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Determining anatomical connectivities between cortical and brainstem pain processing regions in humans: A diffusion tensor imaging study in healthy controls

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Abstract

Neuroimaging methods have so far identified various structures in the brain involved in the processing of pain and its control. However, our understanding of their anatomical connectivities is relatively weak. Diffusion tensor imaging (DTI), a magnetic resonance imaging-based method, allows in vivo mapping of the anatomical connections in the human brain and was used to investigate the white matter connections originating from the periaquaductal grey (PAG) and nucleus cuneiformis (NCF). We performed DTI on 8 healthy right-handed male volunteers. Group analysis showed that tract paths could be defined and their likelihood quantified for connections between the PAG and separately for the NCF, to the prefrontal cortex, amygdala, thalamus, hypothalamus and rostroventral medial medulla bilaterally. The connections identified confirm the existence of an anatomical circuitry for the functionally characterised top-down influences on pain processing via brainstem structures in humans.

Keywords: DTI; PAG; NCF; Pain

1. Introduction

Neuroimaging methods have substantially advanced our understanding of cortical pain processing by defining spatial localisation of function, as well as temporal information regarding the order of neural processing. However, it is less clear how these structures physically interact to bring about pain perception. Generally in humans it is poorly understood how different areas communicate with each other and if there is a direct or indirect physical connection between brain regions. Additionally, there is uncertainty that anatomical connections within the animal brain are equivalent to those found in humans.

Diffusion tensor imaging (DTI) is an MRI-based technique that can map white matter anatomical connections in the living human brain. DTI measures the diffusion of water in different regions of the brain and after subsequent processing, calculates a principal direction of diffusion for water in each imaging voxel. Diffusion direction varies with tissue environment, for example water in white matter tracts has anisotropic diffusion due to the orientational structure of cells. This anisotropic motion of water in white matter tracts allows determination of their anatomical course within the human brain (Conturo et al., 1999; Basser et al., 2000). This enables us to see physical connections between functionally localised brain regions to improve

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our understanding of brain networks. DTI can therefore predict possible relationships between cortical and subcortical areas using diffusion-weighted data. These in turn can be interpreted in light of fMRI and PET results to draw functional conclusions regarding networks.

The PAG and NCF are brainstem nuclei involved in a network mediating anti- and pro-nociception, as largely shown from animal data (Fields and Basbaum, 1999 for a review, Porreca et al., 2002; Gebhart, 2004). Retrograde tracer studies in animals have shown these two nuclei to be connected with each other, as well as to: the rostroventral medial medulla, thalamus, secondary somatosensory and prefrontal cortex (Edwards and de Olmos, 1976; Bragin et al., 1984; Zemlan and Behbehani, 1988; Bernard et al., 1989). Human imaging studies have shown that the PAG and NCF are activated during visceral and somatic pain (Dunckley et al., 2005) and that the NCF plays a key role in the development of experimental secondary hyperalgesia via possible facilitatory pro-nociceptive mechanisms (Zambreanu et al., 2005). Human FMRI and PET studies show the anterior cingulate cortex, insula, primary and secondary somatosensory cortex, thalamus and prefrontal cortex to be significant regions of activation during nociceptive stimulation paradigms (Peyron et al., 2000; Apkarian et al., 2005; Tracey, 2005), and therefore believed to be involved in generating a perception of pain. Moreover, recent studies have shown there to be a significant correlation between cortical, particularly prefrontal cortex, and brainstem areas such as the PAG during the modulation of pain (Lorenz et al., 2003; Valet et al., 2004; Wager et al., 2004). Therefore, it was the aim of this study to assess if the anatomical circuitry between cortical and subcortical structures shown to be involved in nociception in human and animal data exists in healthy human volunteers using DTI.

2. Methods

2.1. Subjects and image acquisition

Eight right-handed male subjects, with median age 24 and range 20–35, were screened for MR compatibility and scanned with ethical approval and informed consent. All MRI images were acquired on a Siemens Sonata 1.5 T MR scanner. Each subject had a T1 weighted $1 \times 1 \times 1$ mm structural image acquired (parameters: repetition time 12 ms, echo time 5.65 ms, and flip angle 90°). The diffusion-weighted images were acquired using echo planar imaging according to the protocol in Johansen-Berg et al. (2004) with a voxel size of $2 \times 2 \times 2$ mm.

The diffusion weighting was isotropically distributed along 60 directions by using a b-value of 1000 s mm^{-2}. For each diffusion-weighted set, five volumes with no diffusion weighting were acquired at points throughout the acquisition. Three diffusion weighted data sets were acquired in total for subsequent averaging to improve the signal-to-noise ratio. Additionally, an optimised $3.0 \times 0.75 \times 0.75$ mm proton density scan was obtained for a single subject (Dunckley et al., 2005; parameters as follows: turbo factor, 3; effective TE, 12 ms; TR, 5 s). This optimised structural image highlights the grey and white matter differences in the brainstem.

2.2. Image processing

All the image processing was completed using the diffusion tools in the FSL software package (FMRIB's software library, http://www.fmrib.ox.ac.uk/fsl/, Smith et al., 2004). The data were corrected for head motion and eddy current artefacts using affine registration to a reference volume (Jenkinson and Smith, 2001). The three volumes were subsequently averaged, to increase the signal-to-noise ratio. Probabilistic tractography was performed as described in Behrens et al. (2003), Johansen-Berg et al. (2004), and Johansen-Berg et al. (2005).

Probabilistic tractography utilizes the non-isotropic diffusion of water in white matter. Water diffusion will mirror neuronal pathways as the myelin sheaths act as barriers to isotropic diffusion. Therefore, for each voxel in the image matrix a diffusion 'tensor' may be calculated. This represents the magnitude of water diffusion along each orientation. The orientation of maximal diffusion is called the principal diffusion direction or PDD. As multiple tracts may exist within an individual voxel and the images acquired will inherently contain noise, there will be a degree of uncertainty associated with the PDD. Uncertainty is smallest in regions of homogeneous white matter. Grey matter and complex regions of white matter have greater uncertainty. The use of Bayes' rule allows us to compute a probability density function (PDF) for the uncertainty of the PDD based on the parameters given in the data (Behrens et al., 2003). Having calculated the PDF, Markov Chain Monte Carlo sampling can be used to identify possible tract directions based on the PDFs. Multiple sampling of the dataset produces connectivity distributions, i.e., the possible tracts that could occur from a particular region of the brain. This method allows prediction of small white matter bundles, which may have a direction of diffusion that differs from the main PDD, which would otherwise be ignored by methods of tractography not incorporating uncertainty.

Probabilistic tractography provides quantification of the likelihood of connectivity between the seed structure and a destination of interest and an image of the tract path between the seed and destination.

To enable us to perform tractography in the native space of the diffusion images, but to examine results in the space of the T1-weighted structural scans, or in MNI space for group analysis, we computed affine transformation matrices between diffusion, T1-weighted and MNI space (Jenkinson and Smith, 2001).

2.3. Defining the structures of interest

Seed masks for the regions of interest for tractography were drawn for both hemispheres for the: (i) prefrontal cortex, (ii) perigenual anterior cingulate cortex, (iii) amygdala, (iv) hypothalamus, (v) thalamus, (vi) rostroventral medial medulla, (vii) periaquaductal grey, and (viii) nucleus cuneiformis. This was done with fslview in FSL (http://www.fmrib.ox.ac.uk/fsl/

fslview/index.html) for each subject individually. These regions were chosen based on their involvement in antinociception in animals (Edwards and de Olmos, 1976; Bragin et al., 1984; Zemlan and Behbehani, 1988; Bernard et al., 1989; Fields and Basbaum, 1999; Porreca et al., 2002; Gebhart, 2004). *Duvernoy's atlas of the Human Brainstem and Cerebellum* (Duvernoy, 1995, pp. 56–72, 70–78) was used to locate the NCF, PAG and RVM. The optimised proton density scan was used to aid location of the PAG and NCF (see Fig. 1).

Seed masks for the amygdala, thalamus and hypothalamus were drawn using the same brainstem and cerebellum atlas and Duvernoy's atlas of Human Brain surface, blood supply and three-dimensional anatomy (Duvernoy, 1999, pp. 336–386). The perigenual ACC and the four subsections of the PFC were drawn using *Talairach Co-Planar Stereotaxic Atlas of the Human Brain* (Talairach and Tournoux, 2000, pp. 42–58, 84–110). The PFC was divided into dorsolateral, dorsomedial, ventrolateral and ventromedial based on the criteria given by Northoff et al. (2004) which are based on Rajkowska and Goldman-Rakic, 1995. The criteria used were:

- Dorsolateral (dlPFC) – The dorsal part of the medial frontal gyrus
- Dorsomedial (dmPFC) – The dorsal part of the superior frontal gyrus
- Ventrolateral (vlPFC) – The ventral part of the inferior frontal gyrus
- Ventromedial (vmPFC) – The ventral part of the superior frontal gyrus, and the frontal pole.

The distinction between dorsal and ventral for the prefrontal cortex was defined as the flat plane that passes through the anterior and posterior poles of the corpus callosum.

The regions of interest were deliberately underestimated during masking. That is, we deliberately excluded areas of mask at the boundaries between anatomical regions. This policy ensures that the mask consisted only of the region of interest. The benefit of this is a reduction in the number of false positive outcomes, as well as increased confidence in the results produced. The cost of this is a slight reduction in the output of the probabilistic tractography. Samples that passed into the ventricular system, in either MNI or structural space due to slight mis-registrations from diffusion space, were excluded.

Masks were drawn on the T1 structural and its MNI space transform and not diffusion-weighted scans, as the former image types provided the necessary spatial resolution and contrast to allow recognition of anatomical structures to process the tractography output. To ensure the chosen structures of interest mapped correctly into diffusion space, we compared the location of the seed masks in structural space and, having transformed them, in native diffusion space. In all eight subjects, the locations of the seed masks in diffusion space agreed with the anatomical maps, and their location in structural space (data not shown).

2.4. Image analysis

Diffusion tractography has often been used to find and or analyse large white matter tracts (Stieltjes et al., 2001; Mori and van Zijl, 2002; Mori et al., 2002; Huang et al., 2005) and probabilistic tractography has brought the possibility of inferring smaller non-dominant pathways. However, even with this technique, studies to date have concentrated on larger pathways.

In this study, the pathways of interest are both small and non-dominant. In order to have confidence in the results, trials were run to find the optimal size for sampling the PDF for probabilistic tractography. The optimal size is one in which the whole range of possible diffusion directions is accurately represented by the sample. This depends on the number of voxels in the seed mask, the resolution of the seed mask and the homogeneity of the white matter tracts in the region. The optimal size was defined as one in which subsequent, sequential increases in sampling rate resulted in $<5\%$ difference in tractography outputs. Sampling was tested with values of 5000, 10,000, 15,000 and 20,000 for connections between the NCF and thalamus, and NCF and prefrontal cortex (data not shown). The difference between outputs of the different sample sizes converged to $<5\%$ with higher sampling rates. Based on these calculations, a sample size of 10,000 was used for quantification of uncertainty or connectivity analyses and a sample size of 15,000 was used for tract image generation.

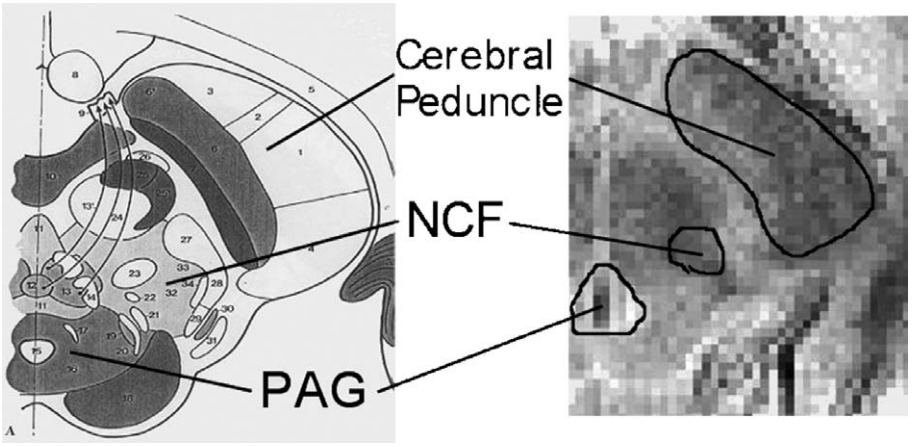


Fig. 1. Midbrain anatomy. (Left) A midbrain section adapted with permission from Duvernoy's Brainstem and Cerebellum atlas (p. 72). (Right) Proton density image showing the equivalent brainstem section and the locations of the NCF and PAG. The cerebral peduncles are highlighted for reference. PAG, periaquaductal grey; NCF, nucleus cuneiformis.

The quantification of tract pathways was performed in native diffusion space for each subject with masks in T1 structural space. T1 structural space was used as opposed to MNI space for the better resolution, as this process depends heavily on the density of voxels in the seed mask. Defining tracts is more successful with a greater number of voxels in the seed mask. Each subject's tract output for each specific tract was summed across the group to get the mean tract connectivity likelihood and the group standard error was calculated for each tract. The subject's individual likelihood of connectivity used here was the average of all the voxels' tract values in the entire seed mask.

DTI remains a relatively novel imaging method and statistical methods are currently not available to calculate the specific significance of an identified tract across subjects. This makes analyses of small tracts difficult. Therefore in addition to the above quantification analysis, the number of individuals in which a successful connection could be defined (*success rate*) was calculated. Success rate is a reliable predictor of tract existence for very small tracts. The ability to define a tract consistently across a group is a very good indicator of its existence and of the robustness of the technique. We considered that a tract was likely to exist if it could be found in at least half the group, i.e., $\geqslant 4/8$ subjects. This is the 'tract defining criterion'. Although this is an arbitrary criterion, in the absence of any statistical methods it is a reasonable criterion we believe.

The generation of tract images (seed mask to waypoint mask tractography) was completed in MNI space for each subject to minimise any potential registration error. This allowed overlay of individual images to show where in the brain across the group the individual subjects had found analogous paths. The criterion mentioned above cannot be applied to the output of image generation. The group output for image generation tests the frequency a voxel was found to be in a group tract. It is possible for all eight subjects to find a tract, but still score a 6/8 in the group image due to the intra-group variability in the specific direction of the pathway.

All parameters for tractography were the same as the connectivity based analysis, with the exception that 15,000 samples per voxel were used to compensate for the reduced number of voxels in the seed mask because of the lower resolution (10,000 samples was borderline for output consistency).

2.5. Controls

A positive control tract from the thalamus to the prefrontal cortex was performed. Positive control tracts originating from the brainstem lack validation, as this is what was under investigation. A theoretical negative control of no output from the tractography was used. A non-theoretical negative control attempted to define a connection from the PAG/NCF to the auditory cortex (which is not known to exist). No output was found, however, interference from the lateral fasciculi could have resulted in this negative result. In addition to this, to show that the tracts originating from the NCF and PAG are separate, a quantitative comparison between the PAG tracts before and after NCF exclusion was made.

3. Results

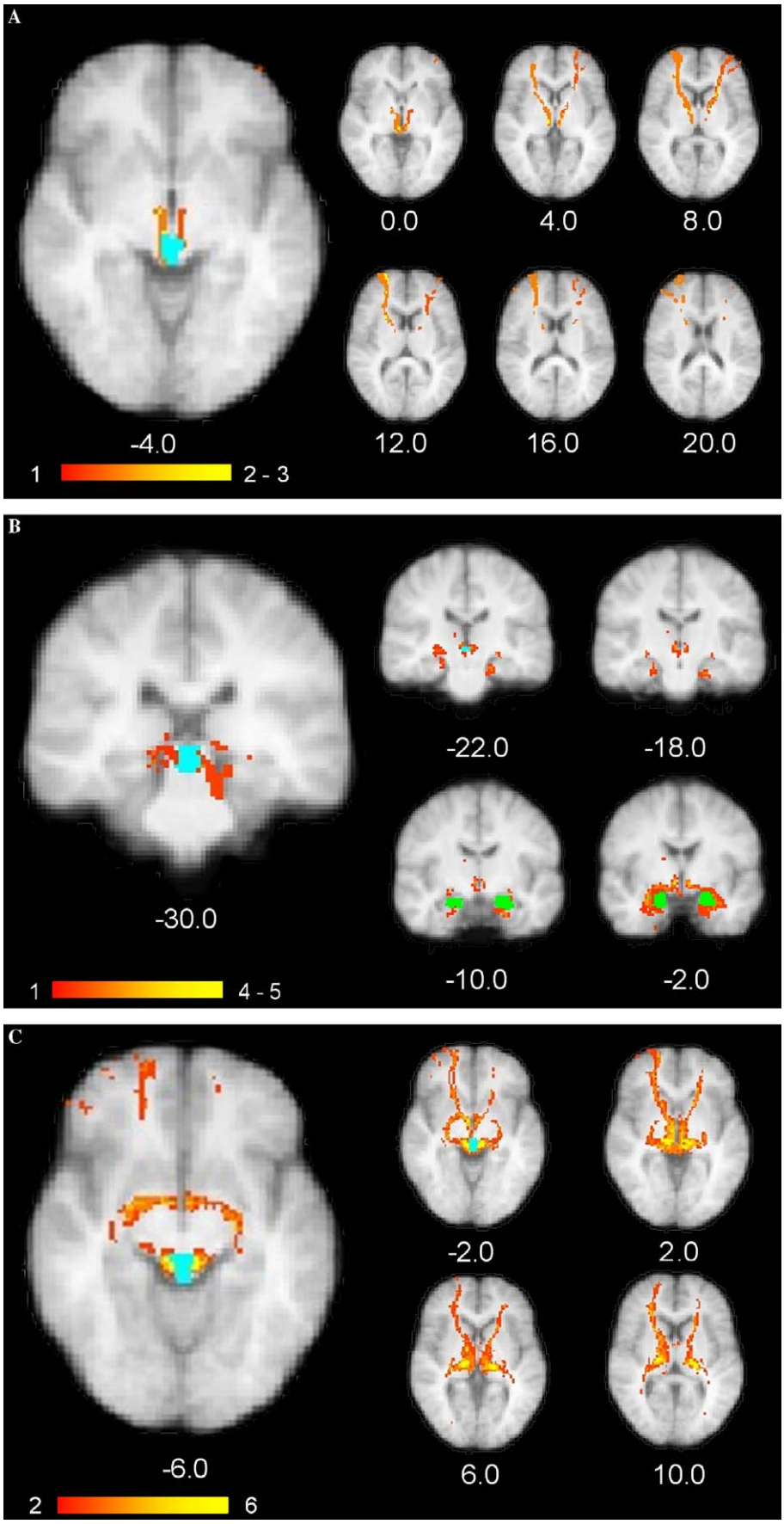
Connections were found originating from both the NCF and PAG that were symmetrical for the left and right hemispheres, and followed similar courses having left the brainstem. Tracts passed through the thalamus and hypothalamus to terminate in cortical regions. Tracts terminated in the prefrontal cortex, amygdala and RVM. Fig. 2 shows the connections from the PAG to the dorsolateral prefrontal cortex (Fig. 2A), amygdala (Fig. 2B) and thalamus (Fig. 2C). Fig. 3 shows the connections the PAG has with the dorsomedial (Fig. 3A), ventromedial (Fig. 3B), and ventrolateral prefrontal cortex (Fig. 3C).

All subjects displayed processable diffusion tensor image sets that were applicable to probabilistic tractography. Additionally, the positive control was found in all eight subjects (Fig. 4). It too shows the symmetry and consistency of the tract path found for the connections originating in the brainstem and provides validation of our data collection and analytical methods.

Table 1 shows the group connectivity values and success rates of tracts originating in the NCF and PAG and passing to the: (i) perigenual anterior cingulate cortex, (ii) amygdala, (iii) hypothalamus, (iv) thalamus, (v) rostroventral medial medulla, (vi) dorsolateral prefrontal cortex, (vii) dorsomedial prefrontal cortex, (viii) ventrolateral prefrontal cortex, (ix) ventromedial prefrontal cortex and (x) connectivity values between the PAG and NCF.

All connections but one satisfied the tract criterion described earlier, which was to have a success rate greater than half the group. Only paths to the perigenual ACC failed to satisfy this criterion, with only 2/8 subjects able to define a connection. In both cases, the tract image was neither symmetrical nor consistent. The connection from the NCF to the RVM narrowly fails the qualifying criterion, in that it satisfies the criterion for

Fig. 2. (A) Connectivity tracts from the periaquaductal grey to the dorsolateral prefrontal cortex. The colour bar shows that the left hemisphere scored 3/8, and the right hemisphere scored 2/8. The tract path originates from the periaquaductal grey (blue), passes through the thalamus to reach the dorsolateral prefrontal cortex. Coordinates correspond to transverse MNI (Montreal Neurological Institute) space. N.B. The colour bars indicate the frequency a voxel was consistently found across the group, not necessarily the number of subjects that defined a tract image. (B) Connectivity tracts from the periaquaductal grey to the amygdala. The colour bar shows that the left hemisphere scored 4/8, and the right hemisphere scored 5/8. The tract path originates from the periaquaductal grey and appears to pass through the hypothalamus (Sagittal −18.0 to +4.0) to reach the amygdala. (C) Connectivity tracts from the periaquaductal grey to the thalamus. The colour bar shows both hemispheres scored 6/8. This tract path originates from the periaquaductal grey passes directly through the thalamus to reach the prefrontal cortex. All voxels with a score <2 have been excluded for clarity.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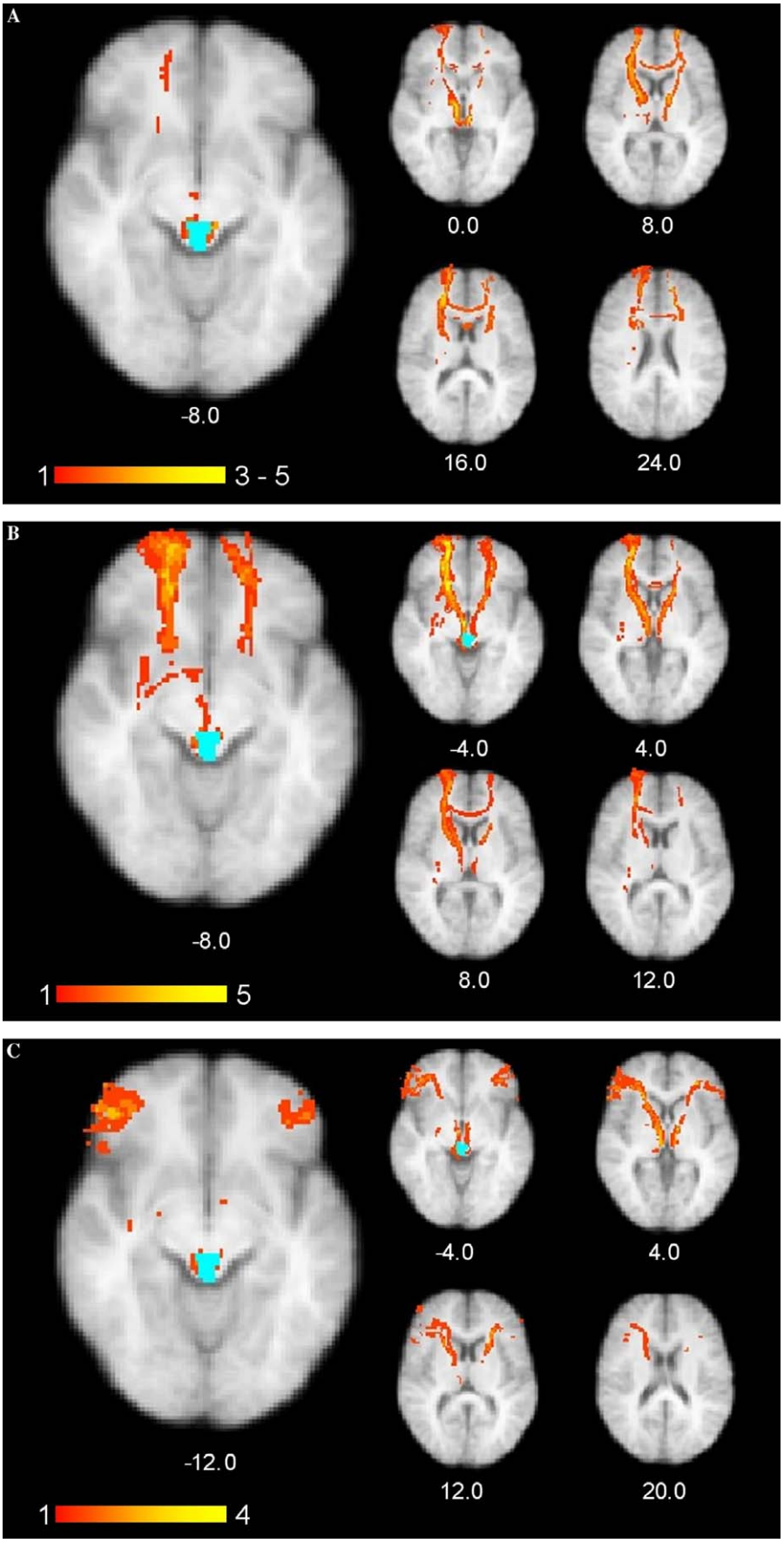
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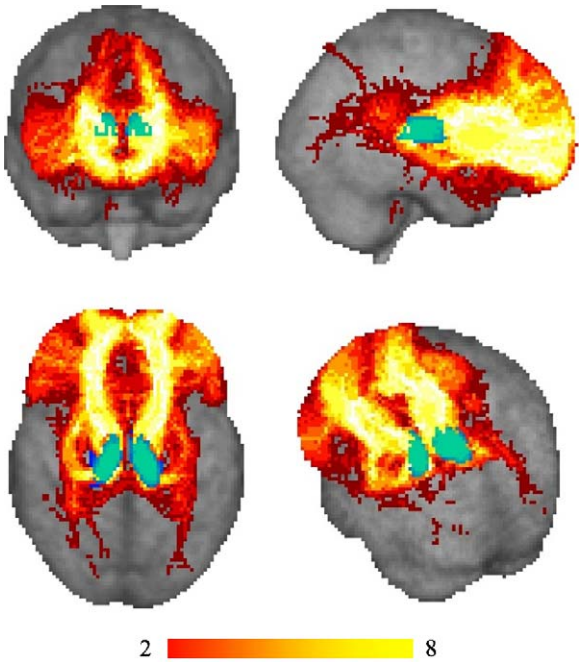

Fig. 4. 3D rendering of the connections between the thalamus and prefrontal cortex in MNI space. Positive control. Thalamus (green). (Top Left) Anterior posterior view. (Top Right) Lateral view. (Bottom Left) Superior inferior view. (Bottom Right) Azimuth 330°, elevation: 37°. All voxels with a score <2 have been excluded for clarity.

the left hemisphere (4/8) but not the right (3/8) due to low signal-to-noise. Nonetheless, a network loop has been defined here linking the PAG, NCF and RVM. The connectivity data replicate the findings displayed in the tract images.

The results from the connectivity analysis and tract images lead to the conclusion that connections from both the PAG and NCF go through (and most likely connect with) the hypothalamus and thalamus, and terminate in the amygdala and four regions of the prefrontal cortex. Likewise, a network exists between the PAG, NCF and RVM.

Due to the close proximity of the PAG and NCF and the locations of the targets, any tract originating in the PAG could pass through the NCF to reach the frontal cortex, amygdala or brainstem. To ensure that the connections originating from the PAG and NCF are separate and therefore independent tracts, a control was run. The connectivity values to all target areas, including the subdivisions of the prefrontal cortex, were compared before and after seeding the PAG with and without excluding the NCF. In both cases, the same connections were found with similar mean values (not shown here). Therefore, it can be said that all tracts originating from the PAG are separate from those of the NCF given this evidence.

4. Discussion

The results of the probabilistic tractography show that there are connections from the PAG, and separately for the NCF to the: prefrontal cortex, amygdala, thalamus and hypothalamus and RVM, agreeing with animal data (Edwards and de Olmos, 1976; Bragin et al., 1984; Zemlan and Behbehani, 1988; Bernard et al., 1989). More recently, the NCF and PAG have been shown to be important in brainstem modulation of pain in humans (Zambreanu et al., 2005), and connections to these nuclei provide an anatomical basis for results of recent studies involving pain control and perception (Lorenz et al., 2003; Valet et al., 2004; Wager et al., 2004).

The amygdala is believed to be involved in coding the uncertainty associated with pain and fear (Bornhovd et al., 2002; Misslin, 2003) and believed to be relevant for planning anti-nociceptive strategies (Bingel et al., 2002). Hypoalgesia induced by opioid stimulation in the amygdala, periaquaductal grey and rostrovental medial medulla suggests that neuronal circuitry between these three regions is involved in planning and mediating antinociception (Helmstetter et al., 1998). In addition to this, bilateral lesions of the amygdala in rhesus monkeys result in a lack of antinociception and fear response, suggesting that fear and antinociception functionally overlap in this region (Manning et al., 2001). If the amygdala has a central role in pain processing, then a physical connection to the brainstem inhibitory network, as described here in man, would help mediate hypoalgesia in relation to uncertainty and fear.

Both the PAG and NCF showed very strong connections to the thalamus. The thalamus is a key area of convergence of ascending spinal nociceptive information (Craig and Dostrovsky, 1999) showing activation in pain studies and decreased activation during nociceptive control. It is possible that the connection from the brainstem to the thalamus could be modulating ascend-

Fig. 3. (A) Connectivity tracts from the periaquaductal grey to dorsomedial prefrontal cortex. The left hemisphere scored 3/8, and the right hemisphere scored 5/8. The tract path originates from the periaquaductal grey (blue), passes directly through the hypothalamus and thalamus and continues to the dorsomedial prefrontal cortex. Coordinates correspond to transverse MNI (Montreal Neurological Institute) space. N.B. The colour bars indicate the frequency a voxel was consistently found across the group, not necessarily the number of subjects that defined a tract image. (B) Connectivity tracts from the periaquaductal grey to ventromedial prefrontal cortex. Both hemispheres scored 5/8. The tract path originates from the periaquaductal grey (blue), passes directly through the hypothalamus and thalamus and continues to the ventromedial prefrontal cortex. Coordinates correspond to transverse MNI space. (C) Connectivity tracts from the periaquaductal grey to ventrolateral prefrontal cortex. Both hemispheres scored 4/8. The tract path originates from the periaquaductal grey (blue), passes through the thalamus to reach the ventrolateral prefrontal cortex. Coordinates are shown in transverse MNI space.

Table 1
List of the connectivity and success rates for tracts originating from the NCF and PAG

	$n = 8$	PACC	Amygdala	Hypothalamus	Thalamus	PFC				Brainstem	
						dlPFC	dmPFC	vlPFC	vmPFC	PAG	RVM
2×2×2 NCF											
Left tract connectivity	Success rate	**3**	**4**	**8**	**8**	**6**	**8**	**6**	**8**	**8**	**4**
	Mean	0.08	0.82	1196.25	5405.61	2.28	16.17	9.45	19.62	705.44	0.34
	Stand. Err	0.06	0.75	353.08	1048.89	2.01	7.64	4.91	5.09	280.78	0.25
Right tract connectivity	Success rate	**1**	**5**	**7**	**8**	**6**	**7**	**6**	**8**	**8**	**3**
	Mean	0.02	0.12	488.78	6037.95	65.01	20.54	65.72	128.95	1015.09	0.06
	Stand. Err	0.02	0.08	449.14	1245.79	64.68	17.99	35.47	139.90	581.04	0.05
	$n = 8$	PACC	Amygdala	Hypothalamus	Thalamus	PFC				Brainstem	
						dlPFC	dmPFC	vlPFC	vmPFC	NCF	RVM
2×2×2 PAG											
Left tract strength	Success rate	**3**	**5**	**8**	**8**	**4**	**5**	**7**	**8**	**8**	**5**
	Mean	1.38	1.04	1464.52	504.85	0.06	1.70	0.44	1.59	155.94	1.71
	Stand. Err	1.06	0.70	474.98	313.74	0.05	1.28	0.43	1.24	58.53	1.87
Right tract strength	Success rate	**3**	**8**	**8**	**8**	**7**	**7**	**7**	**8**	**8**	**6**
	Mean	0.06	1.75	1619.77	1375.28	2.66	10.44	3.53	58.63	158.77	0.06
	Stand. Err	0.06	1.37	302.55	504.90	2.09	8.42	2.07	37.59	102.70	0.03

The mean and standard error (Stand. Err – 95% Confidence Intervals) are out of 10,000. Strength values for Control Thalamus – PFC are: Left mean = 928.70 ± 122.18, Right mean = 1118.77 ± 172.19; Success rate = 8 for control in both hemispheres. PACC, perigenual anterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; vlPFC, ventrolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex; PAG, periaquaductal grey; NCF, nucleus cuneifromis; PFC, prefrontal cortex; Stand err, standard error.

ing nociceptive flow at the thalamic level. This finding warrants further study, particularly as the NCF has recently been shown to be critical for the generation of secondary hyperalgesia in humans (Zambreanu et al., 2005).

We would have expected strong connectivity from the PAG to the perigenual ACC given the correlation found between the two by Valet et al. (2004) and the ACC's role in cognitive and attentional aspects of pain perception (Petrovic and Ingvar, 2002). Two possibilities could account for the lack of connectivity established here. First, it is possible that any attempt at establishing a connection between the ACC and the brainstem was blocked by the large white matter tracts of the corpus callosum. It is particularly challenging to define small tracts within or passing perpendicularly through the large diffusion vectors of white matter bundles due to the complexity of signals and noise (Mori and van Zijl, 2002). Second, it could be that the ACC communicates indirectly with the brainstem via cortico-cortical connections by which it could influence areas such as the PFC to induce reduced pain perception.

Different regions of the prefrontal cortex have been shown to play a role in pain modulation. Valet et al. (2004) showed a correlation between activation within cingulo-frontal regions and brainstem structures during pain processing, supporting the concept that the connection between the PFC and brainstem is important in pain modulation. Lieberman et al. (2004) showed that activation within the right vlPFC was significantly increased after placebo training and therefore concluded to be linked to the placebo-related outcome of diminished pain unpleasantness. The vmPFC may exert inhibitory control on the pain-relevant affective signals (Ingvar and Hsieh, 1999). Remy et al. (2003) showed that there was increased activation of the dmPFC and mid-cingulate and decreased activation in the perigenual ACC, mid-thalamus and insula cortex when performing word repetition or generation during pain compared to without pain. The connections of the vlPFC, vmPFC and dmPFC to the brainstem defined here support the notion that a physical pathway exists in the human brain to drive changes in pain perception in relation to placebo (Wager et al., 2004), cognitive function and pain type and its aversiveness.

The dorsolateral prefrontal cortex has recently been shown to be important in the control of pain perception. The dlPFC correlates negatively with perceived pain intensity (Lorenz et al., 2003) and when subjects were treated with rTMS (regional transcranial magnetic stimulation) over the left dlPFC, there was a significant reduction in chronic pain (Brighina et al., 2004). The right dlPFC has been to shown to have a selective effect of increasing pain tolerance compared to the left dlPFC (Graff-Guerrero et al., 2005) and pain catastrophising behaviour is also linked with increased activity in the dlPFC (Gracely et al., 2004). The question remains as to how these widely found and specific activation changes within PFC regions mediate a change in pain perception. One obvious route is via the descending pain modulatory system within the brainstem that can drive both anti- and pro-nociceptive pathways to elicit a

change in pain perception (Porreca et al., 2002; Gebhart, 2004). The PAG and NCF form part of the brainstem antinociceptive network that control ascending spinal nociceptive flow, and a connection to the dlPFC via the thalamus as shown here in the human brain, supports a top-down role in modulating pain perception. The physical connections determined in this study, between the dlPFC and brainstem therefore provide the necessary circuitry.

Further evidence in studies of pathological pain states supports this conclusion. Apkarian et al. (2004) showed using volume based morphometry, that there is significant reduction in bilateral dlPFC grey matter volume and the right thalamus. This supports the idea that pain pathologies involve changes in thalamocortical connectivity. Grachev et al. (2000) showed that there are changes in the *N*-acetyl aspartate levels, an indicator of changes in neuronal viability and function, in the dlPFC in patients suffering from chronic low back pain. Such losses in connectivity, particularly in tracts implicated in anti-nociception, as found here, could contribute to these pain pathologies.

This study shows the power diffusion tensor imaging has to define human brain anatomical connections. This does not mean it can replace histological techniques, as histology can determine if a tract is direct or indirect and define its polarity, e.g., corticobulbar or bulbocortical, whereas DTI cannot. Nonetheless, the application of probabilistic tractography in this study demonstrates that it is possible to characterise small non-dominant pathways in a qualitative and quantitative manner, as can be done for large dominant tracts (Stieltjes et al., 2001; Mori et al., 2002; Huang et al., 2005). The ability to do this will improve as the resolution at which diffusion images can be taken improves.

Future characterisation of the entire cortical pain processing network in humans is clearly important given the results shown here. In particular how networks change with age and with pathology is a route for investigation, as evidence now suggests that structural deficits in regions involved in the modulation of pain occur in chronic pain syndromes, with the possibility of loss of connectivity. Improving the resolution at which probabilistic tractography can be performed is key to this goal.

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References

Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 2005;9(4):463–84. Epub 2005 Jan 21.

Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, et al. Chronic back pain is associated with decreased prefrontal and thalamic grey matter density. J Neurosci 2004;24(46):10410–5.

Basser PJ, Pajevic S, Pierpaoli C, Duda J, Aldroubi A. In vivo fiber tractography using DT-MRI data. Magn Reson Med 2000;44(4):625–32.

Behrens TE, Woolrich MW, Jenkinson M, Johansen-Berg H, Nunes RG, Clare S, et al. Characterization and propagation of uncertainty in diffusion-weighted MR imaging. Magn Reson Med 2003;50(5):1077–88.

Bernard JF, Peschanski M, Besson JM. Afferents and efferents of the rat cuneiformis nucleus: an anatomical study with reference to pain transmission. Brain Res 1989;490(1):181–5.

Bingel U, Quante M, Knab R, Bromm B, Weiller C, Buchel C. Subcortical structures involved in pain processing: evidence from single-trial fMRI. Pain 2002;99(1–2):313–21.

Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C, Buchel C. Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. Brain 2002;125(Pt 6):1326–36.

Bragin EO, Yeliseeva ZV, Vasilenko GF, Meizerov EE, Chuvin BT, Durinyan RA. Cortical projections to the periaqueductal grey in the cat: a retrograde horseradish peroxidase study. Neurosci Lett 1984;51(2):271–5.

Brighina F, Piazza A, Vitello G, Aloisio A, Palermo A, Daniele O, et al. rTMS of the prefrontal cortex in the treatment of chronic migraine: a pilot study. J Neurol Sci 2004;227(1):67–71.

Conturo TE, Lori NF, Cull TS, Akbudak E, Snyder AZ, Shimony JS, et al. Tracking neuronal fiber pathways in the living human brain. Proc Natl Acad Sci USA 1999;96(18):10422–7.

Craig AD, Dostrovsky JO. Medulla to thalamus. In: Wall Patrick D, Melzack R, editors. Textbook of pain. United Kingdom: Churchill Livingstone; 1999. p. 183–214. ISBN 0-443-06252-8.

Dunckley P, Wise RG, Fairhurst M, Hobden P, Aziz Q, Chang L, et al. A comparison of visceral and somatic pain processing in the human brainstem using functional magnetic resonance imaging. J Neurosci 2005;25(32):7333–41.

Duvernoy HM. Axial (horizontal) sections. In: Duvernoy HM, editor. The human brain surface, blood supply, and three-dimensional sectional anatomy. New York: Springer Wien New York; 1999. p. 336–86. ISBN 3-221-83158-4.

Duvernoy HM, Structure of the brainstem. In: Duvernoy HM, editor. The human brain stem and cerebellum. New York: Springer-Verlag Wien New York; 1995. p. 47, 56–62, and 70–78. ISBN 3-211-82503-7.

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(4):417–31.

Fields HL, Basbaum AI. Central nervous system mechanisms of pain modulation. In: Wall Patrick D, Melzack R, editors. Textbook of pain. United Kingdom: Churchill Livingstone; 1999. p. 309–29. ISBN 0-443-06252-8.

Gebhart GF. Descending modulation of pain. Neurosci Biobehav Rev 2004;27(8):729–37.

Gracely RH, Geisser ME, Giesecke T, Grant MA, Petzke F, Williams DA, et al. Pain catastrophizing and neural responses to pain among persons with fibromyalgia. Brain 2004;127(Pt 4):835–43. Epub 2004 Feb 11.

Grachev ID, Fredrickson BE, Apkarian AV. Abnormal brain chemistry in chronic back pain: an in vivo proton magnetic resonance spectroscopy study. Pain 2000;89(1):7–18.

Graff-Guerrero A, Gonzalez-Olvera J, Fresan A, Gomez-Martin D, Carlos Mendez-Nunez J, Pellicer F. Repetitive transcranial magnetic stimulation of dorsolateral prefrontal cortex increases tolerance to human experimental pain. Brain Res Cogn Brain Res 2005. May 31.

Helmstetter FJ, Tershner SA, Poore LH, Bellgowan PS. Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla. Brain Res 1998;779(1–2):104–18.

Huang H, Zhang J, Jiang H, Wakana S, Poetscher L, Miller MI, et al. DTI tractography based parcellation of white matter: application to the mid-sagittal morphology of corpus callosum. Neuroimage 2005;26(1):195–205.

Ingvar M, Hsieh JC. The image of pain. In: Wall Patrick D, Melzack R, editors. The Textbook of pain. United Kingdom: Churchill Livingstone; 1999. p. 225. ISBN 0-443-06252-8.

Jenkinson M, Smith SM. A global optimisation method for robust affine registration of brain images. Med. Image Anal. 2001;5(2):143–56.

Johansen-Berg H, Behrens TE, Robson MD, Drobnjak I, Rushworth MF, Brady JM, et al. Changes in connectivity profiles define functionally distinct regions in human medial frontal cortex. Proc Natl Acad Sci USA 2004;101(36):13335–40.

Johansen-Berg H, Behrens TE, Sillery E, Ciccarelli O, Thompson AJ, Smith SM, et al. Functional–anatomical validation and individual variation of diffusion tractography-based segmentation of the human thalamus. Cereb Cortex 2005;15(1):31–9.

Lieberman MD, Jarcho JM, Berman S, Naliboff BD, Suyenobu BY, Mandelkern M, et al. The neural correlates of placebo effects: a disruption account. Neuroimage 2004;22(1):447–55.

Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 2003;126(Pt 5):1079–91.

Manning BH, Merin NM, Meng ID, Amaral DG. Reduction in opioid- and cannabinoid-induced antinociception in rhesus monkeys after bilateral lesions of the amygdaloid complex. J Neurosci 2001;21(20):8238–46.

Misslin R. The defense system of fear: behavior and neurocircuitry. Neurophysiol Clin 2003;33(2):55–66. Review.

Mori S, van Zijl PC. Fiber tracking: principles and strategies – a technical review. NMR Biomed 2002;15(7–8):468–80.

Mori S, Frederiksen K, van Zijl PC, Stieltjes B, Kraut MA, Solaiyappan M, et al. Brain white matter anatomy of tumor patients evaluated with diffusion tensor imaging. Ann Neurol 2002;51(3):377–80.

Northoff G, Heinzel A, Bermpohl F, Niese R, Pfennig A, Pascual-Leone A, et al. Reciprocal modulation and attenuation in the prefrontal cortex: an fMRI study on emotional-cognitive interaction. Hum Brain Mapp 2004;21(3):202–12.

Petrovic P, Ingvar M. Imaging cognitive modulation of pain processing. Pain 2002;95(1–2):1–5.

Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis. Neurophysiol Clin 2000;30(5):263–88.

Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. Trends Neurosci 2002;25(6):319–25.

Rajkowska G, Goldman-Rakic PS. 1995. Cytoarchitectonic definition of prefrontal areas in the normal human cortex: II. Variability in locations of areas 9 and 46 and relationship to the Talairach Coodrinate System.

Remy F, Frankenstein UN, Mincic A, Tomanek B, Stroman PW. Pain modulates cerebral activity during cognitive performance. Neuroimage 2003;19(3):655–64.

Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 2004;23(Suppl 1):S208–19.

Stieltjes B, Kaufmann WE, van Zijl PC, Fredericksen K, Pearlson GD, Solaiyappan M, et al. Diffusion tensor imaging and axonal tracking in the human brainstem. Neuroimage 2001;14(3):723–35.

Talairach J, Tournoux P. 2000. Translated by Mark Rayport. Co-Planar Stereotaxic Atlas of the Human Brain. New York: Thieme Medical Publishers, Inc. 1988. p. 42–58 and 84–110. ISBN 3-13-711701-1.

Tracey I. Nociceptive processing in the human brain. Curr Opin Neurobiol 2005;15(4):478–87.

Valet M, Sprenger T, Boecker H, Willoch F, Rummeny E, Conrad B, et al. Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain – an fMRI analysis. Pain 2004;109(3):399–408.

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.

Zambreanu L, Wise RG, Brooks JC, Iannetti GD, Tracey I. A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. Pain 2005;114(3):397–407.

Zemlan FP, Behbehani MM. Nucleus cuneiformis and pain modulation: anatomy and behavioral pharmacology. Brain Res 1988;453(1–2):89–102.