# Functional Imaging in Migraine

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# Abstract

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Migraine is a common disabling condition likely to be associated with dysfunction of brain pathways involved in pain and other sensory modalities. Functional brain imaging in acute migraine has proved challenging due to the logistic problems associated with an episodic condition although it has provided us with important insights into the pathophysiology of the condition. Since the seminal observation of brainstem activation in migraine there has only been a single case substantiating this finding.

Our objectives were, first, to test the hypothesis that brainstem activation could be detected in migraine and to refine the anatomical localisation with higher resolution positron emission tomography (PET) and more advanced analysis techniques. Secondly, we wanted to explore the glyceryl trinitrate (GTN) model of migraine further to determine reliability and reproducibility. Finally, using this model we wished to explore the issue of laterality in migraine and, in particular, how it relates to brainstem activation.

$H_2^{15}O$-labelled PET was used to study acute migraine attacks occurring spontaneously in five migraineurs. Using GTN-triggered migraine twenty-four migraineurs (divided into three groups according to the site of their headache: right/ left/ bilateral) and eight controls were scanned. The data was analysed using statistical parametric mapping (SPM99).

Significant brainstem activation was seen in the dorsolateral pons ($P < 0.05$ after small volume correction) during the migraine state versus the pain-free state in both spontaneous and induced migraine groups. When the induced 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.

Looking at the GTN model, thirty-three of the forty-four patients administered GTN

had a migraine attack fulfilling International Headache Society criteria. Twelve

patients described typical premonitory symptoms, which have not been previously

documented with GTN-induced migraine. A repeat attack was triggered in all subjects

but one and laterality was also remarkably reproducible. In one case a visual aura was

also triggered both times.

Our study shows that GTN-induced triggering is common in our patients, and

remarkably reproducible.

Overall, our findings provide clear evidence for dorsal pontine activation in migraine,

and reinforce the view that migraine is a subcortical disorder involving modulation of

afferent neural traffic. The results also suggest that lateralisation of pain in migraine is

due to lateralised brain dysfunction.

**Ethics approval**

The studies described in this thesis were 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.

# Acknowledgements

# CONTENTS

# Publications

## Papers

1. Afridi SK, Kaube, Goadsby PJ. Occipital activation in glyceryl-trinitrate induced migraine with visual aura. J Neurol Neurosurg Psychiatry 2005; 76:1158-60

2. Afridi SK, Kaube H, Goadsby PJ. Glyceryl trinitrate triggers premonitory symptoms in migraine. Pain 2004;110(3):675-80

3. Afridi SK, Giffin NJ, Kaube H, Friston K, Ward N, Frackowiak RSJ, Goadsby PJ. A PET study in spontaneous migraine. Archives of Neurology 2005; 62:1270-5

4. Afridi SK, Matharu MS, Lee L, Kaube H, Friston K, Ward N, Frackowiak RSJ, Goadsby PJ. A PET study exploring the laterality of brainstem activation in migraine using glyceryl trinitrate. Brain 2005; 128: 932-9

## Other papers arising during thesis period

1. Afridi S, Goadsby PJ. New onset migraine with a brainstem cavernous angioma. J Neurol Neurosurg Psychiatry 2003;74:680-682.

2. Afridi S, Bacon CM, Bowling J, Goadsby PJ. Verapamil and lymphomatoid papulosis in chronic cluster headache. Journal of Neurology 2004;251(4):473-5.

3. Afridi S, Kaube H. Prophylactic therapy of migraine. Current Treatment Options in Neurology 2003 5:431-40

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# List of Abbreviations

| | |
|---|---|
| ATP | Adenosine triphosphate |
| BBB | Blood brain barrier |
| BOLD | Blood oxygen level dependent |
| CGRP | Calcitonin gene-related peptide |
| CNV | Contingent negative variation |
| CSD | Cortical spreading depression |
| cGMP | Cyclic guanosine monophosphate |
| DR | Dorsal raphe |
| DWI | Diffusion weighted imaging |
| FHM | Familial hemiplegic migraine |
| fMRI | Functional magnetic resonance imaging |
| GTN | Glyceryl trinitrate |
| H-MRS | Proton magnetic resonance spectroscopy |
| IDAP | Intensity dependence of auditory evoked potentials |
| IHS | International headache society |
| LC | Locus coeruleus |
| MRI | Magnetic resonance imaging |
| MRS | Magnetic resonance spectroscopy |

| | |
|---|---|
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| NRM | Nucleus raphe magnus |
| PAG | Periaqueductal gray |
| PCr | Creatine phosphate |
| PET | Positron emission tomography |
| Pi | Inorganic phosphate |
| P-MRS | Phosphorous magnetic resonance spectroscopy |
| PVN | Paraventricular nucleus |
| PWI | Perfusion weighted imaging |
| rCBF | Regional cerebral blood flow |
| REM | Rapid eye movement |
| RVM | Rostroventral medulla |
| SC | Subcoeruleus |
| SCN | Supraoptic nucleus |
| SPECT | Single photon emission computerised tomography |
| SPM | Statistical parametrical mapping |
| SUNCT | Short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing |
| SVC | Small volume comparison |
| 5-HT | Serotonin |
| TNC | Trigeminal nucleus caudalis |
| TP | Total phosphate |

# PART I. INTRODUCTION

## Chapter 1. Migraine

### 1.1 An historical perspective

Written accounts of headache have been found in ancient Sumerian, Egyptian and Greek sources. One-sided headache was described in relation to the ancient Egyptian god Horus. Galen, in the second century AD, introduced the term "hemicrania" for unilateral headache following on from Aretaeus of Cappadocia's term "heterocrania". "Hemicrania" was later transformed to the Old English "megrim" and the French "migraine"(Lance and Goadsby, 1998b).

Descriptions of other features of migraine include the Persian Avicenna's observation that sound, light or smell could trigger a headache. Willis, in 1684, described trigger factors including wine, overeating, lying in the sun, passion and long-sleeping. He also comments on "ravenous hunger" as a premonitory symptom. Fordyce, in "De Hemicrania" (1758) also observed premonitory depression and polyuria during the attack as well as the menstrual association.

Early descriptions of aura were provided by Le Pois in 1618 in his description of transient unilateral sensory disturbance preceding the headache. Wepfer (1669) and Vater (1723) observed and described visual aura (Isler and Clifford-Rose, 2000). Fothergill later coined the term "fortification spectra".

Galen thought that the throbbing pain originated from blood vessels and tension pain from tendons or nerves. Willis also linked intracranial vasoconstriction with subsequent dilatation, a concept that was later taken up by Wolff in the early twentieth century. This vascular theory dominated the scene but there were dissenters even in

the nineteenth century. Liveing, in 1873, wrote about migraine as a "nerve-storm" raging within the brain (Liveing, 1873). Hughlings-Jackson (Schiller, 1975) and Gowers (Gowers, 1906) remarked on the similarities between migraine and epilepsy.

Following his investigations into the nature of headache Wolff believed that aura and headache were a result of vascular changes (Wolff, 1948). He attributed aura to cerebral vasoconstriction and ascribed the headache of migraine to dilatation and inflammation of the extracranial arteries with some contribution from the dural or meningeal arteries. For most of the twentieth century migraine was still thought of as a "vascular headache". It has since been shown that this relationship does not hold true (Andersen et al., 1988; Olesen et al., 1990; Olesen et al., 1981) and that the vascular changes may be a consequence rather than a cause of pain (Goadsby and Edvinsson, 1993; Lambert et al., 1984; May et al., 1998b). As a result of increasing research into the field the vascular concept of headache has been transformed into a "neurovascular" concept suggesting that vessel change is driven by nerves.

## 1.2 Current concepts

Migraine is a common disorder affecting an estimated 10-15% of the population (Lipton et al., 2001; Rasmussen and Olesen, 1992; Steiner et al., 2003). The International Headache Society (IHS) created a classification system for headaches in 1998 (Headache Classification Committee of the International Headache Society, 1988) with the aim of standardisation of diagnosis and revised it recently (Headache Classification Committee of The International Headache Society, 2004). Appendix 1 lists some of the current criteria for the diagnosis of migraine.

Migraine can be subdivided into the premonitory phase, the aura phase and the headache phase. Some migraineurs also experience a postdrome and feel tired,

washed out or in other cases euphoric or energetic. This is followed by resolution of the attack.

*Premonitory*

The premonitory phase is experienced by between 7-88% of migraineurs (Blau, 1980; Russell et al., 1996; Waelkens, 1985). The estimates vary probably because the issue has not been systematically studied at a population level. The phase can start up to 48 hours prior to headache onset and can include a number of symptoms (Table 1.1). These have been documented prospectively in a study using an electronic diary (Giffin et al., 2003) which also demonstrated that these symptoms may persist into the headache phase of the attack. It is postulated that there is hypothalamic involvement during this phase since many of the symptoms, such as hunger, thirst and increased urination, are thought to be related to posterior hypothalamic function. A recent study demonstrated evidence of hypothalamic activation following stimulation of the superior sagittal sinus in cats (Benjamin et al., 2004). In a placebo controlled study domperidone was found to prevent the migraine attack when administered during the premonitory phase (Waelkens, 1982). This has led to the suggestion that the dopaminergic system may be involved in or associated with migraine.

**Table 1.1** Premonitory symptoms in migraine

| Psychological | Neurological | General |
|---|---|---|
| Depression | Difficulty concentrating | Neck stiffness |
| Euphoria | Photophobia | Food cravings |
| Irritability | Phonophobia | Thirst |
| Hyperactivity | Dysphasia | Urination |
| | Yawning | Fluid retention |
| | Drowsiness | Sluggishness |

*Aura*

The aura phase involves a transient neurological phenomenon and is present in

approximately 30-40% of migraineurs (Launer et al., 1999; Stewart et al., 1994). The

aura typically precedes the headache by 10 minutes on average (Kelman, 2004) but

can also accompany the headache or, less commonly, follow the headache phase

(Russell and Olesen, 1996). It can, in up to 33.5% of cases (Kelman, 2004), present

without headache. The most common aura symptoms are visual symptoms such as

fortification spectra, photopsia, or scotoma. The disturbance often tends to start at the

centre of the visual field and propagates out towards the periphery over 5-15 minutes.

More complicated visual phenomena such as visual distortions or hallucinations can

also occur. Other symptoms include paraesthesia, dysarthria, dysphasia, weakness,

ataxia and disturbed consciousness.
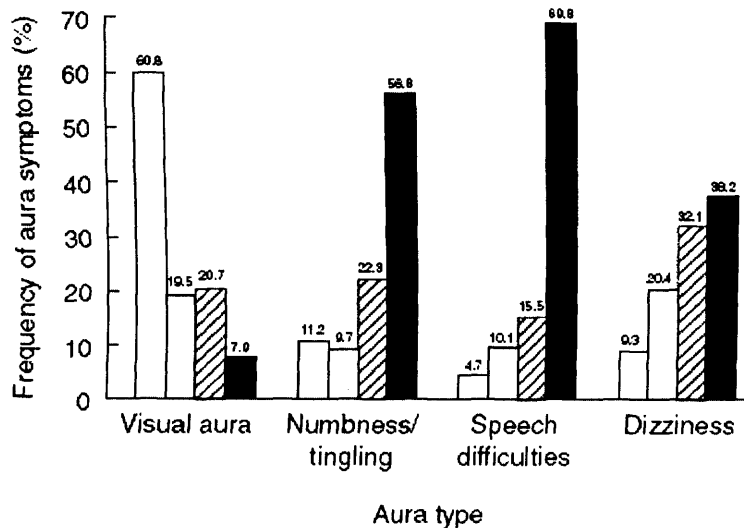


■ absent, ☐ occasional, ▨ frequent, ☐ very frequent.

**Figure 1.1** Frequency of aura symptoms taken from a study of 952 migraine patients

(Kelman, 2004)

Lashley (Lashley, 1941) mapped the progression of his own visual aura, calculating

that cortical function was affected at a rate of ~3mm/minute. Aura is thought to be the

clinical correlate of cortical spreading depression (CSD), first described by Leao

(Leao, 1944). The time course and progression of symptoms correlate well with the

temporal and spatial pattern of CSD. CSD is an electrophysiological phenomenon

producing a slowly (2-6 mm/min) propagating wave of neuronal depolarisation

followed by a suppression of neural activity. It probably starts with a cellular efflux of

potassium, leading to depolarisation and a period of relative electrical silence.

Additional negative ion species, such as glutamate are released. The subsequent

energy-dependent restitution of ion gradients eventually restores normal neuronal

activity. The depolarisation-restoration process takes approximately 1.5 minutes so

the wave is only 5mm deep (James et al., 2001). In animals susceptibility to CSD is

increased by hypoxia and hypoglycaemia as well as by applying solutions with

increased potassium content. Animal studies also reveal behavioural changes induced

by CSD such as contralateral sensory neglect, yawning and drowsiness (Gorji, 2001;

Huston, 1971). Looking at human studies, in addition to the evidence linking CSD

and aura provided by imaging studies (described in chapter 2) including

magnetoencephalography (Bowyer et al., 2001), recent genetic discoveries have

strengthened this proposed link. In a knock-in mouse model carrying one of the

human mutations for familial hemiplegic migraine (*CACNA1A*-R192Q) the mice were

found to have a reduced threshold and increased velocity of cortical spreading

depression (Van Den Maagdenberg et al., 2004). In another group of FHM patients

the mutation is on the *ATP1A2* gene resulting in a loss of Na/K ATPase pump

function (De Fusco et al., 2003). It is proposed that this loss of pump function may

depolarise neurons and lead to impaired clearance of potassium by astrocytes and this, in turn, would facilitate CSD.

CSD does not respect vascular boundaries and results in vascular changes but not ischaemia (Hadjikhani et al., 2001). These will be discussed further in the light of imaging studies in section 2.

In a proportion of patients the aura is prolonged or persistent and in a sub-group of migraineurs the aura defines the condition as "hemiplegic migraine" (see appendix for diagnostic criteria).

*Headache*

The headache in migraine has certain characteristics and associated features:

A.  Headache attacks lasting 4-72 hours and occurring < 15 days/month (untreated or unsuccessfully treated).
B.  Headache has at least two of the following characteristics:
    1.  Unilateral location
    2.  Pulsating quality
    3.  Moderate or severe pain intensity
    4.  Aggravation by or causing avoidance of routine physical activity (i.e., walking or climbing stairs)

Adapted from IHS classification system (Headache Classification Committee of The International Headache Society, 2004).

Despite the origins of the word, migraine is bilateral in approximately one third of patients (Lance and Anthony, 1966). The location of the head pain is variable but typically affects the head, neck and face, most commonly the frontotemporal regions. It has been documented to also affect the limbs in rare cases (Guiloff and Fruns, 1988). Migraineurs also often develop cutaneous allodynia which can extend outside the head to affect the limbs (Burstein et al., 2000).

As to what causes the headache in migraine, this is a point of contention. The current theories will be discussed later.

There are a number of trigger factors which precipitate an attack in susceptible migraineurs. Common triggers include sleep disturbance, stress / relaxation after stress, hormonal changes and eating patterns. It is not known how these factors precipitate an attack.

*Genetics*

It has been observed from twin studies (Svensson et al., 2003) and epidemiological surveys that there is a strong genetic component to migraine. Simple Mendelian patterns of inheritance cannot explain the common versions of migraine, namely migraine with aura and migraine without aura. However, a relatively rare subtype, familial hemiplegic migraine (FHM) has been found to have an autosomal dominant pattern of inheritance. In 1996 the first FHM gene, *CACNA1A*, was identified on chromosome 19p13. It encodes for the pore-forming ($\alpha_{1A}$) sub-unit of Ca(v)2.1 (P/Q) calcium channels. This mutation is thought to account for 75% of FHM families (Kors et al., 2004). A second FHM gene, *ATP1A2*, has recently been identified on chromosome 1 (De Fusco et al., 2003). It encodes for the $\alpha_2$ sub-unit of a sodium-potassium pump. These findings suggest that dysfunction of ion-channels are involved in the pathophysiology of migraine, or at least in hemiplegic migraine.

In addition to the findings in FHM, linkage studies have identified loci on chromosome 4, 6, 11 and 14 which are associated with the more common forms of

migraine (Kors et al., 2004). This reinforces the view that there are a combination of genetic and environmental factors responsible for migraine with and without aura.

There is also evidence, mainly from magnetic resonance spectroscopy studies, to suggest that energy metabolism is dysfunctional in migraine. This will be detailed later. However no mitochondrial mutations have yet been found in migraineurs (Haan et al., 1999; Klopstock et al., 1996; Russell et al., 1997) although patients with mitochondrial disorders such as MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) have migraine as part of their disorder.

## 1.3 The anatomical structures relevant to migraine

Pain can be generated by large intracranial vessels, venous sinuses and by dural vessels (Ray and Wolff, 1940). These vessels are innervated by the ophthalmic division of the trigeminal nerve and the structures in the posterior fossa are innervated by C2 nerve root branches. Stimulation of vascular afferents leads to activation, with Fos protein expression, in the trigeminal nucleus caudalis (TNC) and in the dorsal horn at C1 and C2 (Goadsby and Hoskin, 1997; Kaube et al., 1993). Stimulation of the greater occipital nerve, a branch of C2, also leads to activation in this region (Goadsby et al., 1997). On this functional basis this area has been referred to as "the trigeminocervical complex". The information is then relayed, via the quintothalamic tract, to the ventroposteromedial thalamus, medial nucleus of the posterior complex and the intralaminar thalamus (Zagami and Lambert, 1990). The trigeminal brainstem nuclei also have projections to the hypothalamus (Malick and Burstein, 1998) and other subcortical nuclei (Marfurt and Rajchert, 1991).

*The anatomy of the brainstem monoaminergic systems*

There is a significant body of evidence implicating the involvement of serotonin in migraine. During migraine platelet serotonin levels decrease rapidly and urinary excretion of 5-hydroxyindoleacetic acid, the main metabolite of serotonin (5-HT), increases (Curran et al., 1965). Pharmacological depletion of serotonin with agents such as reserpine can induce a migraine and serotonin itself can abort an attack (Kimball et al., 1960). The triptans and many of the migraine preventives work on 5-HT receptors.

The brainstem serotonergic system is divided into two groups: the rostral group, confined to the midbrain and rostral pons, with major projections to the forebrain, and the caudal group, which extends from the caudal pons to the caudal medulla, with major projections to the caudal brainstem and spinal cord. Most neurons are located along the mid-line of the brainstem. The rostral group accounts for 85% of all serotonergic neurons in the brain and includes the dorsal raphe nucleus which extends from the oculomotor nucleus to the middle of the pons (Hornung, 2003). The rostral group regulates the sleep-wake cycle, affective behaviour, food intake, thermoregulation and sexual behaviour. The caudal group includes the nucleus raphe magnus which projects to the spinal dorsal horn. This is involved in regulation of nociception.

There is limited evidence of differences in platelet and plasma levels of noradrenaline between migraineurs and controls when taking the menstrual cycle into account (D'Andrea et al., 2004). The noradrenergic neurons are separated into dorsal and ventral regions. The nucleus ambiguous, the nucleus of the solitary tract and the dorsal motor vagus nucleus are in the medulla. At the level of the pons the neurons in

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the ventrolateral reticular formation project to the spinal cord and modulate autonomic reflexes and pain sensation. The locus coeruleus (LC), which is situated dorsolaterally in the pons, is composed of 10-15,000 neurons and has extensive projections to the cerebral cortex, hypothalamus and cerebellum and descending projections to the brainstem and spinal cord (Willis and Westlund, 1997). The pericoerulear region receives input from the prefrontal cortex, amygdala, hypothalamus and dorsal raphe (Berridge and Waterhouse, 2003). Locus coeruleus neurons display tonic and phasic discharge activity patterns. The tonic activity is state-dependent: the highest discharge rates are during waking and lowest during sleep. Phasic discharges occur in response to novel stimuli and they habituate with repeated stimulus presentation. The locus coeruleus and its efferent projections are thought to play a role in sleep, vigilance, modulation of sensory neurons and control of cortical activation.

The dopaminergic system is mainly centred around the midbrain and includes the substantia nigra and ventral tegmental area. These provide ascending pathways to the cortex and basal ganglia which are important in the initiation of behavioural responses. Dopaminergic neurons in the hypothalamus send descending pathways to the spinal cord and are thought to be involved in autonomic and endocrine regulation (Kandel et al., 2000).

*Brainstem modulation of nociception and vascular control*

Descending modulation of trigeminal nociception arises from the frontal cortex, insula, amygdala and hypothalamus and extends through the periaqueductal gray

(PAG) and the rostral ventral medulla (RVM) to the superficial lamina of the

trigeminocervical complex (Fields and Basbaum, 1999). The RVM includes the

serotonergic nucleus raphe magnus (NRM) and adjacent reticular formation. The PAG

has excitatory connections with the NRM (Behbehani and Fields, 1979) and it

receives input from the locus coeruleus, nucleus cuneiformis and pontomedullary

reticular formation. Stimulation of the PAG and RVM activates inhibitory

interneurons in the TNC via descending serotonergic pathways (Fields and Busbaum,

1994). The pain-modulating action of the PAG is largely relayed via the RVM and is

abolished by lesions in the NRM or pharmacological depletion of serotonin

(Behbehani and Fields, 1979). Injection of opiods into the RVM or PAG leads to

activation of "off "cells and inhibition of "on" cells resulting in inhibition of

nociception (Morgan and Fields, 1994). The "on" cells are thought to facilitate

nociception and have been shown to be active during the hyperalgesia associated with

naloxone-precipitated opiod withdrawal (Bederson et al., 1990). It is of interest to

note that the activity of these "on" and "off" cells is modulated by 5-HT$_1$ receptor

agonists (Ellrich et al., 2001a; Roychowdhury and Heinricher, 1997).


It is known that the dorsolateral pontine tegmentum is also involved in nociception. It

is directly linked to the PAG and RVM. The region incorporates the subcoeruleus

(SC), the locus coeruleus (LC) and the Kolliker-Fuse nucleus. This area is the major

source of noradrenergic innervation of the spinal cord and stimulation of this region

produces antinociception (Jones, 1991). Electrophysiological experiments have shown

that activation of the dorsolateral pontine tegmentum, either electrically or chemically

can inhibit nociceptive activity in dorsal horn neurons, an effect which is mediated by

α2 adrenoreceptors (Girardot et al., 1987; Yeomans et al., 1992). Electrical

stimulation of the LC/SC produced a reduction in both spontaneous activity and responses of the rat TNC to somatic input, especially nociceptive input (Tsuruoka et al., 2003). Beta-adrenoreceptors are thought to be involved in this trigeminal neuronal inhibition (Sasa et al., 1986). Furthermore, electrical stimulation of this region has been shown to produce analgesia in patients with chronic pain (Young et al., 1992).

The parabrachial nuclei also have interconnections with the trigeminal nuclei and are thought to be involved in suppression of nociceptive firing from the TNC (Chiang et al., 1994).

Ascending nociceptive pathways may also be modulated. The locus coeruleus and dorsal raphe nucleus send noradrenergic and serotonergic projections, respectively to the forebrain which may inhibit nociception. Lesions of these nuclei are associated with a reduced antinociceptive effect of morphine and a reduction of forebrain noradrenaline and serotonin (Sandkuhler and Jensen, 2000).

In addition to modulation of nociception, the brainstem is also involved in influencing vascular changes. Stimulation of the main central noradrenergic nucleus, the locus coeruleus (LC), in animal studies reduces cerebral blood flow but increases external carotid flow in a frequency-dependent manner (Goadsby and Duckworth, 1987; Goadsby et al., 1982). In contrast, stimulation of the dorsal raphe increases cerebral blood flow in the monkey (Goadsby et al., 1985) and also leads to extracranial vasodilatation in the same manner as LC stimulation. This extracranial effect has been shown to be a parasympathetic response mediated via the release of vasoactive intestinal polypeptide (VIP) from the greater superficial petrosal branch of the facial

nerve (Goadsby et al., 1983). This forms the efferent pathway of the "trigeminal-autonomic reflex". The reflex can be activated by trigeminal afferent activation which then, via the superior salivatory nucleus in the pons, results in a cranial parasympathetic response mediated through the pterygopalatine ganglion. Stimulation of the trigeminal ganglion also leads to the antidromic release of substance P and CGRP with a resultant increase in extracerebral blood flow (Lambert et al., 1984; Markowitz et al., 1987).
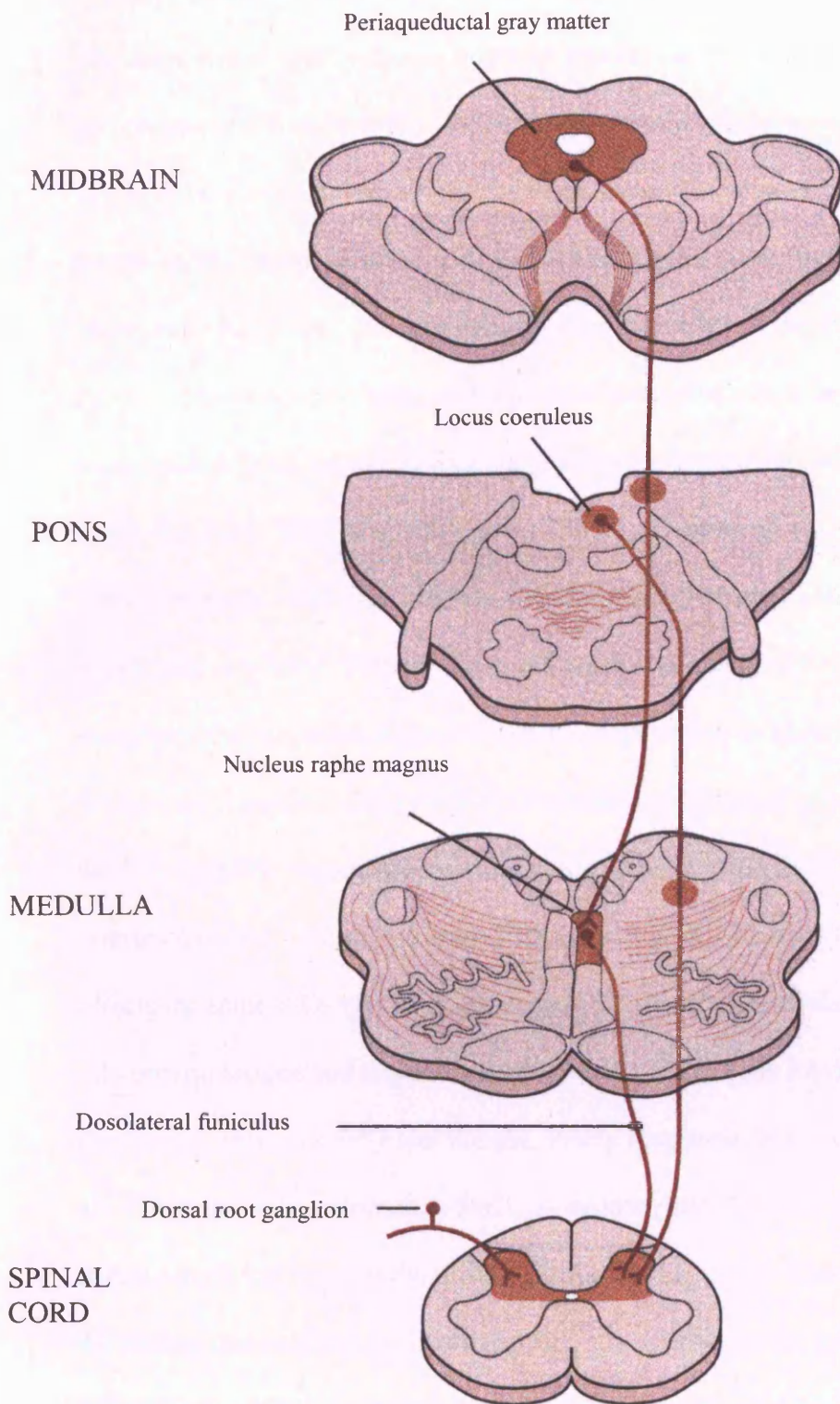
**Figure 1.2** Descending modulation of nociception. Taken from (Kandel et al., 2000).

The labels in the figure, from top to bottom:

Periaqueductal gray matter

MIDBRAIN

Locus coeruleus

PONS

Nucleus raphe magnus

MEDULLA

Dosolateral funiculus

Dorsal root ganglion

SPINAL
CORD

*Evidence for the role of the brainstem in migraine*

The relevance of this system to migraine was first demonstrated clinically by Raskin and colleagues (Raskin et al., 1987). He described a sub-group of 15 patients from 175 with no previous headache history who developed migrainous headaches following the implantation of stimulating electrodes into the periaqueductal gray. This observation has since been reproduced in another series of patients (Veloso et al., 1998). There have also been case reports of new onset migraine following haemorrhage from brainstem (pontine) cavernous angiomata (Afridi and Goadsby, 2003; Goadsby, 2002; Katsarava et al., 2003a). An imaging study revealed that the PAG of a group of migraineurs was found to have abnormally high levels of iron which was correlated with duration of illness (Welch et al., 2001). The PAG has also been shown to modulate trigeminovascular nociception in experimental animals (Knight and Goadsby, 2001) and this modulation is blocked after local blockade of the P/Q-type calcium channel with agatoxin (Knight et al., 2002). The clinical relevance of this finding is that the FHM type 1 gene mutation (*CACNA1A*) also affects the same P/Q- type calcium channels. It has also been shown that dihydroergotamine and triptans bind to receptor sites in the PAG (Goadsby and Gundlach, 1991; Goadsby and Knight, 1997). Moreover, in a recent study administration of naratriptan, a 5-$HT_{1B/1D}$ agonist, into the PAG elicited antinociceptive effects on dural input (Bartsch et al., 2004). This finding, along with the finding that naratriptan modulated neuronal activity in the nucleus raphe magnus (Ellrich et al., 2001a), suggests that triptans may partly work by altering brainstem modulation of nociception in migraine.

There is a significant body of electrophysiological evidence supporting the role of the brainstem in migraine. Contingent negative variation (CNV) is an event related slow cerebral potential appearing in a reaction-time task between a warning and an imperative stimulus. There is an increased amplitude and lack of habituation in patients with migraine (Maertens de Noordhout et al., 1986) which is more pronounced for the early component thought to be modulated by noradrenergic systems. It also appears to be abnormal in non-migraineurs who have a family history of migraine (Siniatchkin et al., 2001). The CNV normalizes after treatment with β-blockers (Maertens de Noordhout et al., 1987) and one study has suggested a significant correlation between CNV amplitude before treatment and therapeutic efficacy of propranolol (Schoenen et al., 1986). This inter-ictal lack of habituation has also been demonstrated with visual-evoked and auditory-evoked potentials (Schoenen, 1998). The lack of habituation reverses during the migraine attack. Habituation is a complex phenomenon which is likely to be affected by the level of cortical preactivation excitability. This excitability is thought to be modulated by the brainstem monoaminergic systems (Hegerl and Juckel, 1993). Interestingly, a recent study showed that fluoxetine, a serotonin reuptake blocker, normalized the VEP habituation pattern in migraineurs (Ozkul and Bozlar, 2002). Another electrophysiological measure found to be abnormal inter-ictally in migraineurs is the intensity dependence of auditory evoked potentials (IDAP) (Wang et al., 1996). This is thought to be related to central serotonergic transmission. It is high in migraineurs which is compatible with low central serotonergic transmission (Hegerl and Juckel, 1993) but it may also be a consequence of lack of habituation (Ambrosini et al., 2003). It, too, is modulated by serotonergic modulators such as dexfenfluramine and triptans (Proietti-Cecchini et al., 1997) and by β-blockers (Sandor et al., 2000). The

increased IDAP normalises just before and during the migraine attack (Judit et al., 2000). It has been proposed that these electrophysiological changes reflect the fluctuations in serotonergic activity throughout the ictal and inter-ictal phases of migraine.

In addition to modulation of nociception, the brainstem serotonergic and noradrenergic systems are involved in the modulation of cortical activity and attentiveness to environmental stimuli (Berridge and Waterhouse, 2003; Matrenza et al., 2004; Parvizi and Damasio, 2003). This may help to explain the so-called associated symptoms of migraine, such as photophobia and phonophobia. The raphe and dorsolateral pontine tegmentum are thought to be involved in sleep and arousal (Jouvet, 1969; Morgane, 1981) . Migraineurs often experience changes in levels of arousal during various phases of a migraine attack (Giffin et al., 2003) and sleep disturbance can trigger a migraine.

There is evidence that the blood-brain barrier (BBB) is abnormal in migraine (Harper et al., 1977). It has been shown that noradrenergic innervation arising from the locus coeruleus is essential for maintaining BBB integrity during some states (Harik and McGunigal, 1984) and that modulation of this system alters BBB permeability in animals (Preskorn et al., 1980).

Neuroimaging has provided us with further evidence for a role for the brainstem. This is detailed in chapter 2.

## 1.4 Explaining the mechanisms of migraine

There are currently two main schools of thought surrounding the pathophysiology of migraine which have incorporated various concepts, including central sensitisation, plasma protein extravasation, cortical spreading depression and brainstem modulation, to different extents. One school maintains that CSD initiates the migraine and that the process is driven by peripheral nerve fibre activation. The other focuses on the pivotal role of the brainstem and maintains that the process is centrally determined.

*A link between CSD and trigeminovascular activation (Figure 1.3)*

Supporters of this theory propose that CSD leads to the release of neurotransmitters, metabolites, potassium and hydrogen ions into the perivascular space resulting in transient hyperemia and vasodilatation of cerebral vessels. The molecules are also thought sensitize perivascular and dural trigeminal afferents (arrow 1 on Fig 1.3) and transmit impulses to the trigeminal ganglia and TNC (arrow 3, 4). It is then thought that inflammatory neuropeptide release (substance P, CGRP) results in plasma protein extravasation and neurogenic inflammation with subsequent sensitization of medullary dorsal horn neurons (Burstein et al., 2000) resulting in head pain. The ipsilateral TNC transmits impulses and stimulates the superior salivatory nucleus and parasympathetic efferents via the sphenopalatine ganglia leading to vasodilatation (trigeminoparasympathetic reflex) and release of nitric oxide or acetylcholine into the dura mater. In a study by the Moskowitz group (Bolay et al., 2002) middle meningeal artery blood flow elevation followed evoked CSD in an experimental model and CSD-induced plasma protein leakage was shown to be blocked by trigeminal denervation.

This theory provides a plausible explanation for the link between migraine aura and headache but does not account for migraine without aura. Also the relationship between aura and headache is not always straightforward. Clinical and experimental observations (Olesen et al., 1990; Selby and Lance, 1960) have shown that aura is not always contralateral to headache.
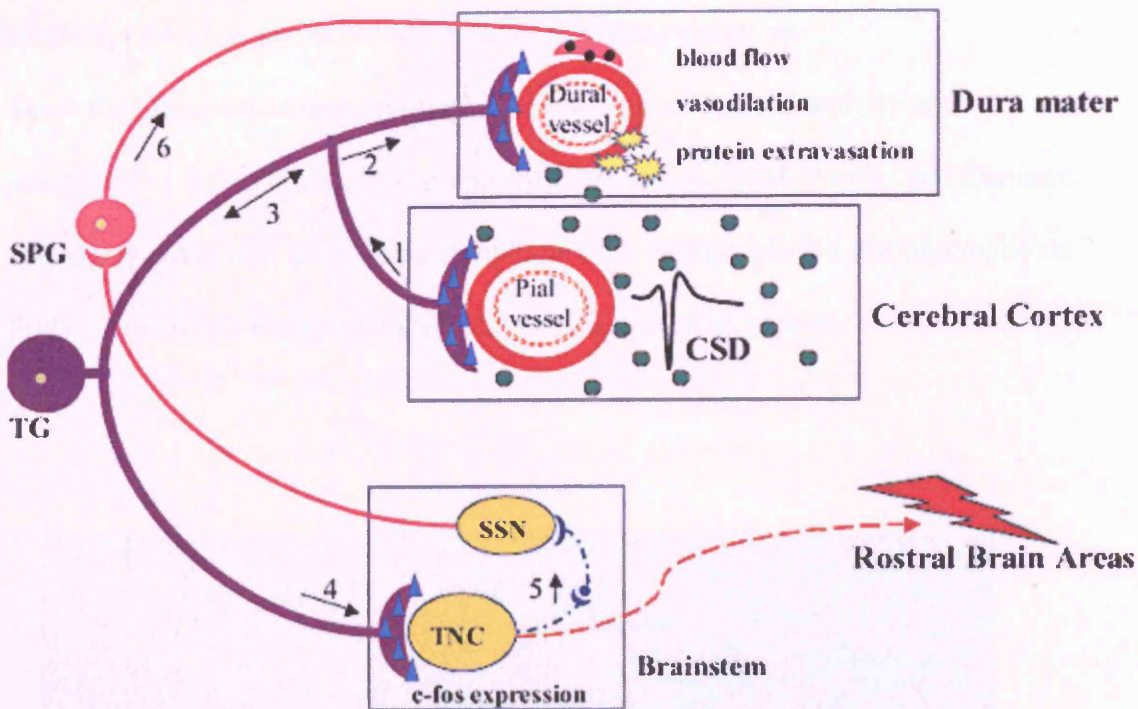


**Figure 1.3** The proposed link between CSD and trigeminovascular activation. Adapted from (Bolay et al., 2002).

*Brainstem theory (Figure 1.4)*

The other theory proposes a more fundamental role for the brainstem in the process. It is proposed that descending pathways from the cortex (stress), thalamus (excessive afferent stimulation, light, noise) or hypothalamus (sleep disturbance) project to the brainstem leading to activation of brainstem nuclei such as the dorsal raphe and locus coeruleus. The vascular changes associated with migraine occur as a result of this brainstem activation, namely LC induced cerebral vasoconstriction and DR stimulated dilatation as well as dilatation of the extracranial circulation mediated via the greater

superficial petrosal nerve (the trigeminoparasympathetic reflex). As a result of

activation of brainstem centres, probably the monoaminergic nuclei and other systems

which are involved in the descending modulation of nociception such as the PAG and

raphe nuclei, there is modulation of sensory input including the input from trigeminal

afferents. Consequently, a normally innocuous stimulus, such as the pulsation of

cerebral vessels, is perceived as a painful throbbing sensation.

Since the brainstem is also involved in modulation of cortical activity and

attentiveness to environmental stimuli (Matrenza et al., 2004; Parvizi and Damasio,

2003) this could also account for symptoms such as photophobia and phonophobia.

Proponents of this theory maintain that CSD is a parallel process.
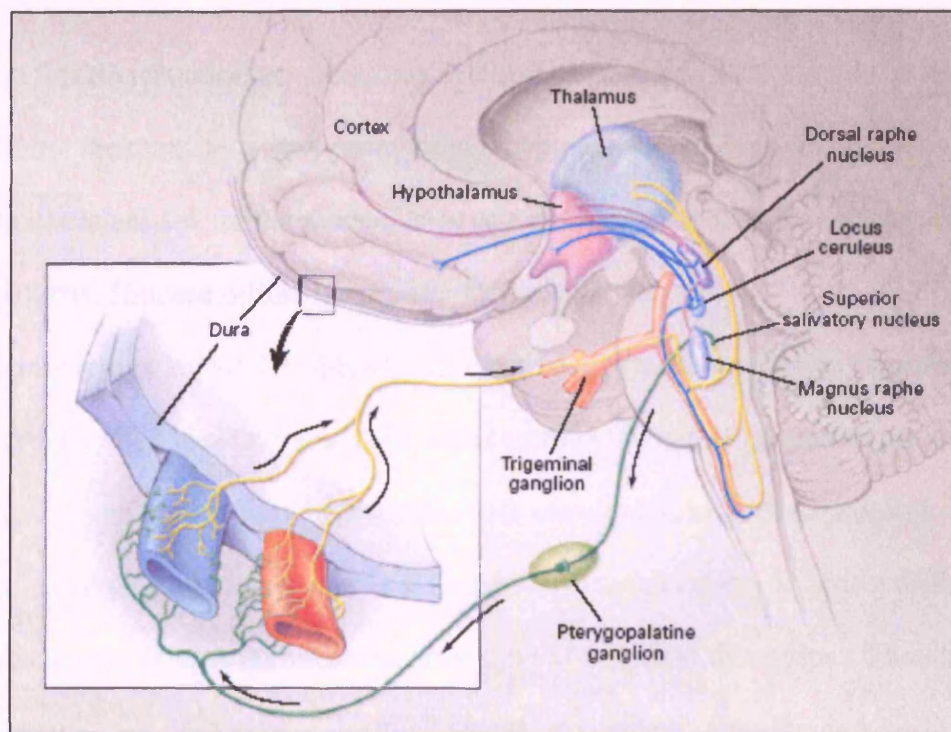


**Figure 1.4** Proposed pathways for brainstem involvement in pathophysiology of

migraine. Taken from (Goadsby et al., 2002).

# Chapter 2. Functional Imaging

## 2.1 Techniques

In 1890 Charles Sherrington demonstrated that stimulation of the brain caused a local

increase in blood flow. Functional imaging is based on the principle that local changes

in cerebral blood flow or metabolism accompany alterations of brain function

(Frackowiak and Friston, 1994). The metabolic changes in neurons and glia that

accompany neurotransmitter release are energy requiring. Most of this energy is used

at or around synapses. Normal brain energy production depends on oxidative

metabolism. Thus, there is a greater local demand for oxygen and glucose with

increased synaptic activity and to meet this demand there is an increase in local blood

flow.

The functional coupling of regional cerebral blood flow (rCBF) and local cerebral

glucose metabolism is well established (Jueptner and Weiller, 1995). The majority of

glucose is needed for the maintenance of membrane potentials and restoration of ion

gradients. Glucose utilization reflects synaptic activity.

The search for regionally specific effects is based on the concept of "functional

segregation". This states that cells with common functional properties are grouped

together and this, in turn, necessitates both convergence and divergence of cortical

connections. An example of this is found in the visual cortex. V2 has a distinctive

cytochrome oxidase architecture, consisting of thick and thin stripes. Directionally

selective cells are found in the thick stripes, exclusively. Retrograde labelling of cells

in V5 is limited to these thick stripes. V5 is a functionally homogenous area,

specialised for visual motion. Thus, if it is the case that neurons in a given area of the

brain share a common responsiveness to some sensorimotor attribute, then this functional segregation is also an anatomical one.

The various functional imaging techniques, described below, are sensitive to different types of changes. The techniques have evolved over time resulting in improvements in spatial and temporal resolution.

## Xenon blood flow studies and single photon emission computed tomography (SPECT)

The earliest attempts at functional imaging involved inhalation or intra-arterial injection of gamma emitting $^{133}$Xenon into the carotid artery. Cerebral blood flow was then detected by a gamma camera consisting of up to 254 stationary detectors covering one hemisphere. The measurements were repeated at 10-20 minute intervals. The numerous regional cerebral blood flow values (from each detector) were stored as a matrix and a flow distribution map created. The resultant spatial resolution was 1-5cm.

Xenon inhalation has also been used in combination with SPECT. SPECT involves rotating gamma camera detectors which results in improved spatial and temporal resolution compared with stationary detectors. Even better spatial resolution can be obtained using intravenous technetium-99m hexamethylpropyleneamine oxime ($^{99m}$Tc HMPAO) as the tracer. This provides a semi-quantitative analysis of regional cerebral blood flow.

## Positron emission tomography (PET)

PET is a tomographic nuclear imaging procedure, which uses positrons as radiolabels and positron-electron annihilation reaction-induced gamma rays to locate the radiolabels. It can provide a quantitative measurement of blood flow and, therefore, represents an improvement since SPECT. In $H_2^{15}O$ PET water is labelled with a positron emitter ($^{15}O$) and injected intravenously into the patient, who is scanned by the tomographic system. The $^{15}O$ is generated in a cyclotron. The tracer has a half-life of 124 seconds. The image is obtained after an interval of 90 seconds during which the $H_2^{15}O$ enters the brain. The scanner detects the spatial and temporal distribution of the radiolabel by detecting gamma rays during the so-called emission scan. The positron emitted by beta decay slows down to a slower speed. This is necessary for the annihilation reaction between the positron and a shell electron of a neighbouring atom to occur. The annihilation reaction produces two gamma rays which travel in almost exactly opposite directions (this is due to the conservation of energy and momentum laws). The two gamma rays are detected by a coincidence counting detection system as they register almost simultaneously on opposing pairs of scintillation detectors. This coincidence event identifies a line upon which the annihilation occurred. Absorption and scatter of photons by intervening tissue needs to be corrected for in order to obtain accurate measurements of tracer concentrations. This "attenuation correction" is performed by means of a transmission scan which is done prior to the emission scan. During this scan a positron-emitting source is placed around the body. From this source one of the annihilation photons has to travel the total distance through body tissues while the second photon is not attenuated at all. This allows attenuation factors to be derived.

The PET scanner consists of multiple detector rings to scan a number of transaxial

planes simultaneously. The detector rings are scintillation crystals (bismuth germinate

or sodium iodide) which fluoresce when struck by ionising radiation. These

scintillations are then converted to electronic signals. The collected raw data are

reconstructed into a cross-sectional image. Usually data are acquired in a three-

dimensional (3D) mode. The PET images are then coregistered with a structural scan,

usually an MRI, to enable accurate anatomical localization.

PET provides relatively good temporal resolution and spatial resolution when

compared with SPECT. The temporal resolution is dependent on the time taken for

the tracer to reach the brain (90 seconds) and on the half-life of the tracer (124

seconds for $^{15}$O). This enables the comparison of brain states such as migraine versus

pain-free but is not fast enough to follow, for example, progression of migraine aura.

The spatial resolution of PET is dependent upon the position and type of detectors.

The resultant nominal resolution for the scanner used in our studies is $6.4 \pm 0.2$ mm.

PET can detect changes in regional CBF of 3-5%.

**Magnetic resonance imaging (MRI)**

This uses a strong magnetic field (B0 field) and gradient fields to localize bursts of

radiofrequency signals coming from a system of spins consisting of reorienting

hydrogen nuclei (protons) after they have been disturbed by radiofrequency pulses

(RF). MR imaging produces high resolution imaging and makes use of the fact that

many nuclei exhibit a property called spin. These spins are orientated in an external

magnetic field. External radiofrequency pulses disturb their orientated state and make

them absorb energy, which is subsequently reradiated. The intensity of the reradiated signal is dependent on the radiating tissue and the pulse sequence used to disturb the spins.

"Relaxation" refers to the process by which spins release the energy of excitation and return to their original configuration. Relaxation times are measured for an entire collection of spins and are statistical or average measurements. The two measures of relaxation which describe energy transfer by the excited spin are T1 and T2. The differences in relaxation times between tissues are primarily responsible for contrast in MRI. The T1 relaxation time, also known as the longitudinal relaxation time or spin-lattice relaxation time, is the time for the magnetization to return to 63% of its original length. It is the mechanism by which the spins give up their energy to the surroundings to return to their equilibrium orientation. T2 relaxation, also known as transverse relaxation or spin-spin relaxation, occurs when spins in the low and high energy state exchange energy but do not lose energy to the surrounding lattice. T2 is the time when the transverse magnetization is 37% of its value immediately after the radiofrequency pulse.

**Functional MRI (fMRI)**

Blood Oxygen Level Dependent (BOLD) imaging allows detection of regional cerebral blow changes with a very high degree of temporal resolution. While oxyhaemoglobin has no substantial magnetic properties, deoxyhaemoglobin is strongly paramagnetic and can thus serve as an intrinsic paramagnetic contrast agent in appropriately performed MR imaging. If perfusion is increased by some means without increasing the local oxygen consumption, the venous oxyhaemoglobin concentration will increase and the deoxyhaemoglobin concentration will decrease.

As a result, there is less paramagnetic influence of free iron on T2* relaxation in a tissue volume containing draining veins and there is a signal intensity increase. This indirect, qualitative measure of perfusion allows localization of brain regions that are activated.

Diffusion weighted imaging (DWI) detects net translational movement of water using diffusion-sensitizing magnetic field gradients to dephase and rephase the protons in water. A regional decrease of diffusion is visible as hyperintensity on DWI. It can detect cell swelling e.g. following ischaemic insults.

Perfusion weighted imaging (PWI) provides estimates of haemodynamic changes based on dynamic imaging during the injection of high-susceptibility paramagnetic contrast medium such as gadolinium. It is sensitive to changes occurring at the microvascular level.

**Magnetic resonance spectroscopy (MRS)**

MRS is primarily employed as a technique which noninvasively monitors biochemistry in vivo. It uses nuclear magnetic resonance for the determination of individual chemical compounds. The underlying principle of MRS is that atomic nuclei are surrounded by a cloud of electrons which very slightly shield the nucleus from any external magnetic field. As the structure of the electron cloud is specific to an individual molecule or compound, the magnitude of this screening effect is also a characteristic of the chemical environment of individual nuclei. In view of the fact that the resonant frequency is proportional to the magnetic field that it experiences, it follows that the resonant frequency will be determined not only by the external applied field, but also by the small field shift generated by the electron cloud. This

shift in frequency is called the chemical shift. It should be noted that chemical shift is

a very small effect, usually expressed in "parts per million" (ppm) of the main

frequency (Table 2.1). In order to resolve the different chemical species, it is therefore

necessary to achieve very high levels of homogeneity of the main magnetic field B0.

Spectra from humans usually require shimming the magnet to approximately one part

in $10^8$.

In the context of human MRS, two nuclei are of particular interest: $^1$H and $^{31}$P. Proton

MR Spectroscopy ($^1$H-MRS) is mainly employed in studies of the brain where

prominent peaks arise from N-acetylaspartate (NAA), choline-containing compounds,

creatine and creatine phosphate (PCr), myo-inositol and, if present, lactate.

Phosphorous 31 MR Spectroscopy ($^{31}$P-MRS) detects compounds involved in energy

metabolism: creatine phosphate (PCr), adenosine triphosphate (ATP) and inorganic

phosphate (Pi) and certain compounds related to membrane synthesis and degradation.

The possibility of determining the intracellular concentrations of high energy (PCr

and ATP) and low energy phosphates (Pi) offers information on the status of energy

metabolism in tissues. The phosphorylation potential, an indication of the free energy

available in tissue, can be calculated from the values of ATP, ADP and Pi. It is also

possible to determine intracellular pH because the inorganic phosphate peak position

is pH sensitive.

**Table 2.1** Chemical shift values for selected resonances.

| Functional group/molecule | Chemical shift (ppm) |
|---|---|
| Lactate, methyl group | 1.3 |
| N-Acetylaspartate (NAA) | 2.0 |
| Choline | 3.2 |
| $\alpha$-ATP | -7.8 |
| $\beta$-ATP | -18.2 |
| $\gamma$-ATP | -2.7 |
| Pi, inorganic phosphate | 5.2 |
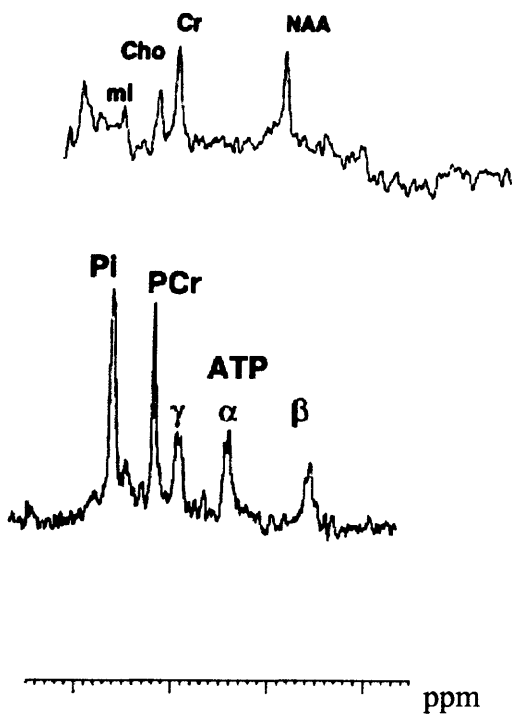


**Figure 2.1** Examples of spectra from $^{1}$H-MRS (top spectrum) and $^{31}$P-MRS.

**Statistical parametrical mapping (SPM)**

Statistical parametric mapping refers to the construction and assessment of spatially extended statistical process used to test hypotheses about neuroimaging data from SPECT/PET and fMRI. It is a voxel-based approach, employing classical inference, to make some comment about regionally specific responses to experimental factors or brain states. Images are spatially normalised into a standard space and smoothed. Parametric statistical models are assumed at each voxel, using the general linear model (GLM) to describe the variability in the data, in terms of experimental and confounding effects, and residual variability. The experimental design and model used to test for specific neurophysiological responses form a mathematical structure called "the design matrix". This is partitioned according to whether the effect is of interest (e.g. an activation) or not (e.g. a nuisance effect). The contribution of each effect (i.e. each column of the design matrix) to the observed physiological responses is estimated using the general linear model and standard least squares. These estimated contributions are known as parameter estimates. Regionally specific effects are framed in terms of differences among these parameter estimates (e.g. an activation effect) and are specified using contrasts. The significance of each contrast is assessed with a statistic that has Student's $t$ distribution under the null hypothesis. For each contrast, or difference in parameter estimates, an SPM {t} statistic is computed for each voxel in the brain. This is transformed into a SPM {Z}. Statistical inferences are then made about local excursions of the SPM {Z} above a specified threshold, using the Gaussian field theory. These pertain to the maximal value and spatial extent of the observed activations. The resulting $P$ values and the SPM {Z} scores are the end point of the analysis.

Global CBF varies both between subjects and within subjects over time. For normal

subjects the global CBF is measured as the mean rCBF over all voxels. The

contribution of global CBF to the variance in regional CBF is removed by a voxel-by-

voxel analysis of covariance (ANCOVA) with global CBF as the confounding

variable. A map is created resulting in condition specific adjusted rCBF values

normalised to a nominal mean global activity of 50ml/100mg/minute.
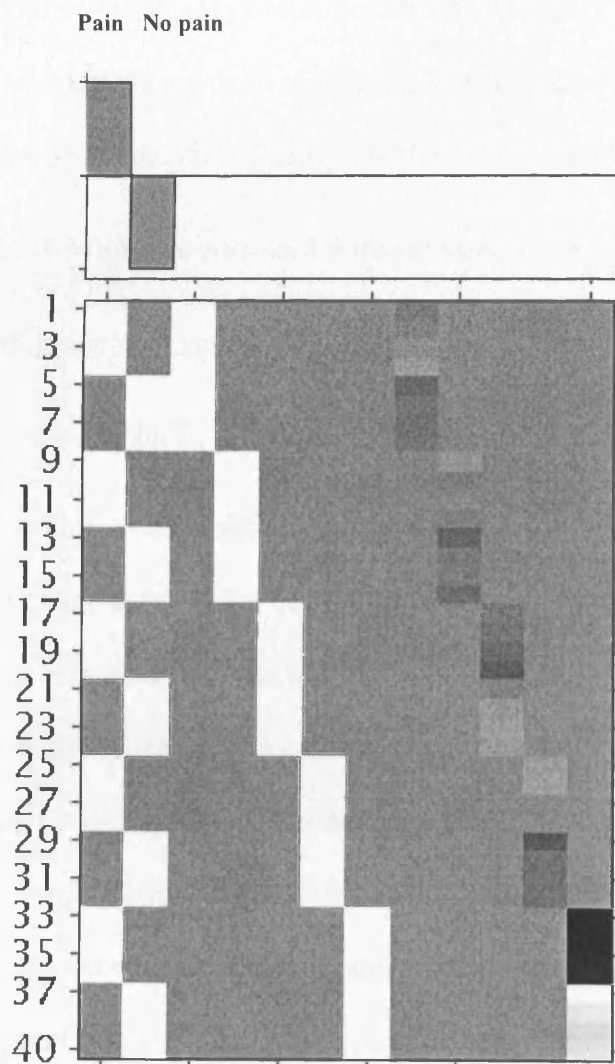


**Figure 2.2** An example of a design matrix incorporating 5 subjects with 2 conditions.

The column above shows the contrast between condition 1 and condition 2.

## 2.2 History of functional imaging in migraine

Functional imaging has been utilised to help characterize the underlying pathophysiology of migraine.

In this section I will first discuss the results from imaging studies investigating migraine with aura and migraine without aura. I will then focus more specifically on the migraine aura and more complicated and rare forms of aura, namely prolonged and persistent aura. Finally I will review the results of MRS studies in migraine.

### 2.2.1 Migraine with and without aura

Migraine with aura

*Xenon / SPECT*

The earliest attempts at functional imaging in migraine tended to focus on the migraine aura. At the time Wolff's theory of migraine was dominant. This proposed that migraine aura was caused by intracerebral vasoconstriction and that the headache was due to reactive vasodilatation of the carotid artery. In the early 1970's Xenon flow imaging showed a reduction in cerebral blood flow during aura and hyperperfusion during the headache (O'Brien, 1971; Skinhoj, 1973). Attacks were triggered by carotid angiography and measurement intervals were between 15 and 30 minutes. In 1981 another Xenon flow study showed that the vascular theory was not sufficient to explain migraine (Olesen et al., 1981). The study involved six migraineurs and used an improved detector unit. It showed that in three of the subjects the unilateral, focal (occipito-parietal) oligaemia during the aura was preceded by hyperemia. In 5 subjects the oligaemia spread anteriorly. What was particularly

interesting was that in 4 subjects severe headache was observed during the oligaemic

phase. This observation was replicated by SPECT studies (Andersen et al., 1988;

Friberg et al., 1991; Lauritzen and Olesen, 1984). The hyperemic phase has also been

shown to persist beyond the headache phase (Andersen et al., 1988; Sakai and Meyer,

1978). Therefore, vasodilatation could not explain the headache component of

migraine.

*fMRI (BOLD)*

A case report of a subject with migraine with aura scanned within 10 minutes of onset

of his spontaneous aura (left, homonymous quadrantanopia) demonstrated an increase

in T2 weighted contrast intensity bilaterally in the occipital cortex, the red nucleus

and the substantia nigra (Welch et al., 1998). Following on from this twenty-six

migraineurs (twenty-three with aura and three without aura) were scanned during

repetitive visual stimulation, using a checkerboard stimulus, in order to trigger a

migraine (Cao et al., 2002). Fifteen subjects (thirteen with aura and two without)

developed either headache or aura or both. In 75% of these, baseline T2-weighted MR

signal intensities increased in the red nucleus, substantia nigra and occipital cortex. In

seven of the subjects signal increases were also detected in other brainstem structures

including locus coeruleus, periaqueductal grey, pons and central midbrain, although

the time course, duration and extent of activation in these structures is not

documented. The same group then studied the occipital cortex in greater depth using

the same stimulus (Cao et al., 1999). In five of twelve subjects the onset of headache

or visual change was preceded by suppression of initial activation. The suppression

propagated into contiguous occipital cortex at a rate of 3-6 mm/minute and was

accompanied by baseline contrast intensity increases that indicated vasodilatation and

hyperoxygenation. Interestingly, one of these five subjects had a diagnosis of migraine without aura. No clear evidence of ischaemia was noted in this study.

In a more detailed study of aura BOLD changes were recorded which suggested CSD was responsible for generating migraine aura (Hadjikhani et al., 2001). The study involved three subjects and five attacks of migraine with aura were studied, two induced by exercise and three spontaneous. Initially, a focal increase in BOLD signal (thought to reflect vasodilatation) developed within the extrastriate visual cortex. This signal then propagated contiguously at a rate of $3.5 \pm 1.1$ mm/min over the occipital cortex, congruent with the retinotopy of the visual percept (Figure 2.3). The BOLD signal then diminished possibly reflecting vasoconstriction. The spreading phenomenon did not cross prominent sulci and were restricted to the hemisphere corresponding to the aura.
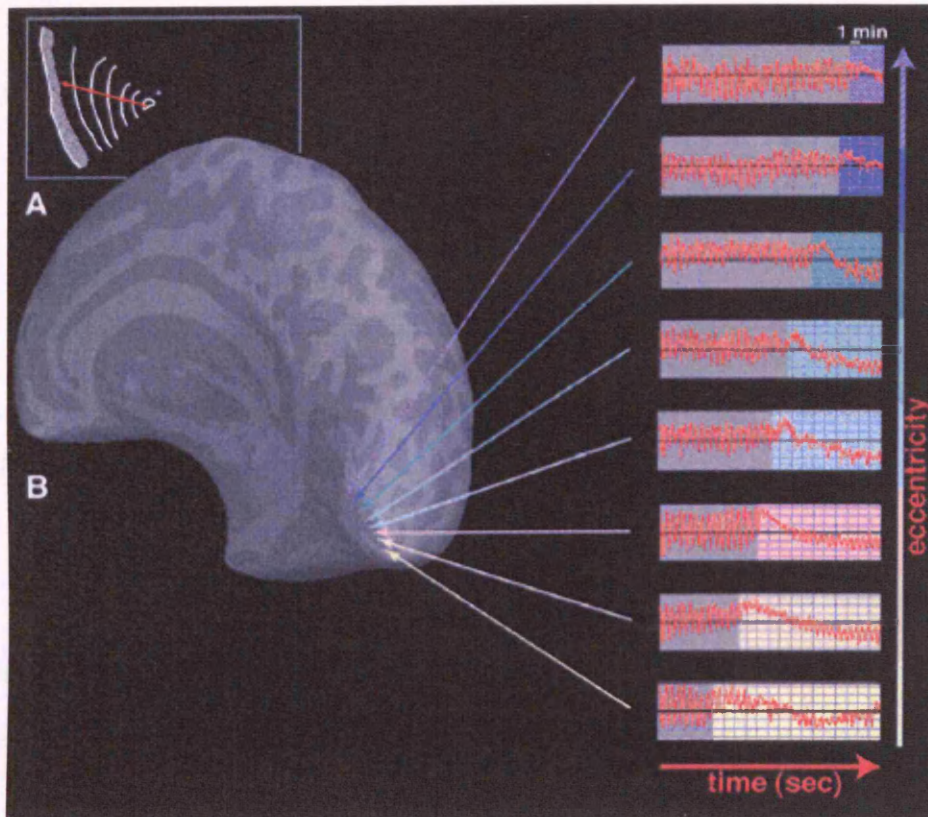
**Figure 2.3** Spreading suppression of cortical activation during migraine aura.

(Adapted from Hadjikhani et al., 2001)

A- A drawing of progression of the visual aura affecting the left hemifield, over

20 minutes.

B- MR signal changes (BOLD response) over time shown on the medial occipital

cortex. Each time course represents a voxel in consecutive areas starting in the

calcarine sulcus and progressing anteriorly.

*DWI/PWI*

PWI abnormalities have been demonstrated in migraine with aura (Sanchez del Rio et al., 1999). During aura, relative cerebral blood flow was found to be decreased (27%) in the contralateral occipital cortex. rCBV was decreased (15%) and mean transit time increased (32%), persisting up to 2.5 hours into the headache phase. No changes in DWI have been observed in migraine with aura (Cutrer et al., 1998).

The level of blood flow reduction which occurs during aura is now clearer due to the advent of PET and MRI. Evidence from these imaging studies has demonstrated that ischaemia does not account for aura nor does it seem that significant ischaemia (>50% decrease in perfusion) is generally provoked by aura.

Migraine without aura

*SPECT*

In contrast to migraine with aura, SPECT studies have failed to show any ictal changes in regional blood flow during migraine without aura. However, Sakai and Meyer reported a global blood flow increase during attacks (Sakai and Meyer, 1978).

*PET*

Bednarczyck studied nine subjects within 13 hours of onset of migraine without aura (Bednarczyk et al., 1998). They observed a 9.9% decrease in global CBF and a 5.2% decrease in CBV persisting for at least 6 hours. Oxygen metabolism and oxygen extraction remained unchanged.

Woods et al reported a case report of a subject with migraine with no previous aura who unexpectedly developed a migraine during her participation in a visual activation paradigm whilst lying in a PET scanner (Woods et al., 1994). The subject described some visual blurring during one of the scans but did not clearly describe any other features of typical aura. The migraine was associated with bilateral hypoperfusion starting in the occipital lobes and spreading anteriorly into the temporal and parietal lobes. The contiguous spread covered areas in the territories of the posterior and middle cerebral artery.

Weiller at al investigated nine subjects with migraine without aura (Weiller et al., 1995). The subjects presented with right-sided spontaneous headaches and were scanned within 6 hours of onset of migraine, prior to having taken any medication. They were scanned during spontaneous migraine attacks, following sumatriptan and inter-ictally. Interestingly, three of the subjects were on migraine preventives ($\beta$-blockers). The study revealed brainstem activation during the migraine which persisted after sumatriptan administration had relieved the pain. The resolution of the PET camera used was not high enough to identify specific nuclei but the foci of maximum increase were around the dorsal midbrain, which contains the dorsal raphe nucleus, and the dorsolateral pons, which contains the locus coeruleus. Activation was also seen in the anterior cingulate, visual and auditory association cortices. A case of a glyceryl trinitrate (GTN)-triggered migraine also revealed brainstem activation, on this occasion in the dorsolateral pons, which again persisted following abortion of the migraine (Bahra et al., 2001).
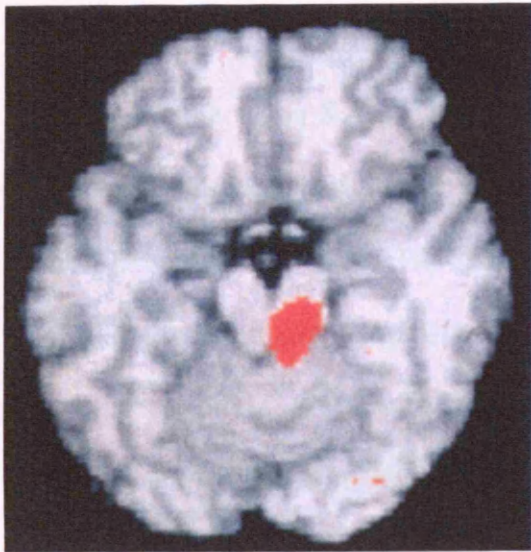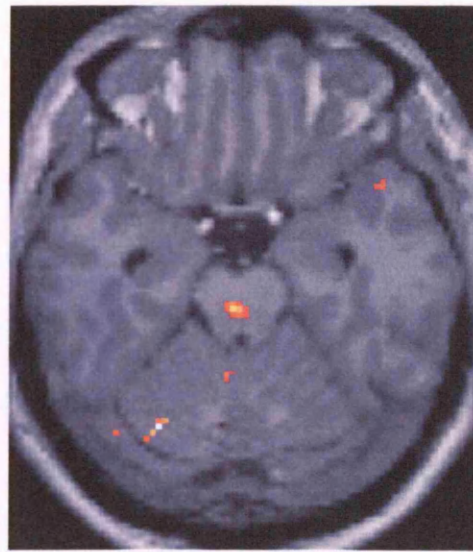
(Weiller et al., 1995)                    (Bahra et al., 2001)

**Figure 2.4** PET scans showing brainstem activation during migraine.

It is important to note that no such brainstem activation was noted in a PET study

which evoked first division trigeminal pain by means of a subcutaneous injection of

capsaicin into the forehead (May et al., 1998b), nor in imaging studies of cluster

headache or SUNCT (May et al., 1998a; May et al., 1999). However, a recent PET

study of hemicrania continua has demonstrated pontine activation (Matharu et al.,

2004b). This will be discussed later.

*fMRI (BOLD)*

There have been no fMRI studies exclusively involving migraineurs without aura.

However, the studies described in the section above on "migraine with aura" included

a mixed group of subjects with both migraine with and without aura.

*PWI/DWI*

No abnormalities have been recorded in either PWI or DWI in migraine without aura.

Inter-ictal changes

In a SPECT study using Xenon and $^{99m}$Tc HMPAO Friberg et al. reported inter-ictal cerebral blood flow asymmetries in almost 50% of migraineurs compared with controls (Friberg et al., 1994). The abnormalities occurred in an equal proportion of migraineurs with aura and those without aura. In a smaller SPECT study no such changes were noted (Lauritzen and Olesen, 1984). It was suggested that this may be because an equivalent regional analysis was not performed in this study. A number of other studies have also reported inter-ictal abnormalities (Lagreze et al., 1988; Levine et al., 1987; Schlake et al., 1990).

High resolution MRI was used to measure non-haem iron in the brainstem of seventeen subjects with episodic migraine (with and without aura) inter-ictally, seventeen controls and seventeen subjects with chronic migraine during headache (Welch et al., 2001). Both migraine groups exhibited increased levels of iron in the periaqueductal gray as compared to controls, with no significant difference between those with and those without aura.

Overall, there appears to be little evidence on the imaging front for a significant difference between the processes involved in migraine with and without aura except when the aura itself is studied.

Chronic migraine

*MRI*

As described above, the periaqueductal gray has been shown to exhibit abnormally high levels of iron in chronic migraineurs during headache (Welch et al., 2001). This appeared to be correlated with increased duration of migraine.

*PET*

A PET study looking at eight chronic migraineurs who had bilateral suboccipital stimulators implanted demonstrated activation in the dorsal rostral pons which persisted when the stimulator was switched on to provide pain relief (Matharu et al., 2004a). This is the same area which has been shown to be activated in episodic migraine (Bahra et al., 2001).

Triggered migraine with and without aura

A PET study by Anderson et al investigated attacks provoked by red wine in 11 subjects with migraine with and without aura. They demonstrated reductions in regional cerebral blood flow (23%) and in oxygen metabolism (22.5%) in an area corresponding to the primary visual cortex. They did not detect any significant increases in blood flow during aura or headache.

## 2.2.2 Prolonged and persistent aura

The migraine aura, itself, has been examined, in detail, in a recent fMRI study (Hadjikhani et al., 2001) described above. These observations support the idea that CSD is responsible for aura. A sub-group of migraineurs, usually those with familial hemiplegic migraine or sporadic hemiplegic migraine, experience a prolonged or persistent form of aura. Prolonged aura was defined in the first IHS classification (Headache Classification Committee of the International Headache Society, 1988) as "aura symptoms lasting between one hour and one week". Persistent aura is defined by the current IHS criteria (Headache Classification Committee of The International Headache Society, 2004) as "aura symptoms persisting for more than a week without radiographic evidence of infarction". The functional deficit can take the form of visual, speech, cognitive, sensory and/or motor disturbance and occasionally altered

consciousness. The pathophysiology behind this form of migraine is not well understood. There are conflicting views as to whether this is a vascular or neuronal phenomenon.

There are a number of small studies, mainly case reports, in which such patients have undergone neuroimaging. The focus has been on detecting any evidence of ischaemia. DWI has, with the exception of two case reports, been normal (Gonzalez-Alegre and Tippin, 2003; Gutschalk et al., 2002; Iizuka T, 2004; Lindahl et al., 2002; Oberndorfer et al., 2004; Smith et al., 2002). In the two case reports there was evidence of decreased diffusion, three weeks into the aura, which affected the contralateral hemisphere (Butteriss et al., 2003; Chabriat et al., 2000). In both cases this eventually normalised. The abnormal diffusion was thought to reflect cerebral oedema rather than ischaemia. MRI with and without Gadolinium enhancement has been abnormal in some case reports, again suggesting oedema or inflammation of the affected hemisphere (Barbour et al., 2001; Butteriss et al., 2003; Crawford and Konkol, 1997; Iizuka T, 2004; Smith et al., 2002).

FDG-PET has been used to look at glucose metabolism and this was found to be reduced in two case reports (Gladstone JP, 2004; Gutschalk et al., 2002). Focussing on perfusion, PWI has mainly demonstrated unilateral hyperperfusion (Lindahl et al., 2002; Oberndorfer et al., 2004; Smith et al., 2002) but was normal in one case study (Gutschalk et al., 2002). In a recent case report of persistent visual aura, however PWI demonstrated decreased perfusion in the relevant hemisphere which normalised when the symptoms resolved (Relja et al., 2005). Similarly, SPECT has also demonstrated unilateral hyperperfusion (Barbour et al., 2001; Gonzalez-Alegre and Tippin, 2003; Iizuka T, 2004; Iizuka, 2004; Oberndorfer et al., 2004). In all but one of these cases the hyperperfusion affected the hemisphere contralateral to the clinical signs.

Magnetic Resonance Angiography (MRA) has been shown to be normal in two case reports (Barbour et al., 2001; Smith et al., 2002). There has only been one case report documenting angiographic evidence of narrowing of intracerebral vessels during an attack of prolonged aura but it must be noted that this case was somewhat atypical and involved a pregnant woman with evidence of thrombophilia whose attack was unlike her previous hemiplegic migraine attacks (Gonzalez-Alegre and Tippin, 2003). Overall, these studies do not suggest an ischaemic aetiology.

### 2.2.3 MRS studies in migraine

$^{31}$P-MRS

The majority of MRS studies in migraine have used $^{31}$P-MRS in order to look for abnormalities in energy metabolism. Most of these involve inter-ictal scanning. Welch's group were the first to apply MRS to migraine subjects (Welch et al., 1989). They studied a group of twenty migraineurs (eight migraineurs with aura and twelve migraineurs without aura) and twenty-seven healthy controls. Eleven migraineurs were studied during an attack (but not during aura) between 3 and 48 hours of headache onset. The remaining nine were studied inter-ictally. In the ictal group they observed a lower mean PCr/Pi and PCr/TP ratio and higher Pi/TP ratio as compared to the controls. When they further characterised the ictal group into migraine with and without aura they found that these observations were true for the migraine with aura group and not significant in the migraine without aura group. These abnormalities were more significant in anterior brain regions but also present in posterior region. Looking at the inter-ictal migraine group they found an increase in the mean Pi/TP ratio as compared to the controls.

Analysis of intracellular pH revealed no significant differences between any of the groups.

Following on from these findings, an Italian group sought to examine both the brain and muscle of migraineurs to determine whether there was a generalised metabolic abnormality in migraine (Barbiroli et al., 1992; Barbiroli et al., 1990; Montagna et al., 1994). They also examined what they termed "complicated forms of migraine" which included migraine with prolonged aura and migraine strokes. Their studies included fifteen subjects with "complicated" migraine, eighteen subjects with migraine with aura, twenty-two with migraine without aura and fifty controls. They examined the occipital region of the brain inter-ictally and also the gastrocnemius muscle. Overall, they observed lower PCr levels in all the migraine groups when compared with the controls (Montagna, 1995). The mean Pi was unchanged, calculated ADP and the rate of maximal ATP biosynthesis were significantly increased and phosphorylation potential significantly decreased in all migraine sub-groups. As for muscle, abnormalities were found when analysing recovery of PCr after exercise in twelve subjects with migraine without aura, thirteen with migraine with aura and all of the "complicated migraine" group.

Essentially the same findings have been described in a case report of migraine with prolonged aura (Sacquegna et al., 1992) and a familial hemiplegic migraine (FHM) family (Uncini et al., 1995). This family study was particularly interesting as it emerged that abnormalities were also present in two clinically asymptomatic members of the family.

Taking the results of all these studies it is evident that abnormalities in energy metabolism exist in migraineurs and these are not exclusive to brain tissue. Such abnormalities have been found inter-ictally and ictally. The possibility of

mitochondrial dysfunction in migraine led to an open-label trial of Coenzyme Q, an

essential element of the mitochondrial electron transport chain, as a migraine

preventative (Rozen et al., 2002) and more recently a randomised controlled trial was

conducted which demonstrated Coenzyme Q was superior to placebo (Sandor et al.,

2005).


*Magnesium*

MRS has also demonstrated significantly lower levels of magnesium in the occipital

region of subjects with migraine when compared with controls (Boska et al., 2002;

Lodi et al., 2001; Welch et al., 1989). The levels appear to be relatively lower in those

with more severe clinical symptoms with subjects with hemiplegic migraine or

complicated migraine aura having lower magnesium levels than those with migraine

without aura. These studies were performed in the inter-ictal period but ictal

abnormalities have also been reported with lower magnesium levels ictally when

compared to controls (Ramadan et al., 1989).


^{1}H-MRS

^{1}H-MRS has been used to look at lactate levels in migraine. Watanabe et al. looked at

the occipital cortex inter-ictally in six subjects with migraine with aura and six

controls (Watanabe et al., 1996). They found relatively higher levels of lactate in five

patients who had experienced a migraine attack in the last two months. One

migraineur had not had a migraine attack for four years. This subject did not

demonstrate a lactate peak. The authors postulated a disturbance in aerobic oxidation

which could arise from mitochondrial dysfunction (or ischaemia).

Other metabolite abnormalities have also been investigated. There has been one study examining eight subjects with migraine with aura inter-ictally and seven controls (Macri et al., 2003). Measurements of metabolite levels in the cerebellum revealed reduced choline values in the migraine group when compared with the control group. Choline is involved in the synthesis of membrane constituents and the neurotransmitter, acetylcholine. It must be noted, however, that the study is relatively small and the results may be affected by the short echo time as this can result in greater spectral overlap.

## 2.3 Pain Processing and Functional Imaging

Functional imaging has been employed with the aim of elucidating the functional anatomy of pain pathways. Human pain is multi-dimensional, consisting of the sensory or discriminative components, the affective components and the cognitive components. The term "pain neuromatrix" has been proposed to explain the network of neural circuits involved in pain processing (Derbyshire, 2000). The human pain system is often divided into the lateral and medial pain systems. The medial pain system projects via the medial thalamic nuclei to prefrontal and anterior cingulate cortices, and parts of insular cortex. The lateral pain system projects via lateral thalamic nuclei to the primary and secondary somatosensory cortices (SI and SII, respectively) and the dorsal insula. The lateral pain system is thought to be responsible for the sensory-discriminative components of pain processing (the location, intensity and duration of the pain), the medial pain system is thought to be involved in the affective, motivational and evaluative components (Treede et al., 1999).

Functional imaging has contributed significantly towards this knowledge of the pain processing systems. Many of the imaging studies used experimental models of pain since the study of spontaneous acute pain is obviously marred by practical difficulties such as getting the patient to the scanner in time. Studies have used tonic and phasic pain stimuli involving heat, cold, chemical and mechanical pain stimuli (Casey et al., 1996; Derbyshire and Jones, 1998; Iadarola et al., 1998) and more recently laser stimuli which is thought to be superior since it allows activation in response to pain without the response to tactile stimulation (Bingel et al., 2002). Chronic pain syndromes have also been studied but these may involve a slightly different set of

neural pathways than those involved in acute pain. PET and f-MRI are the main

modalities used.

Although there are some disparities there are number of consistent findings from these

studies which enable us to derive a reasonably good picture of pain neuroanatomy. I

will focus on these consistent areas in the following section.


*Anterior cingulate cortex (ACC)*

This is one of the most consistently activated areas in pain studies (Ingvar, 1999). It is

part of the medial pain system and is thought to be involved in the affective-

evaluative dimension of pain. It is activated in acute pain, visceral pain and appears to

be tonically activated in chronic pain states (Hsieh et al., 1995). In one study right

anterior cingulate activation has been reported irrespective of the side of pain (Hsieh

et al., 1995). However, this finding has not been replicated (Bingel et al., 2003; Bingel

et al., 2002; Coghill et al., 1994).

In a study using hypnotic suggestion to alleviate the unpleasantness associated with

noxious stimuli without altering the intensity of pain it was found that the activation

in the ACC was reduced whereas the activation in the somatosensory cortex remained

unchanged (Rainville et al., 1997). Similarly, the ACC was also activated in a study

producing the illusion of pain when in fact the stimuli were not noxious (Craig et al.,

1996).

Clinical evidence for the function of the ACC is provided by observations of chronic

pain patients. Following cingulotomy (surgery to cut the white matter tract underlying

the ACC) these patients remained aware of the pain but it no longer bothered them

(Santo et al., 1990).

*Somatosensory cortex*

The primary somatosensory cortex (SI) is involved in the spatially discriminatory aspects of pain stimuli. It is only found to activated in 50% of imaging studies in pain (Peyron et al., 2000) and is activated bilaterally with greater activation contralaterally to the site of painful stimulus (Youell et al., 2004). The secondary somatosensory cortex (SII) is more consistently activated. However, there is a paucity of nociceptive neurons in this region and poorly demarcated receptive fields and activations are generally bilateral (Ingvar, 1999). These observations suggest that it is less likely to be involved in spatial discrimination. It has been proposed that SII may be involved in recognition, learning and memory of painful events (Schnitzler and Ploner, 2000).

*Insula*

The insula is fairly consistently activated in pain studies and, in fact, the insula cortex is the only cortex where stimulation has elicited pain (Ostrowsky et al., 2002). It can be functionally divided into an anterior part, which is the region activated in most pain studies, and a posterior part which can sometimes be difficult to distinguish clearly from the SII cortex. The anterior insula is thought to be involved in the autonomic and affective components of pain. It is also proposed that the insula is involved in intensity coding (Coghill et al., 1999; Peyron et al., 1999). Activation is often bilateral but a recent study looking at hemispheric lateralisation has found that the left side demonstrates greater activation independent of stimulus side suggesting this region is also involved in sensory-discriminative aspects of pain (Youell et al., 2004). There is some suggestion that posterior insula activation is predominantly contralateral (Brooks et al., 2002).

*Thalamus*

The thalamus, despite its role as a relay centre for afferent input into the brain, is less consistently activated in experimental pain studies. It can be divided into medial and lateral parts. The medial thalamus receives input from the ascending pathways from the spinal cord and reticular formation and projects diffusely to the cortex forming part of the medial pain system. The lateral thalamus, including the ventrobasal thalamus, is somatotopically organised and receives input from the ascending tracts and sends fibres to the somatosensory cortex. It forms part of the lateral pain system. The thalamus is bilaterally activated in some studies (Casey et al., 1996; Derbyshire et al., 1998) which suggests that the activation is not merely a sensory response. The bilateral response is thought to reflect activation of the medial nuclei with the lateral nuclei being activated contralaterally (Bingel et al., 2003). It is likely to be involved in both discriminative and attentional networks of pain. The inconsistency of activation in studies may be due to methodological issues of sensitivity.

*Prefrontal cortex*

The dorsolateral prefrontal cortex has often been shown to be activated in pain studies. It is thought to be involved in the cognitive dimension of pain. In a recent fMRI study it was demonstrated that placebo analgesia was associated with increased activity during the anticipation of pain in the prefrontal cortex (Wager et al., 2004).

Other areas of activation which are less consistent in pain imaging studies include the cerebellum, midbrain, hypothalamus and basal ganglia (caudate and lenticular nuclei).

# Chapter 3. Laterality of migraine headache

Unilateral pain has been a hallmark of migraine since Galen (AD 131-201) introduced the term Hemicrania to describe the disorder (Lance and Goadsby, 1998a). Laterality of activation in functional imaging studies of general pain has been discussed, to some extent, in the previous chapter. 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, however, that this study was not designed to look at laterality and so left-sided and right-sided groups were not analysed separately. An MRI study that detected increased iron levels in the periaqueductal gray (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 sixty-four patients in whom electrodes were implanted unilaterally, fifteen 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 (pontine) 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., 2003a).

Autonomic asymmetry has been reported in migraine. One study measured

electrodermal responses to visual and auditory stimuli inter-ictally in unilateral

migraineurs and found that left-sided pain was associated with under-responsiveness

and fast habituation whereas right-sided pain was associated with over-responsiveness

and slow habituation of this sympathetic response (Gruzelier et al., 1987). A recent

study reported parasympathetic but no sympathetic asymmetry in unilateral

migraineurs with left-sided migraineurs displaying a greater parasympathetic response

to an aversive stimulus (Avnon et al., 2004).

Electrophysiology also provides some data regarding laterality. Kaube et al found

facilitation of the nociception-specific blink reflex response predominated on the

headache side only during migraine (Kaube et al., 2002).

# Chapter 4. Glyceryl trinitrate (GTN): a human migraine model

The unpredictable and episodic nature of migraine introduces practical difficulties in research in this field, especially in imaging studies as well as therapeutic intervention studies. The difficulties include getting patients to travel to the research centre whilst suffering with severe pain or nausea and vomiting from migraine. This delays the onset of any therapy in interventional studies and introduces a selection bias in that those most debilitated will not participate in the study as they are unable to travel. It also means that the attack cannot be followed from the very onset. Also, the pathophysiology and clinical manifestations of the headache may change throughout the attack. For example, in migraine there may be a premonitory phase prior to the headache or aura phase. Developing approaches to triggering migraine reliably is important for advancing understanding of the disorder. An effective migraine model will enable the headache to be followed in a controlled environment throughout the attack. The model needs to be reliable, reproducible and validated.

Glyceryl trinitrate (GTN) or nitroglycerine was recognised to trigger very typical headaches in munitions workers (Laws, 1898), and these effects were described in detail some years later (Rabinowitch, 1944). Dalsgaard-Nielsen (1955) suggested GTN administration may be a test for headache classification and certainly migraine-like headache developing following sub-lingual GTN is well recognised (Sicuteri et al., 1987). The method has been refined by the Copenhagen Group (Iversen, 2001; Iversen, 1992; Iversen and Olesen, 1994; Iversen et al., 1989). They conducted a blinded study of controls and subjects with migraine without aura and monitored responses to increasing doses of intravenous GTN (Olesen et al., 1993). They demonstrated a dose-dependent headache response with a ceiling effect and maximum headache score at 0.5μg/kg min of GTN. The migraineurs reported initial, immediate

headaches of greater intensity than the controls and many experienced a second

delayed headache which was labelled as a typical migraine. There was no such

delayed headache in the controls. It was subsequently reported that migraine headache

could also be triggered in some subjects with migraine with aura although the aura

was not triggered (Christiansen et al., 1999).

Sances and colleagues (2004) have recently published a study involving 197

migraineurs on the reliability of the nitroglycerin trigger in migraine and cluster

headache using sublingual GTN. They determined that the sensitivity and specificity

of the test in migraine without aura was 82.1% and 96.2% respectively. In migraine

with aura the test was less sensitive (13.6%) but the specificity was the same.

Interestingly, migraine aura was triggered in three of twenty-two subjects in the

migraine with aura group. A further six subjects developed migrainous headache

without aura similar to those in the study by Olesen's group (Christiansen et al.,

1999).

GTN is highly lipophilic and easily crosses the blood-brain barrier. It is thought to

induce migraine through a nitric oxide (NO) mediated process. The long- acting

nitrate 5-isosorbide mononitrate induces a dose-dependent headache but its

metabolites, apart from NO, are different from those of GTN (Iversen et al., 1992). N-

acetylcysteine, which augments the effects of GTN in the heart by increasing the

formation of or enhancing the effect of NO, also augments the headache response to

GTN (Iversen, 1992). NO is a molecule which is ubiquitous throughout the brain. It

has numerous physiological functions including neurotransmission, regulation of

cerebral blood flow, sensory processing, pain perception and long-term potentiation.

Endogenous NO is generated by the conversion of arginine to citrulline by nitric oxide

synthases of which there are at least three types. Two are constitutive and

calcium/calmodulin dependent: endothelial NOS (eNOS) and neuronal NOS (nNOS) and the third is inducible and calcium/calmodulin independent: inducible NOS (iNOS). The latter produces larger quantities of NO once expressed.

*The role of nitric oxide in migraine*

There is now extensive evidence for the role of nitric oxide in migraine both from human and animal studies (Thomsen and Olesen, 2001). Human studies have shown abnormalities in metabolites of nitric oxide. The physiological actions of NO are mainly mediated by activation of guanylate cyclase and a consequent increase in cyclic guanosine monophosphate (cGMP). Serum cGMP and nitrites have been shown to be raised during migraine (Sarchielli et al., 2000; Stepien and Chalimoniuk, 1998). Platelet levels of nitrate, nitrite and cGMP are also higher during a migraine attack as compared with outside an attack or in healthy controls and they decrease inter-ictally following treatment with propranolol (Shimomura et al., 1999). As described above, clinical studies have demonstrated that a nitric oxide donor such as glyceryl trinitrate can trigger migraine. In a double-blinded study it was demonstrated that the NO synthase inhibitor L-N methylarginine (L-NMMA) hydrochloride was effective in the acute treatment of migraine (Lassen et al., 1998). This suggests an ongoing role for NO in mediating the migraine, not only as an initiator. Furthermore, there is some evidence from an open-label study that the NO-scavenger, hydroxycobalamin has an effect on migraine prophylaxis (van der Kuy et al., 2002). GTN-induced migraine has also been shown to respond to sumatriptan (Afridi et al., 2004; Iversen et al., 1996).

In migraineurs, GTN caused a decrease in the velocity of middle cerebral artery flow during infusion indicating a dilatation of the artery which reversed after the end of the infusion (Thomsen et al., 1994). It has, therefore, been suggested that vasodilatation

of cerebral vessels may be partly responsible for the triggering mechanism of nitric oxide. However, there is evidence to suggest that this is not the case. In a recent study sildenafil was found to induce migraine with a similar time course and success rate to GTN (Kruuse et al., 2003). Sildenafil is a phosphodiesterase 5 inhibitor and consequently is involved in cGMP metabolism. Interestingly, it was not found to produce any significant changes in the middle cerebral artery diameter of the subjects suggesting that vasodilatation of large cerebral vessels is not necessary in order for migraine to develop (Kruuse et al., 2003). However, the immediate GTN-induced headache is thought to be related to vasodilatation and an equivalent headache was not seen following sildenafil.

It is unclear how nitric-oxide, with its short half-life of less than 30 seconds, can trigger a delayed migraine. One possibility is that NO induces neurogenic inflammation. Exogenous NO may also induce endogenous NO production as well as the production of other inflammatory mediators. iNOS may be responsible for the triggered endogenous production. Reuter et al found dose-dependent iNOS mRNA upregulation in rat dura beginning 2 hours after GTN infusion (Reuter et al., 2001). Also, in a placebo controlled study 150mg prednisone was found to reduce the delayed headache induced by GTN in a group of migraineurs (Olesen). It is known that prednisolone inhibits iNOS. It has recently been shown that plasma levels of citrulline, a marker for endogenous NO production, are increased 60 minutes following GTN infusion in patients with IHS defined chronic tension-type headache when compared to healthy controls although this has not yet been demonstrated in migraine (Ashina et al., 2004).

During migraine blood levels of calcitonin gene related peptide (CGRP) are elevated in the external jugular vein (Goadsby et al., 1990). It has been shown that CGRP can

also induce migraine (Lassen et al., 2002). It is possible that CGRP could be the mediator through which nitric oxide induces migraine as GTN has been shown to liberate CGRP from pial arterioles and other vascular beds (Booth et al., 2000; Wei et al., 1992) and raised levels of CGRP are found in GTN triggered migraine (Juhasz et al., 2003). Allodynia has been demonstrated in humans during migraine (Burstein et al., 2000). This is thought to be the result of central sensitisation. Nitric oxide has been shown to be involved in central sensitisation in the spinal cord (Lin et al., 1999; Wu et al., 2001) and to induce hyperalgesia in animals (McMahon et al., 1993; Meller and Gebhart, 1993). The role of NO in central pain modulation has been demonstrated by intra-RVM injection of a NO donor which led to a dose-dependent facilitation of the tail-flick reflex in rats (Urban et al., 1999). Conversely, injection of the NOS inhibitor, L-NAME, attenuated mustard-oil induced hyperalgesia. Subcutaneous GTN has been found to produce a significant increase of nNOS and Fos protein-like immunoreactivity in the trigeminal nucleus caudalis (Pardutz et al., 2000) and brainstem nuclei (Tassorelli and Joseph, 1995) in rats. Administration of the NOS inhibitor L-NAME reduced Fos protein-like expression in the trigeminocervical complex of the cat (Hoskin et al., 1999).

Animal studies have also revealed that cortical spreading depression, which is thought to be responsible for migraine aura in humans, elicits widespread release of NO and increased cortical concentrations of cGMP (Read et al., 2001). The CSD induced increase in cortical NO is reduced when sumatriptan is given as a pre-treatment in cats and rats (Read and Parsons, 2000).

# PART II. METHODS

## Chapter 5. PET data acquisition and analysis

The theoretical basis of PET and SPM is discussed in chapter 2. In this chapter, the practical aspects will be described.

## 5.1. PET

PET scans were performed with an ECAT EXACT HR+ scanning system (CTI Siemens, Knoxsville, TN) in three-dimensional mode with septa retracted. Images were reconstructed by filtered back-projection into 63 image planes (separation 2.4 mm) and into a 128 x 128 pixel image matrix (pixel size 2.1 x 2.1 $mm^2$). The resultant nominal resolution was 6.4 ± 0.2 mm.

The subject's head was placed in the tomogram using a supportive moulding to minimize movement. Markings were made on the face to check for movement in between scans. The subject was asked to lie still with their eyes closed during the scans. The lights in scanning area were dimmed. A transmission scan was performed to ensure the positioning was correct and to allow for attenuation correction. An antecubital vein cannula was used to administer the tracer, 350 mBq of $H_2^{15}O$. The activity was infused into subjects over 20 seconds at a rate of 10 ml/min. The data were acquired in one 90-second frame beginning 5 seconds before the peak of the head curve. The interval between scans was 8 minutes. A maximum of 12 scans per subject was obtained. After reconstruction the dynamic images were transferred to a SPARC SUN system workstation for preprocessing and analysis.

## 5.2. SPM
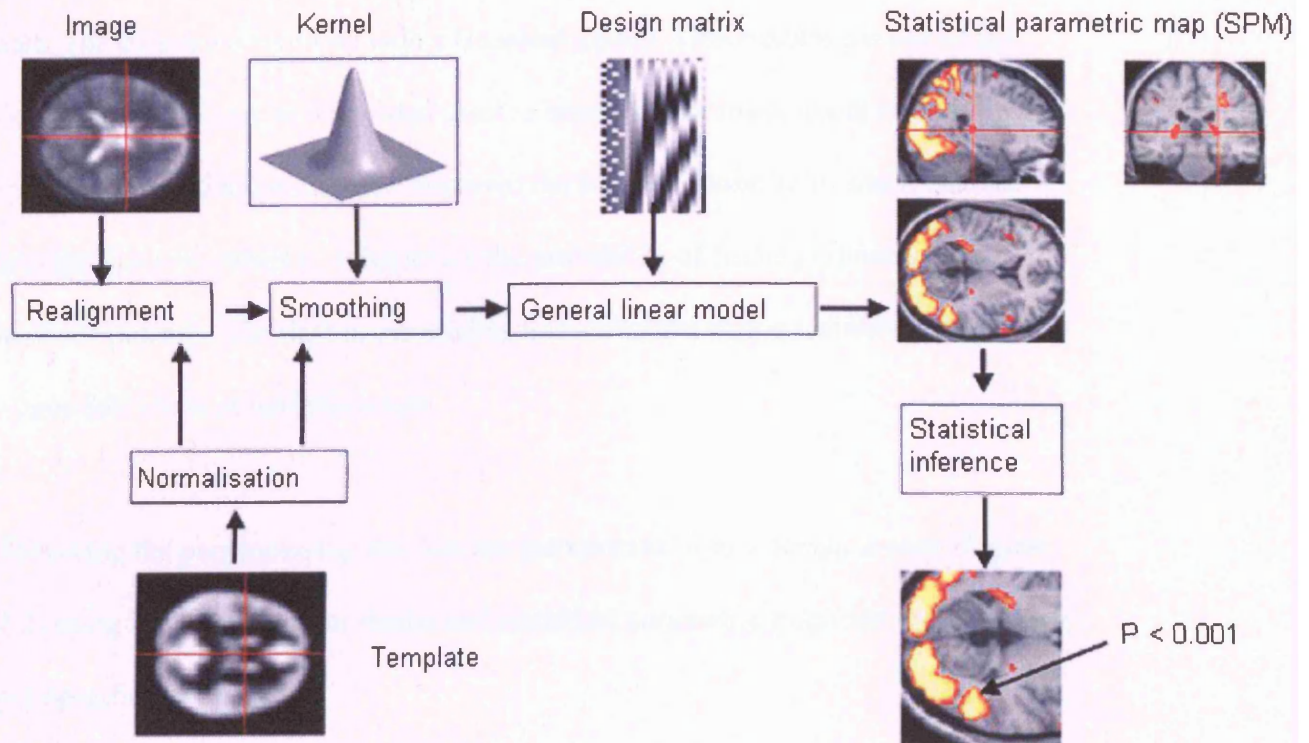


**Fig 5.1** Statistical analysis using SPM.

The raw data undergo preprocessing prior to the statistical analysis. This occurs in

three stages:

1) Realignment and coregistration-this involves correction for head movement

between each scan. Each of the images is realigned and coregistered with reference to

the first scan.

2) Spatial normalisation- this involves warping the image to fit it to a standardised

normal brain template, usually the Talairach and Tournoux brain (Talairach and

Tournoux, 1988) or the Montreal Neurological Institute (MNI) brain. Normalisation

allows intersubject comparisons to be made.

3) Smoothing- this is a process by which data points are averaged with their

neighbours in a series. This has the effect of blurring the sharp edges in the smoothed

data. The data are convolved with a Gaussian kernel. This enables the use of the

Gaussian field theory to allow statistical inferences to be made about regionally

specific effects. Smoothing also improves the signal-to-noise ratio and it enables

averaging across subjects. It improves the probability of finding commonalities

between patients. The data in our studies was smoothed with a Gaussian filter of

10mm full width at half maximum.


Following the preprocessing the data are incorporated into a design matrix (Figure

2.2) using the general linear model and statistical parametric maps are derived using

pre-specified contrasts.

# PART III. CLINICAL STUDIES

## Chapter 6. Exploring GTN triggering of migraine

**Introduction**

Migraine is characteristically episodic making it challenging to perform clinical

studies. There is no recognised model of migraine in animals that encompasses the

syndrome completely (Edvinsson, 1999), so a reliable experimental human model of

migraine is an important goal for those interested in research in this field that can be

difficult to achieve (Levy et al., 2003). The GTN model has the potential to be used in

this manner (see Chapter 4).

There is some debate as to whether triggered attacks are in fact genuine migraine

attacks identical to spontaneous migraine attacks. This issue is crucial to any use of

the model for predicting effects in so-called spontaneous migraine. We set out to

examine the reproducibility of the model, also an essential feature for some

experimental clinical studies, and to document carefully the clinical features of the

period from trigger administration to headache onset. We sought to extend previous

studies of the premonitory phase of migraine (Giffin et al., 2003) and specifically

determine if such symptoms occurred in GTN-triggered migraine.

**Methods**

Patients with migraine without aura ($n = 23$) and migraine with aura ($n = 21$), and 12

healthy controls were recruited as part of a functional imaging study in glyceryl

trinitrate-triggered migraine (Table 6.1). The migraineurs suffered from attacks

fulfilling International Headache Society criteria for migraine with and without aura

(Headache Classification Committee of The International Headache Society, 2004).

They were not taking any preventive medication and the attack frequency was less

than 15 days per month with a minimum of one attack per month. Pregnant women

were excluded and a pregnancy test was performed in women of child-bearing age.

Details of their usual migraine attacks were noted included premonitory symptoms.


Healthy volunteers were recruited as controls on the basis of no personal or family

history of migraine or migrainous headaches and no history of frequent tension-type

headaches. They were excluded if they were on any regular medication.


*Glyceryl trinitrate (GTN) administration*

Each subject received an intravenous infusion of 0.5 µg/kg/min GTN over twenty

minutes via a cannula inserted into the right antecubital vein. This dose has been

successfully used by others (Olesen et al., 1993).The migraineurs were all headache

free for at least 48 hours prior to the initiation of the infusion. Headache intensity and

characteristics were recorded every 5 minutes during the infusion and thereafter every

15 minutes. Subjects were asked to describe any head pain or other associated

symptoms such as nausea, photophobia, phonophobia, worsening of pain on head

movement. In addition they rated the severity of any headache on a verbal rating scale

of 0-10 and the location and nature of the headache was noted. Blood pressure and

pulse were monitored during the infusion. Subjects were monitored for up to four

hours after the infusion and periodically asked to describe any headache. They were

also questioned about premonitory symptoms such as yawning, fatigue, neck stiffness,

thirst, urinary frequency, cravings or mood change (Giffin et al., 2003) . In the

migraine subjects whose attacks were successfully triggered subcutaneous

sumatriptan 6mg s/c was then administered to abort the attack and time taken until

relief was noted. Non-responders, migraineurs who did not develop a migraine, received a follow-up telephone call the next day to document any possible delayed headache.

*Repeat administration of GTN*

Subjects whose attacks were successfully triggered, and the control group, were invited back for a repeat session at least a week later in order to scan them for a functional imaging study. Clinical data were again recorded during the second session. The controls were only studied in the imaging session.

**Results**

*Controls*

Three of the controls developed a headache during the infusion which did not subside after the infusion finished. The headaches persisted for at least two and a half hours and the subjects were then excluded as controls. The clinical features of the attack are documented in Table 6.2. One of these patients was adopted and so a family history of migraine could not be excluded. The second later admitted that she thought her brother has migraines although a family history of migraine was initially denied at the screening stage. The third patient did suffer from featureless headaches approximately two or three times a year. Of the remaining eight controls six experienced a mild, generalised headache during the infusion which then faded within 20 minutes of the end of the infusion. Two experienced no headache at all but described an odd sensation in their head during the infusion.

*Headache*

*Session one*: A migraine attack was successfully triggered in thirty-three out of the

forty-four migraineurs (75%). The trigger rate was 83 percent for migraine without

aura and 67 percent for migraine with aura ($\chi^2$ =1.49, $P$ =0.22). Four subjects

withdrew from the study after the first session.

*Session two*: In twenty-eight out of the twenty-nine of those remaining a migraine was

successfully re-triggered during the second session. Interestingly, in the subject in

whom the migrainous headache was not triggered the second time, a visual aura

without headache was triggered 70 minutes after the start of the infusion. This took

the form of a zig-zag pattern across the visual field, blurring of vision and alteration

of colours. This lasted 20 minutes and was not followed by a headache. His previous

attacks typically involved a bilateral headache with nausea, photophobia and

phonophobia. He very rarely had visual aura.

*Migraine with aura*

Visual aura was triggered in another subject with migraine with aura. In this case he

had both his typical visual aura and migrainous headache and both were successfully

reproduced during the second session with similar latencies of onset, 150 minutes and

160 minutes, respectively, after GTN.

*Latency of migraine*

The latency of onset of the migraine was determined as the time taken from onset of

the infusion to the development of a migraine which fulfilled International Headache

Society criteria for migraine and was typical for the subject. There was no significant difference in latency of onset of migraine during the first and second triggering sessions (Wilcoxon Signed Ranks test $Z = 0.55$, $P = 0.58$). The mean latency for the first session was 142 minutes and for the second session 140 minutes (range 20-330 minutes). Fifty percent of attacks were triggered between 90 and 180 minutes.

*Laterality of triggered attacks*

The laterality of the headache (i.e. left /right-sided /bilateral) was reproduced in all but two out of thirty. The first subject had right sided migrainous headaches more frequently than left-sided. During the first session a left-sided headache was triggered but during the second session she developed a right-sided headache. The second subject who usually has left sided headache developed bilateral headache during the first session but then reverted back to his usual left-sided headache during the second session.

*Premonitory Symptoms*

Fifteen of the thirty-three migraineurs described premonitory symptoms with their usual spontaneous attacks. Symptoms included yawning, tiredness, irritability, neck stiffness, frequency of urination, hunger and low mood. Twelve of these described some of these symptoms after the GTN infusion but prior to the onset of the triggered migraine (Table 6.3). The premonitory symptoms were highly reproducible as was the latency of onset from start of infusion with a mean latency of 53 minutes in session one and 59 minutes in session two from a range of 15-120 minutes. (The data

regarding latency of onset were available for 8 of the 12 subjects with triggered premonitory symptoms).

## Non-responders versus responders

There was a trend towards a greater proportion of subjects with migraine with aura being in the non-responder group but this was not statistically significant ($\chi^2$ =1.49, $P$ = 0.22; Table 6.4).

## Effect of sumatriptan

Sumatriptan 6mg s/c was administered to 59 patients who had been successfully triggered, 32 in session one and 27 in session two. 83% of patients had mild or no headache at 2 hours post treatment. The median time to relief was 30 minutes in session one and 35 minutes in session two. The effect was highly reproducible with all subjects who responded in session one also responding in session two.

**Table 6.1** Subject characteristics.

| Group | Migraine without aura $n = 23$ | Migraine with aura $n = 21$ | Controls $n = 11$ |
|---|---|---|---|
| Successful induction of migraine | 19/23 (83%) | 14/21 (67%) | - |
| Female/Male | 11:12 | 17:4 | 4:7 |
| Mean age (age range) | 46 (25-68) | 44 (27-65) | 38 (21-58) |
| Mean Frequency of attacks/month (range) | 3 (1-8) | 3 (1-12) | - |

**Table 6.2** Glyceryl trinitrate (GTN) triggered headache in controls.

| Subject | Triggered delayed headache | | | | | | |
|---------|---------------------------|------|----------|---------------------|--------|-------------|-------------|
| | Time (hours) | Pain | Severity | Worse with movement | Nausea | Photophobia | Phonophobia |
| 1 | Onset during infusion | Dull ache centred around vertex, worse on movement | 2/10 | + | - | - | - |
| 2 | Onset during infusion | Wave of pain around vertex | 1/10 | - | - | - | - |
| 3 | Onset during infusion | General "muzziness" in whole head | 1/10 | - | - | - | - |

**Table 6.3** Premonitory symptoms in glyceryl trinitrate triggered migraine.

| Subject | Premonitory symptoms in spontaneous attacks | Premonitory symptoms during session 1 | Premonitory symptoms during session 2 |
|---|---|---|---|
| 1 | Neck stiffness, tired | - | - |
| 2 | Neck stiffness, yawning | Neck stiffness | Neck stiffness |
| 3 | Excessively tired, irritable | Excessively tired | Excessively tired |
| 4 | Tired, yawning, neck stiffness | Neck stiffness, tired | Tired, yawning |
| 5 | Yawning, irritable, detached | Yawning | Yawning |
| 6 | Yawning, tiredness | Yawning, tiredness | Yawning, tiredness |
| 7 | Yawning, hunger, neck stiffness, belching | Neck stiffness | Neck stiffness |
| 8 | Frequency of urination, neck stiffness | Frequency of urination, neck stiffness | Frequency of urination, neck stiffness |
| 9 | Frequency of urination, hunger cravings | - | - |
| 10 | Thirst | - | - |
| 11 | Yawning, tired, neck stiffness | Yawning, tired | Yawning, tired |
| 12 | Yawning | Yawning | Yawning |
| 13 | Low mood, tired | Tired | Tired |
| 14 | Yawning, tired | Yawning, tired | Yawning, tired |
| 15 | Yawning, hunger craving | Yawning | Yawning |

**Table 6.4** Comparison of responders and non-responders to glyceryl trinitrate triggered migraine.

| Group | Non-responders $n = 11$ | Responders $n = 33$ |
|---|---|---|
| MWA:MO | 7:4 | 14:19 |
| Female/Male | 8:3 | 27:16 |
| Mean Age | 43 | 45 |
| Mean Frequency of attacks/month | 2.3 | 3 |

*Abbreviations*:
MO, migraine without aura
MWA, migraine with aura
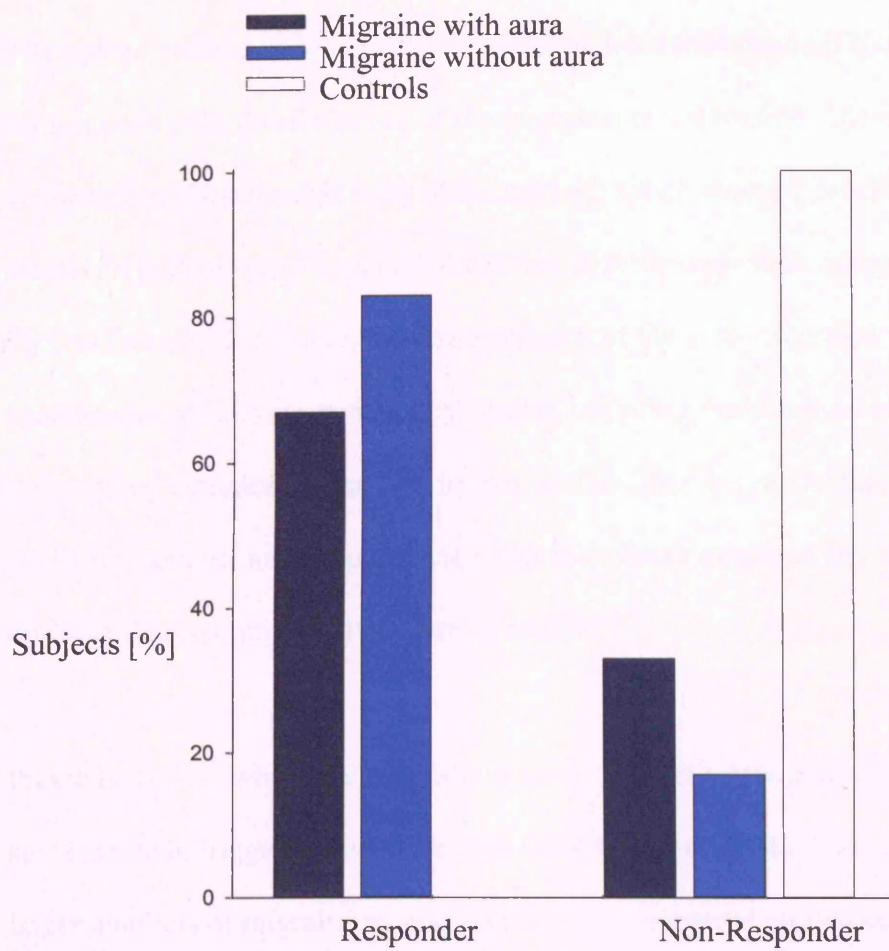
**Figure 6.1** Response to GTN trigger amongst different groups.

**Discussion**

The current study confirms previous work that has established GTN as a potent trigger for migraine with three-quarters of the migraineurs responding. The study characterises the reproducibility of the method, which must be considered excellent: almost all patients have a second attack that is at the same time and with the same clinical features. Moreover, the characteristics of the GTN-triggered versus spontaneous attacks are remarkably similar, including the common experience of premonitory symptoms. The data further confirm that this model has much to offer the study of migraine, and support a view that the attacks triggered are, in essence, no different than spontaneously occurring attacks.

Previous studies, which include those done by Olesen's group, have had a similar success rate in triggering migraine with GTN (Iversen, 2001). Our study involved larger numbers of migraineurs and subjects were triggered on two separate occasions. The latency of development of migraine appeared to be shorter in our study: mean 141 minutes compared to previous reports where the peak intensity was at 236 (Christiansen et al., 2000) or 330 minutes (Thomsen et al., 1994). For ethical reasons we did not let the headache intensity increase to the maximal level and so abortive medication was administered reasonably soon. Another reason for the difference may be due to the fact that in the two previously reported studies (Olesen et al., 1993; Thomsen et al., 1994) patients were observed for one hour and three hours respectively following the start of the infusion whereas we monitored the patients for four and a half hours following the start of the infusion. This would mean that they would have to rely on the questionnaire filled in by the patient at home in order to

determine onset of the migraine whereas in our subjects the migraine developed whilst they were being monitored. Also, in our study they were in a quiet, comfortable environment without distractions and were continuously having to focus on their headache. Sances and colleagues (Sances et al., 2004) have recently published a study on the nitroglycerin trigger in migraine and cluster headache involving 197 migraineurs using sublingual GTN. Their findings on latency of onset were similar to those reported here: 135 minutes and 141 minutes, respectively. Their success rate of induction was also very similar with an overall rate of 77% (82.1% for migraine without aura group and 40.9% for migraine with aura).

*Aura*

Visual aura was triggered in two of the subjects with migraine with aura. One of these subjects was the only one in whom we were unable to trigger the headache the second time. He had recently recovered from an upper respiratory tract infection on the first occasion which may help to explain why he was more susceptible to a typical migraine following the GTN trigger. The other subject always had visual aura preceding his migrainous headache. On both occasions a visual aura was triggered following the GTN infusion (150 minutes and 160 minutes, respectively) followed by a headache. We did not expect this finding. In the study by Olesen's group involving a GTN infusion in 12 patients with migraine with aura (Christiansen et al., 1999) six of them developed migrainous headaches but none developed aura. There had been a case report of new onset migraine with aura in a 46 year old following sublingual GTN for angina (Bank, 2001) but these episodes only occurred following GTN and the patient apparently had no previous history of migraine. However, in the recently published study by Sances and colleagues (Sances et al., 2004) migraine aura was

triggered in three of twenty-two subjects in the migraine with aura group. A further

six subjects developed migrainous headache without aura similar to those in Olesen's

study. The triggering of the aura in our subject may have occurred by chance

however, the fact that it was reproducible makes this less likely, so perhaps the

triggering of aura is rarer but possible.

*Premonitory symptoms*

Premonitory symptoms precede the headache in migraine. In a recent study (Giffin et

al., 2003) 120 migraineurs were given an electronic diary to record non-headache

symptoms. The most common premonitory symptoms were feeling tired and weary

(72% of attacks with warning features), difficulty concentrating (51%), and a stiff

neck (50%). Many of these symptoms were also described by our patients during both

induction sessions. To our knowledge there has not been any previous documentation

of premonitory symptoms following GTN triggering. The finding is important as it

implies that the migraine process is triggered from the earliest point. It also suggests

that after the trigger the neurological processes that follow are identical to those which

occur in spontaneous migraine attacks.

**Conclusion**

To conclude, we have provided further evidence of the reliability and validity of the

GTN model in triggering migraine. The presence of premonitory symptoms extends

the clinical phenotype of the attacks further establishing them as the same as spontaneous migraine. Furthermore the reproducibility in terms of phenotype of attack, laterality and timing should provide considerable confidence in results from the model both in terms of understanding migraine mechanisms and studying its treatment.

# Chapter 7. A PET study in spontaneous migraine

## Introduction

One of the most significant studies in migraine neuroimaging was that of Weiller and

colleagues (Weiller et al., 1995). The study, as described in chapter 2, involved nine

subjects with migraine without aura who were scanned during spontaneous migraine

attacks and following sumatriptan. Interestingly, three of the subjects were on

migraine prophylactics (β-blockers). The study revealed brainstem activation during

the migraine that persisted after sumatriptan administration had relieved the pain. The

resolution of the PET camera used was not high enough to identify specific nuclei but

the foci of maximum increase were around the dorsal midbrain and dorsolateral pons.

It has been difficult to replicate these findings because of the practical logistic

limitations of imaging spontaneous migraine, with only a single GTN-triggered case

thus far in the literature (Bahra et al., 2001). Indeed when acute migraine has been

studied using MRI methods, such as perfusion weighted (Cutrer et al., 1998; Sanchez

del Rio et al., 1999) or BOLD f-MRI (Cao et al., 1999; Hadjikhani et al., 2001; Welch

et al., 1998), the focus has been on patients with migraine with aura or on cortical

changes in patients without aura. These studies have produced fascinating results,

although they were not primarily aimed at further exploring the issue of brainstem

involvement in migraine.


One aim of this study was to replicate and perhaps refine published findings taking

advantage of the advances in PET scanning and analysis methods.

**Methods**

*Patients*

We recruited six subjects, three with migraine with aura and three with migraine without aura as defined by the International Headache Society diagnostic criteria (Headache Classification Committee of The International Headache Society, 2004). All the subjects were female with an age range of 30-55 (Table 7.1). They had a migraine frequency of between one and four per month. One subject was withdrawn from the study as it emerged that she was taking pizotifen. None of the remaining subjects were on any migraine preventives or other medications.

The subjects were scanned within 24 hours of onset of migraine and prior to any abortive medication. No abortive medications had been taken within the preceding 48 hours. The subjects were offered sumatriptan treatment following the scans. Post-treatment scans were not incorporated into the study design. Pain free scans were taken at least 72 hours after a migraine headache. The order of scanning was randomised (ictal versus inter-ictal).

*PET data acquisition and analysis*

An antecubital vein cannula was used to administer the tracer, ~350 mBq of $H_2^{15}O$. The activity was infused into subjects over 20 seconds at a rate of 10 ml/min. The data were acquired in one 90 second frame beginning 5 seconds before the peak of the head curve. The interval between scans was 8 minutes. Each session involved four scans. Attenuation correction was performed with a transmission scan acquired at the beginning of each study.

SPM99 (Wellcome Department of Imaging Neuroscience,

http://www.fil.ion.ucl.ac.uk/spm) was used for data analysis. Images were realigned

with the first as the reference and then co-registered and spatially normalised into the

space defined by the atlas of Talairach and Tournoux (Talairach and Tournoux, 1988).

The normalised images were smoothed with a Gaussian filter of 10 mm full width at

half-maximum since increasing the smoothness of the data increases the sensitivity of

the analysis in a monotonic fashion (Friston et al., 1996). 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. However, our results survived a small volume

correction using a 12 mm radius sphere at $P < 0.05$ centred on the brainstem maxima

as reported (Bahra et al., 2001) . There were three right-sided and two left-sided

attacks. The analysis included data from all five subjects. Analysis was performed

both with and without taking symptomatic lateralisation into account. The first

analysis discounted the side of the attack. In the second analysis the scans of subjects

with left-sided attacks were reflected across the midline for a "flipped" analysis

(Friston, 2003). This was then analysed in a multi-group analysis along with a

spatially transposed version of this second group using a fixed effects model. This

enabled us to detect responses which lateralise in relation to symptoms. Our statistical

model included the main effect of migraine (present versus absent), time (scans 1

through 4) and the migraine by time interaction. This general linear model conforms

to an ANOVA (Friston et al., 1995).

**Results**

Two of the patients had a typical migrainous aura prior to the onset of the headache. All five subjects were scanned within 24 hours of onset of migraine. The mean time from onset to scan was 11 hours. Comparing the scans collected during migraine with those out of the attack revealed significant activations in the rostral, dorsal pons, lateralised to the left (Figure 7.1). After small volume correction the activation was significant at the cluster level ($P = 0.003$, corrected).

Responses were also seen in the right anterior cingulate, posterior cingulate, cerebellum, thalamus, insula, prefrontal cortex and temporal lobes (Table 7.2a, Figure7.3). Interestingly, there was an area of deactivation in the migraine phase also located in the pons, lateralised to the right (Table 7.3, Figure7.2). This was an unexpected finding and reached the recommended level of significance for exploratory analysis ($P < 0.05$, corrected).

*Analysis of flipped data*

After flipping the images so that, effectively, all the migraines were on the right side, the area of activation in the left, dorsal pons remained. A small area in the right anterior pons was also noted which was not seen in the first (unflipped) analysis.

Comparison of the flipped and unflipped data to assess for areas that lateralise with side of pain revealed activation of the thalamus (see Table 7.2b). This was the only area of activation to lateralise with side of pain.

**Table 7.1** Subject characteristics

| Patient | Diagnosis | Age | Nausea | Photophobia | Phonophobia | Worse with movement | Laterality of headache | Attack freq /month | Aura |
|---------|-----------|-----|--------|-------------|-------------|--------------------|------------------------|--------------------|------|
| 1 | MO | 45 | + | + | + | + | Right | 4 | - |
| 2 | MO | 30 | + | - | - | + | Left | 4 | - |
| 3 | MWA | 48 | + | + | + | + | Left | 1 | Visual distortion, clumsy |
| 4 | MWA | 55 | + | + | + | + | Right | 4 | Paraesthesia, incoordination |
| 5 | MO | 54 | + | - | - | + | Right | 1 | - |

MO-Migraine without aura, MWA-Migraine with aura

**Table 7.2** Areas of activation in migraine state compared to inter-ictal state.

**a)** Primary analysis, **b)** Flipped analysis.

The coordinates refer to voxels significant at $P < 0.001$ (uncorrected).

* also significant at the cluster level (cluster size = 90 voxels), $P = 0.003$ (corrected for multiple comparisons across a 12mm radius sphere centred on the brainstem maxima as reported.

**a) Primary analysis**

| Region of activation | Coordinates x,y,z (Talairach andTournoux) | Z score of peak activation |
|---|---|---|
| Anterior cingulate BA24/32(R) | 4,30,19 | 4.66 |
| Posterior cingulate BA23 | 0,-45,34 | Infinite |
| | 0,-20,38 | 7.84 |
| Prefrontal cortex BA9/10 (R) | 6,57,16 | 6.01 |
| Cerebellum- (R) | 12,-67,-15 | 4.95 |
| (L) | -10,-42,-12 | 5.19 |
| Thalamus (R ) | 10,-6,8 | 4.82 |
| Insula (L) | -40,14,3 | 4.10 |
| Dorsal pons (L) | -4,-28,-20 | 4.97* |
| Temporal lobe (L) | -42,12,-24 | 5.21 |
| (R) | 34,18,-18 | 6.19 |

**b) Flipped analysis**

| Region of activation | Coordinates x,y,z (Talairach and Tournoux) | Z score of peak activation |
|---|---|---|
| Anterior cingulate BA24/32 (R) | 2,28,20<br>16,12,28 | 3.81 / 3.95 |
| Posterior cingulate BA23<br>(R) | 0,-20,38<br>2, -44, 34 | 7.84<br>Infinite |
| Prefrontal cortex BA9/10 (R) | 6,49,14 | 5.29 |
| Cerebellum- (R)<br>(L) | 10,-64,-14<br>-10,-44,-14 | 4.29<br>5.09 |
| Thalamus (L) | -8,-4,14 | 3.96 |
| Insula (R)<br>(L) | 44,12,0<br>-36,-14,1 | 3.85<br>3.92 |
| Dorsal pons (L)<br>Anterior pons (R) | -2,-28,-22<br>8,-12,-22 | 4.6<br>3.53 |
| Temporal lobe (L)<br>(R) | -40,12,-24<br>36,14,-14 | 5.45<br>5.83 |

**Table 7.3** Area of deactivation in migraine state compared with inter-ictal state. The coordinates refer to the peak voxels within a cluster $P < 0.05$ (corrected for multiple comparisons across whole brain volume). **Cluster size = 74 voxels.

| Region of activation | T+T coordinates | Z score |
|---|---|---|
| Pons (R ) | 14,-28,-20 | 5.4** |
| **Region of activation after flipping** | **T+T coordinates** | **Z score** |
| Pons (R) | 16,-28,-19 | 5.4 |

**Fig 7.1** Activation in the dorsal pons in the migraine state compared to inter-ictal state.
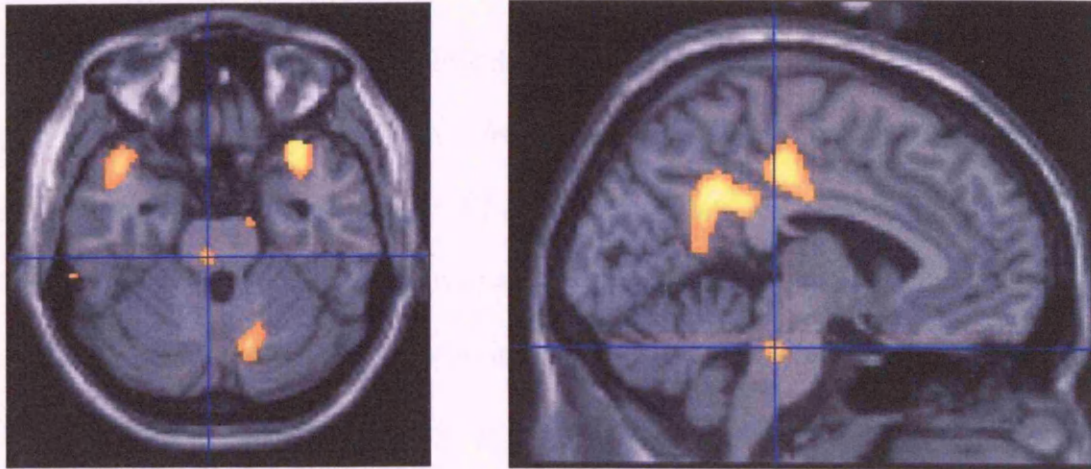


**Fig 7.2** Area of deactivation in migraine state compared to inter-ictal state.
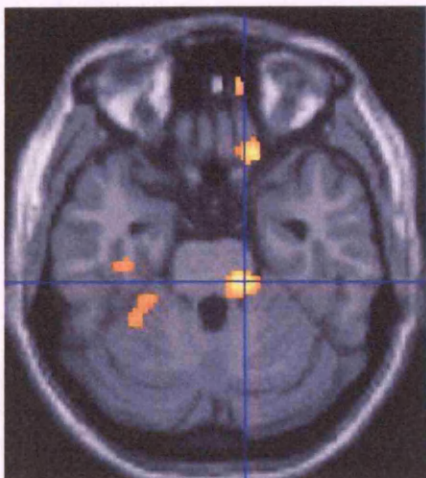


**Fig 7.3** Activation in the thalamus and insula in migraine state.

**Discussion**

These new data supplement and support previous findings of activation of the

dorsolateral pons in migraine. In addition there is activation of other structures
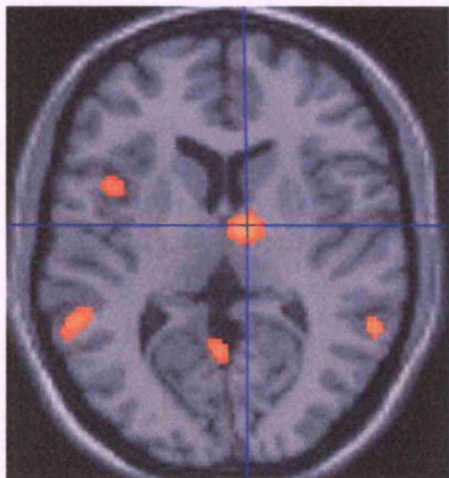
involved in various aspects of pain and related processing in the brain. In addition we

have observed deactivation in the contralateral pons, a new finding in migraine. Our

results are consistent with a view of migraine as primarily a subcortical disorder

involving dysfunction in brainstem areas probably involved in the modulation of

sensory processing.

The areas of activation in the brain reported here include regions thought to be

involved in the central pain matrix (Derbyshire, 2000). The anterior cingulate has

been the most consistently activated area in pain studies. It is thought to be involved

in the affective and evaluative dimension of pain. In particular, the right anterior

cingulate was found to be active irrespective of the afflicted side in a study of patients

with acute cluster headache (Hsieh et al., 1996). The posterior cingulate is relatively

less frequently activated in response to pain (Kwan et al., 2000). The insula is also

consistently activated in experimental pain studies. It has connections with the limbic

system and the autonomic system and is thought to be involved in representing the

emotional aspect of pain. The thalamus, which is the relay centre for afferent input to

the brain and the cerebellum, is less consistently activated in experimental pain

studies. The prefrontal cortex is involved in the cognitive emotional processing of

pain (Ingvar, 1999). Activations in the temporal lobes were also found in the Weiller

study and these were in the auditory association areas.

The area of deactivation was an unexpected finding, not previously reported. It is of

interest to note that it was on the opposite side of the pons to the area activated during

the migraine state. It is possible that as one area of the pons is activated another

region is deactivated. In this regard it is known that the locus coeruleus which is in the

dorsolateral pons and contains neurons that account for 96% of brain noradrenergic

projections (Amaral and Sinnamon, 1977), has such a reciprocal contralateral

inhibitory effect (Buda et al., 1975).

The main area of the brain which was dependent on the anatomical location of pain

was the thalamus. There is evidence that pain processing is lateralized in humans.

This is discussed, to some extent, in section 2.3. Hsieh and colleagues (1995; 1996)

demonstrated that the right anterior cingulate is activated irrespective of side of pain.

PET studies have mainly found bilateral activation of the thalamus (Casey et al.,

1996). However, more recently Bingel and colleagues (Bingel et al., 2003)

demonstrated that the lateral thalamus, but not the medial thalamus, showed a

contralaterally biased representation of painful stimuli. The lateral thalamus is thought

to be somatotopically organized and therefore is involved in localization and

discrimination of stimuli (Ingvar, 1999). This is consistent with our finding of

contralateral activation of the thalamus after the scans were flipped.


Unfortunately the number of patients completing the study was not as many as we had

initially planned, although this does not detract from our positive results there may be

other areas that we have not detected. Unlike previous imaging studies in migraine

our study included subjects with both migraine with and without aura although the

latter group were scanned after the aura had subsided.

## Conclusion

Our data demonstrate activation of the dorsolateral pons on the left side during acute migraine with associated deactivation of the contralateral pons. Areas of activation, such as the anterior cingulate, prefrontal and insula cortex, were seen and are consistent with areas seen during studies involving acute pain. The thalamic activation was found to be lateralized contralateral to the side of pain, which is consistent with known anatomy.

# Chapter 8.  A PET study exploring the laterality of brainstem activation in migraine using GTN

## Introduction

The headache in migraine may be unilateral or bilateral. The largest positron emission tomography (PET) study in migraine to date involved nine migraineurs with right-sided headache (Weiller et al., 1995). The finding of brainstem activation lateralised 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.

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 relatively reliable and effective method of migraine induction (as detailed in previous chapters).

In this study we use 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 $H_2^{15}O$ 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**

*Patients*

Twenty-four migraineurs (aged 26-65, 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 8.1). All of these fulfilled International Headache Society criteria for episodic migraine (Headache Classification Committee of The International Headache Society, 2004) and included eight with migraine with aura and sixteen with migraine without aura. The migraineurs were not on any prophylactic medications. Other medications included the oral contraceptive pill in four of the subjects, HRT in three subjects and thyroxine in two of the subjects. All were successfully scanned during an induced migraine. Eight healthy controls were also studied (aged 21-55, 5 male, 3 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 glyceryl trinitrate

(GTN) over twenty minutes 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 hours prior to the initiation of the infusion. No triptans or

analgesics had been taken in the preceding 48 hours. Headache intensity and

characteristics were recorded every 5 minutes during the infusion and every 15

minutes for up to four hours after the infusion. Subjects were periodically asked 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 was 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 successfully triggered

subcutaneous sumatriptan (6mg) 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 3 consecutive PET scans in each

of the following four conditions (Figure 8.1):

   1)  Pain free

2) During the non-specific headache of a GTN infusion

3) During a migraine headache, or equivalent time delay for controls

4) Pain free following treatment of migraine with subcutaneous sumatriptan 6mg.

The time between conditions two and three (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 three. 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. 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 normalised

into the space defined by the atlas of Talairach and Tournoux (Talairach and

Tournoux, 1988). The normalised 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) (Bahra et al., 2001) . The first analysis was performed using

a model that enabled group by 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 the

group by condition interactions were significant we proceeded to examine the simple

main effects of condition. To examine laterality of 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.

**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 the mean VAS pain severity rating for this headache was 2.5 out of 10 and in the control group it was 1 out of 10. 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 mean VAS pain severity rating in the migraine group at the time of acquisition of "migraine scans" was 5 out of 10.

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 (see Chapter 6).

*PET findings*

Given the previous PET findings in migraine (Weiller et al., 1995) the main focus of

our analysis was on the brainstem 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 (Figure 8.1).

Significant brainstem activation was seen in the dorsal pons and rostral medulla ($P <$

0.05 after small volume correction) (Table 8.2, Figure 8.2). 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 8.2).

*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.

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 (Figure 8.3).

*Migraine with aura versus migraine without aura*

The 24 migraineurs included 8 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 sub-groups.

*Laterality of brain changes in each group*

The brainstem was the main focus for examining lateralised changes in activation with respect to side of pain (refer to Table 8.3 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 (Figure 8.4).

In acute left-sided migraine a small area of activation was seen in left dorsal pons (Figure 8.4).

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 lateralisation*

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. Temporal lobe activation was found in the right and left groups.

Basal ganglia activation (putamen, caudate nucleus) was found in the right sided and bilateral group.

**Figure 8.1 Study Design**



| Condition 1 | Condition 2 | Condition 3 | Condition 4 |
|---|---|---|---|
| Pain free | GTN headache | Migraine | Pain free |

GTN infusion                                    Sumatriptan
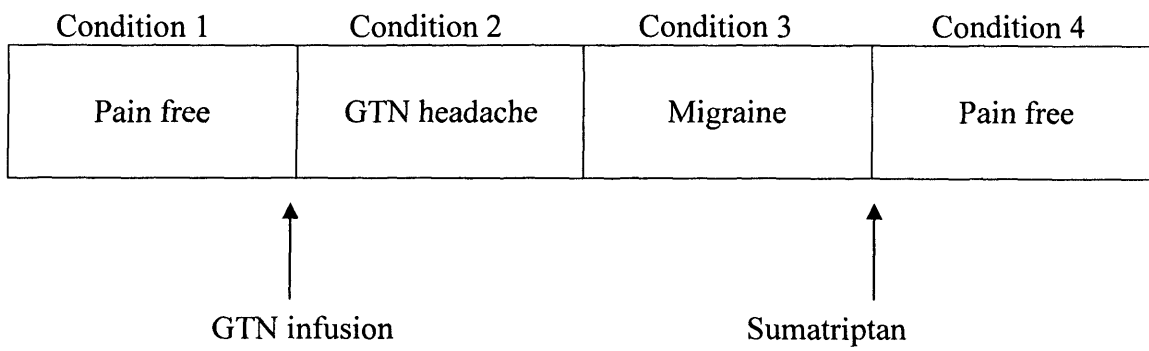
**Figure 8.2**

Activation of the dorsolateral pons in migraine patients during a glyceryl trinitrate (GTN) triggered attack when compared to matched controls administered GTN in the same time course (4,-32,-32).
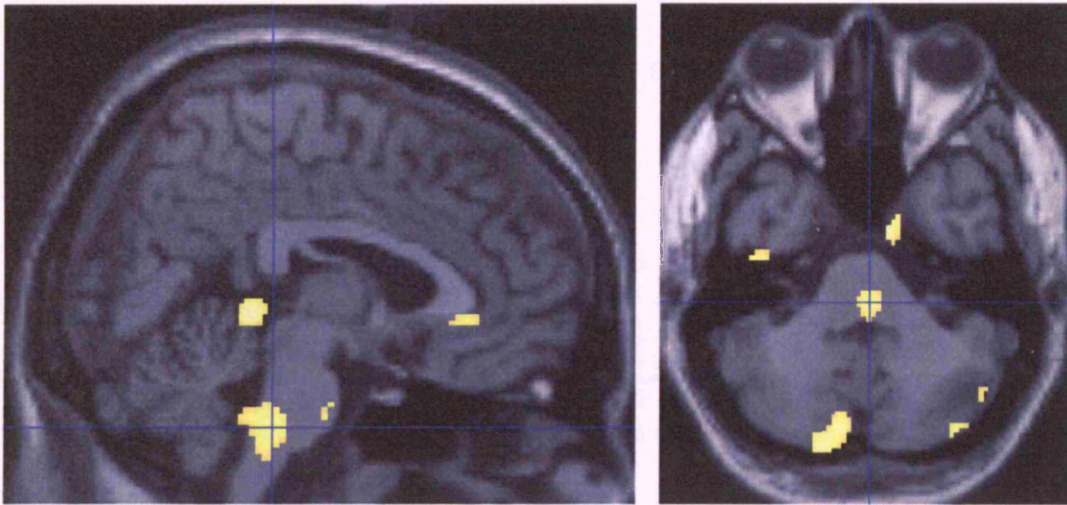
**Figure 8.3**

Activation in migraine patients during GTN infusion and non-specific headache compared to matched controls. Regions of activation demonstrated involve the internal carotid artery and its branches.
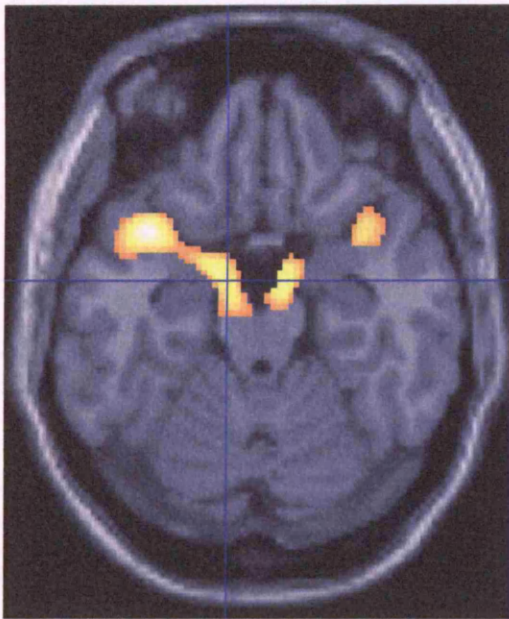
**Figure 8.4**

Activation of the ipsilateral pons in patients with right sided attacks ($n = 8$, a) and left sided attacks ($n = 8$, b).

a
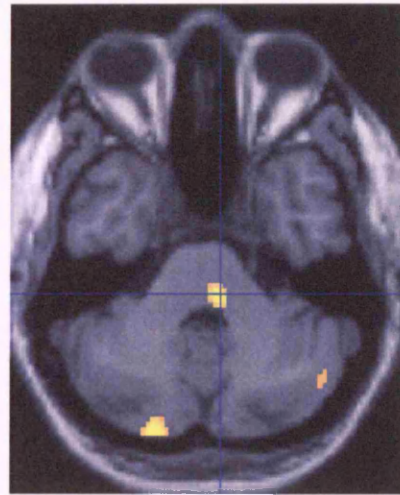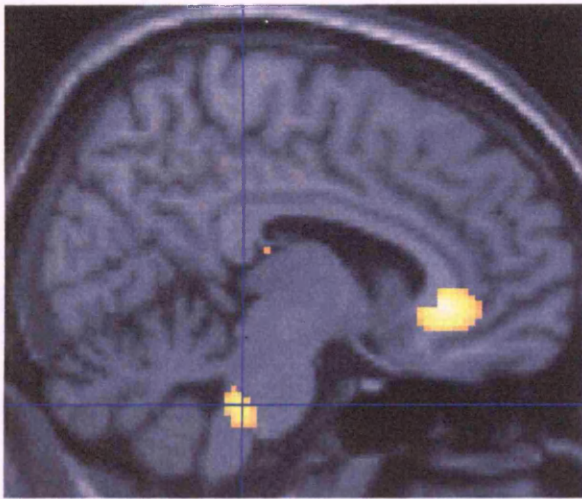


b
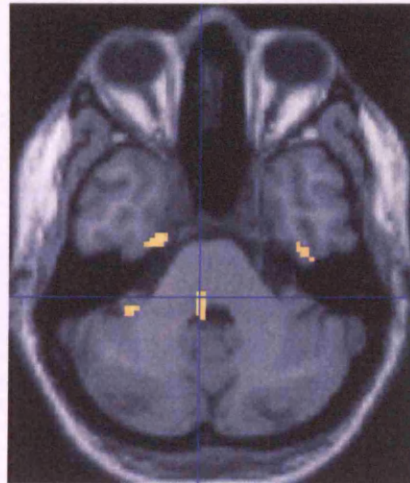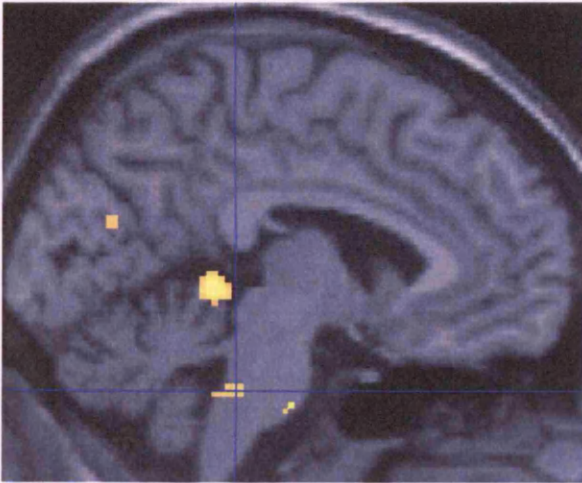
**Table 8.1** Subject characteristics

Right-sided migraine group

| Patient | Diagnosis | Age | Nausea | Photophobia | Phonophobia | Worse with movement | Aura | Attack freq /month |
|---------|-----------|-----|--------|-------------|-------------|---------------------|------|---------------------|
| 1 | MWA | 29 | + | − | + | + | Paraesthesia R arm | 1 |
| 2 | MO | 45 | + | + | + | + | | 1 |
| 3 | MO | 29 | + | − | + | + | | 3 |
| 4 | MO | 48 | + | + | + | + | | 4 |
| 5 | MO | 27 | + | + | − | + | | 2 |
| 6 | MO | 26 | − | + | + | + | | 2 |
| 7 | MO | 60 | − | + | + | + | | 8 |
| 8 | MWA | 28 | + | + | + | + | Visual patterns | 1 |

Left-sided migraine group.

| Patient | Diagnosis | Age | Nausea | Photophobia | Phonophobia | Worse with movement | Aura | Attack freq /month |
|---------|-----------|-----|--------|-------------|-------------|---------------------|------|--------------------|
| 1 | MO | 47 | − | + | + | + | | 1 |
| 2 | MO | 47 | + | + | + | + | | 2 |
| 3 | MO | 28 | + | + | − | − | | 4 |
| 4 | MWA | 54 | + | + | − | − | Visual, speech disturbance | 3 |
| 5 | MO | 65 | − | + | + | − | | 2 |
| 6 | MO | 53 | + | + | + | − | | 4 |
| 7 | MWA | 58 | + | + | + | + | Visual patterns | 4 |
| 8 | MWA | 54 | + | + | + | − | Visual zig-zag pattern | 8 |

Bilateral migraine group

| Patient | Diagnosis | Age | Nausea | Photophobia | Phonophobia | Worse with movement | Aura | Attack freq /month |
|---------|-----------|-----|--------|-------------|-------------|---------------------|------|---------------------|
| 1 | MO | 55 | − | + | + | + | | 4 |
| 2 | MWA | 54 | + | + | + | + | Visual scotoma, paraesthesia | 1 |
| 3 | MWA | 65 | + | + | + | + | Visual -lights | 8 |
| 4 | MO | 51 | − | + | + | + | | 1 |
| 5 | MO | 41 | + | + | + | + | | 6 |
| 6 | MO | 56 | + | + | + | + | | 2 |
| 7 | MO | 59 | + | + | + | − | | 3 |
| 8 | MWA | 58 | + | + | + | + | Visual lines | 8 |

**Table 8.2** Main effect of migraine.

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 section).

BA-Brodmann area

| Area of activation | Coordinates x,y,z | Z score of peak activation |
|---|---|---|
| Dorsal pons* | 4, -32, -32 | 3.95 (P=0.01 SVC) |
| Anterior cingulate BA24 | 10, 30 ,-4 | 3.95 |
| Prefrontal cortex BA10 | -18, 46, -2 | 4.43 |
| Insula- right<br>left | 32, 20,10<br>-60, 4, 8 | 4.62<br>4.3 |
| Cerebellum-right<br>left | 40, -82, -30<br>-24, -66, -14 | 3.62<br>4.8 |
| Putamen | -20, 0 ,12 | 4.95 |

**Table 8.3** Site of brainstem activation in each migraine sub-group. The Talairach and Tournoux coordinates describe the maxima within a cluster ($P < 0.001$). A small volume correction (SVC) was applied (as described in methods section) using a reporting criterion of $P<0.05$ corrected for multiple comparisons.

| Group | Area of activation | Coordinates x,y,z | Z score of peak activation | P value (SVC) |
|---|---|---|---|---|
| Right-sided headache | Dorsal pons (right) | 8,-32,-28 | 4 | 0.02 |
| | Rostral medulla (mid-line) | 0,-32,-40 | 4.25 | |
| Left-sided headache | Dorsal pons (left) | -4,-34,-28 | 3.4 | 0.03 |
| Bilateral headache | Dorsal pons (left) | -4,-32,-20 | 3.9 | 0.01 |

## Discussion

This study demonstrated robust activation of the dorsolateral pons in clinically typical

migraine triggered by glyceryl trinitrate (GTN). By selecting patients with habitually

lateralised attacks, and testing this lateralisation 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 and

triggered migraine (Bahra et al., 2001; Weiller et al., 1995). In both the Weiller study

(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 brain 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.


*Study limitations*

Other than the different design, PET camera and analysis an important difference

between this study and that of Weiller and colleagues (Weiller et al., 1995) is the use

of GTN triggering. Studying triggered attacks allows considerably more experimental

control and permitted us to carefully select patients 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. First, their clinical phenotype is indistinguishable

from spontaneous migraine (Headache Classification Committee of The International Headache Society, 2004; Iversen et al., 1989). Secondly, their response to treatment with triptans is the same (Ferrari et al., 2001; Iversen and Olesen, 1996). 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. Fourthly, the GTN model reproduces premonitory symptoms (Afridi et al., 2004), again a typical feature of spontaneous attacks (Giffin et al., 2003) as well as the laterality of the patients usual migraine attack. Also, nitrergic mechanisms seem fundamental in migraine (Olesen et al., 1994) with 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% (Afridi et al., 2004; Sances et al., 2004; Thomsen et al., 1994) of migraineurs may be responsive to GTN triggering, suggesting that this sub-group represents a reasonably large proportion of the overall migraine population.

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 pain severity rating of 5 out of 10. 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. This is one possible explanation for the difference between our findings and those of the Weiller study (Weiller et al., 1995) in which patients were scanned later. Certainly, the data from our patients with bilateral pain suggest that localisation

of pain is very closely linked to the side of brainstem activation. 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 been subsequently shown to be due to an order effect when an appropriate control group was included (Rosen et al., 1996).

**Conclusion**

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 lateralised 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.

# Chapter 9. Occipital activation in GTN-induced migraine with aura

A small but significant proportion of migraineurs (30-40%) have migraine with aura most commonly in the form of visual disturbances such as scintillating scotoma (Launer et al., 1999; Stewart et al., 1994). Leao (Leao, 1944) first described the phenomenon of spreading depression when he found that noxious stimulation of rabbit cerebral cortex produced a spreading decrease in electrical activity moving at a rate of 2-3 mm/min. This rate corresponds well with the propagation of visual aura (Hadjikhani et al., 2001). It has been proposed that this cortical spreading depression represents the neurobiological basis for migraine aura whereby neuronal activation and suppression are followed by corresponding vascular changes (Lauritzen, 1994).

It has been difficult to image patients with migraine aura because functional brain imaging is technically demanding and spontaneous attacks are unpredictable. While nitric oxide (NO) donors have long been recognised to trigger severe headache (Laws, 1898), and indeed to trigger migraine (Iversen, 2001), it has generally not been recognised to trigger migraine aura even in those who have had it spontaneously (Christiansen et al., 1999).

## Case

A 54 year old male with episodic migraine with aura volunteered to participate in a positron emission tomography (PET) study in migraine. He had a history of migraine with aura as defined by the International Headache Society Criteria (Headache Classification Committee of The International Headache Society, 2004) since his teenage years with a frequency of up to eight attacks per month each lasting up to one

day if left untreated. A typical attack would begin with visual aura in the form of bright, flashing zig-zag shapes moving around his visual field and rarely he experienced macropsia. The visual aura would last 10-20 minutes and would be followed by a left-sided headache with associated nausea, photophobia, phonophobia and aggravation by movement. His attacks were usually aborted within 2 hours using zolmitriptan. He had no other significant medical conditions. His father also suffered with migraine with aura.

*Glyceryl trinitrate triggering*: An intra-venous infusion of 0.5 µg/kg/min glyceryl trinitrate (GTN) was administered over 20 minutes on two occasions separated by one week with the aim of inducing a migraine. On both occasions a migraine was successfully triggered, after 150 minutes and 165 minutes, respectively. The subject underwent PET scanning during the second session of GTN.

A visual aura typical of the subject's previous attacks was also triggered during both sessions of GTN. He described flashing bright zig-zag patterns spreading across his visual field lasting 10 minutes. He was unable to describe any laterality of the visual symptoms. The aura remained unchanged by eye closure. This was then followed by a left-sided migrainous headache. Subcutaneous sumatriptan (6mg) was then administered to the subject and the migraine attack was successfully aborted.

*PET scanning and analysis:* The subject was scanned prior to the infusion, while pain free, and during the aura and headache phases of migraine. He described the onset of the aura and scanning of the aura phase commenced immediately following this description. PET scans were performed with an ECAT EXACT HR+ scanning system

(CTI Siemens, Knoxville, TN) in three-dimensional mode with septa retracted as described in chapter 8. He had his eyes closed throughout the scanning sessions.

The subject had a total of twelve scans, two of which were during the aura. The aura subsided during the second of the scans. The interval between scans was 8 minutes. SPM99 was used for data analysis (Frackowiak and Friston, 1994). Statistical parametric maps were derived with pre-specified contrasts, comparing regional cerebral blood flow during aura versus rest. An uncorrected threshold of $P < 0.001$ was chosen for tabular and graphical reporting.

## Results

Analysis of the PET data revealed right-sided occipital activation when comparing the two scans taken during the visual aura with those taken immediately prior to the GTN infusion (Figure 9.1). The activation was in the primary visual region (V1) of the occipital cortex (Brodmann area 17). This area has a very high degree of retinotopic precision and orientation selectivity (Tootell et al., 1998). No significant activation was observed in the V1 region of 7 other patients with IHS defined migraine with aura who developed migraine without aura following GTN administration (Afridi et al., 2004).

**Figure 9** Right-sided occipital activation during visual aura.

The Talairach and Tournoux (1988) coordinates of peak activation within the cluster

are (14, -82, 8). The Z score at peak activation is 4.4 (uncorrected $P < 0.001$).
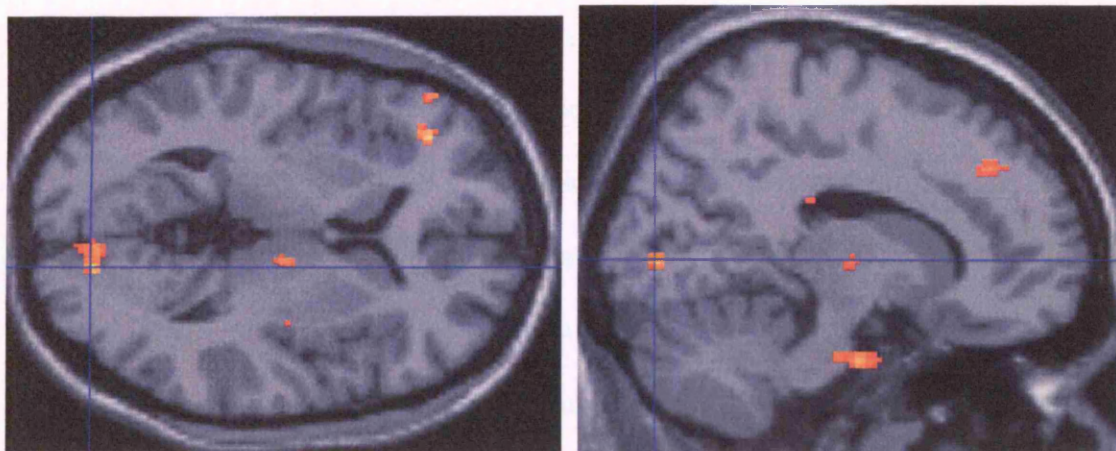
## Discussion

As far as we are aware there have not been any published reports of PET activation in the primary visual cortex during visual aura. Earlier studies using PET and SPECT demonstrated spreading hypoperfusion in the occipital cortex in migraine with aura (Olesen et al., 1981; Woods et al., 1994). More recently functional MRI has been used in migraineurs to investigate occipital cortex activation during visual stimulation with a checker board. In one of these studies an initial increase in blood flow was demonstrated in the extrastriate cortex (V3A) (Hadjikhani et al., 2001). This signal progressed across the occipital cortex (including V1) and was followed by diminution of the signal. The authors proposed that these changes represented cortical spreading depression. In a previous fMRI study (Cao et al., 1999) the process was described in another subject with visually triggered visual aura. In this case it was initiated in the primary visual cortex and then spread anterolaterally. Unfortunately the relatively poor temporal resolution of PET did not allow us to follow the progression of the aura more closely in our case.

Our patient was a participant in a study involving GTN triggering of migraine and was one of two patients in whom migraine aura was triggered (Afridi et al., 2004). Unfortunately the second patient did not undergo PET scanning. The mechanism by which GTN triggers migraine is unclear but it is thought that the exogenous NO from the GTN may induce endogenous NO production. Animal studies have also revealed that cortical spreading depression, which is thought to be responsible for migraine aura in humans, elicits widespread release of NO and increased cortical concentrations of cyclic guanosine monophosphate (Read et al., 2001). The CSD

induced increase in cortical NO is reduced when sumatriptan is given as a pre-treatment in cats and rats (Read and Parsons, 2000).

Other possible causes for the occipital activation in this patient include the possibility that he opened his eyes during the scanning, although the patient denies this and if this was the case then one would expect a much greater pattern of activation in the visual cortex than that observed. Another possibility is that the GTN is responsible for vasodilatation in the occipital cortex. However, the region of activation is localised and unilateral and one would expect a GTN related flow change to be bilateral and generalised. In fact bilateral hypoperfusion rather than hyperperfusion has been documented in the occipital cortex following GTN administration to healthy controls (Bednarczyk et al., 2002). It is possible that some inherent neurovascular asymmetry exists in the brains of migraineurs which may alter the vascular response to GTN. However, this does not explain the lack of significant activation in the V1 region in any of the other patients with migraine with aura who received GTN infusions. It is also of interest to note that activation in V1 has been documented during visual mental imagery in particular when the subjects are required to imagine high resolution detailed images (Kosslyn et al., 2001).

Ours is the first case to show that aura can be triggered reproducibly by GTN making it less likely that the aura was spontaneous or developed by chance in this situation. The fact that the site of activation was contralateral to the headache does not contradict our explanation for the findings since we are proposing that the V1 activation is related to the aura and not the headache. Clinical and experimental observations (Olesen et al., 1990; Selby and Lance, 1960) have shown that aura

symptoms are not always contralateral to headache and consequently aura associated

changes in brain activation may be contralateral to headache. While some take the

view that aura is triggers headache (Moskowitz et al., 2004), others have argued for a

dissociation (Goadsby, 2001).


## Conclusion

We present evidence for the reproducibility of GTN as a trigger of migraine aura and

using PET we have demonstrated activation in the primary visual area of the occipital

cortex during the aura. While the number of patients who can have aura reliably re-

triggered is small, such cases clearly exist and may offer insights into the

neuroanatomical correlates of this fascinating clinical symptom.

# PART IV. DISCUSSION

**How do the brainstem findings relate to the clinical picture?**
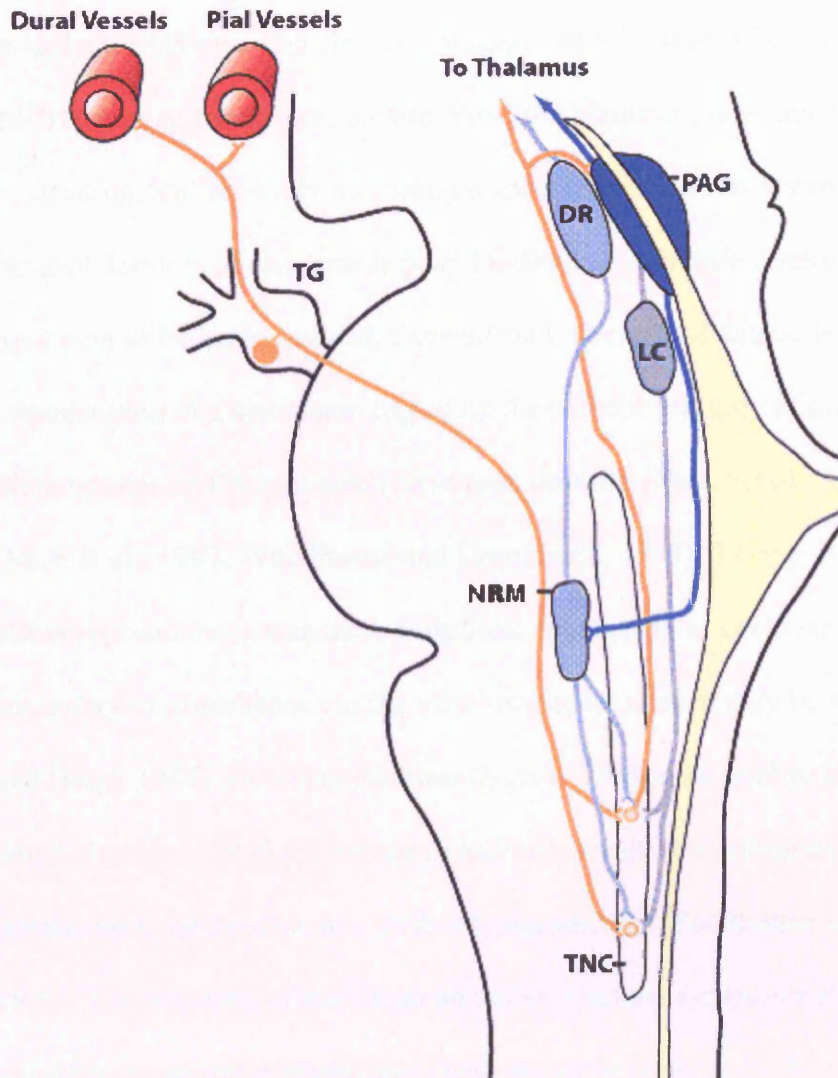


**Figure 10.1** Brainstem structures and pathways involved in migraine

TG-trigeminal ganglion, TNC- trigeminal nucleus caudalis, PAG- periaqueductal gray, DR-dorsal raphe, LC-locus coeruleus, NRM- nucleus raphe magnus, IV-fourth ventricle.

The two PET studies provide further evidence for the involvement of the brainstem in migraine. Weiller et al. initially proposed the brainstem as "a generator" of migraine. It cannot be concluded from these studies whether the brainstem generates or modulates migraine. Our findings suggest that it is possible to relate the anatomical involvement to the clinical picture. First, the brainstem is an integral part of the descending, and probably ascending nociceptive inhibition system and one of the cardinal features of migraine is pain. However, the migraine state does not reflect head pain in isolation. Indeed, there are a number of associated features which require consideration in a discussion regarding the pathophysiology of migraine. Phonophobia and photophobia have been described inter-ictally as well as ictally (Main et al., 1997; Woodhouse and Drummond, 1993). Taking phonophobia, it has been proposed that a transient, peripheral defect such as cochlear dysfunction secondary to disturbances in the vertebro-basilar system may be responsible (Kayan and Hood, 1984). However cochlear dysfunction should lead to loudness recruitment which would result in a decrease in auditory sensitivity during migraine and this was not shown to be the case in a study of migraineurs (Woodhouse and Drummond, 1993). The presence of inter-ictal and often bilateral symptoms also goes against a transient peripheral dysfunction. These are more likely to be the result of a dysfunction of central sensory processing. Brainstem auditory evoked potentials have been shown to be modulated by the serotonergic system (Bank, 1991; Sand and Vingen, 2000) and there is a well described lack of habituation of BAEP in migraineurs inter-ictally (Schoenen, 1998). The brainstem noradrenergic system is known to affect signal-to-noise ratio of sensory processing and this will be discussed later. New onset phonophobia has also been reported as a result of a demyelinating pontine lesion (Weber et al., 2002). It is of interest to note that in this case the

phonophobia was ipsilateral to the pontine lesion. Similarly, taking photophobia as an example, the anatomical dysfunction responsible for this symptom could, in theory, occur at any point from the retina to the occipital cortex. Using Occam's razor one would postulate that the abnormality which results in the various combinations of symptoms in migraine lies in one specific region of the brain. The interaction between pain, photophobia, phonophobia and nausea favours a central dysfunction rather than multiple peripheral defects. Drummond et al. have performed numerous studies looking at the interplay of these symptoms and demonstrated that painful stimulation of the trigeminal system increases photophobia and nausea in migraineurs and also that nausea intensifies facial pain (Drummond and Granston, 2004; Drummond and Woodhouse, 1993). The interplay is suggestive of a central dysmodulation in a region of the brain which could account for such symptoms. The obvious choice would be the brainstem where it is known that modulation of sensory information occurs and pathways exist between the TNC and the medullary nuclei of the solitary tract (Ruggiero et al., 2000). It is interesting to note that painful stimulation from another region of the body (the hand) did not produce the same effect as painful stimulation of the temple suggesting that it is not simply a non-specific response to pain.

If the migraine process was initiated and driven peripherally from the trigeminal fibres then one would not expect there to be any abnormalities inter-ictally. However there is evidence that abnormalities exist during the inter-ictal period both from electrophysiological studies (described earlier) and from clinical studies showing that migraineurs may be more light-sensitive (Drummond, 1997; Drummond and Woodhouse, 1993; Vanagaite et al., 1997), noise-sensitive (Main et al., 1997) and in some cases more pain-sensitive (Marlowe, 1992; Nicolodi et al., 1994; Weissman-

Fogel et al., 2003). This suggests an underlying defect in sensory regulation which becomes exacerbated during the migraine. The brainstem is an ideal candidate for such a region and the work presented in this thesis supports such a notion.

**Arguments against the significance of the brainstem findings**

One argument that has been proposed is that the activation seen in the PET studies in migraine is simply a response to pain. Indeed, activation of the PAG has been described in 37-40% of functional imaging studies of experimental pain (Derbyshire, 2000). However, this activation is restricted to the midbrain and pontine activation has not been described in PET studies of pain although it was found in a recent fMRI study looking at empathy for pain amongst partners (Singer et al., 2004). It may be that with the advent of more sensitive functional imaging techniques pontine activation may be detected increasingly in pain studies, however this remains to be seen. In another fMRI study examining the placebo effect there was no pontine activation during the "pain only"condition but activation was present when opiod and placebo were administered following pain (Petrovic et al., 2002). A problem arises when one compares imaging findings in migraine studies to those in general pain studies which is the fact that these studies may include migraineurs as "healthy controls". Given the difficulty in finding pure non-migraine controls and the prevalence of migraine in the general population it is likely that subjects with migrainous biology would inadvertently have been included in general pain imaging studies as "healthy controls".

Another possible suggestion is that the brainstem activation observed is in the trigeminal nuclei. This is highly unlikely as the activation would tend to involve the TNC which is more caudal. In fact, an area of activation in the rostral medulla was noted *in addition* to the pontine activation in the right-sided induced migraine group (chapter 8). Trigeminal nucleus activation in response to noxious heat stimuli has been mapped using fMRI and the activation is, as expected, in the medulla rather than the pons (DaSilva et al., 2002) (see Fig 10.2). Also previous PET and MRI studies of experimental facial pain (May et al., 1998b), cluster headache (May et al., 1998a) and SUNCT (May et al., 1999) do not demonstrate brainstem activation. However, the recent finding of brainstem activation in hemicrania continua (Matharu et al., 2004b) is noteworthy since the phenotype of this condition shares many common features with migraine and is often clinically difficult to distinguish. Interestingly, in the aforementioned study the activation was also ipsilateral to the side of pain.

One of the arguments against a primary central (brainstem) mediated sensitisation or modulation of gating being responsible for the pain in migraine is that pain and allodynia are often localised and critics of the brainstem theory argue that a general dysfunction of brainstem nociceptive structures would imply a generalised pain state. However, there is evidence for somatotopic organisation of the PAG (Soper and Melzack, 1982) and it has also been shown that there is a differential pattern of response of cells in the RVM depending on the anatomical area stimulated (Ellrich et al., 2001b; Watkins et al., 1980). There is evidence of functional anatomical organisation within the dorsal raphe projection system from animal studies (Kirifides et al., 2001; Waterhouse et al., 1993). Similarly, efferent topography also exists, to some extent, within the locus coeruleus and also some measure of modality-

specificity (Berridge and Waterhouse, 2003; Waterhouse et al., 1993). Furthermore, spatially directed expectation of pain has been shown to induce a specific placebo effect only on the part of the body where pain is expected (Benedetti et al., 1999). This effect was abolished by naloxone indicating that it is an opioid-mediated effect which is targeted towards a particular part of the body. A recent PET study demonstrated the involvement of the brainstem in the placebo response although there was no activation in response to pain only (Petrovic et al., 2002). This gives further credence to the notion that somatotopy exists in the brainstem systems.

It is also possible that the regional specificity of the allodynia or pain is mediated at a higher level than the brainstem i.e. thalamus or cortex. The spread of the pain or allodynia could be a result of increasing receptive fields due to central sensitisation at the third-order level i.e. thalamus. One could postulate that this sensitisation occurs as result of the increasing sensory traffic which is secondary to the disinhibition arising from the brainstem modulatory systems. In rats dorsal raphe stimulation has been shown to modulate the firing rate of thalamic parafascicular neurons to noxious stimuli (Andersen and Dafny, 1983) supporting the view that ascending modulation of nociceptive information also occurs and may be mediated by the brainstem. One could also extend this theory of brainstem-mediated facilitation of thalamic processing to the symptoms of photophobia and phonophobia.

Alternatively, there is an argument that the thalamus itself is a possible candidate for the area of dysfunction in migraine. It is involved in nociceptive pathways and is responsible for integration of sensory information and, as such, could be involved in altering the gating of such information. In fact, it was recently demonstrated that propranolol inhibits trigeminal nociception at thalamocortical neurons (Shields and Goadsby, 2004). However, as the evidence from functional imaging studies of

thalamic involvement in migraine is inconsistent further discussion regarding the role of the thalamus falls beyond the scope of this thesis.

**Laterality**

The ipsilateral findings in our induced migraine PET study are in keeping with the known anatomy of the descending nociceptive pathways, namely the ipsilateral pathways descending from the PAG to the TNC. Kaube et al found facilitation of the nociception-specific blink reflex response predominated on the headache side only during migraine (Kaube et al., 2002).

How does one explain the discrepancy in laterality between the Weiller study and between the two studies described in this thesis? There are a number of differences between Weiller's study and the induced migraine study. First, Weiller only included subjects with migraine without aura whereas we included subjects both with and without aura. Secondly, three of their subjects were taking migraine prophylaxis (β-blockers) at the time of the study. Thirdly, all their subjects had right-sided headache. The lack of left-sided or bilateral migraine subjects makes it difficult to derive inferences regarding laterality. Fourthly, they looked at spontaneous rather than induced migraine and so the likelihood is that the subjects were imaged later on in the attack, as was the case with our spontaneous migraine study. Also, they did not include a control group to account for an order effect. Unlike our induced migraine study neither the Weiller study nor our spontaneous migraine study were designed specifically to look for laterality. It may be possible that the brainstem activation changes from unilateral to bilateral / contralateral as the migraine evolves. In fact, in a clinical report looking at the evolution of allodynia during migraine it was demonstrated that one hour into the migraine the allodynia was ipsilateral to the

headache but this started to affect the contralateral side of the head after two hours (Burstein et al., 2000). One could postulate that the allodynia was a response to the dysfunction of the gating mechanism of the brainstem nociceptive structures leading to sensitisation at the TNC. If this is the case then the development of allodynia on the contralateral side later on in the attack would imply that there was dysfunction of the contralateral brainstem later on in the attack. Clinical observations suggest that side-shift can occur during migraine. In fact even though 62 % of migraineurs are thought to have unilateral headache only 17-21% have side-locked headache (Leone et al., 1993; Selby and Lance, 1960). It is unclear from the Weiller study 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 at any point during the migraine. Our patients were recruited on the basis of their headache laterality and this was closely monitored and documented throughout the study.

The activation seen in functional imaging studies may represent activation of excitatory or inhibitory neurons. In certain parts of the descending nociceptive modulatory pathway the cells are segregated into "on" or "off" cells (Morgan and Fields, 1994). It may be that that this functional segregation enables one group to be preferentially activated during migraine.

Alternatively, if no side shift has occurred, the late activation in the contralateral brainstem may be accounted for by the fact that reciprocal inhibitory pathways exist between right and left locus coeruleus (LC) (Buda et al., 1975). The early dysfunction on the ipsilateral side may lead to loss of inhibition of the contralateral LC which consequently becomes overactive. The ipsilateral LC gradually normalises but central

sensitisation has become established resulting in a continuation of the symptoms which takes longer to resolve.

**Which specific region of the brainstem could be responsible?**

The noradrenergic system, in particular the locus coeruleus (LC) is an ideal candidate. If one considers the functions of this system the relevance to migraine becomes apparent. Noradrenaline acts as a neuromodulator enhancing the signal to noise ratio. It modulates the level of attention and arousal. Higher levels of tonic activity of LC neurons are associated with higher levels of arousal (there is virtual quiescence during REM sleep) and the discharge rates anticipate changes in behavioural state (Foote et al., 1980). Phasic discharges occur in response to novel stimuli and the LC shows plasticity of response as a function of changing significance of stimuli (Sara and Segal, 1991). The LC is involved in modulation of sensory information. Lesions of the LC have been shown to affect auditory event-related potentials in primates (Pineda et al., 1989). In vitro noradrenaline induces a shift in the firing pattern of cortical and thalamic neurons (McCormick, 1989). In a study applying non-noxious electrical stimuli to the trigeminal region of unanaesthetised rats (whisker pad), neuronal activity was recorded from the thalamus and cortex before and during tonic and phasic stimulation of the LC (Berridge and Waterhouse, 2003). The activity was enhanced by LC stimulation in an inverted-U response. In some cases, LC stimulation resulted in responses to otherwise subthreshold synaptic input and in other cases it decreased the mean response latency. These results demonstrate that LC output can optimise information processing within sensory circuits and can simultaneously facilitate stimulus-evoked discharge patterns at multiple levels of an ascending

sensory network. Similarly, in the auditory system both the auditory cortex and cochlear nuclei receive noradrenergic innervation solely from the LC which exerts a synergistic effect at both sites by filtering afferent information in a way which favours stronger signals (Pickles, 1976). A similar effect of the LC on the visual system has been reported (Kasamatsu, 1991) including the noradrenergic modulation of activity in the lateral geniculate nucleus (Funke et al., 1993). This could explain the symptoms of photophobia and phonophobia. The role of the brainstem noradrenergic system in the descending modulation of nociception has already been discussed in chapter 1. The response to stress is also relevant to migraine as stress or relaxation after stress is a classical trigger. Evidence suggests that corticotropin-releasing hormone (CRH) inputs to the LC from the medulla and amygdala may activate the LC in response to physiological and environmental stressors (Van Bockstaele et al., 1996; Van Bockstaele et al., 1998). Fos induction has been demonstrated in the LC and dorsal raphe in response to stress in rats (Ishida et al., 2002). There is also a projection from the hypocretin-containing neurons in the lateral hypothalamus to the LC (Peyron et al., 1998). Both CRH and hypocretin are implicated in stress and arousal-related processes. Hypocretin (orexin) activates LC neurons to increase the frequency of action potentials (Horvath et al., 1999). It is thought to be involved in appetite, neuroendocrine and autonomic regulation, sleep and, more recently, pain. Triggers other than stress may also involve the LC. Nitric oxide is thought to be involved in modulating excitatory activity in the LC (Pineda et al., 1996; Xu et al., 1998). This is discussed in the next section.

There is good evidence for the involvement of the serotonergic system in migraine (see chapter 1). The serotonergic system displays circadian rhythmicity under the

control of the suprachiasmatic nucleus of the hypothalamus. The SCN also receives afferent input from the DR (Zurak, 1997). The DR discharge pattern during the sleep-wake cycle is similar to that of the LC in that it is active during wakefulness and quiescent during REM sleep. There is evidence that selective attention in the auditory system is modulated by serotonin (Ahveninen et al., 2003) and brainstem auditory evoked potentials have been shown to be modulated by the serotonergic system (Bank, 1991; Sand and Vingen, 2000). Another electrophysiological measure, the intensity dependence of auditory evoked potentials (IDAP), which is abnormal inter-ictally in migraineurs (Wang et al., 1996) is also modulated by serotonergic modulators such as dexfenfluramine and triptans (Proietti-Cecchini et al., 1997). The IDAP is thought to be related to central serotonergic transmission. The pivotal role of the brainstem serotonergic nuclei in the descending inhibition of nociception has been described in chapter 1.

There is likely to be considerable overlap between the serotonergic and noradrenergic systems. Indeed, there is a dense innervation of the LC by serotonergic fibres (Pickel et al., 1977) and reciprocal connections have been demonstrated in animals (Kim et al., 2004). It is likely that both systems regulate modulation of sensory input. In rat visual cortex the two function in a complementary manner by modulating single neuron responses to visual stimuli (Waterhouse et al., 1990). Noradrenaline increases the signal-to-noise ratio whereas serotonin decreases it in this example. Both systems are linked to the hypothalamus, serotonin to the suprachiasmatic nucleus and the LC to the lateral hypothalamus and indirectly to the suprachiasmatic nucleus (Aston-Jones et al., 2001). As mentioned above both the DR and the LC demonstrate Fos induction in response to stress (Ishida et al., 2002). The serotonergic system is

involved in mood disorders such as depression. The LC receives inputs from the limbic system (the amygdala) suggesting that mood and emotional status can have an effect on LC activity and the noradrenergic system is thought to have at least a modulatory role in depression. Both systems are also affected by the sleep-wake cycle and there is a well described relationship between sleep and migraine (Sahota and Dexter, 1990). In fact, migraine has been shown to occur in association with stage 3, 4 and REM sleep stages which are when the LC and DR are less active. Both systems also have effects on cerebral vasculature and nociception (as described in chapter1). Together, these observations suggest that the brainstem serotonergic and noradrenergic systems are ideally suited to respond to migraine triggers and to play a significant role in the clinical manifestations of migraine.

**Nitric oxide, the brainstem and migraine**

The role of nitric oxide in migraine has been discussed in chapter 4. Nitric oxide synthase has been found to be distributed throughout the brain. Focussing on the brainstem, it has been demonstrated that in rats the DR provides a source of NO for cortical and subcortical regions including the thalamus and trigeminal nucleus and that serotonin and NO are co-localised in certain regions of the DR (Simpson et al., 2003) although this may be species specific. It is also known that the DR sends projections to the occipital cortex (Wilson and Molliver, 1991). It is possible that these projections may be involved in the GTN-triggering of migraine with aura.

As mentioned earlier, subcutaneous GTN has been found to produce a significant increase of nitric oxide synthase (NOS) and Fos protein-like immunoreactivity in the trigeminal nucleus caudalis (Pardutz et al., 2000) and brainstem nuclei, including the LC (Tassorelli and Joseph, 1995) in rats. The temporal profile of neuronal activation

following GTN shows that neuronal activation begins as early as 60 minutes post-injection in brain areas controlling cardiovascular function and reaches maximum expression 3 hours later in nociceptive structures (Tassorelli and Joseph, 1995). This time course suggests GTN has an initial effect on brain vascular centres which is later followed by the nociceptive effect. Further evidence for this is provided by a study involving GTN administration to rats followed by measurements of monoamine levels (Tassorelli et al., 2002). This showed that at 4 hours there was a decrease in brainstem levels of serotonin. In animal studies serotonin depletion has been shown to result in hypersensitivity of the cranial vascular system to NO (Srikiatkhachorn et al., 2000). Lower basal platelet serotonin concentration appears to increase the susceptibility to develop migraine (Juhasz et al., 2003). The effect of NO on serotonin release has been investigated in a few brain areas with varying results. However in the raphe nuclei (Smith and Whitton, 2000) and hypothalamus (Kaehler et al., 1999) NO exerts an inverse concentration effect on serotonin release: low concentrations decrease serotonin release and high concentrations have the opposite effect. There is further evidence for an interaction between the serotonergic system and the NO system. The 5-HT$_{1B/1D}$ receptor agonist, sumatriptan, attenuates the effect of GTN on cerebral blood flow and on nNOS expression in the TNC and trigeminal ganglion, thus modulating NO synthesis (Suwattanasophon et al., 2003). It is possible that this interaction between NO and serotonin may be responsible for GTN triggered migraine with aura since serotonergic projections to the occipital cortex may also be modulated by NO.

NOS containing neurons have been described in the region of the LC in rats (Xu et al., 1994). NO has a been shown to reduce the amplitude of excitatory post-synaptic

currents in LC neurons (Xu et al., 1998) although another group (Pineda et al., 1996) reported that NO donors cause an increase in firing rate in LC neurons. The discrepancy between these two studies was put down to methodological differences (Xu et al., 1998). Both studies suggest that NO may have a modulatory effect on LC neurons.

As mentioned earlier, there are well described connections between the hypothalamus and brainstem which may be important in the triggering of migraine. Furthermore, many of the typical premonitory symptoms of migraine can be thought of as originating in the hypothalamus. For example, one of the main pathways involved in yawning originates in the paraventricular nucleus (PVN) of the hypothalamus and NO is thought to mediate this in response to activation of the oxytocinergic neurons by dopamine and other substances (Argiolas and Melis, 1998). These neurons are thought to project to other areas including the brainstem. In addition to yawning, it has been postulated that NO is involved in generating circadian rhythms in the suprachiasmatic nuclei which has implications in terms of "spontaneous" triggering of migraine (Golombek et al., 2004). Other common premonitory symptoms of migraine include thirst, polydipsia and polyuria. Following GTN administration in rats Fos activation was seen in the PVN and supraoptic nuclei (SON) of the hypothalamus (Tassorelli and Joseph, 1995). NO has been shown to inhibit the electrical activity of vasopressin and oxytocin neurons in the SON and PVN (Kadekaro, 2004). NOS and its mRNA are expressed in the osmoregulatory networks (Wang and Morris, 1996).

Clearly an important question, which remains as yet unanswered, relates to the mechanism of triggering in migraine. NO has established itself as a reliable and reproducible trigger, although the mechanism for this has not yet been determined. It may be that the hypothalamus is involved in the triggering process although not necessarily exclusively. What appears to be more likely is that the final common pathway is in the brainstem. Certain predisposing conditions may make one more susceptible to triggering; for example, serotonin depletion (Srikiatkhachorn et al., 2000) and oestrogen (Pardutz et al., 2002; Sarchielli et al., 1996) alter the sensitivity to NO. As mentioned earlier, the LC receives a dense serotonergic innervation (Pickel et al., 1977). Under resting conditions, NOS inhibition does not appear to influence serotonin release in the rat LC. However, under conditions of stress NO appears to facilitate serotonin release in this region (Sinner et al., 2001). This finding is interesting as it suggests that a NO-mediated event may only occur under conditions of increased susceptibility.

It is clear that migraineurs and controls respond differently to NO donors. There is evidence that migraineurs exhibit greater intracranial artery sensitivity to NO than controls (Thomsen et al., 1993). In fact, it is likely that this inherent sensitivity extends to the entire trigeminovascular system and not only in relation to nitric oxide (Katsarava et al., 2003b; Weissman-Fogel et al., 2003).
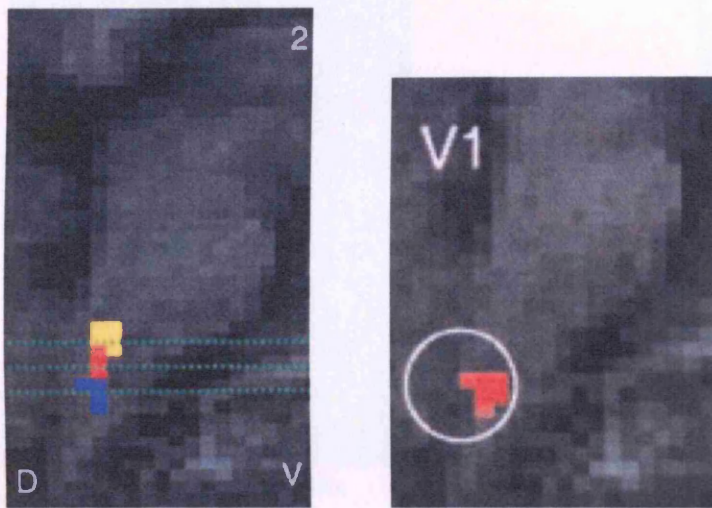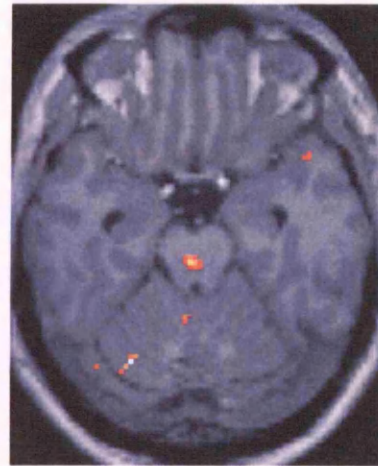
**Figure 10.2** fMRI showing activation in trigeminal nuclei regions in response to

noxious heat stimuli (DaSilva et al., 2002).
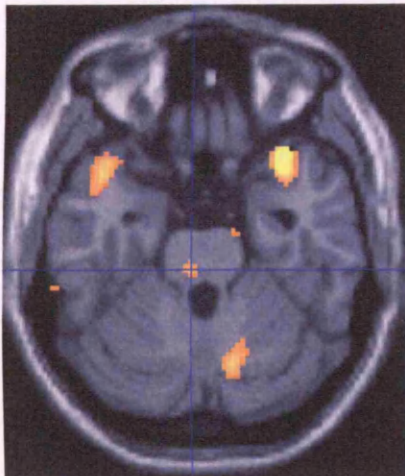
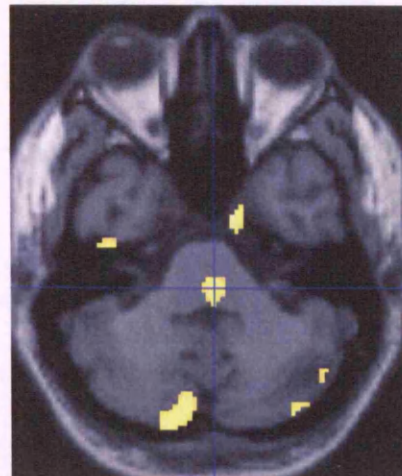Yellow, red, blue correspond to V2,V3,V1 respectively
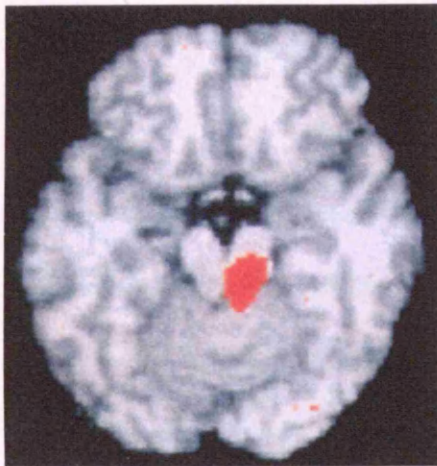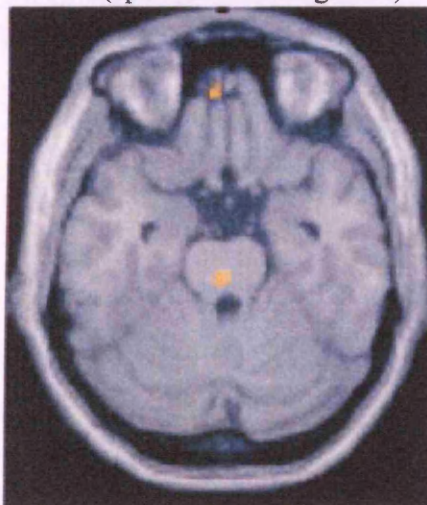
Weiller et al., 1995
(spontaneous migraine)

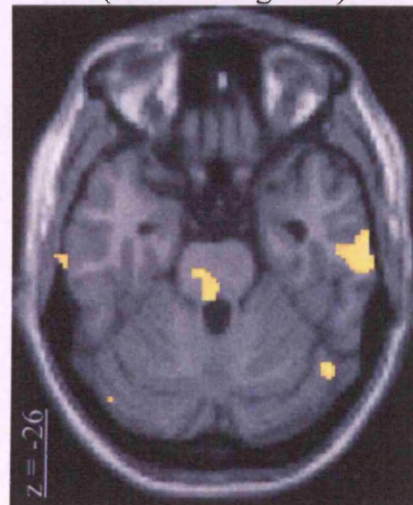Bahra et al., 2001
(induced migraine)

Afridi et al., 2004a
(spontaneous migraine)

Afridi et al., 2005
(induced migraine)

Matharu et al., 2004a
(chronic migraine)

Matharu et al. 2004b
(hemicrania continua)

**Figure 10.3** Brainstem activation in migraine (and hemicrania continua)

# PART V. CONCLUSION

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Migraine is a complex neurobiological disorder which cannot simply be explained by dysfunction in one area of the brain in isolation. The very nature of the complex and intricate pathways involved in neural processing would make this highly unlikely. The PET findings of brainstem activation during migraine presented in this thesis do not necessarily imply a causal link. However when one takes into the account the growing body of evidence which supports a pivotal role for the brainstem in migraine these findings become more significant in such a context. The demonstration of the relationship between laterality of the clinical symptoms and functional neuroanatomy also increases the validity of these findings. This does not exclude the possibility that other regions of the brain, for example the thalamus, may also have an important role to play either as part of the trigeminal sensory and nociceptive pathways or in its own right. Perhaps as the technology advances the picture will become clearer.

The GTN model of migraine has proven to be a reliable and reproducible model which has facilitated the further study of migraine.

To conclude, functional imaging is a valuable tool which, as I hope has been demonstrated, has enhanced our understanding of this complex and challenging condition.

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# APPENDIX

## IHS diagnostic criteria (Headache Classification Committee of The International Headache Society, 2004)

<u>Migraine without aura</u>

*Diagnostic criteria:*

A. At least 5 attacks fulfilling B - D.

B. Headache attacks lasting 4-72 hours and occurring < 15 days/month (untreated or unsuccessfully treated).

C. Headache has at least two of the following characteristics:

    1. Unilateral location

    2. Pulsating quality

    3. Moderate or severe pain intensity

    4. Aggravation by or causing avoidance of routine physical activity (i.e., walking or climbing stairs)

D. During headache at least one of the following:

    1. Nausea and/or vomiting

    2. Photophobia and phonophobia

E. Not attributed to another disorder

<u>Typical aura with migraine headache</u>

*Diagnostic criteria:*

A. At least two attacks fulfilling criteria B – E

B. Fully reversible visual and/or sensory and/or speech symptoms but no motor weakness

C. At least 2 of 3:

1. Homonymous visual symptoms including positive features (i.e. flickering lights, spots, lines and/or negative features (i.e. loss of vision) and/or unilateral sensory symptoms including positive features (i.e. pins and needles) and/or negative features (i.e. numbness)

2. At least one symptom develops gradually over ≥5 minutes and/or different symptoms occur in succession

3. Each symptom lasts ≥5 minutes and ≤ 60 minutes

D. Headache that meets criteria B-D for migraine without aura begins during the aura or follows aura within 60 minutes

E. Not attributed to another disorder


## Persistent aura without infarction

*Description:* Aura symptoms persist for more than 2 weeks without radiographic evidence of infarction.


*Diagnostic criteria:*

A. The present attack in a patient with migraine with aura is typical of previous attacks but one or more aura symptoms persist for more than one week

B. Not attributed to another disorder


## Familial hemiplegic migraine

*Diagnostic criteria:*

A. At least 2 attacks fulfilling B – E

B. Fully reversible motor weakness and at least one of the following other fully reversible aura symptoms: visual, sensory or speech disturbance.

C.    At least two of the following:

    1.    At least one aura symptom develops gradually over ≥ 5 minutes and/or different symptoms occur in succession.

    2.    Each aura symptom lasts ≥ 5 minutes and less than 24 hour.

    3.    Headache that meets criteria B-D for migraine without aura begins during the aura or follows aura within 60 minutes.

D.    At least one first or second-degree relative has migraine attacks with aura including motor weakness (fulfils criteria A, B, C and E).

E.    Not attributed to another disorder.


## Sporadic hemiplegic migraine

*Diagnostic criteria:*

A.    At least 2 attacks fulfilling B – D

B.    Fully reversible motor weakness and at least one of the following other aura symptoms: visual, sensory or speech disturbance.

C.    At least two of the following:

    1.    At least one aura symptom develops gradually over ≥ 5 minutes and/or different symptoms occur in succession.

    2.    Each aura symptom lasts less than 24 hour.

    3.    Headache that meets criteria B-D for migraine without aura begins during the aura or follows aura within 60 minutes.

D.    No first or second degree relative has migraine attacks with aura including motor weakness.

E.    Not attributed to another disorder.