Pain 114 (2005) 397–407

PAIN

www.elsevier.com/locate/pain

A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging

L. Zambreanu[a,b], R.G. Wise[a,b], J.C.W. Brooks[a,b], G.D. Iannetti[a,b], I. Tracey[a,b,*]

[a]*Pain Imaging Neuroscience (PaIN) Group, Department of Human Anatomy and Genetics, Oxford University, South Parks Road, Oxford OX1 3QX, UK*
[b]*Centre for Functional Magnetic Resonance Imaging of the Brain, Department of Clinical Neurology, Oxford University, John Radcliffe Hospital, Headington OX3 9DU, UK*

Received 30 June 2004; received in revised form 7 December 2004; accepted 10 January 2005

Abstract

Animal studies have established a role for the brainstem reticular formation, in particular the rostral ventromedial medulla (RVM), in the development and maintenance of central sensitisation and its clinical manifestation, secondary hyperalgesia. Similar evidence in humans is lacking, as neuroimaging studies have mainly focused on cortical changes. To fully characterise the supraspinal contributions to central sensitisation in humans, we used whole-brain functional magnetic resonance imaging at 3 T, to record brain responses to punctate mechanical stimulation in an area of secondary hyperalgesia. We used the heat/capsaicin sensitisation model to induce secondary hyperalgesia on the right lower leg in 12 healthy volunteers. A paired *t*-test was used to compare activation maps obtained during punctate stimulation of the secondary hyperalgesia area and those recorded during control punctate stimulation (same body site, untreated skin, separate session). The following areas showed significantly increased activation ($Z>2.3$, corrected $P<0.01$) during hyperalgesia: contralateral brainstem, cerebellum, bilateral thalamus, contralateral primary and secondary somatosensory cortices, bilateral posterior insula, anterior and posterior cingulate cortices, right middle frontal gyrus and right parietal association cortex. Brainstem activation was localised to two distinct areas of the midbrain reticular formation, in regions consistent with the location of nucleus cuneiformis (NCF) and rostral superior colliculi/periaqueductal gray (SC/PAG). The PAG and the NCF are the major sources of input to the RVM, and therefore in an ideal position to modulate its output. These results suggest that structures in the mesencephalic reticular formation, possibly the NCF and PAG, are involved in central sensitisation in humans.

Keywords: fMRI; Capsaicin; Hyperalgesia; Central sensitisation; Brainstem

1. Introduction

Neuropathic pain is a chronic condition caused by a primary lesion or dysfunction of the nervous system (Cruccu et al., 2004; Merskey and Bogduk, 1994), which can cause a central sensitisation state. It is a challenging form of pain, particularly difficult to treat, with different aetiologies, location and symptoms. It is characterised by spontaneous, ongoing pain, described as burning, shooting, prickling or electrical, and/or pain in response to innocuous stimuli (allodynia) and exaggerated pain in response to noxious stimuli (hyperalgesia).

Experimental models offer insight into the pathophysiologic mechanisms which underlie the plastic changes occurring in the central nervous system (CNS) during development of neuropathic pain. One such model involves topical application of capsaicin, a vanilloid receptor agonist, which elicits ongoing discharge in C-nociceptors and induces an area of hyperalgesia. Hyperalgesia occurs both at the site of application (primary hyperalgesia) and in the surrounding, untreated area (secondary hyperalgesia). While primary hyperalgesia is peripherally mediated and

* Corresponding author. Address: Pain Imaging Neuroscience (PaIN) Group, Department of Human Anatomy and Genetics, Oxford University, South Parks Road, Oxford OX1 3QX, UK. Tel.: +44 1865 272183; fax: +44 1865 282675.
E-mail address: irene.tracey@anat.ox.ac.uk (I. Tracey).

0304-3959/$20.00
doi:10.1016/j.pain.2005.01.005

is characterised by pain to thermal and mechanical stimuli, secondary hyperalgesia is centrally mediated and is characterised by pain to innocuous mechanical stimuli and increased pain to noxious mechanical stimuli but not to thermal stimulation (Ali et al., 1996).

Since central sensitisation is considered the underlying mechanism of secondary hyperalgesia, the spinal cord has been the principal focus in studies examining mechanisms of hyperalgesia. However, a growing body of evidence, primarily from rat studies, reveals significant contributions from supraspinal sites, particularly in the brainstem reticular formation, in the development and maintenance of central sensitisation and secondary hyperalgesia (Urban and Gebhart, 1999). Indeed, the pain modulatory system within the reticular formation of the brainstem, has a well documented role in supraspinal control and transmission of nociceptive information (Basbaum and Fields, 1984), making it an obvious candidate for involvement in secondary hyperalgesia. Recent evidence suggests that descending facilitatory influences could underlie some chronic pain states (for review see Gebhart, 2004; Porreca et al., 2002). The specific areas involved in chronic nociception in animals are the midbrain periaqueductal gray (PAG) and adjacent nucleus cuneiformis (NCF), the parabrachial nucleus in the rostral pons, and the rostral ventromedial medulla (RVM) (Suzuki et al., 2002; Urban and Gebhart, 1999; Williams and Beitz, 1993). To date, it is not known if these structures are similarly involved in human chronic pain.

In order to fully characterise the supraspinal contributions to secondary hyperalgesia in humans, we recorded whole-brain high-field (3 T) functional magnetic resonance imaging (fMRI) brain responses to punctate stimulation of an area of heat/capsaicin-induced secondary hyperalgesia in healthy subjects, and compared these findings with control stimulation of the same region in a separate imaging session.

2. Methods

2.1. Subjects

Twelve healthy individuals (right-handed, six males and six females) aged 27 ± 4.6 years participated in this study. Subjects were fully briefed on the experimental procedure and underwent comprehensive verbal screening to ensure they did not meet any of the exclusion criteria for magnetic resonance imaging (MRI). Written informed consent was obtained in accordance with the Declaration of Helsinki and the study was approved in full by the local ethics committee.

2.2. Induction of secondary hyperalgesia

In order to induce secondary hyperalgesia to punctate mechanical stimuli, we used the heat/capsaicin sensitisation model, which involves the combined action of heat and capsaicin (Petersen and Rowbotham, 1999). The heat/capsaicin treatment was applied to the anteromedial surface of the right lower leg of the subjects. A computer-controlled Peltier thermode (Medoc TSA-2001, Haifa, Israel) with an area of 9 cm^2 was used to deliver a 45 °C thermal stimulus, lasting 5 min. Thermal stimulation was followed immediately by topical application of 0.075% capsaicin cream (Axsain, Bioglan Laboratories Ltd, UK) to the same skin area for 45 min. This produces a robust area of secondary hyperalgesia, which is stable, reproducible, well-tolerated and has been validated in a number of drug trials (Dirks et al., 2000, 2002; Mikkelsen et al., 2001; Petersen et al., 2001).

The development of secondary hyperalgesia was confirmed by punctate mechanical stimulation with a von Frey probe (26 g, 0.546 mm diameter; Stoelting Co., USA). The hyperalgesic area was defined as the skin region in which punctate stimulation produced a definite change in the quality of the sensation described by the subjects as ‘painful’, ‘burning’, ‘tenderness’, ‘more intense pricking’, ‘more unpleasant’ and/or ‘longer-lasting’. No numerical scale was used; subjects were asked to describe the qualitative perception of von Frey hair stimulation in the presence or absence of heat/capsaicin treatment to confirm that the descriptors mentioned above were reported after treatment only.

2.3. Mechanical stimulation of the area of secondary hyperalgesia

We delivered punctate mechanical stimuli to the right lower leg of the subjects, in two functional magnetic resonance imaging (fMRI) sessions. In one session we delivered mechanical stimuli following heat/capsaicin treatment of the skin, i.e. we stimulated an area of secondary hyperalgesia (now referred to as the hyperalgesia session). In the other session, we delivered identical mechanical stimulation to the same area of skin in the absence of treatment (control session). Sessions were at least 24 h apart and their order was randomised.

As mentioned above, our punctate stimulus was a von Frey filament of 26 g (i.e. 255 mN) and 0.546 mm diameter. This corresponds to 8.5 bar, at which level mostly type I mechano-heat-sensitive A fibres (type I AMH units) (Treede et al., 1998) are activated. However, because the diameter of the punctate probe is larger than 0.2 mm, the stimulus cannot be selective for nociceptor activation (Treede et al., 2002) and tactile Aβ fibres are activated although to a small extent.

For the purpose of delivering mechanical stimuli in the scanner, two square areas were marked on the right lower leg of the subjects: an inner, 3 × 3 cm, square and an outer, larger square, extending 2 cm outside the former in each direction (Fig. 1). In both experimental sessions, we stimulated the outer, larger square (area B, highlighted in grey in Fig. 1). In the hyperalgesia session, the inner (3 × 3 cm) square marked the area of heat/capsaicin treatment, while in the control session the inner square was left untreated. The size of the stimulation area was 40 cm^2.

Previous psychophysical experiments confirmed that the heat/capsaicin stimulation successfully induces a large area of secondary hyperalgesia to punctate stimulation on the leg significantly larger than the secondary area stimulated during the imaging session (Doherty et al., 2003; Zambreanu et al., 2003).

2.4. Functional magnetic resonance imaging

2.4.1. Protocol

All subjects underwent two fMRI sessions, in random order. One of the sessions involved von Frey hair stimulation after

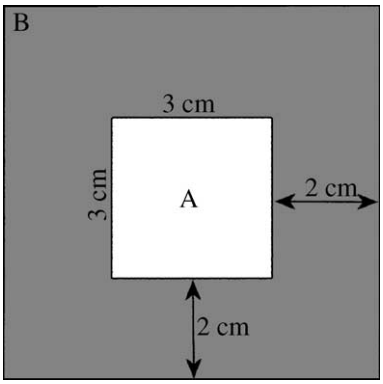

Fig. 1. Area of punctate stimulation. (A) Reference site (white area, 3×3 cm): heat/capsaicin treated in the hyperalgesia session and untreated in the control session. (B) Area of punctate stimulation (grey area, 2 cm outside the reference site in each direction): hyperalgesic following heat/capsaicin treatment and control site in the absence of treatment.

heat-capsaicin treatment (the hyperalgesia session), while the other session (the control session) involved stimulation of the same area in the absence of treatment. For the heat/capsaicin session, the data were acquired approximately 10 min after capsaicin was wiped off the leg.

During each imaging session subjects received 10 punctate stimuli to the pre-defined area (40 cm^2) on the lower right leg (see Fig. 1). The design of the experiment was event-related: one punctate stimulus, lasting 1 s, was delivered every 30 s. At the end of the scan, subjects were asked to describe qualitatively the average sensation elicited by mechanical stimulation and, if any, the sensation in between the stimuli (background sensation). All subjects described the sensation evoked by the von Frey filament as similar to that experienced prior to scanning (where we tested for the development of secondary hyperalgesia).

Online pain ratings were not recorded as we were not expecting a change or modulation of pain perception within imaging session, based on our psychophysical data (Doherty et al., 2003). Also, including an additional event, such as online pain rating can result in signal overlap due to the slowness of the BOLD response (DeYoe et al., 1994), causing saturation of the fMRI signal and decreased ability to detect the fMRI response and statistical power of its amplitude estimate unless the inter-stimulus interval is lengthened (Bandettini and Cox, 2000), which then increases the overall duration of the experiment, causing potential confounds with regard to the duration of secondary hyperalgesia.

2.4.2. fMRI data acquisition

Subjects were scanned in a 3 T human MRI scanner (Oxford Magnet Technology, 1 m bore) using a bird-cage radio frequency coil for pulse transmission and signal reception within a reduced bore gradient coil (Magnex SGRAD MK III). Physiological data (heart rate, pulse oximetry) were digitally recorded every second. A standard whole-brain gradient echo-planar imaging (EPI) sequence was used for functional scans (repetition time (TR) = 3 s; echo time (TE) = 30 ms; 21×6 mm thick axial slices; 214 volumes (the first four were 'dummy' scans), flip angle = 87°, field of view (FOV) = 192×256 mm, matrix = 64×64, voxel size = 3×4×6 mm). In addition, a T1-weighted high-resolution structural scan (64 slices×3 mm) was taken for anatomical overlay of activation.

2.4.3. fMRI image analysis

Analysis of fMRI images to identify regions exhibiting significant stimulus-correlated changes in BOLD (blood oxygen level dependent) signal was carried out in a multi-stage process using the image analysis package FEAT (FMRIB Expert Analysis Tool, www.fmrib.ox.ac.uk/fsl). The following pre-statistic processing was applied: motion correction using MCFLIRT (Motion Correction FMRIB's Linear Image Registration Tool) (Jenkinson and Smith, 2001), spatial smoothing using a Gaussian kernel of 5 mm full-width at half-maximum, non-linear high pass temporal filtering and a high-pass filter cut off of 30 s.

The first level of statistical analysis, the individual subject and session level, was carried out using a general linear modelling (GLM) approach (Friston et al., 1995). A model was constructed using a generalised gamma variate hemodynamic response function (HRF) (Boynton et al., 1996) representing the BOLD signal response to punctate stimulation. The input stimulus function (impulse stimuli representing the punctate stimuli) was convolved with the gamma HRF (mean lag 6 s and full-width-at-half-height 6 s) to yield the regressor for the general linear model. A single contrast, punctate stimulation versus baseline was formed. Group statistics (the higher level analysis) were carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) (Beckmann et al., 2003). A paired analysis was applied on a voxel-wise basis between the stimulus-evoked BOLD signal change in the secondary hyperalgesia condition and the control condition to yield a statistical parametric map. Significantly activated voxels were then thresholded at a Z score of at least 2.3 and a cluster significance threshold of $P < 0.01$ (corrected) was used (Forman et al., 1995; Friston et al., 1994; Worsley et al., 1992).

Registration to T1-weighted high-resolution individual subject images was carried out using FLIRT (FMRIB'S Linear Image Registration Tool) (Jenkinson and Smith, 2001). For the group analysis, registration was carried out onto the MNI (Montreal Neurological Institute) standard brain (Collins et al., 1994).

2.4.4. Localisation of brainstem activation

Specific anatomical landmarks were used to identify the location of the activation clusters in the brainstem, including the superior and inferior colliculi, the cerebral aqueduct, the ponto-mesencephalic junction, the posterior commissure and the midline. Sketches and micrographs adapted from Duvernoy (1995) (see Fig. 4) were used to help identify the location of this activation cluster in relation to the above-mentioned anatomical landmarks and are included to help orient the reader.

3. Results

3.1. Psychophysics

Following the heat/capsaicin treatment, all subjects reported a change in the quality of the sensation elicited by punctate stimulation compared to stimulation in the control condition (no heat/capsaicin treatment). The change in sensation was described as 'painful', 'burning', 'tender-tenderness', 'more intense pricking', 'more unpleasant' and/or 'lasting longer'. This was considered as evidence of development of secondary hyperalgesia. Post-scan

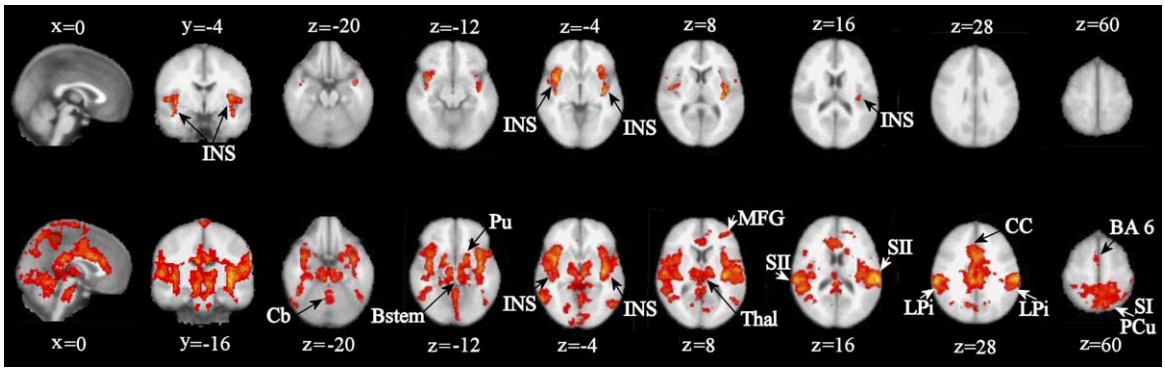

Fig. 2. Brain activation during punctate mechanical stimulation. Top row, in control skin; Bottom row, in the area of secondary hyperalgesia. Group activations registered onto the MNI standard brain in axial view, Z score >2.3, cluster corrected $P<0.01$. Both panels show a midline sagital section ($x=0$), followed to the right by a coronal section and then by a series of seven axial views in infero-superior succession. The y and z coordinates at the top and bottom of the figure indicate the antero-posterior and the superior–inferior location of the sections, respectively. INS, insula; Cb, cerebellum; Bstem, brainstem; Pu, putamen; MFG, middle frontal gyrus; Thal, thalamus; SII, secondary somatosensory cortex; LPi, inferior parietal lobule; CC, cingulate cortex; SI, primary somatosensory cortex; PCu, pre-cuneus (BA7); SMA, supplementary motor area (BA 6). Images are displayed in radiological convention.

interviews confirmed that none of the subjects had ongoing pain during the scanning.

3.2. Imaging results

Control punctate stimulation resulted in activation of the insular cortex bilaterally (see Fig. 2). In contrast, punctate stimulation of the area of secondary hyperalgesia resulted in extensive bilateral activation of the pain matrix: in the cerebellum, brainstem, thalamus, putamen, insula, secondary somatosensory cortices (SII) and inferior parietal lobule (Broadmann Area (BA) 40), as well as in the cingulate cortex, the left (contralateral) middle frontal gyrus (BA 10), the supplementary motor area (BA 6), the left (contralateral) pre-cuneus (BA 7) and the left (contralateral) primary somatosensory cortex (SI), in a region consistent with the representation of the leg on the somatosensory homunculus. The activation map obtained for the group in response to stimulation of the secondary hyperalgesia area is shown in Fig. 2.

The paired comparison (see Section 2.4.3) between functional data obtained during stimulation of the secondary hyperalgesia area and during control stimulation showed activation in several of the major pain processing areas (Peyron et al., 2000b). These included the bilateral insula, the left (contralateral) SI, in a region consistent with the representation of the leg on the somatosensory homunculus, the left (contralateral) SII, the anterior and posterior cingulate cortices (two clusters: BA 32 and BA 23), the bilateral thalamus, the contralateral brainstem and the cerebellum. Activation was also present in the right parietal association cortex with two separate clusters, one in the superior parietal cortex (BA 7) and one in the inferior parietal lobule and the pre-frontal cortex, again with two separate clusters, one in the right middle frontal gyrus (BA 10) and one in BA 6. Fig. 3 displays these group activations registered onto the MNI standard brain in axial view and Table 1 lists the coordinates of these clusters of activations in MNI space.

3.3. Brainstem activation

Two separate brainstem clusters showed significantly increased activation during secondary hyperalgesia compared to control stimulation. We found a distinct, very well-delineated cluster of activation in the lateral part of the mesencephalon, contralateral to the stimulation side. This activation extends throughout the rostro-caudal extension of the midbrain, from the rostral pons to the mesencephalo–diencephalic junction and is immediately adjacent to the ventrolateral PAG. It is bordered dorsally by the inferior colliculus in its caudal part and by the superior colliculus in its rostral part. The region described is consistent with the location of the nucleus cuneiformis (NCF) as described from human anatomical studies by Gioia and Bianchi (1987). In addition, the location is consistent with the same structure identified in animal studies by Edwards and de Olmos (1976) and Zemlan and Behbehani (1988). The sketches and micrographs provided in Fig. 4 (adapted from Duvernoy, 1995) were used to help identify the location of this activation cluster in relation to specific anatomical landmarks (e.g. the superior and inferior colliculi, the cerebral aqueduct, the ponto-mesencephalic junction, the posterior commissure and the midline) and are included to help orient the reader. The NCF, which is part of the reticular formation of the brainstem, has not been previously reported active in any human imaging studies to date.

The other brainstem cluster of activation was located superior to the activation described previously and is

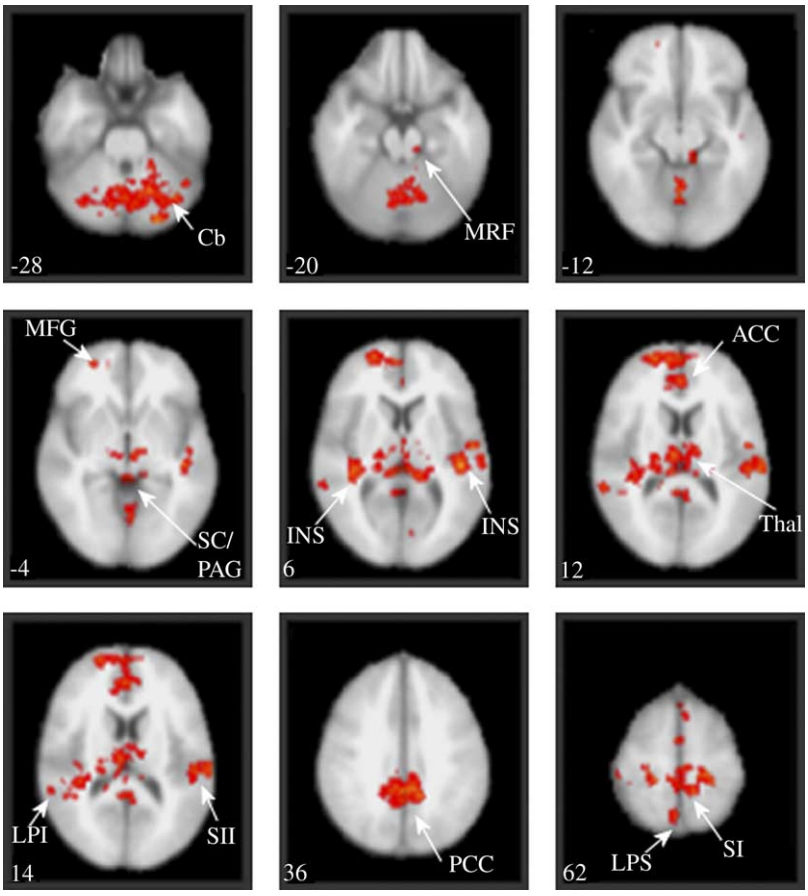

Fig. 3. Paired test between the brain activation maps in response to punctate stimulation of the secondary hyperalgesic area and control site stimulation. Group activations registered onto the MNI standard brain in axial view, Z score >2.3, cluster corrected $P<0.01$. Specific coordinates and Z scores for each cluster are listed in Table 1. Activation was detected in: the cerebellum (Cb), the midbrain reticular formation (MRF) with a lateral cluster corresponding to the location of nucleus cuneiformis (NCF) and a rostral midline cluster consistent with the periaqueductal gray/superior colliculi (SC/PAG), bilateral insula (INS) and thalamus (Thal), anterior and posterior cingulate cortex (ACC and PCC), primary (SI) and secondary (SII) somatosensory cortex, superior (LPS) and inferior (LPI) parietal cortex. The number on the bottom left corner of each panel represents the Z coordinate in standard space, i.e. the superior–inferior location of the slice. Images are displayed in radiological convention.

shown in Fig. 4. It is a midline cluster, extending rostro-audally from the posterior commissure to the upper part of the superior colliculi. It is bordered anteriorly by the third ventricle and extends dorsally as far as the posterior limit of the midbrain, encompassing the superior colliculi (Fig. 3). This corresponds to the anatomical location of the superior colliculi and rostral periaqueductal grey (PAG), whose most rostral region is at the level of the posterior commissure (Behbehani, 1995).

4. Discussion

Using high-field fMRI, we investigated the supraspinal responses to punctate stimulation during heat/capsaicin-induced central sensitisation and compared these with stimulation of normal, untreated skin, in healthy volunteers.

4.1. Central processing of control mechanical stimulation

Control stimulation activated the insular cortex bilaterally (Fig. 2). Activation was present in SII (peak Z scores: 3.7 ipsilateral and 3.4 contralateral), but it did not meet our stringent cluster threshold (corrected $P<0.01$). These areas are involved in processing both painful and non-painful somatosensory inputs (Treede et al., 1999). The lack of statistical significance in contralateral SI and bilateral SII can be explained by the different stimulus used (punctate filament: small size, activating mostly Aδ fibres versus brush: large, moving stimulus, activating only Aβ fibres) and by the stronger sensory input (i.e. higher density and frequency of stimulation) in other studies (Baron et al., 1999; Maihofner et al., 2004).

4.2. Central processing of secondary hyperalgesia

Stimulation of the hyperalgesic area produced extensive activation of the pain matrix (Fig. 2). A paired comparison

Table 1
Brain areas found significantly active for the group (cluster threshold, corrected $P < 0.01$) in the (secondary hyperalgesia condition compared with the control condition (paired test—FLAME)

Anatomical region	Brodmann area	MNI space coordinates of the peak Z score for the cluster			Z score
		x	y	z	
Cerebellum		−18	−60	−28	4.1
Nucleus cuneiformis		−10	−28	−18	3.3
Periaqueductal gray/ superior colliculus		0	−34	−4	3.4
Left insula		−46	−24	4	5.0
Left thalamus		−10	−30	6	3.4
Right thalamus		8	−28	8	3.6
Right insula		38	−26	8	3.5
Left secondary somatosensory cortex		−64	−26	10	3.7
Right inferior parietal	39	62	−40	10	3.5
Anterior cingulate cortex	32	2	44	14	3.5
Posterior cingulate cortex	23	−8	−40	36	4.2
Right parietal lobe	7	4	−58	66	3.1
Right middle frontal gyrus	10	22	62	10	4.0
Supplementary motor area	6	0	2	70	3.8
Left primary somatosensory area	1,2,3	−2	−30	78	4.0

between secondary hyperalgesia and control stimulation showed significantly increased activity in the pre-frontal and parietal association cortices, cingulate cortex, bilateral insula, contralateral SII and SI, bilateral thalamus, brainstem and cerebellum (Figs. 3 and 4, Table 1). These cortical activations are consistent with previous literature (see Table 2), despite numerous methodological differences: in spatial resolution (PET versus fMRI), slice coverage, analysis methods, experimental design (the presence of ongoing background in the study of Witting et al., 2001), choice of stimulus used to elicit hyperalgesia (brush versus punctate) and using capsaicin injection versus topical application.

The insula and SII are among the most consistently reported activations in pain imaging (Peyron et al., 2000b). Laser-evoked potentials in humans have shown bilateral dipolar sources in operculo-insular cortex (Garcia-Larrea et al., 2003) and direct electrical stimulation of the posterior insula evokes pain in humans (Ostrowsky et al., 2002). These areas seem to play a role in clinical pain as well, having been reported as active during clinical allodynia in neuropathic pain (Petrovic et al., 1999; Peyron et al., 1998, 2000a). Similarly, increased activity has been reported in SI during clinical allodynia as well as in experimental secondary hyperalgesia (Table 2). One explanation could be that this reflects increased perceived pain. However, nociceptive neurons in SI are scarce and it is equally likely that SI activation is due to increased attentional drive towards the allodynic stimulus, given that attentional processes can significantly influence somatosensory response (Hofbauer et al., 2001; Johansen-Berg et al., 2000).

The pre-frontal cortex (PFC) is reliably activated in all previous studies of secondary hyperalgesia and is generally linked to cognitive processing. Experimental pain and clinical pain often lead to activity changes in the frontal cortices (Apkarian et al., 2001; Baron et al., 1999; Derbyshire et al., 1997; Hsieh et al., 1995). It has been proposed that the PFC exerts active control on pain perception through top-down influences on the midbrain, via the thalamus and the cingulate (Lorenz et al., 2003; Valet et al., 2004), all of which have been found active in our study. The PFC and the posterior parietal association cortex (PPC) are the most densely interconnected areas of association cortex, they both project to numerous common cortical and subcortical regions and seem to operate in concert in response to stimulation (Derbyshire et al., 1997). Interestingly, the PPC has been demonstrated active in both experimental pain (Coghill et al., 2001; Peyron et al., 2000b) and neuropathic pain (Hsieh et al., 1995; Petrovic et al., 1999; Peyron et al., 1998). It seems likely therefore that activation in the PFC and PPC is linked to hypervigilance and/or increased attention towards pain, as seen in several clinical pain disorders.

During central sensitisation, we found extensive activation of the entire cingulate cortex (Fig. 2). In the paired comparison with the control condition, only two clusters showed significance: one in the perigenual and one in the posterior cingulate cortices (BA 32 and BA 23) (Fig. 3).

The perigenual ACC is thought to encode unpleasantness, the emotional component of pain, and anxiety (Rainville et al., 1997; Vogt et al., 1996). The affective component of experimental pain, and possibly perigenual ACC activation, can be reduced by repeated stimulation, subject training, and habituation to the paradigm in neuroimaging experiments. This area has not been reported active in previous studies of secondary hyperalgesia, which used a greater frequency of stimulation and number of stimuli delivered. In our experiment, the event-related design eliminated that potential bias. Most previous studies have used capsaicin injection to induce secondary hyperalgesia (Baron et al., 1999; Iadarola et al., 1998; Witting et al., 2001) and consequently, the stimulus-evoked pain and anxiety could have been reduced compared to the high levels of pain previously experienced during capsaicin injection. The perigenual ACC has been suggested to exert top-down influences on the brainstem to gate pain modulation during cognitive tasks such as distraction (Bantick et al., 2002; Valet et al., 2004) as well as during placebo and opioid analgesia (Petrovic et al., 2002; Wager et al., 2004). It could be that these top-down influences also play a role in facilitation or pronociception, as well as in antinociception (Tracey and Dunckley, 2004).

Although traditionally implicated in visuospatial attention and evaluative processes, activation of the posterior

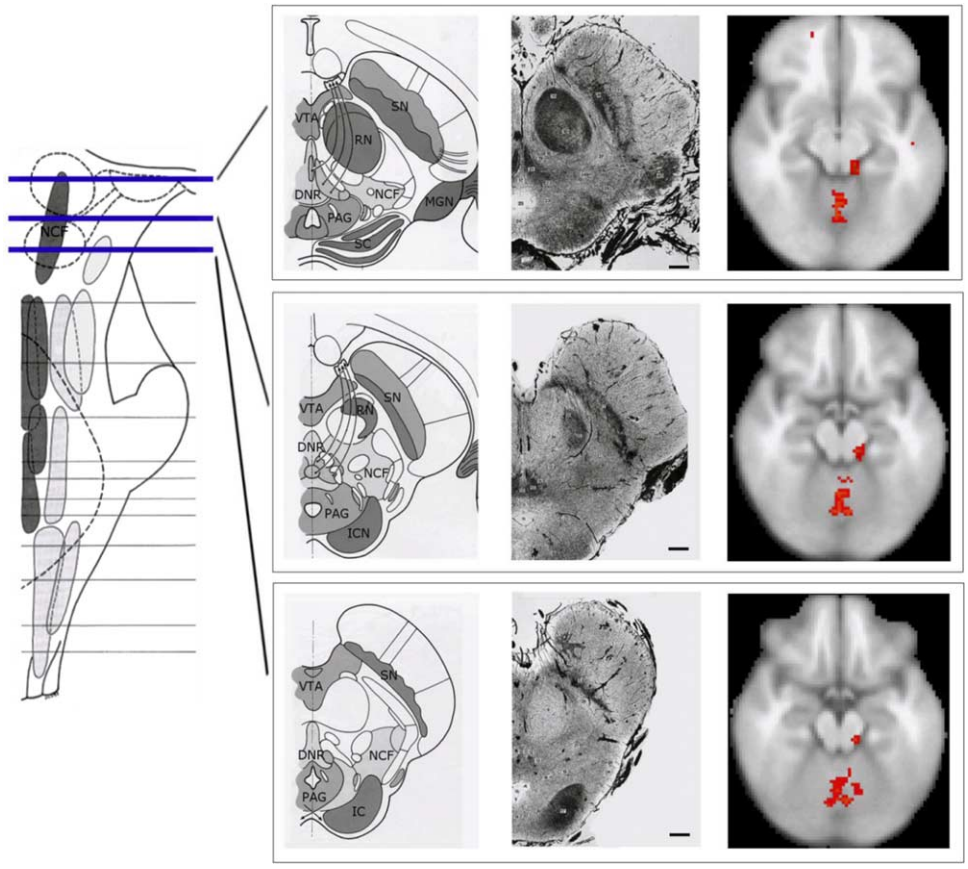

Fig. 4. Localization of the lateral activation cluster in the midbrain reticular formation. The image on the left-hand side of the figure is a coronal section through the brainstem (modified from Duvernoy, 1995) and the superimposed dark blue lines represent the rostro-caudal levels of the three rows of axial sections on the right-hand side of the figure. The left and middle columns of axial sections are adapted sketches and micrographs, respectively, from Duvernoy (1995) (with permission) showing key features of the reticular formation. The column of axial slices on the right-hand side of the figure shows brainstem activation in the paired test between punctate stimulation of the secondary hyperalgesia area and control area. Our cluster of activation lies ventrolateral to the midline periaqueductal gray (PAG) in the mesencephalic tegmentum and is bordered dorsally by the inferior and superior colliculi. This matches the location of the nucleus cuneiformis, as indicated on the column of three sketches. DNR, dorsal nucleus of the raphe; IC, inferior colliculus; ICN, intercollicular nucleus; MGN, medial geniculate nucleus; NCF, nucleus cuneiformis; PAG, periaqueductal gray; RN, red nucleus; SC, superior colliculus; SN, substantia nigra; VTA, ventral tegmental area.

cingulate cortex (PCC) is also reported in human pain studies. Tolle et al. (1999) showed encoding of pain intensity in the PCC. This could explain PCC activation in our study, since with development of secondary hyperalgesia, punctate stimulation becomes painful. Interestingly, activation of the PCC has been reported in chronic pain patients (Derbyshire et al., 2002; Hsieh et al., 1995; Lorenz et al., 1998), however, its role has yet to be fully elucidated. Similarly, cerebellar activation is reported in pain imaging studies, using a wide variety of stimuli (Peyron et al., 2000b) and in one study investigating brush allodynia (Iadarola et al., 1998), but its role in pain processing is unknown. The cerebellum receives nociceptive inputs through the lateral reticular nucleus of the medulla, via the spinoreticular tract (Willis, 1989) and BOLD response in the cerebellum correlates with pain intensity (Helmchen et al., 2003). However, increased cerebellar activity has been often attributed to intention of performing motor response or withdrawal (Peyron et al., 2000b; Saab and Willis, 2003). Studies examining psychological aspects of pain have found activity changes in the cerebellum (Ploghaus et al., 1999; Smith et al., 2002), suggesting a higher cognitive function for the cerebellum in pain processing.

During central sensitisation, activation significantly increased in the midbrain reticular formation in two distinct clusters, the location of which was consistent with the contralateral NCF, rostral PAG and superior colliculi. No previous study of secondary hyperalgesia reported subcortical activations presumably because of reduced brain coverage (Baron et al., 1999; Maihofner et al., 2004) and lower spatial resolution with PET imaging (Iadarola et al., 1998; Witting et al., 2001).

Brainstem structures have a long-recognized capacity for descending modulation of pain. In particular, the RVM has an established role in the development and maintenance of central sensitisation and secondary hyperalgesia in animals

Table 2
Summary of brain activation patterns reported in imaging studies of secondary hyperalgesia in healthy volunteers

Authors	Stimulus & side	SI		SII/LPi		Insula		ACC		PCC		PFC		PP		Thalamus		Brainstem		Cerebellum	
		Ipsi	*Contra*	*Ipsi*	*Contra*	*Ipsi*	*Contra*	*Ipsi*	*Contra*	*Ipsi*	*Contra*	*Ipsi*	*Contra*	*Ipsi*	*Contra*	*Ipsi*	*Contra*	*Ipsi*	*Contra*	*Ipsi*	*Contra*
Control stimulation																					
Iadarola et al. (1998)[a]	Brush; **Left**		+	+	+	+	+	+				+		+							
Baron et al. (1999)[b,*]	Punctate; **Right**		+	+	+																
Witting et al. (2001)[a,†]	Brush; **Right**-C, **Left**-SH																				
Maihofner et al. (2004)[b]	Brush; **Right**-C, **Left**-SH		+	+	+		+								+						
Zambreanu et al. (2005)[b]	Punctate; **Right**					+	+														
Secondary hyperalgesia																					
Iadarola et al. (1998)[a]	Brush; **Left**		+	+	+	+	+	+	+			+	+								
Baron et al. (1999)[b,*]	Punctate; **Right**		+	+	+								+								
Witting et al. (2001)[a,†]	Brush; **Right**-C, **Left**-SH				+	+	+	+	+			+	+		+						
Maihofner et al. (2004)[b]	Brush; **Right**-C, **Left**-SH		+	+	+	+	+						+		+						
Zambreanu et al. (2005)[b]	Punctate; **Right**		+	+	+	+	+	+	+		+		+		+	+	+	+	+	+	+

SI/SII, primary/secondary somatosensory cortex; LPi, inferior parietal lobule; ACC/PCC, anterior/posterior cingulate cortex; PFC, pre-frontal cortex; PP, posterior parietal lobe; Right-C, control stimulation was right-sided; Left-SH, stimulation of secondary hyperalgesia area was left-sided.

[a] PET study.

[b] fMRI study.

* Limited coverage (eight slices, 5–6 mm thick).

† Ongoing pain present during secondary hyperalgesia.

(Urban and Gebhart, 1999). The PAG and the NCF are the major sources of input to the RVM (Basbaum and Fields, 1984; Behbehani and Zemlan, 1986), and hence in an ideal position to modulate its output, i.e. modulate spinal nociception. The PAG and the NCF, like the RVM, have a physiological substrate for bidirectional modulation of pain processing, in that they have functionally distinct classes of cells which either facilitate (on-cells), or inhibit (off-cells) nociception (Fields et al., 1983; Haws et al., 1989; Heinricher et al., 1987).

Rostral PAG stimulation has been reported to produce pain relief in chronic pain patients (Hosobuchi, 1981, 1987) and block mechanical allodynia in animal models of neuropathic pain (Pertovaara et al., 1996). Data on NCF stimulation in humans is lacking but animal studies show a role for the NCF in development of chronic pain. Porro et al. (1991) found the NCF to be the only brainstem structure showing greater activity levels in the late phase of acute nociception compared to the early phase, in a formalin injection model. In addition, Williams and Beitz (1993) investigated brainstem activity in different rat models of chronic pain and found that, as nociception became chronic, activity increased most strikingly in the contralateral NCF. These data support our results showing involvement of midbrain reticular structures, possibly the NCF and PAG, in central sensitisation.

In summary, many of the same brain regions are involved in processing acute experimental pain, pain from models of hyperalgesia and clinical pain. However, an area where differences might exist between these conditions, as suggested by our results, is within specific structures of the brainstem. Our findings support a role for the midbrain reticular formation in central sensitisation in humans. We acknowledge that multiple sites are most likely involved in central sensitisation, and it could be that further differences might be better highlighted by examining the magnitude of activation changes and connectivity between areas of the pain matrix and brainstem structures. However, we propose that a phylogenetically ancient structure, such as the brainstem reticular formation, is more likely to play an important role in mediating this often long-lasting condition commonly found across mammalian species.

Acknowledgements

We wish to acknowledge the Clarendon Fund and McDonnell-Pew Centre for Cognitive Neuroscience (LZ), the Wellcome Trust (RW), Dr Hardwen Trust (JCWB), the Medical Research Council UK (FMRIB Centre) and HEFCE (IT). We would also like to thank Dr Stephen Smith for image analysis advice and Prof. Anthony Dickenson for helpful advice and discussion.

References

Ali Z, Meyer RA, Campbell JN. Secondary hyperalgesia to mechanical but not heat stimuli following a capsaicin injection in hairy skin. Pain 1996; 68:401–11.

Apkarian AV, Thomas PS, Krauss BR, Szeverenyi NM. Prefrontal cortical hyperactivity in patients with sympathetically mediated chronic pain. Neurosci Lett 2001;311:193–7.

Bandettini PA, Cox RW. Event-related fMRI contrast when using constant interstimulus interval: theory and experiment. Magn Reson Med 2000; 43:540–8.

Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I. Imaging how attention modulates pain in humans using functional MRI. Brain 2002;125:310–9.

Baron R, Baron Y, Disbrow E, Roberts TP. Brain processing of capsaicin-induced secondary hyperalgesia: a functional MRI study. Neurology 1999;53:548–57.

Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci 1984;7: 309–38.

Beckmann C, Jenkinson M, Smith S. General multi-level linear modelling for group analysis in FMRI. Neuroimage 2003;20:1052–63.

Behbehani MM. Functional characteristics of the midbrain periaqueductal gray. Prog Neurobiol 1995;46:575–605.

Behbehani MM, Zemlan FP. Response of nucleus raphe magnus neurons to electrical stimulation of nucleus cuneiformis: role of acetylcholine. Brain Res 1986;369:110–8.

Boynton GM, Engel SA, Glover GH, Heeger DJ. Linear systems analysis of functional magnetic resonance imaging in human V1. J Neurosci 1996; 16:4207–21.

Coghill RC, Gilron I, Iadarola MJ. Hemispheric lateralization of somatosensory processing. J Neurophysiol 2001;85:2602–12.

Collins DL, Neelin P, Peters TM, Evans AC. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr 1994;18:192–205.

Cruccu G, Anand P, Attal N, Garcia-Larrea L, Haanpaa M, Jorum E, Serra J, Jensen TS. EFNS guidelines on neuropathic pain assessment. Eur J Neurol 2004;11:153–62.

Derbyshire SW, Jones AK, Gyulai F, Clark S, Townsend D, Firestone LL. Pain processing during three levels of noxious stimulation produces differential patterns of central activity. Pain 1997;73:431–45.

Derbyshire SW, Jones AK, Creed F, Starz T, Meltzer CC, Townsend DW, Peterson AM, Firestone L. Cerebral responses to noxious thermal stimulation in chronic low back pain patients and normal controls. Neuroimage 2002;16:158–68.

DeYoe EA, Bandettini P, Neitz J, Miller D, Winans P. Functional magnetic resonance imaging (FMRI) of the human brain. J Neurosci Methods 1994;54:171–87.

Dirks J, Fabricius P, Petersen KL, Rowbotham MC, Dahl JB. The effect of systemic lidocaine on pain and secondary hyperalgesia associated with the heat/capsaicin sensitization model in healthy volunteers. Anesth Analg 2000;91:967–72.

Dirks J, Petersen KL, Rowbotham MC, Dahl JB. Gabapentin suppresses cutaneous hyperalgesia following heat-capsaicin sensitization. Anesthesiology 2002;97:102–7.

Doherty J, Zambreanu L, Brooks J, Tracey I. A psychophysical study of a secondary hyperalgesia model suitable for fMRI experiments, Pain in Europe IV. In: The European Federation of the IASP Chapters, Prague.

Duvernoy HM. The human brain stem and cerebellum. Surface, structure, vascularization and three-dimensional anatomy with MRI. Wien: Springer; 1995.

Edwards SB, de Olmos JS. Autoradiographic studies of the projections of the midbrain reticular formation: ascending projections of nucleus cuneiformis. J Comp Neurol 1976;165:417–31.

Fields HL, Bry J, Hentall I, Zorman G. The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat. J Neurosci 1983;3:2545–52.

Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA, Noll DC. Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magn Reson Med 1995;33:636–47.

Friston KJ, Tononi G, Reeke Jr GN, Sporns O, Edelman GM. Value-dependent selection in the brain: simulation in a synthetic neural model. Neuroscience 1994;59:229–43.

Friston KJ, Frith CD, Frackowiak RS, Turner R. Characterizing dynamic brain responses with fMRI: a multivariate approach. Neuroimage 1995; 2:166–72.

Garcia-Larrea L, Frot M, Valeriani M. Brain generators of laser-evoked potentials: from dipoles to functional significance. Neurophysiol Clin 2003;33:279–92.

Gebhart GF. Descending modulation of pain. Neurosci Biobehav Rev 2004; 27:729–37.

Gioia M, Bianchi R. The cytoarchitecture of the nucleus cuneiformis. A Nissl and Golgi study. J Anat 1987;155:165–76.

Haws CM, Williamson AM, Fields HL. Putative nociceptive modulatory neurons in the dorsolateral pontomesencephalic reticular formation. Brain Res 1989;483:272–82.

Heinricher MM, Cheng ZF, Fields HL. Evidence for two classes of nociceptive modulating neurons in the periaqueductal gray. J Neurosci 1987;7:271–8.

Helmchen C, Mohr C, Erdmann C, Petersen D, Nitschke MF. Differential cerebellar activation related to perceived pain intensity during noxious thermal stimulation in humans: a functional magnetic resonance imaging study. Neurosci Lett 2003;335:202–6.

Hofbauer RK, Rainville P, Duncan GH, Bushnell MC. Cortical representation of the sensory dimension of pain. J Neurophysiol 2001;86: 402–11.

Hosobuchi Y. Periaqueductal gray stimulation in humans produces analgesia accompanied by elevation of beta-endorphin and ACTH in ventricular CSF. Mod Probl Pharmacopsychiatry 1981;17:109–22.

Hosobuchi Y. Dorsal periaqueductal gray-matter stimulation in humans. Pacing Clin Electrophysiol 1987;10:213–6.

Hsieh JC, Belfrage M, Stone-Elander S, Hansson P, Ingvar M. Central representation of chronic ongoing neuropathic pain studied by positron emission tomography. Pain 1995;63:225–36.

Iadarola MJ, Berman KF, Zeffiro TA, Byas-Smith MG, Gracely RH, Max MB, Bennett GJ. Neural activation during acute capsaicin-evoked pain and allodynia assessed with PET. Brain 1998;121(Pt 5):931–47.

Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. Med Image Anal 2001;5:143–56.

Johansen-Berg H, Christensen V, Woolrich M, Matthews PM. Attention to touch modulates activity in both primary and secondary somatosensory areas. NeuroReport 2000;11:1237–41.

Lorenz J, Kohlhoff H, Hansen HC, Kunze K, Bromm B. Abeta-fiber mediated activation of cingulate cortex as correlate of central post-stroke pain. NeuroReport 1998;9:659–63.

Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 2003;126: 1079–91.

Maihofner C, Schmelz M, Forster C, Neundorfer B, Handwerker HO. Neural activation during experimental allodynia: a functional magnetic resonance imaging study. Eur J Neurosci 2004;19:3211–8.

Merskey H, Bogduk N. Classification of chronic pain. 394. Seattle, WA: IASP Press; 1994. 394 pp.

Mikkelsen S, Dirks J, Fabricius P, Petersen KL, Rowbotham MC, Dahl JB. Effect of intravenous magnesium on pain and secondary hyperalgesia associated with the heat/capsaicin sensitization model in healthy volunteers. Br J Anaesth 2001;86:871–3.

Ostrowsky K, Magnin M, Ryvlin P, Isnard J, Guenot M, Mauguiere F. Representation of pain and somatic sensation in the human insula: a study of responses to direct electrical cortical stimulation. Cereb Cortex 2002;12:376–85.

Pertovaara A, Wei H, Hamalainen MM. Lidocaine in the rostroventromedial medulla and the periaqueductal gray attenuates allodynia in neuropathic rats. Neurosci Lett 1996;218:127–30.

Petersen KL, Rowbotham MC. A new human experimental pain model: the heat/capsaicin sensitization model. NeuroReport 1999;10:1511–6.

Petersen KL, Jones B, Segredo V, Dahl JB, Rowbotham MC. Effect of remifentanil on pain and secondary hyperalgesia associated with the heat—capsaicin sensitization model in healthy volunteers. Anesthesiology 2001;94:15–20.

Petrovic P, Ingvar M, Stone-Elander S, Petersson KM, Hansson P. A PET activation study of dynamic mechanical allodynia in patients with mononeuropathy. Pain 1999;83:459–70.

Petrovic P, Kalso E, Petersson KM, Ingvar M. Placebo and opioid analgesia—imaging a shared neuronal network. Science 2002;295: 1737–40.

Peyron R, Garcia-Larrea L, Gregoire MC, Convers P, Lavenne F, Veyre L, Froment JC, Mauguiere F, Michel D, Laurent B. Allodynia after lateral-medullary (Wallenberg) infarct. A PET study. Brain 1998;121(Pt 2): 345–56.

Peyron R, Garcia-Larrea L, Gregoire MC, Convers P, Richard A, Lavenne F, Barral FG, Mauguiere F, Michel D, Laurent B. Parietal and cingulate processes in central pain. A combined positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) study of an unusual case. Pain 2000a;84:77–87.

Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin 2000b;30:263–88.

Ploghaus A, Tracey I, Gati JS, Clare S, Menon RS, Matthews PM, Rawlins JN. Dissociating pain from its anticipation in the human brain. Science 1999;284:1979–81.

Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. Trends Neurosci 2002;25:319–25.

Porro CA, Cavazzuti M, Galetti A, Sassatelli L. Functional activity mapping of the rat brainstem during formalin-induced noxious stimulation. Neuroscience 1991;41:667–80.

Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC. Pain affect encoded in human anterior cingulate but not somatosensory cortex. Science 1997;277:968–71.

Saab CY, Willis WD. The cerebellum: organization, functions and its role in nociception. Brain Res Brain Res Rev 2003;42:85–95.

Smith KA, Ploghaus A, Cowen PJ, McCleery JM, Goodwin GM, Smith S, Tracey I, Matthews PM. Cerebellar responses during anticipation of noxious stimuli in subjects recovered from depression. Functional magnetic resonance imaging study. Br J Psychiatry 2002;181:411–5.

Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. Nat Neurosci 2002;5:1319–26.

Tolle TR, Kaufmann T, Siessmeier T, Lautenbacher S, Berthele A, Munz F, Zieglgansberger W, Willoch F, Schwaiger M, Conrad B, Bartenstein P. Region-specific encoding of sensory and affective components of pain in the human brain: a positron emission tomography correlation analysis. Ann Neurol 1999;45:40–7.

Tracey I, Dunckley P. Importance of anti- and pro-nociceptive mechanisms in human disease. Gut 2004;53:1553–5.

Treede RD, Meyer RA, Campbell JN. Myelinated mechanically insensitive afferents from monkey hairy skin: heat-response properties. J Neurophysiol 1998;80:1082–93.

Treede RD, Kenshalo DR, Gracely RH, Jones AK. The cortical representation of pain. Pain 1999;79:105–11.

Treede RD, Rolke R, Andrews K, Magerl W. Pain elicited by blunt pressure: neurobiological basis and clinical relevance. Pain 2002;98: 235–40.

Urban MO, Gebhart GF. Supraspinal contributions to hyperalgesia. Proc Natl Acad Sci USA 1999;96:7687–92.

Valet M, Sprenger T, Boecker H, Willoch F, Rummeny E, Conrad B, Erhard P, Tolle TR. Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain—an fMRI analysis. Pain 2004;109:399–408.

Vogt BA, Derbyshire S, Jones AK. Pain processing in four regions of human cingulate cortex localized with co-registered PET and MR imaging. Eur J Neurosci 1996;8:1461–73.

Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, Davidson RJ, Kosslyn SM, Rose RM, Cohen JD. Placebo-induced changes in FMRI in the anticipation and experience of pain. Science 2004;303: 1162–7.

Williams FG, Beitz AJ. Chronic pain increases brainstem proneurotensin/-neuromedin-N mRNA expression: a hybridization-histochemical and immunohistochemical study using three different rat models for chronic nociception. Brain Res 1993;611:87–102.

Willis WD. The origin and destination of pathways involved in pain transmission. In: Wall P, Melzack R, editors. The textbook of pain. London: Churchill-Livingstone; 1989.

Witting N, Kupers RC, Svensson P, Arendt-Nielsen L, Gjedde A, Jensen TS. Experimental brush-evoked allodynia activates posterior parietal cortex. Neurology 2001;57:1817–24.

Worsley KJ, Evans AC, Marrett S, Neelin P. A three-dimensional statistical analysis for CBF activation studies in human brain. J Cereb Blood Flow Metab 1992;12:900–18.

Zambreanu L, Wise R, Tracey I. Imaging secondary hyperalgesia. A functional magnetic resonance imaging study using the heat/capsaicin sensitization model. J Pain 2003;4:39.

Zemlan FP, Behbehani MM. Nucleus cuneiformis and pain modulation: anatomy and behavioral pharmacology. Brain Res 1988; 453:89–102.