Alterations in Functional Connectivity During Different Phases of the Triggered Migraine Attack

Nazia Karsan, PhD, MRCP; Pyari R. Bose, MD, FRCP; Owen O’Daly, PhD; Fernando O. Zelaya, PhD; Peter J. Goadsby, MD, PhD, FRCP

Objective.—To understand the changes in functional connectivity between brain areas of potential importance in migraine during different phases of the attack.

Background.—Migraine is a symptomatically heterogeneous disorder. Understanding the possible changes in brain function and, therefore, neurobiology during different phases of the migraine attack is important in developing disease biomarkers and advancing therapeutics.

Design.—Randomized, double-blind, placebo-controlled, multi-visit experimental study.

Methods.—Subjects aged 18-50 years with migraine with and without aura (≤22 headache days per month) were recruited from across the UK using advertising, from both population and hospital clinic samples (n = 53). Consented subjects were randomized to a 0.5 μg/kg/min nitroglycerin infusion or to placebo over 20 minutes across different study visits, during the period February 2015-July 2017.* All subjects were exposed to a nitroglycerin infusion at least on 1 occasion at screening.** For those subjects who consented to participate in imaging visits (n = 25), structural T1, T2 and FLAIR sequences and resting state blood oxygen level dependant contrast (rsBOLD) time series, using a multiecho EPI sequence, were conducted over 30-40 minutes at baseline and rsBOLD during premonitory symptoms and migraine headache on a 3T General Electric MR750 MRI scanner. For the placebo visit, the imaging was conducted at the same times following infusion in the absence of symptoms.

Results.—Montreal Neurological Institute (MNI) coordinates were used to characterize identified brain areas of connectivity change. Using repeated measures ANOVA models with time (visit number) and trigger substance (nitroglycerin/placebo) as factors, significant positive functional coupling was found between the thalami bilaterally and the right precuneus and cuneus regions during the nitroglycerin-triggered premonitory phase (T = 3.23, peak connectivity change at [−6, −68, 40] for left thalamus, P = 0.012 and [−4, −68, 40] for right thalamus, P = 0.019). The nitroglycerin-triggered premonitory phase was associated with a change in the direction of connectivity from positive to negative between the pons and the limbic lobe (T = 3.47, peak connectivity change at [2, 8, 50], P < 0.001). The headache phase of the nitroglycerin-triggered migraine attack was associated with ongoing negative functional coupling between the pons and the cingulate and frontal cortices, and positive functional coupling between the pons and the cerebellar tonsils and medulla (T = 3.47, peak connectivity change at [−8, −52, −58], P = 0.007).

From the Headache Group, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, London, UK (N. Karsan, P.R. Bose, and P.J. Goadsby); Department of Neuroimaging, Centre for Neuroimaging Sciences, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, London, UK (O. O’Daly and F.O. Zelaya); NIHR-Wellcome Trust King’s Clinical Research Facility, SLaM Biomedical Research Centre, King’s College Hospital, London, UK (N. Karsan, P.R. Bose, and P.J. Goadsby).

Address all correspondence to N. Karsan, Headache Group, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King’s College London, London, UK, email: nazia.karsan@kcl.ac.uk

[*Correction added on July 22, 2020 after first online publication: This sentence was revised from, “Consented subjects had a 0.5 μg/kg/min nitroglycerin infusion...”.]

[**Correction added on July 22, 2020 after first online publication: This sentence was revised from, “… at least on 1 occasion at screening.”]
Conclusions.—Understanding the functional reorganization between subcortical and cortical brain areas in different phases of the migraine attack provides novel insights into the abnormal sensory processing and integration during migraine, as well as functional correlation with clinical symptoms displayed during each phase.

Key words: migraine, premonitory, headache, fMRI, connectivity, neuroimaging

Abbreviations: ASL arterial spin labeling, CBF cerebral blood flow, CGRP calcitonin gene-related peptide, FWE family wise error-corrected, LC locus coeruleus, NRM nucleus raphus magnus, NTG nitroglycerin, PACAP38 pituitary adenylate cyclase activating polypeptide 38, PBN parabrachial nucleus, pCASL pseudocontinuous arterial spin labeling, ROI region of interest, RVM rostroventral medulla, VTA ventral tegmental area

(Headache 2020;60:1244-1258)

INTRODUCTION

Migraine is a common\(^1\) and symptomatically heterogeneous brain disorder, which encompasses a variety of painful and painless symptoms.\(^2,3\) Research into the different “phases” of the migraine attack has increased over the last few decades, owing to the increasingly recognized spectrum of symptoms patients report,\(^4,9\) from the earliest premonitory phase hours to days before pain onset,\(^9\) through to the postdrome following headache resolution.\(^10\) Understanding neurobiological mediation of the various phases of the attack and the symptoms encountered during each phase is vital to increasing understanding of the mechanisms behind attack initiation and abortion, and, therefore, in developing disease biomarkers and effective therapeutics.

Studying the entirety of a migraine attack is logistically challenging, in particular with functional neuroimaging, given the early manifestation and variable duration of premonitory symptoms and the inability of patients to associate these symptoms with impending headache at times. For this reason, among others, experimental migraine triggers such as intravenous nitroglycerin (NTG), have been developed and used in migraine research since the 1980’s.\(^11\) NTG has also been used to study provoked MWoA attacks with functional neuroimaging,\(^12,15\) in particular as it has the ability to trigger premonitory symptoms in some migraineurs.\(^16\)

Functional neuroimaging studies have consistently demonstrated dorsal pontine activation during both spontaneous \(^17\) and provoked migraine attacks,\(^12,14\) and the hypothalamus has been shown to be activated during spontaneous migraine headache.\(^18\) More recently, studies have looked at the premonitory phase in particular with functional imaging methods. A study using H\(_2\)\(^15\)O PET to measure cerebral perfusion identified early involvement of the hypothalamus and brainstem structures during the premonitory phase.\(^13\) Imaging studies using alternative MR approaches have provided supportive evidence for early hypothalamic and brainstem dysfunction prior to headache onset, and have suggested altered functional connectivity of some of these regions prior to headache and during headache.\(^19,22\) The involvement, with differing functional neuroimaging modalities, of key cortical and subcortical brain areas already recognized to be involved in migraine nociception, during painless symptoms, is interesting and alludes to early and widespread functional reorganization within the brain during migraine.

Conflict of Interest: The authors report no relevant conflicts of interest related to this work. Separate to this work, NK has received speaker honoraria from Novartis Pharmaceuticals and Teva Pharmaceuticals, and writer honoraria from Continuum. PB has received speaker honoraria from Teva Pharmaceuticals and writer honoraria from Continuum. OOD and FOZ report no disclosures. PJG reports, over the last 36 months, grants and personal fees from Amgen and Eli-Lilly and Company, grant from Celgene, and personal fees from Alder Biopharmaceuticals, Allergan, Autonomic Technologies Inc, Biohaven Pharmaceuticals Inc, Clexio, Electrocore LLC, eNeura, Epalex, Impel Neuropharma, MundiPharma, Novartis, Teva Pharmaceuticals, Trigemina Inc, WL Gore, and personal fees from MedicoLegal work, Massachusetts Medical Society, Up-to-Date, Oxford University Press, and Wolters Kluwer; and a patent magnetic stimulation for headache assigned to eNeura without fee.

Funding: This work was partially funded by the Migraine Trust, and a Guarantors of Brain/Association of British Neurologists Clinical Research Training Fellowship.
In this study, we set out to increase understanding of various phases of the migraine attack with functional neuroimaging during NTG-triggered attacks, using resting state seed-based functional connectivity approaches, with predefined seeds identified based on the previous studies discussed. We also wanted to explore if the areas of altered connectivity identified and their networks could feasibly functionally correlate with the detailed phenotype of symptoms displayed by subjects during various phases of the attack following exposure to NTG. Aspects of the work were presented in preliminary form at the 19th International Headache Congress, Dublin 5-8th September 2019.23

METHODS

Subject Recruitment.—The study was advertised to identify potentially eligible subjects aged 18-50 years with migraine with or without aura, as defined by the International Classification of Headache Disorders (ICHD)-3beta, which was in use at the time that the study was conducted.24 The term premonitory is used throughout, as it was preferred to prodrome in ICHD-1, 2 and 3beta, and in the recent literature; we expect it will remain so.25 Subjects with ≤22 headache days per month with typical premonitory symptoms prior to spontaneous migraine attacks and no other significant medical comorbidities were screened and consented. The headache day criteria were used to ensure that study visits on “crystal clear” days could be planned. Single agent oral migraine preventive therapy at a stable dose was allowed, to aid recruitment. [*Correction added on July 22, 2020 after first online publication: This sentence was revised from, “... as defined by the current International Classification of Headache Disorders (ICHD)-3beta.”]

Three hundred and thirty subjects were prescreened via email, or telephone, or both. Fifty-three attended a screening visit and were agreeable to participation in the study. The most common reasons for prescreen failure were frequency of headache >22 days/month (n = 140), administration of more than 1 migraine preventive therapy (n = 52) and being on other excluded CNS-acting medications (n = 36).

Sample Size.—Based on other resting state studies,26 we aimed for 20 subjects to complete the imaging visits in the study, to allow demonstration of altered functional connectivity patterns between the seeds identified and other brain areas.

Ethical Approval.—The study was approved by the Camden and Islington National Health Service Ethics Committee (reference 14/LO/2241). Written informed consent was obtained from all subjects prior to inclusion and enrollment in the study, according to the Declaration of Helsinki.

Subject Screening.—Fifty-three subjects were invited to a screening visit, where following informed consent, a detailed migraine history was taken. This included extended phenotyping of spontaneous migraine attacks, medication history and ensuring no medical or pharmaceutical contraindications to any of the study drugs. An ECG was performed to exclude cardiac contraindications to the study drugs.

Nitroglycerin (NTG) Infusion.—Each subject was exposed to a 0.5 µg/kg/min NTG infusion over 20 minutes following consent, to identify those who developed premonitory symptoms and headache in a reasonable time scale to allow planning of scanning sessions.

Subjects were symptomatically and hemodynamically assessed before the infusion and at 5-minute intervals during the infusion, with questioning regarding the evolution of any typical premonitory symptoms, such as yawning, neck stiffness, tiredness, concentration, or mood change, as well as headache and associated symptoms (see Fig. 1).

The premonitory phase was defined as the presence of at least 3 symptoms typical for the subject following completion of the NTG infusion in the absence of any migraine headache, which were consistently present on 2 separate epochs of enquiry. Migraine headache was defined as per the criteria for experimentally provoked migraine, occurring after premonitory symptoms.27

Imaging Visits.—Twenty-five subjects triggered both premonitory symptoms and headache at the screening visit following NTG infusion and were agreeable to attend further imaging visits. All subjects had to be completely pain-free and acute treatment-free for at least 12 hours prior to an imaging visit, to limit any residual effects of any prior headache or medications on imaging.** A double-blind randomized crossover design was used, to allow each subject to have imaging following NTG infusion and after placebo on separate visits. Randomization was performed using the
*RAND random number generator in Microsoft Excel. [**Correction added on July 22, 2020 after first online publication: This sentence was revised from, “…pain-free and treatment-free…”].

All drug preparation and administration on imaging visits was performed by an unblinded investigator who was not involved in the final imaging analysis, and out of sight of the subject, to ensure maintenance of a double-blind design.

For NTG imaging visits, the infusion was administered as for the screening visit. For the placebo visit, a 0.9% normal sodium chloride infusion (clear and colorless) was administered in the same volume over 20 minutes, and the symptomatic and hemodynamic questioning was identical to the triggered visit. For timing the scans following placebo, subjects were scanned at the same times following infusion at which they developed premonitory and headache symptoms during the screening visit.

**Imaging Acquisition.**—The baseline scan was performed on arrival at all visits following the screening visit, following an MRI safety check and review of migraine diary. The premonitory scan was conducted in the presence of 3 or more premonitory symptoms on the symptom questionnaire on the NTG-triggered visit. This phase was purposely defined as the presence of 3 or more symptoms for the study, to minimize any doubt about the presence of 1 or more potentially vague premonitory-like symptoms, and to increase confidence that these symptoms were truly due to the migraine attack. The headache scan was performed in the presence of moderate-severe headache, meeting classification for a pharmacologically induced migraine headache on the NTG-triggered visit. Where possible, to limit the pain severity, duration and scanning tolerability for subjects, headache was not allowed to escalate beyond moderate and imaging was performed for the headache stage as soon as the headache reached this level, and headache treatment was promptly administered as soon as the scan was completed. The headache resolution scan was performed after headache treatment and resolution of migraine headache, although

![Image of Table 1](image-url)
mild residual head discomfort or movement sensitivity were accepted. The headache resolution data will be reported elsewhere.

**Imaging Conduct.**—All scans were performed on a 3 Tesla GE MR750 MRI scanner within the Clinical Research Facility at King’s College Hospital, with the capability for parallel image acquisition. The MRI scanner was fitted with a receive-only 8-channel, phased-array head coil. Structural MRI data were collected using the three-dimensional T1-weighted weighted inversion recovery prepared gradient echo sequence (MP-RAGE) pulse sequence (TR 7.3ms, TE 3.02ms, TI 400ms, flip angle 11, FOV 270mm, slice thickness 1.2mm, axial slices 196, matrix 256 x 256). Structural brain imaging was acquired for every subject at the baseline scanning session at each imaging visit. This imaging allowed broad screening for any secondary cause for a headache disorder in the subjects and provided structural imaging for spatial normalization during imaging preprocessing. The structural sequences in the study took approximately 10 minutes to acquire during the baseline scanning session.

All imaging was conducted such that circadian variability in image acquisition times was minimized, given the evidence that there is circadian variability in cerebral blood flow. All baseline and premonitory scans were acquired before 1200, and all headache and headache resolution scans before 1600. Imaging time in total per study visit did not exceed 2.5 hours.

Functional BOLD time series were acquired with a multi-echo EPI sequence (TR 2500ms, flip angle 80, matrix size 64 × 64, in-plane resolution 3.75mm, FOV 240 mm, 32 axial slices, slice thickness 3mm with 4mm gap), at each scanning session. Four echoes were acquired with the shortest possible echo times; TE 12ms, 38ms, 44ms and 60ms. Each multiecho time series was acquired over 8 minutes 10 seconds. Pulse and respiratory data were acquired using scanner-integrated photoplethysmograph and respiratory bellow. During the resting state data acquisition, subjects were asked to fixate on a cross they were able to see on the wall while in the scanner by looking in a mirror.

**Seed Selection.**—The seeds selected for seed-based correlation analysis of the multiecho BOLD time series were the left thalamus, right thalamus, hypothalamus, pons and midbrain, as well as a mask of areas such as the anterior cingulate cortex, orbitofrontal cortices, which emerged of interest from a prior perfusion-based investigation conducted by the same authors. These areas were selected because of other studies in migraine implicating them as uniformly important in migraine pathophysiology.

In particular, the hypothalamus was of interest in the premonitory phase based on previous imaging investigations, and the dorsal pons and midbrain (in particular the ventral tegmentum) have been emerging as particular areas of interest in migraine. Additiona[l the thalamus is a large sensory relay nucleus within the brain, with roles in pain and sensory modulation via thalamocortical pathways, as well as a demonstrated role in migraine suggested by imaging, therefore, the thalami were identified as seeds of interest. Through identification of both cortical and subcortical seeds of interest, we sought to identify altered functional networks and subcortical-cortical network integrity throughout a migraine attack.

Additionally, the thalamus is a large sensory relay nucleus within the brain, with roles in pain and sensory modulation via thalamocortical pathways, as well as a demonstrated role in migraine suggested by imaging, therefore, the thalami were identified as seeds of interest. Through identification of both cortical and subcortical seeds of interest, we sought to identify altered functional networks and subcortical-cortical network integrity throughout a migraine attack.

The seeds chosen were created as anatomical masks using WFUPickatlas within SPM. The images of each seed are shown in Figure 2A-G. The numbers in white on the left of each image correspond to the MNI location of each slice.

MNI coordinates from the literature for the dorsolateral pons [4, −20, −20] were also tested for spatial constriction. Based on the previous literature regarding resting state fMRI in migraine, some areas of altered connectivity have emerged; namely altered functional connectivity with the amygdala in migraine, as well as altered hypothalamic and brainstem connectivity with the spinal trigeminal nuclei in the preictal phase. For this reason, the spinal trigeminal nuclei and the limbic...
lobe were also tested using spherical small volume correction against each seed. For the region of the spinal trigeminal nuclei, MNI coordinates were taken from a previous study [4, −40, −55]. An 8mm radius sphere (VOI) was used for the ROI analysis for the dorsolateral pons and spinal trigeminal nuclei areas. Rather than testing the amygdalae and insular cortices separately, an anatomical limbic lobe mask was made in WFUPickatlas and used for small volume correction. These ROI’s were tested for each model within the imaging analysis.

Data Processing.—The resting state time series were preprocessed by: realignment of the images and resetting of the origin, motion correction, de-spiking, slice timing correction, de-noising, T1 segmentation, co-registration and normalization, signal regression followed by band pass filtering, spatial smoothing and subsequent connectivity analyses by calculation of voxel-wise Pearson’s correlation coefficients.

Multiple Comparison Correction.—For all exploratory whole brain analyses (voxel-wise analyses), a family wise error (FWE)-corrected $P$ value for multiple comparisons of <0.05 was considered significant on the basis of cluster extent. A cluster-forming threshold of uncorrected $P < 0.001$ was used for the BOLD data.

For all hypothesis-driven analyses presented (region of interest (ROI) analyses), a peak FWE-corrected $P$ value for multiple comparisons of < 0.05 was considered significant on the basis of the response amplitude within an independently derived ROI.

Statistical Analysis.—A repeated measures $2 \times 2$ ANOVA was used to interrogate the effect of the interaction of time (the time between visits) and trigger (NTG or placebo) on functional connectivity ($n = 21$). Post hoc exploratory paired $t$-tests were also used to identify additional areas of changes in functional connectivity between conditions with the larger subject cohort ($n = 25$), and further ANOVA analyses were conducted on restricted but more homogeneous subject cohorts (migraine with and without aura and sub-
jects on and not on single agent migraine preventive therapy).

**Primary Outcome.**—Given both bilateral and unilateral headache on either side at baseline or following NTG triggering was accepted in this study, the laterality of any imaging findings was not interrogated. The primary outcome was areas of altered connectivity relative to each seed (following NTG) compared to baseline, in the entire subject cohort, during both the premonitory and headache phases which withstood comparison to scans acquired at similar time points following placebo. [Correction added on July 22, 2020 after first online publication: The preceding sentence was revised from, “... in the entire subject cohort, and when contrasting subjects with and without aura at baseline, and subjects on and not on migraine preventive therapy, which withstood comparison to scans...”.] All other analyses were exploratory.

All images displayed in this study were generated using the xjview toolbox, available within SPM (http://www.alivelearn.net/xjview).

**RESULTS**

**Triggering Rates.**—Among the 53 recruited subjects, the median headache days at baseline were 8 (IQR 5-12). Forty-four (83%) developed a migraine headache following the infusion. The majority (52/53, 98%) had typical premonitory symptoms preceding the headache. Of the 44, 33 met the study criteria to attend a second visit (75%), and 25 completed the active NTG-triggered imaging visit, while 21 completed both active and placebo imaging visits.

Twenty-five subjects were included in primary and secondary outcome analyses (21 female, 4 male, 15 MWA, 10 MWoA, 8 on single agent migraine preventive therapy, 19 right-handed). During the imaging visits, the most common premonitory symptoms reported were tiredness, photophobia and concentration difficulty.

**Triggering of Aura.**—Typical migraine aura was triggered by NTG in 7 subjects at the screening visit. Six subjects went on to develop delayed migraine headache following the onset of aura. Four of these 7 subjects who went on to have imaging experienced symptomatic typical aura in the scanner, of which 1 of these was pure typical visual aura. In all subjects, the same aura symptoms triggered had been experienced before in association with a spontaneous migraine attack. There was a lifetime history of aura in 15 of the 25 subjects who entered the imaging phase of the study (60%).

We have previously reported the phenotype of premonitory, aura and headache symptoms triggered in this cohort. 40

**Areas of Connectivity Change- Premonitory Phase.**—Baseline vs NTG-Triggered Premonitory Phase: Active Premonitory vs Placebo “Premonitory” (2 × 2 ANOVA).—When this model was tested in the 21 subjects who had both active and placebo baseline and premonitory data, significant findings were present.
for both thalamic seeds (peak connectivity change at $[-6, -68, 40]$ for left thalamus, $P = 0.012$ and $[-4, -68, 40]$ for right thalamus, $P = 0.019$), but not for any other seeds using whole brain or region of interest analyses. There was a reduction in functional connectivity between the thalami bilaterally and the right precuneus and cuneus regions. The spatial extent of this cluster of connectivity change is shown in Figure 3.

This analysis was repeated with the subjects on single agent oral preventive therapy removed from the model ($n = 12$), but no significant results were found. The analysis was also repeated with subjects with ($n = 11$) and without baseline aura ($n = 10$) analyzed separately, in case the involvement of visual cortex and precuneus via the thalamus could be related to an aura effect, but again no statistically significant results were found.

With ROI analysis, there was a positive result for a change in direction of functional connectivity between the pons seed and the limbic lobe from positive coupling at baseline to negative coupling during the NTG-triggered premonitory phase (peak change at $[4, 8, 46]$, $P = 0.008$). The plots for the direction of this change are shown in Figure 4.

$Baseline \ vs \ Premonitory$ ($Paired\ t-test$).—There was 1 cluster of altered functional connectivity in the premonitory phase compared to baseline for the pons seed which reached the threshold for statistical significance at a whole brain analysis level (peak connectivity change at $[2, 8, 50]$, $P < 0.001$). This cluster was centered in the frontal lobe comprising medial frontal and superior frontal gyri and the cingulate gyrus.* Weakly positively correlated BOLD time series at baseline switched to strongly negatively correlated time series in the premonitory phase (a change in the direction of connectivity between these regions from positive functional coupling to negative functional coupling). The spatial extent of this cluster is shown in Figure 5. [*Correction added on July 22, 2020 after first online publication: This sentence was revised from “This cluster was centered in the frontal lobe and cingulate lobes, comprising medial frontal …”.]*

No other seeds showed significant connectivity change with whole brain or ROI analysis for this comparison.

$Baseline \ vs \ NTG-Triggered\ Headache\ Phase:\ Active\ Headache \ vs \ Placebo \ “Headache”\ (2 \times \ 2\ ANOVA)$.—There was no significant evidence of a time-by-treatment interaction in functional connectivity of any of the seed regions within this model on whole brain analysis.

$Baseline \ vs \ Headache$ ($Paired\ t-test$).—There was 1 cluster of altered connectivity in the headache phase
compared to baseline between the pons seed and a cluster involving the cerebellum and medulla, which reached the threshold for statistical significance at a whole brain analysis level. There was a change in the direction of functional connectivity from negative at baseline to positive during NTG-triggered migraine headache (peak connectivity change at [−8, −52, −58], $P = 0.007$). The spatial extent of this cluster is shown in Figure 6.

With region of interest analysis, there was a reduction in functional connectivity between the pons and the mask of regions made from prior perfusion results during NTG-triggered migraine headache compared to baseline (peak change over middle frontal gyrus, [38, 0, 54], $P = 0.024$), with a change in the direction of functional connectivity from strongly positive at baseline, to weakly negative during headache (a loss of functional coupling). The
direction of connectivity change for this contrast is seen in Figure 7.

**Interictal Variability in Connectivity.**—Many migraine functional imaging studies are conducted on the same day, without repeated measures designs for increased statistical power. Given the design of this study, we acquired baseline BOLD time series on 2 occasions (on the NTG and the placebo visits). This allowed us to compare the baseline resting state connectivity between brain regions of interest across 2 visits prior to any drug exposure.

Given the variability in baseline connectivity that was observed using the resting state data in the ANOVA models, the significant clusters of results on the whole brain analyses for each comparison were taken, and an anatomical binary mask was made for each significant cluster. The mean connectivity change was then extracted using this mask, to assess if the variability was reduced using mean connectivity change over the cluster rather than over the peak coordinates, where the results are likely to be most extreme.

Despite using mean connectivity change values over the cluster of significance rather than peak change, there remained a difference in baseline connectivity relative to each seed between the 2 visits (active and placebo) and the direction of connectivity change within the cluster relative to the seed between the baseline and premonitory scans on each visit remained unchanged.

**DISCUSSION**

In this study, we have demonstrated alterations in functional connectivity between key areas of brain involved in migraine biology, with distinct results during the premonitory phase and during headache, with most results withstanding comparison to placebo within rigorous statistical models.

The finding of reduced connectivity between the thalami and the region of the precuneus and cuneus in the premonitory phase, alludes to altered thalamocortical connections and functional reorganization of such networks during this phase, therefore, suggesting that sensory pathways between the thalamus and the precuneus (a region of the default mode network known to play a role in sensory integration) and the cuneus (an area of visual cortex also thought to be involved in multisensory integration and cognitive processing) are implicated during premonitory symptoms. A previous study has demonstrated reduced functional connectivity between the posterior thalamus and the area of the precuneus in MWoA, while another has suggested structural changes in the precuneus with disease chronification. In addition, the precuneus/cuneus regions may be implicated in allodynia in migraine, with 1 study demonstrating reductions in functional connectivity between these areas and posterior thalamus correlating with allodynia severity in MWoA. These thalamocortical pathways are likely involved in
mediating sensory sensitivities in migraine—including allodynia and photophobia, as well as mediating other symptoms such as visuospatial and cognitive dysfunction, in particular during the premonitory phase.

We found negative coupling between the pons and the limbic lobe, as well as with frontal cortical regions and cingulate gyrus during the premonitory phase and during migraine headache relative to baseline. A previous migraine study has also found reduced connectivity between the pons and other brainstem regions with sensory and motor cortices. While many roles for the pons in the premonitory phase, and indeed in headache could be postulated, particularly relevant to this altered connectivity is the superior salivatory nucleus (SSN) within the pons, which has anatomical connections to limbic and other cortical regions. This nucleus is involved in mediating cranial autonomic symptoms, as well as pain, and via these connections may also be involved in feeding and sleep. There may, therefore, be cortical modulation of pontine and other subcortical pathways regulating sensory and autonomic symptoms during the migraine attack. There are several other pontine nuclei which could feasibly be involved in mediating premonitory symptoms including altered sleep-wake cycle and autonomic function via noradrenergic connections of the locus coeruleus (LC), providing a possible link between premonitory sleep disturbance and pain. Another is the parabrachial nucleus (PBN), which is also involved in both homeostatic and pain mechanisms, via calcitonin gene-related peptide (CGRP), cholecystokinin and other neurotransmitters, and via connections to limbic and other cortical regions, therefore, having a possible role in feeding, cravings, temperature control and arousal in migraine. Whether there is top-down inhibition of pontine activity via the cortex in migraine or an inverse relationship remains a question, but clearly both areas are implicated. Limbic and frontal sensory cortices and their connections are likely involved in mediating the emotional and cognitive responses seen during a migraine attack, as well as sensory modulation and the perception of sensory sensitivity.

The findings in general support the literature regarding ictal alterations in functional connectivity between the pons and the spinal trigeminal nuclei region, although no alterations in pontine connectivity with these regions was demonstrated during this study in the premonitory phase, and there were no statistically significant premonitory alterations in connectivity relative to the hypothalamus. Altered hypothalamic-brainstem connectivity was suggested to be a potential early driver for migraine attacks based on the other studies performed to date looking at the premonitory or preictal migraine brain. The results from this study support functional reorganization of networks between subcortical structures known to be important in migraine, with cortical regions before pain, and during different phases of the migraine attack. The subsequent positive functional coupling between the pons and the medulla and cerebellar tonsils, and the spinal trigeminal nuclei in the headache phase is likely to mediate the nociceptive component of the migraine attack, while ongoing alterations in connectivity between the pons and cortical regions continue to mediate non-painful symptoms, cranial autonomic symptoms, homeostatic dysregulation, and the affective components of migraine.

The emergence of subcortical structures such as the thalamus, pons, and medulla in such imaging studies, further alludes to multiple neurotransmitters being...
involved in migraine, with aminergic networks likely being involved in the premonitory phase. While dopaminergic neurons in the hypothalamus may be responsible for yawning,61 arousal changes could be mediated via noradrenergic neurons in the LC.53 altered feeding could be mediated via the PBN,62 the thalamus could be responsible for mood symptoms, pain, sensory sensitivities,32,33,35,63 and mood and cognitive change are likely mediated via limbic pathways (see Table 1). All these areas also have roles in pain, therefore, providing a neurobiological and functional imaging correlate between premonitory symptoms and pain in migraine.

These connectivity results suggest that there is altered subcortical-cortical network integrity in the premonitory phase, in diencephalic, brainstem and cortical areas of the brain of interest in migraine, particularly those involved in cognition, autonomic control, and homeostatic functions. Some of these alterations continue into headache, with other changes likely accounting for nociception and headache initiation and maintenance during the headache phase.

Novel connectivity results relative to those in previous studies in the preictal or premonitory phase of migraine are demonstrated, implicating functional reorganization of ponto-cortical and thalamocortical networks in the premonitory and headache phases. We also demonstrate that interictal differences in connectivity do occur, and we have tried to account for these in this study and these should be considered in other studies going forward.

### LIMITATIONS

While brain imaging changes were compared following NTG and placebo, we did not expose a healthy control arm to the study paradigm. This would be an important next step. In addition, the subject cohort was heterogeneous and included subjects with and without aura and those on 1, or no, oral preventive agents. This was to aid recruitment, although the sample sizes were inadequate to analyze each group individually. Ideally, we would use such a study paradigm to compare groups of this kind. The interictal variabilities in baseline connectivity could be in part due to the wide range of headache days at baseline allowed into the study. This was to again aid recruitment and because it was felt that there was unlikely to be a biological difference in the subjects within the range of headache days we studied. In addition, subjects were in their own environment for the 2 week intervals between study visits and could, therefore, be exposed to environmental and exogenous factors which could affect the connectivity. The use of NTG itself, and the triggering of similar symptoms with placebo is an issue,40 and we cannot completely exclude a drug effect on the imaging, although premonitory scans were conducted on average 36 minutes following NTG, and headache scans 117 minutes following NTG, therefore, we would expect any perfusion effects of NTG in the brain to have passed by this point.64 [Correction added on July 22, 2020 after first online publication: In the preceding sentence, a citation to reference 40 was added.] Acquiring data for a healthy control arm would help answer these questions.

In conclusion, we have shown significant negative functional coupling between the thalami bilaterally and the right precuneus and cuneus regions during the nitroglycerin-triggered premonitory phase. In addition, in the nitroglycerin-triggered premonitory phase there was a change in the direction of connectivity from positive to negative between the pons and the limbic lobe. The headache phase of the nitroglycerin-triggered migraine attack was associated with ongoing negative functional coupling between the pons and the cingulate and frontal cortices, and positive functional coupling between the pons and the cerebellar tonsils and medulla. Taken together with the functional imaging literature in migraine, the data continue to contribute to a narrative of migraine as a distinct, neuronal network disorder in

### Table 1.—Summary of Symptoms Experienced During Migraine and Where They May Be Mediated in the Brain (NTS = nucleus tractus solitarius)

| Symptom During Migraine Attack | Area of Brain Identified in Study Which Could Be Implicated |
|--------------------------------|-------------------------------------------------------------|
| Cravings                      | PBN                                                         |
| Thirst                        | PBN                                                         |
| Mood change                   | Cingulate and limbic cortex                                 |
| Concentration difficulty      | Cingulate and limbic cortex                                 |
| Nausea                        | NTS in medulla                                              |
| Photophobia                   | Thalamus, cuneus                                            |
| Alldynia                      | Thalamus, precuneus                                         |
| Altered arousal               | LC, PBN                                                     |
| Headache                      | Pons, spinal trigeminal nuclei, thalamus                    |

---
which brain regions normally concerned with sensory processing alter in function in such a way as to produce the complex, disabling symptoms that patients experience.

Acknowledgments: The authors would like to thank all the patients and volunteers who participated in this study.

STATEMENT OF AUTHORSHIP

Category 1

(a) Conception and Design

Nazia Karsan, Pyari R. Bose, Fernando O. Zelaya, Owen O’Daly, Peter J. Goadsby

(b) Acquisition of Data

Nazia Karsan, Pyari R. Bose

(c) Analysis and Interpretation of Data

Nazia Karsan, Pyari R. Bose, Owen O’Daly, Fernando O. Zelaya

Category 2

(a) Drafting the Manuscript

Nazia Karsan

(b) Revising It for Intellectual Content

Nazia Karsan, Pyari R. Bose, Owen O’Daly, Fernando O. Zelaya, Peter J. Goadsby

Category 3

(a) Final Approval of the Completed Manuscript

Nazia Karsan, Pyari Bose, Owen O’Daly, Fernando O. Zelaya, Peter J. Goadsby

REFERENCES

1. GBD 2016 Headache Collaborators. Global, regional, and national burden of migraine and tension-type headache, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2018;17:954-976.

2. Karsan N, Goadsby PJ. Biological insights from the premonitory symptoms of migraine. Nat Rev Neurol. 2018;14:699-710.

3. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition. Cephalalgia. 2018;38:1-211.

4. Giffin NJ, Ruggiero L, Lipton RB, et al. Premonitory symptoms in migraine: An electronic diary study. Neurology. 2003;60:935-940.

5. Kelman L. The premonitory symptoms (prodrome): A tertiary care study of 893 migraineurs. Headache. 2004;44:865-872.

6. Quintela E, Castillo J, Munoz P, Pascual J. Premonitory and resolution symptoms in migraine: A prospective study in 100 unselected patients. Cephalalgia. 2006;26:1051-1060.

7. Cuvelier JC, Mars A, Vallee L. The prevalence of premonitory symptoms in paediatric migraine: A questionnaire study in 103 children and adolescents. Cephalalgia. 2009;29:1197-1201.

8. Laurell K, Artto V, Bendtsen L, et al. Premonitory symptoms in migraine: A cross-sectional study in 2714 persons. Cephalalgia. 2016;36:951-959.

9. Karsan N, Prabhakar P, Goadsby PJ. Characterising the premonitory stage of migraine in children: A clinic-based study of 100 patients in a specialist headache service. J Headache Pain. 2016;17:94.

10. Giffin NJ, Lipton RB, Silberstein SD, Olesen J, Goadsby PJ. The migraine postdrome: An electronic diary study. Neurology. 2016;87:309-313.

11. Iversen HK, Olesen J, Tfelt-Hansen P. Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics. Pain. 1989;38:17-24.

12. Bahra A, Matharu MS, Buchel C, Frackowiak RS, Goadsby PJ. Brainstem activation specific to migraine headache. Lancet. 2001;357:1016-1017.

13. Maniyar FH, Sprenger T, Monteith T, Schankin C, Goadsby PJ. Brain activations in the premonitory phase of nitroglycerin-triggered migraine attacks. Brain. 2014;137:232-241.

14. Afridi SK, Matharu MS, Lee L, et al. A PET study exploring the laterality of brainstem activation in migraine using glyceryl trinitrate. Brain. 2005;128:932-939.

15. Christiansen I, Thomsen LL, Daugaard D, Ulrich V, Olesen J. Glyceryl trinitrate induces attacks of migraine without aura in sufferers of migraine with aura. Cephalalgia. 1999;19:660-667.

16. Afridi SK, Kaube H, Goadsby PJ. Glyceryl trinitrate triggers premonitory symptoms in migraineurs. Pain. 2004;110:675-680.

17. Afridi SK, Giffin NJ, Kaube H, et al. A positron emission tomographic study in spontaneous migraine. Arch Neurol. 2005;62:1270-1275.
18. Denuelle M, Fabre N, Payoux P, Chollet F, Geraud G. Hypothalamic activation in spontaneous migraine attacks. *Headache*. 2007;47:1418-1426.

19. Schulte LH, May A. The migraine generator revisited: continuous scanning of the migraine cycle over 30 days and three spontaneous attacks. *Brain*. 2016;139:1987-1993.

20. Marciszewski KK, Meylakh N, Di Pietro F, et al. Changes in brainstem pain modulation circuitry function over the migraine cycle. *J Neurosci*. 2018;38:10479-10488.

21. Schulte LH, May A. The migraine generator re-visited: two discrete patterns of premonitory symptoms. *Cephalalgia*. 2018:38:32-33.

22. Meylakh N, Marciszewski KK, Di Pietro F, et al. Increased resting-state dmri connectivity in patients with migraine: a resting-state functional connectivity analysis study. *PLoS One*. 2014;9:e103929.

23. Kagan R, Kainz V, Burstein R, Noseda R. Circadian and homeostatic modulation of functional connectivity in migraine with aura. *J Neurosci*. 2016;36:1987-1993.

24. Ashina M, Hansen JM, Olesen J. Pearls and pitfalls in human pharmacological models of migraine: 30 years’ experience. *Cephalalgia*. 2013:33:540-553.

25. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fmri data sets. *NeuroImage*. 2003;19:1233-1239.

26. Coppola G, Di Renzo A, Petolicchio B, et al. Increased neural connectivity between the hypothalamus and cortical resting-state functional networks in chronic migraine. *J Neurol*. 2020;267:185-191.

27. Schulte LH, May A. The migraine generator revisited: two discrete patterns of premonitory symptoms. *Cephalalgia*. 2018:38:32-33.

28. Hodkinson DJ, O’Daly O, Zunszain PA, et al. Circadian and homeostatic modulation of functional connectivity and regional cerebral blood flow in humans under normal entrained conditions. *J Cereb Blood Flow Metab*. 2014;34:1493-1499.

29. Karsan N, Bose P, Zelaya FO, Goadsby PJ. Alterations in cerebral blood flow associated with the premonitory phase of migraine. *Cephalalgia*. 2018;38:36.

30. Weiller C, May A, Limroth V, et al. Brain stem activation in spontaneous human migraine attacks. *Nat Med*. 1995;1:658-660.

31. Schulte LH, Sprenger C, May A. Physiological brain-stem mechanisms of trigeminal nociception: An fMRI study at 3T. *NeuroImage*. 2016;124:518-525.

32. Noseda R, Borsook D, Burstein R. Neuropeptides and neurotransmitters that modulate thalamo-cortical pathways relevant to migraine headache. *Headache*. 2017;57(Suppl. 2):97-111.

33. Noseda R, Kainz V, Borsook D, Burstein R. Neurochemical pathways that converge on thalamic trigeminovascular neurons: potential substrate for modulation of migraine by sleep, food intake, stress and anxiety. *PLoS One*. 2014;9:e103929.

34. Kagan R, Kainz V, Burstein R, Noseda R. Hypothalamic and basal ganglia projections to the posterior thalamus: possible role in modulation of migraine headache and photophobia. *Neuroscience*. 2013;248:359-368.

35. Noseda R, Kainz V, Jakubowski M, et al. A neural mechanism for exacerbation of headache by light. *Nat Neurosci*. 2010;13:239-245.

36. Magon S, May A, Stanekwitz A, et al. Morphological abnormalities of thalamic subnuclei in migraine: A multicenter MRI study at 3 tesla. *J Neurosci*. 2015;35:13800-13806.

37. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*. 2003;19:1233-1239.

38. Maldjian JA, Laurienti PJ, Burdette JH. Precentral gyrus discrepancy in electronic versions of the Talairach atlas. *NeuroImage*. 2004;21:450-455.

39. Hadjikhani N, Ward N, Boshyan J, et al. The missing link: enhanced functional connectivity between amygdala and viscerceptive cortex in migraine. *Cephalalgia*. 2013;33:1264-1268.

40. Karsan N, Bose PR, Thompson C, Newman J, Goadsby PJ. Headache and non-headache symptoms provoked by nitroglycerin in migraineurs: A human pharmacological triggering study. *Cephalalgia*. 2020;33:103929.

41. Tessitore A, Russo A, Giordano A, et al. Disrupted default mode network connectivity in migraine without aura. *J Headache Pain*. 2013;14:89.

42. Zhong X, Shi H, Ming Q, et al. Whole-brain resting-state functional connectivity identified major depressive disorder: A multivariate pattern analysis in two independent samples. *J Affect Disord*. 2017;218:346-352.
43. Minoshima S, Foster NL, Petrie EC, Albin RL, Frey KA, Kuhl DE. Neuroimaging in dementia with Lewy bodies: metabolism, neurochemistry, and morphology. *J Geriatr Psychiatry Neurol*. 2002;15:200-209.

44. Wang T, Zhan W, Chen Q, et al. Altered resting-state ascending/descending pathways associated with the posterior thalamus in migraine without aura. *NeuroReport*. 2016;27:257-263.

45. Hubbard CS, Khan SA, Keaser ML, Mathur VA, Goyal M, Seminowicz DA. Altered brain structure and function correlate with disease severity and pain catastrophizing in migraine patients. *eNeuro*. 2014;1:e20.14.

46. Wang T, Chen N, Zhan W, et al. Altered effective connectivity of posterior thalamus in migraine with cutaneous allodynia: A resting-state fMRI study with Granger causality analysis. *J Headache Pain*. 2015;17:17.

47. Coppola G, Di Renzo A, Tinelli E, et al. Thalamo-cortical network activity between migraine attacks: Insights from MRI-based microstructural and functional resting-state network correlation analysis. *J Headache Pain*. 2016;17:100.

48. Zhang J, Su J, Wang M, et al. The sensorimotor network dysfunction in migraineurs without aura: A resting-state fMRI study. *J Neurol*. 2017;264:654-663.

49. Spencer SE, Sawyer WB, Wada H, Platt KB, Loewy AD. CNS projections to the pterygopalatine parasympathetic preganglionic neurons in the rat: A retrograde transneuronal viral cell body labeling study. *Brain Res*. 1990;534:149-169.

50. May A, Goadsby PJ. The trigeminovascular system in humans: pathophysiologic implications for primary headache syndromes of the neural influences on the cerebral circulation. *J Cereb Blood Flow Metab*. 1999;19:115-127.

51. Robert C, Bourgeais L, Arreto CD, et al. Paraventricular hypothalamic regulation of trigeminovascular mechanisms involved in headaches. *J Neurosci*. 2013;33:8827-8840.

52. Ramos JM, Castillo ME, Puerto A. Effects of atropine injection on food-associated drinking in rats with superior salivary nucleus lesions. *Behav Neural Biol*. 1989;52:422-429.

53. Vila-Pueyo M, Strother LC, Kefel M, Goadsby PJ, Holland PR. Divergent influences of the locus coeruleus on migraine pathophysiology. *Pain*. 2019;160:385-394.

54. Balaban CD, Jacob RG, Furman JM. Neurologic bases for comorbidity of balance disorders, anxiety disorders and migraine: neurotherapeutic implications. *Expert Rev Neurother*. 2011;11:379-394.

55. Mehnert J, Schulte L, Timmann D, May A. Activity and connectivity of the cerebellum in trigeminal nociception. *NeuroImage*. 2017;150:112-118.

56. Goadsby PJ, Holland PR, Martins-Oliveira M, Hoffmann J, Schankin C, Akerman S. Pathophysiology of migraine: A disorder of sensory processing. *Physiol Rev*. 2017;97:553-622.

57. Edelmayer RM, Vanderah TW, Majuta L, et al. Medullary pain facilitating neurons mediate allodynia in headache-related pain. *Ann Neurol*. 2009;65:184-193.

58. Akerman S, Holland PR, Goadsby PJ. Diencephalic and brainstem mechanisms in migraine. *Nat Rev Neurol*. 2011;12:570-584.

59. Ellrich J, Messlinger K, Chiang CY, Hu JW. Modulation of neuronal activity in the nucleus raphe magnus by the 5-HT(1)-receptor agonist naratriptan in rat. *Pain*. 2001;90:227-231.

60. Kros L, Angueyra Aristizabal CA, Khodakhah K. Cerebellar involvement in migraine. *Cephalalgia*. 2018;38:1782-1791.

61. Argiolas A, Melis MR. The neuropharmacology of yawning. *Eur J Pharmacol*. 1998;343:1-16.

62. Scott TR, Small DM. The role of the parabrachial nucleus in taste processing and feeding. *Ann N Y Acad Sci*. 2009;1170:372-377.

63. Burstein R, Jakubowski M, Garcia-Nicas E, et al. Thalamic sensitization transforms localized pain into widespread allodynia. *Ann Neurol*. 2010;68:81-91.

64. Abrams J. Pharmacology of nitroglycerin and long-acting nitrates. *Am J Cardiol*. 1985;56:12a-18a.