Resting-state PAG connectivity in migraine

Altered functional MRI resting-state connectivity in periaqueductal gray networks in migraine

Caterina Mainero[1], MD, PhD, Jasmine Boshyan[1], Nouchine Hadjikhani[1,2], MD, PhD.

[1]Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA

[2]Brain Mind Institute, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland

Correspondence to: Nouchine Hadjikhani

A. A. Martinos Center for Biomedical Imaging

Bldg149 13th Street, Room 2301

Charlestown, MA 02129

E-mail: nouchine@nmr.mgh.harvard.edu

Phone: (617) 724-5625

Fax: (530) 309-4973

Keywords: periaqueductal gray, connectivity, resting-state, functional MRI, migraine

Abstract word count= 250

Text word count=2870

References=41

Tables= 5

Figures=3

Supplemental data= 2 Tables, 1 Figure

Resting-state PAG connectivity in migraine

Summary

Objective: The periaqueductal gray matter (PAG), a known modulator of somatic pain transmission, shows evidence of interictal functional and structural abnormalities in migraineurs, which may contribute to hyperexcitability along spinal and trigeminal nociceptive pathways, and lead to the migraine attack. The aim of this study was to examine functional connectivity of the PAG in migraine.

Methods: Using resting-state functional MRI, we compared functional connectivity between PAG and a subset of brain areas involved in nociceptive/somatosensory processing and pain modulation in 17 subjects with migraine, during a pain free state, versus 17 gender- and age-matched controls. We also assessed the relation between intrinsic resting-state correlations within PAG networks and the average monthly frequency of migraine attacks, as well as allodynia.

Results: Our findings show stronger connectivity between the PAG and several brain areas within nociceptive and somatosensory processing pathways in migraineurs versus controls. In addition, as the monthly frequency of migraine attacks worsens, the strength of the connectivity in some areas within these pathways increases while a significant decrease in functional resting-state connectivity between the PAG and brain regions with a predominant role in pain modulation (prefrontal cortex, anterior cingulate, amygdala) can be evidenced. Finally, migraineurs with a history of allodynia exhibit significantly reduced connectivity between PAG, prefrontal regions and anterior cingulate compared to migraineurs without allodynia.

Conclusions: These data reveal interictal dysfunctional dynamics within pain pathways in migraine manifested as an impairment of the descending pain modulatory, likely leading to loss of pain inhibition, and hyperexcitability primarily in nociceptive areas.

Background

Current concepts of migraine suggest that a broad sensory processing dysfunction characterizes this common neurological disorder, and identify the periaqueductal gray matter (PAG), a known modulator of somatic pain transmission, as one of the key areas in the pathophysiology of migraine headache. The relevance of the PAG involved in the pathogenesis of migraine is further supported by reports of subjects without headache who developed migraine-like episodes after stereotactic placement of electrodes in this area of brainstem[1, 2]. Abnormalities in the PAG of migraineurs are often paralleled by structural changes in functionally and/or anatomically connected brain regions involved in pain processing and modulation[3-9].

A prevailing theory in the pathogenesis of the migraine attack is that hyperexcitability develops along the trigeminovascular pathway, probably facilitated by a dysfunction of the descending pain modulatory circuits. Functional MRI (fMRI) studies of the interictal phase of migraine show hypofunction of pain modulatory circuits to thermal stimuli, and a specific involvement of the nucleus cuneiformis (NCF) in the brainstem[10], a structure extensively connected to the PAG and sharing its modulatory effects on pain perception. Interictal altered dynamics within pain pathways might therefore contribute to the development of the migraine attack. Task-fMRI findings, however, reflect task-related increases of neuronal metabolism that are small, less than 5% compared to the metabolism of the brain at rest[11]. Thus, it is critical to take into account brain activity that occurs in the absence of external stimulation in order to understand better how pain networks function and adapt in migraineurs. Clarifying this aspect will have important implications for understanding the pathogenesis of migraine, and for disclosing similarities and potential differences with other chronic pain conditions.

During migraine attacks, in about two-thirds of patients, allodynia, the perception of pain to normally innocuous stimulation, may manifest[12]. Migraineurs with allodynia show a significant drop in pain thresholds to mechanical and thermal stimulation of cephalic and extracephalic skin, a phenomenon not observed in the absence of migraine[12, 13]. Experimental studies have

demonstrated that the neurophysiological correlate of allodynia is sensitization along the

trigemino-vascular pathway, an increased afferent barrage for an unchanged peripheral stimulus[14,

15]. Speculations exist whether this sensitization can be indirectly activated through pain

modulatory neurons in the brainstem.

We used resting-state fMRI, a technique that allows to identify correlations during rest

between remote brain areas (functional connectivity) through their highly correlated low

frequency spontaneous fluctuations[16], to investigate intrinsic connectivity within the PAG in 17

patients with episodic migraine during a pain free period (i.e. the interictal phase) and in 17

gender- and age-matched healthy controls. Given that connectivity within pain pathways may be

dysfunctional outside episodes of migraine pain, even in the absence of external pain stimulation,

and that accumulating evidence suggests that this might lead to the development of the migraine

attack, we focused our approach on a subset of brain areas involved in the processing and

modulation of somatosensory and pain signals. We tested the hypotheses that: 1) intrinsic

connectivity within the PAG and either somatosensory/pain processing and modulatory pathways

would be dysfunctional in migraineurs relative to age-matched healthy subjects; and that 2) these

alterations would be associated with disease severity as measured by the frequency of migraine

attacks per month. A further aim was to assess functional connectivity differences within PAG

circuits between patients who reported allodynia during the migraine attack and patients without

allodynia.

Methods

Subjects. Demographic and clinical characteristics of migraineurs are shown in Table 1.

Seventeen subjects with migraine (15 females, mean±SD age= 32.4±8.2 years, median, range

disease duration= 16.6, 1-30 years), eight with migraine with aura (MWA), and nine with

migraine without aura (MWoA), as defined by the IHS diagnostic criteria[17], were included in the

study. All patients were migraine free for at least 72 hours at the time of MRI, had no other

Resting-state PAG connectivity in migraine

medical or psychiatric condition than migraine, no history of medications overuse, and without any prophylactic or chronic medication for at least 1 year prior to study entry. All migraineurs included in the study had a normal neurological examination.

Seventeen gender- and age-matched healthy subjects (15 females, mean±SD age= 31.9±8.45 years) with no history of migraine or other headache types, and free from any chronic medical condition and any chronic treatment were included as controls.

The local ethics committee of our Institution approved all experimental procedures of the study, and written informed consent was obtained from each study participant.

Acquisition. All subjects underwent anatomical and functional scanning on a 3 Tesla Tim Trio scanner (Siemens, Erlangen) with a 32-channel Siemens head-coil. Single-shot EPI images (47, $3x3x3mm^3$ slices, TR/TE=300/30ms, flip angle 90°, 160 volumes) were acquired for functional resting state analysis. During resting state all subjects were asked to keep their eyes closed. Anatomical data consisted of a high-resolution structural 3D scan with a magnetization-prepared rapid acquisition with multiple gradient echoes (MEMPR) sequence resolution (0.9 x 0.9 x 0.9 mm^3, TI=1200 ms, TR=2530 ms, flip angle=7°, TE=1.7+n.1.88 ms where n=0, .., 3, FoV=230 mm, bandwidth=651 Hz/px). We also acquired in each control and in 16 out of 17 migraineurs T2 turbo-spin-echo (TSE) weighted images (TR/TE=11810/95 ms, flip angle=120°, matrix size= 320×328, 63 2 mm thick slices, 2 averages) for identification of white matter (WM) lesions. None of the subjects enrolled in the study showed brain WM hyperintensities. Finally, none of the patients developed a migraine during the entire scan and in the following hour during which they underwent neurological examination.

Post-processing. Images were processed using FMRIB software library (FSL, http://www.fmrib.ox.ac.uk/fsl/). Data were motion corrected to the middle frame in each series

using MCFLIRT [18] and spatially smoothed with a 6mm FWHM 3D Gaussian kernel using FSL's

SUSAN. Non-brain areas of the scan were removed using BET [19]. Grand-mean intensity

normalization of the entire 4D dataset by a single multiplicative factor was performed, as well as

high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with

sigma=60.0s). Each time series was prewhitened [20]. Independent Component Analysis (ICA)

using MELODIC was run to remove structured noise from the data. Registration of functional

and anatomical data was performed using FLIRT. Nonlinear registration using FNIRT was

applied between the subject's structural and the standard space (the MNI_2mm brain). Seeds were

manually selected in standard space based on the anatomy. The right and left PAG were selected

as regions of interest (peak MNI coordinates: left PAG= -2; -28; -6; right PAG= 4; -28; -6, with 3

mm radius). We chose this location as a seed based on our previous findings of interictal

fractional anisotropy changes in the PAG in another population of migraineurs. The preprocessed

time series were then fitted with a linear model consisting of regressor representing the extracted

time courses. Average time courses were extracted from these seeds using FSL's featquery

function. The preprocessed time series were then fitted with a linear model consisting of regressor

representing the extracted time courses. A temporal derivative of that regressor was entered into

the model, to allow the general linear model (GLM) to compensate for latency offsets in the

hemodynamic response of different brain regions, and to compensate for slice timing differences

in the acquisition. In addition, motion correction parameters were used as distractor regressors.

The spatially normalized effect size and standard error volumes served as input to a mixed effect

group analysis in FSL's feat based on the method described by Beckman et al., [21]. The modeled

group effect size and standard error were then divided to produce a volume whose voxels were T

scores, subsequently transformed to Z scores. Images were thresholded using clusters determined

by Z>2.3 and a corrected cluster significance threshold of p=0.05, including at least 20

contiguous voxels [22].

Resting-state PAG connectivity in migraine

The thresholded Z maps were then sampled on the cortical surface of an individual subject using the inverse coordinate transformation between this individual's native space and the group MNI space. Within- and between-group comparisons of correlation effect size were performed using one sample t-test in each group and unpaired t-test in controls versus migraineurs. Correlation analysis with the monthly frequency of migraine was performed by including the mean number of migraine attacks of each patient as a regressor. Since we did not detect laterality differences between the two seeds used, results for left and right PAG were grouped together.

Results

Results of intrinsic functional connectivity of the PAG in migraineurs and controls are shown in Table A - Supplemental data and Figure 1. In both groups, we found predominantly positive correlations between PAG and nearby structures including the brainstem (mostly the midbrain), thalamus, putamen, parahippocampus, amygdala (only in controls), cerebellum, and with distant regions including the anterior cingulate, frontal and temporal cortex, and left insula (only in controls), although correlation with these cortical areas was more prevalent in migraineurs than in controls. Migraineurs showed additional positive correlations in the precentral and postcentral cortex, and angular gyri. There were no negative correlations in migraineurs, while controls showed significant negative correlations between the PAG and the caudal brainstem and central opercular cortex.

The comparison between migraineurs and controls revealed in patients significantly increased functional resting-state correlations (Z>2.3, corrected cluster p=0.05) between the PAG and several cortical regions primarily involved in nociception and somatosensory processing compared to age-matched controls (Table 2, Figure A – Supplemental data). Seeds with a 3 mm radius were selected in a subset of these areas using the peak MNI coordinates in Table 2. Average time courses were extracted from each seed using FSL's featquery function and correlation analysis with both right and left PAG time courses was performed using linear

regression. The resulting r-values were entered into single sample t-tests with the null hypothesis of 0 for the mean r-value of the group using Bonferroni correction for multiple comparisons (Table B – Supplemental data). We did not find any increased intrinsic connectivity between PAG and other pain regions in controls compared to migraineurs.

In patients we found that the higher was the monthly frequency of migraine attacks, the greater was the connectivity between the PAG and some of the brain areas that showed significantly higher correlations compared to controls (Table 3, Fig.2). We observed a similar positive correlation between the monthly frequency of migraine episodes and resting state correlations between the PAG the nucleus NCF and the hypothalamus. (Fig.2)

Interestingly, an increased monthly frequency of migraine attacks was associated with reduced functional connectivity between the PAG and several regions of the prefrontal cortex, anterior cingulate, amygdala, medial thalamus (Table 4, Fig. 2).

Subjects with migraine were asked to fill out a questionnaire to determine if they usually developed allodynia during the migraine attack. Five subjects reported a history of either cephalic or extra-cephalic allodynia during migraines. The comparison of functional resting-state connectivity in these patients (mean±SD age= 35.6±7.2 years) versus five gender- ad age-matched migraineurs with no history of allodynia (mean±SD age= 36.8±5.9 years) showed that migraineurs who develop pain to normally innocuous stimulation have significantly reduced connectivity between PAG, prefrontal regions, anterior cingulate, and anterior insula compared to subjects without allodynia (Table 5, Fig. 3).

Discussion

Our findings demonstrate an interictal increase in resting-state intrinsic connectivity between the PAG and both nociceptive and sensory processing pathways in migraineurs relative to age and gender- matched controls. Connectivity in some of these pathways was stronger as the monthly

Resting-state PAG connectivity in migraine

frequency of migraine attacks increased. Conversely, the greater the number of attacks was, the lower the functional resting-state connectivity appeared to be between the PAG and several brain regions with a predominant role in pain modulation (prefrontal cortex, anterior cingulate, amygdala). Since the occurrence of the next migraine attack was not recorded, whether the observed association between migraine frequency and changes in PAG connectivity reflects either an adaptive mechanism or an abnormal status in cortical excitability that predisposes to a migraine attack needs to be further clarified. Excitability in the visual cortex has been shown to increase interictally in pediatric MWoA, although it tends to decrease or normalize in the peri-ictal period[23].

Previous neuroimaging findings indicate that the brainstem covers a crucial role in acute migraine[24], and positron emission tomography (PET) studies report metabolic changes in either the dorsal rostral brainstem or in the locus coeruleus and dorsal raphe nucleus of migraineurs[25, 26]. Dysfunction of the regulation of the brainstem pain-inhibiting circuitry, of which the PAG is best known, may provide an explanation for many of the facets of migraine headache.

Compared to healthy subjects, migraineurs showed, in the absence of any pain, significantly greater intrinsic connectivity between PAG in several brain regions including thalamus, posterior parietal cortex (angular and supramarginal gyri), anterior insula, S1, M1, and partly S2. While S1, S2, anterior insula, and thalamus are part of the fundamental core network found active by several neuroimaging studies in different pain conditions activity in other regions including the posterior parietal cortex and M1 has also been reported in nociception[27]. These findings could reflect a condition of hyperexcitability of pain pathways within the central nervous system (CNS), which is thought to represent a crucial event in the pathophysiology of migraine. Transcranial magnetic stimulation (TMS), and magneto-encephalography (MEG) studies have shown generalized interictal changes of excitability in the cerebral cortex in migraine with alteration of excitability in the motor and visual cortices [28-33]. In addition, in patients with unilateral migraine, in a headache-free condition, diffuse hypersensitivity of the peripheral nerves has been measured,

leading to further evidence to the presence of a state of hyperexcitability of the CNS of migraineurs[34]. Interestingly, central hyperexcitability does not seem to be a feature of chronic either tension type or cervicogenic headaches, which are characterized by different pathogenetic and clinical features than migraine[35].

A systematic investigation of intrinsic connectivity patterns of the PAG by resting-state fMRI in a large cohort of healthy subjects reports that PAG activity is positively correlated with surrounding subcortical brain regions including midbrain tegmentum, substantia nigra, raphe nucleus, thalamus, and hypothalamus, as well as with cortical regions including the anterior cingulate, and anterior insula[36]. In our study, in some of these areas (anterior insula, NCF in the midbrain, and hypothalamus) the strength of intrinsic connectivity with PAG positively correlated with disease severity as measured by the number of migraine episodes per month. Additional areas involved in nociception and somatosensory processing including S2 and the posterior parietal cortex also show increased resting-state connectivity with PAG with increasing in the monthly frequency of migraine attacks.

The finding that hypothalamic intrinsic correlations with PAG are related to the frequency of migraine attacks extends previous PET data that have first described activation of the hypothalamus in migraineurs during the headache phase, which persisted after headache remission with sumatriptan[37]. While specific hypothalamic activations have been consistently observed in cluster headache, and linked to the autonomic manifestations of trigeminal autonomic cephalalgias, the role of the hypothalamus in migraine has not been clarified yet[24]. The hypothalamus is integrated in the hypothalamic-pituitary-adrenal (HPA) axis, which is also involved in mood disorders such as stress and anxiety. In addition to pain modulation, the PAG is well known to participate in mood and emotion regulation, which may be carried out through a pathway involving the HPA axis also[36]. In this context, although subjects with depression were excluded from our study, an increase in the intrinsic correlations between PAG and hypothalamus in relation to the frequency of migraine attacks can be viewed as a part of a stress/anxiety

response of the brain to worsening of the disease. Behavioral data assessing anxiety levels in patients with migraine can help to clarify this aspect.

In migraineurs, we also found that the greater the frequency of migraine episodes was, the lower the resting-state functional connectivity appeared to be between PAG and several brain foci, mostly located in the bilateral prefrontal (medial, dorsomedial) cortex, but also in the anterior cingulate and amygdala, both involved in descending pain modulation and nociception[27], and in somatosensory processing regions including the angular gyrus (posterior parietal cortex), right S1, and M1. The pattern, however, was clearly dominated by a widespread reduced connectivity between PAG and prefrontal cortex that correlated with the increase in migraine attacks frequency. Pain studies have emphasized the role of prefrontal areas in controlling the functional interactions among key nociceptive brain regions in order to modify the perceptual correlates of pain, specifically by driving endogenous pain-inhibitory mechanisms[27, 38]. Our findings could therefore indicate an interictal dysfunction of the descending inhibitory system that in turns contributes to the development of migraines. TMS and PET imaging demonstrated that in patients with chronic migraine increase in visual cortical excitability is accompanied by brainstem activation and inhibition of specific cortical areas including the medial frontal and parietal cortex, and somatosensory area, suggesting a potential dysfunction of inhibitory pathways[39]. Interestingly, this pattern of significantly reduced connectivity between PAG, prefrontal cortex, and anterior cingulate was also observed in migraineurs with allodynia compared to migraineurs without allodynia, corroborating the hypothesis that pain modulatory neurons might be involved in the development of allodynia.

Taken together, our data demonstrate the presence of interictal dysfunctional dynamics within PAG networks in episodic migraine. Since resting brain activity is either increased or decreased within multiple networks in other chronic pain conditions including fibromyalgia[40] and diabethic neuropatic pain[41], future studies can clarify whether the changes that we observed within the PAG networks can be interpreted as a "brain signature" of migraine; or rather they are shared by other

chronic pain conditions.

Resting-state PAG connectivity in migraine

References

1.   Raskin NH, Hosobuchi Y, Lamb S. Headache may arise from perturbation of brain. Headache. 1987;27:416-420

2.   Veloso F, Kumar K, Toth C. Headache secondary to deep brain implantation. Headache. 1998;38:507-515

3.   Rocca MA, Ceccarelli A, Falini A et al. Diffusion tensor magnetic resonance imaging at 3.0 tesla shows subtle cerebral grey matter abnormalities in patients with migraine. J Neurol Neurosurg Psychiatry. 2006;77:686-689

4.   DaSilva AF, Granziera C, Tuch DS et al. Interictal alterations of the trigeminal somatosensory pathway and periaqueductal gray matter in migraine. Neuroreport. 2007;18:301-305

5.   DaSilva AF, Granziera C, Snyder J, Hadjikhani N. Thickening in the somatosensory cortex of patients with migraine. Neurology. 2007;69:1990-1995

6.   Kim JH, Suh SI, Seol HY et al. Regional grey matter changes in patients with migraine: a voxel-based morphometry study. Cephalalgia. 2008;28:598-604

7.   Schmidt-Wilcke T, Ganssbauer S, Neuner T et al. Subtle grey matter changes between migraine patients and healthy controls. Cephalalgia. 2008;28:1-4

8.   Schmitz N, Admiraal-Behloul F, Arkink EB et al. Attack frequency and disease duration as indicators for brain damage in migraine. Headache. 2008;48:1044-1055

9.   Valfre W, Rainero I, Bergui M, Pinessi L. Voxel-based morphometry reveals gray matter abnormalities in migraine. Headache. 2008;48:109-117

10.   Moulton EA, Burstein R, Tully S et al. Interictal dysfunction of a brainstem descending modulatory center in migraine patients. PLoS One. 2008;3:e3799

11.   Raichle ME, Mintun MA. Brain work and brain imaging. Annu Rev Neurosci. 2006;29:449-476

Resting-state PAG connectivity in migraine

12.   Burstein R, Yarnitsky D, Goor-Aryeh I et al. An association between migraine and

         cutaneous allodynia. Ann Neurol. 2000;47:614-624

13.   Lipton RB, Bigal ME, Ashina S et al. Cutaneous allodynia in the migraine population.

         Ann Neurol. 2008;63:148-158

14.   Burstein R, Cutrer MF, Yarnitsky D. The development of cutaneous allodynia during a

         migraine attack clinical evidence for the sequential recruitment of spinal and supraspinal

         nociceptive neurons in migraine. Brain. 2000;123 (Pt 8):1703-1709

15.   Strassman AM, Raymond SA, Burstein R. Sensitization of meningeal sensory neurons

         and the origin of headaches. Nature. 1996;384:560-564

16.   Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with

         functional magnetic resonance imaging. Nat Rev Neurosci. 2007;8:700-711

17.   IHC. The International Classification of Headache Disorders: 2nd edition. Cephalalgia.

         2004;24 Suppl 1:9-160

18.   Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and

         accurate linear registration and motion correction of brain images. Neuroimage.

         2002;17:825-841

19.   Smith SM, Zhang Y, Jenkinson M et al. Accurate, robust, and automated longitudinal and

         cross-sectional brain change analysis. Neuroimage. 2002;17:479-489

20.   Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal autocorrelation in univariate

         linear modeling of FMRI data. Neuroimage. 2001;14:1370-1386

21.   Beckmann CF, Jenkinson M, Smith SM. General multilevel linear modeling for group

         analysis in FMRI. Neuroimage. 2003;20:1052-1063

22.   Worsley KJ, Chen JI, Lerch J, Evans AC. Comparing functional connectivity via

         thresholding correlations and singular value decomposition. Philos Trans R Soc Lond B

         Biol Sci. 2005;360:913-920

Resting-state PAG connectivity in migraine

23. Siniatchkin M, Reich AL, Shepherd AJ et al. Peri-ictal changes of cortical excitability in children suffering from migraine without aura. Pain. 2009;147:132-140

24. May A. New insights into headache: an update on functional and structural imaging findings. Nat Rev Neurol. 2009;5:199-209

25. Bahra A, Matharu MS, Buchel C et al. Brainstem activation specific to migraine headache. Lancet. 2001;357:1016-1017

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

27. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. Neuron. 2007;55:377-391

28. Aurora SK, Wilkinson F. The brain is hyperexcitable in migraine. Cephalalgia. 2007;27:1442-1453

29. Bowyer SM, Mason KM, Moran JE et al. Cortical hyperexcitability in migraine patients before and after sodium valproate treatment. J Clin Neurophysiol. 2005;22:65-67

30. Wang X, Xiang J, Wang Y et al. Identification of abnormal neuromagnetic signatures in the motor cortex of adolescent migraine. Headache. 2010;50:1005-1016

31. van der Kamp W, Maassen VanDenBrink A, Ferrari MD, van Dijk JG. Interictal cortical hyperexcitability in migraine patients demonstrated with transcranial magnetic stimulation. J Neurol Sci. 1996;139:106-110

32. van der Kamp W, MaassenVanDenBrink A, Ferrari MD, van Dijk JG. Interictal cortical excitability to magnetic stimulation in familial hemiplegic migraine. Neurology. 1997;48:1462-1464

33. Hoffken O, Stude P, Lenz M et al. Visual paired-pulse stimulation reveals enhanced visual cortex excitability in migraineurs. Eur J Neurosci. 2009;30:714-720

Resting-state PAG connectivity in migraine

34.   Fernandez-de-las-Penas C, Arendt-Nielsen L, Cuadrado ML, Pareja JA. Generalized

mechanical pain sensitivity over nerve tissues in patients with strictly unilateral migraine.

Clin J Pain. 2009;25:401-406

35.   Uthaikhup S, Sterling M, Jull G. Widespread sensory hypersensitivity is not a feature of

chronic headache in elders. Clin J Pain. 2009;25:699-704

36.   Kong J, Tu PC, Zyloney C, Su TP. Intrinsic functional connectivity of the periaqueductal

gray, a resting fMRI study. Behav Brain Res. 2010;211:215-219

37.   Denuelle M, Fabre N, Payoux P et al. Hypothalamic activation in spontaneous migraine

attacks. Headache. 2007;47:1418-1426

38.   Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral

prefrontal cortex in pain modulation. Brain. 2003;126:1079-1091

39.   Aurora SK, Barrodale PM, Tipton RL, Khodavirdi A. Brainstem dysfunction in chronic

migraine as evidenced by neurophysiological and positron emission tomography studies.

Headache. 2007;47:996-1003; discussion 1004-1007

40.   Napadow V, LaCount L, Park K et al. Intrinsic brain connectivity in fibromyalgia is

associated with chronic pain intensity. Arthritis Rheum;62:2545-2555

41.   Cauda F, D'Agata F, Sacco K et al. Altered resting state attentional networks in diabetic

neuropathic pain. J Neurol Neurosurg Psychiatry;81:806-811

Table 1. Demographic and clinical characteristics of migraineurs.

Patient#	Age (years)	Gender	Disease duration (years)	Migraines frequency (per month)	Aura	Allodynia
MIG113	18	Female	8	3	No	No
MIG108	23	Female	17	3	Yes	No
MIG110	24	Female	13	3	No	No
MIG116	24	Female	2.5	4	Yes	No
MIG118	24	Female	12	1	No	No
MIG117	29	Female	12	1	Yes	Yes
MIG105	30	Female	25	4	No	No
MIG119	31	Female	16	5	No	Yes
MIG107	33	Female	20	5	Yes	Yes
MIG111	34	Female	20	1	Yes	No
MIG112	34	Female	8.5	3	No	No
MIG109	37	Female	30	1	No	No
MIG104	38	Male	22	1	Yes	Yes
MIG106	39	Female	1	3	Yes	No
MIG101	42	Female	15	2	No	No
MIG102	44	Male	30	15	No	No
MIG120	47	Female	30	3	Yes	Yes

Table 2. Peak locations and significance of brain areas with increased connectivity with the periaqueductal gray (PAG) during resting state fMRI in 17 patients with migraine compared to 17 gender- and age-matched controls.

Location	PAG	
	MNI coordinates (x, y, z)	Z
R Ventrolateral prefrontal cortex	28, 58, -16	2.49
R Supramarginal gyrus	56, -44, 34	2.93
	64, -36, 44	2.83
	52, -40, 26	2.73
R Anterior insula	40, 14, -10	2.3
R Precentral gyrus (M1)	42, 0, 56	2.9
R Postcentral gyrus (S1)	46, -34, 52	2.45
R Thalamus	10, -14, 4	2.55
L Angular gyrus	-56, -52, 44	3.24
L Supramarginal gyrus/Parietal operculum	-58, 52, 44	3.17
(S2)	-10, 10, 52	3.0
L Precentral gyrus (M1)	-44, 8, 28	2.53
	-44, 6, 28	2.32

R=right; L=left; M1=primary motor cortex; S1=primary somatosensory cortex; S2=secondary somatosensory cortex Z>2.3, corrected cluster significance of p=0.05 corresponding to at least 20 contiguous voxels.

Table 3. Brain areas where resting state connectivity with the periaqueductal gray (PAG)

positively correlates with the frequency of migraine attacks in 17 patients with migraine.

Location	PAG	
	MNI coordinates (x, y, z)	Z
R Supramarginal gyrus	68, -38, 38	3.03
	60, -44, 50	2.84
R Ventral prefrontal cortex	20, 64, -16	3.14
R Anterior Insula	42, 16, 0	3
L Supramarginal gyrus / Parietal operculum (S2)	-56, 44, 26	2.78
	-54, -44, 28	2.6
L Nucleus Cuneiformis	-4, -38, -22	2.47
	-6, -38, 20	2.83
Hypothalamus	2, -18, -14	2.34

R=right; L=left; S2=secondary somatosensory cortex; PAG= periaqueductal gray; Z>2.3,

corrected cluster significance of p=0.05 corresponding to at least 20 contiguous voxels.

Table 4. Brain areas where resting state connectivity with the periaqueductal gray (PAG) negatively correlates with the frequency of migraine attacks in 17 patients with migraine.

Location	PAG	
	MNI coordinates (x, y, z)	**Z**
R Dorsomedial prefrontal cortex	16, 56, 34	-3.29
R Postcentral gyrus (S1)	36, -24, 52	-2.79
R Precentral gyrus (M1)	-8, -28, 58	-2.99
R Anterior Cingulate	6, 52, 16	-2.83
R Parahippocampal gyrus/Amygdala	16, 2, -28	-3.08
L Dorsomedial prefrontal cortex	-14, 48, 42	-3.91
L Medial prefrontal cortex	-10, 68, -10	-2.61
L Precentral gyrus (M1)	-50, -4, 38	-3.14
L Angular gyrus	-40, -52, 14	4.13
L Medial Thalamus	-8, -8, 16	2.54

R=right; L=left; M1=primary motor cortex; S1=primary somatosensory cortex; Z>2.3, corrected cluster significance of p=0.05 corresponding to at least 20 contiguous voxels.

Table 5. Brain areas showing significantly decreased connectivity with the periaqueductal gray (PAG)

during resting state fMRI in five patients with allodynia compared to age-matched patients without allodynia.

Location	PAG	
	MNI coordinates (x, y, z)	Z
R Dorsolateral prefrontal cortex	36, 50, 6	-2.8
R Lateral prefrontal cortex	44, 46, -14	-2.61
R Anterior Cingulate	12, 36, 24	-2.54
L Dorsomedial prefrontal cortex	-18, 62, 20	-2.55
L Medial prefrontal cortex	-12, 62, -22	-3.00
L Anterior Insula	-34, 16, 10	-2.15

 R=right; L=left; Z>2.3, corrected cluster significance of p=0.05 corresponding to at least 20
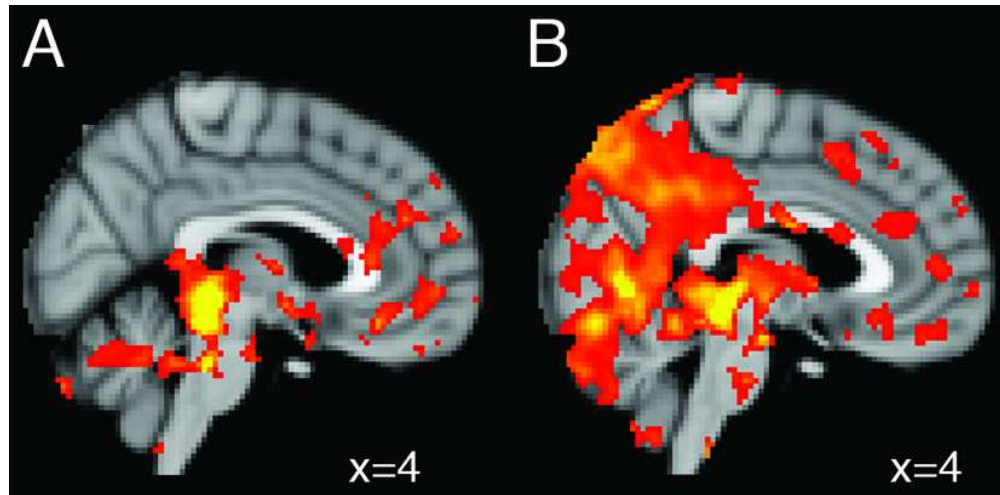
contiguous voxels.

Figure 1: Sagittal sections of the MNI brain illustrating representative brain regions that show positive functional resting state connectivity with the periaqueductal gray in (A) 17 healthy subjects and in (B) 17 age- and gender-matched patients with migraine, interictally. Statistical threshold corresponded to Z>5.0
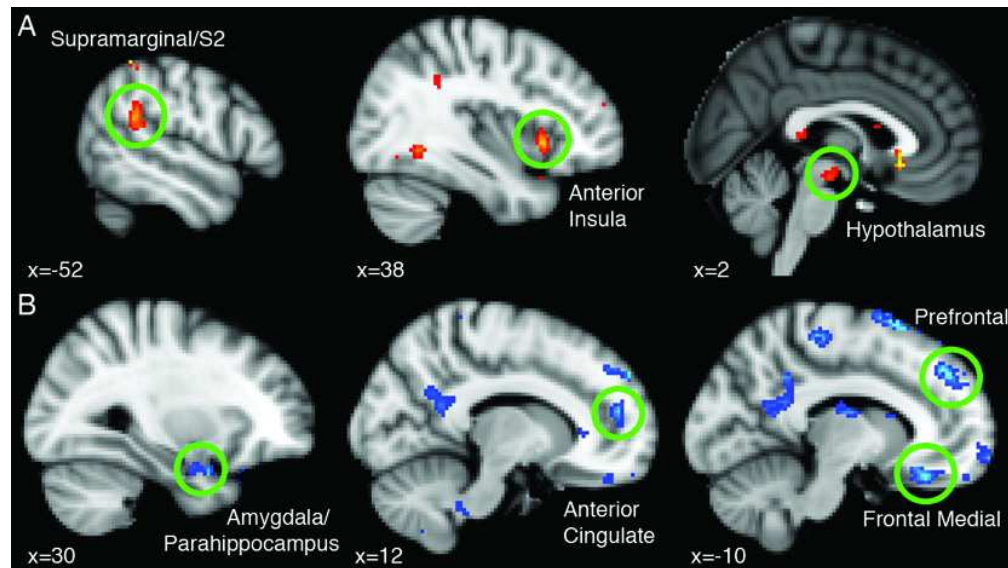70x34mm (300 x 300 DPI)

Figure 2: Sagittal sections of the MNI brain showing in 17 patients with migraine, interictally, A) brain areas where increased functional resting-state connectivity with the periaqueductal gray (PAG) positively correlates with the frequency of migraine attacks; B) brain regions where decreased functional connectivity with the PAG is associated with an increased frequency of migraine attacks. Statistical threshold corresponded to Z>2.3 and a corrected cluster significance of p=0.05, including clusters with at least 20 contiguous voxels.
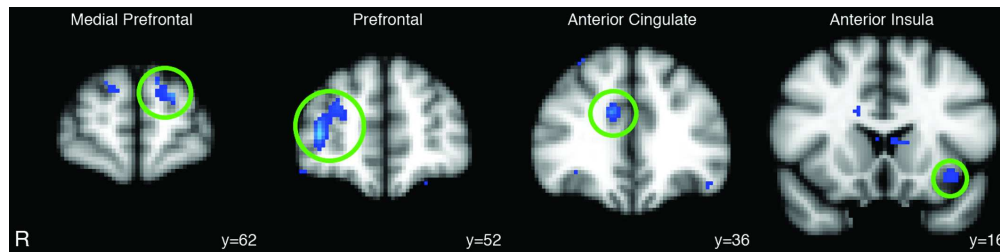70x39mm (300 x 300 DPI)

Figure 3: Coronal views of the MNI showing brain areas (Z>2.3 and a corrected cluster significance of p=0.05 corresponding to at least 20 contiguous voxels) of reduced functional resting-state connectivity with the periaqueductal gray in five migraineurs with a history of allodynia compared to five age- and gender-matched migraineurs without any history of allodynia development during the migraine attack. R= right.
180x44mm (300 x 300 DPI)

Table A-Supplemental data. Results of functional connectivity during resting state in 17 migraineurs and

17 gender- and age-matched controls demonstrating correlations in the brain with the periaqueductal gray.

Location	Migraineurs		Controls	
	MNI coordinates (x, y, z)	Z	MNI coordinates (x, y, z)	Z
Superior frontal gyrus			16, 26, 50	3.57
Middle frontal gyrus	36, 0, 62	4.06	-44, 14, 40	4.04
	34, -2, 46	4.04	40, 0, 50	3.73
	-36, 16, 36	3.64		
Inferior frontal gyrus	44, 12, 24	3.98	-50, 14, -2	3.84
Frontal pole	-24, 4, -4	5.27	-36, 42, -4	3.85
	26, 60, 6	3.89	36, 42, -8	3.53
Orbitofrontal cortex	14, 30, -16	4.55	-30, 28, -6	5.14
	-26, 14, -22	3.94	26, 8, -22	3.73
Angular gyrus	-64, -56, 18	4.73		
Postcentral gyrus	-8, -36, 70	3.61		
Precentral gyrus	6, -14, 80	3.94		
	-52, 6, 32	3.61		
	32, -18, 66	3.51		
Cingulate cortex	10, 32, 40	4.26	-2, -24, 30	3.49
	2, 50, 6	3.17	0, -44, 24	3.33
			8, 38, 26	3.37
Insula			-38, 10, -14	4.06
Central opercular cortex			46, 2, 8	-2.99
Middle temporal gyrus			64, -14, 26	3.75
Temporal pole	38, 14, -40	4.95		
	-38, 8, -42	4.86		
Inferior temporal gyrus	-52, -26, 20	4.19	46, -16, 30	4.1
Temporal occipital fusiform	42, -52, 12	4.32		
	44, -52, 12	4.21		
Parahippocampal gyrus	10, 32, 40	4.26	-28, -14, 36	4.3
			16, 4, -22	3.77
Amygdala			-18, -2, -16	3.72
Thalamus	10, -25, 6	3.9	2, -6, 8	3.8
	-6, -16, 8	3.4	-16, -16, 2	3.27
Putamen	24, -2, 12	3.66	-24, 2, 4	3.66
			24, 18, 0	3.65
Brainstem	6, -28, 8	6.83	8, -28, -6	6.73
	-6, -30, -8	6.29	-2, -30, -6	6.52
			4, -40, -44	-3.06
Cerebellum	46, -66, 36	5.36	34, -42, 48	4.58
	-24, -58, -60	4.04	16, -46, 42	4.09
	-50, -60, -42	3.88	-40, -50, -60	3.96
	14, -48, -56	3.73	-16, -52, 44	3.89
Vermis			2, -68, -26	3.54

Z>2.3, corrected cluster significance of p=0.05 corresponding to at least 20 contiguous voxels.

Table B-Supplemental data. Significance values from one-sample t-test in 17 migraineurs and 17 gender- and age-matched controls of r-values derived from the correlation analysis between resting state fMRI time courses in the periaqueductal gray and time courses in somatosensory and pain processing brain regions.

	Migraineurs		Controls	
	L PAG	R PAG	L PAG	R PAG
Brain Area	**T value**	**T value**	**T value**	**T value**
L Angular	3.03**	3.54**	0.66	1.11
L Supramarginal	3.16**	3.70**	0.20	0.71
R Supramarginal	4.03***	4.57***	1.95	3.28**
R Postcentral	4.11***	4.88	2.21*	1.88
R Insula	5.25***	6.70***	1.81	2.82*
R Thalamus	3.75**	3.07**	0.10	0.29

*=p<0.05; **=p<0.01; ***=p<0.001 by paired t-test; L= left; R= right; PAG= periaqueductal gray

Legend to Figure A – Supplemental data

Figure A. Sagittal sections of the MNI brain illustrating brain regions of significantly increased functional resting-state connectivity with the periaqueductal gray in 17 subjects with migraine, interictally, relative to 17 age- and gender-matched healthy controls. Statistical threshold corresponded to Z>2.3 and a corrected cluster significance of p=0.05, including clusters with at least 20 contiguous voxels.
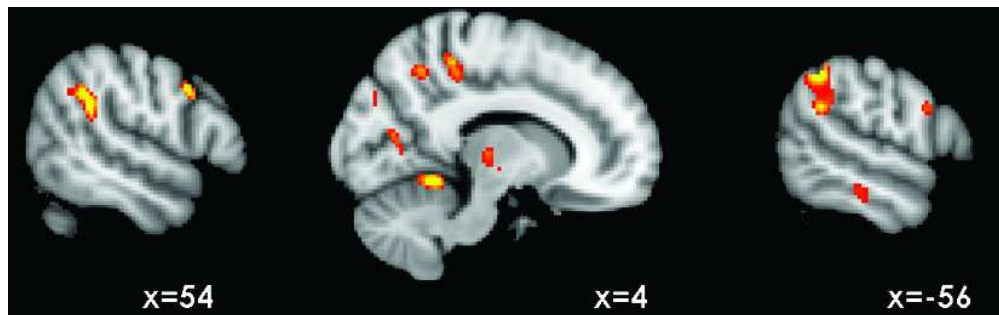
Figure A: Sagittal sections of the MNI brain illustrating brain regions of significantly increased functional resting-state connectivity with the periaqueductal gray in 17 subjects with migraine, interictally, relative to 17 age- and gender-matched healthy controls. Statistical threshold corresponded to Z>2.3 and a corrected cluster significance of p=0.05, including clusters with at least 20 contiguous voxels.
70x21mm (300 x 300 DPI)