Research article

NMR IN BIOMEDICINE

Received: 28 April 2012, Revised: 2 December 2012, Accepted: 9 December 2012, Published online in Wiley Online Library: 24 January 2013

(wileyonlinelibrary.com) DOI: 10.1002/nbm.2917

Alterations of regional spontaneous neuronal activity and corresponding brain circuit changes during resting state in migraine without aura

Ting Xue[a†], Kai Yuan[a†], Ping Cheng[a], Ling Zhao[b], Limei Zhao[a], Dahua Yu[a,c], Tao Dong[a], Karen M. von Deneen[a], Qiyong Gong[d], Wei Qin[a*] and Jie Tian[a,e*]

Although previous resting-state studies have reported abnormal functional cerebral changes in patients with migraine without aura (MwoA), few have focused on alterations in both regional spontaneous neuronal activity and corresponding brain circuits in MwoA patients during rest. Eighteen MwoA patients and 18 age- and gender-matched healthy controls (HC) were recruited in the current study. Baseline cerebral alterations were investigated using amplitude of low-frequency fluctuation (ALFF) and region of interest (ROI)-based functional connectivity (FC) analyses. Compared with HC, MwoA patients showed decreased ALFF values in the left rostral anterior cingulate cortex (rACC) and bilateral prefrontal cortex (PFC) as well as increased ALFF values in the right thalamus. FC analysis also revealed abnormal FCs associated with these ROIs. In addition, ALFF values of the left rACC correlated with duration of disease in MwoA. Our findings could lead to a better understanding of intrinsic functional architecture of baseline brain activity in MwoA, providing both regional and brain circuit spontaneous neuronal activity properties. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: migraine without aura (MwoA); amplitude of low-frequency fluctuation (ALFF); functional connectivity (FC); resting state; functional magnetic resonance imaging (fMRI)

INTRODUCTION

Migraine is a common chronic disorder typically characterized by recurrent attacks of disabling headaches (1). According to the International Headache Society (IHS) guidelines (2), migraine is divided into two major subtypes: migraine without aura (MwoA) and migraine with aura (MA). MwoA is the most common form of migraine (3–6) characterized by unilateral and pulsating severe headache lasting 4-72 h and often accompanied by nausea and phono-and photophobia. Given the heavy burden that migraine places on the individual and society, it is imperative to develop a better understanding of migraine pathophysiology. Neuroimaging has helped increase our knowledge about the neural mechanisms underlying migraine. For instance, researchers found that defects in the brainstem descending modulatory circuits could contribute to the onset of migraine (7,8). Alternatively, other studies have confirmed the importance of cortically-spread depression (9) as the pathophysiological mechanism of migraine aura (10,11). Converging evidence of structural (12–16) and functional cerebral abnormalities (17–19) in patients with migraine has also been accumulated, such as in the prefrontal cortex (PFC), the rostral anterior cingulate cortex (rACC), the somatosensory cortex, the

* *Correspondence to: W. Qin and J. Tian, Life Sciences Research Center, School of Life Sciences and Technology, Xidian University, Xi'an, Shaanxi 710071, China. Tel: +86 29 81891070, Fax: +86 29 81891060. E-mail: wqin@xidian.edu.cn and tian@ieee.org*

a *T. Xue, K. Yuan, P. Cheng, L. Zhao, D. Yu, T. Dong, K. M. Deneen, W. Qin, J. Tian Life Sciences Research Center, School of Life Sciences and Technology, Xidian University, Xi'an, Shaanxi, 710071, China*

b *L. Zhao The 3rd Teaching Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan, 610075, China*

c *D. Yu Information Processing Laboratory, School of Information Engineering, Inner Mongolia University of Science and Technology, Baotou, Inner Mongolia, 014010, China*

d *Q. Gong Department of Radiology, Huaxi MR Research Center (HMRRC), Center for Diagnostic Imaging, West China Hospital of Sichuan University, China*

e *J. Tian Institute of Automation, Chinese Academy of Sciences, Beijing 100190, China*

† *Both authors contributed equally to this article*

Abbreviations used: *fMRI, functional magnetic resonance imaging; bOLD, blood-oxygen level-dependent; MwoA, migraine without aura; MA, migraine with aura; HIS, International Headache Society; HC, healthy controls; SPM, Statistical Parametric Mapping; ALFF, amplitude of low-frequency fluctuation; FC, functional connectivity; ROI, region of interest; MNI, Montreal Neurological Institute; PCC, posterior cingulated cortex; MPFC, medial prefrontal cortex; IPL, inferior parietal lobe; rACC, rostral anterior cingulate cortex; PFC, prefrontal cortex; OFC, orbitofrontal cortex.*

orbitofrontal cortex (OFC) and insula (12–19). All these findings demonstrate that migraine is associated with altered central processing of pain stimulus and recurrent migraine attacks can result in selective damage of brain areas involved in pain processing.

Recently, Yu *et al*. employed the regional homogeneity method to analyze temporal homogeneity of regional functional magnetic resonance imaging (fMRI) blood-oxygen level-dependent (BOLD) signals in migraine patients (20). They found focal functional changes in the rACC, PFC, OFC and supplementary motor area, inferring that abnormalities in these regions might be related to functional impairments in pain processing. With the application of the functional connectivity (FC) approach, Mainero *et al*. investigated the resting-state brain function at the level of altered functional integration within the periaqueductal gray matter networks in migraine (21), a key region involved with nociceptive processing. They revealed interictal dysfunctional dynamics within pain pathways in migraine, attributing such abnormalities to the impairment of the descending pain modulatory circuits. However, to our knowledge, few migraine studies have investigated the alterations in both regional spontaneous neuronal activity and corresponding brain circuits during resting state.

An alternative way of measuring regional spontaneous neuronal activity during resting-state fMRI is to examine amplitude of low frequency fluctuation (ALFF) (22). ALFF investigates low frequency BOLD signal fluctuation from the aspect of regional brain activity during rest, reflecting intensity of regional spontaneous brain activity (23,24). From such an examination, a result of abnormal ALFF can tell us which area is abnormal in migraine patients. However, regional brain dysfunctions alone are not sufficient to explain the pathophysiology of migraine (25). Recent resting-state fMRI studies (21,26) emphasized the role of disrupted functional circuitry in migraine, providing evidence that dysfunctional connectivity within pain pathways outside episodes of migraine pain might lead to the development of migraine attacks (27). Therefore, as a complement to the findings of the ALFF analysis, a functional connectivity analysis was also applied to elucidate central processing of the migraine system from an integrated perspective.

ALFF and FC have been widely employed in several brain disorders and provide some important information for the understanding of these diseases such as in Alzheimer's disease (28), heroin addiction (29–32), schizophrenia (33,34) and depression (35). We believe that the application of ALFF and FC approaches could lead to a better understanding of resting-state brain activity in migraine, providing both regional and brain circuit spontaneous neuronal activity properties. Given that MwoA is the most frequent form of migraine, we restricted our sample to this subtype. We first investigated alterations in regional spontaneous neuronal activity during resting state in MwoA patients by applying the ALFF approach (22). Next, ROI-based FC analysis was applied to assess brain circuit changes in MwoA. We hypothesized that: *(i)* MwoA patients would have altered resting-state ALFF values or FCs in the pain-processing brain areas; and *(ii)*, alterations in brain function would be associated with clinical indicators of migraine (duration or attack frequency).

METHODS

Subjects

The project was approved by the Medical Ethics Committee of the West China Hospital at Sichuan University. Before the start of the experiment, all participants received a complete description of the study and provided written informed consent. The inclusion criteria for MwoA were as follows: *(i)* fulfill the diagnostic criteria of the International Headache Society for migraine without aura (2);*(ii)* had not suffered from a migraine attack at least 72 h prior to testing (36); and *(iii)*, did not have a migraine precipitated during or on the day following the scan. Exclusion criteria for both MwoA and HC were: *(i)* existence of additional psychiatric or neurological disorders; *(ii)* alcohol, nicotine or drug abuse; *(iii)* taking any drugs affecting the central nervous system; *(iv)* having any physical illness such as a brain tumor, hepatitis or epilepsy as assessed according to clinical evaluations and medical records; *(v)* claustrophobia; and *(vi)*, for the HC, they should either have no personal or family history of migraine or other headaches. Prior to scanning, urine drug screening was performed on all subjects to exclude the possibility of substance abuse. Eighteen adult individuals (13 females; 5 males; mean age, 32.6 $\pm$ 10.3 years) with MwoA and 18 HC of comparable age and gender distribution (13 females; 5 males; mean age 32.4 $\pm$ 11.8 years) were recruited in the current study. All participants were right-handed according to the Edinburgh Handedness Inventory (37). To minimize variability due to hormonal influences on cortical excitability, all female subjects were always recorded mid-menstrual cycle (all female subjects completed the fMRI scan between the 10th and 14th days of their menstrual cycles) (38). Subjects with MwoA rated the pain intensity of their average migraine as 5.9 $\pm$ 1.7 on a 0-10 scale derived from attacks in the past 4 weeks, with 10 being the most intense pain imaginable. Other clinical information such as attack frequency and duration of disease was also recorded by a structured interview and self-reports (Table 1).

Data acquisition

All fMRI studies were performed on a 3-T GE scanner (EXCITE, GE Signa, Milwaukee, WI, USA) using an eight-channel phase-array head coil in the Huaxi MR Research Center. Subjects lay supine with their heads snugly fit to foam pads to reduce head motion and ear plugs were used to minimize scanner noises. Prior to the functional run, a high-resolution structural image of each subject was obtained using a 3D MRI sequence with a voxel size of 1 mm^3 employing an axial fast spoiled gradient recalled sequence (TR = 1900 ms; TE = 2.26 ms; matrix, 256 $\times$ 256; field of view, 256 $\times$ 256 mm^2). For all participants, structural information was examined by two experienced radiologists to exclude the possibility of clinically silent lesions. Next, functional BOLD signals

Table 1. Clinical details of patients with migraine without aura and healthy controls (mean $\pm$ standard deviation)

Clinical details	MwoA Patients	Healthy controls
Age (years)	32.6 $\pm$ 10.3	32.4 $\pm$ 11.8
Sex (F, female; M, male)	13 F, 5 M	13 F, 5 M
Disease duration (years)	10.2 $\pm$ 6.1	—
Attack frequency (times)*	4.9 $\pm$ 2.5	—
Duration of migraine (h)*	14.4 $\pm$ 6.1	—
Pain intensity (0-10)*	5.9 $\pm$ 1.7	—

*Information on migraine attacks during the past 4 weeks. MwoA, migraine without aura.

NMR IN BIOMEDICINE

were acquired with an echo-planar imaging sequence. Thirty continuous slices were obtained with TR = 2000 ms; TE = 30 ms; flip angle = 90°; field of view, $240 \times 240\,mm^2$; matrix, 64×64, 180 volumes. During the entire functional scanning, all participants were asked to keep their eyes closed, to stay awake and not to focus on anything in particular. After scanning, all subjects confirmed that they remained awake during the entire procedure.

Data preprocessing

Data preprocessing and statistical analyses were performed using Statistical Parametric Mapping 5 software (SPM5, http://www.fil.ion.ucl.ac.uk/spm/software/spm5). For each participant, the first 10 volumes were discarded for scanner calibration and for subjects to get used to the scanning environment (34). The remaining 170 volumes were corrected for acquisition delay between slices and aligned to the first image of each session for motion correction. The standard Montreal Neurological Institute (MNI) template provided by SPM5 was used for spatial normalization with a resampling voxel size of $3 \times 3 \times 3$ mm. No subjects had head motion exceeding 1 mm of movement or 1° rotation in any direction. Next, the functional images were spatially smoothed with a 4-mm full-width at half maximum Gaussian kernel. Finally, imaging data were first temporally filtered (band pass, 0.01-0.08 Hz) to remove the effects of very low-frequency drift and high-frequency noise (e.g., respiratory and cardiac rhythms) (23,39).

ALFF analysis

ALFF analyses were performed using REST V1.7 software (40) with a procedure similar to that described previously (22,32,34). Rather than directly measure the magnitude of fMRI BOLD signal time series in time domain, ALFF calculates the voxel-wise average amplitude of specific frequency bands (0.01 ~ 0.08 Hz) in the frequency domain of the original fMRI signal. Specifically, the preprocessed filtered time series was transformed to the frequency domain by using fast Fourier transform (FFT, parameters: taper percent = 0, FFT length = shortest), obtaining the power spectrum as a result. Since the power of a given frequency is proportional to the square of the amplitude of the frequency component of the original time series in the time domain, the square root was calculated at each frequency of the power spectrum. Then, the averaged square root obtained across 0.01 - 0.08 Hz at each voxel was taken as the ALFF value. For standardized purposes, the ALFF of each voxel was divided by the global mean of the ALFF value. The normalization procedure was analogous to that used in PET studies (41). Previous functional imaging studies have revealed that certain brain regions consistently show greater activity during resting state than during tasks (42), including the posterior cingulate cortex (PCC), precuneus, inferior parietal lobe (IPL) and medial prefrontal cortex (MPFC). These regions constitute a network (default mode network) supporting a default mode of brain function. To examine whether standardized ALFF in these regions also demonstrated higher values than the expected global mean value of 1, we performed a one-sample *t*-test within each group (MwoA and HC).

As described above, ALFF measures the magnitude of spontaneous fluctuations in fMRI BOLD signals. It may reflect intensity of regional spontaneous brain activity (22–24). More importantly, it has been demonstrated that abnormal ALFF could be implicated in brain dysfunction related to various disorders such as attention deficit hyperactivity disorder (22), schizophrenia (34), mild cognitive impairment (43), epilepsy (44) and low-grade hepatic encephalopathy (45). In the current study, from such an examination, a result of abnormal ALFF can tell us which area is abnormal in the migraine. Therefore the ALFF may reflect, at least to some extent, different aspects of spontaneous brain activity in MwoA patients.

ROI-based FC analysis

Prior to FC analysis, several sources of spurious variances including head motion parameters, global mean BOLD signals and average BOLD signals in ventricular and white matter regions were removed from the preprocessed data with linear regression. ROIs were anatomically defined based on the fact that these regions showed abnormal ALFF values; that is, the left anterior cingulate cortex, right thalamus and bilateral prefrontal cortex. In addition, it is worth mentioning that MwoA patients also showed increased ALFF values in the right insula ($p < 0.005$, uncorrected). However, it did not survive with multiple corrections ($p < 0.05$, corrected). Previous studies have demonstrated that the insula is generally involved in pain processing (46,47). It has been activated in several PET studies (48–50) and task-related fMRI studies (17,18). We performed a connectivity analysis of the right insula to examine whether functional interactions related to this area would be altered in MwoA patients. Anatomically defined ROIs were created using WFU-Pick Atlas software (http://fmri.wfubmc.edu/cms/software, Department of Radiology, Wake Forest University School of Medicine, Winston Salem, North Carolina, USA) (51). As a result, a total of 5 ROIs were defined. For each ROI, a seed reference time course was obtained by averaging the time series of all voxels in the ROI. Correlation analysis was performed between the seed reference time course and time series from the whole brain in a voxel-wise way.

Statistics

To explore the within-group ALFF patterns (to evaluate whether ALFF differed from the value of 1) (41), a one-sided one-sample *t*-test [$p < 0.05$, family-wise error (FWE) corrected] was performed on the individual normalized ALFF maps for each group (MwoA and HC). Then, a two-sample *t*-test was performed to elucidate ALFF differences between the two groups after controlling for age and gender. To protect against false positive activations, we used a double-threshold Monte Carlo simulation approach; that is, combing a voxel-based threshold with a minimum cluster size (52) for controlling the overall significance level (the probability of a false detection for the entire functional volume). A single voxel threshold was set at $p < 0.005$ and a minimum cluster size of 351 mm^3 was used to correct for multiple comparisons. This yielded a corrected threshold of $p < 0.05$ (see program AlphaSim by D. Ward in AFNI software). Parameters were: single voxel uncorrected p value = 0.005, FWHM = 4 mm, with whole brain mask, 5000 iterations. (see http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf). Activations exceeding this double threshold (individual uncorrected voxel threshold $p < 0.005$, minimum cluster size of 351 mm^3) were therefore considered to be activated at a threshold of $p < 0.05$ corrected for multiple comparisons. For regions in which MwoA patients showed abnormal ALFF properties, ALFF values were extracted, averaged and regressed against the pathological indicators reflected by duration of the disease and migraine attack frequency. Because we performed multiple statistical tests on each

pathological indicator (duration and frequency), we considered the question of statistical significance. We used the Bonferroni correction adjustment of the alpha level by dividing the conventional alpha of 0.05 by the number of tests performed, yielding a Bonferroni corrected *P* value of 0.05/4.

For ROI-based FC analysis, a seed reference time course was obtained for each ROI by averaging the time series of all voxels in the ROI. Correlation analysis was performed between the seed reference time course and time series from the whole brain in a voxel-wise way. The correlation coefficients were transformed into *z* values using Fisher's transformation to improve normality (53). Then, individual *z* values were entered into a two-sample *t*-test to determine group differences of FC between the MwoA and HC. Multiple comparisons were corrected using the same method as in the group of ALFF comparisons.

RESULTS

The regional ALFF results for both groups (MwoA and HC) are shown in Figure 1 (one-sample *t*-test, $n = 18$, $p < 0.05$, FWE corrected). Significant ALFF values in both groups were found in the PCC and adjacent precuneus. Additionally, several other neural regions such as the MPFC and IPL also exhibited higher ALFF values than the mean ALFF value. It should be noted that the result of the one-sample *t*-test could not provide information on differences between the two groups. Results of the two-sample *t*-test showed significant ALFF differences between MwoA and HC (Fig. 2) ($p < 0.05$, corrected). Areas showing decreased ALFF values in the MwoA group included the bilateral PFC and left rACC, while the right thalamus showed increased ALFF values. Furthermore, correlation analysis demonstrated that there was a significant negative correlation between average ALFF values of the left rACC ($r = -0.6213$, $p = 0.0059$, Bonferroni corrected) and duration of disease.

ROI-based FC analysis revealed increased FCs between the left rACC (ROI1) and bilateral frontal lobe and left parietal lobe; right thalamus (ROI2) and bilateral caudate, left temporal lobe and right putamen; left PFC (ROI3) and right precuneus and bilateral parietal lobe; right PFC (ROI4) and bilateral parietal lobe and left temporal lobe; and right insula (ROI5) and left temporal pole, right frontal lobe and left parietal lobe (Fig. 3).

DISCUSSION

Resting-state fMRI is a noninvasive imaging technique used to measure spontaneous brain activity as low-frequency fluctuations in BOLD signals (54). During the resting state, correlated spontaneous fluctuations occur within spatially distinct and functionally related groups of cortical and subcortical regions, consisting of the human brain's intrinsic functional networks (55). Variations in intrinsic functional networks can influence task performance in real life (56,57). Furthermore, the resting-state method has been extensively used to reveal the intrinsic typical and atypical functional architecture of the brain (54,58). Changed features during resting state can serve as a marker to reflect the progress of multiple diseases such as heroin addiction (29–31), Alzheimer's disease (59) and schizophrenia (60). Thus, it is critical to take into account spontaneous brain activity in the absence of external stimulation to better understand how the brain functions and adapts in migraineurs. Previous studies have revealed abnormal regional and inter-regional resting-state properties in migraine separately; however, few studies have investigated whether brain regions with abnormal regional resting-state properties show dysfunctional connectivity in migraine patients. In other words, they failed to assess the regional alterations in both regional spontaneous neuronal activity and corresponding brain circuits during rest. Therefore, ALFF and ROI-based FC methods were employed in the current study to provide scientific evidence for the above-mentioned issues.

Consistent with previous ALFF findings (22,61,62), the results of the one-sample *t*-test showed that several brain areas exhibited significantly higher ALFF values (Fig. 1), including the PCC, MPFC, precuneus and IPL. These areas are the major regions of the default mode network identified in previous resting-state PET (41) and fMRI studies (63). Our findings demonstrate that ALFF is a convincing measure of resting-state spontaneous neuronal activity and can provide both the nature and extent of signal changes underlying spontaneous neuronal activity (32).

Most importantly, compared with HC, we found that MwoA patients showed altered regional brain activity in a set of brain areas. Specifically, ALFF values decreased in the left rACC and bilateral PFC as well as increased in the right thalamus (Fig. 2). Increased FCs were also observed between several pain-related brain areas and ROIs (Fig. 3). The brain regions showing atypical function

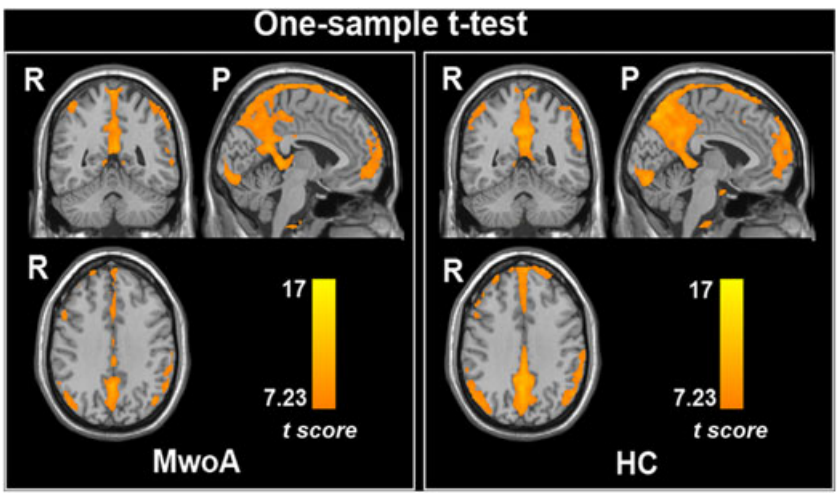

Figure 1. ALFF group analysis results in the MwoA and HC groups ($p < 0.05$, FWE corrected).

NMR IN BIOMEDICINE

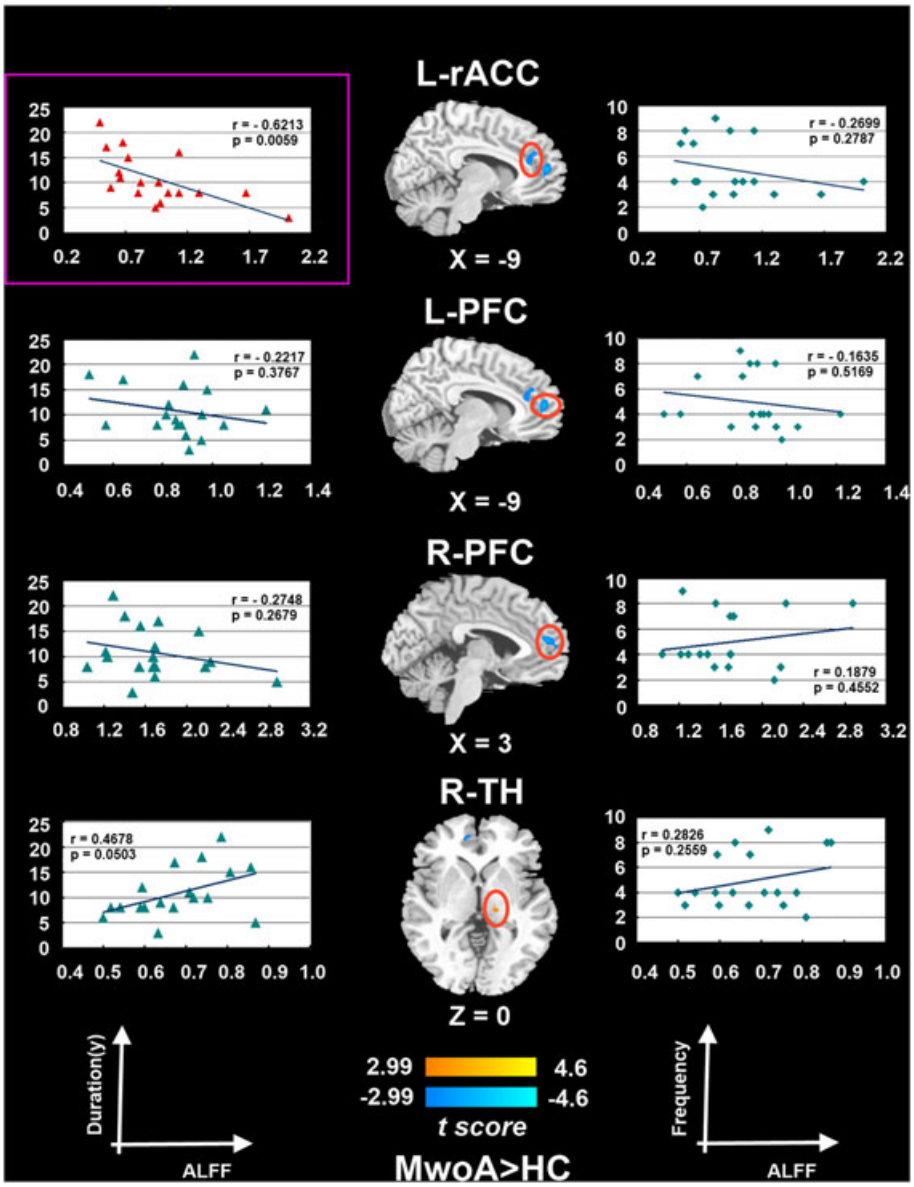

Figure 2. Left column: correlation analysis between duration of disease and ALFF difference. There was a significant negative correlation between average ALFF values of left rACC and duration of disease. The correlation was significant when the alpha level was adjusted with the Bonferroni correction ($r = -0.6213$, $p = 0.0059$, Bonferroni). Middle column: regions showing ALFF differences between MwoA and HC groups (MwoA > HC, $p < 0.05$, corrected). Right column: correlation analysis between frequency of migraine attacks and ALFF difference.

herein were similar to the brain areas reported in previous structural (12,13) and task-based migraine studies (17–19), which were mainly involved in pain-processing (11,64). We speculated that resting-state functional abnormalities in MwoA patients could be associated with functional impairments in pain-processing. Moreover, ALFF values of the left rACC were negatively correlated with duration of disease. Our results suggest that ALFF properties of rACC become abnormal as a function of time, which may reflect the process of migraine.

The PFC and ACC are major cortical areas involved in the 'medial pain system,' which is thought to be responsible for the 'affective-cognitive-evaluative' aspects of pain (65,66). As shown by previous neuroimaging studies (67,68), these two brain areas are the most common active regions responding to pain stimulus. rACC has been recognized to encode the affective component of pain such as unpleasantness, suffering and other negative effects (69,70). It is also thought to be important in endogenous pain control, which is mediated by endogenous opioid systems (71). Using PET, Petrovic *et al.* confirmed that opioid analgesia is associated with increased activity in the rACC (72). We speculated that the decreased resting-state ALFF values of the rACC in MwoA patients could be related to functional impairments in the long-term pain affective response and endogenous analgesia.

The PFC is generally considered to be involved in cognitive and attention processing of painful stimuli (73). Additionally, it has been demonstrated that the PFC can influence the descending pain modulatory system, notably through the modulation of brainstem structures participating in pain modulation (71,74). Decreased ALFF values in the PFC possibly suggest efficiency reduction in descending modulatory function in pain processing induced by long-term migraine attacks. In the present study, altered FCs were observed in the cingulo-frontal brain regions. Previous

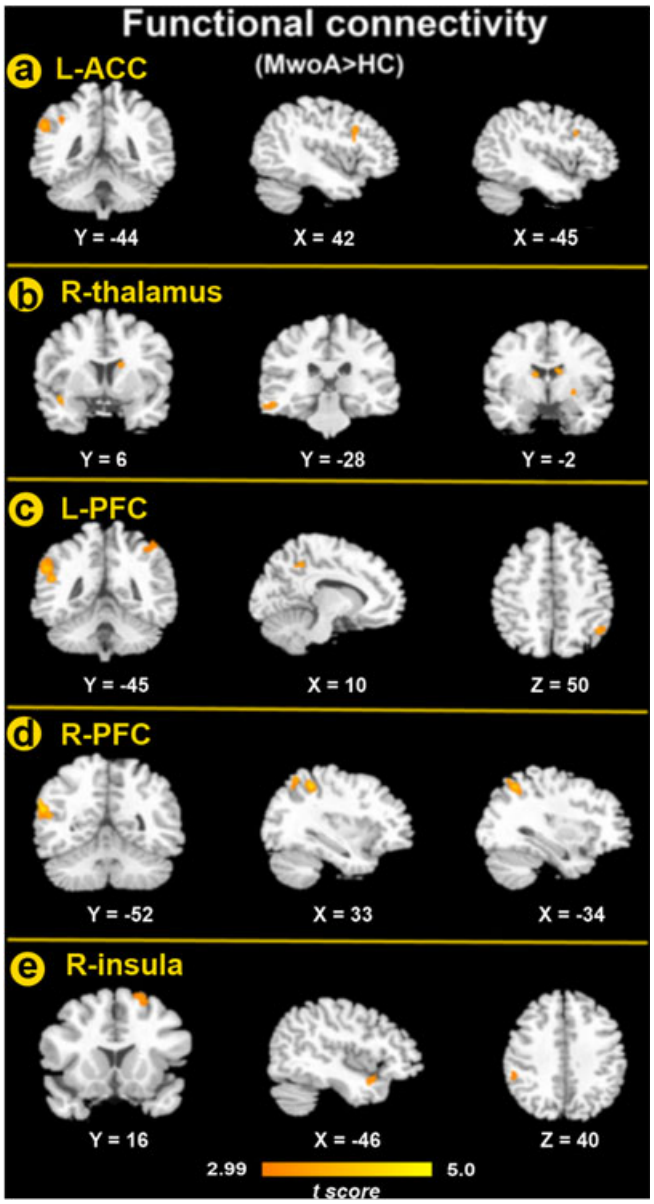

Figure 3. Functional connectivity differences in the left rACC, left PFC, right PFC, right thalamus and right insula between MwoA and HC groups (MwoA > HC, $p < 0.05$, corrected).

neuroimaging studies support the putative pain modulating capacity of the cingulo-frontal regions (72), since pain attenuation under cognitive modulation was associated with a relative activation increase in this area. Moreover, attention and emotional control of pain processing has been suggested as a major role of the cingulo-frontal brain regions (72,75). Our findings of dysfunction in the cingulo-frontal regions might suggest disturbed interaction among these areas caused by frequent pain processing.

Increased ALFF values were found in the right thalamus. In addition, dysfunctional interaction patterns were also observed between the right thalamus and the bilateral caudate in MwoA patients. Functional neuroimaging studies of human beings have demonstrated that the thalamus can be activated in spontaneous attacks of migraine and in models of trigeminal nociceptive stimulation (68,76,77). As a critical multifunctional relay center, the thalamus is believed to play an important role in the transmission of nociceptive inputs to cortical structures (77). It receives important modulatory inputs from the brainstem monoaminergic centers (8,48). Thus, abnormal ictal activity in these areas could directly alter nociceptive information processing in the thalamus. We speculated that the interictal atypical function in the thalamus could be implicated in long-term ongoing transmission of nociceptive information induced by frequent migraine attacks. Moreover, one recent study reported that triptans medication was effective in migraine via its inhibitory action on thalamic neuronal firings in response to trigeminovascular stimuli (78), suggesting that the thalamus could be an additional site where migraine pain can be modulated by reducing thalamic neuron activity.

Previous studies demonstrated that patients with common or classic migraine headaches and spontaneously occurring cluster headaches have significant elevations in blood flow within the caudate nucleus compared to subjects without headaches (76,79). Evidence from both animal and human studies has also demonstrated that pain reactivity can be decreased by caudate stimulation (80,81). Given the results of previous investigations (48,68,76,77,79–81), we speculate that the atypical function of the thalamus and dysregulation between the thalamus and caudate could be related to deficits in pain modulation induced by frequent migraine attacks.

It should be noted that the specificity of our findings was only limited to MwoA, the most common subtype of migraine. Previous studies have demonstrated that MwoA and MA, another major subtype of migraine, are distinct clinical entities (5,6). Differences in regional cerebral blood flow (82) and platelet content of 5-HT (serotonin) (83) during attacks of MwoA and MA also imply that these two forms of migraine can have different causes. With respect to resting-state fMRI signatures of MA, we cannot provide information regarding ALFF and FC alterations in the current study. Further studies will be needed to determine relevant resting-state fMRI signatures in MA. Another limitation of this study is that, although ALFF was effective in detecting low-frequency fluctuations, it could be sensitive to possible artifactual findings in the vicinity of blood vessels and the cerebral ventricles (84), which could be responsible for the noise in the image.

CONCLUSIONS

With the application of resting-state ALFF and FC approaches, we found that MwoA patients have altered resting-state spontaneous neuronal activity in pain-processing areas, including the left rACC, bilateral PFC and right thalamus, as well as altered FCs associated with these areas. Our findings revealed interictal dysfunctional patterns in both regional spontaneous neuronal activity and inter-regional interactions between spatially remote brain areas, providing additional evidence that brain functional alterations induced by migraine attacks are not limited to focal changes but also expressed at the level of altered functional integration within distributed brain areas involved in pain-processing.

Acknowledgements

This paper was supported by the Project for the National Key Basic Research and Development Program (973) under Grant Nos. 2011CB707702 and 2012CB518501; the National Natural Science Foundation of China under Grant Nos. 30930112, 30970774,

81000640, 81000641, 81101036, 81101108, 31150110171, 30901900, 81271644, 81030027, 31200837 and 61179019; the Fundamental Research Funds for the Central Universities; the Natural Science Foundation of Inner Mongolia under Grant No. 2012MS0908; and the Innovation Fund Project of Inner Mongolia University of Science and Technology Nos. 2010NC030 and 2010NC037.

REFERENCES

1. Kruit MC, van Buchem MA, Hofman PAM, Bakkers JTN, Terwindt GM, Ferrari MD, Launer LJ. Migraine as a risk factor for subclinical brain lesions. JAMA 2004; 291(4): 427-434.
2. Headache Classification Subcommittee of the International Headache Society. The International Classification of Headache Disorders, 2nd. Cephalalgia 2004; 24(Suppl 1): 1-160.
3. Launer LJ, Terwindt GM, Ferrari MD. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology 1999; 53(3): 537-542.
4. Sakai F, Igarashi H. Prevalence of migraine in Japan: a nationwide survey. Cephalalgia 1997; 17(1): 15-22.
5. Rasmussen BK, Olesen J. Migraine with aura and migraine without aura: an epidemiological study. Cephalalgia 1992; 12(4): 221-228.
6. Russell M, Rasmussen B, Fenger K, Olesen J. Migraine without aura and migraine with aura are distinct clinical entities: a study of four hundred and eighty- four male and female migraineurs from the general population. Cephalalgia 1996; 16(4): 239-245.
7. Welch KM, Nagesh V, Aurora SK, Gelman N. Periaqueductal gray matter dysfunction in migraine: cause or the burden of illness? Headache 2001; 41(7): 629-637.
8. Bahra A, Matharu MS, Buchel C, Frackowiak RS, Goadsby PJ. Brainstem activation specific to migraine headache. Lancet 2001; 357(9261): 1016-1017.
9. Leao AAP. Spreading depression of activity in the cerebral cortex. J. Neurophysiol. 1944; 7(6): 391-396.
10. Bowyer SM, Tepley N, Papuashvili N, Kato S, Barkley GL, Welch KM, Okada YC. Analysis of MEG signals of spreading cortical depression with propagation constrained to a rectangular cortical strip. II. Gyrencephalic swine model. Brain Res. 1999; 843(1-2): 79-86.
11. Schwedt TJ, Dodick DW. Advanced neuroimaging of migraine. Lancet Neurol. 2009; 8(6): 560-568.
12. Schmidt-Wilcke T, Ganssbauer S, Neuner T, Bogdahn U, May A. Subtle grey matter changes between migraine patients and healthy controls. Cephalalgia 2008; 28(1): 1-4.
13. Kim JH, Suh SI, Seol HY, Oh K, Seo WK, Yu SW, Park KW, Koh SB. Regional grey matter changes in patients with migraine: a voxel-based morphometry study. Cephalalgia 2008; 28(6): 598-604.
14. Rocca MA, Pagani E, Colombo B, Tortorella P, Falini A, Comi G, Filippi M. Selective diffusion changes of the visual pathways in patients with migraine: a 3-T tractography study. Cephalalgia 2008; 28(10): 1061-1068.
15. Szabo N, Kincses ZT, Pardutz A, Tajti J, Szok D, Tuka B, Kiraly A, Babos M, Voros E, Bomboi G, Orzi F, Vecsei L. White matter microstructural alterations in migraine: a diffusion-weighted MRI study. Pain 2012; 153(3): 651-656.
16. DaSilva AF, Granziera C, Tuch DS, Snyder J, Vincent M, Hadjikhani N. Interictal alterations of the trigeminal somatosensory pathway and periaqueductal gray matter in migraine. Neuroreport 2007; 18(4): 301-305.
17. Aderjan D, Stankewitz A, May A. Neuronal mechanisms during repetitive trigemino-nociceptive stimulation in migraine patients. Pain 2010; 151(1): 97-103.
18. Eck J, Richter M, Straube T, Miltner WH, Weiss T. Affective brain regions are activated during the processing of pain-related words in migraine patients. Pain 2011; 152(5): 1104-1113.
19. Russo A, Tessitore A, Esposito F, Marcuccio L, Giordano A, Conforti R, Truini A, Paccone A, d'Onofrio F, Tedeschi G. Pain processing in patients with migraine: an event-related fMRI study during trigeminal nociceptive stimulation. J. Neurol. 2012; 259(9): 1903-1912.
20. Yu D, Yuan K, Zhao L, Dong M, Liu P, Wang G, Liu J, Sun J, Zhou G, Deneen K. Regional homogeneity abnormalities in patients with interictal migraine without aura: a resting-state study. NMR Biomed. 2012; 25(5): 806-812.
21. Mainero C, Boshyan J, Hadjikhani N. Altered functional magnetic resonance imaging resting-state connectivity in periaqueductal gray networks in migraine. Ann. Neurol. 2012; 70(5): 838-845.
22. Zang YF, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M, Tian LX, Jiang TZ, Wang YF. Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. Brain Dev. 2007; 29(2): 83-91.
23. Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magn. Reson. Med. 1995; 34(4): 537-541.
24. Zou Q, Wu CW, Stein EA, Zang Y, Yang Y. Static and dynamic characteristics of cerebral blood flow during the resting state. Neuroimage 2009; 48(3): 515-524.
25. Sprenger T, Borsook D. Migraine changes the brain: neuroimaging makes its mark. Curr. Opin. Neurol. 2012; 25(3): 252-262.
26. Maleki N, Becerra L, Nutile L, Pendse G, Brawn J, Bigal M, Burstein R, Borsook D. Migraine attacks the Basal Ganglia. Mol. Pain 2011; 7: 71.
27. Mainero C, Boshyan J, Hadjikhani N. Altered functional magnetic resonance imaging resting-state connectivity in periaqueductal gray networks in migraine. Ann. Neurol. 2011; 70(5): 838-845.
28. Sorg C, Riedl V, Mühlau M, Calhoun VD, Eichele T, Läer L, Drzezga A, Förstl H, Kurz A, Zimmer C. Selective changes of resting-state networks in individuals at risk for Alzheimer's disease. Proc. Natl. Acad. Sci. USA 2007; 104(47): 18760-18765.
29. Yuan K, Qin W, Dong M, Liu J, Liu P, Zhang Y, Sun J, Wang W, Wang Y, Li Q. Combining spatial and temporal information to explore resting-state networks changes in abstinent heroin-dependent individuals. Neurosci. Lett. 2010; 475(1): 20-24.
30. Yuan K, Qin W, Dong M, Liu J, Sun J, Liu P, Zhang Y, Wang W, Wang Y, Li Q, Zhao L, von Deneen KM, Liu Y, Gold MS, Tian J. Gray matter deficits and resting-state abnormalities in abstinent heroin-dependent individuals. Neurosci. Lett. 2010; 482(2): 101-105.
31. Yuan K, Qin W, Liu JX, Guo QA, Dong MH, Sun JB, Zhang Y, Liu P, Wang W, Wang YR, Li QA, Yang WC, von Deneen KM, Gold MS, Liu YJ, Tian J. Altered small-world brain functional networks and duration of heroin use in male abstinent heroin-dependent individuals. Neurosci. Lett. 2010; 477(1): 37-42.
32. Jiang GH, Qiu YW, Zhang XL, Han LJ, Lv XF, Li LM, Lin CL, Zhuo FZ, Hu SY, Tian JZ. Amplitude low-frequency oscillation abnormalities in the heroin users: A resting state fMRI study. Neuroimage 2011; 57(1): 149-154.
33. Zhou Y, Liang M, Tian LX, Wang K, Hao YH, Liu HH, Liu ZN, Jiang TZ. Functional disintegration in paranoid schizophrenia using resting-state fMRI. Schizophr. Res. 2007; 97(1-3): 194-205.
34. Hoptman MJ, Zuo XN, Butler PD, Javitt DC, D'Angelo D, Mauro CJ, Milham MP. Amplitude of low-frequency oscillations in schizophrenia: a resting state fMRI study. Schizophr. Res. 2010; 117(1): 13-20.
35. Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, Mathews VP, Kalnin A, Lowe MJ. Activity and connectivity of brain mood regulating circuit in depression: a functional magnetic resonance study. Biol. Psychiatry 2005; 57(10): 1079-1088.
36. Moulton EA, Becerra L, Maleki N, Pendse G, Tully S, Hargreaves R, Burstein R, Borsook D. Painful heat reveals hyperexcitability of the temporal pole in interictal and ictal migraine States. Cereb. Cortex 2011; 21(2): 435-448.
37. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971; 9(1): 97-113.
38. Salimpoor VN, Benovoy M, Larcher K, Dagher A, Zatorre RJ. Anatomically distinct dopamine release during anticipation and experience of peak emotion to music. Nat. Neurosci. 2011; 14(2): 257-355.
39. Lowe MJ, Mock BJ, Sorenson JA. Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. Neuroimage 1998; 7(2): 119-132.
40. Song XW, Dong ZY, Long XY, Li SF, Zuo XN, Zhu CZ, He Y, Yan CG, Zang YF. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS One 2011; 6(9): e25031.
41. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc. Natl. Acad. Sci. USA 2001; 98(2): 676-682.
42. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc. Natl. Acad. Sci. USA 2003; 100(1): 253-258.
43. Han Y, Wang J, Zhao Z, Min B, Lu J, Li K, He Y, Jia J. Frequency-dependent changes in the amplitude of low-frequency fluctuations in amnestic mild cognitive impairment: a resting-state fMRI study. Neuroimage 2011; 55(1): 287-295.

44. Zhang Z, Lu G, Zhong Y, Tan Q, Chen H, Liao W, Tian L, Li Z, Shi J, Liu Y. fMRI study of mesial temporal lobe epilepsy using amplitude of low-frequency fluctuation analysis. Hum. Brain Mapp. 2010; 31(12): 1851-1861.
45. Chen HJ, Zhu XQ, Jiao Y, Li PC, Wang Y, Teng GJ. Abnormal baseline brain activity in low-grade hepatic encephalopathy: a resting-state fMRI study. J. Neurol. Sci. 2012; 318(1-2): 140-145.
46. Henderson LA, Gandevia SC, Macefield VG. Somatotopic organization of the processing of muscle and cutaneous pain in the left and right insula cortex: A single-trial fMRI study. Pain 2007; 128(1-2): 20-30.
47. Coghill RC, Talbot JD, Evans AC, Meyer E, Gjedde A, Bushnell MC, Duncan GH. Distributed processing of pain and vibration by the human brain. J. Neurosci. 1994; 14(7): 4095-4108.
48. Afridi SK, Giffin NJ, Kaube H, Friston KJ, Ward NS, Frackowiak RS, Goadsby PJ. A positron emission tomographic study in spontaneous migraine. Arch. Neurol. 2005; 62(8): 1270-1275.
49. Afridi SK, Matharu MS, Lee L, Kaube H, Friston KJ, Frackowiak RSJ, Goadsby PJ. A PET study exploring the laterality of brainstem activation in migraine using glyceryl trinitrate. Brain 2005; 128: 932-939.
50. Matharu MS, Bartsch T, Ward N, Frackowiak RSJ, Weiner R, Goadsby PJ. Central neuromodulation in chronic migraine patients with suboccipital stimulators: a PET study. Brain 2004; 127: 220-230.
51. 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(3): 1233-1239.
52. Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA, Noll DC. Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magn. Reson. Med. 1995;33(5):636-647.
53. Zar JH. *Biostatistical analysis*. Prentice Hall: Upper Saddle River, New Jersey; 1996.
54. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci. 2007; 8(9): 700-711.
55. Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD. Neurodegenerative diseases target large-scale human brain networks. Neuron 2009; 62(1): 42-52.
56. Fox MD, Snyder AZ, Vincent JL, Raichle ME. Intrinsic fluctuations within cortical systems account for intertrial variability in human Behavior. Neuron 2007; 56(1): 171-184.
57. Hesselmann G, Kell CA, Eger E, Kleinschmidt A. Spontaneous local variations in ongoing neural activity bias perceptual decisions. Proc. Natl. Acad. Sci. USA 2008; 105(31): 10984-10989.
58. Greicius M. Resting-state functional connectivity in neuropsychiatric disorders. Curr. Opin. Neurol. 2008; 21(4): 424-430.
59. Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc. Natl. Acad. Sci. USA 2004; 101(13): 4637-4642.
60. Lui S, Deng W, Huang XQ, Jiang LJ, Ma XH, Chen HF, Zhang TJ, Li XL, Li DM, Zou L, Tang HH, Zhou XHJ, Mechelli A, Collier DA, Sweeney JA, Li T, Gong QY. Association of Cerebral Deficits With Clinical Symptoms in Antipsychotic-Naive First-Episode Schizophrenia: An Optimized Voxel-Based Morphometry and Resting State Functional Connectivity Study. Am. Psychiatric Assoc. 2009; 166(2): 196-205.
61. Yang H, Long XY, Yang Y, Yan H, Zhu CZ, Zhou XP, Zang YF, Gong QY. Amplitude of low frequency fluctuation within visual areas revealed by resting-state functional MRI. Neuroimage 2007; 36(1): 144-152.
62. Yan CG, Liu DQ, He Y, Zou QH, Zhu CZ, Zuo XN, Long XY, Zang YF. Spontaneous brain activity in the default mode network is sensitive to different resting-state conditions with limited cognitive load. PLoS One 2009; 4(5): e5743.
63. Fransson P. How default is the default mode of brain function? Further evidence from intrinsic BOLD signal fluctuations. Neuropsychologia 2006; 44(14): 2836-2845.
64. May A. Neuroimaging: visualising the brain in pain. Neurol. Sci. 2007; 28: 101-107.
65. Vogt BA, Sikes RW, Vogt LJ. Anterior cingulate cortex and the medial pain system. In: Gabriel M, editor. *Neurobiology of cingulate cortex and limbic thalamus: a comprehensive handbook*. Birkhauser: Boston; 1993. p 313-344.
66. Albe-Fessard D, Berkley KJ, Kruger L, Ralston HJ, 3rd, Willis WD, Jr. Diencephalic mechanisms of pain sensation. Brain Res. 1985; 356 (3): 217-296.
67. Zhuo M. Cortical excitation and chronic pain. Trends Neurosci. 2008; 31(4): 199-207.
68. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur. J. Pain 2005; 9(4): 463-484.
69. Craig A. Functional neuroimaging of an illusion of pain. Nature 1996;384:258-260.
70. Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC. Pain affect encoded in human anterior cingulate but not somatosensory cortex. Science 1997; 277(5328): 968-971.
71. Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, Davidson RJ, Kosslyn SM, Rose RM, Cohen JD. Placebo-induced changes in fMRI in the anticipation and experience of pain. Science 2004; 303(5661): 1162-1167.
72. Petrovic P, Kalso E, Petersson KM, Ingvar M. Placebo and opioid analgesia-imaging a shared neuronal network. Science 2002; 295(5560):1737-1740.
73. Casey KL. Forebrain mechanisms of nociception and pain: Analysis through imaging. Proc Natl Acad Sci USA. 1999; 96(14): 7668-7674.
74. Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 2003; 126(5): 1079-1091.
75. Villemure C, Bushnell MC. Cognitive modulation of pain: how do attention and emotion influence pain processing? Pain 2002; 95(3): 195-199.
76. Kobari M, Meyer JS, Ichijo M, Imai A, Oravez WT. Hyperperfusion of cerebral cortex, thalamus and basal ganglia during spontaneously occurring migraine headaches. Headache 1989; 29(5): 282-289.
77. Kupers R, Kehlet H. Brain imaging of clinical pain states: a critical review and strategies for future studies. Lancet Neurol. 2006; 5(12): 1033-1044.
78. Shields KG, Goadsby PJ. Serotonin receptors modulate trigeminovascular responses in ventroposteromedial nucleus of thalamus: a migraine target? Neurobiol. Dis. 2006; 23(3): 491-501.
79. Kawamura J, Meyer JS, Terayama Y, Weathers S. Cerebral hyperemia during spontaneous cluster headaches with excessive cerebral vasoconstriction to hyperoxia. Headache 1991; 31(4): 222-227.
80. San Pedro EC, Mountz JM, Mountz JD, Liu HG, Katholi CR, Deutsch G. Familial painful restless legs syndrome correlates with pain dependent variation of blood flow to the caudate, thalamus, and anterior cingulate gyrus. J. Rheumatol. 1998; 25(11): 2270-2275.
81. Lineberry CG, Vierck CJ. Attenuation of pain reactivity by caudate nucleus stimulation in monkeys. Brain Res. 1975; 98(1): 119-134.
82. Olesen J. Cerebral and extracranial circulatory disturbances in migraine: pathophysiological implications. Cerebrovasc. Brain Metab. Rev. 1991; 3(1): 1.
83. Ferrari MD, Odink J, Tapparelli C, Van Kempen GM, Pennings EJ, Bruyn GW. Serotonin metabolism in migraine. Neurology 1989; 39 (9): 1239-1242.
84. Zuo XN, Di Martino A, Kelly C, Shehzad ZE, Gee DG, Klein DF, Castellanos FX, Biswal BB, Milham MP. The oscillating brain: Complex and reliable. Neuroimage 2010; 49(2): 1432-1445.