Coupling of long projecting vasoactive dopaminergic afferents to negative fMRI signals in the striatum

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Introduction Functional magnetic resonance imaging (fMRI) nowadays is an indispensable tool for studying brain functions. Positive fMRI signals are conventionally related to the increased cerebral blood flow/volume in the activated brain areas that increase tissue oxygenation. Interestingly, negative fMRI signals associated with decreased cerebral blood flow/volume have been observed from time to time. But the representation of these signals remains to be investigated. An often reported negative fMRI response occurs in the striatum following noxious stimulation [1]. Administration of a dopaminergic D2/D3 antagonist abolished this negative fMRI response [2]. Hence it is believed that dopaminergic neurotransmission is coupled to the striatal negative fMRI signals. Nevertheless, the dopaminergic system mainly involves a long projecting pathway with the cell bodies located in the substantia nigra (SN) sending projections to the striatum. If the negative fMRI response is directly coupled to dopaminergic neurotransmission, inactivating the remote soma site should affect the signals significantly. In this scenario, the local striatal neurons may not be necessarily coupled to the negative response. To clarify the source coupled to the negative fMRI signals in the striatum, the striatum or SN was selectively inhibited by a Na^+ channel blocker, bupivacaine, as a local anesthetic without interrupting afferent activity. The negative fMRI response triggered by noxious electrical stimulation as previously described was assessed using cerebral blood volume (CBV)-weighted fMRI. The findings yielded from the present study offer insights to the representation of negative fMRI signals that involve the activation of a long projecting vasoactive neurotransmitter pathway.

Materials and Methods Adult male SD rats (n=10; 300-350 g body weight) were initially anesthetized by 5% isoflurane and then stereotaxically injected with bupivacaine hydrochloride (37.5 mg in 5 mL of saline). The striatum received a 3 uL microinjection of 5% bupivacaine at Bregma=0.5 mm, lateral=3.2 mm, depth=4.5 mm and the SN received two 1 uL microinjections at Bregma=-5.4 mm, lateral=1.8 mm, depth=6.8 mm and Bregma=-6.2 mm, lateral=2.5 mm, depth=6.0 mm. The volume and injection positions were decided based upon the size and shape of the target areas. A pilot study showed that bupivacaine injection into either left or right hemisphere did not change the effects of bupivacaine on fMRI signal changes. For the noxious electrical stimulation elicited by a current with an intensity of 10 mA, two needle electrodes were inserted under the skin of left forepaw. α-chloralose (70 mg/kg) was then injected intraperitoneally for subsequent anesthesia. fMRI was performed on a 4.7 T Biospec 47/40 spectrometer (Bruker, Germany) with a FLASH (Fast / Low Angle SHot) sequence [TR/TE=150/15 ms; flip angle=22.5°; NEX=1; FOV=2.56 cm^2; slice thickness=1.5 mm; matrix=128x64 (zero-filled to 128x128)]. Iron oxide particles (Feridex; 22.4 mg/kg) were intravenously injected as contrast agent. A series of 60 images were acquired during each stimulation paradigm. The first, middle, and last 20 time points corresponded to the off, on, and off statuses of the stimulation paradigm, respectively. Images were analyzed using a custom-built ISPMER data processing system. Correlation maps were produced by plotting the correlation coefficient (CC) between the image signals and the off–on–off stimulation paradigm on a pixel-by-pixel basis.

Results and Discussion Figure 1A illustrates the experimental design on a horizontal view. Immediately before fMRI experiments, bupivacaine was injected into the left striatum (left panel) or the left SN (right panel) of the rat brain in order to selectively inhibit neuronal activation. During the off–on–off stimulation paradigm, 60 axial sequential images were acquired covering the striatum (Bregma=~1.0 mm, as indicated by bold black line). Figure 1B demonstrates the representative results of CBV-fMRI out of the ten rats. The purple/blue pixels indicate increased CBV (i.e. vasodilation) while the yellow/red pixels mean decreased CBV (i.e. vasoconstriction). As shown in Figure 1B (left), injection of bupivacaine into the left striatum for inactivating local striatal neurons did not reduce the striatal negative fMRI signals of interest as compared with the contralateral side. Interestingly, injection of bupivacaine into the left SN for inactivating the soma site of the nigrostriatal pathway effectively reduced the negative fMRI signals in the left striatum (white arrow in Figure 1B (right)). This result implied that the striatal negative fMRI response following electrical stimulation is directly coupled to the remote area of the nigrastriatal pathway without involving the local striatal neurons. No differences in cortical hemodynamics were seen regardless of bupivacaine injections into either striatum or SN. Figure 2A delineated the regions of interest (ROIs) for quantifying the temporal fMRI signal changes in the somatosensory cortex (blue), left striatum (black) and right striatum (red). The quantitative results in Figure 2B showed lower signal intensity (i.e. dilated vessels with more iron oxide) in the cortex and higher signal intensity (i.e. constricted vessels with less iron oxide) in the striatum during electrical stimulation. The signal intensity in the left striatum was lessened only when bupivacaine was injected into the left SN. The numbers of pixels with significant fMRI signal changes were also counted and graphed in Figure 3. The results showed that injection of bupivacaine into the left SN, but not left striatum, significantly diminished the pixel numbers with negative fMRI signals in the left striatum (Figure 3A). By contrast, the pixel numbers in the right striatum (Figure 3A) or cortex (Figure 3B) was similar irrespective of the bupivacaine injection site. The quantitative results support that negative fMRI signals in the striatum following stimulation is coupled with SN dopaminergic activity but not local neuronal activation. Therefore, caution is necessary when interpreting negative fMRI responses as the representation of local neuronal activity or inactivity.

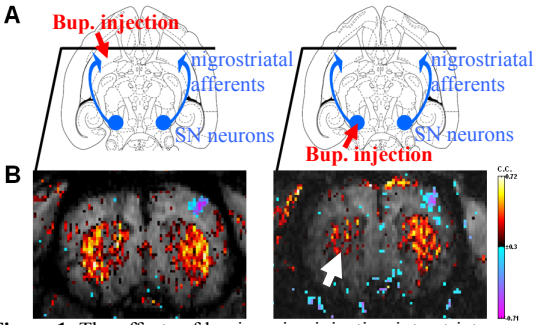

Figure 1. The effects of bupivacaine injection into striatum or SN on the forebrain hemodynamics during noxious electrical stimulation. **(A)** Diagrams of the experimental design. Immediately prior to fMRI, bupivacaine was injected into striatum (left panel) or SN (right panel). Red arrow, bupivacaine injection site; blue dot, SN neurons; blue arrow, nigrostriatal pathway; black line, location of images in (B). **(B)** Images of CBV-fMRI showing the forebrain hemodynamics during stimulation. Purple/blue pixel, increased CBV; yellow/red pixel, decreased CBV. The decreased CBV in the striatum was suppressed (white arrow) when activation of remote SN was inhibited by bupivacaine.

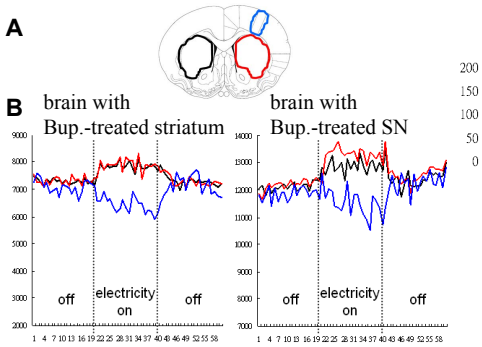

Figure 2. The temporal changes of CBV-fMRI signals in the forebrain before, during and after stimulation. **(A)** Illustration of regions of interest (ROIs) for quantification. Black circle, ROI for striatum at the side with bupivacaine; red circle, ROI for striatum at the side without bupivacaine; blue circle, for cortex. **(B)** Graphs of fMRI signal changes. X axis, a series of 60 images acquired during stimulation paradigm; Y axis, signal intensities measured form corresponding ROIs. Left and right graphs, the rat brains with bupivacaine injection into striatum and SN, respectively.

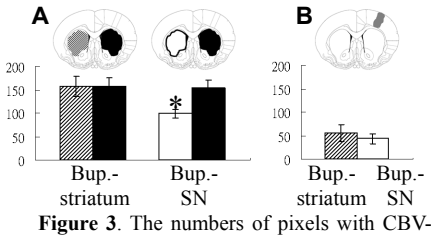

Figure 3. The numbers of pixels with CBV-fMRI signal changes in the forebrain during stimulation. Upper diagrams indicate the ROIs while lower graphs indicate the counted pixel numbers. **(A)** The numbers of pixels with fMRI signal changes in the striatum. Left column shows the results from the brains with Bup. injection into left striatum whereas right column shows the results from the brains with Bup. injection into left SN. **(B)** The numbers of pixels with fMRI signal changes in the cortex. Left bar and right bar are the pixel numbers counted from the brains with Bup.-injected left striatum and Bup.-injected left SN, respectively.

References 1. Morrow et al., (1998) Pain. 75:355-65.
2. Shih et al., (2009) J Neurosci. 29:3036-44.