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A voxel-based analysis of brain activity in high-order trigeminal pathway in the rat induced by cortical spreading depression

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ABSTRACT

Cortical spreading depression (SD) is a self-propagating wave of depolarization that is thought to be an underling mechanism of migraine aura. Growing evidence demonstrates that cortical SD triggers neurogenic meningeal inflammation and contributes to migraine headaches via subsequent activation of trigeminal afferents. Although direct and indirect evidence shows that cortical SD activates the trigeminal ganglion (peripheral pathway) and the trigeminal nucleus caudalis (TNC, the first central site of the trigeminal nociceptive pathway), it is not yet known whether cortical SD activates the high-order trigeminal nociceptive pathway in the brain. To address this, we induced unilateral cortical SD in rats, and then examined brain activity using voxel-based statistical parametric mapping analysis of FDG-PET imaging. The results show that approximately 40 h after the induction of unilateral cortical SD, regional brain activity significantly increased in several regions, including ipsilateral TNC, contralateral ventral posteromedial (VPM) and posterior thalamic nuclei (Po), the trigeminal barrel-field region of the primary somatosensory cortex (S1BF), and secondary somatosensory cortex (S2). These results suggest that cortical SD is a noxious stimulus that can activate the high-order trigeminal nociceptive pathway even after cortical SD has subsided, probably due to prolonged meningeal inflammation.

Introduction

Migraine is a neurovascular disorder characterized by unilateral and throbbing headache, lasting 4 to 72 h. The migraine headache is thought to be triggered by intracranial neurogenic inflammation that activates trigeminal nociceptors in meningeal blood vessels (Goadsby and Edvinsson, 1993; Moskowitz and Macfarlane, 1993). Moskowitz and colleagues have proposed that pro-inflammatory peptides, such as substance P and calcitonin gene-related peptide (CGRP), released from trigeminocervical nerve terminals in response to meningeal nociceptive stimuli, induce vasodilation and plasma protein extravasations, which causes headache via stimulation of trigeminal afferents (Markowitz et al., 1987; Moskowitz, 1984). Consistent with this theory, vasogenic leakage (Arnold et al., 1998; Iizuka et al., 2006) and CGRP increase in the jugular vein (Goadsby et al., 1990) have been reported during migraine attack. However, the initial event that activates trigeminocervical nerve terminals to mobilize pro-inflammatory peptides is still unclear.

In one-third of migraine patients, attacks are preceded by transient neurological symptoms, such as a scintillating scotoma, a type of migraine aura in which a spot of flickering light appears almost near the center of the visual field and then gradually expands outward (Lauritzen, 1994; Rasmussen and Olesen, 1992). Increasing evidence suggests that a neurovascular phenomenon of cortical spreading depression (SD) may contribute to the initial phase of migraine attack, such as plasma protein leakage (Bolay et al., 2002; Gursoy-Ozdemir et al., 2004; Markowitz et al., 1987). Cortical SD is a self-propagating wave of transient neuronal/glial membrane depolarization that is accompanied by temporal elevation of the cerebral blood flow (CBF) throughout the cerebral cortical hemisphere at a rate of 2–5 mm/min (Cui et al., 2003; Leao, 1944). The rate of spread correlates with the observed spread of the typical migraine visual aura, the scintillating scotoma (Lashley, 1941; Milner, 1958). A neurovascular event closely resembling cortical SD has been shown with fMRI during the migraine visual aura (Hadjikhani et al., 2001). Moreover, a spreading oligemia has been observed during migraine attack at a similar velocity as cortical SD (Lauritzen et al., 1983; Olesen et al., 1981). Meanwhile, the involvement of cortical SD in trigeminal nociception has been supported by indirect evidence for a cortical SD-induced increase in c-Fos expression in the ipsilateral trigeminal nucleus caudalis (TNC) (Moskowitz et al., 1993). More recent electrophysiological experiments show cortical SD-evoked neuronal activity in the ipsilateral trigeminal ganglion (Zhang et al., 2010) and in the TNC (Zhang et al., 2011). However, it remains unknown whether cortical SD activates the high order trigeminal nociceptive pathway in the brain to be engaged in pain transmission and sensation.

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Non-invasive functional brain imaging techniques, such as positron emission tomography (PET), provide a global overview of human brain function. With recent advances in the spatial resolution of PET, this technique is increasingly used in studies of rodent brain function (Endepols et al., 2010; Jang et al., 2009; Kobayashi et al., 2013; Sung et al., 2009). Recently, we developed a small-animal neuroimaging method combining 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) PET imaging with statistical parametric mapping (SPM) analysis to evaluate regional brain activity in the entire rat brain. FDG is taken up by active brain regions and remains within the system for at least an hour (Schiffer et al., 2007). Therefore, FDG-PET imaging could provide a fascinating method for evaluating brain activity free from the effect of anesthesia, as image scanning can be conducted after FDG uptake without anesthesia. Using the FDG-PET imaging method, here we provide a new line of evidence that the cortical SD, a presumed initial event of migraine headache, significantly activates the migraine-related pain matrix in higher brain regions, as a result of prolonged meningeal inflammation which has been demonstrated previously (Bolay et al., 2002; Cui et al., 2009; Gursoy-Ozdemir et al., 2004).

Materials and methods

All experimental protocols were approved by the Ethics Committee on Animal Care and Use of RIKEN Center for Life Science Technologies and were performed in accordance with the *Principles of Laboratory Animal Care* (NIH Publication No. 85-23, revised 1985).

Animal preparation for generation of SD

Male Sprague–Dawley rats (SLC, Hamamatsu, Shizuoka, Japan), weighing approximately 300 g, were used. In order to generate cortical SD in the rat cerebral hemisphere, the head of each rat was fixed in a stereotaxic apparatus (Type 1430, David Kopf, Tujunga, CA, USA) under 1.5% isoflurane anesthesia. A thermocouple probe was connected with a thermo-controller and inserted into the rectum to maintain the body temperature at 37 °C. A small burr hole was drilled in the skull at the left side of the frontal cortex (2.0–3.0 mm anterior and 2.0–3.0 mm lateral to the bregma). A glass micropipette (internal diameter of the tip, 50 μm) was inserted 1 mm below the cortical surface through the burr hole for subsequent microinjection. Two hours after the insertion, microinjection of 1 M KCl was performed (at a rate of 0.2 μl/min for 1 min) every 10 min for a period of 2 h (12 injections; 2.4 μl total volume). Sham controls were injected with 1 M NaCl at an analogous rate, duration, and frequency.

Because autotomy (self-attack and mutilation of the denervated limb) has been observed in some neuropathic pain model animals (Jaggi et al., 2011; Kim and Chung, 1992; Wall et al., 1979), all rats that we used were monitored for autotomy behavior through the entire period of the experiment. No signs of overt autotomy, such as wounds or scars, were observed in the forehead or areas above the eye, the representative cutaneous receptive fields of the ophthalmic division of the trigeminal nerve, which also densely innervates meningeal blood vessels.

Cerebral blood flow recording

A laser Doppler flowmetry probe (LDF, Type FLO-N1, Omega Wave, Tokyo, Japan) was attached to a micromanipulator and stereotactically placed in the ipsilateral parietal cortex (5 mm posterior and 2.0–3.0 mm lateral to the bregma) over the skull for recording changes in cerebral blood flow (CBF). The absolute value from LDF does not mean actual perfusion units; therefore relative change in CBF is displayed (the data are normalized to pre-level). At the end of CBF recording, all surgical incisions were carefully sutured, and the animals were treated with antibiotics. After awakening from anesthesia, the animals returned to their home cage to recover.

PET scanning

All PET scans were performed using microPET Focus220 (Siemens Co., Ltd, Knoxville, TN, USA) designed for high resolution imaging of small laboratory animals. This scanner has a spatial resolution of 1.4 mm in full width at half maximum at the center of the field of view (FOV). The transaxial FOV is 19 cm and the axial FOV is 7.6 cm.

Results from our preliminary study showed that FDG uptake was higher in the caudal part of the left versus right brainstem after cortical SD in the left cerebral hemisphere. We tested a wide range of post-induction periods (0–60 h after cortical SD, 2–4 animals per time point), and the most obvious difference in L/R ratio was observed around 40 h after induction of cortical SD (Supplementary Table 1). The increased L/R ratio that was occasionally observed shortly after cortical SD induction might have been due to the long-term operation under anesthesia for induction of cortical SD. Based on these preliminary results, FDG-PET scans were performed approximately 40 h after induction of cortical SD in the SD-induced ($n = 14$) and sham control rats ($n = 8$). Prior to PET scanning, each rat received a tail vein cannulation under anesthesia with a mixture of 1.5% isoflurane and nitrous oxide/oxygen (7:3). After more than an hour of recovery, the freely-moving rat received intravenous injection of ^{18}F-FDG (70–75 MBq/0.4 ml) in the home cage. After a 45-min uptake period, rats were anesthetized with a mixture of 1.5% isoflurane and nitrous oxide/oxygen (7:3) and positioned in the gantry of a PET scanner. A thermocouple probe was inserted into the rectum to monitor rectal temperature. During the PET scan, body temperature was maintained at about 37 °C with a heating blanket. Fifty-five minutes after ^{18}F-FDG injection, a 30-min emission scan was performed with 400–650 keV as the energy window and 6 ns as the coincidence time window. Emission data were acquired in the list mode. The acquired data were sorted into a single sinogram. The data were reconstructed by standard 2D filtered back projection (FBP) with Ramp filter and cutoff frequency at 0.5 cycles per pixel, or by a statistical maximum a posteriori probability algorithm (MAP), 12 iterations with point spread function (PSF) effect. The reconstructed image pixel size was 0.38 mm transaxially with a 0.79 mm slice thickness. Compared with FBP, MAP reconstructed images have been shown to result in improved spatial resolution and noise properties on small animal PET images, an advantage for image co-registration. Meanwhile FBP reconstructed images were used for quantification and statistical analysis.

Image analysis

For voxel-based statistical analysis, individual MAP reconstructed FDG images were coregistered to a FDG template image using a mutual information algorithm with Powell's convergence optimization method implemented with PMOD software package (version 3.2, PMOD Technologies, Ltd., Zurich, Switzerland). The FDG template image was made from 10 age-matched satellite rats prior to the experiment, as previously described (Schweinhardt et al., 2003) with slight modification. Briefly, individual FDG images from normal rats were coregistered to the respective images and then these images were averaged to make an FDG rat brain template. Subsequently, the FDG template was transformed into the space of a MRI reference template, which was placed in Paxinos and Watson stereotactic space. The transformation parameters obtained after individual MAP images to FDG template were applied to each FBP reconstructed FDG image. For using the default parameter setting in SPM, the voxel size of template was scaled by a factor of 10 to close the human brain size. Because the Paxinos stereotactic space has a 0.12 mm slice thickness, the final voxel size resampled at $1.2 \times 1.2 \times 1.2$ mm. To enhance the statistical power, each FBP image was spatially smoothed with an isotropic Gaussian kernel (6-mm FWHM).