Optogenetic manipulation of VTA dopaminergic neurons and global patterns of functional neural connectivity

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Introduction: Dopaminergic dysregulation plays a central, but still ill-defined role in the pathogenesis of major neuropsychiatric disorders such as schizophrenia, with the majority of antipsychotic medication targeting D1/D2 receptors[1,2]. The Ventral Tegmental Area (VTA), comprised of approximately 60% dopamine (DA) neurons, projects extensively with several cortical and subcortical brain areas important in reward, motivation and cognition. Recent advances in recombinase-driver rat lines make it possible to utilize optogenetic strategies to selectively activate dopaminergic neurons within the VTA[3]. Here, we coupled optogenetic stimulation techniques with cerebral blood volume (CBV)-weighted fMRI technology in an *in vivo* rat model to selectively activate DA neuron cell bodies in the ventral midbrain. This project aims to investigate whether the selective activation of DA neurons within the midbrain alters the functional connectivity between these neurons and their postsynaptic target regions, as well as global patterns of brain connectivity to provide clinically relevant insight into the effects of dopaminergic dysregulation on neural circuit mechanisms in the intact brain.

Methods: To target DA neurons within the midbrain, tyrosine hydroxylase (TH)-Cre adult Long Evans rats were microinjected into the ventral midbrain with a Cre-inducible adeno-associated virus carrying the gene encoding channelrhodopsin-2 (ChR2), a light-gated cation channel fused to an enhanced yellow fluorescent protein (EYFP) (TH^{VTA}::ChR2 rats) or only EYFP (TH^{VTA}::control rats) (quadruple injections of 1 µl (in mm from bregma): -5.8 and -6.2 anterior/posterior, ±0.7 medial/lateral, -8.4 and -7.4 dorsal/ventral). Chronic optical fibers were stereotactically implanted bilaterally above the VTA to permit light delivery (5 ms pulse width, 473-nm wavelength, 10-mW light pulses) at 10, 20, 30, and 40 Hz to selectively activate DA neurons within this region[4]. The effect of stimulus frequency was tested in a pseudo-random manner. Two to five repeated trials were performed for each frequency. fMRI experiments were performed 5-6 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 intraperitoneally 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. The D1 receptor antagonist, SCH23390 (0.6 mg/kg) was injected intravenously to explore its effects on CBV signal responses observed in TH^{VTA}::ChR2 rats. 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 maps using correlation coefficient (CC) method with reference to the optogenetic stimulation paradigm. Bonferroni correction was applied to adjust for the multiple comparisons of fMRI maps by dividing the significance level ($p<0.05$) by the number of brain voxels.

Results and Discussion: To genetically target the expression of ChR2-EYFP into midbrain tyrosine-hydroxylase (TH)-positive DA neurons, we introduced a Cre-inducible adeno-associated virus encoded by ChR2 fused to an enhanced yellow fluorescent protein (ChR2-EYFP) into the VTA of TH-Cre adult rats **(Fig 1A)**. 5-6 weeks following surgery, we observed expression of ChR2-EYFP within the VTA and confirmed colocalization with TH via immunohistochemistry **(Fig 1B)**. Transient optogenetic activation of DA neurons within the midbrain caused significant regional CBV increases in downstream targets of the VTA including the dorsal and ventral striatum in TH^{VTA}::ChR2 rats, whereas TH^{VTA}::control rats displayed no significant CBV increases within these regions **(Fig 2 A,B,D)**. One-way ANOVA followed by Bonferroni multiple-comparison tests revealed a frequency-dependent effect in TH^{VTA}::ChR2 rats with significantly lower CBV responses observed at 10 Hz in comparison to those found at 20, 30, and 40 Hz ($p<0.05$). We next investigated whether intravenous application of the DA D1 receptor antagonist SCH23390 would modulate the CBV responses displayed in the TH^{VTA}:: ChR2 rats. Intravenous application of SCH23390 significantly attenuated the optical stimulation mediated CBV responses within these forebrain targets **(Fig 2C,D).**

Conclusions and Future Directions: This study demonstrates significant CBV increases in the dorsal and ventral striatum following optogenetic activation of DA neurons within the VTA. These data suggest that aberrant DA neuromodulation may alter the neuronal activation patterns seen between these neurons and their postsynaptic target regions, providing mechanistic insight into how DA signaling alters global patterns of brain connectivity. Our future work will analyze existing resting-state fMRI datasets to identify network connectivity changes following optogenetic modulations.

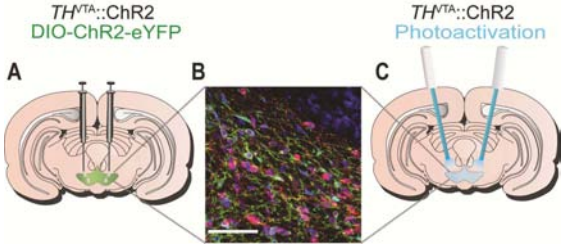

Figure 1. (A) To target DA neurons within the midbrain, TH::Cre rats were microinjected into the ventral midbrain with a Cre-inducible AAV carrying the gene encoding ChR2. (B) Confocal image of a coronal section showing co-expression of ChR2-EYFP and TH following viral injection into the VTA of a TH::Cre rat (red = TH; green = ChR2-EYFP; blue = NeuroTrace; scale bar = 100 µm). (C) Chronic optical fibers were implanted bilaterally dorsal to the VTA to permit light delivery to selectively activate DA neurons within this region.

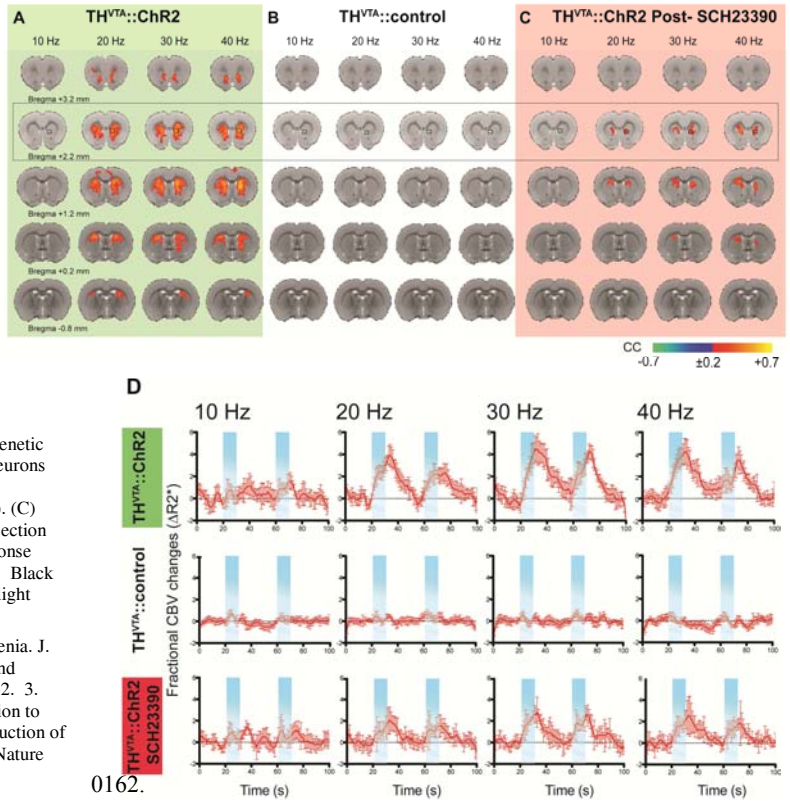

Figure 2. (A) Correlation coefficient maps correlating CBV signal changes and optogenetic stimulation paradigm with a temporal delay of 5 s. Optical stimulation of VTA DA neurons increases fractional CBV changes in forebrain targets in TH^{VTA}::ChR2 rats (n=6) (B) TH^{VTA}::control rats displayed no significant CBV increases within these regions (n=6). (C) CBV signal increases displayed in TH^{VTA}::ChR2 rats were attenuated following IV injection of the D1 receptor antagonist SCH23390 (0.6 mg/kg) (n=4). (D) Averaged CBV response traces to optical stimulation in coronal brain slices found +2.2 mm anterior to bregma. Black boxes found on the CC maps indicate the approximate ROIs. Blue bars indicate blue light stimulation. Error bars represent the SEM.

References: 1. Knable & Weinberger, Dopamine, the prefrontal cortex and schizophrenia. J. Psychopharmacol. 1997; 11, 123-131. 2. Finley, Mesoprefrontal dopamine neurons and schizophrenia: role of developmental abnormalities. Schizophr Bull. 2001; 27: 431-442. 3. Witten et al, Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. Neuron. 2011; 72: 721-733. 4. Sparta et al, Construction of implantable optical fibers for long-term optogenetic manipulation of neural circuits. Nature Protocols. 2012; 7:12-23.