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**The migraine postdrome
A clinical and neuroimaging study**

Bose, Pyari Raghavan

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THE MIGRAINE POSTDROME - A CLINICAL AND NEUROIMAGING STUDY

A thesis submitted to King's College London for the degree of MD in the Faculty of Clinical
Neurosciences

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PUBLISHED PAPERS AND ABSTRACTS

Bose P, Karsan N, O'Daly O, Zelaya F, Goadsby PJ. ALTERATIONS IN CEREBRAL BLOOD FLOW DURING THE POSTDROME PHASE OF A MIGRAINE ATTACK CAPTURED WITH ARTERIAL SPIN LABELLED (ASL) MRI. Cephalalgia. 2017;37 2017, Vol. 37(1S) 1–24

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Bose P, Kader S, Goadsby, P. J. An audit of the migraine postdrome-how common are the symptoms in a clinic cohort? Cephalalgia 2016, Vol. 36(1S) 67.

ABSTRACT

Migraine is a common and disabling neurological disorder. The postdrome phase of migraine, is poorly defined and studied, in comparison to the preceding phases. It has also not been defined in the International Classification of Headache disorders. Hence there exists a vital need to understand the pathophysiology involved in this phase.

No imaging study has evaluated the postdrome phase. Preceding phases of migraine have been studied, but with PET imaging as the functional imaging modality. However, there are various limitations of PET which pose several logistical challenges in studying the postdrome phase. Arterial Spin Labelling (ASL) MRI is a novel MRI technique that measures tissue perfusion without ionizing radiation.

Previous diary studies have shown the extent of postdrome amongst migraineurs to vary from 61-80%. A clinical audit carried out within our clinical cohort of patients showed that 86% of subjects reported postdrome symptoms.

The primary hypothesis behind the ASL MRI study is that, brain areas with increased neuronal activations in the premonitory phase, remain persistently activated in the postdrome phase. The reasoning behind this is, the broadly similar nature of symptoms that patients complain of, in both these phases. A nitroglycerin induced migraine model was applied to study the activations.

In this prospective imaging study involving 16 subjects, voxel based analysis showed a near global reduction in rCBF in the postdrome phase compared to the premonitory phase with a peak reduction over the left superior temporal gyrus ($P<0.001$).

This study has shown that the biology of neural activations in the premonitory and postdrome phase are different and not due to persistent activation of similar neural networks. The symptoms experienced by subjects in the postdrome are associated with a significant, near global reduction in cerebral blood flow. In chapter 7, a subgroup analysis was carried out to see if there was any seasonal variation to nitroglycerin induced migraine. The analysis showed that the triggering rates were lowest during winter, which may have a profound impact on experimental migraine research.

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ABBREVIATIONS LIST

analysis of covariance (ANCOVA), 83
ASL (Arterial Spin Labeled), 24
blood oxygenation level-dependent (BOLD), 15
Calcitonin gene related peptide (CGRP), 6
CAS (Cranial Autonomic Symptoms), 6
Cortical spreading depression (CSD), 15
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volume of Interest, VOI, 85, 88

CHAPTER 1

INTRODUCTION

1 INTRODUCTION

Migraine is a common and disabling disorder affecting an estimated 12% of the world population (2015). Four non-obligatory phases have been described in migraine. The postdrome phase is the least studied amongst the various phases of migraine and described only recently (Selby, 1983; Kelman, 2006; Bose and Goadsby, 2016). The various phases of migraine often overlap in symptomatology but generally comprise of specific symptoms prior to the onset of head pain such as thirst, cravings and yawning, which are collectively called the premonitory symptoms (Giffin *et al.*, 2003), the migraine aura, which is characterised by brief transient focal neurological symptoms and only occurs in one-third of patients, the migraine headache or attack phase when a throbbing headache is experienced and finally, the postdrome phase, a combination of various non-headache symptoms that patients often experience after the throbbing headache has ceased, and before they return to back to normal function (Giffin *et al.*, 2016).

Premonitory symptoms occur prior to the onset of migraine headache, and can include symptoms like fatigue, excessive yawning and excessive micturition. An electronic diary study by Giffin *et al.*, had previously shown that premonitory symptoms are highly predictive of an impending attack and there tends to be a large amount of inter-individual variability but not intra-individual variability in these symptoms (Giffin *et al.*, 2003). The postdrome phase of migraine has been largely neglected in the literature, and tends to comprise of cognitive difficulties, fatigue and poor attention (Blau, 1991; Kelman, 2006; Quintela *et al.*, 2006; Giffin *et al.*, 2016). The paucity of literature surrounding this phase and its significant burden for patients, in terms of returning to normal function, highlights the importance of developing a greater understanding of the mechanisms that underlie this phase (Ng-Mak *et al.*, 2011). In a cohort of 827 patients, Kelman found that 68% described postdrome symptoms (Kelman, 2006). A prospective electronic study by Giffin *et al.*, noted that 55% of subjects returned to normal function within 6 hours whereas 7% required over 24 hours to return to normal (Giffin *et al.*, 2016). No imaging study has looked into the postdrome phase. Given that there are some similar symptoms experienced by subjects in the premonitory phase and postdrome phase (Giffin *et al.*, 2003; Giffin *et al.*, 2016), it seems pertinent to know whether this is the consequence of activation of similar brain areas. Understanding the pathophysiology involved in the postdrome will ultimately lead to an enhanced understanding of the underlying central nervous system mechanisms and potential new therapeutic strategies.

One of the earliest observations that allude to the postdrome, was made by Liveing who documented in his book 'On megrim, sick-headache, and some allied disorders', published in 1873,

that sleep resolved migraine attacks in some patients (Liveing, 1872). Selby was amongst the few neurologists who designated the postdrome as a characteristic feature of migraine. In his book 'Migraine and its variants', Selby used the term 'post-headache phase' to describe what is now called the postdrome phase. He described it as the "anticlimactic act of the migraine drama where pain and nausea has settled down but patients are left with a difficult to describe prostration and malaise yet typical of migraine" (Selby, 1983). One of the earliest authors to speculate on the pathophysiological basis of the postdrome was Blau who suggested that the postdrome may be due to a slow decline in migraine processes or, potentially an inverse mechanism with respect to the premonitory symptoms (Blau, 1991) .

Novel neuroimaging modalities have pushed the frontiers in the understanding of neurological disorders and have also shed further light into the possible links between brain structures and brain networks that could be involved in the pathophysiology of disease. Functional imaging, including functional MRI (fMRI) and positron emission tomography (PET), have been increasingly used in migraine and other pain states and has alluded to areas of brain activation that are thought to be key structures in the initiation and propagation of the headache and pain states (Maniyar *et al.*, 2014a; Hodkinson *et al.*, 2015b; Schulte and May, 2016b).

1.1 Migraine- A brief historical perspective

Headache has been a part and parcel of human experience since antiquity. The earliest document descriptions of migraine appear in ancient Egyptian papyrus and Babylonian cuneiform tablets over 6000 years ago (Walsh *et al.*, 1982; Weatherall, 2012). The descriptions of migraine and the varying treatments carried out were just as diverse as the various cultures from pre-historic times. Perhaps the diversity in treatments of headache disorders carried out around the world, even in this current age, depend on various cultural, social and geographical traditions (Levin, 2008; Finger *et al.*, 2010). Certain accounts narrated in ancient religious texts like the Old Testament, could be attributed to migraine due to some key pertinent aspects (Borsook, 2012).

The earliest documentation of the migraine aura comes from the writings of the Hippocratic School of medicine 200 BC (Borsook, 2012). The prominent Greek physician, Galen of Pergamon first used the term 'hemicrania' which later gave rise to the term migraine. Aretaeus of Cappadocia, also a prominent Greek physician, wrote about headaches in the early second century and divided it into three types namely, short lived, persistent or recurrent (Borsook, 2012). This was the first clear headache classification recorded (Martelletti *et al.*, 2011).

The Islamic medicine writers of the 11th to 13th centuries reinforced the ideas of the earlier Greco-Roman physicians. The renowned Persian physician of the time, Avicenna made significant contributions to neurology including in the field of headache and migraine (Zargaran *et al.*, 2016).

Thomas Willis (1621-1675) can be arguably called the father of the modern era of neurology. In his book *De Anima Brutorum*, he dedicates two chapters to the topic of headache. His work dealt with headache etiology, mechanisms and classifications. It was heavily based on his 'animal spirits' concept (Eadie, 2003). Thomas Willis may have been the first to hypothesize the neurovascular theory of migraine by speculating that it was caused by increased arterial blood flow in the meninges (Willis, 1668; Eadie, 2003; Akkermans, 2015).

Premonitory symptoms of migraine have been long recognized (Gowers, 1970). The postdrome phase as an entity is a relatively newly described phase of migraine (Selby, 1983). To get a historical perspective regarding the neural activations in these phases, one must start with the vascular versus neurogenic theory, which is an oft-recurring theme amongst neurologists and has been debated as early as the 19th century. The notable proponents of these theories in the 19th century include the

British neurologists Peter Wallwork Latham (Latham, 1872), who favoured the 'vasomotor theory' and Edward Liveing (Liveing, 1872) who favoured the 'nerve storm' theory. These theories can be considered the prototypical vascular and neurogenic theories of migraine pathogenesis. Various contemporary neurologists of the time, like Gowers (Gowers and Princess Marina Hospital. Library, 1886), undermined the theory proposed and terminologies used by Liveing (Gowers, 1906; Weatherall, 2012). Furthermore, Harold Wolff a 20th century pioneer in headache research, performed ground breaking research further promoting the vascular theory of migraine (Akkermans, 2015).

Over time, with the help of various scientific technological breakthroughs, it has been shown that migraine is initiated by a primary neuronal activation that sets off a cascade of changes intracranially and extracranially that account for the symptoms (Cutrer, 2006). Vascular changes like extracranial arterial dilatation that was hypothesised to be the cause of pain in patients suffering from migraine without aura (Tunis and Wolff, 1953) can be considered an epiphenomenon and not the primary trigger (Amin *et al.*, 2013). Advances in the understanding of the key brain areas involved in migraine pathogenesis, using functional imaging techniques have helped to characterise further the premonitory and headache phases of migraine (Maniyar *et al.*, 2014a). The evolving improvement in our understanding of migraine-specific anatomical pathways and molecular pathways have led to the development of novel treatments strategies with furthermore on the horizon (Diener *et al.*, 2015; Karsan and Goadsby, 2015; Goadsby *et al.*, 2017). Therapeutic agents targeting calcitonin gene-related peptide receptor, 5-HT_{1F} receptor and neuromodulatory options offer promise in the treatment of migraine (Karsan *et al.*, 2017; Nazia Karsan, 2017) and have ushered in, a new era in migraine management.

As science progresses, from the ancient Egyptian civilization to the present times, we are now in a unique position to study what happens to the brain during different phases of migraine including the postdrome, using innovative technological advances. In time, as physicians and scientists, we hope to see the bigger picture when it comes to understanding the migraine pathophysiology.

1.2 Migraine anatomical considerations and pathophysiology

Migraine is thought to be due to a primary brain dysfunction. Neuronal activation leads to a cascade of changes intracranially and extracranially that account for the symptoms. Dysfunction of subcortical structures may modulate the perception and activation of the trigeminovascular system.

1.2.1 Key structures

The brain itself is largely insensitive to pain. The key structures involved in the perception of headache include the large intracranial vessels and dura mater (Feindel *et al.*, 1960), the peripheral terminals of the trigeminovascular system that innervate these structures, the caudal portion of the trigeminal nucleus, which extends into the dorsal horns of the upper cervical spinal cord and receives input from the first and second cervical nerve roots (the trigeminocervical complex) and the pain modulatory systems in the brain that receive input from trigeminal nociceptors (Feindel *et al.*, 1960; McNaughton FL, 1977; Akerman *et al.*, 2011).

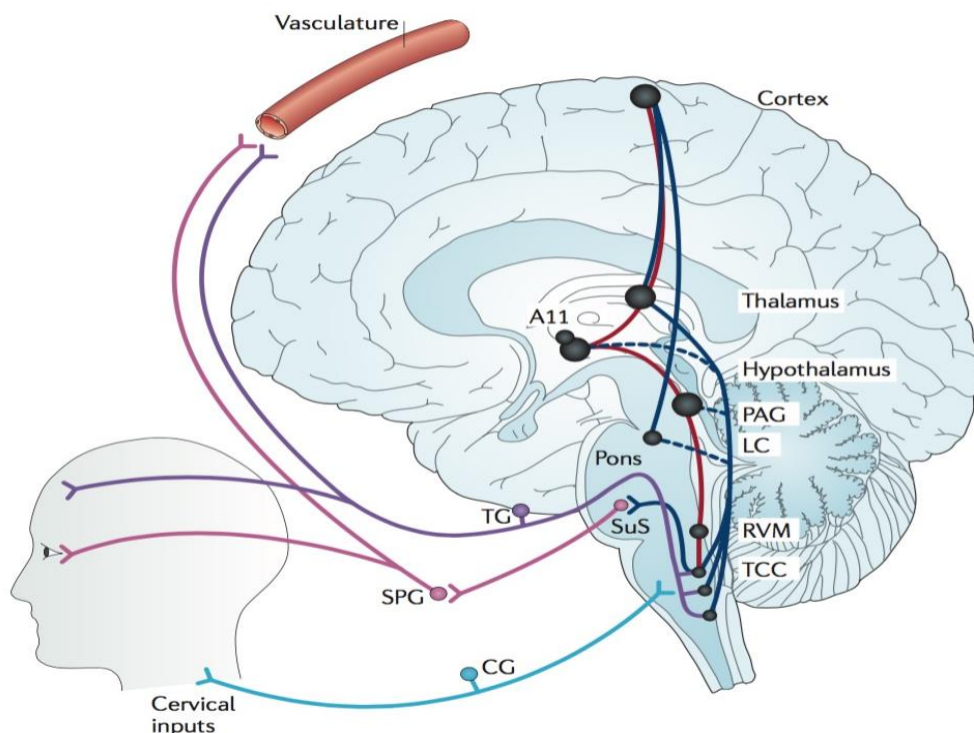


Figure 1. Neuronal Pathways involved in Migraine pathophysiology

Sensory afferents from the head and neck travel as trigeminal afferents through the trigeminal ganglion (TG) or as afferents from the greater occipital nerve through the cervical ganglion (CG), and synapse on second-order neurons in the trigeminocervical complex (TCC). Neurons in the TCC project through the quintothalamic tract, decussate in the brainstem and form synapses within

the thalamus. There is also a reflex connection from neurons of the TCC to neurons in the superior salivatory nucleus (SuS) in the pons, and from there to the cranial vasculature through the sphenopalatine ganglion (SPG; pterygopalatine), which provides parasympathetic outflow to the cranium. Activation of the trigeminal autonomic reflex contributes to the cranial autonomic symptoms of many primary headaches. Second-order neurons in the TCC also send direct projections to various structures in the brainstem, including the locus coeruleus (LC) and periaqueductal grey (PAG), and to higher structures including the hypothalamus and thalamus, which in turn send ascending signals to the cortex. There are also descending projections from the cortex to thalamic and hypothalamic structures, as well as to the LC. Descending modulation of TCC neurons takes place via hypothalamic nuclei, including the A11 dopaminergic nucleus, and there is direct descending modulation of TCC neurons from projections from the PAG that pass through the rostral ventromedial medulla (RVM). Trigeminal sensory connections to the periphery and the TCC (shown by purple fibres), the parasympathetic connection to the head and orofacial vasculature (shown by pink fibres) and the occipital nerve projection to the peripheral face and neck, and central projections to the TCC (shown by light blue fibres) are included. Ascending connections through the quintothalamic tract and to the SuS (shown by dark blue fibres) and the likely ascending projections to the LC, PAG and hypothalamic nuclei (shown by dashed lines) are shown. The descending projections to the TCC that control its activity (shown by red fibres) are also indicated. With kind permission (Akerman *et al.*, 2011) (See Page 142 for permissions to use figure).

Neuroimaging studies have pointed to the hypothalamus as the key driving force for migraine and hypothalamic activation has been noted even before the onset of headache (Maniyar *et al.*, 2014a; Schulte and May, 2016b). What causes the hypothalamic and other brain areas to be activated in migraine, is not fully understood. There are bidirectional connections between the key brainstem areas activated in migraine and diencephalic areas involved in pain processing like the hypothalamus and the thalamus (Davis and Dostrovsky, 1988; Malick and Burstein, 1998; Malick *et al.*, 2000; Burstein *et al.*, 2010; Robert *et al.*, 2013).

1.2.2 The role of neuropeptides

Various neuropeptides have been postulated to play a dominant role in the migraine pathophysiology. Calcitonin gene-related peptide (CGRP) appears to be a key molecule involved and studies have shown that CGRP levels can increase during migraine attacks (Goadsby *et al.*, 1990; Goadsby and Edvinsson, 1993b) and migraine can be induced by giving a CGRP infusion (Lassen *et al.*, 2002). Therapeutic interventions that target CGRP and its receptors look promising in the management of migraine (Olesen *et al.*, 2004; Connor *et al.*, 2011; Bigal *et al.*, 2015; Goadsby *et al.*, 2017). Other key neuropeptides speculated to play a dominant role in the pathophysiology of migraine include vasoactive intestinal peptide (VIP) (Dickson and Finlayson, 2009; Couvineau and Laburthe, 2012), nociceptin (NOP) (Bartsch *et al.*, 2002; Ertsey *et al.*, 2005) pituitary adenylate-cyclase activating polypeptide (PACAP) (Arimura, 1992; Schytz *et al.*, 2009), neuropeptide Y

(NPY)(Goadsby and Edvinsson, 1993a), substance P (SP)(Chang *et al.*, 1971; Moskowitz, 1993), somatostatin (SST)(Vecsei and Widerlov, 1988; Vecsei *et al.*, 1992; Bartsch *et al.*, 2005) and the orexins (OXs)(Cady *et al.*, 2014; Hoffmann *et al.*, 2015). It is thought that neuropeptides like CGRP, PACAP and PGI₂ may trigger a migraine cascade at a molecular level by releasing nitric oxide (Lassen *et al.*, 2002; Schytz *et al.*, 2010). Though it is known that these dominant neuropeptides play a key role in the migraine process, it remains to be elucidated as to their precise molecular cascade that maybe responsible for a migraine attack.

1.2.3 Cortical spreading depression

Cortical spreading depression (CSD) is a wave of neuronal and glial depolarization that maybe induced either physically or by chemical stimuli. This wave, once initiated, spreads across the cerebral cortex autonomously. The speed at which this wave spreads, is similar to the observed speed of the migraine aura (Lashley, 1941; Leao, 1947; Lauritzen, 1994) and hence thought to be the experimental phenomenon linked to migraine aura. The concept was first highlighted by Leao and was a serendipitous observation whilst he was working on his PhD thesis at Harvard. Leao first demonstrated the phenomenon in rabbits (Leao, 1947). Currently, cortical spreading depression is thought to cause the aura of a migraine attacks, and some think, activate trigeminal nerve afferents and also considered to alter blood-brain barrier permeability by virtue of activation of matrix metalloproteinases and its upregulation (Moskowitz *et al.*, 1993; Hadjikhani *et al.*, 2001; Gursoy-Ozdemir *et al.*, 2004).

There was a lot of scepticism regarding the concept of cortical spreading depression being a migraine trigger when it was raised first (Takano and Nedergaard, 2009). Experimental data (Gursoy-Ozdemir *et al.*, 2004) and functional neuroimaging (Hadjikhani *et al.*, 2001) have helped to allay the initial scepticism. In a study using blood oxygenation level-dependent (BOLD) MRI imaging, Hadjikhani and colleagues showed brain activation changes that suggested the presence of cortical spreading depression (CSD) in human subjects and thereby proposed that CSD generates aura in the human visual cortex. Whether there is a link between CSD and brain activations during the premonitory phase, remains to be studied further.

1.2.4 Direct clinical evidence

The first direct clinical evidence that showed involvement of brainstem structures in migraine generation was reported by Raskin and colleagues (1987). Patients ($n=175$) underwent implantation of an electrical pain stimulation system, commonly for intractable lower back ache. The electrodes were implanted in the somatosensory area of the thalamus or the periaqueductal gray region (PAG). Out of these 175 patients, 15 who did not typically suffer from headaches before, developed headache with what the authors describe as 'florid migrainous' features. Of these subjects, 13 had electrodes inserted into the periaqueductal gray region and the headaches persisted following explantation of the electrodes (Raskin *et al.*, 1987). Subsequently, activation of the ventrolateral periaqueductal gray (vlPAG) was shown by PET imaging during a spontaneous migraine attack (Weiller *et al.*, 1995). Activation of the periaqueductal gray has also been shown using PET imaging in NTG triggered migraine attacks (Maniyar *et al.*, 2014a).

Another key brain structure involved in the migraine pathophysiology is the hypothalamus as shown by various functional imaging studies (Denuelle *et al.*, 2007; Maniyar *et al.*, 2014a). Several symptoms seen in the premonitory phase of migraine like sleepiness (Goder *et al.*, 2001) reduced alertness (Dalkvist *et al.*, 1984), mood changes, food craving, thirst (Blau, 1980; Giffin *et al.*, 2003) could be attributed to hypothalamic activation (Rao and Pearce, 1971). Due to the broadly similar nature of symptoms seen in the postdrome, it would be vital to find out if the symptoms are associated with persistent hypothalamic activation. Other key areas found to be activated in the premonitory phase include the midbrain tegmental area, dorsal pons and cortical areas like occipital, temporal and prefrontal cortex (Antonaci *et al.*, 1997).

1.2.5 The migraine postdrome: A definition

For the purpose of the study, the migraine postdrome was defined as the time between headache resolution and feeling completely back to normal. Headache resolution was defined as cessation of troublesome throbbing headache (Giffin *et al.*, 2016). While individual attack profiles vary amongst individuals, the average postdromal duration has been seen to vary from 18 – 25 hours. This prolonging of abnormal behavioural states thus significantly prolongs the burden of migraine and represents a key disabling attack feature (Blau, 1991; Ng-Mak *et al.*, 2011). A factor that has profound relevance to the lack of understanding and research into the postdrome phase, is the absence of a definition in the International Classification of Headache disorders. (2013). Hence it is crucial to have a classification to drive further research into this important but neglected phase of migraine.

1.2.6 Key structures that may be involved in the postdrome

The dysfunction of neuromodulatory structures in the brainstem is thought to be a core component in the pathophysiology of migraine (Akerman *et al.*, 2011). As the major noradrenergic nucleus, the locus coeruleus has a vital role in the regulation of cortical function and is known to modulate responses to afferent traffic (Aston-Jones and Cohen, 2005). This area has been shown to be activated during acute migraine attacks in positron emission tomography (PET) studies (Afridi *et al.*, 2005; Weiller *et al.*, 1995) and could play a dominant role in the postdrome.

There is evidence to support the view that the brain in migraineurs is hyperexcitable to a variety of stimuli. This suggests that neuronal depolarisation, which is the presumed initiating event in migraine aura and possibly in migraine without aura, is more easily triggered (Mayevsky *et al.*, 1996; Strong *et al.*, 2002; Fabricius *et al.*, 2006). Due to the hyperexcitability, a lower level of transcranial magnetic stimulation of the occipital cortex is required to produce visual phosphenes in migraineurs compared with non-migraineurs (Olesen *et al.*, 1981; Ebersberger *et al.*, 2001; Hadjikhani *et al.*, 2001). Genetic mutations can increase neuronal excitability through a variety of mechanisms (Ophoff *et al.*, 1996; Goadsby, 2007; Goadsby *et al.*, 2009). The cortical excitability may indicate chronicity process in the disease (Stankewitz and May, 2009).

Previous studies have shown activations of posterolateral hypothalamus, midbrain tegmental area, periaqueductal grey, dorsal pons and various cortical areas like frontal, temporal and occipital

regions in the premonitory phase (Maniyar *et al.*, 2014a). The symptoms that patients complain of are broadly similar in the premonitory and postdromal phase (Giffin *et al.*, 2003; Giffin *et al.*, 2016). Based on the similarity of symptoms, one can hypothesize that there may be a shared neural network that is active in the postdrome and premonitory phases. It will further our understanding of the migraine pathophysiology when we know which brain areas are activated for the onset and development of the postdromal symptoms and if indeed they are similar to the networks known to be activated in the premonitory phase.

Another potential neuroanatomical explanation for postdrome symptoms is diffuse cortical and subcortical involvement given the multitude of symptoms patients describe in the postdrome (Kelman, 2006; Quintela *et al.*, 2006; Pascual, 2011; Bose and Goadsby, 2016; Giffin *et al.*, 2016). Blau suggested that the involvement of the whole brain, especially the frontal lobes and the hypothalamus as vital structures in the pathophysiology of the postdrome after evaluating postdrome symptoms within his cohort of subjects (Blau, 1991).

Hence due to the multifaceted presentation of postdrome symptoms, any number of neural networks could potentially be involved and it is vital to study and identify the key regions that are involved in this phase.

1.3 NTG triggered human migraine model

There are several practical difficulties in studying spontaneous migraine mainly due to various logistical challenges. The unpredictability of the attacks means that the subject may not be in the vicinity of the research centre whilst having a migraine attack. Secondly, if the subject does get an attack, there may be difficulties in travelling to the research centre whilst in pain or whilst having some of the other associated symptoms like photophobia or nausea. Finally, the window of opportunity to capture the brain activations using functional imaging maybe lost in transit.

NTG was first synthesized by the Italian chemist Ascanio Sobrero in 1847, whilst he was working under Théophile-Jules Pelouze, a renowned chemist of the time, at the University of Turin. At the time of discovery, Sobrero observed the ability of NTG to induce a violent headache (Tfelt-Hansen and Tfelt-Hansen, 2009; Schytz *et al.*, 2010). Alfred Nobel, who was a colleague of Sobrero, realized the explosive potential of NTG and utilized it in the invention of dynamite. Dynamite was a commercial success and dynamite factories sprung up all over the world. Workers in these factories were frequently noted to have a headache which was then thought to be NTG induced (Tfelt-Hansen and Tfelt-Hansen, 2009).

Based on these observations, various groups explored migraine provocation models using NTG. Models using oral (Schnitker and Schnitker, 1947), percutaneous (Dalsgaard-Nielsen, 1949) and sublingual (Peters, 1953) NTG preparations were tried with varying successes. The wide range of bioavailability of these models had the effect of large inter subject variability of symptoms (Tfelt-Hansen and Tfelt-Hansen, 2009). In order to limit this wide variability, the concept of intravenous infusion of NTG (Iversen *et al.*, 1989) was tested and has since become the most widely used human migraine provocation model (Thomsen *et al.*, 1994; Christiansen *et al.*, 1999; Afridi *et al.*, 2004b; Sances *et al.*, 2004; Afridi *et al.*, 2005a).

The exact mechanisms through which NTG induces a migraine is not known. The widely accepted understanding is that NTG gets converted to nitric oxide in the body. This in turn activates intracellular soluble guanylate cyclase and catalyses the formation of cyclic guanosine monophosphate (cGMP). The upregulation of intracellular cGMP may trigger off a cascade of changes centrally and peripherally, leading to migraine though there is no firm evidence at the present moment (Olesen *et al.*; Schoonman *et al.*, 2008; Olesen *et al.*, 2009; Schytz *et al.*, 2010).

NTG triggering induces a biphasic response in migraine patients and in healthy subjects with a family history of migraine. There is an initial NTG induced non-specific featureless headache which is dissimilar to the subject's usual migraine and is short lasting. Later on, subjects develop a delayed headache that is similar to their usual migraine attacks. Studies have shown that a migraine attack maybe be triggered in 75% of migraineurs using NTG (Afridi *et al.*, 2004b). Clinically, spontaneous and triggered migraine attacks are indistinguishable (Afridi *et al.*, 2004b) and functional imaging studies in spontaneous migraine (Afridi *et al.*, 2004a; Schytz *et al.*, 2010; Schulte and May, 2016b) and NTG induced migraine attacks show similar areas of neural activation (Afridi *et al.*, 2005c; Maniyar *et al.*, 2014a).

NTG triggering is therefore an appropriate human migraine provocation model as waiting for subjects' spontaneous attacks would be impractical. This model has been successfully used in the past to study brain activations during premonitory phase and migraine, using PET imaging (Afridi *et al.*, 2004b; Maniyar *et al.*, 2014a; Maniyar *et al.*, 2014b). The findings of such studies have shown that triggering with NTG produces headache and premonitory symptoms which are phenotypically similar to subjects' spontaneous attacks in a reproducible manner and it is uncommon for aura to be triggered using this method (Afridi *et al.*, 2004b).

Functional imaging of NTG triggered migraine attacks, gives us the unique opportunity to study cerebral blood flow alterations in the brain to assess if similar brain structures are being activated during these different phases, giving rise to the similar nature of symptoms.

1.4 Migraine functional imaging

Understanding the key brain areas involved in migraine should help unravel the pathophysiology further and potentially identifying newer therapeutic targets. Previous functional imaging studies have advanced our knowledge regarding the pathophysiology of migraine (Afridi *et al.*, 2005c; Maniyar *et al.*, 2014a; Schulte and May, 2016b) and functional neuroimaging can also be used to assess migraine experimental correlates. For example, in the 1940s, Leao first described the bioelectrical phenomenon of cortical spreading depression (CSD) (Leao, 1947; Hadjikhani *et al.*, 2001). Using blood oxygenation level-dependent (BOLD) MRI signal techniques, Hadjikhani *et al.*, provided data to support the hypothesis that an electrophysiological event such as CSD generates the aura in human visual cortex which was challenging to test before the advent of functional neuroimaging (Hadjikhani *et al.*, 2001) (See Page 20).

Functional neuroimaging techniques are indirect measures of neuronal activations. They work broadly on the principle that increased neuronal activity would require nutrients and the accompanying 'functional hyperaemia' would service this purpose. The concept of functional hyperaemia was first advanced by Roy and Sherrington (Roy and Sherrington, 1890) who demonstrated using animal models, that to meet the nutritional demands of the brain tissue secondary to brain activity, there is an increase in cerebral blood flow. This phenomenon is broadly known as 'neuro-vascular coupling' (Attwell *et al.*, 2010). Commonly used functional imaging techniques include Positron Emission Tomography (PET) and fMRI (functional magnetic resonance imaging). Currently no reliable biomarker exists to predict or diagnose migraine attacks. Development of functional imaging techniques may one day serve as a diagnostic and predictive tool. It may also serve to assess treatment responses to therapeutic agents, by observing their effects on the brain activations or alterations in regional cerebral blood flow.

Choosing the right functional imaging modality for a study depends on certain factors that need to be considered and also how the chosen imaging modality will address these factors. For example, PET imaging and whole brain fMRI modalities are associated with poor spatial resolution when it comes to assessing the brainstem (Schulte and May, 2016a). The cerebral blood flow images extracted from PET and ASL are similar in qualitative aspects but ASL is superior in spatial resolution. With improvements in ASL techniques, even better temporal and spatial resolutions can be expected in the future (Zhang *et al.*, 2014).

Not all centres are equipped with the technology or the technical staff with expertise to carry out functional imaging during spontaneous migraine attacks. Capturing spontaneous migraine attacks using functional imaging requires multi-disciplinary resources and the unpredictability of migraine attacks adds to the complexity. Using a provoked migraine model mitigates some of these logistical issues (Iversen *et al.*, 1989). One could argue whether the spontaneous and chemically triggered migraines attacks are one and the same. The argument can be considered at three levels, namely, clinical, radiological and molecular. Clinically, triggered patients would develop the symptoms required to make a diagnosis of migraine using the International Classification of Headache Disorders (ICHD)-3beta criteria (Headache Classification Committee of the International Headache, 2013). Hence, it would not be possible to tell the difference between a spontaneous migraine and a triggered migraine clinically unless one is given the triggering history. However, it must be noted that in most provocation studies, for ethical reasons, the migraine headache is treated and not allowed to sustain at peak intensity for long periods of time, with abortive treatment so the total duration of the attacks is truncated (Afridi *et al.*, 2004b). At a radiological, level, once again, the areas activated in spontaneous and triggered migraine appear to be broadly similar (Weiller *et al.*, 1995; Afridi *et al.*, 2005b; Afridi *et al.*, 2005c; Maniyar *et al.*, 2014a). Now what may be most useful in the future to differentiate or connect together, triggered and spontaneous migraine would be to understand what happens at a molecular level.

Human models (Thomsen and Olesen, 2001) have shown evidence of the involvement of nitric oxide (NO) acting via cyclic guanosine monophosphate (cGMP) pathways in generating migraine (Stepien and Chalimoniuk, 1998). In the NTG human migraine provocation model, it is thought that NTG gets converted to nitric oxide in the body and this sets off a cascade of changes responsible for the generation of migraine (Olesen *et al.*, 2009). Other provocation models using calcitonin gene-related peptide (CGRP) (Lassen *et al.*, 2002), pituitary adenylate cyclase activating polypeptide-38 (PACAP38) (Schytz *et al.*, 2009), sildenafil (Kruuse *et al.*, 2003), histamine (Lassen *et al.*, 1995), dipyridamole (Kruuse *et al.*, 2006), vasoactive intestinal peptide (VIP) (Rahmann *et al.*, 2008), prostaglandin I₂ (PGI₂) (Wienecke *et al.*, 2009) have all been used in various studies, provoking attacks varying from 0-83% of the subjects enrolled in the studies (Schytz *et al.*, 2010). No study has assessed the migraine postdrome in a human migraine provocation model using NTG as the triggering agent and ASL MRI as the imaging modality to study the alterations in regional cerebral blood flow.

1.5 Arterial spin-labeled (ASL) perfusion MRI

Tissues in the body have varying nutritional and oxygen demands. The demands are met by the delivery of the oxygen and nutritional needs by blood and this process is called perfusion. An investigation of perfusion due to various physiological states or pathological states can be utilized to understand the processes involved, using perfusion based imaging techniques. The most widely used perfusion based imaging technique until recently has been PET (Positron Emission Tomography) scanning. However, there are several drawbacks in using PET scanning. It is an expensive technique and many hospitals do not have the financial resources to acquire and maintain the infrastructure needed for PET scans. The amount of radiation exposure is substantial with an estimated subject dose of 25 mSv (millisievert) per whole body ^{18}F -FDG PET/CT examination, independent of clinical protocol (Brix *et al.*, 2005). There is also the associated cancer risk with repeat PET imaging that limits its widespread use and repeatability (Wen *et al.*, 2013). Arterial Spin-labeled (ASL) perfusion MRI is a fMRI technique where the subjects arterial blood is used as an endogenous marker for perfusion imaging by magnetically labeling blood water. It is analogous to PET cerebral blood flow measurements which use radio decay rate of ^{15}O and the subsequent detection of gamma rays to deduce brain activations. However, in ASL MRI, the modified decay rate of longitudinal magnetisation from T1 relaxation, induced by the magnetically labelled arterial water is used to determine regional CBF (Detre *et al.*, 2012). As it does not involve the use of radioactive tracers, radiation related adverse effects and limitations do not apply here.

Drawbacks of PET Imaging
Expensive Substantial radiation risk Extensive Infrastructure requirements Associated cancer risk Limitations in repeatability Not suitable for quantitative assessments Use of contrast agents that may require hepatic or renal metabolism

Table 1. Drawbacks of PET Imaging

1.5.1 Core Concepts of ASL MRI

ASL utilizes the principle that the longitudinal magnetization of arterial blood water can be manipulated, by which, it will differ from the static tissue magnetization. Using the difference in magnetization between labelled and non-labelled images, a subtractive comparison can be made. The difference between the image intensity in each voxel is converted to CBF in physiological units in ml/g/min (Detre *et al.*, 2009) using a suitable mathematical model. The technique has been validated in humans using H₂¹⁵O PET (Ye *et al.*, 2000).

1.5.2 ASL Methods

There are several labeling approaches that can be used for ASL (Alsop *et al.*, 2015; Haller *et al.*, 2016), most commonly:

Pulsed ASL

Here, a short adiabatic inversion pulse is used to label the arterial blood and the labeling pulses are usually located inferior to the brain. The labeling pulses are usually in the region of 10 msec and they are meant to invert the blood water instantaneously. Once labeling is done, a sufficient post labeling delay period is necessary. This period is also called the inflow time. This is the period where the instantaneously inverted blood water moves into the brain and loses its 'magnetic' label gradually through longitudinal T1 relaxation. Due to this property, the signal to noise ratio (SNR) is intrinsically lower than that of pseudocontinuous ASL (PCASL).

Pseudocontinuous ASL (PCASL)

Unlike the comparatively short pulse duration of approximately 10 msec in pulsed ASL, in PCASL, the labelling period is made up of multiple very short trains of pulses, each lasting approximately 1 msec, for a total duration of 1.5-4 seconds. With this train of short pulses, the labelled blood is inverted in more or less a steady state manner (Haller *et al.*, 2016). If phase shifts of 180° are made on each second pulse, we are in the position to acquire non-labelled control images. The difference between labelled and control images can be converted to CBF in physiological units. The advantage of PCASL over PASL is that it offers better signal to noise ratio (SNR). This new ASL method was introduced in 2008 (Dai *et al.*, 2008).

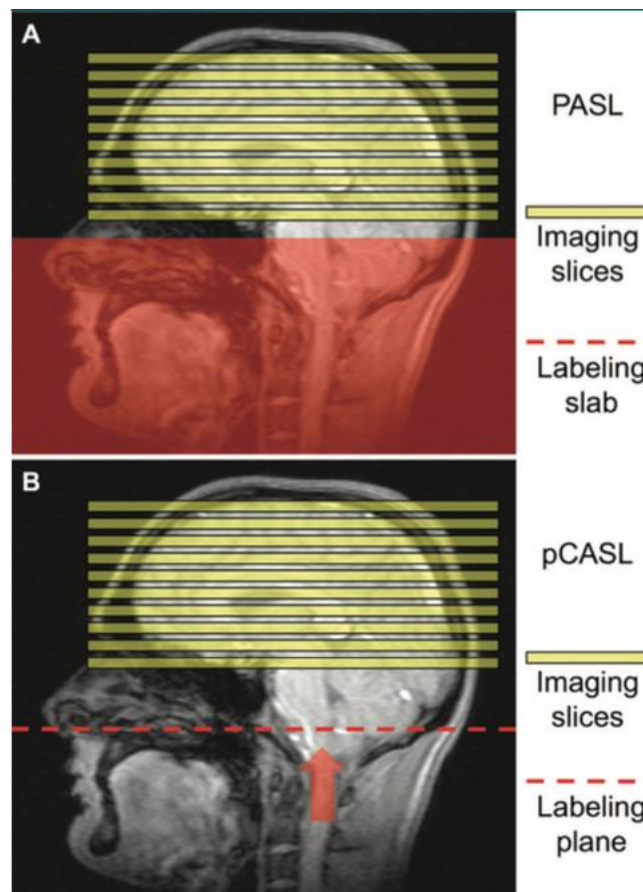


Figure 2. ASL labeling schemes A. PASL- Inversion slab places proximal to imaging volume to label blood in the arterial feeding vessels supplying the brain. The pulse is short (approximately 10 msec) and the blood is inverted simultaneously. **B. In PCASL,** the inflowing arterial blood is continuously inverted as it flows through the labelling plane by means of a process known as flow induced adiabatic inversion. The PCASL labelling pulse train is typically applied for a period of 1-2 seconds. For permissions to use figure, see page 142.

1.5.3 Using PCASL over other ASL techniques in migraine research

PCASL has higher SNR when compared to PASL and hence it is better suited for migraine studies to analyse regions of the brain undergoing alterations in cerebral blood flow during various phases of a migraine attack.

1.5.4 Post-label delay time

An important aspect to grasp when understanding ASL is the concept of post-label delay time. There should be a sufficient post labelling time following the labelling pulse to allow for the labelled blood to enter the brain tissue. The post-label delay time is critically important, because, if the post delay

time is set longer than the longest period of transit from the tagging plane and the volume images, then the ASL signal becomes impervious to the alterations in the arterial blood water arrival time. The recommended post-label delay time for an adult population is 2000 ms (Alsop *et al.*, 2015).

CHAPTER 2

OUTLINES OF STUDIES AND AIMS

2 OUTLINES OF STUDIES AND AIMS

2.1 The migraine postdrome clinical audit

2.1.1 Introduction

Studies have shown that 68-81% of migraineurs have postdrome symptoms (Kelman, 2006; Giffin *et al.*, 2016). Previous studies into the postdrome had several methodological flaws. For example, they were limited by retrospective data collection, absence of diaries, scarcity of patients and heterogeneous classification (Quintela *et al.*, 2006). We performed a clinical audit to gauge the impact of the migraine postdrome within our clinical cohort. It is worth noting that a prospective diary study into the postdrome (Giffin *et al.*, 2016) addressing all the methodological flaws and aided by an electronic diary study, was published only after this clinical audit was underway. Permission for the audit was granted by the audit department at King's College Hospital, London. All data were retrieved from clinic records dated from August 2014 to August 2016 following approval by the audit department. Clinic records of 100 patients under the care of Professor Peter J. Goadsby at King's College Hospital, London, were examined to obtain data against an audit collection tool. Migraine diagnosis was made using ICHD-3 (beta) criteria (Headache Classification Committee of the International Headache, 2013).

For the purpose of this audit, the migraine postdrome was defined as the time between headache resolution and feeling completely back to normal. Headache resolution was defined as cessation of troublesome throbbing headache (Giffin *et al.*, 2016)

Category	Symptoms
Neuropsychiatric symptoms	Mood changes, concentration trouble, sleep disturbance (insomnia and hypersomnolence)
Sensory symptoms	Head soreness, photophobia, phonophobia, speech disturbance
Gastrointestinal symptoms	Nausea, flatulence, constipation, vomiting, anorexia, food craving, abdominal pain, diarrhea
General systemic symptoms	Tiredness, urination, fluid retention

Figure 3. Broad Classification of Postdrome symptoms (Bose and Goadsby, 2016)

2.1.2 Background behind the audit

Migraine is the 6th most disabling disorder worldwide (2017) and affecting an estimated 15% of the UK adult population (Steiner *et al.*, 2003). In 2003, it was estimated that in the United Kingdom, approximately 25 million work days were lost due to migraine each year (Steiner *et al.*, 2003). This would impact the patients' health, social and financial commitments and have a negative impact on the economy due to loss of productivity.

While its pathophysiology is beginning to be unravelled, the least studied component of the

different migraine phases, remains the postdrome (Bose and Goadsby, 2016). The four main phases of a migraine attack: premonitory, aura, headache, and postdrome, are well described in textbooks (Lance *et al.*, 2005) whereas the postdrome is not yet defined in the International Classification of Headache Disorders (Headache Classification Committee of the International Headache, 2013). Although the symptoms here may have classically been thought of as less dramatic than the pain of the headache phase, the symptoms are no less disabling. In a study involving 34 patients, postdrome symptoms were reported to impact the ability to work, to affect family interactions and social life, and to cause cognitive impairment (Ng-Mak *et al.*, 2011). A larger study involving 827 patients reported that 68% of patients experienced postdrome symptoms, with an average duration of 25.2 hours (Kelman, 2006). In a prospective electronic diary study, among 120 evaluable patients, 97 (81%) reported at least one non-headache symptom in the postdrome thereby contributing to the distress and disability of the subjects (Giffin *et al.*, 2016). Blau described 255 symptoms in the postdrome for a cohort of 40 patients with an average duration of 18 hours (Blau, 1991). The most common symptoms reported in this study included tiredness, head soreness, cognitive difficulties, hangover, mood changes, and dizziness. However, it was difficult to distinguish between certain symptoms for example, a subdued mood, depressed mood, bad mood, and introverted mood. To rectify this, Quintela and colleagues (Quintela *et al.*, 2006) assigned postdrome symptoms into four groups: neuropsychiatric, sensory, gastrointestinal (digestive as per the authors), and general symptoms which is a very useful way to classify postdrome symptoms.

Hence, the postdrome phase of a migraine attack can prolong disability for patients. It is essential to fill this knowledge gap regarding the migraine postdrome and get this phase defined and included in the International Classification of Headache Disorders (Headache Classification Committee of the International Headache, 2013). Though research studies have looked into the extent of postdrome previously (Quintela *et al.*, 2006; Giffin *et al.*, 2016), the purpose of carrying out a clinical audit was to evaluate the extent of the problem within our clinical patients and to see if the problem was as common as reported by other studies.

2.1.3 Migraine postdrome clinical audit aims

The aims of the audit were to explore using a clinical audit: The migraine postdrome as it presents and to categorize the disabling symptoms patients report.

Following the identification of the group of patients who experienced postdrome, the next step was to ask the question: what variables affected the presence of the postdrome phase.

The variables considered were: attack duration (in hours), migraine type, medication overuse*, age (in years), gender, co-morbidities, lateralisation of pain, CAS (Cranial Autonomic Symptoms), photophobia, phonophobia, osmophobia, motion sensitivity.

*The definition for medication overuse was dependent upon the pharmacological class of medication. The definitions used for this project were based on the guidelines of the International Headache Society (Headache Classification Committee of the International Headache, 2013). The following was taken as medication overuse: Triptans, opioids: 10 days per month for a period of 3 months and over the counter (OTC) medications like paracetamol and NSAIDs: 15 days per month of sustained use.

2.2 THE MIGRAINE POSTDROME ARTERIAL SPIN LABELED (ASL) MRI IMAGING STUDY (POSTD)

The study was carried out in accordance with the World Medical Association Declaration of Helsinki, 1964 (Rickham, 1964), the Research Governance Framework for Health and Social Care (2nd edition, 2005), the Data Protection Act (1998) and the principles of Good Clinical Practice (GCP). Study approval was granted by the Health Research Authority – Camden & King’s Cross Research Ethics Committee (REC) Ethics (Ref: 14/LO/2241 UKCRN ID: 18439) and the King’s College Hospital Research Office (Hospital study code - KCH15-027). The principal aim of this study was to investigate alterations in cerebral blood flow during the postdromal stage of a migraine attack using a NTG triggered human migraine model.

2.2.1 Primary hypothesis:

The primary hypothesis behind this study was that the brain areas identified as having increased cerebral blood flow (CBF) in the premonitory phase of migraine, such as the hypothalamic region, and the attack phase, such as the dorsolateral pons based on previous studies (Maniyar *et al.*, 2014a), continue to be active in the postdrome phase. We suspected this based on the similar nature of symptoms reported in the premonitory phase and the postdrome (Giffin *et al.*, 2003; Maniyar *et al.*, 2014a; Giffin *et al.*, 2016).

2.2.2 Aims of POSTD Imaging study

2.2.2.1 Primary aim

To study the alterations in cerebral blood flow during the postdrome phase of a migraine attack using Arterial spin labelled (ASL) MRI, relative to the baseline and premonitory phases.

2.2.2.2 Secondary aims

- a. To study changes in attention, fatigue and sleepiness during the postdrome phase compared to baseline.
- b. Clinical phenotyping of the subjects’ symptoms and clinical features during different phases of the migraine attack in response to NTG triggering.

CHAPTER 3

PREVIOUS ASL STUDIES IN MIGRAINE

3 PREVIOUS ASL STUDIES IN MIGRAINE

ASL MRI techniques have been used extensively to quantify several pain states and has been validated as a reliable and reproducible technique (Howard *et al.*, 2011; Howard *et al.*, 2012; Hodgkinson *et al.*, 2015a). The use of ASL in migraine studies is relatively new. Kato and colleagues used ASL MRI to study a single patient during 3 stages: One hour into a migraine attack, 30 minutes following treatment with 10mg of rizatriptan and finally, once the subject was attack free. The normalized images attained during the migraine headache stage, showed significant relative hypoperfusion on the bilateral medial thalamic regions that included the hypothalamus, and there was significant relative hyperperfusion in the frontal cortex when these images were compared to the pain free period. When images were acquired within 30 minutes of treatment with rizatriptan, they showed a recovered significant relative perfusion in the hypothalamus when the images were compared to the migraine headache scans (Kato *et al.*, 2010).

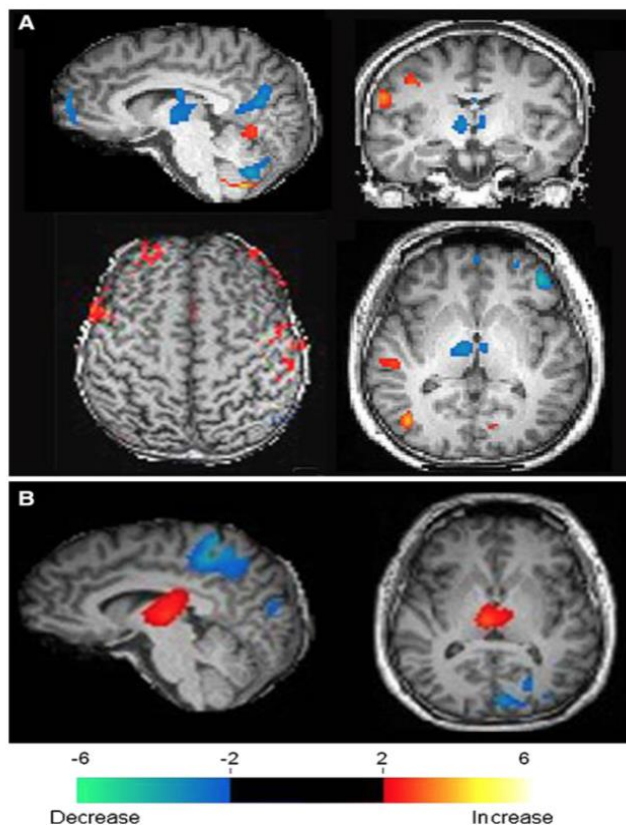


Figure 4. Normalized ASL images during migraine attack show significant relative hypoperfusion in bilateral thalamic areas including hypothalamus, posterior cingulate, and cerebellum, and significant relative hyperperfusion in the frontal convexity as compared to the migraine-free state. b Normalized ASL images acquired 30 min after rizatriptan administration demonstrated recovered significant relative perfusion in the hypothalamus and its surrounding areas as compared to during migraine attack. a, b Two-tail view. Colour bar is Z-score (SD) (For Permission to use figure, please see page 142).

Pollock and colleagues published a case series involving 11 subjects, showing regional cortical hyperperfusion during migraine attacks. They concluded that ASL perfusion studies have significant potential in evaluating symptomatic migraines in the future (Pollock *et al.*, 2008).

ASL techniques have also been used to show an altered pattern of regional cerebral blood flow involving the primary somatosensory cortex in the interictal phase of migraineurs when compared to age and sex matched healthy controls (Hodkinson *et al.*, 2015b). Whether these changes suggest adaptation or maladaptation to multiple migraine attacks, remain unclear.

These studies show that ASL is a versatile tool to investigate perfusion changes in migraine with an unprecedented level of spatial detail. They also offer potential to assess therapeutic response in migraine (Hodkinson *et al.*, 2015a).

CHAPTER 4

METHODS

4 METHODS

4.1 Methods - Migraine postdrome clinical audit

The clinic records of 100 random patients under the care of a UK headache tertiary care centre, King's College Hospital, London were examined to obtain data against an audit collection tool. Permission for clinical audit was granted by the hospital clinical audit department. All the records obtained were for patients under the care of Professor Peter Goadsby. Migraine diagnosis was made using ICHD-3(beta) criteria (Headache Classification Committee of the International Headache, 2013). Predetermined data based on previous studies (Blau, 1991; Kelman, 2006) was extracted and analysed. This included symptoms in the postdrome, such as neuropsychiatric, gastrointestinal, sensory and general systemic (Quintela *et al.*, 2006; Kelman, 2007; Bose and Goadsby, 2016). The presence of co-morbidities including depression, anxiety, hypertension and fibromyalgia, and overuse with medications such as simple analgesics, triptans, opioids, and cannabinoids were extracted (See Table 2, Figure 5,6 and 7 for audit data extraction tools used). The data regarding the basic demographics of the cohort was extracted using Microsoft Excel (Microsoft Office, 2016). Statistical analysis of the whole data set (both postdrome and non-postdrome group) was assessed together using Statistical Package for the Social Sciences (IBM SPSS statistical package, version 23 for Mac).

AUDIT EXTRACTION TOOL
Age (years)
Gender
Headache duration (hours) (continuous also set)
Migraine Type
Lateralization of Pain
Site of Attack
Severity of Head Pain (out of 10)
Symptoms during Attack
Postdrome presence
Neuropsychiatric symptoms
Sensory symptoms
Allodynia
Gastrointestinal symptoms
General Systemic Symptoms
Cranial Autonomic Symptoms (CAS)
Co-morbidities
Medication overuse

Table 2. Extraction tool used to extract basic patient demographics and symptoms during migraine attack

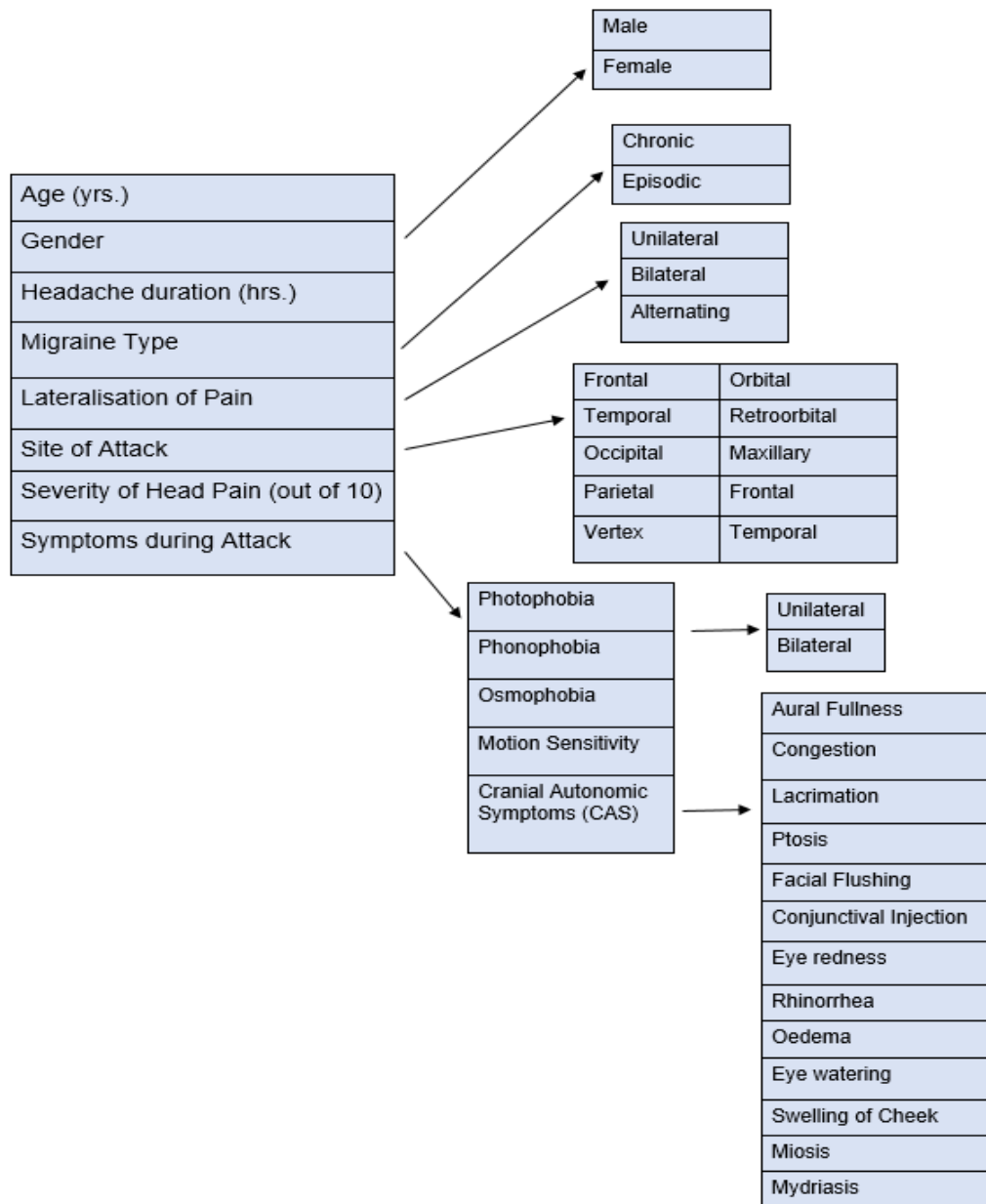


Figure 5. Tool used to extract basic patient demographics and symptoms during migraine attack

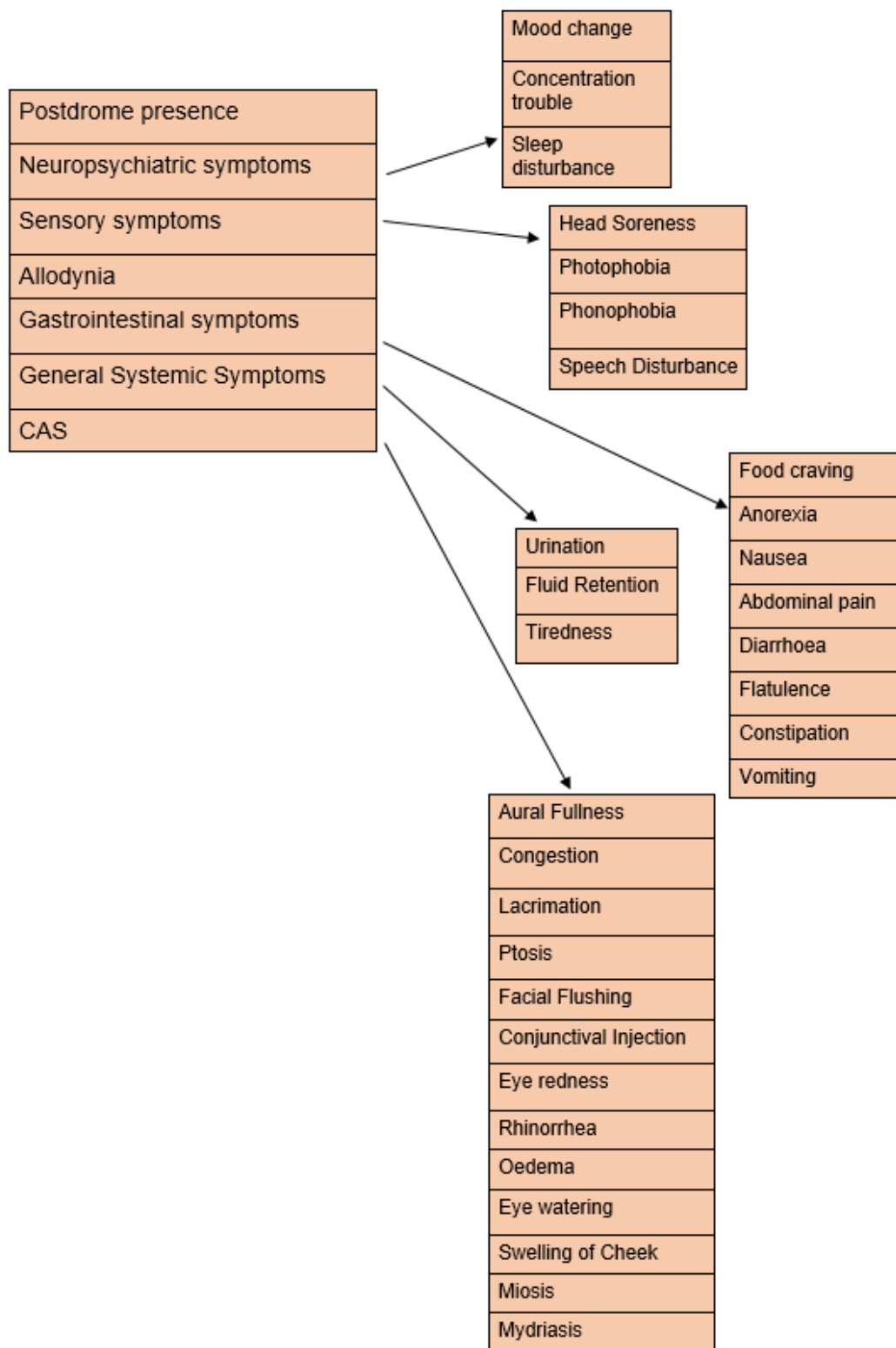


Figure 6. Extraction tool used to identify postdrome presence and the symptoms experienced during this phase

Other Considerations

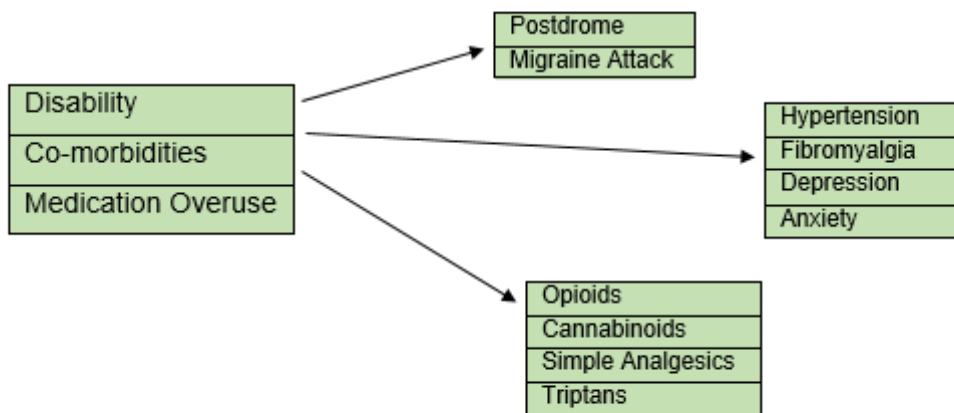


Figure 7. Other variables accounted for during information extraction

4.1.1 Statistical analysis

Data was then exported (IBM SPSS Statistical software package, Version 23 for Mac) for analysis using the most appropriate statistical tests for the extracted data.

The statistical analysis was performed to assess the variables and their association with the presence of postdrome). The tests used were:

Independent samples *t*-test (for continuous variables) or Chi Square test (for categorical variables).

The Fisher's Exact Test was reported instead of the Chi-square Tests when one or more cells had expected cell counts of less than five.

The Kolmogorov-Smirnov test was also utilized and A two-sided significance level of 5% was applied to all hypothesis tests. The Kolmogorov-Smirnov test (KS-test) tries to determine if two datasets differ significantly and has the advantage of making no assumption about the distribution of data.

4.2 Methods- POSTD Imaging study

4.2.1 Study population

The target population for the study was 60 subjects aged between 18 and 50 years of age with a diagnosis of migraine with or without aura.

4.2.2 Recruitment sites

Relevant permission was obtained to recruit subjects through King's College Hospital, Denmark Hill, Guy's Hospital, Royal London Hospital, Basildon Hospital, St George's Hospital, Croydon University Hospital, Frimley Park Hospital, Kingston Hospital and St Peter's Hospital. Subjects were also recruited through The Migraine Trust and Migraine Action websites. An advertisement was also placed in a local newspaper (Metro). King's College London sends out a research bulletin to staff and students on a fortnightly basis, which also served as a recruitment route. Subjects received a patient information sheet (PIS) and gave permission to be contacted by me, after having adequate time to consider participation. Once they agreed to participate, they were given a headache diary. Subjects who contacted after reading the information leaflet, underwent a telephone screening to review if they met the inclusion criteria (see Page 147). Those who were eligible, were invited for a NTG triggering visit. Once they attended the first visit, informed consent was taken and the subject entered the study, after ensuring that all relevant questions were answered and issues addressed.

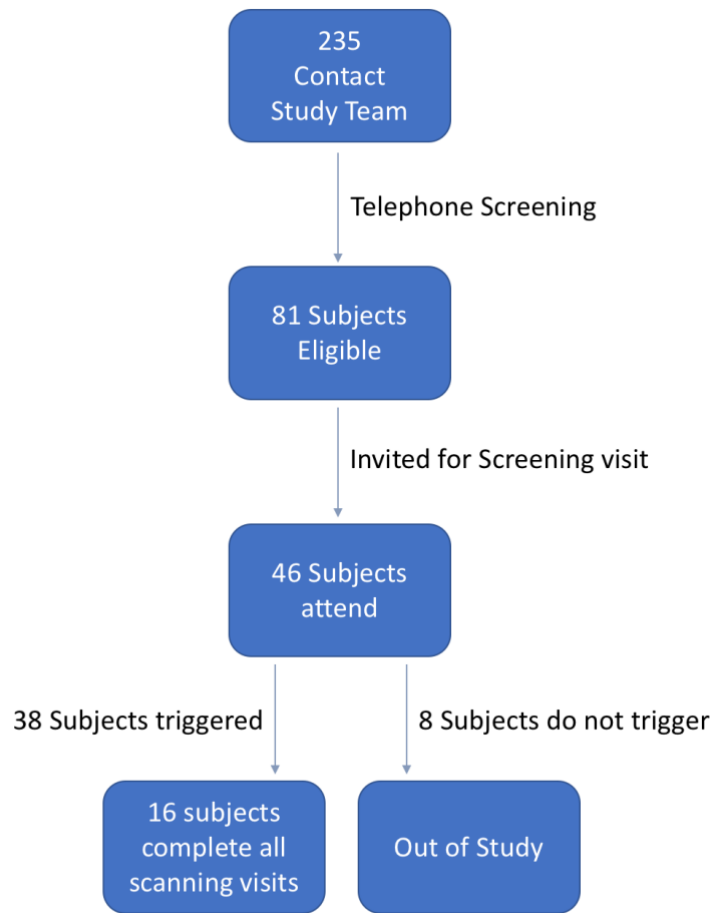


Figure 8. Recruitment flowchart. In total, 235 subjects contacted the study team. Following telephone screening, 81 subjects were found to be eligible and were invited to attend a screening visit. 46 subjects attended the screening visit. 16 subjects completed all study visits.

4.2.3 Inclusion and exclusion criteria for the study

Subjects needed to meet all the inclusion criteria to be eligible for enrolment into the study. Key inclusion criteria were: subjects with a history of migraine with or without aura and between the age group 18-50 years. For a detailed list of inclusion and exclusion criteria, please see Appendix B (Page 147).

4.2.4 Lifestyle guidelines whilst enrolled in the study

The presence of lifestyle factors that could potentially affect cerebral blood flow or functional network connectivity were assessed. Subjects were asked to abstain from caffeine-containing products for 12 hours prior to each visit as this could potentially affect cerebral blood flow (Field *et al.*, 2003). Similarly, subjects were asked to abstain from taking NSAIDs (non-steroidal anti-inflammatory drugs) or paracetamol for 12 hours prior to each visit. They were also asked not to smoke more than five cigarettes per day or consume more than six cups of caffeinated drinks per day for the duration of the study. They were asked to abstain from the use of tobacco or nicotine containing products for four hours prior to admission until discharge for each visit and were asked to abstain from taking alcohol for 24 hours prior to each visit. Alcohol and nicotine are known to alter the resting state Functional Network Connectivity in the brain (Kelman, 2004; Vergara *et al.*, 2016, 2017). Subjects were asked to maintain a normal sleep routine for the duration of the study.

4.2.5 Study endpoint

Alterations in rCBF using pCASL with 3D fast spin echo (FSE) readout across baseline, premonitory, migraine headache and postdrome phase.

4.2.6 Study design

The study design involved three visits to the Clinical Research Facility, King's College Hospital, London, of which the first visit was a screening visit.

Visit 1 (screening/no scanning)

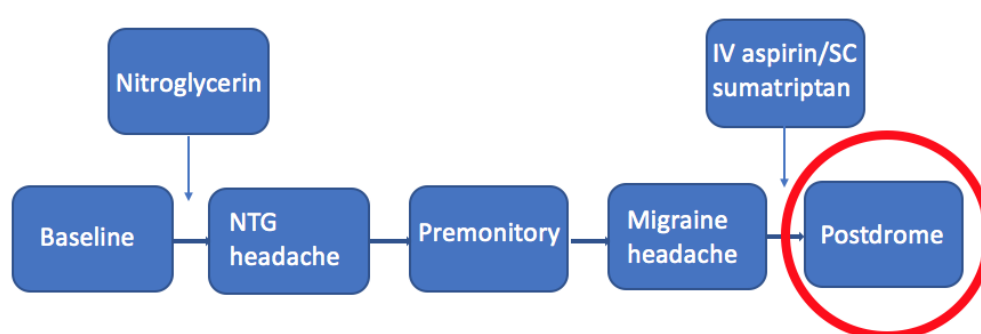


Figure 9. Visit 1 Flow chart

Visit 1 (screening visit) comprised familiarization, phenotyping, physical examination, ECG examination and a test infusion of NTG to assess and confirm how many of the recruited subjects developed premonitory symptoms, migraine headache and postdromal symptoms in response to NTG triggering (via oral questioning for establishing stage of attack and onset of postdrome), as migraine headache development in response to NTG is not universal to all migraineurs (Afridi *et al.*, 2004b). Subjects who did not trigger in response to NTG, or who did not display symptomatology compatible with the study arm timelines were excluded from further visits at this point. Subjects were instructed to consume only a light breakfast prior to each study visit. The subjects were provided meals at the end of completion of each study visit. During each visit following the triggering infusion, subjects were kept nil by mouth but were given intravenous 5% dextrose-normal saline infusion to maintain hydration and to prevent feelings of hunger. Additionally, intravenous fluids are part of standard care for acute migraine. Preventing oral food intake during each study visit would minimize vomiting and subsequent complications which maybe present once a migraine is triggered. Food and oral intake (Field *et al.*, 2003; Frank *et al.*, 2012) have been shown to have an effect on cerebral blood flow, and this would make it difficult to interpret whether changes seen on imaging are actually related to migraine processes itself.

Visit 1 established the approximate timeline from start of NTG infusion to development of

premonitory symptoms, migraine headache and postdrome following treatment for each successfully triggered subject, so that these timelines could be used to plan scanning times for further study visits. Previous studies had shown that this timeline for patients is reproducible on separate occasions using NTG triggering (Afridi *et al.*, 2004b). Subject symptoms during the postdromal phase were meticulously analysed. Their current treatments and their responses to various treatments were reviewed and formed the basis for the decision with regards to which treatment to use to treat the NTG induced migraine attack (i.e. 6mg subcutaneous sumatriptan or 1-gram intravenous aspirin).

Subjects were given intravenous aspirin or subcutaneous sumatriptan to treat the migraine headache post triggering. Once the headache was treated, the subjects were assessed for the presence of postdromal symptoms.

During Visit 1, subjects were also offered the chance to familiarise themselves with the MRI scanner environment by having the screening visit in the vicinity and by having the opportunity to lie in a mock scanner, if desired. However, none of the subjects requested access to the mock scanner during the first visit.

We attempted to characterize the neuropsychiatric symptoms encountered during the postdromal phase of a migraine attack. During subsequent visits following the screening visit, subjects underwent validated questionnaires to assess sleepiness (Kaida *et al.*, 2006), attention (Robertson *et al.*, 1997; Dillard *et al.*, 2014) and fatigue (Fisk and Doble, 2002) during baseline stage of a non-triggered (placebo) visit or during the postdrome phase of a NTG triggered subsequent visit. A one-off Migraine Impact on Daily Activities Scale (MIDAS) was also conducted at baseline prior to any triggering, at Visit 1.

The validated questionnaires to compare the sleepiness, attention and fatigue for the baseline versus the postdrome phase were administered on two different visits to minimize any practice (Goldberg *et al.*, 2015) and order (Cole *et al.*, 2016) effects. For the same reason, the Sustained Attention Response Task (SART) (Manly *et al.*, 2002) was chosen, a computer based validated questionnaire to assess attention span, that shows multiple random numbers and asked the subject to click the space bar for every random number that flashes except for the number '3,' as a test to measure attention span. The randomness of the numbers that flash on the computer screen, meant that it avoided any order or practice effect.

The questionnaires took about 20 minutes in total, to complete and were administered only once at each subsequent study visit after Visit 1 and were administered either at the baseline phase of a non-triggered visit or postdrome phase of a triggered visit.

The subjects were telephoned prior to each study visit to ensure that they were headache and

symptom free prior to attending for the next visit and to ensure that they were still willing to attend for the visit and partake in the study. They were warned to monitor closely migraine headache onset prior to attending for each study visit and were instructed to reschedule the study visit if they had a headache or active menstruation.

Participants assigned to the study underwent two further visits, a triggered and a non-triggered visit, in addition to Visit 1, but the order, triggered vs non-triggered visit, at which they attended these visits were randomised. Randomisation was determined via a computerised code using Microsoft Excel, after formal written consent was taken.

One of these visits (triggered visit) involved triggering migraine with an infusion of NTG and treating with sumatriptan 6mg subcutaneous injection or 1 gram intravenous aspirin, when there was moderate to severe pain on a verbal rating scale (score of 2-3)(Skovlund and Flaten, 1995), and the other visit (non-triggered) involved a placebo infusion (normal saline) and subcutaneous sumatriptan 6mg injection or intravenous 1 gram aspirin, given at the same treatment time based on information derived from the first visit (screening visit). All the drug agents used for infusion in the study (NTG/placebo) were administered intravenously through a cannula placed on the dorsum of the hand or antecubital fossa.

Visit 2/3 (Triggered visit)

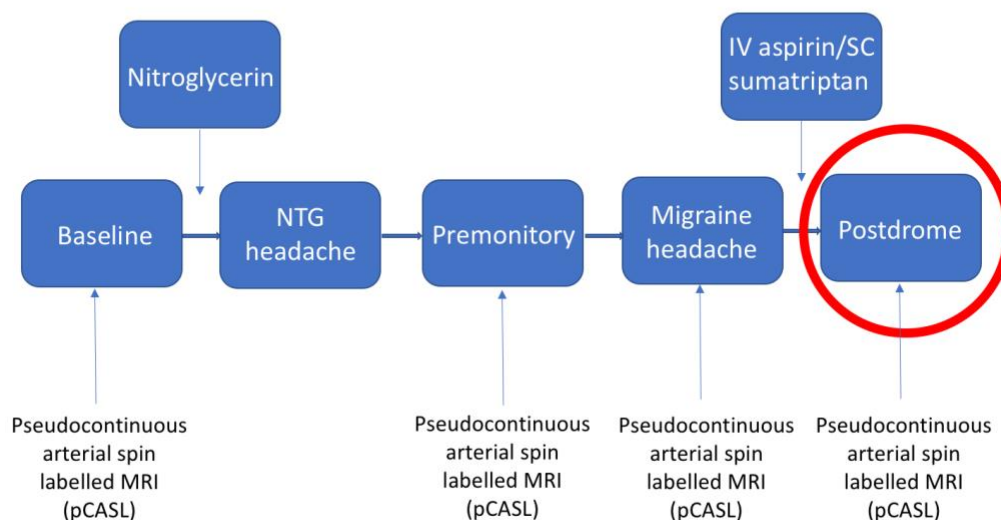


Figure 10. Sequence of events at Visit 2/3- Triggered visit

Visit 2/3 (Non-triggered)

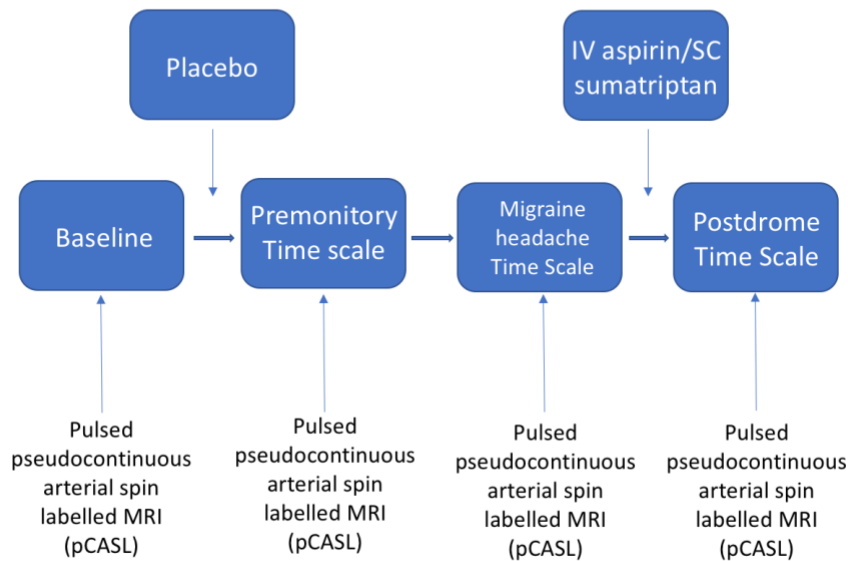


Figure 11. Sequence of events at Visit 2/3- Non-triggered visit

Before and after each scan series, pain intensity was assessed using a verbal rating scale. This is the suggested scale from the International Headache Society, using scores of 0-3 to score pain, with 0 being pain free and 3 being severe pain (Skovlund and Flaten, 1995). The purpose of a non-triggered visit was to control for any potential impact on brain imaging from diurnal alterations in rCBF (Hodkinson *et al.*, 2014), by imaging the same subjects throughout the day in the absence of headache. Hodkinson *et al.* had previously shown that the homeostasis of the hypothalamic-pituitary-adrenal axis has a role in modulating functional integrity of the default mode network (DMN) and hence when using functional imaging, to assess disease processes, it is important to factor in changes related to circadian rhythm when assessing alterations in cerebral blood flow (Hodkinson *et al.*, 2014). The purpose of administering subcutaneous sumatriptan or intravenous aspirin during the non-triggered scanning visit was to control for any effects sumatriptan or aspirin may have on cerebral blood flow which may affect end analysis and to ensure that the alterations in regional cerebral blood flow seen in the postdrome phase of a triggered visit is not a drug effect. Imaging was performed at both of these visits (triggered and non-triggered).

During Visit 2 and 3, patients, were infused with either NTG or normal saline, depending whether it was a triggered or non-triggered visit respectively, and underwent four scans per visit; a baseline scan, 'premonitory' scan, 'migraine headache' scan and a 'postdrome' scan. At each scanning point, subjects underwent pCASL examinations over approximately 30-45 minutes. Visits 2-3 occurred at a

minimum of 2-week interval from each other and from Visit 1 to allow a pain free and recovery period prior to re-triggering. Subjects had to be headache free for 24 hours prior to each study visit. This was assessed through a detailed headache diary that each subject was asked to keep. Before the infusion, subjects were assessed for headache symptoms via oral questioning to ensure symptom freedom. Following NTG/placebo infusion, the onset of headache symptoms was assessed at periodic intervals initially every 5 minutes until the end of infusion in twenty minutes and then less frequently, every 10–15 minutes and finally during the postdrome. Further detailed questioning with regards to typical premonitory symptoms, yawning, mood changes and frequency of micturition, occurred at intervals to establish what stage of an attack subjects were in. Any changes in symptoms from baseline was considered positive. During moderate to severe migraine, as determined the verbal rating scale, subjects were treated with sumatriptan 6 mg subcutaneous injection or 1-gram intravenous aspirin. If the subject received a placebo infusion in the non-triggered visit, they still received sumatriptan 6mg subcutaneous or 1-gram intravenous aspirin injection at the same time of moderate to severe migraine headache onset derived from Visit 1 (NTG triggered visit). The subject was blind to which agent (placebo or NTG) they are receiving as the infusion during visit 2 and 3. This was to ensure unbiased analysis of the study data and to avoid a placebo response.

4.2.7 Sample size

The target completed sample size for this study was determined to be for 16 patients to go through to study completion (to go through from visits 2-3, after having been identified as suitable from Visit 1. Based on normative CBF data sets collected in many ASL based studies (Howard *et al.*, 2011), we have been able to ascertain that with a standard deviation of 10% (from a grey matter mean of 50 ml/blood/100gm tissue/minute), 16 subjects are needed to observe a change of 5 ml blood/100gm tissue/min (effect size 0.8 using G-power). ASL based publications from this institution confirm these numbers (Howard *et al.*, 2011; Howard *et al.*, 2012; Hodkinson *et al.*, 2013; Hodkinson *et al.*, 2014; Hodkinson *et al.*, 2015a).

4.2.8 Procedures involved during each study visit

4.2.8.1 Visit 1 (Screening, Phenotyping and Familiarisation Visit)

During this visit, a very thorough medical history was taken with characterisation of migraine, premonitory and postdrome symptoms. The medication history was noted to ensure there was no contraindication to any of the study drugs used. A pregnancy test was performed on all female subjects of childbearing age to exclude any risk of imaging and any risk from study drugs, whilst they were pregnant. Randomisation of study visits 2 and 3 (triggered and non-triggered visits) was also performed at this stage.

4.2.8.2 Visits 2-3 - Triggered and Non-triggered visits

Each subject was contacted on the day prior to the study visit to verify attendance and to assess for the presence of any ongoing or paroxysmal pain or the onset of any premonitory symptoms prior to the study visit. A pregnancy test was performed on all female subjects of childbearing age to exclude any risk of imaging and any risk from study drugs whilst they were pregnant, for each study visit. (For Diagram see Page 51).

ASL measurements were conducted during each study visit and total measurement sequences lasted approximately 30-45 minutes per individual scanning session.

4.2.8.3 Study treatments

During the visits, NTG or a placebo (normal saline) infusion was administered through a syringe driver, aspirin 1 gram given as an IV bolus or 6mg sumatriptan administered subcutaneously by a trained medical professional.

In between scanning sessions, subjects were monitored for the occurrence of a non-specific NTG triggered featureless headache, premonitory symptoms, migraine headache and postdromal symptoms. No questioning or pain scoring were performed during scanning to prevent any interference with the imaging sequences and to avoid task related changes in rCBF during imaging.

4.2.8.4 Prior to discharge

Subjects' observations and pain scores were reassessed to ensure safety for discharge and rescue medication advice were given. Nausea and/or vomiting was also controlled through administration of appropriate anti-emetic therapy if needed. They were advised to be accompanied by someone when they left the hospital after being treated with sumatriptan unless they felt completely normal.

4.2.9 Assessments

All of the patient questionnaires, apart from the MIDAS which was completed once only during visit 1, were completed at one stage of an attack at each study visit in each study arm after visit 1 (screening visit). The stage at which they were performed was determined by randomisation and occurred at baseline during the non-triggered placebo visit and during the postdrome in the triggered visit.

4.2.9.1 Migraine related disability

The MIDAS (Migraine Disability Assessment Score) was conducted at baseline during visit 1 just once to determine the functional impact of the disorder on the subjects' lives. The Migraine Disability Assessment (MIDAS) score is used to quantify headache-related disability. This is a validated scale for assessment of migraine impact on the activities of daily living. The reliability and internal consistency of the MIDAS scores are high, as tested in a population-based sample of headache sufferers (Stewart *et al.*, 1999).

4.2.9.2 Attention

Attention was tested using a computer-based programme, SART. Permission from the authors was granted to use this test (Robertson *et al.*, 1997). This is a validated measure of attention and concentration, used widely in psychology assessments.

4.2.9.3 Sleepiness and alertness

Sleepiness and alertness was assessed with the validated Karolinska Sleepiness Scale (KSS) (Akerstedt *et al.*, 2014). Permission from the author was kindly granted to use this test.

4.2.9.4 Fatigue

Fatigue was assessed using a daily fatigue impact scale (D-FIS) (Fisk and Doble, 2002). This is a validated, widely used questionnaire to semi-quantitatively assess fatigue.

4.2.9.5 Perceived pain intensity

Estimates of perceived pain intensity was acquired using a previously used and validated Verbal rating scale, as per the International Headache Society guidelines for drug treatments (Skovlund and Flaten, 1995). Perceived pain intensity was measured during monitoring periods throughout each scanning visit.

4.2.9.6 Premonitory, migraine headache and postdromal questionnaire

Oral questioning regarding premonitory, migraine headache and postdromal symptoms occurred throughout each study visit to help ascertain which phase of migraine the subject was experiencing. These included questioning about typical premonitory and postdromal symptoms such as cravings, photophobia, phonophobia, thirst, frequency of micturition, yawning, mood changes, fatigue, concentration changes and character of headache questions including site, laterality, severity and character of pain. Answers involved the subjects' individual experiences and any change from baseline involving 3 or more symptoms was taken as positive. These were asked at 5-10 minutely intervals following the initiation of NTG or placebo infusion, for 20 minutes and every 15-20 minutes thereafter, to ascertain which stage of an attack subjects were in. The approximate timeline was acquired for each subject from visit 1 and from previous studies, there is usually minimal variability to this on multiple triggering sessions as per previous studies (Afridi *et al.*, 2004b).

4.2.10 NTG triggering

Subjects underwent NTG triggering in the Clinical Research Facility at King's College Hospital, London. The dose for NTG infusion has been established based on previous other studies and was set at 0.5mcg (microgram)/kg/minute over 20 minutes, administered through an intravenous syringe driver pump (Iversen *et al.*, 1989; Afridi *et al.*, 2004b). No analgesics was prescribed, unless the subject reached moderate to severe migraine headache as per the verbal rating scale, at which point rescue medication (either 1-gram intravenous aspirin or 6mg subcutaneous sumatriptan) was administered. All subjects were treated following the scanning session at the onset of moderate-severe migraine headache during the second triggered visit.

4.2.11 MRI

MRI examinations were conducted by qualified radiographers within the scanning facility at the Clinical Research Facility, King's College Hospital, London and all imaging data were collected using a 3T whole-body MRI scanner with capability for parallel image acquisition. MRI measurements were performed at visit 2 and 3. No MRI scan was performed at visit 1. All imaging took place between 0900-1700, to account for standardization of scanning time of day and diurnal variability (Hodkinson *et al.*, 2014). Total time of imaging per visit did not exceed 2.5 hours. The 3 T whole-body MRI scanner (GE MR750) was fitted with a receive-only 8-channel, phased-array head coil. For image registration purposes, a high resolution T2-weighted Fast Spin Echo (FSE) image was acquired. Perfusion measurements were made using a pseudocontinuous ASL (pCASL) sequence (Dai *et al.*, 2008). Labelling was performed using a train of Hanning RF pulses; 500 μ s duration, peak-to-peak gap 1500 μ s, and a total labelling duration of 1.5 s. After a post-labelling delay of 1.5 s, the image was acquired with a 3D FSE inter-leaved spiral readout (8 shots, TE/TR = 32/5500 ms, ETL = 64, 3 tag-control pairs). Pre-saturation of the image volume, followed by selective inversion pulses for background suppression, was also acquired in order to minimise the static signal. A proton density reference images was used for the computation of the CBF maps in physiological units (ml blood per 100 g of tissue per min). Each ASL took 6 minutes to acquire. Two were collected in each scanning period to increase SNR. Participants were instructed to lie still with their eyes open. Because of the genuine three-dimensional encoding and readout of this multi-shot technique, and the refocusing of magnetic susceptibility induced signal distortions, the maps of CBF obtainable with this technique are of good image quality and exquisite spatial resolution (3.6 x 3.6 x 3mm). Quantification of rCBF using pCASL was repeated multiple times within a session for determination of the temporal stability of CBF, and to gain an insight into the temporal variation of the premonitory, migraine headache and postdrome states.

4.2.12 Withdrawal

If a subject withdrew from the study and also withdrew consent for disclosure of future information, no further evaluations were performed, and no additional data were collected. None of the subjects enrolled in the study withdrew consent.

4.2.13 Research Related Injury

A medically important research related injury is any untoward medical occurrence that: Results in death or is life-threatening (immediate risk of death or results in persistent or significant disability/incapacity. No subject involved in the study sustained a medically important research related injury.

4.2.14 Medication handling

All medication was cross checked for name, dose, batch number and expiry date with a research nurse or a medical doctor. All drug administration was checked by two individuals, including the drug administrator and a research nurse or a medical doctor. All drug batch numbers and expiry dates, as well as drugs administered, times and doses were recorded in the source file.

After drug administration, and after completion of each study visit, all sharps were disposed of safely, and all contaminated needles and syringes, including used sumatriptan cartridges were disposed of as soon as possible.

4.2.15 Data analysis

4.2.15.1 Data pre-processing

All analyses were conducted using analysis toolboxes available in Statistical Parametric Mapping (SPM version 12) ([http:// www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)). Each of the rCBF time-series was normalised into a standard reference space (MNI) to allow for comparisons across individuals. Data was also spatially smoothed to accommodate for gyral variability across subjects and to improve SNR.

4.2.15.2 Statistical analysis

4.2.15.2.1 First level

An average of the two CBF maps (see Page 57) for each time point (i.e., premonitory, migraine headache and postdrome) was computed for each individual.

4.2.15.2.2 Second Level

Individual subject data from first level analyses provided inputs to paired *t*-test analyses. Paired data for the following six scan comparisons were done:

A- Baseline scans of all subjects compared with premonitory scans of all subjects.

B- Baseline scans of all subjects compared with migraine headache scans of all subjects.

C- Baseline scans of all subjects compared with postdrome scans of all subjects.

D- Premonitory scans of all subjects compared with postdrome scans of all subjects.

E- Migraine headache scans of all subjects compared with postdrome scans of all subjects and

F- Non-triggered (placebo) visit scan comparison, which is a comparison of all scans done at the time onset of migraine headache derived from screening visit, with the scan done at the comparable time of postdrome, derived from screening visit following administration of aspirin/sumatriptan to assess qualitatively alterations in rCBF related to aspirin/sumatriptan (See Page 52).

Voxel-wise contrasts of parameter estimates were computed, resulting in several whole brain maps reflecting the changes in rCBF following triggering and onset of the different migraine phases and also following treatment across subjects. After appropriate statistical thresholding (cluster-corrected $\alpha < 0.05$, derived from an uncorrected voxel *p*-value of 0.01), the resulting statistical parametric maps indicated local changes in rCBF that differ during the premonitory and headache phases of the attack, as well as following recovery of headache, in the postdrome phase. For exploratory purposes a small volume correction was applied with sphere set at 12mm radius of voxel of interest (VOI) to analyse regions of special interest, that did not pass significance at a whole brain level.

In addition to the voxel-wise, unbiased analysis, rCBF data were extracted from anatomically defined ROIs in hypothalamus, midbrain, pons, thalamus, insula and anterior cingulate cortices. Functional imaging data support the role of these regions in the pathophysiology of migraine (Weiller *et al.*, 1995; Maniyar *et al.*, 2014a; Schulte and May, 2016a). With the exception of the hypothalamus, anatomical ROIs in MNI template space were derived from the Harvard-Oxford Cortical and Subcortical Atlas (<http://www.fmrib.ox.ac.uk/fsl/>) and Juelich Histological Atlases (Amunts *et al.*, 2005). Due to the relatively small size of hypothalamus, ROI extraction was performed manually in each subject. Summary measures for each subject in each ROI was used as conventional univariate endpoint. A paired *t*-test was performed for each ROI and comparisons were made between premonitory vs postdrome rCBF data.

4.2.16 Data handling, record keeping, quality control and quality assurance

During the study conduct, data was reviewed to ensure that the protocol and Good Clinical Practices were being followed.

CHAPTER 5

RESULTS

5 RESULTS

5.1 Results of migraine postdrome clinical audit

5.1.1 Demographic characteristics

The age range in this cohort varied from 17 to 77 years ($n = 100$), with a female preponderance ($n = 85$). The mean age was 46 [± 13].

5.1.2 Postdrome reported

A postdrome was reported in 86% of patients with an average duration of 45 hours. The duration of postdrome was however only noted in three cases. The majority (72%) of postdrome sufferers within our cohort were chronic migraineurs with mostly bilateral headache (46%). Medication overuse was present in 44% of patients with 39% reporting postdromal symptoms. Simple analgesics (26%) were the most overused medication followed by triptans (16%).

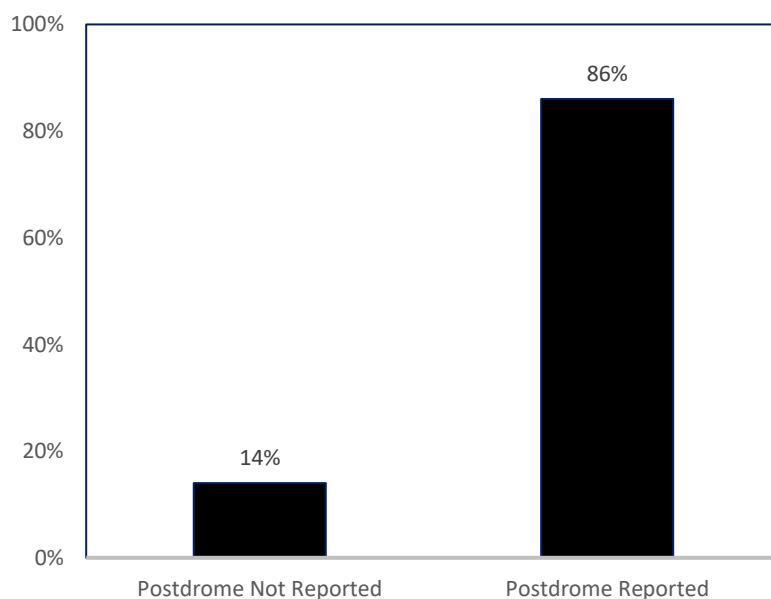


Figure 12. Postdrome presence in audit sample of migraine patients ($n=100$)

5.1.3 Postdrome Symptoms.

The most prevalent symptom was tiredness (77%) followed by neuropsychiatric (39%) and gastrointestinal (7%) symptoms. The most prevalent gastrointestinal symptom was food craving (4%) followed by an aversion to food (2%). Sensory symptoms (2%) such as photophobia and head soreness were also reported in the postdrome. Twenty-six percent of the cases who developed postdrome symptoms, specifically mentioned in clinic that their symptoms were disabling. However, for 74% of patients presenting with postdrome, it was not recorded whether the symptoms were disabling or not. A breakdown of the neuropsychiatric symptoms highlights difficulties in concentration (31%) as the most common followed by mood changes (26%). 18 patients exhibited both concentration trouble and mood changes.

Symptom presence in the postdrome group (%)	
Neuropsychiatric	39
Sensory	2
Allodynia	0
Gastrointestinal	7
General Systemic	77
Cranial Autonomic Symptoms (CAS)	0

Table 3. Postdrome symptoms

5.1.4 Headache Characteristics

The mean headache duration in this cohort was 57 [± 13] hours. Continuous headache was reported in 51%. Chronic migraine was diagnosed in 83% (Headache Classification Committee of the International Headache, 2013) and 17% had episodic migraine.

Migraine Type (%)	
Chronic	83
Episodic	17
Lateralisation of Pain (%)	
Unilateral	21
Bilateral	51
Alternating	28
Site of Attack (%)	
Frontal	58
Temporal	46
Occipital	35
Parietal	30
Vertex	23
Orbital	21
Retroorbital	19
Maxillary	14

Table 4. Headache characteristics

5.1.5 Associated features

Photophobia was bilateral in 86% cases. Phonophobia was bilateral in 75%. Phonophobia was not present in 22%. Osmophobia was present in 45%. Motion sensitivity was present in 84%. Various cranial autonomic symptoms (CAS) were reported in the headache phase. At least one CAS was present in 63%.

Symptoms during Attack (%)	
Photophobia (%)	
Unilateral	3
Bilateral	86
Not present	11
Phonophobia (%)	
Unilateral	3
Bilateral	75
Not present	22
Osmophobia (%)	
Present	45
Not present	55
Motion Sensitivity (%)	
Present	84
Not present	16

Table 5. Sensory features of migraine headache

Cranial autonomic symptoms (CAS)(%)	
Aural fullness	27
Congestion	25
Lacrimation	22
Ptosis	15
Facial flushing	15
Conjunctival injection	11
Eye redness	12
Rhinorrhoea	10
Oedema	7
Eye watering	6
Swelling of cheek	1
Miosis	0
Mydriasis	0

Table 6. Cranial autonomic symptoms reported during migraine headache

5.1.6 Co-morbidities

Patients having postdromal symptoms had documented evidence of certain co-morbidities like depression, hypertension, anxiety and fibromyalgia. Medication overuse was present in 44% of the total cases. Medication overuse was found in 39% of those with postdromal symptoms.

Co-morbidities (%)	
Hypertension	11
Fibromyalgia	5
Depression	13
Anxiety	7

Table 7. Co-morbidities in postdrome group

Medication Overuse (%)	
Opioids	13
Simple Analgesics	29
Triptans	17

Table 8. Medication overuse

5.1.7 Statistical analysis

Based on the basic demographic results and previous literature, it was decided to continue analysis on the following variables to determine any association with the presence of a postdrome.

5.1.7.1 Attack Duration

The headache/attack duration (in hours) in the population followed a normal distribution. The Kolmogorov-Smirnov test was not significant at the 5% level and thus an independent samples *t*-test was carried out assuming equal variances. This showed no differences in the headache duration (in hours) distribution between the postdrome ($n= 86$) and non-postdrome ($n= 14$) group ($P = 0.254$).

5.1.7.2 Migraine Type (Chronic vs Episodic)

There was no difference in the presence of a postdrome amongst chronic vs episodic ($P=0.702$).

5.1.7.3 Medication Overuse

There was no difference in the presence of a postdrome amongst those with and without medication overuse (Pearson Chi-Square, 0.454, $df=1$, $P=0.501$). There was also no difference in the presence of medication overuse amongst males and females ($P= 0.821$).

5.1.7.4 Age

As the age in the entire cohort followed a normal distribution and as the Kolmogorov-Smirnov test was not significant at the 5% level, an Independent Samples *t*-test was carried out assuming equal variances. This showed no differences in age distribution between the postdromal ($n=86$) and non-postdromal ($n=14$) group ($P=0.935$).

5.1.7.5 Gender

There was no difference in the presence of postdrome amongst males and females ($P=0.437$).

5.1.8 CO-MORBIDITIES

5.1.8.1 Hypertension

There was no difference in the presence of a postdrome amongst those with hypertension ($n=8$) and those without ($n=78$) ($P=0.182$).

5.1.8.2 Fibromyalgia

There was no difference in the presence of a postdrome amongst those with fibromyalgia ($n=4$) and those without ($n=82$) (Fisher's exact test, $P=0.537$).

5.1.8.3 Depression

There was no difference in the presence of a postdrome amongst those with depression ($n=13$) and those without ($n=73$) (Fisher's exact test, $P=0.537$).

5.1.8.4 Anxiety

There was no difference in the presence of a postdrome amongst those with anxiety ($n=6$) and those without ($n=80$) (Fisher's exact test, $P=1.000$).

5.1.8.5 Lateralisation of Pain during headache phase

There was no difference in the presence of a postdrome amongst those with unilateral, bilateral or alternating headache (Fisher's exact test, $P=0.198$).

5.1.8.6 CAS during headache phase

There was no difference in the presence of a postdrome amongst those with CAS during migraine and those without (Pearson Chi-Square, 0.496, $df = 1$, $P = 0.481$).

5.1.8.7 Photophobia during headache phase

There was no difference in the presence of a postdrome amongst those with unilateral/bilateral/or no photophobia during headache phase and those without (Fisher's exact test, $P=0.376$).

5.1.8.8 Phonophobia during headache phase

There was no difference in the presence of a postdrome amongst those with phonophobia during migraine attack and those without (Fisher's exact test, $P=0.231$).

5.1.8.9 Osmophobia during headache phase

There was no difference in the presence of a postdrome amongst those with osmophobia during migraine attack ($n=38$) and those without ($n=38$) (Pearson Chi-Square, 0.164, $df=1$, $P=0.685$).

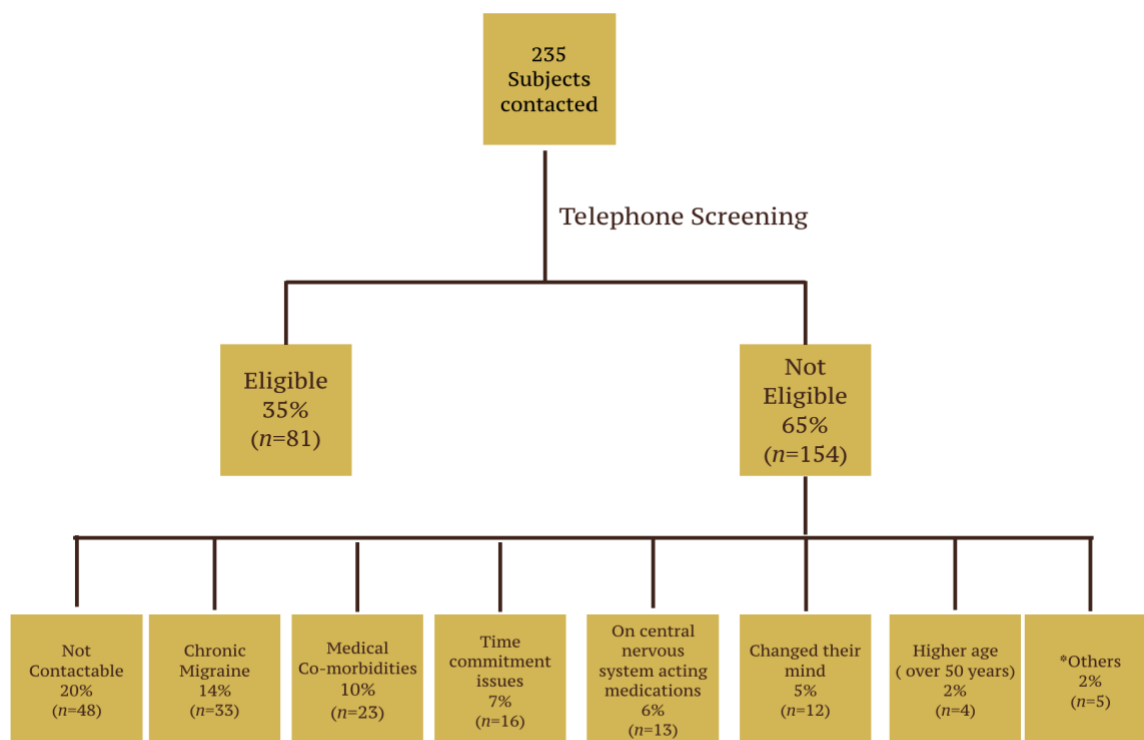
Motion Sensitivity during headache phase

There was no difference in the presence of a postdrome amongst those with motion sensitivity during migraine attack and those without (Fisher's exact test, $P=0.231$).

5.2 POSTD Study clinical results

5.2.1 Study recruitment

Approval for the study was granted on 27th February 2015. The first subject was enrolled on 15th April 2015. The study was completed on 8th December 2016 after hitting the completion target of 16. The enrolment period for each research subject included a screening plus NTG triggering visit. Subjects who had a successful triggered migraine on the first study visit were invited for two further scanning visits. Subjects who did not develop a migraine following NTG triggering, were excluded from further participation in the study.



*Others (n=5) included one subject living abroad, two subjects with titanium jaw plates, one subject with study drug allergies and one subject on recreational drugs .

Figure 13. Screening figures.

Two hundred and thirty-five subjects contacted to take part in the study. Eighty-five percent were female (n=199) and 15 were male (n= 36). Following telephone screening, 35% were eligible to take part (n=81).

Out of the 81 eligible subjects, 46 subjects proceeded to attend the screening and triggering visit (Visit 1). Thirty-eight subjects had a successful NTG triggered migraine attack. Out of the 38 subjects, one subject had a short time interval between premonitory symptoms and migraine headache (less than 10 minutes) which would have rendered imaging difficult and hence had to be

excluded. One subject had a titanium jaw plate which would have affected the quality of the ASL images and hence was excluded. Thirty-six subjects proceeded in the study and were invited for a further two visits that involved ASL MRI scans. Sixteen subjects completed all the study visits (three in total) and thereby meeting the study completion target. ASL MRI scans from these subjects over the course of triggered attacks during the study visits were procured and the alterations in the cerebral blood flow during different phases of a migraine attack were analysed.

5.2.2 Demographics

There was a total of 14 females and 2 male subjects, excluding any gender analysis. The age range of the study cohort was 18-49 years with a mean age of 35.

5.2.3 Migraine Characteristics

Ten subjects had migraine without aura, 4 subjects had migraine with aura and 2 subjects had chronic migraine as per the ICHD-3beta criteria (Headache Classification Committee of the International Headache, 2013). The subjects had between 1-22 headache days per month with a mean of 9 headache days per month. The subjects had suffered from migraine from between 3 years to 39 years with a mean duration of 20 years.

5.2.4 Handedness

Twelve subjects were right handed and 4 were left handed.

5.2.5 Migraine Associated features

Of the subjects, 94% (15/16) gave a history of photophobia with their attacks. Similarly, 94% (15/16) reported phonophobia with their attacks, while 81% (13/16) reported nausea or vomiting with their attacks. Osmophobia and movement sensitivity was reported in 81% (13/16) with their attacks. At least one CAS was reported in 69% (11/16) with their migraine attacks, while 50% (8/16) reported cranial allodynia with their attacks. All the patients reported the presence of premonitory and postdrome symptoms with their attacks.

Photophobia (%)	
Present	94
Not present	6
Phonophobia (%)	
Present	94
Not present	6
Nausea or vomiting (%)	
Present	81
Not present	19
Osmophobia (%)	
Present	81
Not present	19

Table 9. Baseline migraine associated features.

5.2.6 Migraine Triggers

Subjects in the study reported various potential triggers for their migraine attack (see Table 10). None of the female subjects in this cohort reported menstruation as a trigger for their migraine attacks.

Stress	56
Bright light	50
Lack of sleep	50
Alcohol	25
Loud sound	19
Strong smells	19
Oversleeping	19
Stormy weather	19
Dehydration	13
Missing meals	6
Exertion	6

Table 10. Triggering factors reported for migraine headache as a percentage

5.2.7 Acute Medications

One subject took 2.5 mg frovatriptan prn for acute migraine attacks. One subject took almotriptan 12.5mg prn for acute migraine attacks. Six subjects took 50 mg of sumatriptan prn for acute migraine attacks. One subject took aspirin prn for acute migraine attacks. Ten subjects took 1-gram paracetamol prn for acute migraine attacks. Seven subjects took 400mg ibuprofen prn for acute migraine attacks.

5.2.8 Current Preventives

Five subjects were on migraine preventives at the time of the study. They were asked to continue with the drugs for the duration of the study and advised not to make any dose adjustments lest the adjustments lead to any alteration in rCBF values in ASL. Two subjects were on pizotifen. One subject each was taking amitriptyline, candesartan and propranolol.

5.2.9 Previous Preventives

Two subjects had taken gabapentin in the past for prevention. Four subjects were on Amitriptyline in the past. One subject was on pizotifen in the past. Four subjects were on propranolol in the past.

5.2.10 Family History

Out of the 16 subjects in the study, 14 (88%) reported a family history of migraine.

5.2.11 MIDAS scores

The MIDAS scores of subjects taking part in the study varied from 11 to 201, with a mean score of 33.

5.2.12 NTG dose

From previous studies, the recommended NTG dose to trigger a migraine was calculated to be 0.5 microgram/kg/minute over 20 minutes (Iversen *et al.*, 1989). The total NTG dose used in the study varied from 500-980 micrograms with a mean dose of 726 micrograms.

Patient ID	Gender	Age (years)	Headache Days/Month	Duration of Disease in Years	Acute Medication	Preventative Medication	Past Preventatives	Family History
1	F	49	14	29	sumatriptan	None	propranolol	Yes
2	F	49	22	39	almotriptan ibuprofen paracetamol	None	gabapentin propranolol	Yes
3	M	26	1	6	aspirin paracetamol	None	propranolol	Yes
4	F	46	14	21	frovatriptan paracetamol ibuprofen	None	None	Yes
5	F	36	4	24	rizatriptan	candesartan	None	Yes
6	F	35	5	22	sumatriptan	pizotifen	None	Yes
7	F	24	6	11	sumatriptan paracetamol ibuprofen	amitriptyline	None	Yes
8	F	41	15	30	paracetamol naproxen	None	gabapentin amitriptyline	Yes
9	F	25	4	3	sumatriptan	propranolol	pizotifen	Yes
10	F	43	2	23	rizatriptan naproxen	None	None	No
11	M	42	6	22	sumatriptan	None	None	Yes
12	F	18	14	7	sumatriptan	None	amitriptyline propranolol	Yes
13	F	37	10	23	paracetamol ibuprofen	pizotifen	amitriptyline	No
14	F	27	10	15	paracetamol ibuprofen	None	None	Yes
15	F	30	7	20	paracetamol ibuprofen	None	None	Yes
16	F	27	5	17	paracetamol ibuprofen	None	None	Yes

Table 11. Baseline migraine characteristics of completed subjects

5.2.13 Premonitory phase

Patients receiving NTG to trigger a migraine typically report a bimodal headache pattern. Firstly, they report a dull band like featureless headache related to NTG which settles in a few minutes and then from previous studies, approximately 75% of subjects go on to develop a migraine headache (Afridi *et al.*, 2004b). The first triggered visit did not involve imaging whereas subjects underwent triggering (or placebo infusion) and imaging on the subsequent two visits. In our study, subjects developed a NTG headache within 1-6 minutes from the onset of NTG infusion (Mean onset 4 mins; SE ± 1.5) during the first triggered visit and within 1-10 minutes from the onset of the NTG infusion (Mean onset 4 mins; SE ± 2.3) at the second triggered visit. The mild NTG headache lasted for 17 ± 10 mins (mean $\pm$ SE) during the first triggered (non-scanning visit) and 18 ± 8 min (mean $\pm$ SE) during the second triggered (scanning) visit. For the purposes of the study, we set the onset of premonitory phase as the time of onset of three or more premonitory symptoms. The median onset of premonitory symptoms occurred at 20 minutes (IQR=8-40) at the first triggered visit and at 15 minutes (IQR=5-28) at the second triggered visit.

5.2.14 Postdrome Onset

At the onset of moderate to severe headache at the first triggered visit, subjects received either 6mg sumatriptan subcutaneously or 1-gram intravenous aspirin to render them headache free and induce the postdrome phase. The postdrome was induced within a median time of 150 minutes (IQR=135-192) from the onset of NTG infusion at the first triggered visit and 212 minutes (IQR=184-265) from the onset of NTG infusion at the second triggered visit.

5.2.15 Premonitory symptoms

During all the visits, subjects were asked regarding various premonitory symptoms after the initiation of NTG triggering. Subjects did not report premonitory, headache or postdrome symptoms on the non-triggered placebo visit.

Premonitory symptoms	Percentage (%)
Neck Stiffness	69%
Fatigue	69%
Photophobia	66%
Concentration difficulty	66%
Thirst	50%
Nausea	50%
Yawning	44%
Movement sensitivity	34%
Phonophobia	31%,
Increased urinary frequency	19%
Irritability	19%
Speech difficulty	19%
Visual Blurring	17%
Depressed Mood	13%
Gastric Discomfort	6%
Elevated Mood	6%
Food Craving	6%

Table 12. Premonitory symptoms reported in the 16 subjects across two NTG triggered visits

5.2.16 Postdrome Symptoms

Once the throbbing headache ceased, subjects developed multiple non-headache related symptoms, with tiredness, concentration difficulty and head discomfort the most common reported symptoms (see Table 13).

POSTDROME SYMPTOMS	PERCENTAGE (%)
Tiredness	90%
Concentration difficulty	72%
Head Discomfort	63%
Hangover	50%
Generalised weakness	47%
Feeling of relief	44%
Photophobia	38%
Malaise	28%
Elevated mood	22%
Thirst	22%
Movement sensitivity	22%
Cranial Allodynia	22%
Eye Discomfort	13%
Internal vertigo	13%
Nausea	13%
Phonophobia	13%
Gastric Discomfort	10%
Depressed Mood	10%
Nasal Stuffiness	7%
Feeling of Dread	3%
Feeling Drunk	3%

Table 13. Postdrome symptoms of 16 subjects over two NTG triggered visits.

5.2.17 Cognitive Assessments in the Postdrome

Much of the focus in migraine research and management has been centred around the throbbing pain. However various non-headache symptoms experiences in the postdrome, contribute to the disability and suffering of migraineurs (Bose and Goadsby, 2016). Various studies have shown that cognitive symptoms are commonly reported in the postdrome (Kelman, 2006; Giffin *et al.*, 2016). Although studies exist that look into various cognitive domains during a migraine attack (Gil-Gouveia *et al.*, 2016), the cognitive symptoms and dysfunction reported in the postdrome have not been objectively assessed. Previous studies relied on retrospective assessment of postdrome symptoms using diary based self-reporting or prospective assessment using self-reported electronic diaries (Giffin *et al.*, 2005; Kelman, 2006) whereas we carried out a prospective and objective assessment of cognitive symptoms in the postdrome.

5.2.17.1 Sustained Attention to response test (SART- See Also Page 50)

Migraineurs commonly complain of non-headache symptoms, including cognitive symptoms in the postdrome (Bose and Goadsby, 2016). An electronic diary study of the migraine postdrome by Giffin *et al.*, showed that 81% of the subjects evaluated had at least one non-headache symptom in the postdrome. Concentration difficulty was reported in 56% of the attacks (Giffin *et al.*, 2016). To study the impact of cognitive symptoms in the postdrome as a secondary objective, attention was assessed using a sustained attention to response test (SART). Subjects were shown single digits on a laptop presented serially but employing a random sequence. Each digit was presented for a duration of 250 ms. This was followed by a diagonal cross mask which was of a total duration of 900 ms. The subjects were asked to press the spacebar of the laptop for each number. The number 3 served as a 'no-go' target and subjects were asked not to press the space bar when the number 3 flashed on the screen. The probability of number 3 flashing was set by the software as 1 in 9 (0.11) which the subject was not aware of. Subjects were given the opportunity to have a short practice session to get to grips with the keyboard before the launch of the scoring session. The total task duration was approximately nine minutes. Subjects were scored at baseline during their placebo scanning visit and during the postdrome during their triggered scanning visit to assess if there was a significant difference between attention at the baseline state compared to attention in the postdrome state. Nine out of sixteen subjects underwent the SART test at both the baseline state during a non-triggered visit and during the postdrome phase of a triggered visit.

5.2.17.1.1 SART Errors of commission

This aspect of the test was to assess how well the subjects pressed the keys when the numbers apart from number 3 flashed (i.e. the 'no-go' target).

The values of errors of commission during the baseline state ranged from 0-25% with mean=7, (SE±8). The values of errors of commission during postdrome state ranged from 0-15% with mean=5, (SE±8).

5.2.17.1.2 SART Errors of omission

This aspect of the test was to assess how well the subjects refrained from pressing the keys when the number 3 flashed (i.e. the 'no-go' target). The values of errors of omission during the baseline state ranged from 1-25% with mean=18, (SE±8). The values of errors of omission during the postdrome state ranged from 12-25% with mean=21 (SE±6).

5.2.17.1.3 SART Mean reaction time

The values of mean reaction time during baseline state ranged from 293.76 to 605.66 ms with mean=454 ms (SE±113). SART mean reaction time during postdrome state ranged from 254.24 to 628.71 with mean=484 ms (SE±128).

5.2.17.1.4 Statistical Analysis of SART results

A paired-samples *t*-test was conducted to compare errors of commission between subjects at the baseline state compared to the postdrome state. There was no significant statistical difference in the scores for baseline state (mean=7, SE±8) and postdrome state (mean=5, SE±6); $t(8) = 0.696$, $p = 0.50$.

A paired-samples *t*-test was conducted to compare errors of omission between subjects at the baseline state compared to the postdrome state. There was no significant statistical difference in the scores for baseline state (mean=18, SE±8) and postdrome state (mean=21, SE±6); $t(8) = 0.652$, $p = 0.53$.

A paired-samples *t*-test was conducted to compare mean reaction time between subjects at the baseline state compared to the postdrome state. There was no significant statistical difference in the scores for baseline state (mean=454, SE±113) and postdrome state (mean=484, SE±128); $t(8) = 1.3$, $p = 0.23$.

5.2.17.2 Karolinska sleepiness scale (KSS)

With newer studies highlighting the postdrome phase, the awareness of its existence and the common symptoms linked to it is advancing (Kelman, 2006; Giffin *et al.*, 2016). A common symptom experienced in the postdrome is somnolence. Edward Liveing made the observation that sleep resolved migraine in his masterpiece *On Megrim* (Liveing, 1872). In a study of 50 migraineurs, it was noted that sleep was a common way of aborting a migraine attack (Blau, 1982). Several other studies looking in to symptoms described in the migraine postdrome have not specifically documented sleep, but tiredness is the most common symptom expressed by patients in the postdrome (Kelman, 2006; Giffin *et al.*, 2016). Lack of sleep is also reported as a migraine trigger.

The impact of sleepiness on the postdrome phase of migraine was assessed using a sleepiness indicator. Using a validated subjective nine-point Likert-type sleepiness scale (Kaida *et al.*, 2006) is a simple way of assessing sleepiness and avoids the use of intrusive and costly equipment. Previous studies using this scale have found a U-shaped diurnal pattern to sleep- i.e., a high sleepiness score in the morning and late evening (See Page 82). Sleepiness in the postdrome phase has not been assessed before using a sleepiness scale. The Karolinska Sleepiness scale (KSS) is a 9 point Likert-type scale which value ranges from 'Extremely alert' (score 1) to 'Very sleepy, great effort to keep awake, fighting sleep' (score 9). A comparison of the sleepiness scores of subjects between the baseline state and the postdrome state was done.

1	Extremely alert
2	Very alert
3	Alert
4	Rather alert
5	Neither alert nor sleepy
6	Some signs of sleepiness
7	Sleepy, but no effort to keep awake
8	Sleepy, some effort to keep awake
9	Very sleepy, great effort to keep awake, fighting sleep

Figure 14. The Karolinska Sleepiness scale.

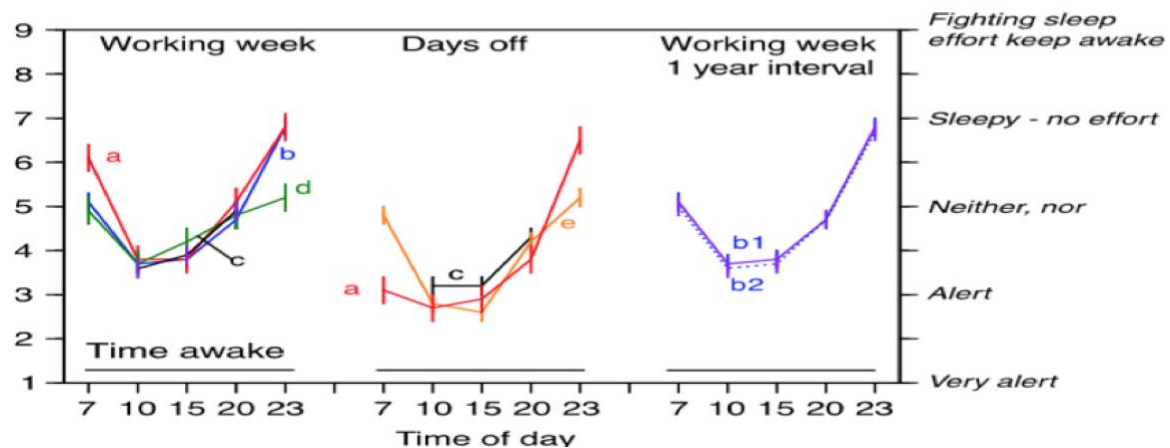


Figure 15. Karolinska Sleepiness Scale (KSS) values from a working day starting at approximately 08:00 hours (a–d) and a day off (a, c, e). All are daytime workers, except for those in study (e), who are rotating shift workers on the second day off. A comparison of working days with a 1.2-year interval (b1, b2) for the same group. Mean $\pm$ standard error. Waking span indicated at the bottom. The right y-axis provides the verbal labels of the KSS. (For Permissions to use figure, please see page 142)

5.2.17.2.1 Results

Nine out of sixteen subjects had their sleepiness scale assessed during the baseline of a non-triggered (placebo) visit and also the postdrome phase during a triggered visit. The KSS score during the baseline of a non-triggered placebo visit varied from 2-7 with a median score of 3 (IQR=2-5). The KSS score during the postdrome phase of a NTG triggered visit varied from 5-8 with a median score of 8 (IQR=7-8). A Wilcoxon Signed-ranks test was conducted to compare KSS scores between subjects at the baseline state compared to the postdrome state. The test indicated that KSS scores were significantly higher in the postdrome state (median=8) than at the baseline (median= 3), ($Z = 2.54$, $p=0.01$).

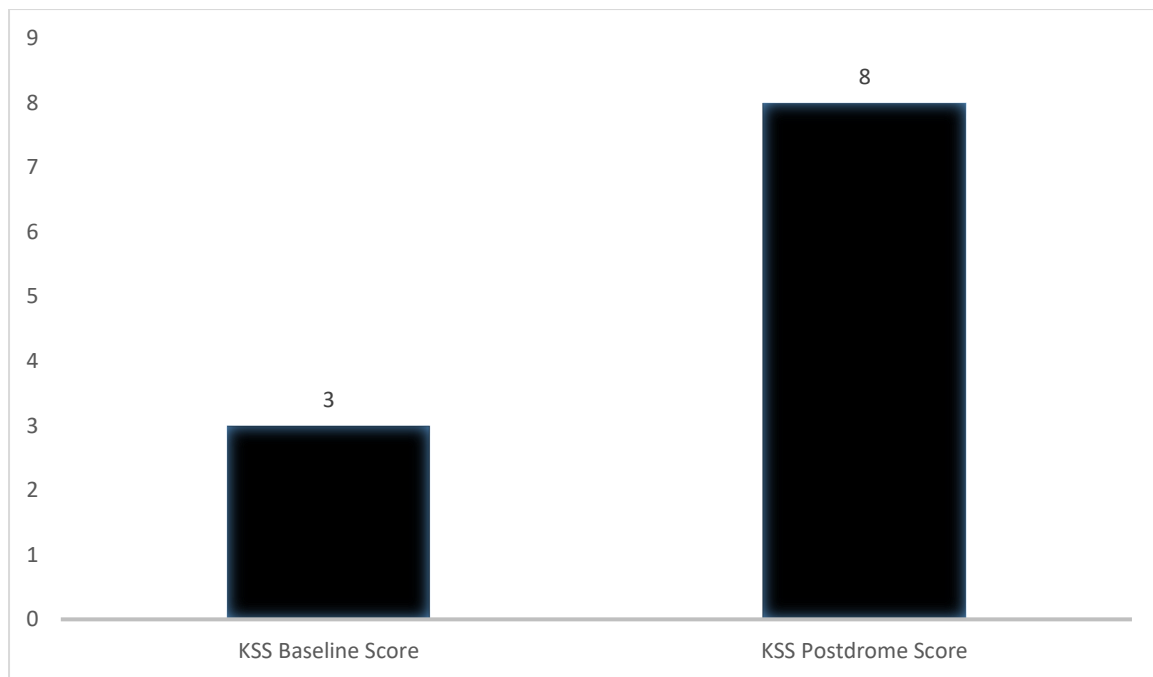


Figure 16. Median KSS scores at baseline of non-triggered visits and at postdrome phase of triggered visits

5.2.17.3 Daily Fatigue Impact Scale (DFIS)

Tiredness or fatigue is a common symptom reported in the postdrome phase of migraine (Kelman, 2006; Bose and Goadsby, 2016). Though it is a common symptom, because of the difficulty for patients to define the symptom, it is poorly understood. A validated questionnaire to objectively assess fatigue was used to compare the difference between the baseline state and the postdrome state.

	No problem 0	Small problem 1	Moderate problem 2	Big problem 3	Extreme problem 4
1. Because of fatigue, I feel less alert.					
2. Because of fatigue, I have to reduce my workload or responsibilities.					
3. Because of fatigue, I am less motivated to do anything that requires physical effort.					
4. Because of fatigue, I have trouble maintaining physical effort for long periods.					
5. Because of fatigue, I find it difficult to make decisions.					
6. Because of fatigue, I am less able to finish tasks that require thinking.					
7. Because of fatigue, I feel slowed down in my thinking.					
8. Because of fatigue, I have to limit my physical activities.					

Figure 17. Daily Fatigue Impact Scale (DFIS)

For each factor affected by fatigue (i.e. alertness, workload, motivation for physical effort, maintenance of physical effort, decision making, thinking tasks, thinking slow down, limitation of physical activity), subjects could score between 0-4 (0-no problem, 1-mild problem, 2-moderate problem, 3-big problem and 4- extreme problem). A Wilcoxon Signed-ranks test to see if there was a statistically significant difference between the baseline state and the postdrome state.

5.2.17.3.1 Fatigue impacting alertness

The median DFIS alertness score during the baseline of a non-triggered placebo visit was 1 (IQR=0-2).

The median score during the postdrome phase of a NTG triggered visit was 2 (IQR=1-2).

A Wilcoxon Signed-ranks test was conducted to compare DFIS alertness scores between subjects at the baseline state compared to the postdrome state. The test indicated that DFIS scores were not statistically significant in the postdrome state (median=2) compared to the baseline (median= 1), ($Z = 1.73$, $p=0.08$).

5.2.17.3.2 Fatigue impacting workload

The median DFIS workload score during the baseline of a non-triggered placebo visit was 0 (IQR=0-1). The median score during the postdrome phase of a NTG triggered visit was 2 (IQR=1-2).

A Wilcoxon Signed-ranks test was conducted to compare DFIS workload scores between subjects at the baseline state compared to the postdrome state. The test indicated that DFIS scores were statistically significant in the postdrome state (median=2) compared to the baseline (median= 0), ($Z = 2.16, p=0.03$).

5.2.17.3.3 Fatigue impacting motivation

The median DFIS motivation score during the baseline of a non-triggered placebo visit was 0 (IQR=0-1). The median score during the postdrome phase of a NTG triggered visit was 2 (IQR=1-2).

A Wilcoxon Signed-ranks test was conducted to compare DFIS motivation scores between subjects at the baseline state compared to the postdrome state. The test indicated that DFIS scores were statistically significant in the postdrome state (median=2) compared to the baseline (median= 0), ($Z = 1.98, p=0.04$).

5.2.17.3.4 Fatigue impacting maintenance of physical effort

The median DFIS maintenance of physical effort score during the baseline of a non-triggered placebo visit was 1 (IQR=0-1). The median score during the postdrome phase of a NTG triggered visit was 1 (IQR=1-2). A Wilcoxon Signed-ranks test was conducted to compare DFIS maintenance of physical effort scores between subjects at the baseline state compared to the postdrome state. The test indicated that DFIS scores were not statistically significant in the postdrome state (median=1) compared to the baseline (median= 1), ($Z = 1.73, p=0.08$).

5.2.17.3.5 Fatigue impacting decision making

The median DFIS decision making score during the baseline of a non-triggered placebo visit was 0 (IQR=0-1). The median score during the postdrome phase of a NTG triggered visit was 1 (IQR=1-2).

A Wilcoxon Signed-ranks test was conducted to compare DFIS decision making scores between

subjects at the baseline state compared to the postdrome state. The test indicated that DFIS scores were statistically significant in the postdrome state (median=1) compared to the baseline (median=0), ($Z = 2.46, p=0.01$).

5.2.17.3.6 Fatigue impacting thinking tasks

The median DFIS thinking task score during the baseline of a non-triggered placebo visit was 0 (IQR=0-1). The median score during the postdrome phase of a NTG triggered visit was 2 (IQR=1-2). A Wilcoxon Signed-ranks test was conducted to compare DFIS thinking tasks scores between subjects at the baseline state compared to the postdrome state. The test indicated that DFIS scores were not statistically significant in the postdrome state (median=2) compared to the baseline (median=0), ($Z = 1.89, p=0.06$).

5.2.17.3.7 Fatigue slowing down thinking

The median DFIS thinking slowdown score during the baseline of a non-triggered placebo visit was 1 (IQR=0-2). The median score during the postdrome phase of a NTG triggered visit was 2 (IQR=1-2). A Wilcoxon Signed-ranks test was conducted to compare DFIS slowing down of thinking scores between subjects at the baseline state compared to the postdrome state. The test indicated that DFIS scores were not statistically significant in the postdrome state (median=2) compared to the baseline (median=1), ($Z = 0.99, p=0.32$).

5.2.17.3.8 Fatigue limiting physical activities

The median DFIS physical activities limiting score during the baseline of a non-triggered placebo visit was 0 (IQR=0-1). The median score during the postdrome phase of a NTG triggered visit was 1 (IQR=1-2). A Wilcoxon Signed-ranks test was conducted to compare DFIS slowing down of thinking scores between subjects at the baseline state compared to the postdrome state. The test indicated that DFIS scores were statistically significant in the postdrome state (median=1) compared to the baseline (median=0), ($Z = 2.06, p=0.04$).

CHAPTER 6

POSTD STUDY IMAGING RESULTS

6 POSTD Study Imaging Results

Sixteen subjects (14 female, 2 male) with a history of migraine (Headache Classification Committee of the International Headache, 2013) completed all imaging visits. Twelve subjects were right handed and 4 were left handed (For clinical characteristics of the subjects included in the analysis, see Page 74).

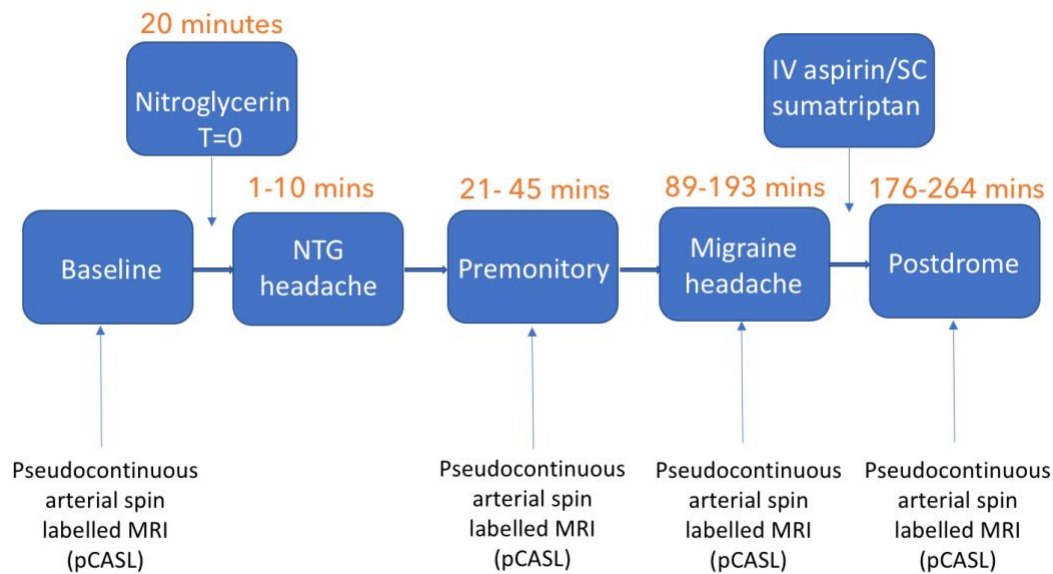


Figure 18. Time line for scans after initiation of NTG infusion. Subjects underwent 4 scans at each study visit: baseline, premonitory, migraine headache and postdrome scan. T_0 being the time of initiation of the NTG infusion.

Imaging data were processed using SPM version 12 (<http://www.fil.ion.ucl.ac.uk/spm>) implemented in MATLAB (Mathworks, Inc, Sherborn, MA, USA). The high-resolution structural images were used to align the CBF data to MNI152 standard space [average T1 brain image constructed from 152 normal subjects at the Montreal Neurological Institute (MNI), Montreal, QC, Canada]. Spatial normalization was performed by co-registering the CBF image to the T1 image. The spatial normalization parameters required to warp the T1 image to MNI space were estimated (via SPM unified segmentation) and these transformation parameters were applied to the CBF map. The data were spatially smoothed using an 8 mm full-width at half-maximum (FWHM) Gaussian kernel to accommodate for gyral variability across subjects. Group-level voxel-wise changes in regional CBF were calculated under the framework of the general linear model (GLM) using a random effects independent two-sample *t*-test. Significant clusters were displayed with a probability threshold of $p < 0.05$, corrected for multiple comparisons using family-wise error rate (FWE). Global cerebral blood

flow covariate correction was done using analysis of covariance (ANCOVA) and grey matter masking was also applied due to the low signal-to-noise ratio of ASL in white matter.

6.1 Voxel Based Analysis

Subjects underwent a total of 4 scans each during visit 2 and 3 one of which was a triggered visit and the other a placebo visit in a randomised order. The scans done at the triggered visit were done at baseline, onset of three or more premonitory symptoms, onset of moderate to severe migraine headache and finally at the onset of postdrome. Scans were done at the non-triggered visits based on time scales derived from the screening visit, for comparison of the scan done at time scale of migraine headache derived from screening visit, with scan done at time scale of postdrome derived from screening visit following administration of aspirin/sumatriptan.

The scans were compared as follows:

1. Baseline scans of all subjects compared to premonitory phase.
2. Baseline scans of all subjects compared to migraine headache.
3. Baseline scan of all subjects compared to postdrome phase.
4. Postdrome scans of all subjects compared to premonitory phase.
5. Migraine headache scans of all subjects compared to postdrome.
6. Non-triggered (placebo) visit scan comparisons where, comparison of all scans done at the time onset of migraine headache derived from screening visit, with the scan done at the comparable time of postdrome, derived from screening visit following administration of aspirin/sumatriptan to assess qualitatively alterations in rCBF related to aspirin/sumatriptan and diurnal variation (Hodkinson *et al.*, 2014) (See Page 52, 59 and Figure 19).

Cluster statistics were used to identify statistically significant changes in CBF. Clusters were corrected for multiple comparison based on cluster extent (p 0.05), from a cluster-forming threshold $T > 2.84$

Scan comparisons (Triggered visits)

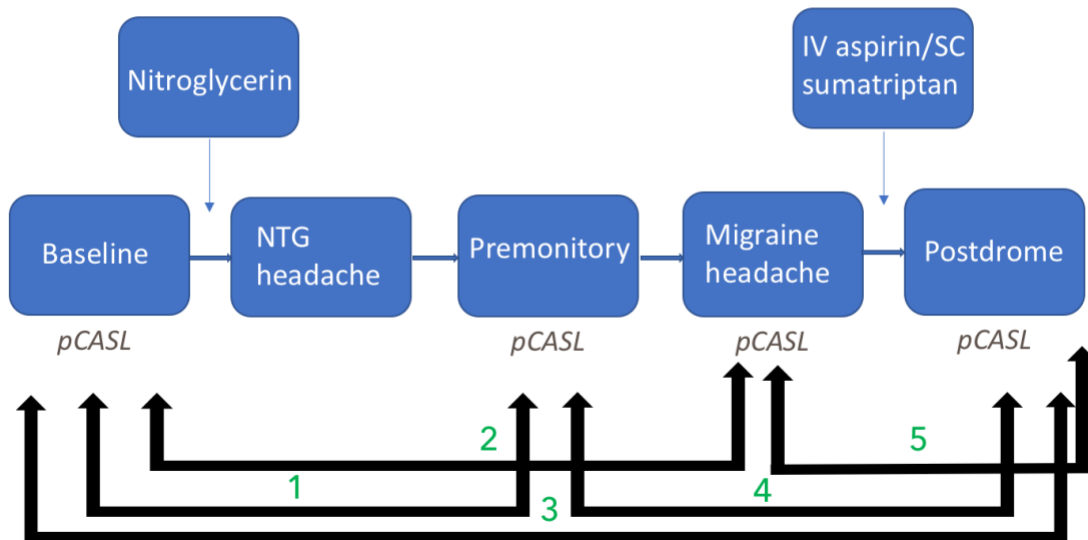


Figure 19. Triggered visit scan comparisons. 1. Baseline scans of all subjects compared to premonitory phase 2. Baseline scans of all subjects compared to migraine headache. 3. Baseline scan of all subjects compared to postdrome phase. 4. Postdrome scans of all subjects compared to premonitory phase. 5. Migraine headache scans of all subjects compared to postdrome.

Scan Comparison (Non-triggered)

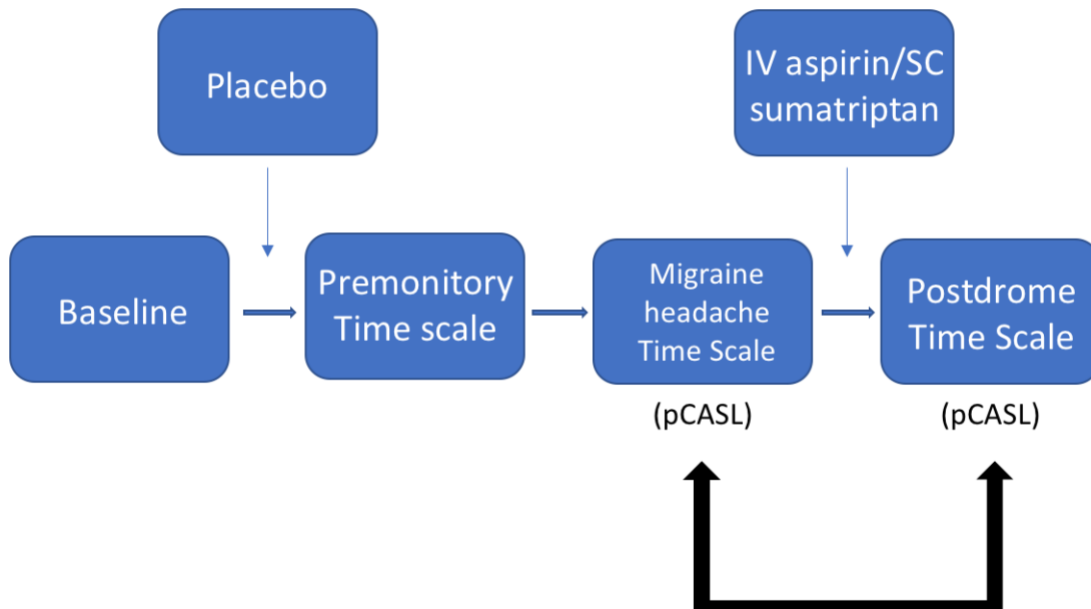


Figure 20. Non-triggered (placebo) visit scan comparison to qualitatively assess alterations in rCBF related to aspirin/sumatriptan and diurnal variation

6.1.1 Baseline scans compared to premonitory phase scans:

There was increased rCBF over the right and left superior frontal, medial frontal, bilateral anterior cingulate with peak increase over the left medial frontal gyrus ($P < 0.001$) at a whole brain analysis level in the premonitory phase compared to the baseline state. There was also a reduction in rCBF in the premonitory phase over the right middle occipital gyrus, right middle temporal gyrus, lingual gyrus and cuneus, with a peak reduction over the right inferior occipital gyrus ($P = 0.018$) compared to the baseline state. With small volume correction, (sphere set at 12mm radius volume of Interest, VOI), there was increased rCBF over the posterior cingulate and superior frontal gyrus with a peak increase over the posterior hypothalamus ($P = 0.046$). No areas of reduction in rCBF were seen in the premonitory phase with small volume correction.

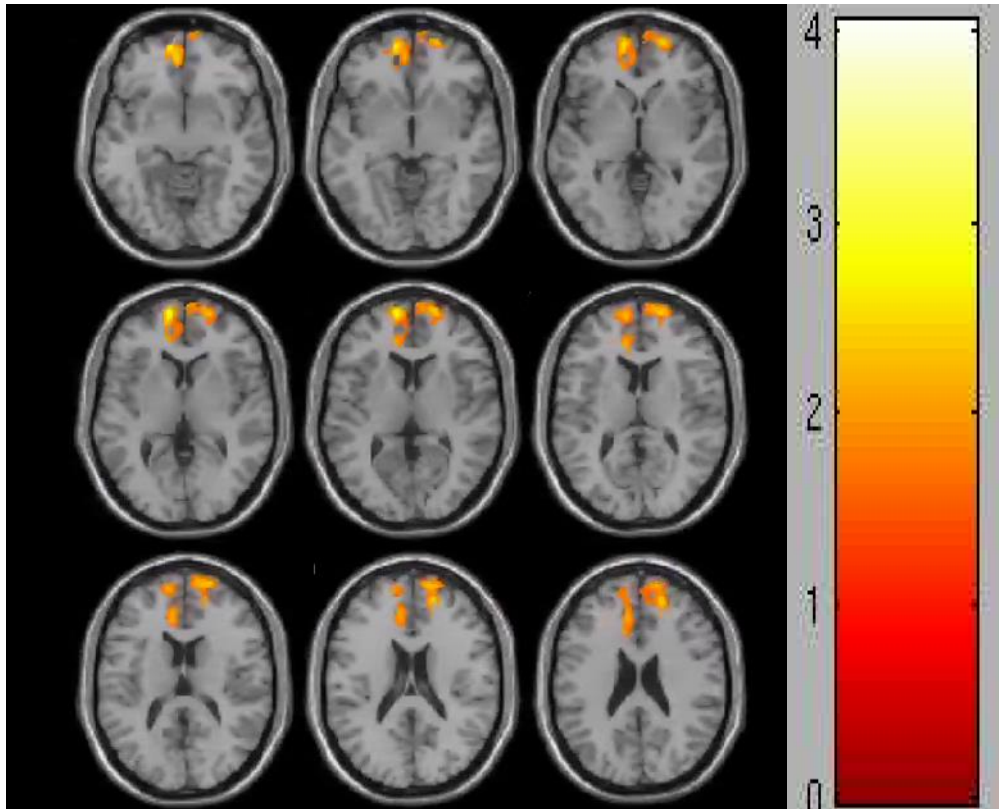


Figure 21. Increased rCBF in the premonitory phase compared to baseline at a whole brain level (Axial sections). The colour bar on the right indicates the colour coding of the T -scores.

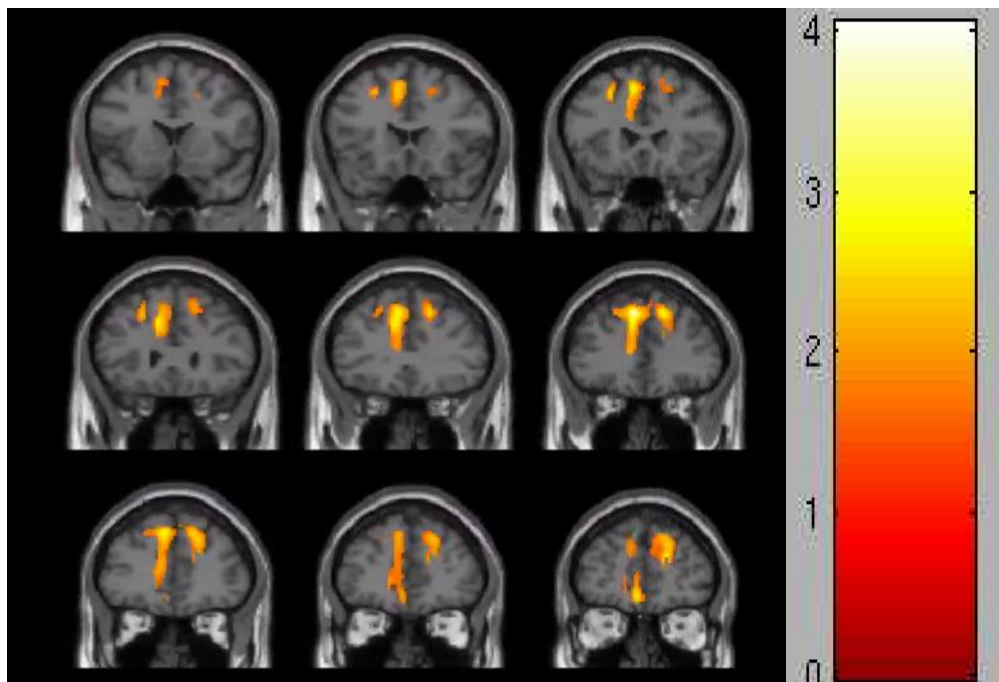


Figure 22. Increased rCBF in the premonitory phase compared to baseline at whole brain level (Coronal sections). The colour bar on the right indicates the colour coding of the T -scores.

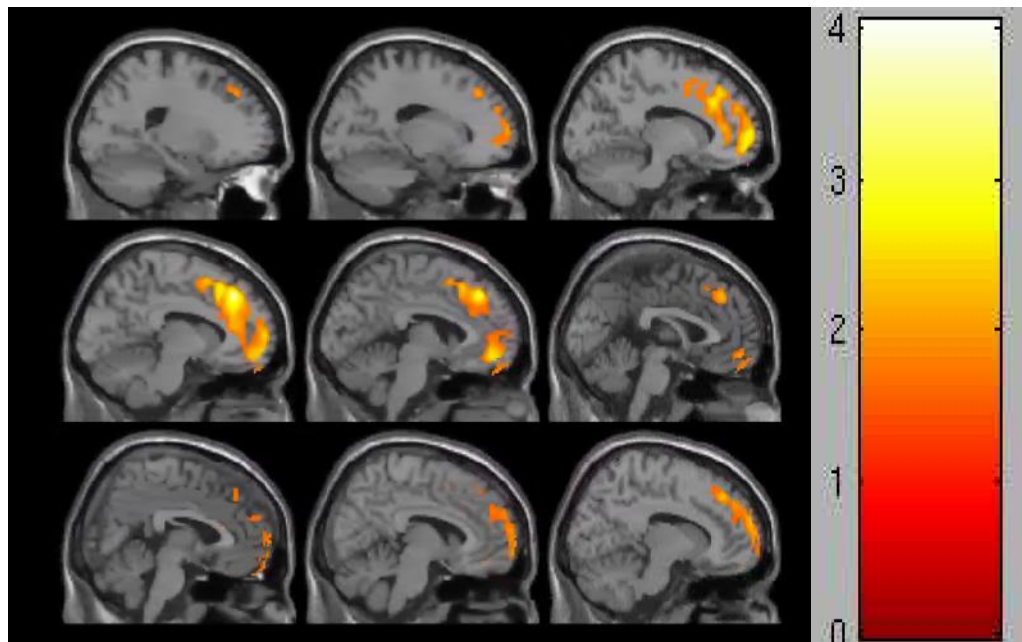


Figure 23. Increased rCBF in the premonitory phase compared to baseline at whole brain level (Sagittal sections). Figure 21, 22 and 23 show increased rCBF over the right and left superior frontal, medial frontal, bilateral anterior cingulate with peak increase over the left medial frontal gyrus ($P < 0.001$) at a whole brain analysis level. The colour bar on the right indicates the colour coding of the T-scores.

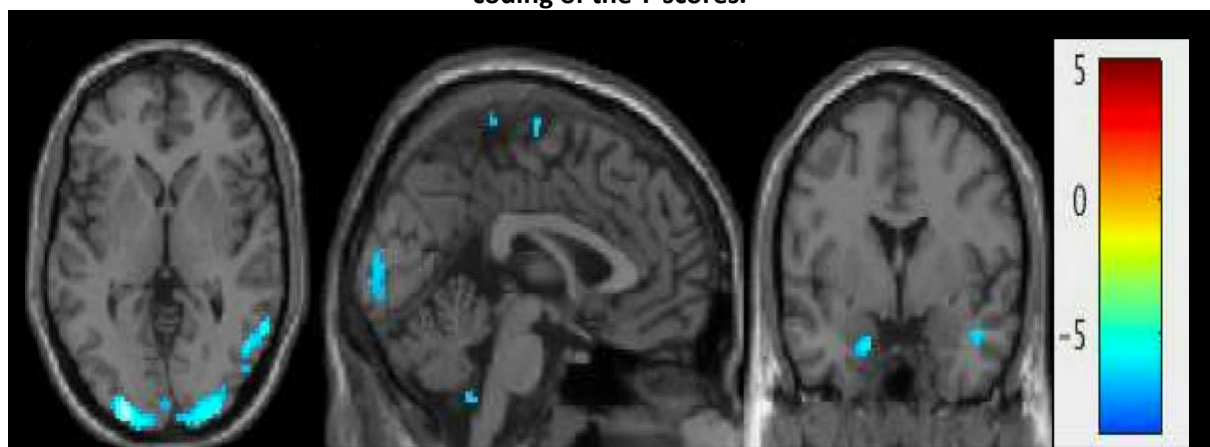


Figure 24. Reductions in rCBF the premonitory phase compared to baseline at a whole brain level. (Axial, sagittal and coronal sections) over the right middle occipital gyrus, right middle temporal gyrus, lingual gyrus and cuneus, with a peak reduction over the right inferior occipital gyrus ($P = 0.018$). The colour bar on the right indicates the colour coding of the T-scores.

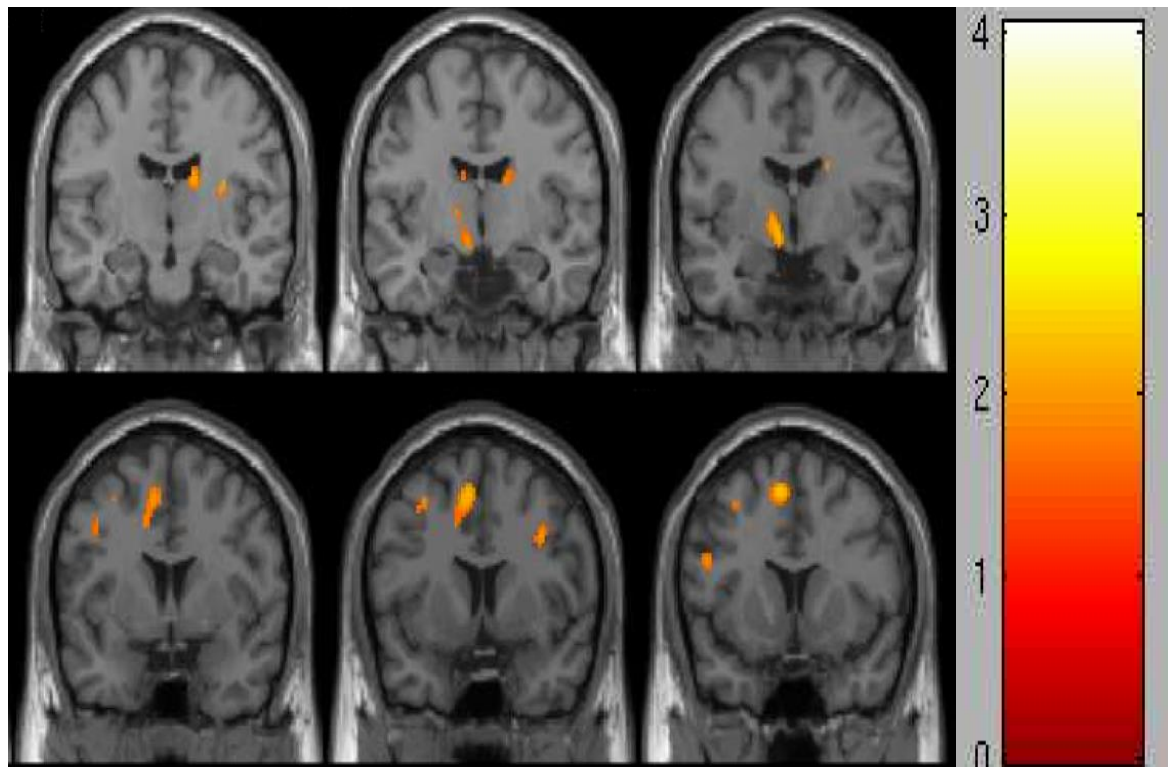


Figure 25. Increased rCBF in the premonitory phase compared to baseline following small volume correction (Coronal sections). The colour bar on the right indicates the colour coding of the T-scores.

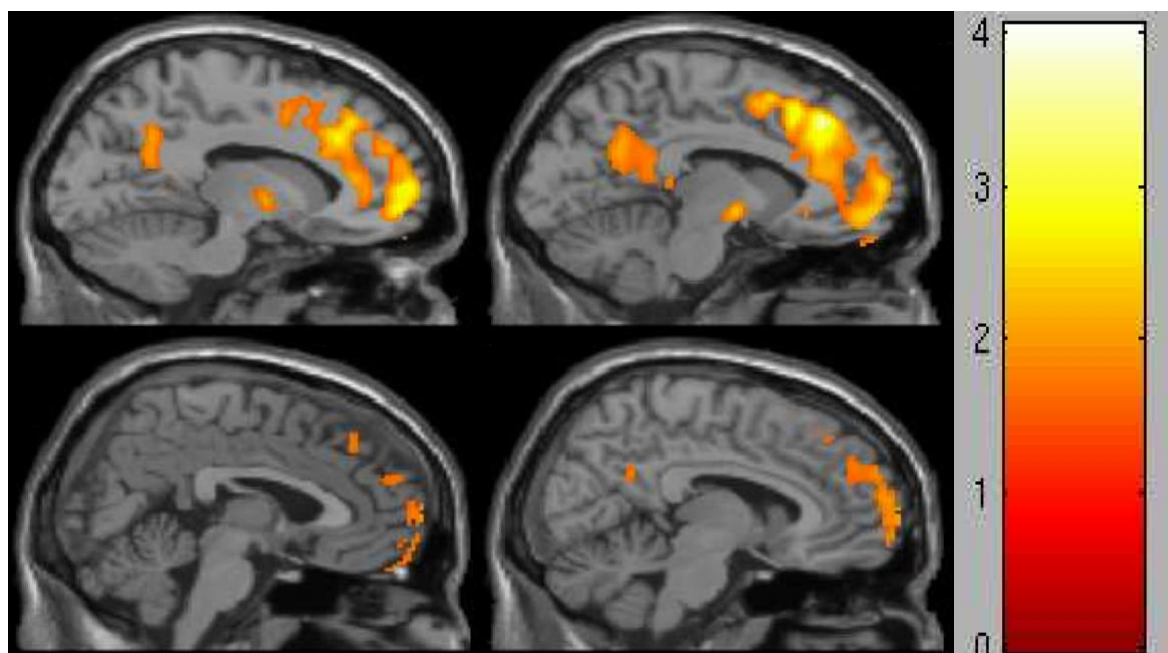


Figure 26. Increased rCBF in the premonitory phase compared to baseline following small volume correction (Coronal sections). Figures 25 and 26- Following small volume correction, (sphere set at 12 mm radius volume of Interest, VOI), additional areas of increased rCBF along with those seen at a whole brain level analysis, were picked up over the posterior cingulate and posterior hypothalamus with a peak increase over the posterior hypothalamus ($P=0.046$). The colour bar on the right indicates the colour coding of the T-scores.

6.1.2 Baseline scans compared to migraine headache scans:

There was increased rCBF over the medial frontal gyrus, superior frontal gyrus, inferior frontal gyrus, midbrain, hypothalamus, thalamus and cingulate gyrus with peak increase over the right superior frontal gyrus ($P=0.001$) at a whole brain analysis level, in the migraine headache phase compared to baseline scans. No further significant changes were observed following small volume correction.

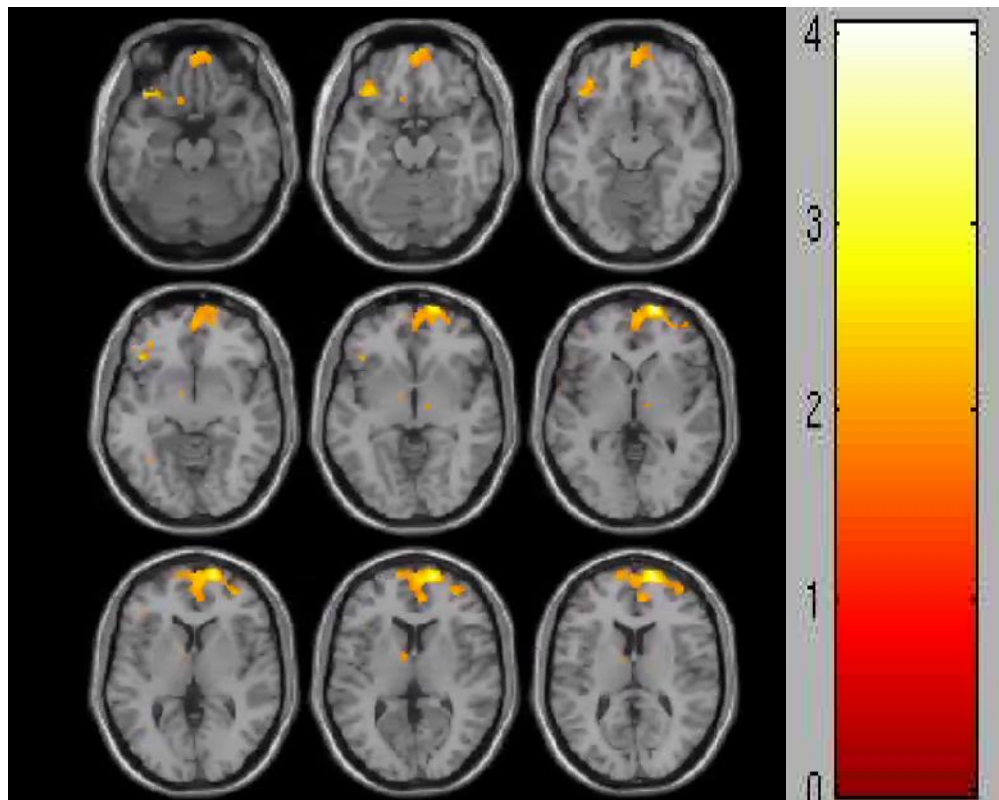


Figure 27. Increased rCBF in the migraine headache phase compared to baseline at whole brain level (axial sections). The colour bar on the right indicates the colour coding of the T-scores.

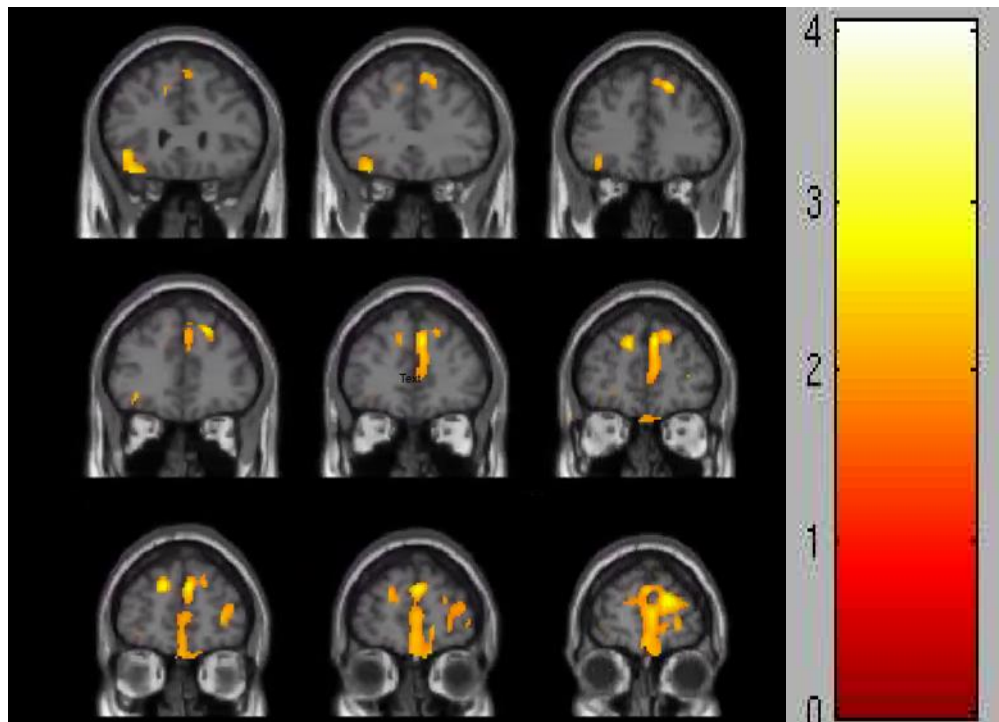


Figure 28. Increased rCBF in the migraine headache phase compared to baseline at whole brain level (Coronal sections). The colour bar on the right indicates the colour coding of the *T*-scores.

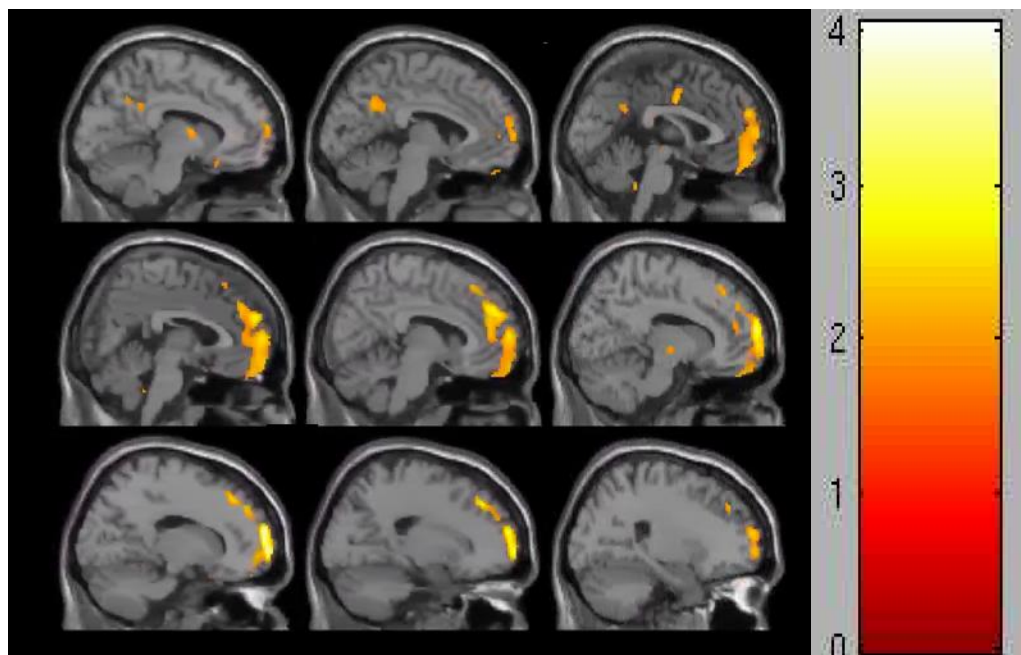


Figure 29. Increased rCBF in the migraine headache phase compared to baseline at whole brain level (Sagittal sections). Figures 28, 29 and 30 show increased rCBF over the medial frontal gyrus, superior frontal gyrus, inferior frontal gyrus, midbrain, hypothalamus, thalamus and cingulate gyrus with peak increase over the right superior frontal gyrus ($P=0.001$) compared to baseline scans. The colour bar on the right indicates the colour coding of the *T*-scores.

6.1.3 Baseline scans compared to postdrome phase scans:

There was a reduction in rCBF over the superior temporal gyrus, middle temporal gyrus, superior frontal, medial frontal gyrus, Insula, precentral gyrus, inferior frontal gyrus, inferior temporal gyrus, lingual gyrus, cingulate gyrus with peak reduction over the Left precentral gyrus ($P < 0.001$) at a whole brain analysis level in the postdrome phase compare to baseline. Small volume correction did not yield additional areas. No increase in rCBF were observed.

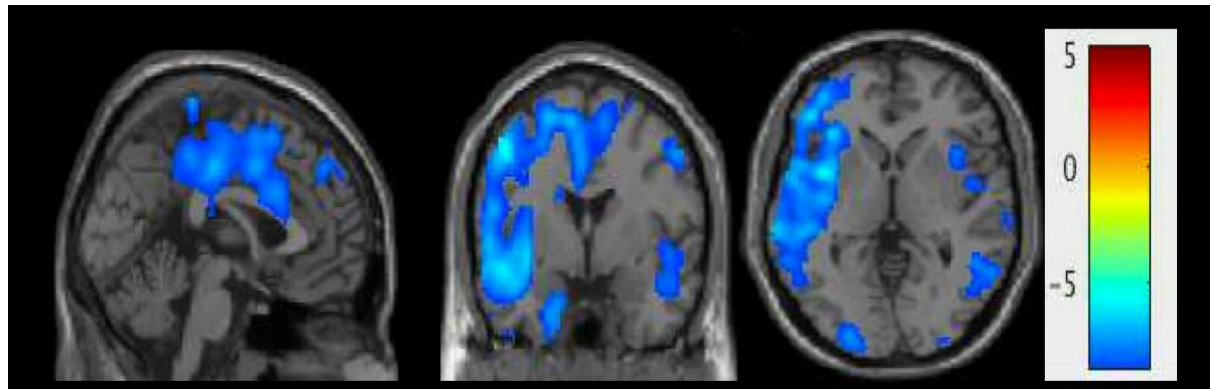


Figure 30. Reduction in rCBF in the postdrome phase compared to baseline at whole brain level and following small volume correction. (Sagittal, coronal and axial sections). Figures show reduction in rCBF over the superior temporal gyrus, middle temporal gyrus, superior frontal, medial frontal gyrus, Insula, precentral gyrus, inferior frontal gyrus, inferior temporal gyrus, lingual gyrus, cingulate gyrus with peak reduction over the Left precentral gyrus ($P < 0.001$) at a whole brain analysis level. Small volume correction did not yield additional areas. The colour bar on the right indicates the colour coding of the T -scores.

6.1.4 Postdrome scans compared to premonitory scans:

There was a reduction in rCBF over the superior frontal gyrus, medial frontal gyrus, middle frontal gyrus, putamen, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, thalamus, hypothalamus, midbrain, posterior cingulate, anterior cingulate, claustrum and a peak reduction in rCBF over the left superior temporal gyrus ($P<0.001$) at a whole brain analysis level in the postdrome phase compared to premonitory phase. Small volume correction showed additional areas of reduction in rCBF over the frontal medial orbital gyrus, Insula, caudate, with peak reduction in rCBF over the left medial globus pallidus ($P=0.027$, sphere set at 12mm radius of VOI) along with areas with reductions seen in rCBF at a whole brain level analysis. There was a preponderance of the reductions in rCBF to the left cerebral hemisphere, but this study was not designed to look at laterality of the changes.

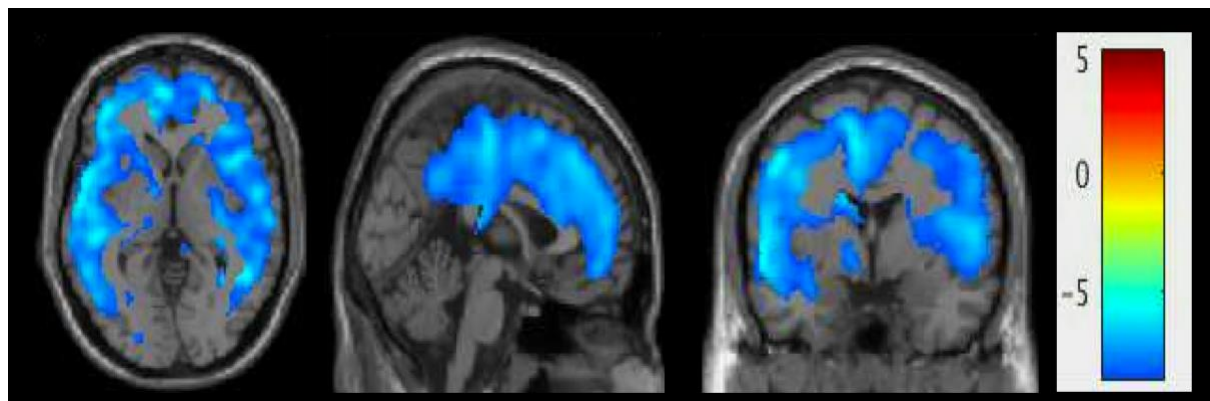


Figure 31. Reduction in rCBF in the postdrome phase compared to the premonitory phase at a whole brain level (Axial, sagittal and coronal sections). Figures 34 show reduction in rCBF over the superior frontal gyrus, medial frontal gyrus, middle frontal gyrus, putamen, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, thalamus, hypothalamus, midbrain, posterior cingulate, anterior cingulate, claustrum and a peak reduction in rCBF over the left superior temporal gyrus ($P<0.001$) at a whole brain analysis level in the postdrome phase compared to the premonitory phase. The colour bar on the right indicates the colour coding of the T -scores.

6.1.5 Postdrome scans compared with migraine headache:

There was a reduction in rCBF over the pons, midbrain, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, cingulate gyrus, precuneus, inferior parietal lobule, superior frontal gyrus, medial frontal, inferior frontal gyrus, precentral gyrus, post central gyrus and a peak reduction in rCBF over the left superior temporal gyrus ($P<0.001$) at a whole brain analysis level in the postdrome phase compared to migraine headache phase. Small volume correction did not yield any additional areas of change in rCBF.

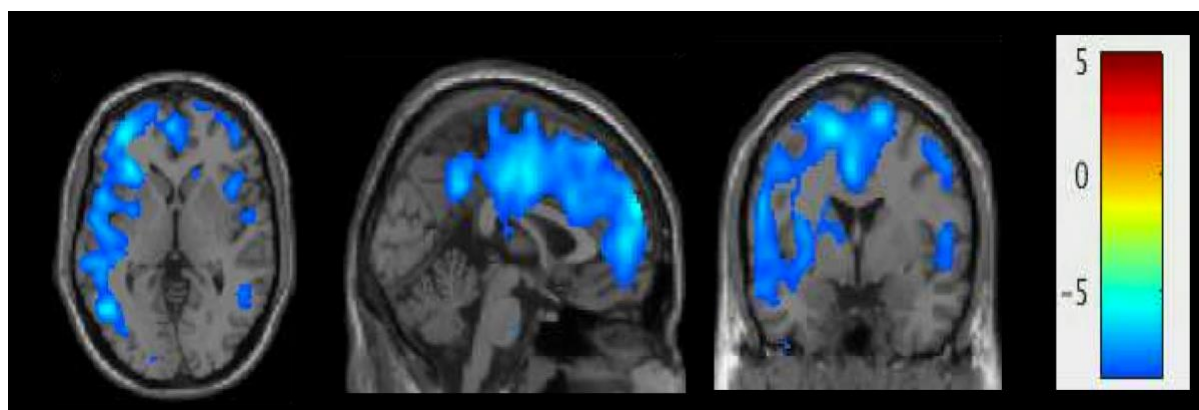


Figure 32. Reduction in rCBF in the postdrome phase compared to the headache phase at a whole brain (Axial, sagittal and coronal sections). Figures show a reduction in rCBF over the pons, midbrain, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, cingulate gyrus, precuneus, inferior parietal lobule, superior frontal gyrus, medial frontal, inferior frontal gyrus, precentral gyrus, post central gyrus and a peak reduction in rCBF over the left superior temporal gyrus ($P < 0.001$) at a whole brain analysis level in the postdrome phase compared to migraine headache phase. Small volume correction did not yield any additional areas of change in rCBF. The colour bar on the right indicates the colour coding of the T -scores.

6.1.6 Non-triggered (placebo) visit scan comparisons

Comparison of all scans done at time scale of migraine headache derived from screening visit, with all scans done at time scale of postdrome derived from screening visit following administration of aspirin/sumatriptan on the placebo visit (See Figure 20). Reduction in rCBF was seen in the posterior cingulate on the placebo visit at corresponding time to postdrome phase compared to migraine headache phase, from triggered visit but does not reach significance ($P = 0.142$).

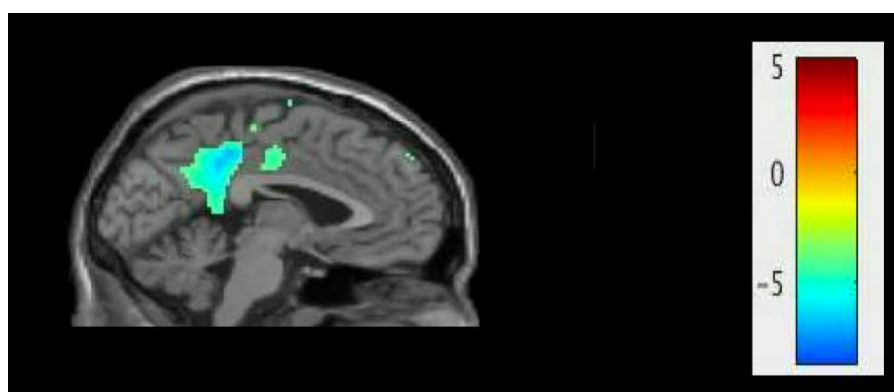


Figure 33. The corresponding time scale of migraine headache scan compared to the postdrome scan on the non-triggered placebo visit. Reduction in rCBF was seen in the posterior cingulate on the placebo visit at corresponding time to postdrome phase compared to migraine headache phase, from triggered visit but does not reach statistical significance ($P = 0.142$). The colour bar on the right indicates the colour coding of the T -scores.

Phase Compared	Peak Brain Regions Involved	MNI Co-ordinates			T-score	P-value, FDR corrected, significant <0.05
		x	y	z		
Baseline vs Premonitory Scan	Left medial frontal gyrus	-6	36	42	6.92	<0.001
Baseline vs Migraine Headache Scan	Right superior frontal gyrus	14	64	18	4.16	0.001

Table 14. MNI co-ordinates of areas with increased peak regional cerebral blood flow at whole brain level

Phase Compared	Peak Brain Regions Involved	MNI Co-ordinates			T-score	P-value, FWE corrected, significant <0.05
		x	y	z		
Baseline vs Premonitory Scan	Posterior Hypothalamus	-8	-4	-8	3.92	0.046

Table 15. MNI co-ordinates of areas with increased peak regional cerebral blood flow following small volume correction (with sphere set at 12mm radius of VOI)

Phase Compared	Peak Brain Regions Involved	MNI Co-ordinates			T-score	P-value, FDR corrected, significant <0.05
		x	y	z		
Baseline vs Premonitory Scan	Right inferior occipital gyrus	34	-86	-18	5.22	0.018
Baseline vs Postdrome Scan	Left precentral gyrus	-46	-6	50	9.55	<0.001
Migraine headache vs Postdrome Scan	Left superior temporal gyrus	-46	-54	16	8.78	<0.001
Premonitory vs Postdrome Scan	Left superior temporal gyrus	-12	34	26	8.66	<0.001

Table 16. MNI co-ordinates of areas with reduced peak regional cerebral blood flow at whole brain level

Phase Compared	Peak Brain Regions Involved	MNI Co-ordinates			T-score	P-value, FWE corrected, significant <0.05
		X	Y	Z		
Baseline vs Postdrome	Left precentral gyrus	-46	-6	50	9.55	<0.001
Premonitory vs postdrome	Left medial globus pallidus	-10	-2	0	3.89	0.027

Table 17. MNI co-ordinates of areas with reduction in peak regional cerebral blood flow following small volume correction (with sphere set at 12mm radius of VOI).

6.2 Region of interest (ROI) analysis

Certain key brain regions were selected for further investigation, based on previous functional imaging studies in migraine; and on the relevance of those functional areas for the perception of pain. The key brain areas include the hypothalamus, the pons, the midbrain, the thalamus, the anterior cingulate cortex and the insula (Maniyar *et al.*, 2014a; Hodgkinson *et al.*, 2015b; Schulte and May, 2016b). Mean CBF from these key areas during the baseline, premonitory, migraine headache and postdrome phases were extracted and paired *t*-test comparisons were done to see if there were any significant alterations in rCBF. Results were tested at a significance threshold < 0.05 .

6.2.1 Hypothalamus

No statistically significant changes in rCBF were seen in the hypothalamus with ROI analysis in the premonitory, migraine headache or postdrome phases.

Paired samples	Phases Compared	Mean rCBF	N	Std. Deviation	Std. Error mean	<i>p</i> -value
Pair 1	a. Baseline	46.49	16	6.80954	1.70238	.197
	b. Premonitory	48.18	16	8.32212	2.08053	
Pair 2	a. Baseline	46.49	16	6.80954	1.70238	.426
	b. Headache	47.26	16	6.77143	1.69286	
Pair 3	a. Baseline	46.49	16	6.80954	1.70238	.894
	b. Postdrome	46.63	16	6.16587	1.54147	
Pair 4	a. Headache	48.18	16	8.32212	2.08053	.173
	b. Postdrome	46.63	16	6.16587	1.54147	
Pair 5	a. Premonitory	47.26	16	6.77143	1.69286	.373
	b. Postdrome	46.63	16	6.16587	1.54147	

Table 18. ROI analysis hypothalamus

6.2.2 Pons

No statistically significant changes in rCBF was seen in the pons with ROI analysis in the premonitory, migraine headache or postdrome phases.

Paired samples	Phases Compared	Mean rCBF	N	Std. Deviation	Std. Error mean	<i>p</i> -value
Pair 1	a. Baseline	44.4368	16	7.01249	1.75312	.788
	b. Premonitory	44.0424	16	10.28714	2.57178	
Pair 2	a. Baseline	44.4368	16	7.01249	1.75312	.963
	b. Headache	44.4697	16	6.56484	1.64121	
Pair 3	a. Baseline	44.4368	16	7.01249	1.75312	.529
	b. Postdrome	43.8677	16	6.13102	1.53275	
Pair 4	a. Headache	44.4697	16	6.56484	1.64121	.367
	b. Postdrome	43.8677	16	6.13102	1.53275	
Pair 5	a. Premonitory	44.0424	16	10.28714	2.57178	.911
	b. Postdrome	43.8677	16	6.13102	1.53275	

Table 19. ROI analysis pons

6.2.3 Midbrain

No statistically significant changes in rCBF was seen in the midbrain with ROI analysis in the premonitory, migraine headache or postdrome phases.

Paired samples	Phases Compared	Mean rCBF	N	Std. Deviation	Std. Error mean	<i>p</i> -value
Pair 1	a. Baseline	48.0671	16	7.44398	1.86099	.265
	b. Premonitory	49.1182	16	9.40729	2.35182	
Pair 2	a. Baseline	48.0671	16	7.44398	1.86099	.736
	b. Headache	48.3470	16	8.01662	2.00415	
Pair 3	a. Baseline	48.0671	16	7.44398	1.86099	.758
	b. Postdrome	47.7573	16	7.01158	1.75289	
Pair 4	a. Headache	48.3470	16	8.01662	2.00415	.418
	b. Postdrome	47.7573	16	7.01158	1.75289	
Pair 5	a. Premonitory	49.1182	16	9.40729	2.35182	.253
	b. Postdrome	47.7573	16	7.01158	1.75289	

Table 20. ROI analysis midbrain

6.2.4 Left thalamus

No statistically significant changes in rCBF was seen in the left thalamus with ROI analysis in the premonitory, migraine headache or postdrome phases.

Paired samples	Phases Compared	Mean rCBF	N	Std. Deviation	Std. Error mean	p-value
Pair 1	a. Baseline	46.7497	16	7.81486	1.95372	.093
	b. Premonitory	48.5809	16	9.68338	2.42084	
Pair 2	a. Baseline	46.7497	16	7.81486	1.95372	.450
	b. Headache	47.3642	16	8.40972	2.10243	
Pair 3	a. Baseline	46.7497	16	7.81486	1.95372	.824
	b. Postdrome	46.5753	16	6.96955	1.74239	
Pair 4	a. Headache	48.5809	16	9.68338	2.42084	.067
	b. Postdrome	46.5753	16	6.96955	1.74239	
Pair 5	a. Premonitory	47.3642	16	8.40972	2.10243	.248
	b. Postdrome	46.5753	16	6.96955	1.74239	

Table 21. ROI analysis left thalamus

6.2.5 Right thalamus

No statistically significant changes in rCBF was seen in the right thalamus with ROI analysis in the premonitory, migraine headache or postdrome phases.

Paired samples	Phases Compared	Mean rCBF	N	Std. Deviation	Std. Error mean	p-value
Pair 1	a. Baseline	45.4107	16	7.24481	1.81120	.158
	b. Premonitory	46.9095	16	8.79185	2.19796	
Pair 2	a. Baseline	45.4107	16	7.24481	1.81120	.379
	b. Headache	46.3842	16	7.97035	1.99259	
Pair 3	a. Baseline	45.4107	16	7.24481	1.81120	.664
	b. Postdrome	45.8432	16	6.48616	1.62154	
Pair 4	a. Headache	46.3842	16	7.97035	1.99259	.543
	b. Postdrome	45.8432	16	6.48616	1.62154	
Pair 5	a. Premonitory	46.9095	16	8.79185	2.19796	.391
	b. Postdrome	45.8432	16	6.48616	1.62154	

Table 22. ROI analysis right thalamus

6.2.6 Anterior cingulate cortex

Statistically significant reduction in rCBF was seen in anterior cingulate cortex (ACC) during the postdrome phase compared to the migraine headache ($P=0.011$). The mean rCBF in the ACC during the migraine headache phase was 55 ± 8 ml/min/100ml tissue and mean rCBF in the ACC during the postdrome phase was 53 ± 7 ml/min/100ml tissue.

Statistically significant reductions in rCBF were seen in anterior cingulate cortex (ACC) during the postdrome phase compared to the premonitory phase ($P=0.002$). The mean rCBF in the ACC during the premonitory phase was 58 ml/min/100ml tissue ($SE \pm 9$) and mean rCBF in the ACC during the postdrome phase was 53 ml/min/100ml tissue ($SE \pm 7$).

Paired samples	Phases Compared	Mean rCBF	N	Std. Deviation	Std. Error mean	<i>p</i> -value
Pair 1	a. Baseline	54.5048	16	8.06952	2.01738	.066
	b. Premonitory	57.8716	16	9.32448	2.33112	
Pair 2	a. Baseline	54.5048	16	8.06952	2.01738	.709
	b. Headache	54.9251	16	7.92511	1.98128	
Pair 3	a. Baseline	54.5048	16	8.06952	2.01738	.115
	b. Postdrome	52.6568	16	6.70438	1.67609	
Pair 4	a. Headache	54.9251	16	7.92511	1.98128	.011
	b. Postdrome	52.6568	16	6.70438	1.67609	
Pair 5	a. Premonitory	57.8716	16	9.32448	2.33112	.002
	b. Postdrome	52.6568	16	6.70438	1.67609	

Table 23. ROI analysis anterior cingulate cortex

6.2.7 Insula

Statistically significant reductions in rCBF were seen in the Insula in the postdrome phase compared to the baseline ($P=0.03$). The mean rCBF in the Insula during the baseline phase was 57 ± 10 ml/min/100ml tissue and mean rCBF in the Insula during the postdrome phase was 54 ± 7 ml/min/100ml tissue.

Statistically significant reductions in rCBF were seen in the insula in the postdrome phase compared to the migraine headache phase ($P=0.009$). The mean rCBF in the Insula during the migraine headache phase was 57 ± 2 ml/min/100ml tissue and mean rCBF in the Insula during the postdrome phase was 54 ± 7 ml/min/100ml tissue.

Statistically significant reductions in rCBF were seen in the Insula in the postdrome phase compared to the premonitory phase ($P=0.002$). The mean rCBF in the Insula during the premonitory phase was 59 ml/min/100ml tissue ($SE \pm 10$) and mean rCBF in the Insula during the postdrome phase was 54 ml/min/100ml tissue ($SE \pm 7$).

Paired samples	Phases Compared	Mean rCBF	N	Std. Deviation	Std. Error mean	<i>p</i> -value
Pair 1	a. Baseline	56.7447	16	9.89417	2.47354	.307
	b. Premonitory	58.5857	16	9.80808	2.45202	
Pair 2	a. Baseline	56.7447	16	9.89417	2.47354	.857
	b. Headache	56.5265	16	9.02621	2.25655	
Pair 3	a. Baseline	56.7447	16	9.89417	2.47354	.030
	b. Postdrome	53.8688	16	7.24383	1.81096	
Pair 4	a. Headache	56.5265	16	9.02621	2.25655	.009
	b. Postdrome	53.8688	16	7.24383	1.81096	
Pair 5	a. Premonitory	58.5857	16	9.80808	2.45202	.002
	b. Postdrome	53.8688	16	7.24383	1.81096	

Table 24. ROI analysis Insula

CHAPTER 7

SEASONAL VARIATION IN NTG TRIGGERING

7 Seasonal variation in NTG Triggering

Since the introduction of intravenous NTG for triggering migraine, studies have shown that migraine can be induced in about 75% of migraineurs. No study has looked into the variability of NTG induced migraine in association with weather changes.

Weather has a profound influence on our day to day life. It has an effect on our function, clothing, commute to work and more importantly, on illnesses that may affect us. Seasonal variation has been reported in various neurological and psychological illnesses (Seregi *et al.*, 2016; Sipila *et al.*, 2016; Watad *et al.*, 2016; Wynchank *et al.*, 2016) . Why there should a seasonal variation in illnesses has not been fully elucidated but several factors either on their own or in combination can be considered. Firstly, temperature has an effect on nerve conduction velocity perhaps by differential altering of the kinetics of sodium/potassium channel gating mechanisms (Kiernan *et al.*, 2001). Secondly, differential seasonal expression of genes may affect human immunity and physiological functions (Dopico *et al.*, 2015). We already know that weather influences the attacks or symptoms of various disorders including neurological disorders. For example, patients with multiple sclerosis can get worsening symptoms in the heat (Uhthoff's phenomenon) (Tsolaki *et al.*, 2011).

The hypothalamus may be a driver in the circannual and circadian periodicity seen in various disorders especially trigeminal autonomic cephalalgias, though this is not established (Holle *et al.*, 2011). Also, neuroimaging studies have shown activation of the hypothalamus even before the onset of headache in migraine (Maniyar *et al.*, 2014a; Schulte and May, 2016b) and hence it is conceivable that the hypothalamus could be a key driver in the initiation and also maintenance of periodicity in various pain states.

Seasonal variation in migraine attack frequency has been reported (Caperell and Pitetti, 2014; Shin *et al.*, 2015; Pakalnis and Heyer, 2016) . There may be a select group of migraineurs sensitive to temperature changes associated with weather and also, the impact maybe more with certain types of migraine, like migraine with aura rather than migraine without aura (Alstadhaug *et al.*, 2005).

We carried out a subgroup analysis of subjects taking part in the POSTD imaging study, to see if the weather had any effect on NTG triggered migraine. There were 46 subjects included in this analysis. Sixteen subjects completed all the imaging study visits. However, there were subjects who had underwent NTG triggered visits and were at various stages of the study or did not have a migraine

triggered by NTG. The period of this subgroup analysis started from April 2015 and ended in November 2016.

7.1 Methods

46 subjects were included in this subgroup analysis. There was a total of 82 NTG triggering visits within this subgroup ($n = 46$; mean age 35 years, range 18-49 years, 38 females).

Based on simplicity and consistency, the Met Office, UK, meteorologically divides seasons over three monthly intervals. March, April and May constitute Spring. June, July and August constitute Summer. September, October and November constitute Autumn. December, January and February constitute Winter.

7.2 Results

7.2.1 NTG triggering in Spring

This period covers NTG triggering visits between April-May 2015 and April-May 2016. There was a total of 16 NTG triggered visits. Subjects successfully had a triggered migraine in 12 visits and did not trigger in four visits. The NTG trigger success rate was 75%.

7.2.2 NTG triggering in Summer

This period covers NTG triggering visits between June-August 2015 and June-August 2016. There was a total of 26 NTG triggered visits. Subjects successfully had a triggered migraine in 25 visits and did not trigger during one visit. The NTG trigger success rate was 96%.

7.2.3 NTG triggering in Autumn

This period covers NTG triggering visits between September-November 2015 and September-November 2016. There was a total of 28 NTG triggered visits. Subjects successfully had a triggered migraine in 25 visits and did not trigger in 3 visits. The NTG trigger success rate was 89%.

7.2.4 NTG triggering in Winter

This period covers NTG triggering visits between December-February 2015. There was a total of 13 NTG triggered visits. Subjects successfully had a triggered migraine in 4 visits and did not trigger in 9 visits. The NTG trigger success rate was 31%.

The overall number of NTG trigger visits across all the weathers was 82. Subjects did not have a migraine triggered by NTG in 17 visits. The net positive NTG trigger rate was 79% (65/82).

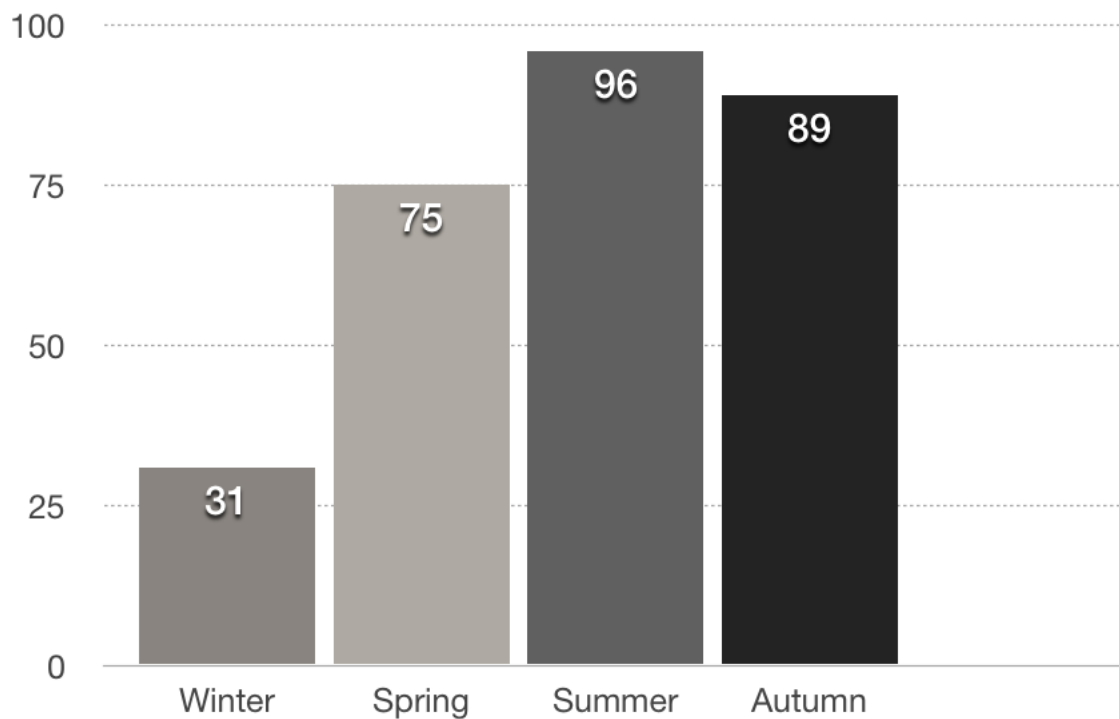


Figure 34. NTG triggering rates in Winter, Spring, Summer and Autumn as a percentage.

A generalised estimating equations analysis was conducted using SPSS version 23 to predict if there was any effect of season on NTG triggering rates. The Wald Chi square test demonstrated that summer has a statistically significant effect on NTG triggering rates compared to winter ($P=0.034$). Exp(B) value indicates that when NTG triggering occurs in summer as compared to winter, the odds ratio is 12.5. Hence summer increases NTG trigger rate by a factor of 12.5 when compared to winter. (Wald $\chi^2 = 5.1$, $P = 0.034$, with $df = 1$). Spring did not have a statistically significant effect on NTG triggering rates compared to winter (Wald $\chi^2 = 0.39$, $P = 0.53$, with $df = 1$). Autumn did not have a statistically significant effect on NTG triggering rates compared to winter (Wald $\chi^2 = 1.49$, $P = 0.22$, with $df = 1$).

7.3 Discussion

This data clearly shows that NTG trigger rates are lowest during winter. The reasons behind this need further exploration. Perhaps weather changes have a profound effect on the metabolism of NTG impacting its nitric oxide (NO) donation, or there may be another seasonally induced variance such as hydration, mood, etc. that causes these differences. Another possibility is that weather changes can affect the conduction of nerve impulses, leading to a slowdown during winter of the propagation of molecular cascades triggered by NTG. Not all subjects may be susceptible, but there could be a select group of subjects who are exquisitely sensitive to weather changes when it comes to NTG induced migraine.

This data may have profound implications for the conduction of future migraine research studies by means of NTG triggering. Some of the possible issues to consider are:

1. The issue of wasted resources for the subjects and physicians involved, when a subject does not trigger a migraine attack during cooler weather.
2. It can drive up the cost of research projects due to wasted visits.
3. It may lead to prolongation of migraine provocation model based research studies and the non-triggered subjects will need to be replaced.
4. There may also be weather-related effects on the speed of headache resolution after treatment with sumatriptan or aspirin, which are also worth further investigation.

Further studies specifically designed to assess the impact of weather on NTG triggering, controlling for potential confounding factors like preventive medications, migraine frequency etc., need to be done to assess this further. It would be interesting to assess if there is a difference in brain activations depending on the weather with a designated imaging protocol. This knowledge would be vital to plan and allocate resources accordingly in both imaging based and non-imaging based migraine provocation studies.

CHAPTER 8

DISCUSSION

8 Discussion

8.1 The migraine postdrome clinical audit

Whether patients experienced the postdromal phase or not was well set out in the clinic records. This is an indication that clinicians are asking about this phase following the headache pain. It must be noted though that this may be because the case notes assessed in this audit were from a tertiary neurology referral centre and the physicians involved in the clinic under the supervision of Professor Goadsby are likely to be more aware of the postdrome. Also, the patients referred to the centre may have a more frequent attacks or severe migraine attacks. This audit also cannot conclusively state that a postdrome or postdromal symptoms were not present in the non-postdrome group because even though they did not explicitly appear in the clinic records, there is still a chance that during clinic the postdrome phase was not addressed due to various factors like recall bias and lack of postdromal symptoms entry in patient headache diaries (Quintela *et al.*, 2006). The issues can be addressed by prospective data collection and an electronic prospective diary study of postdrome symptoms was published after this clinical audit was undertaken (Giffin *et al.*, 2016). Underlying all of the questions about postdrome is the fact that some patients are not aware of the postdromal phase, as a distinct phase describing the post headache experience. This may be due to them not experiencing this phase, or more likely they have not heard of this terminology and therefore have not been able to name their experience after the headache has dissipated. Therefore, without being specifically asked by their neurologist, they may not volunteer symptoms. Once they are prompted to speak about their experience following headache subsidence, they are able to distinguish between these two phases. One being the true headache pain and the other being perhaps a duller head discomfort with various other non-headache related symptoms, but nonetheless, just as, if not a more debilitating phase of feeling exhausted and 'hung-over'. This was highlighted by Kelman (Kelman, 2006) who narrated one description of the 827 patients in the cohort: 'The calm, after the storm'. Due to this lack of awareness, not all postdrome is reported, even when asked. Additionally, in rare cases where attacks were very frequent, the distinguishing of episodic and chronic migraine was problematic in terms of dissecting the postdrome from premonitory symptoms. This does bring into question whether similar brain areas are active in both these phases, given the striking resemblance of many of the symptoms in both these phases. Disability and postdrome duration were not well recorded in the clinic records. This is an indication for neurologists to ask more specifically about the postdrome duration and disability aspects to aid better management plans and also to gauge the wider extent of the problem. Better knowledge of

the extent of the problem and its impact will guide future research into its pathophysiology and possible therapeutic interventions.

There is scarcity of papers in the medical literature regarding the postdrome and much could be achieved with prospective studies and careful clinical observations. Population based studies are essential to understand the wider impact of the problem. Clear classification is an essential requirement to guide future research into the condition. Incorporation of this phase into clinical trials may help to understand the wider potential of therapeutic agents because although treatments like triptans may relieve headache symptoms, it appears that they do not alter the fundamental brainstem mechanisms responsible for the initiation and propagation of migraine (Weiller *et al.*, 1995; Akerman *et al.*, 2011). Understanding the pathophysiology behind the postdrome may hold the key in understanding the overall biology of migraine and to manage symptoms of patients better.

8.2 THE MIGRAINE POSTDROME ARTERIAL SPIN LABELED (ASL) MRI IMAGING STUDY (POSTD)

This study has revealed the activations of key brain areas like the superior frontal, medial frontal, anterior cingulate, posterior cingulate and posterior hypothalamus in the premonitory phase do not remain persistently activated in the postdrome phase. Though these structures appear integral to initiation and propagation of neural activations which could potentially modulate inhibitory effect on the trigeminocervical complex (TCC) in a top-down manner, the findings from this study suggest other mechanisms responsible for postdrome symptoms. Activation of the hypothalamus in the premonitory phase has been demonstrated in previous studies both with PET (Maniyar *et al.*, 2014a) and BOLD MRI studies (Schulte and May, 2016b), which shows the consistency of ASL technique and the reliability of the involvement of this brain area. The role of the hypothalamus in the initiation of migraine is clearly a subject of further research. It also remains to be elucidated whether activation of hypothalamus is specific to the migraine process or whether it can occur in other pain conditions, heralding the onset of acute severe pain by merely being a component of nociceptive pathways.

The common symptoms experienced by subjects in the premonitory phase during the study include neck stiffness, fatigue, photophobia, concentration difficulty, thirst, nausea, yawning, movement sensitivity, phonophobia, increased urinary frequency, irritability, speech difficulty, visual blurring, depressed mood, gastric discomfort, elevated mood, and food craving. Hypothalamic activation may explain some of the symptoms by virtue of its dopaminergic connections and possibly related to reduction in vasopressin (Melis and Argiolas, 1999; Krowicki and Kapusta, 2011). Involvement of the frontal brain regions may explain the various other symptoms experienced in the premonitory phase like concentration difficulty, irritability, speech difficult, and mood changes (Thompson-Schill *et al.*, 2009).

This study showed that there was increased rCBF over the frontal gyrus, midbrain, hypothalamus, thalamus and cingulate gyrus during the migraine headache phase. The brain areas activated in the migraine headache phase have similarly been seen in previous neuroimaging studies of both spontaneous and provoked migraine models (Weiller *et al.*, 1995; Afridi *et al.*, 2005b; Kato *et al.*, 2010). Previous voxel-based morphometry studies in migraine subjects, have shown significant gray matter reduction in brain areas that are thought to be involved in pain modulation such as the temporal gyrus, parietal Operculum, frontal gyrus, and precentral gyrus. Also, chronic migraineurs were shown to have a reduction in gray matter mainly in the ACC (Kim *et al.*, 2008; Valfre *et al.*, 2008). This study was not designed to assess the reduction of grey matter but has picked up some of the relevant pain structures key to migraine headache as described by previous studies. Whether

repeated attacks will have any effect on grey matter alterations in rCBF or structural integrity remains to be studied with ASL MRI with a designated protocol.

The common postdrome symptoms reported by subjects in this study cohort were: tiredness, concentration difficulty, head discomfort, neck stiffness, hangover, generalised weakness, feeling of relief, photophobia, elevated mood, malaise, thirst, movement sensitivity, cranial allodynia, eye discomfort, internal vertigo, nausea, phonophobia, gastric discomfort, depressed mood, nasal stuffiness, feeling of dread and feeling drunk. Some of the symptoms complained by subjects in this phase were similar to the ones reported in the premonitory stage and hence it would be reasonable to assume that the brain activations in both these phases maybe similar. However, this study has conclusively shown that postdrome symptoms are associated with near global reduction in rCBF over the superior frontal gyrus, medial frontal gyrus, middle frontal gyrus, putamen, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus, thalamus, hypothalamus, midbrain, posterior cingulate, anterior cingulate, claustrum, with a peak reduction in rCBF over the left superior temporal gyrus at a whole brain analysis level in the postdrome phase compared to the premonitory phase. There was a preponderance of the reductions in rCBF to the left cerebral hemisphere, but this study was not designed to look at laterality of the changes. What this has demonstrated for the first time in literature, is that, the symptoms experienced in the postdrome phase are related to a near global reduction in rCBF and there was no brain area found to be activated in this stage. The multitude of symptoms experienced in this phase can now be explained by a near global brain shut down as it tries to 're-boot' itself from a migraine headache attack. It is worth stressing that this near-global reduction is independent of the effects of the treatment as evidenced by the results obtained from the non-triggered visit (See Figure 33). This reduction is also present even when the global CBF over the whole brain is added as a covariate, showing the significance of the perfusion characteristics of the substrates involved.

Global reduction in CBF has been shown to be associated with chronic fatigue syndrome, a complicated and controversial disease where fatigue/tiredness is a prominent feature (Yoshiuchi *et al.*, 2006; Biswal *et al.*, 2011). An ASL study looking into chronic fatigue syndrome found reduction in CBF in a global distribution especially involving the frontal, parietal and temporal regions (Yoshiuchi *et al.*, 2006; Biswal *et al.*, 2011). However, given the prolonged duration of fatigue in chronic fatigue syndrome as opposed to postdrome, the pathophysiological basis for both these conditions are likely to be different (Clayton, 2015). Moreover, in the postdrome phase additional areas were involved like the insula, lingual gyrus and cingulate gyrus were involved when baseline scans were compared

to postdrome, putamen, thalamus, hypothalamus, midbrain and posterior cingulate when postdrome scans were compared to premonitory scans. This indicates involvement of a different and widespread neural network in postdrome as opposed to chronic fatigue syndrome. CBF changes associated with mood changes, robustly induced in healthy adolescents has been reported. The authors reported increased rCBF in the limbic regions following happy mood induction procedures (Mikita *et al.*, 2015). The POSTD study has shown significant involvement of limbic structures in the postdrome that can explain some of the mood changes reported in the postdrome. To further study the neural networks associated with mood changes in the postdrome, it would require a designated protocol and careful patient selection of those having prominent mood changes within the postdrome phase.

The pathophysiological basic of the postdrome, hence cannot be explained by persistent activation of similar networks seen in the postdrome phase and other mechanisms need to be considered. The locus coeruleus is a brainstem noradrenergic nucleus located in the dorsal pontine tegmentum. This nucleus provides the major source of norepinephrine (NE) to the cerebrum, brainstem, cerebellum and spinal cord. The existence of reciprocal circuits between this nucleus, the neocortex, diencephalon, limbic system, and spinal cord emphasise its widespread impact within the neuraxis (Mai and Paxinos, 2012). The locus coeruleus noradrenergic system is one of the first systems that gets involved during a stressful event. It is involved in a broad range of physiological and psychological events like pain processing, behavioural modification and stress reactivity (McCall *et al.*). Functional imaging studies have shown activation of the dorsal pons in premonitory and migraine headache phases (Weiller *et al.*, 1995; Afridi *et al.*, 2004a; Afridi *et al.*, 2005c; Maniyar *et al.*, 2014a). This activation might include the locus coeruleus (Maniyar *et al.*, 2014a), leading to widespread vasoconstriction mediated by an α_2 -adrenoceptor mechanism (Goadsby *et al.*, 1982, 1983, 1985). The near global reductions in rCBF seen in the postdrome can be explained by widespread vasoconstriction via α_2 -adrenoceptor mechanism through activation of brain stem nuclei. This may serve as a pain modulatory mechanism but as a consequence, lead to the protean postdromal symptoms resulting from a near global reduction in rCBF.

Another mechanism that can potentially explain the reductions in rCBF in the postdrome is the phenomenon of cortical spreading depression (CSD). This bioelectrical phenomenon was first described by Leao (AAP, 1944; Leao, 1947) who demonstrated a wave of spreading suppression of spontaneous EEG activity when electrically stimulating rabbit cortex. CSD usually silences spontaneous and evoked electrical activity for 5-15 minutes (AAP, 1944). However, in certain

pathophysiological states like hypoglycaemia, hypoxia and ischemia, CSD can occur spontaneously and can be prolonged in nature (Kraig and Nicholson, 1978). There is increased susceptibility to CSD when astroglial function is hampered (Largo *et al.*, 1997). Electrophysiological studies demonstrate that in migraineurs, a cortical and possibly subcortical dysfunction may explain increased susceptibility to CSD (Schoenen *et al.*, 2003; Lauritzen *et al.*, 2011). CSD is preceded by a fast network of oscillations (AAP, 1944) suggesting brief hyperexcitability (AAP, 1944). This is followed by complete suppression of neuronal activity, lasting several minutes, followed by complete recovery (Leao, 1947). Hadjikhani *et al.*, used functional imaging to support CSD as the generator of migraine aura (Hadjikhani *et al.*, 2001). Persistent hypoperfusion following CSD has been demonstrated and hence corroborates the notion that the perfusion changes of migraine may be pathophysiologically related to spreading depression (Lauritzen, 1984). The CSD phenomenon can potentially explain the neuronal activations initially seen in the premonitory and migraine headache phase followed by the persistent near global hypoperfusion seen in the postdrome. However, this is an area that needs further research.

8.3 Cognitive assessments in the POSTD Imaging study

The numbers used to study attention in this study are small, but within the timeframe of this MD project; and given the recruitment resources available, it was not possible to increase it. Larger numbers may be needed to get a statistically significant difference in assessing the postdrome phase using the SART test. Nevertheless, the test is based on sound principles and avoids practice and order effect. Future studies specifically designed to assess attention in postdrome phase using the SART test maybe needed to objectively assess attention.

The number of patients used to study sleepiness in this study, once again is small. The scale has not been used to assess sleepiness in the various migraine states before. From previous studies, the typical KSS values for healthy alert individuals would be between 3 and 4. Various factors like, the time of the day, medications, disease states, stress etc. can affect the scores (Akerstedt *et al.*, 2014). In this study, the median KSS score (8) during the postdrome phase was significantly higher ($P=0.01$) than the median baseline KSS score (3). This would fit with the common clinical scenario and as often seen in clinical studies into the postdrome, where sleepiness is commonly reported. The scale is easy to self-administer by subjects and is quick to perform. This scale holds promise as a reliable and simple tool to assess sleepiness over the various phases of migraine.

Fatigue in the postdrome had a statistically significant effect on reduction of workload, motivation, decision making and limitation of physical activity. The DFIS is useful to assess the short-term impact of fatigue in the postdrome phase of migraine and larger studies specifically designed to assess fatigue may provide further evidence of reliability and reproducibility of this scale in assessing fatigue.

CHAPTER 9

CONCLUSIONS

9 CONCLUSIONS

9.1 Migraine postdrome clinical audit

The data convincingly demonstrates that the postdromal phase has a profound impact on migraineurs within our clinical cohort. The disability related to migraine is not limited to the headache phase and includes the postdrome. Although the presence of the postdrome was regularly asked about in clinic, its duration was not. Duration should be routinely recorded going forward. While over half of the postdrome sufferers experienced at least one cranial autonomic symptom during their migraine, none were documented during the postdrome. This may be due to lack of direct questioning regarding presence of autonomic symptoms in the postdrome phase. Understanding pathophysiology of the migraine postdrome will inform how the brain behaves as the attack subsides and may inform interventions to shorten its duration. Functional neuroimaging may give us further clues regarding the neural networks involved in the postdrome to understand the biology of the postdrome better and the improve management of migraineurs.

9.2 POSTD Imaging study

ASL MRI is a promising imaging technique to understand the neural processes involved in migraine. Due to its non-invasive character, it offers several practical advantages like repeatability, safety, complete avoidance of nuclear tracers, less requirement of a sophisticated infrastructure like PET and the ability to make quantitative assessments.

The study shows that the symptoms experienced in the postdrome phase are associated with a near total reduction in rCBF and not due to activation of similar areas as those seen in the premonitory phase, thereby disproving my original working hypothesis which proposed that the activations brain regions seen in the premonitory phase would remain activated, thus giving rise to the postdrome symptoms. The biology behind this can potentially be explained by widespread vasoconstriction through a α_2 -adrenoceptor mechanism or CSD. Clearly this is an area that needs further research to explore the underlying mechanisms and neural networks involved.

A significant finding is that the neural activity in regions normally associated with pain perception, starts before the onset of throbbing headache (i.e. in the premonitory phase) and persists beyond the cessation of the throbbing headache (i.e. the postdrome phase) whereas the mainstay of acute migraine treatments traditionally focusses on the cessation of the throbbing pain. The throbbing pain may represent only the tip of the iceberg when it comes to migraine symptomatology. We must broaden our scope of treatment beyond the onset and cessation of the throbbing pain, and target the neural activity, which may have a bigger impact on symptom resolution in migraine i.e.- targeting the root cause rather than the tip of the problem. One can anticipate that the brain activations detected during the course of a migraine attack, particularly during the symptomatic but pain free stages before and after the throbbing headache cessation, may be an appropriate target for therapeutics research.

9.3 Study limitations

The limitations of this study can be broadly discussed under the following categories:

1. Limitations of the imaging technique used (i.e. ASL MRI)
2. Limitations in the study design.

ASL has an intrinsic low signal to noise ratio (SNR) though PCASL offers the best SNR. The relative sensitivity to motion artefacts is high (Petcharunpaisan *et al.*, 2010; Grade *et al.*, 2015). ASL does not give information regarding cerebral blood volume or mean transit time. So it is currently not possible to say whether alterations in rCBF with ASL could be attributed to either higher cerebral blood volume or increased mean transit time or both (Pollock *et al.*, 2009). Improvements in ASL techniques are likely to produce better spatial and temporal resolutions in the future (Zhang *et al.*, 2014).

One could argue whether the spontaneous and triggered migraines are one and the same (See Page 27). Clinically, and radiologically there are several similarities of triggered and spontaneous migraine attacks. What happens at a molecular level remains to be elucidated and may help differentiate if indeed the attacks are similar or not.

The lack of a control arm in the study design (healthy volunteers receiving nitroglycerin) leads to the question, what proportion of the observed alterations in CBF are related to NTG. Although it would be useful to have such a control arm, it is unlikely that the alterations in rCBF are due to nitroglycerin because of the short half-life of nitroglycerin and because similar alterations in migraine headache phase have been observed in spontaneous migraine attacks in previous studies.

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APPENDIX B - INCLUSION AND EXCLUSION CRITERIA FOR STUDY

Inclusion criteria for the study

Subjects needed to meet all the following inclusion criteria to be eligible for enrolment into the study:

1. Subjects had to be willing to travel to and from King's College Hospital, London, during the course of a day for the study visits.
2. Subjects had to have a diagnosis of migraine with or without aura, as per the ICHD-3 beta criteria (Headache Classification Committee of the International Headache, 2013).
3. Subjects were aged between 18-50 years. Subjects over 50 years were not included in the study to minimise any atherosclerotic changes in the cerebral blood vessels affecting the regional cerebral blood flow (Powers and Zazulia, 2010) as age is a risk factor for atherosclerotic disease (Cefalu and Wagner, 1997).

Exclusion criteria for study

Presence of any of the following factors meant that the subjects were excluded from the study:

1. If a subject had a previous history of an allergic response to NTG, sumatriptan or aspirin.
2. If a subject was on concurrent anticoagulation with warfarin or a novel anticoagulant.
3. If a subject was on current treatment with methotrexate.
4. If a subject had a history of past or current upper gastrointestinal haemorrhage or gastrointestinal ulceration.
5. If a subject had a history of thrombocytopenia or haemorrhagic diathesis.
6. If a subject gave a history of significant vomiting as part of their migraine attacks.
7. If a subject had a ICHD3 beta criteria (Headache Classification Committee of the International Headache, 2013) diagnosis of medication overuse headache.
8. If a subject had continuous or daily headache as it would not be possible to administer NTG to trigger a migraine and will also make it difficult to interpret the alterations in cerebral blood flow.
9. If a subject was pregnant or breastfeeding, they were excluded.
10. If a subject had active menstruation at the time of the study visit, this was rescheduled.
11. If a subject had aortic stenosis or significant hypotension (systolic blood pressure <90mmHg or <100mmHg and symptomatic) precluding intravenous NTG infusion.
12. If a subject had obstructive airways disease with previous exacerbation post aspirin, cardiac failure, renal failure or liver failure.

13. If a subject used NSAIDs, aspirin or paracetamol analgesia within 24 hours of each visit, then the study visit was rescheduled.
14. If a subject used regular (greater than 10 days per month) tramadol, codeine or other opioid drugs.
15. Subjects who smoked more than five cigarettes a day or drank more than six cups of caffeinated drinks a day were excluded.
16. Subjects with history of significant mental health disorders were excluded.
17. Any person unable to lie still within the environment of the fMRI scanner for the required period to perform the study were excluded.
18. When subjects had MRI contraindications (metal implants, pacemaker, brain aneurysm clips etc.), they were excluded. Subjects with titanium jaw plate were excluded as it would affect the quality of the images.
19. Any subject unable to understand and follow my instructions were excluded.
20. Any subject with a history or current use of drugs of abuse were excluded.
21. Shift workers with altered circadian rhythm were excluded from the study.
22. Any other condition that in the opinion of the investigator would make the subject unsuitable for the study were excluded.