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Altered functional connectivity of the insula in a rat model of recurrent headache

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Abstract

Migraine is a pain disorder accompanied by various symptoms. The insula, a "cortical hub," is involved in many functions. Few studies have focused on the insula in migraine. We explored the resting-state functional connectivity between the insula and other brain areas in rats subjected to repeated meningeal nociception which was commonly used as animal model of migraine. Inflammatory soup was infused through supradural catheters in conscious rats. The rats were subdivided based on the frequency of the inflammatory soup infusions. Magnetic resonance imaging data were acquired on rats 21 days after inflammatory soup infusion and functional connectivity seeded on the insula was analyzed. In the low-frequency inflammatory soup group, magnetic resonance imaging was performed again 1 h after the glyceryl trinitrate injection following baseline scanning. The cerebellum showed increased functional connectivity with the insula in the inflammatory soup groups. The insula showed increased functional connectivity with the medulla and thalamus in the ictal period in the low-frequency inflammatory soup rats. In the high-frequency inflammatory soup group, several areas showed increased functional connectivity with the insula, including the pons, midbrain, thalamus, temporal association cortex, and retrosplenial, visual, and sensory cortices. Our findings support the hypothesis that the headache phase of migraine depends on the activation and sensitization of the trigeminovascular system, and that the chronification of migraine may be related to higher brain centers and limbic cortices. The insula may be a new target for treatment of migraine.

Keywords

Migraine, insula, functional connectivity, chronification

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Introduction

Migraine is a type of primary headache. It is a highly prevalent and complex brain disorder.[1,2] Chronic migraine (CM), defined as headaches occurring on 15 or more days/month for more than three months, is the most troublesome form of headache and imposes a greater burden.[3,4] Clinical and basic research have consistently supported the prevailing view that the headache phase of migraine depends on the activation and sensitization of the trigeminovascular system, which in turn activates different areas of the brainstem and forebrain, resulting in pain and other migrainous symptoms.[5] In addition, functional magnetic resonance imaging (fMRI) studies in patients with migraine have shown a common effect of sensory regulatory system in response to sensory stimuli as well as impaired habituation to stimuli interictally.[6] Despite extensive studies aimed to disambiguate specific and nonspecific components of migraine, a migraine-specific brain region has not yet been identified. Therefore, important brain regions involved in migraine are sought to provide a possible neuromodulation target for migraine.

In addition to its identity as a pain disorder, migraine (especially CM) comprises a wide range of sensory, physiological, psychological, and cognitive symptoms. The insula, a "cortical hub" buried within the lateral sulcus, is involved in many functions ranging from sensory processing to representation of feelings and

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emotions, autonomic and motor control, emotion, homeostasis (e.g., error detection), awareness, goal-directed cognition, and complex social functions (e.g., empathy).[7,8] Presumably, the insula serves as a cortical hub, which processes the complex symptomology of migraine.

Structural changes in the insula vary among migraineurs, with a thicker posterior insula in female migraineurs and reduced gray matter volume in migraineurs.[9,10] In fMRI studies, the insula had greater connectivity with the default mode network in patients who had migraine without aura and increased connectivity with the amygdala in patients who had migraine with aura and those who had migraine without aura.[11,12] There were significant correlations between the number of years with CM and the strength of functional connectivity (FC) between the anterior insula and mediodorsal thalamus, as well as between anterior insula and periaqueductal gray matter.[13] Migraineurs with severe allodynia had stronger FC between the periaqueductal gray matter and the insula, as well as between the nucleus cuneiform and the insula.[14] All these findings indirectly suggest that the insula has an important role in the pathophysiology of migraine of migraine. The function of the insular cortex may be related to its dense connectivity with an extensive network of cortical and subcortical brain regions. However, few studies have focused on the insula in patients with migraine.

FC has emerged as a tool for measuring the spatiotemporal correlations between distinct regions of cortex in the resting brain.[15] Seed-based or region of interest (ROI) analysis is used to calculate correlations of a particular point with other voxels of the brain, which provides a more precise and detailed look at specific connectivity characteristics in brain areas of interest. In this study, we performed fMRI of the insula to investigate its resting-state FC with other brain regions in migraine model rats. We chose the commonly used animal model of migraine, via infusion of inflammatory soup (IS) to stimulate meningeal afferents. Episodic migraine (EM) and CM animal models were prepared through IS infusions at different frequencies.[16] We hypothesized that altered FC of the insula with brain areas related to trigeminovascular pain pathways, pain modulation, and cognitive and emotion processing would be present in EM and CM, and that these atypical FCs would be affected differently in EM and CM.

Materials and methods

Animals

Twenty-four specific-pathogen-free Sprague Dawley adult male rats were used (weight 180–220 g; 6–7 weeks of age). The experimental procedures were approved by the Committee of Animal Use for Research and Education of the Laboratory Animal Center of the Chinese PLA General Hospital (Beijing, P. R. China) and followed the ethical guidelines for the study of experimental pain in conscious animals.[17] The rats were housed individually in a temperature ($22 \pm 2^{\circ}C$) and light-controlled (12/12 h light/dark) environment for three weeks prior to the surgery.

Groups

The rats were randomly divided into two experimental groups and corresponding control groups ($n = 6$ for each group): low-frequency infusion of IS to mimic EM (LF-IS; 10 μL, once every four days for three weeks) and high-frequency infusion of IS to mimic CM (HF-IS; 10 μL, daily for three weeks).[18,19] The IS contained histamine (2 mM), 5-HT (2 mM), bradykinin (2 mM), and PGE2 (0.2 mM) in sterile saline. The rats in the IS groups exhibited nociceptive behavior (i.e., face-grooming and quiescent behaviors) after IS infusion; notably, the high-frequency IS (HF-IS) group showed a significantly greater decline in the periorbital tactile threshold compared with the high-frequency control (HF-Con) group. We described these findings in detail in previous studies.[18,20]

Surgical procedures

PE10 tubes were implanted for the dural IS/saline infusion, as described previously.[18] Briefly, the rats were anesthetized to a deep surgical plane with pentobarbital (50 mg/kg, intraperitoneal). Two PE10 tubing (62310, RWD Life Science Co., Ltd., Shenzhen, P. R. China) were oriented and secured in two previously drilled troughs (8- to 10-mm long, 2-mm wide and ~0.5-mm deep, 3–4 mm lateral to midsaggital suture) aimed at the posterior sagittal sinus. The catheters were fixed to the skull using dental cement and the side exposed the skull were sealed. All surgical tools were sterilized. All rats recovered one week after surgery for IS/saline infusion.

fMRI acquisition

To mimic the interictal migraine state, the MRI was conducted on rats 24 h after the last infusion of IS/saline. Functional images were acquired on a 7.0 T Bruker Pharma Scan system (Bruker BioSpin, Ettlingen, Germany). We obtained the ictal migraine state MRI data 1 h after the glyceryl trinitrate (0.1 mg/kg, ip) injection in the LF-IS group. Animals were placed in the prone position in the scanner and anesthetized with isoflurane/oxygen mixture (5% isoflurane for induction and 1.5% for maintenance) through a gas mask. Respiration rate was monitored and

maintained at a rate of 40–50 breaths per minute throughout the scans. The resting-state functional images were collected with the same parameters as in our previous research.[20]

First, high-resolution anatomical T2-weighted images (T2WI) were obtained using a 2 D-RARE sequence. The parameters were set as: repetition time (TR) = 6200 ms, echo time (TE) = 24 ms, flip angle =180°, field of view (FOV) = 35×35 mm^2, matrix size = 256×256, slice thickness = 0.3 mm, slice gap = 0 mm, and 20 min scanning time. Second, functional images were obtained with a gradient echo-planar imaging (EPI) sequence (TR = 2,000 ms, TE = 27.1 ms, flip angle = 90, number of segments = 2, slice thickness = 1 mm, matrix = 128×128) axially for total time 13 min 20 s.[21]

Data analysis

All functional data processing and analysis was performed by an experienced professor majored in MRI technology who was blinded to the treatment group. fMRI data were analyzed by using the "spmratIHEP" toolbox within the SPM8 software (http://www.fil.ion.ucl.ac.uk/spm), which contains structural and functional rat brain template, as well as the atlas of Paxinos and Watson.[22]

The fMRI data of all individual rats were subjected to the following processing steps in spmratIHEP as used in previous studies.[20] Briefly, (1) removing the first 10 volumes, (2) using slice timing to correct the acquisition time differences, (3) realignment: remove the head motion and create a mean image over the 150 realigned volumes, (4) a spatial smoothing step with full width at half-maximum (FWHM) Gaussian kernel set to $2 \times 4 \times 2$ mm3 was performed to reduce noise, and (5) registering the realigned volumes to the Paxinos and Watson space of a rat brain by normalization with the EPI template. The registered fMRI images were finally resliced to $1.0 \times 1.5 \times 1.0$ mm^3 voxels.

Using DPARSF (http://rfmri.org/DPARSF), data were finally band-pass filtered at 0.01–0.08 Hz to reduce low-frequency signal drifts and high-frequency components of physiologic noise. The effect of head movement was further corrected using DPARSF. An ROI analysis was performed seeded on insula. The anatomical boundaries of the insula seeds are shown in Figure 1. A voxel-wise two-sample t-test was performed to estimate differences the seed-based connectivity between the IS group and the corresponding control group. FC with significance was determined based on a voxel-level height threshold of $P < 0.005$ (uncorrected) and a cluster-extent threshold of 100 contiguous voxels.

Results

The IS-group rats showed significantly increased FC between the insula and cerebellum compared with the control-group rats ($P < 0.005$, uncorrected, extent threshold k = 100 voxels; details shown in Figures 2 to 4 and Table 1). With the exception of the cerebellum, the medulla and thalamus showed a relative increase in FC with the insula in the ictal period in low-frequency IS (LF-IS) rats, compared with low-frequency control (LF-Con) rats ($P < 0.005$, uncorrected, extent threshold k = 100 voxels; details shown in Figure 3 and Table 1). Compared with the HF-Con group, the HF-IS group showed relatively greater FC with the insula in several areas, these areas included the pons, midbrain, thalamus, and temporal association cortex, as well as the retrosplenial, visual, and sensory cortex ($P < 0.005$, uncorrected, extent threshold k = 100 voxels; details shown in Figure 4 and Table 1).

Discussion

To obtain a more comprehensive understanding of the role the insula plays in migraine, we investigated the

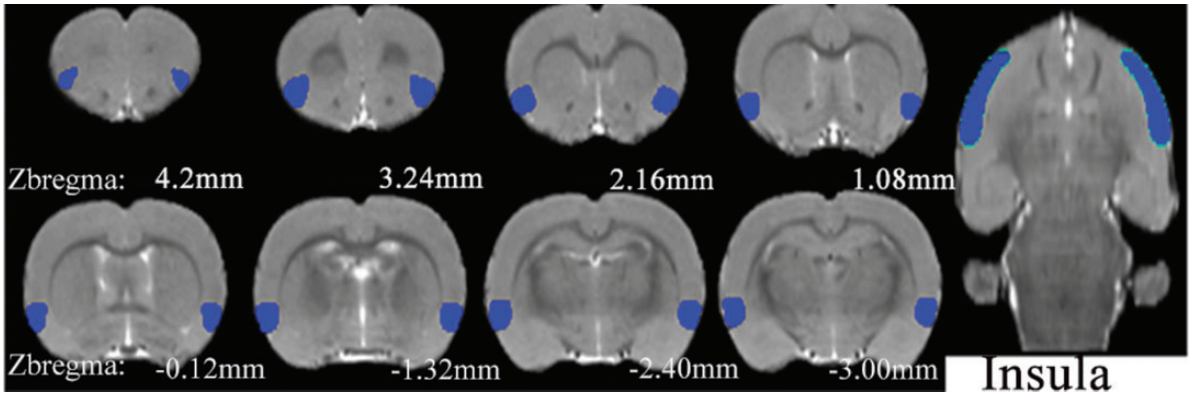

Figure 1. The insula seeds across the rats imposed on the T2-weighted MRI template and on the rat atlas structures. The anatomical boundaries for each rat were based on the atlas of Paxinos and Watson.

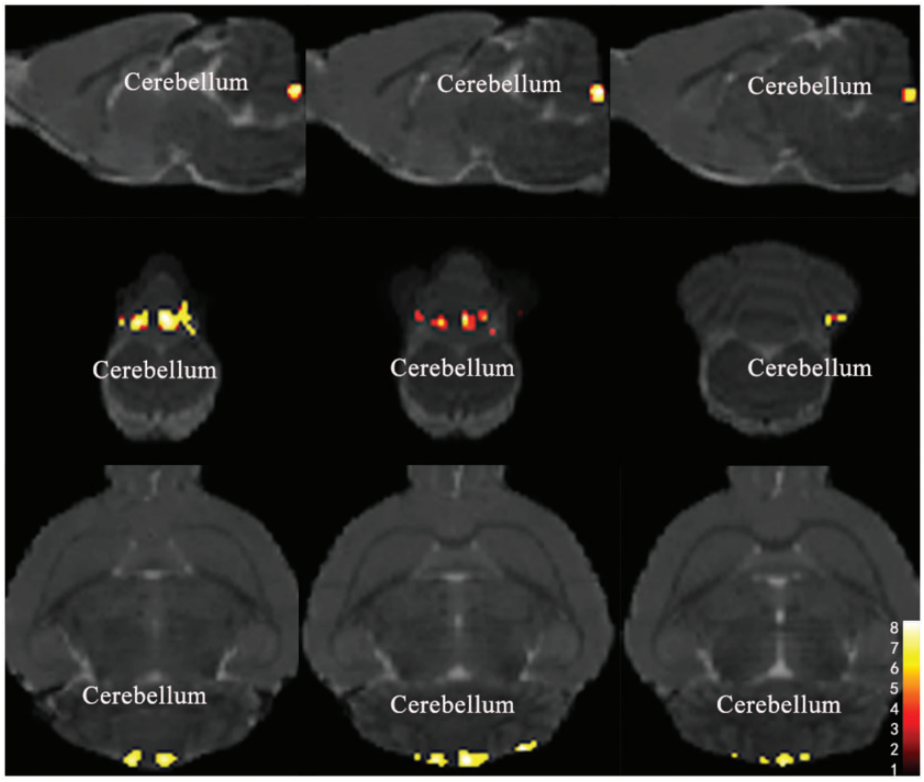

Figure 2. Increased functional connectivity was found in cerebellum (shown in red) with the insula in the LF-IS group compared with the LF-Con group (day 21) ($P < 0.005$, uncorrected, extent threshold k = 100 voxels). Detail of the cluster shown is reported in Table 1.

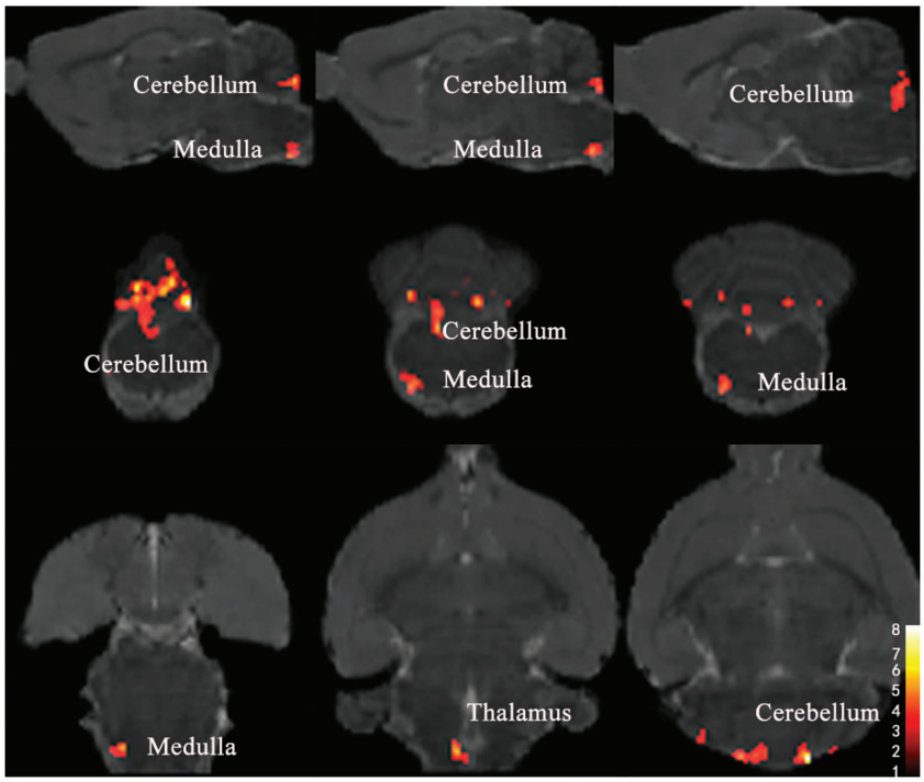

Figure 3. In ictal phase in LF-IS group, the medulla, thalamus, and cerebellum shown significantly increased FC with insula compared with LF-Con group (day 21), ($P < 0.005$, uncorrected, extent threshold k = 100 voxels). Details of the clusters shown are reported in Table 1.

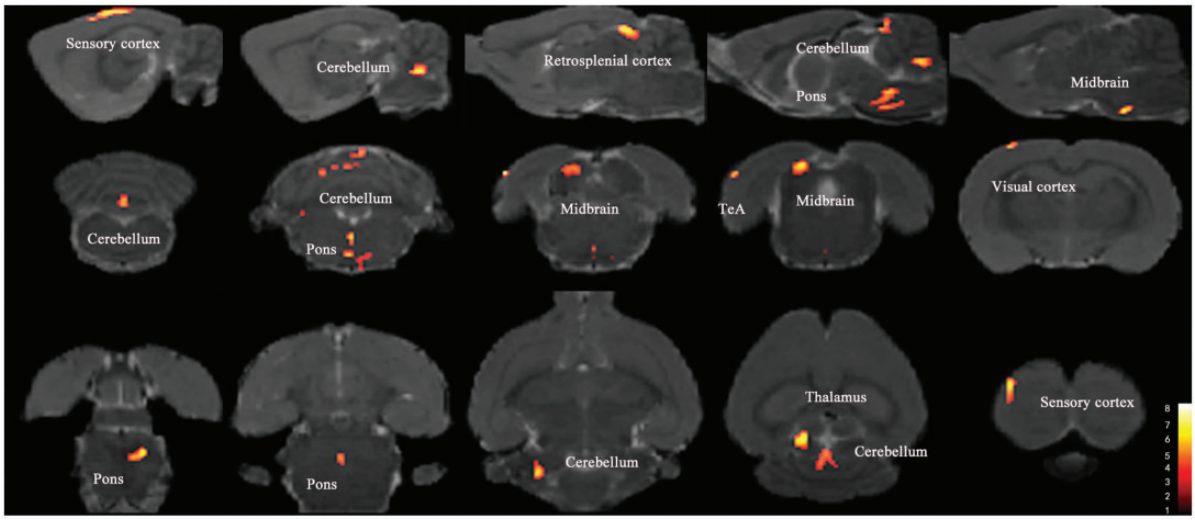

Figure 4. Altered FC with insula in the HF-IS group compared with the HF-Con group, colored voxels (day 21), ($P < 0.005$, uncorrected, extent threshold k = 100 voxels). Details of the clusters shown are reported in Table 1.

Table 1. Increased functional connectivity between the insula and other brain regions in rats induced by dural inflammatory stimulation.

Cluster or region of interest	Coordinates of peak(s) voxel (*x, y, z*)	Peak *T* value	Effect direction
Cerebellum	0.3, 5.2, –14.8	4.39	LF-IS>LF-Con
	–1.0, 5.4, –14.5	4.01	(interictal stage)
	–1.1, 4.6, –14.5	8.15	LF-IS>LF-Con
	1.5, 5.5, –14.5	8.51	(ictal stage)
	–0.01, 1.8, –9.5	4.18	HF-IS>HF-Con
	–0.1, 5.3, –12.8	4.61	(interictal stage)
Medulla	–2.2, 9.5, –13.8	4.64	LF-IS>LF-Con (ictal stage)
Pons	1.2, 9.3, –9.2	5.51	HF-IS>HF-Con (interictal stage)
Midbrain	–1.9, 2.3, –8.3	4.48	HF-IS>HF-Con (interictal stage)
Thalamus	–2.2, 9.5, –13.6	4.63	LF-IS>LF-Con (ictal stage)
	–3.1, 5.8, –11.2	4.98	HF-IS>HF-Con
	–1.6, 1.8, –7.80	6.13	(interictal stage)
Temporal association cortex	–6.0, 2.4, –8.5	6.41	HF-IS>HF-Con (interictal stage)
Retrosplenial cortex	–1.6, 1.8, –7.8	6.14	HF-IS>HF-Con (interictal stage)
Visual cortex	–3.4, –0.2, –4.4	3.45	HF-IS>HF-Con (interictal stage)
Sensory cortex	–4.0, –0.1, –2.0	4.71	HF-IS>HF-Con (interictal stage)

Effect direction >, increased functional connectivity with the insula; HF, high frequency; LF, low frequency; IS, inflammatory soup; Con, control.

altered FC with other brain regions in EM and CM model rats, focusing on the insula. In the LF-IS group, the results showed increased FC of the insula with subcortical areas related to the trigeminovascular pain pathway in the ictal stage. In the HF-IS group, the results showed central pain pathways (including the pons, midbrain, thalamus, and sensory cortex), pain modulation area (visual cortex), and cognitive processing area (such as the retrosplenial and temporal association cortex) were involved in migraine.

A cortico-cortical evoked potential study found that the insula is a "hub" that has involved in many roles, that is, sensation, saliency processing and language, as well as auditory, visual, vestibular and limbic functions through multimodel network with the cerebral cortex.[23] In human studies, the somatosensory cortices, insula, cingulate cortex, and thalamus were consistently activated in response to acute pain and are presumed to have an important role in the affective aspects of pain processing and sensory discrimination.[24] The only significant

positive correlation between the absolute cerebral blood flow changes and the intensity of tonic pain experienced was observed in the contralateral dorsal posterior insula in healthy subjects, suggesting that the dorsal posterior insula serves a fundamental function in human pain.[25] In patients with chronic pain, the insula showed a discrepant activation pattern and anatomical changes, which largely reflected dysfunction of the pain modulation system involving the insula that encompassed connectivity with the central executive network and default mode network.[26] A study of healthy subjects found that excitatory and inhibitory neurotransmitter levels and the ratio of glutamate/GABA levels in the posterior insula were related to individual differences in pain sensitivity.[27] Increased glutamate levels and reduced GABA levels were found within the insula of patients with fibromyalgia; insula glutamate was also greater in patients with diabetic neuropathy.[28,29] The altered balance of excitatory and inhibitory neurotransmitters in the insula may contribute to its hyperreactivity in chronic pain.

Migraine is a pain disorder involving a wide range of sensory, physiological, psychological, and cognitive symptoms. It is characterized by altered neural processing in the central nervous system and impaired habituation, even during the interictal phase. A study examining default mode network–insula connectivity during migraine attacks found that migraine patients showed stronger FC between the medial prefrontal cortex and bilateral insula. Concurrently, the strength of the atypical connectivity was negatively correlated with pain intensity. These contrasting findings with respect to other chronic extracephalic pain disorders may serve as a hallmark of acute migraine head pain.[30] In the interictal stage, migraineurs without aura showed increased connectivity between the primary visual cortex and the right dorsal anterior insula, the primary auditory cortex and the right dorsal anterior insula, and the dorsal pons and bilateral anterior insula. These results support the hypothesis that migraineurs exhibit heightened, anomalous interictal regional connectivity between the networks involved in processing upstream sensory information and those that represent the salience of such stimuli.[31] In an fMRI study in EM, patients showed greater amplification from Sp5 to the posterior insula and hypothalamus compared to healthy controls. In addition, habituation was not observed to repetitive sensory stimulation in the posterior insula of migraine subjects. Furthermore, the habituation slope in the posterior insula demonstrated correlated with reduced habituation in EM. These findings highlight the important role of the insula in mechanisms supporting altered sensory processing in EM.[32] In the LF-IS group, our study found increased FC between subcortical areas (i.e., medulla and thalamus) and the insula in the ictal stage, further demonstrating the important role of the insula in migraine; moreover, this finding supported the view that the headache phase of migraine depends on the activation and sensitization of the trigeminovascular system.

Repeated migraine attacks may lead to central sensitization. Consistent with the results of human CM studies, we found increased FC between the insula and the pons, between the insula and the midbrain, and between the insula and the thalamus in the interictal stage in the HF-IS group; these findings suggested that the insula participates in the sensory-discriminative, cognitive, and integrative domains of the pain experience.[13] Moreover, increased FC between the insula and the sensory cortex, as well as between the insula and the visual cortex, was found in the HF-IS group, implying that the chronification of migraine is due to the abnormal processing of sensory information and dysfunctional pain modulation. Due to its multidimensional link, the insula affects the pain experience by affecting the recognition or valence of a given sensory input, but not by altering sensory perception thresholds.[7] Psychological and cognitive disorders are more obvious in patients with CM. Clinical and basic studies have demonstrated that migraine especially CM is comorbidity with depression and anxiety.[33] The insula acts as a core region affected by many psychiatric disorders; these include, but are not limited to, anxiety disorders, addiction, depression, schizophrenia, and autism. In our study, we also found increased FC between the insula and the retrosplenial association cortex, as well as between the insula and the temporal association cortex in the HF-IS group. These two regions are mainly involved in memory, cognition, and emotional processing; they are related to dementia, schizophrenia, and addiction.[34,35] Atypical FC with the insula in the HF-IS group may be involved in the cognitive dysfunction observed in CM. In addition to its participation in pain, the insula can serve as an indicator of pain progression and remission. An fMRI study showed reduced activation in the right anterior insula during occlusion over the course of therapy for patients with craniomandibular disorders. Correlation analysis between the pain score and reduced fMRI activation identified the right anterior insula and left posterior insula, which highlights the impact of the insula on the internal monitoring of temporomandibular joint pain.[36]

Insula involves lots of brain functions especially advanced functions, so some have argued that the human insular cortex is unique and corresponding functions are exclusively human. As modern technologies available for detecting function in animal models, they found many of the functional characteristics of the insula are shared between rodents and humans.[7] Our findings support the important role of the insula in the

pathogenesis of both EM and CM. More longitudinal clinical and basic research studies should examine the insula in migraine. The relationship between the changes in imaging findings and the underlying pathologies is needed to be identified in basic researches and further explore the pathogenesis of migraine.

Conclusion

We investigated the atypical resting-state FC between the insula and other brain areas in rats subjected to repeated meningeal nociception in the ictal and interictal periods. We found increased FC of the insula with second- and third-order neurons in the trigeminovascular pain pathway in the ictal phase, supporting the hypothesis that the headache phase of migraine depends on the activation and sensitization of the trigeminovascular system. The involvement of brain areas important in pain modulation and in emotional and cognitive processing in the interictal stage in the HF-IS group suggested that higher brain centers and limbic cortices are related to the chronification of migraine. The insula as a "cortical hub" participating in the headache and chronification of migraine may serve as a new therapeutic target in migraine.

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Author contributions

All authors reviewed the manuscript. Guarantor of integrity of entire study: SY, WT, and ZJ. Designed and supervised experiments, data acquisition, and analysis: ZJ and DZ. Raised the mice and manuscript drafting: ZJ. Manuscript editing: SY. Manuscript final approval: all authors.

Declaration of Conflicting Interests

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