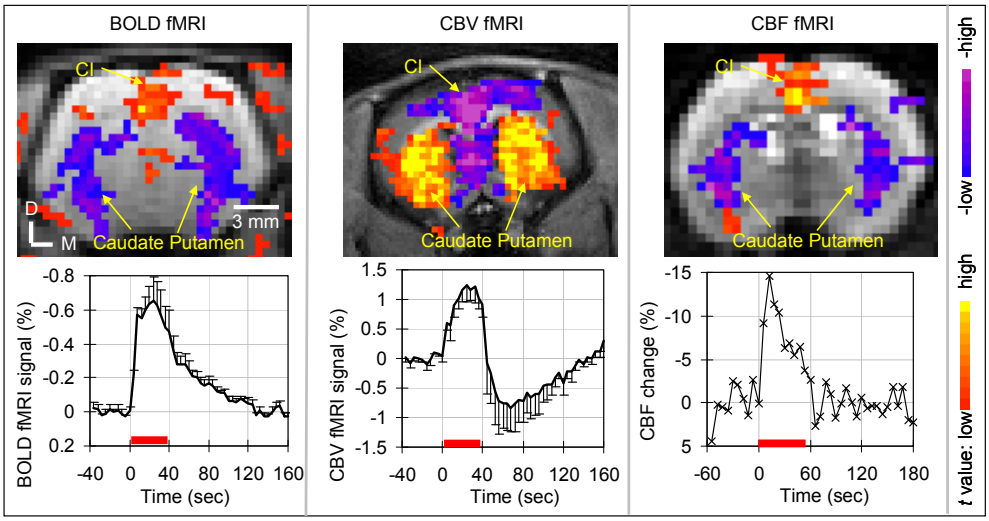
BOLD, CBV, and CBF fMRI of caudate putamen in rat brain during noxious electrical stimulation: its negative hemodynamic response to neural activities

F. Zhao[1], D. Welsh[1], M. Williams[1], A. Coimbra[1], M. O. Urban[2], R. Hargreaves[2], J. Evelhoch[1], and D. S. Williams[1]

[1]Imaging Department, Merck Research Laboratories, West Point, PA, United States, [2]Neuroscience Department, Merck Research Laboratories, West Point, PA, United States

[INTRODUCTION] In the central nervous system, neuronal activity generally leads to localized *increases* in cerebral blood flow (CBF), cerebral blood volume (CBV), and cerebral metabolic rate of oxygen (CMRO2). These responses cause the local venous blood oxygenation level to *increase*. While the blood oxygenation level changes can be measured by BOLD fMRI (*1*), CBV changes can be independently measured by a CBV fMRI technique, typically using an intravascular contrast agent such as USPIO (*2*), and CBF changes can be independently measured using arterial spin labeling (ASL) (*3*). Previous studies have reported BOLD (5) and CBV (4) *decreases* in rat caudate putamen (CPu) during noxious electrical stimulation (NES) of paws, and such BOLD and CBV decreases in hemodynamics have still been attributed to an *increase* in local neural activity (4). To further understand the specific hemodynamic response in this anatomical structure and its temporal characteristics, BOLD, CBV, and CBF fMRI studies were all performed in rats in a brain slice containing the CPu.

[METHODS] The animal protocol was approved by the IACUC of Merck Research Laboratories. The Sprague-Dawley rats (Taconic Farms Inc, Hudson, NY, USA) were initially anesthetized with isoflurane in a mixture of O_2 and N_2 gases (3:7). A bolus of 0.05 mg/kg medetomidine (dormitor) was then injected subcutaneously, followed by continuous subcutaneous infusion at the rate of 0.15 mg/kg/h. Isoflurane was then reduced to 0.4% and continuously delivered throughout the experiment. All MRI measurements were performed on a 7 T Bruker Biospec system. A 2 cm diameter surface coil positioned on the top of the brain was used as the RF receiver, while an actively-decoupled 72 mm diameter volume coil was used as the RF transmitter. An optimum electrical pulse train delivered to forepaws (2 ms, 5 mA, 40 Hz) (*5*) was used for NES. For BOLD and CBV fMRI, T2*-weighted images were acquired using a single-shot GE EPI with phase-encoding in the dorsal-ventral direction; matrix size = 64 × 64; TE=11 ms; FOV = 2.5 cm × 2.5 cm. Each run consisted of 10-**10**-30 image acquisitions (boldface represents stimulation on) with TR=4 sec. CBV fMRI was acquired after administration of USPIO (dextran-coated, iron core size is ~5 nm), synthesized in-house. CBF fMRI was carried out using ASL (*3*). Briefly, the volume coil was used to label the arterial spin in the neck region, labeled and control spin-echo EPI images were acquired alternately: matrix size = 64 × 64; TE=19 ms; FOV = 2.5 cm × 2.5 cm; labeling time = 3 sec; TR = 6.07 sec per pair). Forty pairs were acquired in one fMRI run with 10-**10**-20 pair acquisitions scheme.



[RESULTS & DISCUSSION] Figure shows the activation maps for the three fMRI techniques and the time courses of the averaged fMRI signals (CBF percentage change) over the activated pixels within the caudate putamen. D: Dorsal; M: Medial. Red bars under the time courses indicate the stimulation period. Activation maps are displayed as statistical t-value maps. During NES, the CPu shows a negative BOLD fMRI response (blue/purple) indicating a decrease in local venous blood oxygenation level, a positive CBV fMRI response (red/yellow) indicating a decrease in the local blood volume, and a negative CBF fMRI response (blue/purple) indicating a decrease in local CBF. Previous studies have shown that pain causes an increase of neural activity in the CPu, as evidenced by the increase in metabolic rate of glucose using 2DG autoradiography (*6*), and by the increase in neural activity using electrophysiology (*4*). Hence the observed *decreases* of BOLD, CBV and CBF in CPu during NES are probably resulting from an *increase* in neural activity. During the stimulation, the fMRI responses across BOLD, CBV and CBF are consistent; a decrease of BOLD signal is consistent with the expected increase in CMRO2 and the observed decrease in CBF, while CBV and CBF decreases also are consistent with one another because they are closely coupled. Interestingly, comparing the time courses, after the stimulation is stopped, CPu shows no post-stimulus polarity change in BOLD and CBF signals, whereas a post-stimulus polarity change in the CBV fMRI signal is clear. After stopping the stimulus, the continuously decreased BOLD signal can be rationalized as a continued elevation of post-stimulus CMRO2 and/or an increase in post-stimulus venous CBV; both effects can also explain the observed post-stimulus decrease in CBV fMRI signal since CBV fMRI signal depends on both the change in CBV and the change in blood oxygenation level (*2*). However, since no post-stimulus CBF increase concomitant with the CBV increase was observed, we believe that the post-stimulus CBV fMRI signal decrease and the continuously post-stimulus decreased BOLD fMRI signal are caused by an elevated post-stimulus CMRO2. Overall, these results give a valuable observation about the specific hemodynamic response in the CPu: the neural activity during nociception causes decreases in CBV and CBF, and an estimated increase in CMRO2 which lasts >2 minutes after stopping the NES.

[Reference] 1. S. Ogawa, et al., PNAS 1990. 2. R. P. Kennan, et al., MRM, 1997. 3. D. S. Williams, et al., PNAS 1992. 4. Y. Y. Shih et al., J Neurosci 2009. 5. F. Zhao et al., Neuroimag 2008. 6. J. Mao, et al., J Neurosci 1993.