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Fluctuating regional brainstem diffusion imaging measures of microstructure across the migraine cycle

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Authors: Kasia K. Marciszewski[1], Noemi Meylakh[1], Flavia Di Pietro[1], Vaughan G. Macefield[2], Paul M. Macey[3] and Luke A. Henderson[1]

[1]Department of Anatomy and Histology, Sydney Medical School, University of Sydney, Sydney, NSW, Australia, 2006; [2]School of Medicine, Western Sydney University, Sydney, Australia; [3]UCLA School of Nursing and Brain Research Institute, University of California, Los Angeles, California 90095, United States.

Corresponding author: Luke A. Henderson, Department of Anatomy and Histology, F13, University of Sydney, Australia. lukeh@anatomy.usyd.edu.au (email); +612 9351 7063 (Tel) +612 9351 6556 (Fax)

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ABSTRACT

The neural mechanisms responsible for the initiation and expression of migraines remain unknown. Though there is growing evidence of changes in brainstem anatomy and function between attacks, very little is known about brainstem function and structure in the period immediately prior to a migraine. The aim of this investigation is to use brainstem-specific analyses of diffusion weighted images to determine if the brainstem pain processing regions display altered structure in individuals with migraine across the migraine cycle, and in particular immediately prior to a migraine. Diffusion tensor images (29 controls, 36 migraineurs) were used to assess brainstem anatomy in migraineurs compared with controls. We found that during the interictal phase, migraineurs displayed greater mean diffusivity in the region of the spinal trigeminal nucleus, dorsomedial/dorsolateral pons and midbrain periaqueductal gray matter/cuneiform nucleus. Remarkably, the mean diffusivity returned to controls levels during the 24-hour period immediately prior to a migraine, only to increase again within the three following days. Additionally, fractional anisotropy was significantly elevated in the region of the medial lemniscus/ventral trigeminal thalamic tract in migraineurs compared with controls over the entire migraine cycle. These data show that regional brainstem anatomy changes over the migraine cycle, with specific anatomical changes occurring in the 24 hours prior to onset. These changes may contribute to the activation of the ascending trigeminal pathway by either an increase in basal traffic or by sensitising the trigeminal nuclei to external triggers, with activation ultimately resulting in perception of head pain during a migraine attack.

SIGNIFIANCE STATEMENT

It has been hypothesised that modulation of brainstem pain pathways may be critical for the initiation of migraine attacks. There is some evidence that altered brainstem function, possibly involving increased astrocyte activation, occurs immediately prior to a migraine

attack. We sought to obtain evidence to support this theory. Using diffusion tensor imaging, we found that immediately prior to a migraine, mean diffusivity decreased in the spinal trigeminal nucleus, dorsomedial/dorsolateral pons and midbrain periaqueductal gray matter/ nucleus cuneiform. Mean diffusivity then increased again immediately following the migraine attack. Decreased mean diffusivity before a migraine is consistent with increased astrocyte activation, since astrocyte processes enlarge during activation. These changes may underlie changes in brainstem function that are essential for the generation of a migraine.

INTRODUCTION

Migraine is a common, debilitating disorder characterised by headaches and often accompanied by aura, nausea, and sensitivity to light and sound. Despite these well-characterised symptoms, the exact mechanisms underlying the initiation and maintenance of migraine head pain are still hotly debated. To date, human brain imaging investigations have revealed that during a migraine attack, activity increases in brain regions such as the cingulate cortex, insula, thalamus, hypothalamus and dorsal pons (Weiller *et al.* 1995; Bahra *et al.* 2001; Denuelle *et al.* 2004; Afridi *et al.* 2005). In addition, a number of studies have identified anatomical, sensitivity and resting activity pattern changes between migraine attacks, i.e. in the interictal period (Chong & Schwedt 2015; Mathur *et al.* 2016; Chong *et al.* 2017; Porcaro *et al.* 2017; Marciszewski *et al.* 2018a). These findings highlight the apparent brain dysfunction in migraineurs even when in a pain-free state.

A recent review has proposed that these observed changes in brain function are not stable, but dynamic in nature (May 2017). Some have suggested that functional brain changes actually trigger a migraine from basal firing (Goadsby & Akerman 2012). Others have suggested that the brain fluctuates between a state where the effectiveness of endogenous analgesic mechanisms is too great to allow incoming noxious inputs to evoke head pain, and a state where incoming inputs can activate central pathways and evoke head pain (Akerman *et al.* 2011; Borsook & Burstein 2012). Consistent with these hypotheses, it has recently reported that during the interictal phase, migraineurs display reduced grey matter density and increased free water movement within brainstem pain-modulating regions including the midbrain periaqueductal gray matter (PAG), dorsolateral pons (dlPons), medullary raphe and spinal trigeminal nucleus (SpV) (Marciszewski et al., 2018a). Furthermore, it was recently shown that immediately prior to a migraine resting infra-slow oscillatory activity increases in these same brainstem regions and returns to controls levels shortly after the migraine and it was speculated that these oscillatory changes may result from transient increases in astrocyte activation and its associated gliotransmission (Meylakh

et al. 2018). Given there is some evidence that astrocytes may play a role in aspects of migraine such as the propagation of cortical spreading depression (Nedergaard *et al.* 1995) and that a genetic form of migraine, Familial Hemiplegic Migraine, is associated with astrocyte dysfunction (Benarroch 2005), it is not unreasonable to suggest that astrocytes may also play a critical role in migraine pathophysiology via actions within the brainstem.

It was recently reported that pain sensitivity to noxious stimuli in migraineurs is dramatically decreased in the 24 hour period prior to a migraine, and this decrease is associated with increased functional magnetic resonance imaging (fMRI) signal intensity within the SpV and reduced PAG-SpV connectivity (Marciszewski *et al.* 2018b). This altered brainstem function may result from altered neural-glial interactions, though evidence of astrocyte activation in migraineurs, particularly in the period immediately prior to a migraine is lacking. This is likely due to the fact that it is not possible to predict when an individual will have a migraine and thus examining them in the 24-hour period before an attack is exceptionally difficult. Local free water movement, as measured by diffusion tensor imaging, is a method by which microstructural alterations can be examined in living humans. Such diffusion measures can be altered by numerous processes that change local anatomy, including oedema, inflammation, demyelination and alterations in cell numbers and/or morphology. Furthermore, astrocyte activation, which is associated with their processes enlarging considerably, would result in a decrease in local free water movement.

The aim of this investigation is to use diffusion tensor imaging (DTI) to determine if the brainstem displays microstructural alterations throughout the migraine cycle. More specifically we aim to explore changes in the 24-hour period prior to a migraine. We hypothesise that immediately prior to a migraine, mean diffusivity will decrease, consistent with an increase in astrocyte size, in areas of the brainstem that process and modulate pain such the PAG, dlPons, medullary raphe and SpV. Furthermore, that decrease will be reversed during the period immediately following a migraine and return to interictal levels.

METHODS

Subjects

Thirty-six subjects with migraine (8 males, mean [±SEM] age: 30.6 ±1.7 years) and 29 age and gender matched pain-free controls (5 males, age: 31.9±2.3 years) were recruited for the study. All subjects were recruited from the general population using an advertisement. Migraine subjects were diagnosed according to the criteria laid out by the International Classification of Headache Disorders (ICHD), 3rd edition, sections 1.1 and 1.2 (ICHD-3β 2013) Seven migraineurs reported aura associated with their migraines and the remaining 29 reported no aura. Of the 36 migraineurs, 31 were scanned during the interictal period (7 males, age 30.1±1.9 years), that is, between 72 hours after and 24 hours prior to a migraine attack; 13 during the 24-hour period immediately prior to a migraine (4 males, age 26.0±2.4 years), and 15 within the 72-hour period following a migraine (4 males, age 31.5±2.5 years). For subjects scanned prior to an attack, there was no predicting factor that they were within 24 hours of a migraine. Eleven migraineurs were scanned during the interictal period and period immediately prior to a migraine. In addition, 7 of these 10 subjects were also scanned during the period immediately after a migraine.

All migraine subjects indicated the pain intensity (6-point visual analogue scale; 0 = no pain, 5 = most intense imaginable pain) and facial distribution (drawing) of pain they commonly experience during a migraine attack. Each subject described the qualities of their migraines and indicated any current treatments used to prevent or abort a migraine once started. Exclusion criteria for controls were the presence of any current pain or chronic pain condition, current use of analgesics, and any neurological disorder. Exclusion criteria for migraineurs were any pain condition other than migraine, and any other neurological disorder. Informed written consent was obtained for all procedures according to the Declaration of Helsinki 7th revision and local Institutional Human Research Ethics Committees approved the study.

144

145 ***MRI acquisition***

146 Subjects lay supine on the bed of a 3T MRI scanner (Philips Achieva, Neuroscience
147 Research Australia, Sydney) with their head immobilised in a fitting 32-channel head coil.
148 With all subjects relaxed and at rest, in each subject a high-resolution T1-weighted
149 anatomical image set covering the entire brain was collected (turbo field echo; field of view
150 250x250mm, matrix size=288x288, slice thickness=0.87mm, repetition time=5600ms, echo
151 time=2.5ms, flip angle 8°). Following this, two high-resolution DTI image sets covering the
152 entire brain were collected using a single-shot multisection spin-echo echo-planar pulse
153 sequence (repetition time=8788ms; flip angle=90°, matrix size 112x112, field of view
154 224x224mm, slice thickness=2.5mm, 55 axial slices). For each slice, diffusion gradients
155 were applied along 32 independent orientations with b=1000s/mm^2 after the acquisition of
156 five b=0 s/mm^2 (b0) images. Two DTI acquisitions were averaged to improve signal-noise
157 ratios.

158

159 ***Image analysis***

160 *DTI analysis*

161 Using SPM12 software (Friston *et al.* 1994), the two DTI image sets from each
162 subject were realigned based on the b0 images, and the diffusion tensors calculated from
163 the images using a linear model (Basser & Pierpaoli 1996). Mean diffusivity (MD), axial
164 diffusivity, radial diffusivity and fractional anisotropy (FA) whole-brain maps were then
165 derived and co-registered to each individual subject's T1-weighted image. Using brainstem-
166 specific isolation software (SUIT toolbox)(Diedrichsen 2006), a unique mask of the brainstem
167 was manually created for each subject's MD map. Using these masks, the brainstem was
168 isolated from the MD, axial diffusivity, radial diffusivity and FA maps, spatially normalised, re-

sliced to the SUIT template in Montreal Neurological Institute (MNI) space, and spatially smoothed using a 3 mm full-width-at-half-maximum (FWHM) Gaussian filter.

Statistical analyses

Using a voxel-by-voxel analysis, significant differences in MD and FA values were determined between (i) controls (n=29) and migraineurs during the interictal period (n=31), (ii) controls and migraineurs during the period immediately *prior to* a migraine (n=13), and (iii) controls and migraineurs during the period immediately *following* a migraine (n=15; all comparisons $p<0.05$, false discovery rate corrected at a voxel level, minimum cluster size 5 contiguous voxels). Age and gender were included as nuisance variables and a brainstem mask that excluded cerebrospinal fluid as well as the cerebellum was applied to each analysis.

Since we found significant MD increases during the interictal period that were eliminated immediately prior to a migraine, we extracted MD values from those significant clusters for all three migraine periods. Significant MD differences between controls and the period immediately prior to and following migraine were then determined for these clusters (two-tailed, two-sample t-test, $p<0.05$). In addition, the axial diffusivity, radial diffusivity and FA values from each of the significant clusters were extracted and plotted and significant differences between controls and each migraine period as well as between migraine periods themselves were determined (two-tailed, two-sample t-test, $p<0.05$). We also found a cluster of significant FA change that was present during all three migraine phases. We extracted the MD, axial diffusivity, radial diffusivity and FA values from significant cluster of the control versus interictal analysis for all three migraine periods and determined significance relative to controls and between migraine phases (two-tailed, two-sample t-test, $p<0.05$). Significant MD and FA differences between controls and the interictal period were not assessed a

second time since these were already established as significant with the voxel-based statistics, thus avoiding the issue of "double-dipping".

To explore changes in individual migraineurs throughout the migraine cycle, in 11 migraineurs that were scanned during more than one period, we plotted MD and FA values for each significant cluster identified in the original group analysis. Significant MD and FA differences between each of the three migraine periods were then determined using paired t-tests (two-tailed $p<0.05$). Additionally, for all migraineurs, MD and FA values relative to the time until next migraine were plotted and a line of best fit applied for each significant cluster determined in the original group analysis to explore whether MD and FA increased or decreased as a migraine event approached. Finally, for each cluster, significant relationships between MD and FA and migraine characteristics were determined (Pearsons correlation, $p<0.05$).

RESULTS

Migraine Characteristics

Using a self-report questionnaire, migraineurs reported the most common location of their migraines over the past 12 months. In 12 of the 36 migraineurs, headaches were more common on the right side, in six they were more common on the left, and in the remaining 18 they were most often bilateral. Migraine subjects most frequently described their migraine pain as "throbbing", "pulsating", and/or "sharp", in nature. They indicated that "stress", "lack of sleep", and "dehydration" most often triggered migraine attacks. The mean (±SEM) estimated frequency of migraine attacks was 1.3±0.1 per month, mean length of time since the onset of migraine attacks (years suffering) 16.2±1.9 years, and mean pain intensity of migraines was 3.8±0.1 on a 6-point visual analogue scale. Although 24 of the 36 migraineurs were taking some form of daily medication (mostly the oral contraceptive pill), none of the migraine subjects was taking prophylactic medication prescribed for migraine.

Diffusivity measures

Group comparisons:

The DTI analysis revealed that compared to controls, migraineurs show regional differences in brainstem MD throughout the migraine cycle (Figure 1, Table 1). Consistent with our previous report, during the interictal period migraineurs showed increased MD compared with controls in regions encompassing the left SpV, left dlPons, right dorsomedial/dlPons and the region of the midbrain PAG and including the region of the cuneiform nucleus (CNF) (Figure 1, Table 1). Strikingly, this MD increase during the interictal period was absent during the 24-hour period prior to a migraine, with no significant difference between controls and migraineurs in this period. The MD increase then returned to above control levels in the dm/dlPons and PAG/CNF in the 72-hour period immediately following a migraine. Extraction of MD values from the clusters displaying a significant increase during the interictal period confirmed this pattern of MD change, i.e., MD increases during the interictal, no MD difference immediately prior to migraine and MD increase again immediately following a migraine (Figure 2, Table 2). In no brainstem region was MD significantly lower in migraineurs compared with controls.

Extraction of axial and radial diffusion values from these brainstem regions resulted in an interesting pattern of change (Table 2). Within the SpV, as with MD, both axial and radial diffusivity was significantly increased only during the interictal phase and within the PAG/CNF axial and radial diffusivity was significantly increased during the interictal phase but unlike MD, it was not increased during the phase immediately following a migraine. Interestingly, within the left and right dlPons and dmPons regions, whilst axial diffusivity was significantly increased during the interictal and immediately following migraine phases, radial diffusivity was not significantly different during any migraine phase compared with controls and FA was not different during either phase or compared with controls.

Analysis of FA revealed a different pattern of change to that of the other diffusion measures. Migraineurs displayed a significant increase in FA in the area encompassing the ventral trigeminothalamic tract/ventral tegmental area (VTT/VTA) during all three phases (Figure 1). Extraction of FA values from this region confirmed that FA was significantly increased in migraineurs and that this increase was apparent during all migraine phases (Figure 2, Table 2).

Individual migraineur comparisons:

Plots of MD values in the 11 migraineurs that were scanned during at least two of the three migraine periods revealed that the pattern of MD changes was consistent in individual subjects. That is, MD was lower immediately prior to a migraine compared with both the interictal and immediately following a migraine periods (Figure 3). Whilst the left dlPons did not show a significant difference between phases, paired t-tests revealed that the left SpV, right dlPons and left PAG/CNF displayed significantly reduced MD during the phase immediately prior to a migraine compared with both the interictal and immediately following a migraine phases. More specifically, of the 11 migraineurs, MD decreased immediately prior to a migraine compared to the interictal period in 10 migraineurs within the left SpV, left PAG/CNF and right dm/dlPons and in 9 migraineurs in the left dlPons. Additionally, of the 7 migraineurs scanned during all three phases, all showed a MD decrease immediately prior to a migraine compared with both the interictal and immediately after a migraine within the left SpV, and 5 migraineurs for the left dlPons, right dm/dlPons and PAG/CNF. In contrast to MD, consistent with the group analysis, FA was relatively consistent between the migraine phases although it was significantly increased during the immediately prior to compared with the immediately following migraine phase.

Furthermore, plots of MD and FA values over the migraine cycle revealed that within the SpV, right and left dlPons and PAG/CNF, MD appeared to remain relatively stable

across the interictal period before decreasing immediately prior to the migraine and then increasing again after the migraine (Figure 4). In contrast, the elevated FA in the VTT/VTA remained relatively stable across the migraine cycle and did not change immediately prior to a migraine. Finally, in all migraine subject groups, MD or FA values in these clusters during the interictal phase were not significantly correlated to migraine frequency (MD: left SpV r=0.03, left dlPons r=-0.13, right dm/dlPons r=0.15, left PAG/CNF: r=0.01; FA: VTT/VTA: r=0.09; all $p>0.05$), years suffering (MD: left SpV r=0.11, left dlPons r=-0.09, right dm/dlPons r=-0.30, left PAG/CNF r=0.14; FA: VTT/VTA r=0.14; all $p>0.05$), or the intensity of migraine pain (left SpV r=0.31, left dlPons r=-0.11, right dm/dlPons r=-0.03, left PAG/CNF r=0.09; FA: VTT/VTA r=-0.03; all $p>0.05$).

DISCUSSION

This study demonstrates that migraine is associated with changes in regional anatomy that fluctuate over the migraine cycle in a number of brainstem regions. More specifically, during the long interictal period, migraineurs display increased free water movement compared with controls in areas that process orofacial pain, such as the SpV, dlPons and PAG/CNF. Remarkably, immediately prior to a migraine attack, this increase in diffusivity reduces to control levels before increasing again in the period immediately following migraine. It is clear from these data that in episodic migraineurs, regional brainstem microstructural changes occur throughout the migraine cycle, and that there are specific anatomical changes in the 24 hours prior to onset.

A number of migraine studies have used DTI to identify anatomical changes in large fibre bundles such as the corpus callosum, internal capsule and corona radiata, although these studies did not explore changes in diffusion within grey matter or within fibre bundles in the brainstem (Neeb *et al.* 2015; Zhang *et al.* 2017). Whilst a previous investigation used a region of interest approach to find that migraineurs show greater MD compared to controls

within the red nucleus (Kara *et al.* 2013), no study has specifically explored the brainstem, particularly at different times over the migraine cycle. Consistent with a previous study, we found significant MD increases in SpV, dlPons and PAG/CNF (Marciszewski *et al.* 2018a) and furthermore, we show that during the 24-hour period immediately prior to a migraine, these structural changes disappear so that migraineurs are no different from controls in the preictal period. However, these structural changes then return during the 72-hour period following the migraine event. Furthermore, we found increased FA in the area encompassing the ascending VTT/VTA in migraineurs during all phases.

It has been argued by many that the headache period of migraine results from activation of trigeminal afferents in brain meninges and large cerebral arteries and these afferents terminate in the SpV and upper cervical dorsal horn (Liu *et al.* 2004, 2008; Olesen *et al.* 2009). Whilst the nature of the cellular changes underlying such diffusion changes is unclear, MD changes can be associated with oedema, vascular injury, inflammation, demyelination, cell count, and cellular morphology; as such our findings could therefore reflect several underlying biological changes (Alexander *et al.* 2007; Hutchinson *et al.* 2017). The dynamic nature of the changes reported here suggest that they reflect processes that are not permanent or static in nature, but that can instead change relatively rapidly, at least over the period of a day. Since MD changes can result from dynamic processes such as gliosis (Sierra *et al.* 2011), and there is evidence that migraine is associated with altered glial function (Nedergaard *et al.* 1995; Benarroch 2005), it is possible that the MD decreases immediately prior to a migraine result from astrocyte activation and associated expansion of astrocytic processes. Indeed, a recent preclinical epilepsy investigation linked microstructural changes in astrocytic processes with altered measures of diffusivity (Salo *et al.* 2017).

Consistent with the idea that migraine is associated with astrocyte activation, it was recently shown that immediately prior to a migraine, resting infra-slow oscillatory activity (0.03-0.06Hz) increases in these same brainstem regions (Meylakh *et al.* 2018). Astrocytes

can exhibit infra-slow calcium oscillations that can propagate among surrounding astrocytes and it has been proposed that in pathological situations, enhanced calcium-wave synchrony and amplitude may occur which can alter local neural function (Parri & Crunelli 2001; Crunelli *et al.* 2002; Cunningham *et al.* 2006; Halassa *et al.* 2007; Lorincz *et al.* 2009). This raises the prospect that immediately prior to a migraine, astrocyte activation results in decreased mean diffusivity and increased infra-slow oscillatory activity resulting in altered sensitivity within brainstem regions that receive and process orofacial noxious information. Whether such a sensitivity change is adequate to evoke head pain from basal levels of neural traffic or simply to facilitate an incoming trigger to activate higher brain centres to produce head pain remains to be determined.

The hypothesis that the brainstem pain processing sites become more sensitive as a migraine approaches was supported by Stankewitz and colleagues and Lee and colleagues, who reported that orofacial noxious and non-noxious stimuli evoked greater SpV signal intensity increases as a migraine attack approaches, although no significant change in the perceived intensity of orofacial stimuli (Stankewitz *et al.* 2011; Lee *et al.* 2017). Similarly, a recent study by Marciszewski and colleagues also reported that noxious stimuli evoked dramatic SpV activation increases, particularly in the 24-hour period prior to a migraine (Marciszewski *et al.* 2018b). However, despite the increase in SpV activation, during acute orofacial stimuli, individuals' reported pain intensity ratings decreased as a migraine approached. This appears at odds with the idea that brainstem pain-processing circuits become more sensitive as a migraine approaches although there are a number of potential explanations: i) since these data reveal that both the anatomy and function of brainstem pain processing circuits are dynamic, it is possible that these pathways may change again at the onset or during a migraine itself, ii) since preclinical studies have shown convergence of dural-sensitive and facial cutaneous afferents in SpV (Burstein *et al.* 1998; Ellrich *et al.* 1999), a decrease in noxious cutaneous afferent drive onto second-order convergent SpV neurons may result in an overall increase in dural afferent input sensitivity, iii) changes in

descending brainstem modulatory inputs onto the SpV may evoke a heightening of dural afferent input sensitivity at the expense of inputs from other orofacial structures. Whilst these ideas are highly speculative, it is unlikely that the alterations in SpV anatomy and function are involved in other functions to the same degree as the processing of orofacial noxious afferents.

Whilst our data imply that the processes involved in migraine attack onset may be astrocytic in nature, whether astrocyte activity is specifically driving migraine initiation or simply a symptom of another process cannot currently be discerned. The gradual increase in MD in brainstem pain-related regions over the interictal period suggests that changes are occurring throughout the interictal period that then dramatically reverse immediately prior to a migraine to control levels. Whilst brainstem functional measures such as SpV signal intensity changes and infra-slow oscillations were at control levels during the interictal period and dramatically increased immediately prior to a migraine, MD was above control levels during the interictal period and reduced to control levels prior to migraine. This implies that brainstem anatomy is not simply changing prior to a migraine but is altered throughout the long interictal period.

Several reports suggest reduced endogenous analgesic ability in migraine (Sandrini *et al.* 2006; de Tommaso *et al.* 2007) whilst others report no change (Perrotta *et al.* 2010; Teepker *et al.* 2014). This inconsistency may reflect subtle variations in endogenous analgesic responsiveness across the migraine cycle and it might be that endogenous analgesic ability gradually increases over the interictal period which is consistent with MD increases in pain-processing and modulating regions across the interictal period. Additionally, none of the regional microstructural changes we detected were correlated to migraine properties such as migraine frequency, intensity or duration, suggesting that the changes are not cumulative over time, and is consistent with the idea that they may be dynamic in nature. Whilst these results are in line with some migraine studies (Chen *et al.* 2016; Uggetti *et al.* 2017), others have reported significant linear relationships between

anatomical measures and migraine frequency (Kruit *et al.* 2009; Mainero *et al.* 2011), intensity (Russo *et al.* 2012) and years suffering (Liu *et al.* 2012; Schwedt *et al.* 2013; Rocca *et al.* 2014; Chong & Schwedt 2015); however, none of these studies explored the brainstem.

In addition to diffusion changes associated with brainstem nuclei, we found FA increases in migraneurs that encompassed the VTT/VTA. These FA increases were not dynamic and occurred at all migraine phases, indicated a constant underlying change, possibly in the ascending trigeminal pain pathway from SpV to the thalamus. Whilst it has been suggested that altered radial diffusion within a fibre tract may represent increases in response to demyelination (Song *et al.* 2005) and axial diffusivity to axonal damage (Song *et al.* 2003; Loy *et al.* 2007), we found no change in axial or radial diffusivity associated with the increase in FA; although it is unclear how accurately the directional diffusivities relate to specific pathologies. Furthermore, whilst we found primarily changes in axial and not radial diffusivity in brainstem nuclei, what these changes represent remains unknown, although it is not inconsistent with altered astrocyte activation.

There are a number of methodological and subject-related limitations of this study. The spatial resolution of human DTI is relatively low and thus it is difficult to precisely localize each brainstem cluster with respect to small brainstem nuclei. However, the location of each cluster was defined using brainstem atlases and placed the changes into context with respect to the existing human and preclinical research. Secondly, although we managed to scan 13 migraineurs immediately prior to a migraine, only 7 migraineurs were scanned during all three phases and we did not scan migraineurs during a migraine attack itself. However, we did used population-based statistics with thresholds corrected for multiple comparisons and although larger subject number are always preferable, we are confident our results are robust. Future studies in which the migraine phase itself was also explored would further increase our understanding of anatomical changes across the migraine cycle. Thirdly, this is a largely a cross-sectional study and mounting evidence of relatively rapid

brainstem changes, scanning individual migraineurs over the course of several weeks while measuring indices of brain anatomy, activity, and sensitivity would provide more precise evidence supporting this hypothesis. Furthermore, although the diffusivity changes reported here are dynamic in nature, i.e. over a period of days, it remains unknown if such changes are dynamic enough to be altered by transient activation of cell populations.

Overall, our findings suggest that migraine is associated with anatomical changes within brainstem structures involved in trigeminal noxious transmission and endogenous analgesia. More importantly, these anatomical changes alter over the migraine cycle specifically during the 24-hour period prior to a migraine attack. We speculate that these anatomical changes reflect astrocyte activation that alters local neural function by the release of gliotransmitters, which either trigger or alter the sensitivity of the brainstem so that an external trigger induces a migraine attack. Future investigations exploring brainstem resting activity, evoked activity and anatomy over the migraine cycle may provide evidence supporting such a proposal. If dynamic changes in brainstem function and structure do occur, we may be in a position to modify these changes and potentially prevent the triggering of a migraine attack.

REFERENCES

Afridi S.K., Giffin N.J., Kaube H., Friston K.J., Ward N.S., Frackowiak R.S. & Goadsby P.J. (2005) A positron emission tomographic study in spontaneous migraine. *Arch Neurol* **62**, 1270-5.

Akerman S., Holland P.R. & Goadsby P.J. (2011) Diencephalic and brainstem mechanisms in migraine. *Nat Rev Neurosci* **12**, 570-84.

Alexander A.L., Lee J.E., Lazar M. & Field A.S. (2007) Diffusion tensor imaging of the brain. *Neurotherapeutics* **4**, 316-29.

Bahra A., Matharu M.S., Buchel C., Frackowiak R.S. & Goadsby P.J. (2001) Brainstem activation specific to migraine headache. *The Lancet* **357**, 1016-7.

Basser P.J. & Pierpaoli C. (1996) Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B* **111**, 209-19.

Benarroch E.E. (2005) Neuron-astrocyte interactions: partnership for normal function and disease in the central nervous system. *Mayo Clin Proc* **80**, 1326-38.

Borsook D. & Burstein R. (2012) The enigma of the dorsolateral pons as a migraine generator. *Cephalalgia* **32**, 803-12.

Burstein R., Yamamura H., Malick A. & Strassman A.M. (1998) Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons. *J Neurophysiol* **79**, 964-82.

Chen Z., Chen X., Liu M., Liu S., Ma L. & Yu S. (2016) Nonspecific periaqueductal gray lesions on T2WI in episodic migraine. *J Headache Pain* **17**, 101.

Chong C.D., Plasencia J.D., Frakes D.H. & Schwedt T.J. (2017) Structural alterations of the brainstem in migraine. *Neuroimage Clin* **13**, 223-7.

Chong C.D. & Schwedt T.J. (2015) Migraine affects white-matter tract integrity: a diffusion-tensor imaging study. *Cephalalgia* **35**, 1162-71.

449 Crunelli V., Blethyn K.L., Cope D.W., Hughes S.W., Parri H.R., Turner J.P., Toth T.I. &
450 Williams S.R. (2002) Novel neuronal and astrocytic mechanisms in thalamocortical
451 loop dynamics. *Philos Trans R Soc Lond B Biol Sci* **357**, 1675-93.
452 Cunningham M.O., Pervouchine D.D., Racca C., Kopell N.J., Davies C.H., Jones R.S.,
453 Traub R.D. & Whittington M.A. (2006) Neuronal metabolism governs cortical network
454 response state. *Proc Natl Acad Sci U S A* **103**, 5597-601.
455 de Tommaso M., Difruscolo O., Sardaro M., Libro G., Pecoraro C., Serpino C., Lamberti P. &
456 Livrea P. (2007) Effects of remote cutaneous pain on trigeminal laser-evoked
457 potentials in migraine patients. *J Headache Pain* **8**, 167-74.
458 Denuelle M., Fabre N., Payoux P., Chollet F. & Geraud G. (2004) Brainstem and
459 hypothalamic activation in spontaneous migraine attacks. *Cephalalgia* **24**, 775-814.
460 Diedrichsen J. (2006) A spatially unbiased atlas template of the human cerebellum.
461 *NeuroImage* **33**, 127-38.
462 Ellrich J., Andersen O.K., Messlinger K. & Arendt-Nielsen L. (1999) Convergence of
463 meningeal and facial afferents onto trigeminal brainstem neurons: an
464 electrophysiological study in rat and man. *Pain* **82**, 229-37.
465 Friston K.J., Holmes A.P., Worsley K.J., Poline J.P., Frith C.D. & Frackowiak R.S. (1994)
466 Statistical parametric maps in functional imaging: a general linear approach. *Hum*
467 *Brain Mapp* **2**, 189-210.
468 Goadsby P.J. & Akerman S. (2012) The trigeminovascular system does not require a
469 peripheral sensory input to be activated--migraine is a central disorder. Focus on
470 'Effect of cortical spreading depression on basal and evoked traffic in the
471 trigeminovascular sensory system'. *Cephalalgia* **32**, 3-5.
472 Halassa M.M., Fellin T. & Haydon P.G. (2007) The tripartite synapse: roles for
473 gliotransmission in health and disease. *Trends Mol Med* **13**, 54-63.
474 Hutchinson E.B., Schwerin S.C., Avram A.V., Juliano S.L. & Pierpaoli C. (2017) Diffusion
475 MRI and the detection of alterations following traumatic brain injury. *J Neurosci Res*
476 **96**, 612-25.

ICHD-3β (2013) International Classification Committee of the International Headache Society: International Classification for Headache Disorders (ICHD)-3β Manual. *Cephalalgia* **33**, 629-808.

Kara B., Kiyat Atamer A., Onat L., Ulusoy L., Mutlu A. & Sirvanci M. (2013) DTI findings during spontaneous migraine attacks. *Clin Neuroradiol* **23**, 31-6.

Kruit M.C., Launer L.J., Overbosch J., van Buchem M.A. & Ferrari M.D. (2009) Iron accumulation in deep brain nuclei in migraine: a population-based magnetic resonance imaging study. *Cephalalgia* **29**, 351-9.

Lee J., Lin R.L., Garcia R.G., Kim J., Kim H., Loggia M.L., Mawla I., Wasan A.D., Edwards R.R., Rosen B.R., Hadjikhani N. & Napadow V. (2017) Reduced insula habituation associated with amplification of trigeminal brainstem input in migraine. *Cephalalgia* **37**, 1026-38.

Liu J., Zhao L., Li G., Xiong S., Nan J., Li J., Yuan K., Von Deneen K.M., Liang F., Qin W. & Tian J. (2012) Hierarchical alteration of brain structural and functional networks in female migraine sufferers. *PLoS one* **7**, ePub.

Liu Y., Broman J. & Edvinsson L. (2004) Central projections of sensory innervation of the rat superior sagittal sinus. *Neuroscience* **129**, 431-7.

Liu Y., Broman J. & Edvinsson L. (2008) Central projections of the sensory innervation of the rat middle meningeal artery. *Brain Res* **1208**, 103-10.

Lorincz M.L., Geall F., Bao Y., Crunelli V. & Hughes S.W. (2009) ATP-dependent infra-slow (<0.1 Hz) oscillations in thalamic networks. *PLoS One* **4**, e4447.

Loy D.N., Kim J.H., Xie M., Schmidt R.E., Trinkaus K. & Song S.K. (2007) Diffusion tensor imaging predicts hyperacute spinal cord injury severity. *J Neurotrauma* **24**, 979-90.

Mainero C., Boshyan J. & Hadjikhani N. (2011) Altered functional MRI resting-state connectivity in periaqueductal gray networks in migraine. *Ann Neurol* **70**, 838-45.

Marciszewski K.K., Meylakh N., Di Pietro F., Macefield V.G., Macey P.M. & Henderson L.A. (2018a) Altered brainstem anatomy in migraine. *Cephalalgia* **38**, 476-86.

504 Marciszewski K.K., Meylakh N., Di Pietro F., Mills E.P., Macefield V.G., Macey P.M. &
505 Henderson L.A. (2018b) Changes in brainstem pain modulation circuitry function over
506 the migraine cycle. *J Neurosci*.
507 Mathur V.A., Moayedi M., Keaser M.L., Khan S.A., Hubbard C.S., Goyal M. & Seminowicz
508 D.A. (2016) High frequency migraine is associated with lower acute pain sensitivity
509 and abnormal insula activity related to migraine pain intensity, attack frequency, and
510 pain catastrophising. *Front Hum Neuroci* **10**, 489.
511 May A. (2017) Understanding migraine as a cycling brain syndrome: reviewing the evidence
512 from functional imaging. *Neurological Sciences* **38 Supple 1**, 125-20.
513 Meylakh N., Marciszewski K.K., Di Pietro F., Macefield V.G., Macey P.M. & Henderson L.A.
514 (2018) Deep in the brain: Changes in subcortical function immediately preceding a
515 migraine attack. *Hum Brain Mapp* **39**, 2651-63.
516 Nedergaard M., Cooper A.J. & Goldman S.A. (1995) Gap junctions are required for the
517 propagation of spreading depression. *J Neurobiol* **28**, 433-44.
518 Neeb L., Bastian K., Villringer K., Gits H.C., Israel H., Reuter U. & Fiebach J.B. (2015) No
519 microstructural white matter alterations in chronic and episodic migraineurs: a case-
520 control diffusion tensor magnetic resonance imaging study. *Headache* **55**, 241-51.
521 Olesen J., Burstein R., Ashina M. & Tfelt-Hansen P. (2009) Origin of pain in migraine:
522 evidence for peripheral sensitisation. *Lancet Neurol* **8**, 679-90.
523 Parri H.R. & Crunelli V. (2001) Pacemaker calcium oscillations in thalamic astrocytes in situ.
524 *Neuroreport* **12**, 3897-900.
525 Perrotta A., Serrao M., Sandrini G., Burstein R., Sances G., Rossi P., Bartolo M., Pierelli F.
526 & Nappi G. (2010) Sensitisation of spinal cord pain processing in medication overuse
527 headache involves supraspinal pain control. *Cephalalgia* **30**, 272-84.
528 Porcaro C., di Lorenzo G., Seri S., Pierelli F., Tecchio F. & Coppola G. (2017) Impaired
529 brainstem and thalamic high-frequency oscillatory EEG activity in migraine between
530 attacks. *Cephalalgia* **37**, 915-26.

531 Rocca M.A., Messina R., Colombo B., Falini A., Comi G. & Filippi M. (2014) Structural brain
532 MRI abnormalities in pediatric patients with migraine. *J of Neurol* **261**, 350-7.
533 Russo A., Tessitore A., Giordano A., Corbo D., Marcuccio L., De Stefano M., Salemi F.,
534 Conforti R., Esposito F. & Tedeschi G. (2012) Executive resting-state network
535 connectivity in migraine without aura. *Cephalalgia* **32**, epub.
536 Salo R.A., Miettinen T., Laitinen T., Grohn O. & Sierra A. (2017) Diffusion tensor MRI shows
537 progressive changes in the hippocampus and dentate gyrus after status epilepticus in
538 rat - histological validation with Fourier-based analysis. *NeuroImage* **152**, 221-36.
539 Sandrini G., Rossi P., Milanov I., Serrao M., Cecchini A.P. & Nappi G. (2006) Abnormal
540 modulatory influence of diffuse noxious inhibitory controls in migraine and chronic
541 tension-type headache patients. *Cephalalgia* **26**, 782-9.
542 Schwedt T.J., Schlaggar B.L., Mar S., Nolan T., Coalson R.S., Nardos B., Benzinger T. &
543 Larson-Prior L.J. (2013) Atypical resting-state functional connectivity of affective pain
544 regions in chronic migraine. *Headache* **53**, 737-51.
545 Sierra A., Laitinen T., Lehtimaki K., Rieppo L., Pitkanen A. & Grohn O. (2011) Diffusion
546 tensor MRI with tract-based spatial statistics and histology reveals undiscovered
547 lesioned areas in kainate model of epilepsy in rat. *Brain Struct Funct* **216**, 123-35.
548 Song S.K., Sun S.W., Ju W.K., Lin S.J., Cross A.H. & Neufeld A.H. (2003) Diffusion tensor
549 imaging detects and differentiates axon and myelin degeneration in mouse optic
550 nerve after retinal ischemia. *Neuroimage* **20**, 1714-22.
551 Song S.K., Yoshino J., Le T.Q., Lin S.J., Sun S.W., Cross A.H. & Armstrong R.C. (2005)
552 Demyelination increases radial diffusivity in corpus callosum of mouse brain.
553 *Neuroimage* **26**, 132-40.
554 Stankewitz A., Aderjan D., Eippert F. & May A. (2011) Trigeminal nociceptive transmission in
555 migraineurs predicts migraine attacks. *J Neurosci* **31**, 1937-43.
556 Teepker M., Kunz M., Peters M., Kundermann B., Schepelmann K. & Lautenbacher S.
557 (2014) Endogenous pain inhibition during menstrual cycle in migraine. *Eur J Pain* **18**,
558 989-98.

559 Uggetti C., Squarza S., Longaretti F., Galli A., Di Fiore P., Reganati P.F., Campi A.,
560 Ardemagni A., Cariati M. & Frediani F. (2017) Migraine with aura and white matter
561 lesions: an MRI study. *Neurol Sci* **2017**, 11-3.
562 Weiller C., May A., Limmroth V., Juptner M., Kaube H., Schayck R.V., Coenen H.H. & Dlener
563 H.C. (1995) Brain stem activation in spontaneous human migraine attacks. *Nature*
564 *Medicine* **1**, 658-60.
565 Zhang J., Wu Y.-L., Su J., Yao Q., Wang M., Li G.-F., Zhao R., Shi Y.-H., Zhao Y., Zhang Q.,
566 Lu H., Xu S., Qin Z., Cui G.-H., Li J., Liu J.-R. & Du X. (2017) Assessment of gray
567 and white matter structural alterations in mgiraine without aura. *J Headache Pain* **18**,
568 74.

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570

571 **Table 1:** Montreal Neurological Institute (MNI) coordinates, cluster size and t-score for
572 regions of significant increases in mean diffusivity and fractional anisotropy in migraineurs
573 compared with controls.

Brain region	MNI Co-ordinate			cluster size (voxels)	t-score
	x	y	z		
interictals>controls					
Mean diffusivity					
left midbrain periaqueductal gray matter/cuneiform nucleus	-6	-28	-3	12	4.41
right dorsolateral pons	6	-36	-21	5	3.49
left dorsolateral pons	-4	-32	-13	13	3.41
left spinal trigeminal nucleus	-4	-40	-47	7	3.43
Fractional anisotropy					
ventral trigeminothalamic tract/ventral tegmental area	-2	-20	-9	10	3.74
Immediately prior to migraine>controls					
Fractional anisotropy					
ventral trigeminothalamic tract/ventral tegmental area	6	-20	-5	65	5.51
immediately following a migraine>controls					

Mean diffusivity					
left midbrain periaqueducal gray matter/cuneiform nucleus	-6	-30	-4	17	3.54
right dorsomedial/dorsolateral pons	6	-34	-19	24	4.16
left dorsolateral pons	-4	-32	-23	6	3.44
Fractional anisotropy					
ventral trigeminothalamic tract/ventral tegmental area	4	-18	-5	85	5.77

574

575 **Table 2:** Diffusion values for clusters displaying significant increases in mean diffusivity or
576 fractional anisotropy during the interictal phase in migraineurs compared with controls. Mean
577 diffusivity, axial diffusivity and radial diffusivity values are expressed as $x10^{-3}$. The grey
578 shaded boxes indicate a significant difference compared with controls. dlPons: dorsolateral
579 pons; dmPons: dorsomedial pons; VTT/VTA: ventral trigeminothalamic tract/ventral
580 tegmental area; PAG: midbrain periaqueductal gray matter; CNF: cuneiform nucleus; SpV:
581 spinal trigeminal nucleus.

582

Brain region	***Diffusivity parameter***	***controls***	***interictal***	***Immediately prior to migraine***	***Immediately following migraine***
left PAG/CNF	mean diffusion	0.86±0.01	0.92±0.01	0.89±0.01	0.93±0.02
	axial diffusivity	1.07±0.02	1.14±0.01	1.12±0.02	1.14±0.02
	radial diffusivity	0.75±0.01	0.81±0.01	0.79±0.02	0.81±0.02
	fractional anisotropy	0.28±0.01	0.27±0.01	0.27±0.01	0.27±0.01
left dlPons	mean diffusion	0.77±0.01	0.82±0.01	0.79±0.01	0.82±0.01
	axial diffusivity	1.37±0.02	1.44±0.01	1.40±0.02	1.45±0.02
	radial diffusivity	0.47±0.01	0.51±0.01	0.49±0.01	0.50±0.02
	fractional anisotropy	0.63±0.01	0.61±0.01	0.61±0.01	0.63±0.01
right dl/dmPons	mean diffusion	0.83±0.01	0.89±0.1	0.86±0.01	0.90±0.01
	axial diffusivity	1.52±0.02	1.61±0.02	1.58±0.02	1.64±0.02
	radial diffusivity	0.49±0.01	0.54±0.02	0.55±0.03	0.53±0.02
	fractional anisotropy	0.66±0.01	0.65±0.01	0.65±0.02	0.66±0.01
SpV	mean diffusion	0.73±0.01	0.79±0.01	0.76±0.1	0.78±0.1
	axial diffusivity	1.04±0.02	1.10±0.01	1.06±0.01	1.08±0.01
	radial diffusivity	0.59±0.01	0.63±0.01	0.61±0.01	0.62±0.01

	fractional anisotropy	0.37±0.01	0.36±0.01	0.37±0.01	0.36±0.01
VTT/VTA	mean diffusion	0.90±0.02	0.87±0.02	0.84±0.02	0.87±0.01
	axial diffusivity	1.26±0.02	1.25±0.02	1.25±0.02	1.29±0.02
	radial diffusivity	0.72±0.02	0.67±0.02	0.64±0.03	0.67±0.01
	fractional anisotropy	0.41±0.01	0.45±0.01	0.47±0.02	0.47±0.01

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585

586 **Figure Legends:**

587

588 **Figure 1: A)** Regional mean diffusivity and **B)** fractional anisotropy differences in
589 migraineurs during the interictal (n=31), immediately prior to migraine (n=13), and
590 immediately following a migraine (n=15) compared with controls (n=29). Significantly
591 different clusters are overlaid onto axial brainstem template images and significant increases
592 in migraineurs are represented by a t-value with a hot colour scale. Slice locations are
593 indicated at the upper left of each axial slice in Montreal Neurological Institute space.
594 Compared to controls, migraineurs have increased mean diffusivity during the interictal and
595 immediately following migraine periods in the region of the left spinal trigeminal nucleus
596 (SpV), left and right dorsolateral pons (dlPons) and in the region encompassing the midbrain
597 periaqueductal gray matter and nucleus cuneiform (PAG/CNF). In addition, migraineurs
598 display an increase in fractional anisotropy in the region of the ventral trigeminothalamic
599 tract/ventral tegmental area (VTT/VTA) during all three phases compared with controls.

600

601 **Figure 2:** Plots of mean (±SEM) mean diffusivity, axial diffusivity, radial diffusivity and
602 fractional anisotropy values in migraineurs compared with pain-free controls in the left spinal
603 trigeminal nucleus (SpV), left and right dorsolateral pons (dlPons), left midbrain
604 periaqueductal gray matter and cuneiform nucleus (PAG/CNF) and in the ventral
605 trigeminothalamic tract/ventral tegmental area (VTT/VTA). Consistent with the voxel-by-voxel
606 analysis, MD was significantly increased during the interictal and immediately following a
607 migraine phase but not immediately prior to a migraine. In addition, axial diffusivity showed a
608 largely similar pattern of difference in migraineurs whereas radial diffusion was only different
609 during the interictal phase in the PAG/CNF and SpV. In contrast, FA was significantly
610 increased in the area of the VTT/VTA in migraineurs during all three phases. #$p<0.05$ voxel-
611 by-voxel analysis; *$p<0.05$ two-sample t-tests.

612 **Figure 3:** Plots of mean diffusivity and fractional anisotropy in 11 migraineurs that were
613 scanned during at least two of the three migraine phases. Note the consistency of mean
614 diffusivity change which decreases significantly during the period immediately prior to
615 migraine in the left spinal trigeminal nucleus (SpV), right dorsolateral pons (dlPons) and left
616 midbrain periaqueductal gray matter and nucleus cuneiform (PAG/CNF). In contrast,
617 fractional within the area of the ventral trigeminothalamic tract/ventral tegmental area
618 (VTT/VTA) was significantly greater during the period immediately prior to compared with
619 immediately following a migraine. *$p<0.05$ paired t-tests.

620

621 **Figure 4:** Plots of mean (±SEM) mean diffusivity and fractional anisotropy changes in
622 migraineurs over the migraine cycle in the left spinal trigeminal nucleus (SpV), left and right
623 dorsolateral pons (dlPons), left midbrain periaqueductal gray matter and cuneiform nucleus
624 (PAG/CNF) and in the ventral trigeminothalamic tract/ventral tegmental area (VTT/VTA).
625 Values are averaged for those migraineurs scanned at least 30 days (n=18), 30-10 days
626 (n=6), 9-2 days (n=7), and 1 day prior to their next migraine (n=13), as well as 1-3 days
627 following a migraine (n=15). Note how mean diffusivity remains relatively stable over the
628 interictal period and falls dramatically immediately prior to a migraine before recovering. In
629 contrast, fractional anisotropy remains stable over all three migraine phases.

630