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Negative cerebral blood volume fMRI response coupled with Ca^{2+}-dependent brain activity in a dopaminergic road map of nociception

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ABSTRACT

Decreased cerebral blood volume/flow (CBV/CBF) contributes to negative blood-oxygen-level-dependent (BOLD) functional MRI (fMRI) signals. But it is still strongly debated whether these negative BOLD or CBV/CBF signals are indicative of decreased or increased neuronal activity. The fidelity of Ca^{2+} signals in reflecting neuronal excitation is well documented. However, the roles of Ca^{2+} signals and Ca^{2+}-dependent activity in negative fMRI signals have never been explored; an understanding of this is essential to unraveling the underlying mechanisms and correctly interpreting the hemodynamic response of interest. The present study utilized a nociception-induced negative CBV fMRI response as a model. Ca^{2+} signals were investigated in vivo using Mn^{2+}-enhanced MRI (MEMRI), and the downstream Ca^{2+}-dependent signaling was investigated using phosphorylated cAMP response-element-binding (pCREB) immunohistology. The results showed that nociceptive stimulation led to (1) striatal CBV decreases, (2) Ca^{2+} increases via the nigrostriatal pathway, and (3) substantial expression of pCREB in substantia nigra dopaminergic neurons and striatal neurons. Interestingly, the striatal negative fMRI response was abolished by blocking substantia nigra activity but was not affected by blocking the striatal activity. This suggests the importance of input activity other than output in triggering the negative CBV signals. These findings indicate that the striatal negative CBV fMRI signals are associated with Ca^{2+} increases and Ca^{2+}-dependent signaling along the nigrostriatal pathway. The obtained data reveal a new brain road map in response to nociceptive stimulation of hemodynamic changes in association with Ca^{2+} signals within the dopaminergic system.

Introduction

Functional MRI (fMRI) has become a widely used approach for exploring brain activity in vivo (Logothetis, 2008). The aspects of neural activation and electrophysiology represented by fMRI signals have attracted immense attention (Logothetis, 2010). Among the most elusive fMRI responses are the negative blood-oxygen-level-dependent (BOLD) fMRI signals, which are dominated by decreased cerebral blood volume/flow (CBV/CBF) (Boorman et al., 2010; Schafer et al., 2012; Shih et al., 2011). This hemodynamic change is opposite to the conventional fMRI response (Bianciardi et al., 2011; Bressler et al., 2007; Kastrup et al., 2008; Liu et al., 2011; Shmuel et al., 2002; Smith et al., 2004; Zeharia et al., 2012) associated with elevated BOLD signals due to increased CBV/CBF and oxygen utilization. Negative BOLD fMRI signals are physiologically meaningful (Devor et al., 2007; Mishra et al., 2011; Schridde et al., 2008; Shih et al., 2009; Shmuel et al., 2006), strongly correlated with the spatial, temporal, and quantitative characteristics of the applied stimulus (Bressler et al., 2007; Shmuel et al., 2002; Smith et al., 2004), and closely associated with overt behaviors (Boorman et al., 2010; Grimm et al., 2009; Kastrup et al., 2008). Nevertheless, it is strongly debated whether the negative BOLD fMRI signals are representative of decreased or increased neuronal activity, due to divergence in the evidence from electrophysiological recordings (Bressler et al., 2007; Mishra et al., 2011; Schridde et al., 2008; Shmuel et al., 2006).

The fidelity of Ca^{2+} signals in reflecting neuronal activity is well documented (Berridge, 1998; Berridge et al., 2000). Positive BOLD fMRI responses have recently been found to be temporally and physiologically related to Ca^{2+} increases during neural activation (Schulz et al., 2012). In contrast, the relationship of a negative fMRI response—in the form of decreased BOLD signals or CBV/CBF—to Ca^{2+} signals and Ca^{2+}-dependent cascades is not understood. A second advantage of studying Ca^{2+} signals is that mapping these signals would help identify the circuitry (Yates, 2011) responsible for generating an observed fMRI

Abbreviations: MEMRI, Mn^{2+}-enhanced MRI; pCREB, phosphorylated cAMP response-element-binding; SN, substantia nigra; CBV, cerebral blood volume; T1WIs, T1-weighted images; FLASH, fast/low-angle shot; TR, repetition time; TE, echo time; FA, flip angle; FOV, field of view; slth, slice thickness; T2*WIs, T2*-weighted images; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; sO_2, oxygen saturation; CC, correlation coefficient; TH, tyrosine hydroxylase; DARPP-32, dopamine- and cyclic-AMP-regulated 32-kDa phosphoprotein.

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response. Such anatomical information is critical to determine the role of input and/or output activity in the formation of fMRI signals, which is another long-debated issue (Logothetis, 2007; Nir et al., 2007, 2008; Viswanathan and Freeman, 2007).

The hypothesis tested in the present study is that negative fMRI signals are accompanied with alterations in Ca^{2+} signals and Ca^{2+}-dependent cascades in the involved pathway. The model used was a commonly reported negative CBV fMRI response in the striatum induced by nociceptive electrical stimulation administered to the forepaw, regardless of the anesthetic being used (Pawela et al., 2010; Shih et al., 2009, 2011; Zhao et al., 2008). Ca^{2+} signals associated with this negative CBV response were investigated in vivo using Mn^{2+}-enhanced MRI (MEMRI), and the downstream Ca^{2+}-dependent signaling was investigated immunohistologically (Chen et al., 2008; Hsu et al., 2008; Lin and Koretsky, 1997; Silva, 2012; Silva et al., 2004). Dopaminergic neurotransmission via the activation of the D2/D3 receptor is probably responsible for the negative CBV response (Chen et al., 2012; Shih et al., 2009). Therefore, in MEMRI, the dopaminergic soma site, the substantia nigra (SN), was infused with Mn^{2+} as a surrogate marker of Ca^{2+}. The anterograde transport of Mn^{2+} to the striatum along the nigrostriatal pathway was monitored. The Ca^{2+}-dependent signaling focused on the phosphorylated cAMP response-element-binding (pCREB) protein, which is a key transcriptional factor dependent upon Ca^{2+} levels in dopaminergic neurotransmission (Brami-Cherrier et al., 2002; Neve et al., 2004; Shi and McGinty, 2011; Wu et al., 2001). The role of the input and output activity in triggering the negative CBV signals was then investigated by local blockage of the SN or the striatum, respectively, with bupivacaine (Kim and Richardson, 2008; Marvel et al., 2004; Stark, 1979; Tabatabai and Booth, 1990; Tabatabai et al., 1989). pCREB immunostaining was used to confirm the blocked activity. The obtained findings reveal a dopaminergic road map of nociception where the striatal negative CBV fMRI response is coupled with its input activity and Ca^{2+}-dependent signaling.

Materials and methods

Animals

In total, 38 adult male Sprague–Dawley rats (8–10 weeks old, weighing 280–350 g; National Laboratory Animal Center, Taiwan) were used in this study. The rats were housed in a specific-pathogen-free environment with a 12:12-hour light:dark cycle and controlled humidity and temperature. The rats were allowed to access food and water ad libitum. All procedures were approved by the Institute of Animal Care and Utilization Committee at Academia Sinica, Taipei, Taiwan.

Concomitant CBV fMRI and MEMRI

The MRI experiments were performed using a 4.7-T spectrometer (Biospec 47/40, Bruker, Germany) with a 72-mm volume coil as the RF transmitter and a quadrature surface coil placed on the head as the receiver. The animals were initially anesthetized with 5% isoflurane in oxygen at a flow rate of 5 L/min, and maintained with 2% isoflurane in oxygen at a flow rate of 1 L/min throughout the surgery. The body temperature was maintained at 37 °C using a warm-water blanket. For MEMRI, an Mn^{2+} solution (0.15 μL, 100 mM; Sigma, MO, USA) was infused stereotaxically at a nonneurotoxic dosage into the left SN [anterioposterial (AP) from bregma = −5.8 mm, mediolateral (ML) = 1.6 mm, dorsoventral (DV) = 7 mm] (n = 14, comprising 9 rats in the noxious stimulation group and 5 rats in the sham group). Each rat was then fixed in a customized holder so as to minimize motion artifacts. For CBV fMRI, the femoral vein was cannulated with polyethylene catheters (PE-50, Becton Dickinson, CA, USA) for the injection of the contrast agent [monocrystalline iron oxide nanoparticles (MIONs)], while the femoral artery was cannulated for monitoring the arterial blood pressure (IX-214, iWorx, NH, USA). After the surgical procedures, α-chloralose (50 mg/kg/hour; Sigma) was injected intravenously in place of isoflurane to maintain the anesthesia.

For MEMRI, T1-weighted images (T1WIs) were acquired using a FLASH (fast/low-angle shot) sequence, with a repetition time (TR) of 200 ms, echo time (TE) of 4.3 ms, flip angle (FA) of 60°, field of view (FOV) of 2.56 cm × 2.56 cm, slice thickness (slth) of 1.5 mm, 68 excitations, and an acquisition matrix of 256 × 128 zero-filled to 256 × 256. The scan time for each T1WI was approximately 30 min. There was a designated 5-hour interval between the baseline and second time point of MEMRI during which CBV fMRI was performed synchronously with the nociceptive stimulation. The interval not only allowed execution of the CBV fMRI experiments but also the clearance of MIONs from the bloodstream. Starting from the second time point (i.e., after CBV fMRI), T1WIs of MEMRI were acquired every 30 min until 13 h after Mn^{2+} infusion. The temporal data set established the MEMRI signal time course.

CBV fMRI used MIONs at 15 mg Fe/kg body weight as the contrast agent (Martinos Center for Biomedical Imaging, MA, USA). T2*-weighted images (T2*WIs) of CBV fMRI were acquired using a FLASH sequence with a TR of 150 ms, TE of 15 ms, FA of 22.5°, FOV of 2.56 cm × 2.56 cm, slth of 1.5 mm, 1 excitation, acquisition matrix of 128 × 64 zero-filled to 128 × 128, and temporal resolution of 9.6 s. The noxious nociceptive electrical stimulation was administered to the rat by first inserting two needle electrodes under the skin of the left forepaw, fixing them with surgical tape, and then applying electrical stimulation at 10 mA with a 3-Hz square wave and a 0.5-ms pulse duration using a constant-current stimulator (model 2100, A-M Systems, WA, USA). The stimulation involved an off–on–off paradigm, which would be correlated on a pixel-by-pixel basis with the corresponding image signals to generate the CBV correlation maps. A series of 60 T2*WIs was acquired for each stimulation paradigm, with the first, middle, and last 20 time points corresponding to the off, on, and off statuses of the nociceptive stimulation paradigm, respectively. This stimulation paradigm was repeated eight times. The individual stimulation episodes were separated by 15 min to avoid adaptation.

Monitoring of physiological variables

Physiological variables including the arterial pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), oxygen saturation (sO_2), and heart rate were monitored in another group of rats (n = 5) under identical experimental conditions. The animals were placed inside the MR scanner and underwent the same experimental paradigm except for image acquisition. Arterial pH, pCO_2, and pO_2 were measured in a 0.2-mL blood sample taken from the femoral artery (ABL5, Radiometer America, OH, USA), and sO_2 and the heart rate were assessed using the MouseOx system (STARR Life Sciences, PA, USA). All of the recorded physiological variables are listed in Table 1. The values were within normal ranges reported by other groups (Sicard and Duong, 2005).

Image analysis of CBV fMRI

The methods used for image analysis in this study have been described previously (Chen et al., 2012; Shih et al., 2009, 2011). Briefly, images were analyzed using a custom-built data processing system. Correlation maps were generated by color coding the correlation coefficient (CC) between the image signals and the off–on–off stimulation paradigm on a pixel-by-pixel basis. The thresholds for the CC were $r = \pm 0.25$ with $p < 0.01$, based on the results of several previous fMRI studies (Shih et al., 2009; Shmuel et al., 2002). The CBV fMRI signals averaged from two axial image slices (the AP levels from bregma = 0.7 mm and −0.8 mm) were plotted as the percentage change at each time point relative to the signal at the first time point. A linear baseline correction was applied to all individual time courses (Keilholz et al., 2006). The group time course was obtained by averaging the individual time courses, and it was expressed as mean and standard-error values. For statistical analysis, the signal changes over the