Optogenetic modulation of the mouse default mode network with a single tapered fiber

Poster #ZZ25

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Background and Aims

- The default mode network (DMN) is a functional network of the human brain widely studied with fMRI due to its association with higher cognitive processes and frequent dysregulation in human brain disorders[1]
- However, due to the spatial limitations of traditional optogenetic flat-faced fibers[2], reliable manipulation of the widely distributed contituents of this network have so far been prevented
- Here, we describe network-scale manipulation of the rodent medial prefrontal cortex (mPFC, a key node of the DMN[3,4]) via single tapered fiber (TF[5]) optogenetic stimulation

Methods

All experiments were carried out in accordance with Italian regulations governing animal welfare and protection (DL 26/214, EU 63/2010, Ministero della Sanità, Roma). Animal research protocols were reviewed and consented to by the animal care committee of the Istituto Italiano di Tecnologia and the Italian Ministry of Health (authorization 752/2019 PR to A. Gozzi).

Experimental groups
In adult experimental mice, channel rhodopsin (ChR2) was expressed under one of the following promoters:
- **Thy1** (n=8 [TF], n=4 [FF]): transgenic mice (B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J; Jax #7612; expressing channelrhodopsin-2 [ChR2] under the Thy1 promoter)
- **CamkIIa** (n=9)**:** viral injection (AAV5-CamkIIa-hChR2(H134R)-EYFP, Addgene #26969) into the PFC of C57Bl6/J

Opsin free controls (n=8) were either littermates of Thy1-ChR2, or injected with a control virus

Optic fiber implantation
Mice were implanted in the mPFC with either:
- **A single tapered fiber** (TF) in the mPFC at a 15º angle (AP +1.9 mm; ML +/- 0.6mm; DV –2.1 mm), or
- **Two flat fibers** (FF, Doric lenses, d=200um) bilaterally in the mPFC at an 8º angle (AP +1.9 mm; ML +/- 0.5mm; DV –2.1 mm)

fMRI acquisition
Anesthetic: Optogenetic fMRI (ofMRI[6]) scans were acquired under artificial ventilation and light anesthesia using either:
- **Med-iso:** i.a. medetomidine bolus [0.05 mg/kg] and infusion [0.1 mg/kg/h] + 0.5% isoflurane
- **Halothane:** 0.8%

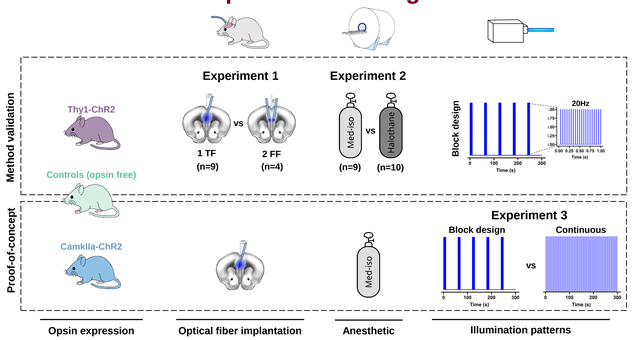
Blood pressure was measured via femoral artery catheterization

Scan parameters: Blood oxygen level dependent (BOLD) images were acquired on a 7T scanner (Bruker) using an echo planar imaging gradient echo (EPI-GE) sequence with the following parameters: TE=15ms, TR=1s, flip angle=60º, NR=390, matrix size=98x98, slice number=18, field of view=2.3x2.3x9.9mm.

Optical stimulation: All stimulation paradigms were applied at a nominal power of 12mW (6mW/FF). One of two types of temporal illumination patterns were used:
- **Block design:** 15ms pulses at a frequency of 20Hz for 10s, followed by 50s of rest
- **Continuous:** 10ms pulses at a frequency of 10 or 1Hz for the entire 5 minute scan.

Analyses:
- **Block design:** evoked response was modelled with a GLM[7] (hemodynamic response function [HRF, Fourier basis set] convolved with a boxcar)
- **Continuous:** Global functional connectivity, as defined by the correlation of each voxel with the global signal[3]

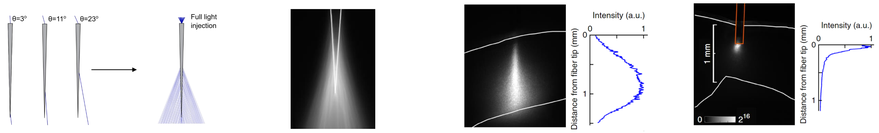
Experimental Design



What are tapered fibers?

For fibers with tapered tips, light emission takes place along the length of the TF

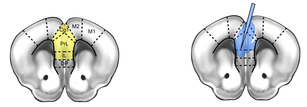
allowing for more homogeneous energy delivery when compared to traditional FF



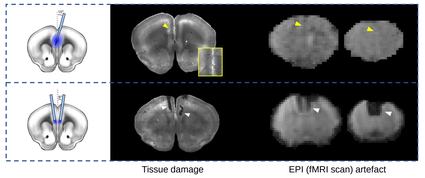
Pisanello et al., 2017, Nature Neuroscience

Why do we want to use tapered fibers?

Network level manipulations

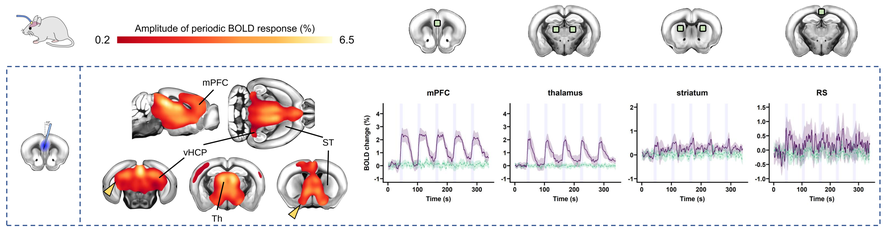


Minimal tissue damage and susceptibility artefacts

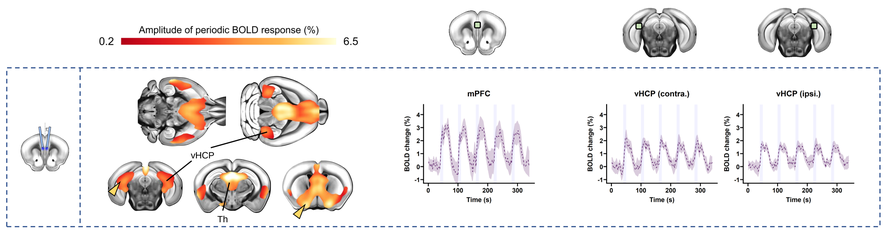


Experiment 1: Network-level stimulation of the DMN with a single TF

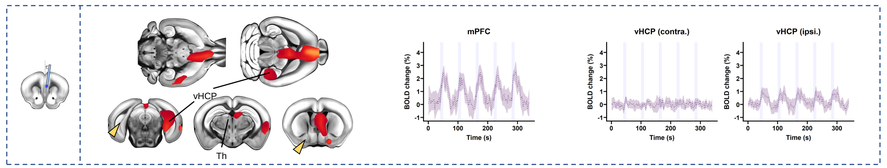
Bilateral evoked response from a single TF



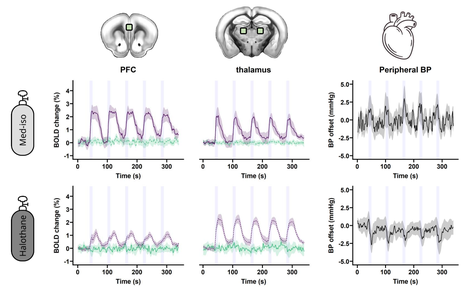
Similar to bilateral stimulation with two flat fibers



and distinct from the unilateral stimulation of one flat fiber



Experiment 2: fMRI response is not explained by peripheral BP changes

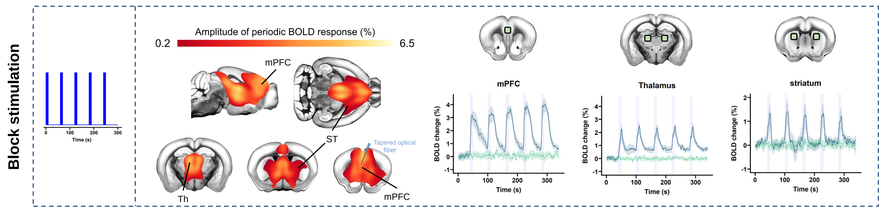


Pattern of activation reflects axonal output of mPFC

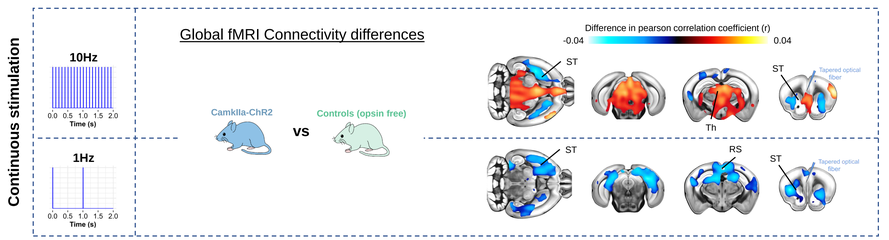
Mapped from the mouse brain connectome (Coletta et al., 2020, Sci. Advances)

Experiment 3: Engagement of DMN components is frequency dependent

Evoked response from block stimulation can be predicted by mPFC projections



Continuous stimulation engages different components of the DMN in a frequency dependent manner



Conclusions

Tapered optical fibers (TFs) offer homogeneous illumination of large cortical volumes while minimizing damage and artefacts. The set of experiments presented here demonstrate the power of using a single tapered fiber to stimulate the mPFC, a key node of the DMN. While block stimulation paradigms produce BOLD changes in regions that are structurally connected, we believe that rhythmic stimulation of this node can be exploited to understand network communication.

References

[1]Buckner et al. Ann N Y Acad Sci, 2008, [2]Christie et al. NeuroImage, 2013, [3]Sforazzini et al. NeuroImage, 2014 [4]Whitesell et al. Neuron, 2022, [5]Pisanello et al., Nat. Neurosci, 2017, [6]Lee et al. Nature, 2010, [7]Bates et al. J. Stat. Softw, 2015

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