding to moderate to severe pain. Subjects were blind to the applied temperature of the thermode. This temperature (T_{PAIN}) was then used in the first part of the fMRI study.

Scanning was performed using a General Electric 1.5 tesla LX/NVi scanner with version 8.4 of the operating system software. Subjects were placed in the standard quadrature head coil, and the head restrained by suitable placement of foam padding. Following acquisition of localiser images, twenty four 6 mm thick axial oblique slices were prescribed parallel to the AC-PC line for functional imaging. fMRI data were obtained with a gradient echo (GRE) echo-planar imaging (EPI) sequence: with 64 by 64 matrix, 19 cm field of view, TE/TR = 40/3000 ms and flip angle 90°.

The thermode was attached to the subject's right palm, and an MRI compatible potentiometer placed in their left hand to provide on-line rating of pain experience using a VAS. VAS data were recorded using an analog-to-digital converter (ADC-11, Pico technology, UK) connected to a laptop computer and in-house software, and the relative position of the potentiometer used to generate a value between 0 and 100 on a linear VAS. The minimum position of the VAS was labelled with "no pain" and the maximum value labelled with "worst pain imaginable". The VAS was back-projected on to a screen visible to subjects in the scanner room using LCD projector.

Stimuli were applied using a block design (Brooks et al. 2002) with an inter-stimulus interval of 30 seconds. Experiments commenced with a baseline epoch (stimulus temperature 35°C), followed by 9 seconds of painful stimulation (at the predetermined value, T_{PAIN} , the pain epoch). Immediately after stimulation the VAS was displayed on the screen for 9 seconds, followed by a 12 second baseline epoch. Experiments consisted of 10 cycles of alternating rest – stimulation – rating, giving a total scan time of 300 seconds, during which 100 brain volumes were acquired.

Experiment 2: experimental thermal hyperalgesia

Following the first experiment subjects were removed from the scanner and underwent the capsaicin sensitisation procedure (Petersen and Rowbotham 1999). Briefly, the thenar eminence of the right hand was first stimulated using the thermode, whose temperature was maintained at a constant 45°C for 5 minutes. Following thermal stimulation, capsaicin was applied topically to the same area and covered with a Tegaderm® dressing. The dressing remained in place for approximately 45 minutes, after which the capsaicin was removed and the skin cleaned. Subsequently, thermal pain thresholds were reassessed with subjects blind to the applied temperature. The new temperature giving rise to a VAS rating of between 5 and 7 was noted (T_{CAPS}), and subjects replaced in the scanner.

Post-capsaicin fMRI was performed as in the first part of the experiment, with one modification, namely that the applied temperature during the pain epochs was now T_{CAPS} .

Data analysis

By using statistical parametric mapping (SPM99: <http://www.fil.ion.ucl.ac.uk/spm>) software, functional imaging data were first motion corrected using a 6 parameter rigid body transformation, normalized using a 12 parameter affine transformation, and smoothed using a 9 mm full width half maximum (FWHM) gaussian kernel. Changes in BOLD signal intensity were modelled using the general linear model approach (Worsley et al. 1992; Friston et al. 1994; Worsley et al. 1996), and motion parameters included as covariates of no interest. Representative group activation maps were

determined for pre- and post-capsaicin scans separately using a random effects model (one-sample t-test) and reported at an uncorrected $p < 0.001$. To determine the specificity of observed difference in group activation maps, ROI analysis was performed (Smith et al. 2000). Using bilateral ROIs previously defined in the anterior/middle/posterior insula (Brooks et al. 2002) and in prefrontal (Iadarola et al. 1998; Witting et al. 2001) and parietal cortex (Witting et al. 2001), regional activity in response to stimulus lateralisation and experimental condition (pre-capsaicin versus post-capsaicin) was recorded. Means signal amplitudes from each of the ROIs were analysed using a repeated measures general linear model (GLM) approach in SPSS software (SPSS Inc., Chicago), and within subject factors defined for “experiment” (pre- versus post-capsaicin), “side” (left versus right hemisphere) and “location” (corresponding to the 5 different ROIs). A level of significance of $p < 0.05$ was adopted.

RESULTS

The average temperature T_{PAIN} used for pre-capsaicin fMRI was 47.2 (SD=2.4) °C, whereas the average post-capsaicin temperature T_{CAPS} was 44.9 (3.1) °C. Whilst there was a significant difference between T_{PAIN} and T_{CAPS} (paired t-test, $p=0.01$), psychophysics data acquired outside the scanner demonstrated remarkable similarity between VAS scores (pre = 63 (SD=8) and post = 63 (7), paired t-test, $p=0.92$). Online recording of VAS data confirmed this finding (pre = 60 (16) and post = 62 (15), $p=0.77$). No subject reported any significant spontaneous pain following application of capsaicin cream. The main change caused by capsaicin sensitisation was the generation of thermal hyperalgesia, as evidenced by the significant differences in T_{PAIN} and T_{CAPS} , whilst pain ratings remained constant.

The different patterns of brain activity following thermal stimulation pre- and post-capsaicin may be seen on Fig. 1. Post-capsaicin, increased activity was observed in bilateral prefrontal cortex and ipsilateral (right) parietal regions. The specific locations, Brodmann areas and Talairach coordinates of activated brain regions are given in Table I. By using a repeated measures GLM and ROI data, significant interactions were found between “experiment” (pre- versus post-capsaicin) and “location” (of the ROIs), and between “side” (hemisphere) and “location”, but not “side” and “experiment”. Further comparisons were thus made using paired t-tests, see Table II. No significant difference between activation pre- and post-capsaicin was found for the insula ROIs, whereas the prefrontal and parietal ROIs were significantly more active post-capsaicin. In addition, significant rightward asymmetry of the BOLD response was observed in anterior insula and prefrontal cortex (post-capsaicin). The only region exhibiting leftward asymmetry was the posterior insula, which has previously been reported (Brooks et al. 2002). The average BOLD signal amplitude recorded from each subject is shown for those regions exhibiting a dependence on capsaicin sensitisation, namely, prefrontal and parietal ROIs, see Fig. 2.

DISCUSSION

Previous experiments using intradermal capsaicin injection have demonstrated similar patterns of activity to those observed in the current study. Using PET, Iadarola et al. imaged spontaneous pain caused by capsaicin injection and compared it to activation maps obtained by light brushing of the treated area after the injection pain had subsided. Their subjects reported experiencing allodynic pain to light brushing which was associated with activation across the pain matrix. However, when comparing

brush evoked touch and allodynia conditions, the main difference was bilateral activation of superior frontal gyrus. Similar findings were reported by Baron et al using fMRI to study mechanical allodynia, whilst Witting et al. using PET reported the main difference between allodynic and capsaicin pain as being increased activity in contralateral posterior parietal cortex (BA 7). In a PET study of mechanical allodynia in patients with mononeuropathy (Petrovic et al. 1999), the largest observed differences during allodynia were found in primary somatosensory cortex, however, increased blood flow was also found in contralateral posterior parietal cortex (BA 7). Additional evidence supporting this observation comes from a PET study of patients with lateral-medullary (Wallenberg) infarct (Peyron et al. 1998), who when comparing mechanical/cold allodynia to identical stimulation of the unaffected side, found increased activation in inferior parietal cortex.

CONCLUSIONS

By using a model of neuropathic pain, which synergistically combines mild warming of the skin and topical application of 0.075% capsaicin ointment to produce cutaneous heat hyperalgesia, we have demonstrated significant modulation of the cortical pain network with fMRI. When compared to activation maps produced by an intrinsically painful hot stimulus, the brain response to heat hyperalgesia showed significantly increased activity in ipsilateral prefrontal (BA 10) and bilateral parietal (BA 40) regions. By recording VAS pain levels inside and outside of the scanner, we were able to discount the possibility that the observed change in brain activation was due to altered subjective pain experienced post-capsaicin. The similarity between the pattern of activity recorded in this study and those previously reported in experimental studies

on hyperalgesia and clinical case studies suggests that these areas may be specifically involved in processing of altered sensory input, such as that seen after tissue damage.

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FIGURE CAPTIONS

Figure 1: Sagittal and axial sections through the MNI average brain, with pre- and post-capsaicin activation maps superimposed. Activations in 4 main areas are shown: (a) anterior cingulate gyrus, (b) anterior insula, (c) prefrontal cortex, and (d) parietal lobe. Note that activations in (c) and (d) are only associated with post-capsaicin activation maps. The activation maps correspond to the output from a second level analysis, thresholded at an uncorrected $p < 0.001$.

Figure 2: Regions of interest (ROI) analysis for parietal (A) and frontal (B) activation. By defining ROIs in prefrontal and parietal cortices we were able to extract the amplitude of the BOLD fMRI response for each region. Bar graphs show plots of mean “activation” for each ROI (in either the right or left hemisphere) and for each condition (pre- or post-capsaicin). Error bars represent $2 * \text{standard error on the mean}$. Dark and light grey corresponds to pre- and post-capsaicin experiments, respectively. A significant increase in activation was found for left and right parietal ROIs and for the right prefrontal ROI – which was the largest activation increase. P-values were calculated using a two tailed paired t-test.

Table I: Description and Talairach coordinates for main regions of activity observed following “physiologic” pain (pre-capsaicin) and after capsaicin sensitisation (post-capsaicin). Identification of activated regions was based on the stereotaxic atlas of Talairach and Tournoux (Talairach and Tournoux 1988). Only activated regions with cluster size greater than 10 voxels are reported.

Experiment	Region	Talairach coordinates			Brodmann
		X (R-L)	Y (A-P)	Z (I-S)	Area (BA) [†]
PRE- CAPSAICIN	Anterior insula	36	18	7	
	Anterior insula	-33	6	-1	
	Cingulate gyrus	3	-28	29	23
	Medial frontal gyrus	3	17	52	6
	Inferior frontal gyrus	-45	-1	30	44
	Middle frontal gyrus	51	8	47	6/8
	Inferior parietal lobule	36	-45	46	40
	Cerebellum	-27	-60	-25	
	Cerebellum	36	-68	-19	
	Thalamus	0	-3	8	
Superior frontal gyrus	33	48	28	9	
POST- CAPSAICIN	Anterior insula	39	20	-9	
	Anterior insula	-39	20	-6	
	Middle frontal gyrus	42	47	12	10
	Middle frontal gyrus	-45	45	17	46
	Inferior parietal lobule	42	-44	55	40
	Cingulate gyrus	3	17	41	32
	Cerebellum	-12	-80	-24	

[†]Where appropriate.

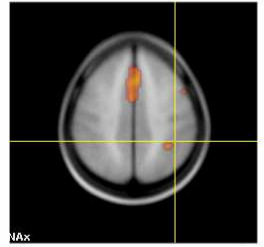
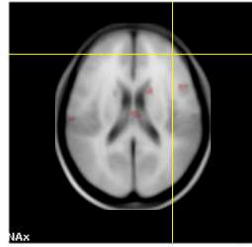
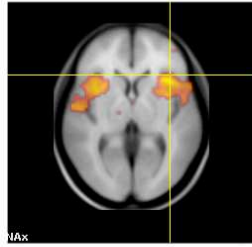
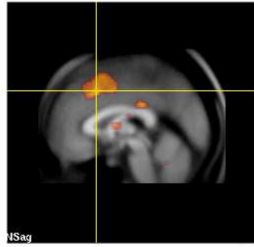
Table II: Results of paired t-tests for each region of interest, examining the main effects of “experiment” (pre versus post-capsaicin) and “side” (left versus right hemisphere). Capsaicin sensitisation has the greatest effect in the right hemisphere prefrontal and parietal ROIs. Asymmetrical BOLD response was found primarily in anterior insula, and is in keeping with neuroanatomical data (Craig 1998) and our previous study (Brooks et al. 2002). Activity within posterior insula is contralateral to the stimulation site for pre-capsaicin stimulation only, whilst for prefrontal cortex, rightwards asymmetry was observed following capsaicin sensitisation.

Region of interest	“Experiment” ¹		“Side” ^{1,2}	
	Right	Left	Pre	Post
Anterior insula	0.579	0.986	0.025 (R>L)	0.034 (R>L)
Middle insula	0.620	0.780	0.932	0.874
Posterior insula	0.732	0.900	0.043 (L>R)	0.107
Prefrontal	0.017	0.353	0.150	0.003 (R>L)
Parietal	0.016	0.049	0.188	0.492

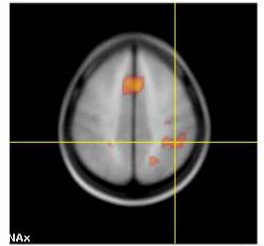
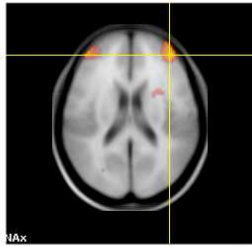
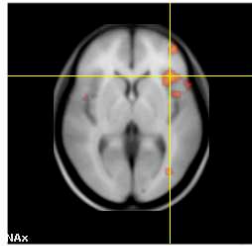
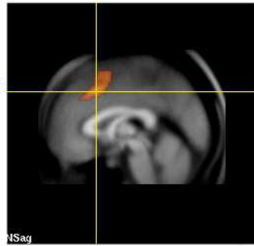
¹Note that, in the column labelled “experiment” right and left refer to laterality of the ROI, whilst in the column labelled “side” comparisons were made between left and right ROIs, and are distinguished by the experimental condition during data collection i.e. pre- or post-capsaicin.

²Where a significant asymmetry of BOLD response was found, the nature of the asymmetry is indicated.

PRE



POST



(a)

(b)

(c)

(d)

