Hunting the Source of a Unique Negative fMRI Signal in the Striatum Using Optogenetics

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Introduction: Although neurovascular coupling stands as a central tenet of fMRI, studies of the rodent striatum suggest that the relationship between hemodynamics and neuronal activity may not always be straightforward[1-3]. Recent studies have shown that negative fMRI responses in the striatum are evoked by peripheral sensory stimulation[1-7], which are correlated with increased neuronal activity and dependent upon D2 dopamine receptor signaling[1,6,7]. Conversely, a recent optogenetic study from our group has demonstrated selective stimulation of midbrain dopaminergic neurons induces striatal CBV *increases*, which are blocked by a D1 dopamine receptor antagonist[8]. These collective findings suggest that striatal hemodynamics may present in a dichotomous fashion, with activity between D1 and D2 receptor-expressing striatal projection neurons evoking opposing hemodynamic responses (CBV increases and decreases, respectively). To further shed light on the unique neurovascular coupling in the striatum, we employed a cutting-edge optogenetic fMRI approach to address two critical questions of whether vasodilation occurs in the striatum during: **1)** selective stimulation of striatal glutamatergic input from the motor cortex, and **2)** antidromic stimulation of D1 receptor-expressing striatal outputs at the substantia nigra pars reticulata (SNr). Based on the well-known "input" theory of fMRI responses[9] and our prior work[1,6,7], we anticipated that both stimulations would evoke CBV increases. Surprisingly, we were wrong – as detailed below, both manipulations reliably evoked striatal CBV decreases.

Methods: To target either deep-layer motor cortex (n=8 in total) or the striatonigral projection pathway (n=6 in total), wildtype Sprague-Dawley rats were microinjected with an adeno-associated virus carrying the gene encoding channelrhodopsin-2 (ChR2), a light-gated cation channel fused to an enhanced yellow fluorescent protein (EYFP) or only EYFP (all under the calcium/calmodulin kinase IIa promotor). Motor cortical injections were 1 µl in volume, while striatum was double injected at 0.5 µl volume; coordinates were as follow (in mm from bregma): Motor Cortex: +3.0 anterior/posterior, ±2.5 medial/lateral, -1.5 dorsal/ventral; Striatum: 0.0 and 1.5 anterior/posterior, ±3.0 and 2.8 medial/lateral, -4.5 and 4.4 dorsal/ventral. Chronic optical fibers were stereotactically implanted above deep-layer motor cortex (0.5mm dorsal to cortical microinjection), or above the substantia nigra pars reticulata (to target striatonigral projections; coordinates: -5.5 anterior/posterior, ±2.2 medial/lateral, -7.2 dorsal/ventral). Light was delivered to selectively stimulate deep-layer motor cortex of striatonigral projections at a range of frequencies (20-40Hz) under the following parameters: 5 ms pulse width, 473-nm wavelength, 12-15mW light pulses. The effect of stimulus frequency was tested in a pseudo-random manner. Stimulation paradigms were as follows (in seconds): Motor Cortex Experiment: 20OFF, 10ON, 30OFF, 10ON, 30OFF; Striatonigral Experiment: 120OFF, 40ON X 5 epochs. fMRI experiments were performed 4-6 weeks after surgery. Each rat was endotracheally intubated and ventilated with ~1.5% isoflurane and medical air. The ventilation rate and volume were adjusted to maintain end-tidal CO2 (EtCO2) within a range of 2.6-3.2% and oxygen saturation (SpO2) above 96%. Rectal temperature was maintained at 37±0.5°C. Dexmedetomidine (0.1 mg/ml) and pancuronium bromide (1.0 mg/ml) were infused intravenously for duration of scan. For CBV-weighted MRI, a tail-vein catheter was used to deliver monocrystalline iron oxide contrast agent at a dose of 30 mg Fe/kg. Single shot, single sampled GE-EPI sequences (BW= 300 kHz, TR= 1000 ms, TE= 8.107 ms, 80x80 matrix, FOV= 2.56 x 2.56 cm2, slice thickness= 1 mm) were acquired using a Bruker 9.4T MR scanner and home-made surface coil. Automatic co-registration using SPM codes were applied to realign time-series data within subjects and then again across subjects. Data were then averaged across subjects in order to provide group-averaged fMRI responses. Data were analyzed using our established pipelines detailed elsewhere[1,2,5-8].

Results: Transient optical stimulation of both deep-layer motor cortex and striatonigral afferents elicited a negative CBV response within the dorsal striatum of ChR2 animals **(Fig a & b)**. Additionally, stimulation of the motor cortex induced robust local CBV increases **(Fig a).** Interestingly, striatonigral afferent stimulation required higher frequency stimulation than motor cortex; consistent striatonigral responses were only noted at 40Hz. No CBV changes were noted in control animals infected with EYFP alone (motor cortex and striatonigral path) under any stimulation condition; we are thus confident that our responses do not reflect laser/heating artifacts.

Discussion: This study provides a cautionary tale for global acceptance of the "input" theory of fMRI signals. As predicted by this theory, stimulation of glutamatergic corticostriatal afferents would be expected to generate vasodilation, via activation of input fibers[9]. However, as our experiments with optogenetic stimulation of motor cortex show, these activities induce paradoxical vasoconstriction in the striatum; thus, the input theory of BOLD is rejected in this case. Equally mysterious are the vasoconstrictive responses observed during optogenetic stimulation of the striatonigral pathway (D1). Previous work from our group has demonstrated striatal vasodilation mediated by the D1 receptors[8], which are exclusively expressed on striatal neurons projecting to the SNr[10]. Thus, we anticipated that antidromic stimulation would evoke spiking and local vasodilation in the striatum, mimicking dopamine-induced activation in these cells. Surprisingly, this optogenetic manipulation also induced vasoconstriction. One potential explanation for the observed responses is through local release of GABA, as roughly 95% of striatal neurons release this inhibitory transmitter[10].

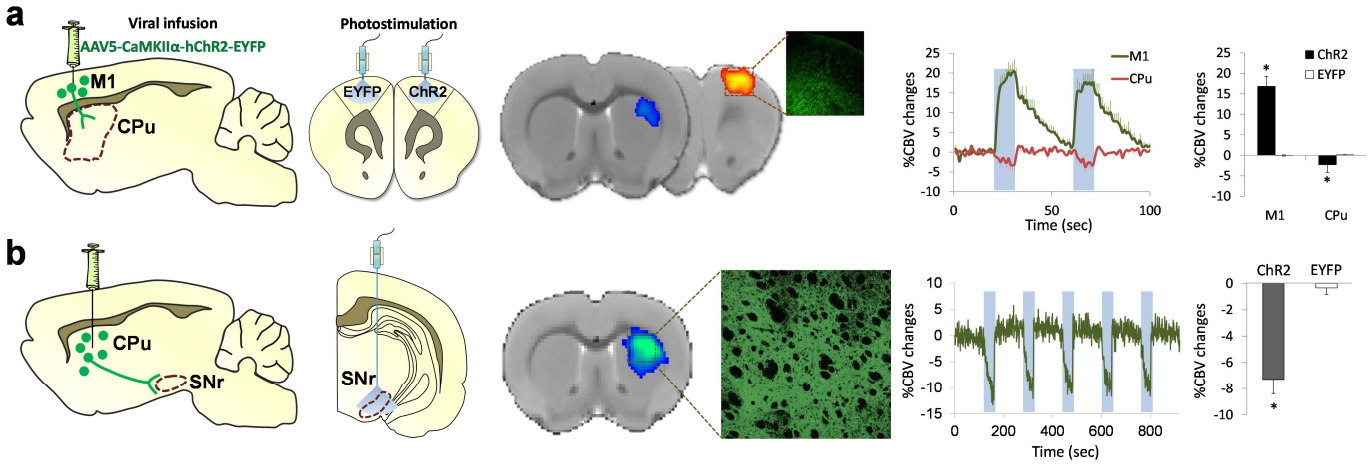

Fig (a): Optogenetic stimulation of glutamatergic projection neurons within motor cortex generates local vasodilation, as well as vasoconstriction in the downstream striatum (ChR2: n=5, EYFP control: n=3). **(b)**: Optogenetic stimulation of striatal projection neurons distally within the terminal field of the SNr induces vasoconstriction in the striatum (ChR2: n=4, EYFP control: n=2). *denotes significant difference from EYFP control (P<0.05).

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