A PET study exploring the laterality of brainstem activation in migraine using glyceryl trinitrate

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Summary
Migraine is a common disabling condition likely to be associated with dysfunction of brain pathways involved in pain and other sensory modalities. A cardinal, indeed signature, feature of the disorder that led to its name is that the pain may be lateralized. H215O-labelled PET was used to study 24 migraineurs and eight healthy controls. The migraineurs were divided into three groups according to the site of their headache: right, left or bilateral. In each group, a migraine was induced using a glyceryl trinitrate (GTN) infusion. The subjects were scanned at predefined points: pre-infusion, during GTN, during migraine and post-migraine. SPM99 software was used to analyse the data. Significant brainstem activation was seen in the dorsal lateral pons (P < 0.05 after small volume correction) during the migraine state versus the pain-free state when comparing migraineurs with controls. When each group was analysed separately, to investigate laterality, it was found that the dorsal pontine activation was ipsilateral in the right-sided and left-sided groups and bilateral in the bilateral headache group with a left-sided preponderance. Consistent with previous work, the activation persisted after pain was controlled by sumatriptan. These results suggest that lateralization of pain in migraine is due to lateralized brain dysfunction.

Keywords: migraine; brainstem; PET; glyceryl trinitrate; laterality

Abbreviations: GTN = glyceryl trinitrate; PAG = periaqueductal grey

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Introduction
Migraine is a common disorder affecting an estimated 10–15% of the population (Rasmussen and Olesen, 1992; Lipton et al., 2001). The headache in migraine may be unilateral or bilateral. The largest PET study in migraine to date involved nine migraineurs with right-sided headache (Weiller et al., 1995). The finding of brainstem activation lateralized to the left raised the question of whether activation is always contralateral to the side of headache. Exploring this issue would provide an important insight into the functional anatomy of primary headache, in particular migraine (Goadsby et al., 2002).

Because of the episodic nature of migraine, it is challenging to perform clinical studies, in particular functional imaging studies. The glyceryl trinitrate (GTN) model of migraine has become established as a reliable and effective method of migraine induction. Laws (1898) described very clear cases of migraine and one of cluster headache triggered by GTN in munitions workers, and the effects were described in detail some years later (Rabinowitch, 1944). Dalsgaard-Nielsen (1955) suggested GTN administration may even be a test, and certainly migraine-like headache developing after sublingual GTN is a well recognized phenomenon (Sicuteri et al., 1987). The technique has been refined by the Copenhagen Group (Iversen et al., 1989; Iversen, 1992, 2001; Iversen and Olesen, 1994) and appears to be a very robust and reproducible way to trigger typical migraine that seems no different from a patient’s usual attacks (Afridi et al., 2004).

In this study, we used the intravenous GTN model to enable the study of a migraine attack from its earliest point and to follow it through to its resolution after therapeutic intervention. To our knowledge, there have been no H215O PET studies of GTN-induced migraine with the exception of a single case report (Bahra et al., 2001). PET has been
used to study healthy controls following administration of intravenous GTN and this revealed regional activation in areas of the large intracranial vessels and the anterior cingulate cortex (Bednarczyk et al., 2002). We were particularly interested in a comparison between the PET findings in induced migraine and earlier PET findings of brainstem involvement in spontaneous migraine (Weiller et al., 1995). Further, we sought to extend previous work to examine the laterality of brain changes in migraine since this is such a key feature of the characteristic clinical picture.

Methods
The study was approved by the joint Ethics Committee of the National Hospital for Neurology and Neurosurgery (UCLH NHS Trust) and the Institute of Neurology (UCL), London, UK.

Patients
Twenty-four migraineurs (aged 26–65 years, 10 male, 14 female) were divided into three groups according to the location of their usual migraine headache: eight right-sided, eight left-sided and eight with bilateral headache (Table 1). All of these fulfilled International Headache Society (IHS) criteria for episodic migraine (Headache Classification Committee of The International Headache Society, 2004) and included eight with migraine with aura and 16 with migraine without aura. All 24 subjects had responded to GTN triggering of their migraine on one prior occasion at least 1 week earlier (Afridi et al., 2004b). The migraineurs were not on any prophylactic medications. Other medications included the oral contraceptive pill in four of the subjects, hormone replacement therapy in three subjects and thyroxine in two of the subjects. All were scanned successfully during an induced migraine. Eight healthy controls were also studied (aged 21–55 years, five male, three female). The controls were recruited on the basis of no personal or family history of migrainous headaches and no history of frequent tension-type headaches. They were excluded if they were on any regular medication. Pregnant women were excluded from the study and a pregnancy test was performed in women of child-bearing age.

Glyceryl trinitrate infusions
Each subject received an intravenous infusion of 0.5 μg/kg/min GTN over 20 min via a cannula inserted into the right antecubital vein. This dose has been validated in previous studies (Iversen, 2001). The migraineurs were all headache free for at least 48 h prior to the initiation of the infusion. No triptans or analgesics had been taken in the preceding 48 h. Headache intensity and characteristics were recorded every 5 min during the infusion and every 15 min for up to 4 h after an infusion. Subjects were asked periodically to describe any headache or other associated symptoms such as nausea, photophobia, phonophobia, or worsening of pain on head movement. In addition, they rated the severity of any headache on a verbal rating scale of 0–10 (0 = no pain, 10 = most severe pain imaginable) and the location and nature of any headache were noted. Blood pressure and pulse were monitored during infusions.

Subjects were also questioned about premonitory symptoms, such as yawning, fatigue, neck stiffness, thirst, frequency of urination, cravings or mood change (Giffin et al., 2003). In the migraine subjects whose migraines were triggered successfully, subcutaneous sumatriptan (6 mg) was administered to abort the attack and the time taken for relief was noted. These subjects were invited back for a repeat session at least a week later in order for them to undergo PET scanning.

PET scanning
The subjects (migraineurs and controls) underwent three consecutive PET scans in each of the following four conditions (Fig. 1): (1) pain free; (2) during the non-specific headache of a GTN infusion; (3) during a migraine headache, or equivalent time delay for controls; and (4) pain free following treatment of migraine with subcutaneous sumatriptan 6 mg.

The time between conditions 2 and 3 (the latency of onset of migraine) varied, and this delay was reproduced for the control scans as the controls did not have headache during condition 3. This enabled the controls’ scans to be yoked to the migraineurs’ scans. During each scan, the subjects were again asked to rate their headache using a scale of 0–10 (0 = no pain, 10 = most severe pain imaginable) and to describe the nature and location of the headache along with non-headache features including premonitory symptoms. All subjects were asked to close their eyes during scanning.

Data acquisition and analysis
PET scans were performed with an ECAT EXACT HR+ scanning system (CTI Siemens, Knoxville, TN) in three-dimensional mode with septa retracted. An antecubital vein cannula was used to administer the tracer, ~350 MBq of H215O. The activity was infused into subjects over 20 s at a rate of 10 ml/min. The data were acquired in one 90 s frame beginning 5 s before the peak of the head curve. The interval between scans was 8 min. Attenuation correction was performed with a transmission scan acquired at the beginning of each study. Images were reconstructed by filtered back-projection into 63 image planes (separation 2.4 mm) and into a 128 × 128 pixel image matrix (pixel size 2.1 × 2.1 mm2). SPM99 (Wellcome Department of Imaging Neuroscience, http://www.fil.ion.ucl.ac.uk/spm) was used for data analysis (Frackowiak and Friston, 1994). Images were realigned with the first as the reference and then co-registered, and finally spatially normalized into the space defined by the atlas of Talairach and Tournox (1988). The normalized images were smoothed with a Gaussian filter of 10 mm full width at half-maximum. Statistical parametric maps were derived with pre-specified contrasts, comparing regional cerebral blood flow during headache versus rest. An uncorrected threshold of P < 0.001 was chosen for tabular and graphical reporting. In areas with a prior anatomical hypothesis, the reporting criterion was P < 0.05 applying a small volume correction for multiple non-independent comparisons using a 12 mm radius sphere centred on the brainstem maxima as reported (coordinates: −2, −28, −22) (Weiller et al., 1995). The first analysis was performed using a model that enabled group × condition interactions. This allowed us to discount order and other non-specific effects shared with the controls. The PET scans from the left-sided group were reflected through the sagittal plane (flipped) to analyse all the migraine subjects as one group (incorporating right-sided, flipped left-sided and bilateral migraineurs) in a comparison with the control group. This technique enabled us to look for the main effect of migraine in terms of contralateral and ipsilateral effects over all subjects (as opposed to left and right effects). Having
established that the group × condition interactions were significant, we proceeded to examine the simple main effects of condition. To examine laterality of regional cerebral blood flow (rCBF) changes with respect to side of pain, a migraine versus pain-free contrast (condition 3 versus condition 1) was then performed for each group of patients separately. Finally, to test for bilateral changes, a conjunction analysis was performed. This was done by comparing the migraine versus pain-free contrast in a statistical model that included both flipped and unflipped scans for each of the migraine groups. The conjunction analysis tests for the presence of significant changes in rCBF in the flipped and unflipped scans simultaneously, therefore a bilateral effect will appear as a significant conjunction (Friston, 2003). The model also included time (scan number) as a nuisance variable, which was modelled separately for each group.

Table 1  Subject characteristics

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Symbols in italics represent characteristics of the studied attack. MWA = migraine with aura; MO = migraine without aura; Y = yes; N = no.
Results
Clinical findings
All of the migraineurs and six of the controls experienced a very mild, non-specific headache during the GTN infusion. In the migraine group, this eventually developed into a typical migraine headache fulfilling IHS criteria for migraine (Headache Classification Committee of The International Headache Society, 2004), whereas in the control group the headache subsided within a few minutes of the end of the GTN infusion.

The migraine group included migraineurs with and without aura. The GTN method of inducing migraine has been shown to induce migraine without aura in subjects who have migraine with aura (Christiansen et al., 1999). This was the case in seven of our eight subjects with migraine with aura. However, in one of them, a typical visual aura was induced along with the headache. It is also of interest that a significant number (50%) of the migraineurs described typical premonitory symptoms, such as yawning, tiredness, irritability, neck stiffness, frequency of urination, hunger and low mood following the GTN trigger (Afridi et al., 2004).

PET findings
Given the previous PET findings in migraine, the main focus of our analysis was on the brainstem (Weiller et al., 1995; Bahra et al., 2001; Matharu et al., 2004) although other areas of activation were also observed and are reported.

Main effect of migraine over all groups
The main effect of migraine was obtained by comparing condition 3 versus 1 in all 24 migraine subjects with condition 3 versus 1 in the control subjects (Fig. 1).

Significant brainstem activation was seen in the dorsal pons and rostral medulla (P < 0.05 after small volume correction) (Table 2, Fig. 2). Following abortion of the migraine with sumatriptan, the dorsal pons remained activated.

Other areas of activation present in the migraine state included the anterior cingulate, bilateral insula, bilateral cerebellar hemispheres, prefrontal cortex and the putamen (Table 2).

Main effect of GTN
This was obtained by comparing condition 2 versus 1 in all 24 migraine subjects with condition 2 versus 1 in the control subjects.

Table 2 Main effect of migraine

<table>
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<th>Area of activation</th>
<th>Coordinates</th>
<th>Z score of peak activation</th>
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<tr>
<td>Dorsal pons*</td>
<td>4 -32 -32</td>
<td>3.95 (P = 0.01 SVC)</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>10 30 -4</td>
<td>3.95</td>
</tr>
<tr>
<td>BA24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prefrontal cortex</td>
<td>-18 46 -2</td>
<td>4.43</td>
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<td>BA10</td>
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<tr>
<td>Right insula</td>
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</tr>
<tr>
<td>Left insula</td>
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<td>4.3</td>
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<tr>
<td>Right cerebellum</td>
<td>40 -82 -30</td>
<td>3.62</td>
</tr>
<tr>
<td>Left cerebellum</td>
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<td>4.8</td>
</tr>
<tr>
<td>Putamen</td>
<td>-20 0 12</td>
<td>4.95</td>
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Migraine group pooling right-sided, bilateral and flipped left-sided migraineurs. The Talairach and Tournoux coordinates describe the maxima within a cluster defined as the voxel with the highest Z-score (P < 0.001, uncorrected). *A small volume correction (SVC) was applied to the brainstem area (as described in Methods). BA = Brodmann area.

During the GTN infusion, the migraineurs experienced a mild, non-migrainous headache which faded as soon as the infusion was complete. No activation in the dorsal pons was detected. However, activation was seen in the anterior cingulate and in regions corresponding to the internal carotid and basilar arteries (Fig. 3).

Migraine with aura versus migraine without aura
The 24 migraineurs included eight with a diagnosis of migraine with aura and 16 with migraine without aura. Analysis of the data revealed activation in the dorsal pons in both subgroups.

Laterality of brain changes in each group
The brainstem was the main focus for examining lateralized changes in activation with respect to side of pain (refer to Table 3 for coordinates of activation).

In acute right-sided migraine, activation was seen in the right dorsal pons and in the rostral medulla near the mid-line (Fig. 4).

In acute left-sided migraine, a small area of activation was seen in left dorsal pons (Fig. 4).

In acute migraine with bilateral pain, activation was seen in the left dorsal pons. A formal conjunction analysis with flipped scans (see Methods) revealed a bilateral component to the dorsal pontine activation in this group.

Other areas of significant activation examined for lateralization
Right anterior cingulate activation, bilateral insular and bilateral cerebellar activations were consistently seen in the migraine groups during headache (uncorrected P < 0.001).
Bilateral prefrontal activation was found in all groups except in the right-sided group where it was left-sided only. Occipital lobe activation was seen in the left-sided group and temporal lobe activation was found in the right and left groups. Basal ganglia activation (putamen and caudate nucleus) was found in the right-sided and bilateral group.

Discussion

This study demonstrated robust activation of the dorsolateral pons in clinically typical migraine triggered by GTN. By selecting patients with habitually lateralized attacks, and testing this lateralization in a pre-scanning session, we showed that patients with right-sided pain activate the right dorsolateral pons and patients with left-sided pain activate the left dorsolateral pons. By comparison, patients with bilateral pain activate the left side of the dorsolateral pons predominantly, but a conjunction analysis reveals that in fact there are bilateral activations present. Brainstem activation has been reported previously in spontaneous migraine (Weiller et al., 1995; Afridi et al., 2005) and triggered migraine (Bahra et al., 2001). In both the study of Weiller et al. (1995) and our current study, activation persists after pain control is achieved with sumatriptan. The data suggest that unilateral pain, one of the hallmark features of migraine, results from an asymmetrical brainstem dysfunction. We infer that the persistence of activation after pain control reflects an underlying abnormality in the disorder and not simply a response to pain.

Migraine, the brainstem and functional imaging

There is a growing body of evidence implicating brainstem mechanisms in the genesis of migraine. A PET study by Weiller et al. (1995) involved nine subjects with right-sided migraine without aura. They were scanned during spontaneous migraine attacks and following sumatriptan. The study revealed brainstem activation which lateralized
to the left during migraine and persisted after sumatriptan administration had relieved the pain. The spatial resolution of the PET camera was not high enough to identify specific nuclei, but the foci of maximum activations were around the dorsal–rostral midbrain and dorsolateral pons. Given smoothing procedures in PET analyses, this study was not designed specifically to assess lateralization with respect to side of symptoms. A case study of a GTN-triggered migraine also revealed brainstem activation in the dorsolateral pons (Bahra et al., 2001). The dorsolateral pons was also shown to be active during a study of patients with chronic migraine (Matharu et al., 2004). Recently, functional MRI has been used to investigate migraine. In a study by Cao et al. (2002), migraine was triggered by a visual stimulus in 12 migraineurs, 10 of whom had migraine with aura. Some of these patients were taking migraine prophylactics. Increased activations were found in the red nucleus and substantia nigra before the onset of migraine symptoms in eight symptomatic subjects. This was followed by signal change in the occipital cortex. In seven of these subjects BOLD (blood oxygen level-dependent) signal increases were also seen in other brainstem areas including dorsolateral pons, basilar pons, pontine tegmentum, medial longitudinal fasciculus, periaqueductal grey (PAG) and central midbrain, although it was not clear at what time point and for how long these structures remained abnormal. In contrast, brainstem activation has not been noted in other primary headaches, such as cluster headache (May et al., 1998a, 2000). It was also notably absent in a PET study where capsaicin was injected into the forehead of healthy subjects to induce experimental pain (May et al., 1998b).

**Fig. 4** Activation of the ipsilateral pons in patients with right-sided attacks (n = 8, A) and left-sided attacks (n = 8, B).

### The laterality question in migraine

Unilateral pain has been a hallmark of migraine since Galen (AD 131–201) introduced the term *hemicrania* to describe the disorder (Lance and Goadsby, 1998). However, as far as we are aware, there have been no imaging studies specifically designed to explore the issue of laterality in migraine. A PET study of GTN-triggered cluster headache involving nine subjects, five with left-sided and four with right-sided attacks, demonstrated bilateral insula activation, contralateral thalamic and anterior cingulate activation with ipsilateral hypothalamic activation (May et al., 1998a). It must be noted that this study was not designed to look at laterality and so left-sided and right-sided groups were not analysed separately, although lateralized deep brain stimulation techniques have been proposed as a treatment strategy for chronic cluster headache (Franzini et al., 2003). An MRI study that detected increased iron levels in the PAG region of migraineurs did not find any side-to-side differences in PAG iron and therefore could not be used to make any inferences about laterality (Welch et al., 2001). The study reporting PAG-stimulating electrodes triggering migraine referred to patients implanted bilaterally with no previous headache history, so laterality was not discernible (Raskin et al., 1987). In a further series of 64 patients in whom electrodes were implanted unilaterally, 15 reported post-implantation headache and accompanying symptoms, such as lacrimation, visual blurring and nasal congestion, that were exclusive to, or worse on the ipsilateral side (Veloso et al., 1998). There are three case reports of new onset migraine following haemorrhage. Following a brainstem (ponsine) cavernous angioma, ipsilateral migraine was
reported (Afridi and Goadsby, 2003), whereas contralateral migraine was reported following a dorsal midbrain bleed (Goadsby, 2002) and in a further case report of a pontine cavernoma (Katsarava et al., 2003).

A recent abstract of a PET study in spontaneous migraine showed activation in the midline pons (Denuelle et al., 2004). This study involved six subjects with migraine without aura, and data from the left-sided migraine patients were flipped and analysed together with the data from the right-sided migraine patients, thus making it difficult to make inferences about laterality. Our group has also conducted a study of spontaneous migraine using a similar method of analysis which demonstrated activation of the dorsal pons (Afridi et al., 2005). Neither of the aforementioned studies was designed to look at laterality. Considering other pain studies, right anterior cingulate activation has been reported irrespective of the side of pain (Hsieh et al., 1995). However, this finding has not been replicated (Coghill et al., 1994; Bingel et al., 2002, 2003).

**Study limitations**

Other than the different design, PET camera, selection of patients and analysis, an important difference between this study and that of Weiller et al. (1995) is the use of GTN triggering. Studying triggered attacks allows considerably more experimental control and permitted us to select patients carefully with habitually right, left or bilateral pain, and to pre-test their responses. In addition, we used a non-headache group to control for order or time effects. There are several reasons to conclude that GTN-triggered attacks are migraine and not more nor less. First, their clinical phenotype is indistinguishable from spontaneous migraine (Iversen et al., 1989; Headache Classification Committee of The International Headache Society, 2004). Secondly, their response to treatment with triptans is the same (Iversen and Olesen, 1996; Ferrari et al., 2001). Thirdly, triggering of migraine is itself a fundamental feature of the disorder, so that a so-called spontaneous attack, triggered for example by missed sleep, is not differentiated in clinical practice; indeed it is typical of the disorder. Fourthly, the GTN model reproduces premonitory symptoms (Afridi et al., 2005), again a typical feature of spontaneous attacks (Giffin et al., 2003), as well as the laterality of the patient’s usual migraine attack. Nitrergic mechanisms seem fundamental in migraine (Olesen et al., 1994), with the blockade of its synthesis aborting acute migraine (Lassen et al., 1997). However, we cannot exclude some difference that cannot be accounted for by our study design.

Our migraine subjects were pre-selected on the basis of response to GTN as a trigger of migraine. It is possible that this may introduce some selection bias. However, studies of the GTN model suggest that 75–80% (Thomsen et al., 1994; Afridi et al., 2005; Sances et al., 2004) of migraineurs may be responsive to GTN triggering, suggesting that this subgroup may represent a reasonably large proportion of the overall migraine population. Moreover, it is not clear whether if one re-challenged migraineurs how many would respond at another time. Another issue that needs consideration is the timing of attacks. Since we induced migraine, we could study attacks early, acquiring imaging data as soon as patients had reasonably developed headache with a mean of pain severity of 5/10. This is one possible explanation for the difference between our findings and a previous study in which patients were scanned later in the attack (Weiller et al., 1995). It is possible that the activation switches sides during an attack if pain develops further, although one could speculate that this was a response to the initial homolateral dysfunction. In fact, in a clinical report looking at the evolution of allodynia during migraine it was demonstrated that 1 h into the migraine the allodynia was ipsilateral to the headache, but this started to affect the contralateral side of the head after 2 h (Burstein et al., 2000). Clinical observations suggest that in some cases the migraine switches sides or goes from a unilateral to a bilateral phenotype (Selby and Lance, 1960). Unlike our induced migraine study, the study of Weiller et al. was not designed specifically to look for laterality and did not include any patients with left-sided or bilateral headache. It is unclear from the study of Weiller et al. to what extent laterality of headache was examined in the subjects and whether they were subjects who had side-locked headache or whether they experienced side shift or any bilateral component to the headache. Our patients were recruited on the basis of their headache laterality, and this was closely monitored and documented throughout the study. Certainly, the data from our patients suggest that localization of pain is very closely linked to the side of brainstem activation. These findings are in keeping with the known anatomy of the descending nociceptive inhibition pathways from the PAG and serotonergic raphe nuclei to the trigeminal nucleus caudalis. Lastly, by including a control group matched for time and GTN administration, it seems unlikely that the effect we report is a non-specific effect of GTN or an order effect. The latter is crucial since brain activation in other pain states, such as hypothalamic activation with cardiac pain (Rosen et al., 1994), has subsequently been shown to be due to an order effect when an appropriate control group was included (Rosen et al., 1996).

This study provides an insight into the neuroanatomical changes associated with migraine. The data reinforce a view of migraine as a brain disturbance and suggest that lateralized change in the brain, particularly in the dorsolateral pontine tegmentum, may answer the age old question of why the head hurts on just one side. Understanding the neurobiology of migraine is crucial to a rational explanation of the clinical phenomenon and development of better management strategies.

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References