Spatiotemporal Dynamics of Brain Function During the Natural Course in a Dental Pulp Injury Model

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Short Report

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Abstract

Purpose Toothache, a common disorder afflicting most people, shows distinct features at different clinical stages. This study aimed to depict metabolic changes in brain and investigate the potential mechanism involved in the aberrant affective behaviors during the natural process of toothache.

Methods We investigated the spatiotemporal patterns of brain function during the natural course of toothache in a rat model of dental pulp injury (DPI) by using positron emission tomography (PET).

Results Glucose metabolism peaked on the 3rd day and gradually decreased in several brain regions after DPI, which was in line with the behavioral and histological results. PET imaging showed visual pathway was involved in the regulation of toothache. Meanwhile, the process of emotional regulation underlying toothache was mediated by N-methyl-D-aspartic receptor subunit 2B (NR2B) in the caudal anterior cingulate cortex (cACC).

Conclusion Our results revealed the spatiotemporal neurofunctional patterns during toothache process and preliminarily elucidated the role of NR2B in cACC in the regulation of toothache-related affective behaviors.

Introduction

Toothache is considered to be the highest in the degree of pain and often leads to some emotional symptoms such as anxiety- and depression-related behaviors [1]. Many studies have confirmed that the neural conduction pathway of the toothache is the trigeminal nerve-thalamo-cortical circuitry, which included the brainstem trigeminal complex, thalamus and cerebral cortex [2, 3]. The signal of nociceptive stimulus in the dental pulp is transmitted from the periphery to the central nervous system through the trigeminal ganglion, and then converges to the trigeminal spinal nucleus, which relays nociceptive information to the thalamus, and further transduces nociceptive information to the cerebral cortex [2–4]. However, the relationship between toothache and emotional reaction is not clear. The dynamic spatio-temporal changes of brain function involved in the natural process of toothache also have not been clearly studied. Here, for the first time to our knowledge, we non-invasively investigated the dynamic metabolic changes of brain activities longitudinally using PET molecular imaging approach [5] in a rat model of dental pulp injury (DPI), and further explored the potential mechanisms underlying the aberrant affective behaviors during the toothache natural course.

Materials And Methods

Animals

Adult male Sprague-Dawley (SD) rats (240-260g) (*n* = 60) were randomly assigned to the DPI group and the Sham group for natural process investigation (1st, 2nd, 3rd, 7th, 14th day respectively, total of 10 groups, n = 6 in each group). For intervention study, the rats (n = 48) were randomly assigned to four

groups (including the Sham, DPI, DPI + NS and DPI + APV treated, n = 12 in each group). We performed DPI on part of the rats by mechanically exposing the dental pulp as previously described [6], which produces pulpal inflammation (pulpitis) followed by necrosis of the pulpal tissues. A double-guide cannula was embedded into the cACC [7], then rats were allowed to recover for 7-10 days before intervention to evaluated the wound healing and weight recovery. All the procedures are provided in the Supplementary Material.

Behavioral assessment

Preoperative physiological and behavior baseline were defined by using the average body weight values, duration time, frequency of the face grooming and the time of desperate resting behavior from the last 3 days and the average water and food intake measures from the last 2 days before the surgery. These parameters were continued to be recorded every day from 1 to 14 days of operation [8] (Fig. 1A).

HE staining and immunostaining

We extracted the left maxillary first molars of rats at each time period and performed hematoxylin and eosin (HE) staining to observe the progress of inflammation, then cut the medulla oblongata and the cACC tissues for immunostaining and immunofluorescence staining, respectively.

Western Blot

The total protein of cACC tissue was extracted to conduct western blotting assay. Total protein extracts (25 μg) were loaded to perform western blotting analysis with antibody against NR2B and p-NR2B. Protein bands were detected by enhanced chemiluminescence (ECL, Millipore, USA) and imaged with a Bio-Rad chemiDoc XRS+ imaging system (USA) [9].

PET imaging and image analysis

I*n vivo* 2-deoxy-2-[^{18}F] fluoro-D-glucose (^{18}F-FDG) PET scanning were performed at different time periods after DPI surgery. The images were reconstructed using a modified back projection algorithm and analyzed using the AMIDE (version 9.2; Stanford University) and Statistical Parametric Mapping (SPM) software. We adopted two independent *t*-tests to evaluate regional metabolic differences between baseline and post-stimulation PET images. Statistical significance was determined when P value < 0.01 and cluster Ke > 100 [10]. The lesion-to-normal (L/N) ratio was used for semi-quantitative analysis by using PMOD (v.3.902, PMOD Technologies Ltd.). The regional cerebral metabolism rate (rCMR) of each ROI was calculated as the lesion-to-pons (L/P) ratio.

Statistical analysis

Data were expressed as mean ± SD. A value of P < 0.05 was considered as significant. Statistical analyses were performed by Student's t-test and one-way and two-way analysis of variance (ANOVA) and the SPSS software (version 22.0, SPSS Inc.).

Results

Behavioral assessment and histology of DPI

We observed the drinking, diet and average weight gain of DPI rats decreased greatly in comparison with the Sham group, while the duration and frequency of facial grooming and the frozen time in forced swimming test (as a model of depressive-like behavior) [11] were increased significantly, especially during the first 3 days in DPI. All these parameters returned to the baseline levels on the 14th day after surgery (Fig. 1A). Notably, our results of HE staining showed that the inflammation appeared gradually from the crown to the root pulp in DPI, and the acute (days 1-3) and chronic inflammation (days 4-17) and finally dental pulp necrosis (on the 14th day) after surgery were also identified (Fig. 1B).

Glucose metabolism in the rat brain after DPI

The analysis of the SPM and PMOD results showed that the main manifestations were the increased absorption of ^{18}F-FDG in the brain, including ventroposterior medial thalamic nucleus (VPM), corpus callosum (cc), hippocampus (HPC), striatum, somatosensory cortex (S1&S2), motor cortex (M1&M2), cingulate cortex (Cg), retrosplenial dysgranular cortex (RSD), prefrontal cortex (PFC) and lateral geniculate nucleus (LGN), while periaqueductal dray (PAG) and superior colliculus inhibited absorption in the process of toothache (Fig. 1C, 2A and 2B, SI Appendix, Fig. S2, Table S1, S2 and S3). The accumulation of ^{18}F-FDG peaked on the 3rd day after DPI in a wide range of brain regions, and then decreased gradually from 7 to 14 days after DPI surgery.

cACC participation in emotional regulation

In order to further clarify the potential contribution of cACC to toothache, APV (also known as AP5, (2R)-amino-5-phosphonovaleric acid, or (2R)-amino-5-phosphonopentanoate), a selective NMDA receptor antagonist, was applied to reduce the expression of NR2B and block the neuronal response of cACC [12]. The optimal concentration of APV was determined by autoradiography (SI Appendix, Fig. S3). After APV administration in the cACC, we observed significantly decreased duration of grooming and freezing ($P < 0.01$, Fig. 2C), reduced expression of NR2B and p-NR2B, and downregulated rate of glucose metabolism ($P < 0.05$, Fig. 2D and E).

Discussion

In this study, the behavioral, histological and metabolic dynamic changes of toothache were longitudinally evaluated for up to 14 days in a rat DPI model. Interestingly, a perfect correlation among the behavioral, histological examinations and PET imaging was identified during the natural process of toothache, and therefore, our study provided a comprehensive understanding for the neurofunctional patterns underlying toothache. More importantly, the nerve conduction pathways related to toothache, especially the emotional reaction to toothache are identified. The related features provide rich resources to inspect toothache-induced brain changes.

The upload of toothache stimulation signal caused transient and large-scale reactive activation of rat brain nuclei, and there was a relationship between the functional connectivity of each nucleus and the strength of pain signals. According to previous literatures [13] and the results of the current study (Fig. 2F and G), the ascending pathway of toothache is postulated to be conducted through the trigeminal nerve complex to PAG, cc and VPM, and then to the subcortical area (nucleus accumbens, striatum, hippocampus and amygdala) and cerebral cortex (S1&S2, M1&M2, PFC, Cg, RSD). Among them, pain signals cross cc and PAG, which activate most of the brain regions bilaterally, thus regulating the transmission of pain in the left maxillary teeth. Different from previous studies [14], the visual signals related to VPM, superior colliculus and LGN, as well as RSD may also be involved in the transmission of toothache. Among them, the superior colliculus and LGN can make a visual reflex response to tactile information [15], thus reducing perception and emotion caused by pain, suggesting that the signals of toothache might activate the eye branch when ascending through the trigeminal ganglion, which explains why some patients with acute toothache often experience symptoms of eye discomfort and even tears [16]. The RSD is involved in the control of spatial navigation, episodic memory and pain related emotional behaviors [17]. It is speculated that RSD might be related to the cognitive and spatial memory of toothache in rats. Here, PET showed, for the first time, that the degree of activation and inhibition of the 12 brain regions involved in toothache is closely related to the changes of its pain intensity, and may also be related to the cognition and memory of toothache in rats. The changes of brain network caused by toothache are very complex and elusive and need to be further investigated.

Toothache induces anxiety- and depression-related symptoms, thus may cause the patients to miss the best time window to get treatments. Therefore, it is of great significance, to understand how the toothache related emotion was regulated in order to alleviate the negative emotions. ACC, including rostral anterior cingulate cortex (rACC) and cACC, is central to pain processing. rACC participates in the process of somatic pain emotional response through NR2B activation. According to our results, we chose to intervene the cACC at 3rd day after DPI. APV, an antagonist of NMDA receptor, can block the synaptic response of ACC neurons [9]. We found that the glucose metabolism level of bilateral cACC but not rACC increased significantly 3 days after DPI. Interestingly, APV reversed the emotional response related to toothache. These results suggest that cACC but not rACC, may mediate the emotional reaction to toothache by phosphorylating NR2B receptor, which is the difference between emotional regulation of toothache and somatic pain. This might provide a novel insight for relieving the pain affect induced by toothache.

Conclusion

Our research results indicate changes in rat brain function and toothache-related nerve conduction circuits during toothache, and we found that NR2B in cACC might mediate the aberrant emotional behaviors during the toothache natural course. This may provide a certain basis for the clinical prevention and treatment of toothache-related emotional symptoms.

Declarations

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Conflict of interest The authors declare no conflicts of interest.

Ethical approval Animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Zhejiang University School of Medicine (Protocol No. #ZJU20190015). The operational processes were under the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) and National Research Council‘s Guide for the Care and Use of Laboratory Animals.

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Figures

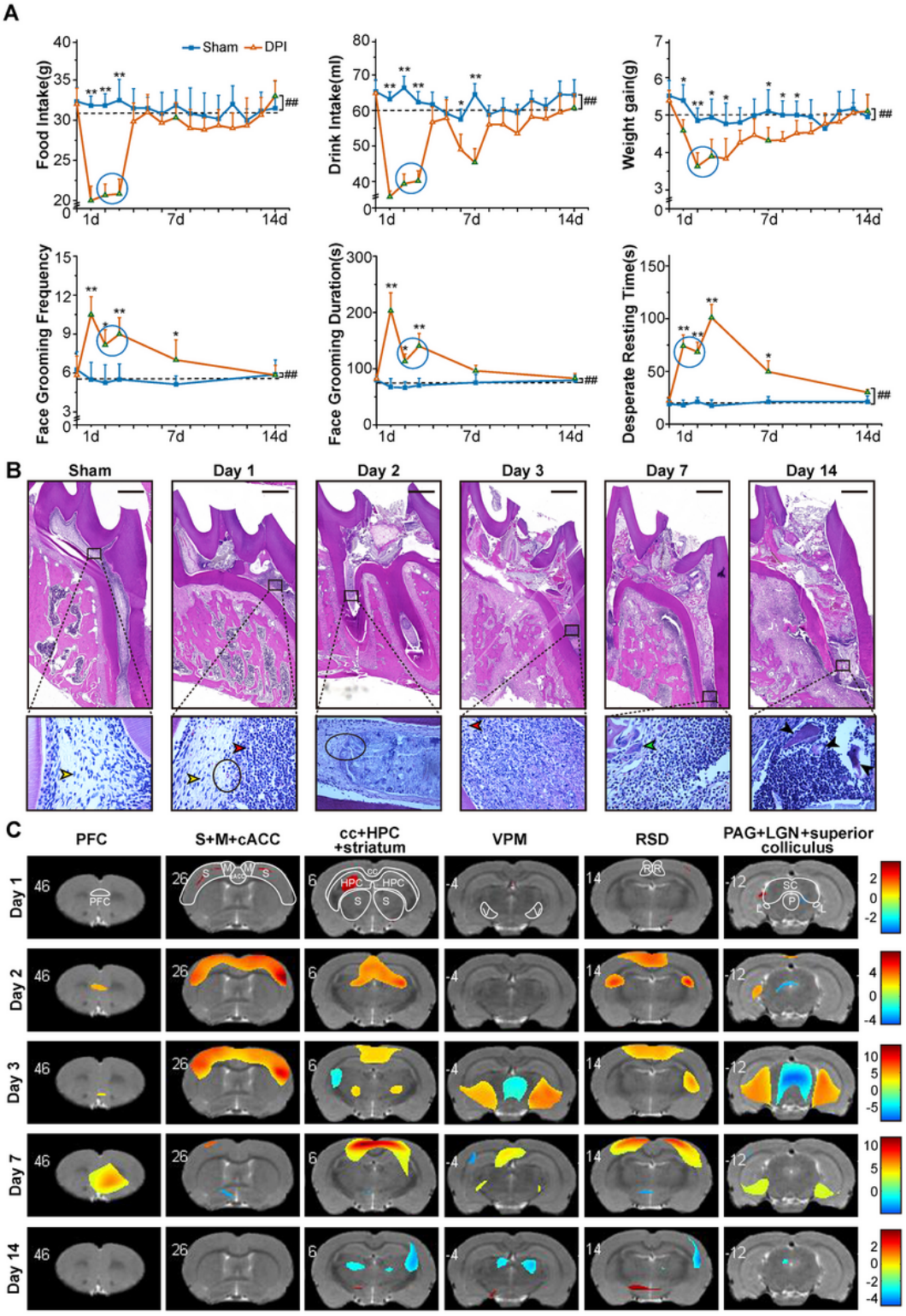

Figure 1

Establishment of DPI verified by behavioral assessment, Histology and PET molecular imaging. (**A**) Behavioral changes of food intake, drink intake, average weight gain, face grooming frequency, face grooming time and freezing time in DPI. (**B**) HE staining images showed the development changes of pulpitis during the natural course of DPI (Bar = 250 μm). The yellow arrows represent normal pulp cells, the red arrows indicate neutrophils, the green arrows indicate osteoclasts, the black circles represent the

border of normal pulp and inflammatory tissue, and the black arrows indicate necrotic tooth tissue (400 ×). (**C**) Brain regions that showed significant glucose metabolism changes on day 1, 2, 3, 7 and 14 after DPI operation in rats (Data are shown as mean ± SD, $n = 6$ in each group; $P < 0.01$, d: day).

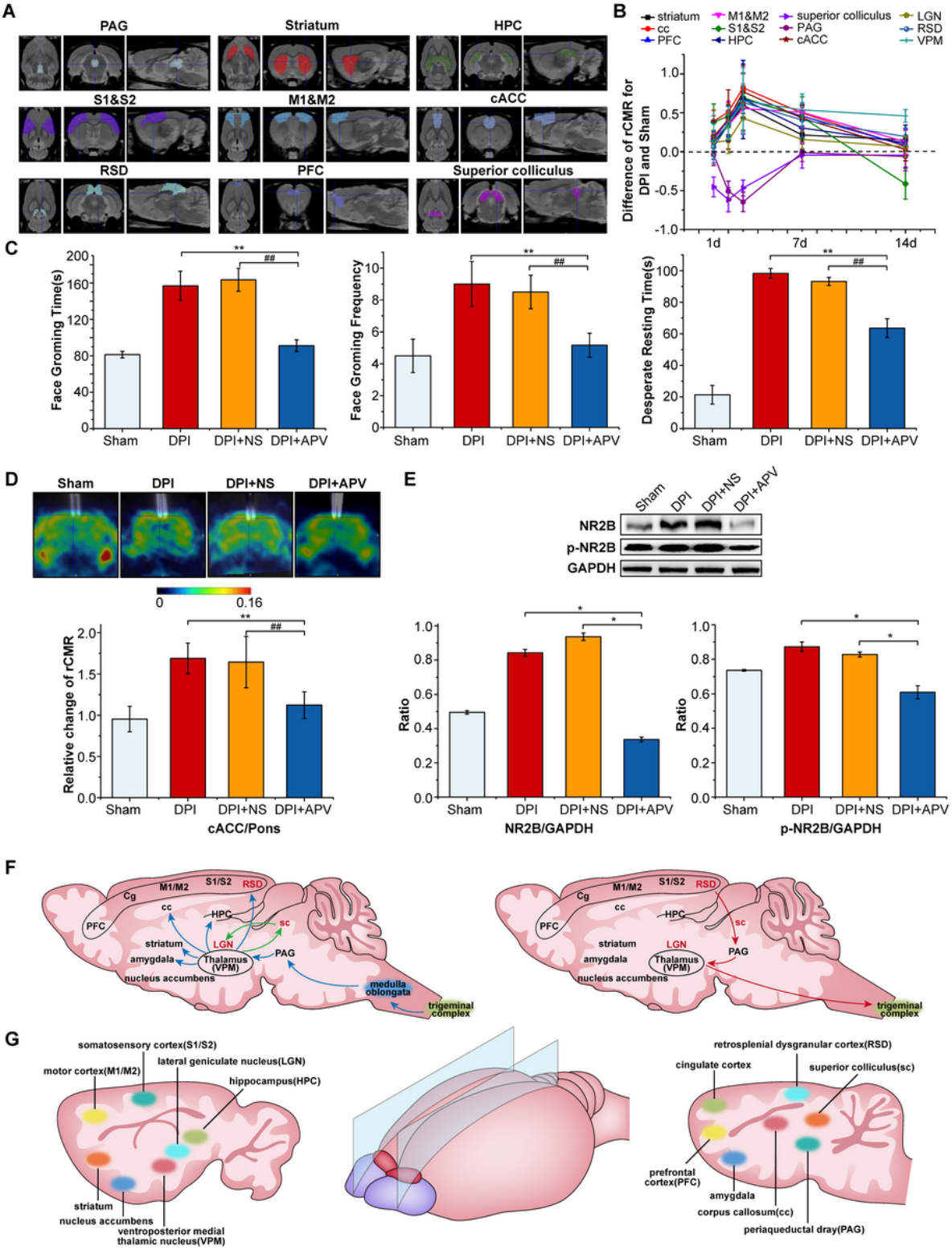

Figure 2

Nerve conduction pathway related to toothache in DPI. (**A**) Representative transverse (left), coronal (middle), and sagittal (right) PET images demonstrated alternation of glucose metabolism. (**B**) The rCMR of each ROI of the 12 brain regions after DPI by PMOD. The *P*. value of relative change of rCMR in each group were shown in Table S2. (Two independent samples T test; n = 6 in each group, d: day). (**C-E**) Face grooming time and frequency, and desperate resting time, glucose metabolism after injecting APV in cACC region, and the expression of NR2B and p-NR2B proteins were analyzed in DPI+APV group compared to DPI and DPI+NS groups. (Data are shown as mean ± SD, **, $^{\#\#}$: $P < 0.01$, one-way ANOVA test. DPI: dental pulp injury; NS: normal saline). (**F**) Schematic of major ascending (bottom-up) pathways from the medulla oblongata to the brain that are activated by noxious stimuli related to toothache (left) and descending (top-down) pathways that modulate transmission of ascending nociceptive signals (right). (**G**)Schematic of the location of brain regions that showed significant glucose metabolism changes in sagittal view after DPI.

Supplementary Files

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