Silenced but still connected: chemogenetic inactivation of the prefrontal cortex results in brain-wide functional hyper-synchronization

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NO – PHOTO / RECORD

Background and Aims

- Neuroimaging measurements of functional coupling and connectivity are often employed as an index of reciprocal inter-regional communication [1]. However, direct testing of this hypothesis has been lacking.
- Here we combine chemogenetics and mouse fMRI (*chemo-fMRI* [2]) to disambiguate the neural elements necessary for brain-wide functional connectivity.
- We report that pan-neuronal inhibition of the mouse medial prefrontal cortex, a hub-like integrative region, results in paradoxical cortico-cortical resting-state fMRI (rsfMRI) *overconnectivity.*

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 857/2017 PR to A. Gozzi).

Viral Injections

Chemogenetic inhibition: 300 nL of virus (AAV8-hSyn-hM4D(Gi)-mCherry; and AAV8-hSyn-GFP) solution was injected bilaterally in PFC (AP:+1.7 ML:+- 0.3 lateral, DV: −1.7).
Chronic inhibition: 2 µL of virus (AAV8-hSyn-MYC-mKir2.1(E224G/Y242F)-IRES-eGFP; and AAV8-hSyn-GFP) solution was injected bilaterally in PFC (coordinates above).

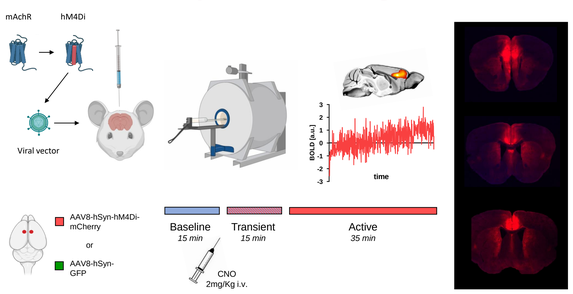
Resting-state fMRI

Animal preparation: Adult male mice (hM4Di, n=15, and GFP, n=19; Kir2.1, n=16 and GFP, n=19) were imaged at 7 tesla under halothane-induced sedation (0.75%) as in [3] and artificial ventilation using a single-shot EPI sequence (TR/TE 1200/15 ms, flip angle 30°, FOV 2×2 cm, matrix 100×100, 24 coronal slices, slice thickness 0.50 mm, 500 volumes).
Acquisition timeline and drug administration: Chemogenetic inhibition: CNO (2 mg/kg, Sigma Aldrich) was administered i.v. Timeseries were split in three parts (baseline: 15 min; transient effect: 15 min; active period: 35 min). *Chronic inhibition:* 35 min of acquisition upon induction of halothane sedation. *Functional connectivity analyses:* We calculated connectivity maps for all subjects and mapped voxel-wise inter-group differences between the targeted PFC regions, and its long-range targets [4]. To quantify the contribution of distinct thalamic subregions to overall group differences, we used a clustering algorithm to partition voxels within each region based on their PFC connectivity profile [5].

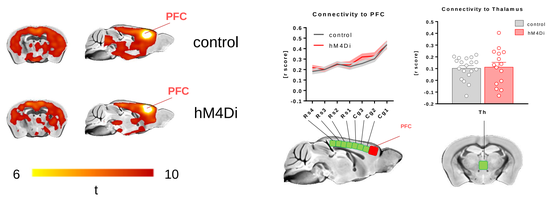
Electrophysiology

Animal preparation: Mice (hM4Di, n=5 and GFP, n=5) were sedated with halothane and spontaneous activity was measured using a 16-channel shank electrode inserted in the PFC (Neuronexus). *Drug administration:* CNO (2 mg/kg, Sigma Aldrich) was administered i.v., 15 min after the beginning of the recordings *LFP* were extracted from the raw recordings by bandpass filtering the neuronal signal between the frequencies of 1 and 250 Hz. [6] *MUA*: the signal was high pass filtered at 100 Hz using a Butterworth fourth-order filter, and bandpass filtered in the range of 400-3000 Hz using a Kaiser window filter. The absolute value of the signal was then taken. Finally, it was low-pass filtered at 250 Hz and resampled at 500 [6]

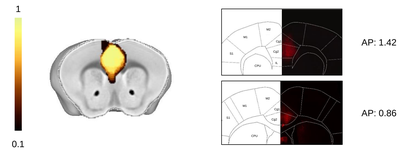
Experimental Design



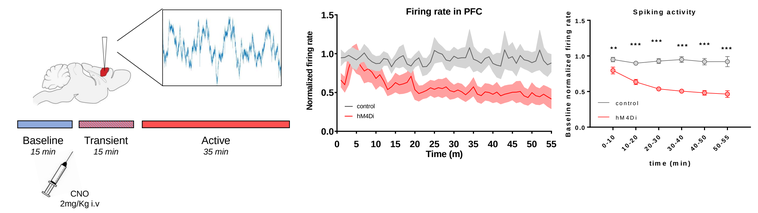
hM4Di expression does not alter baseline functional connectivity

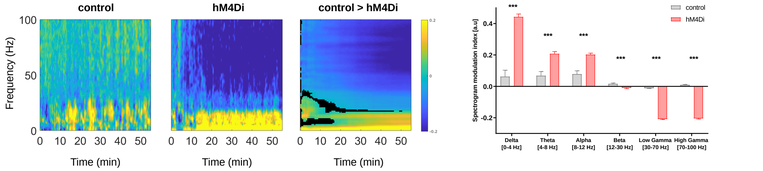


Between-subject localization of viral expression

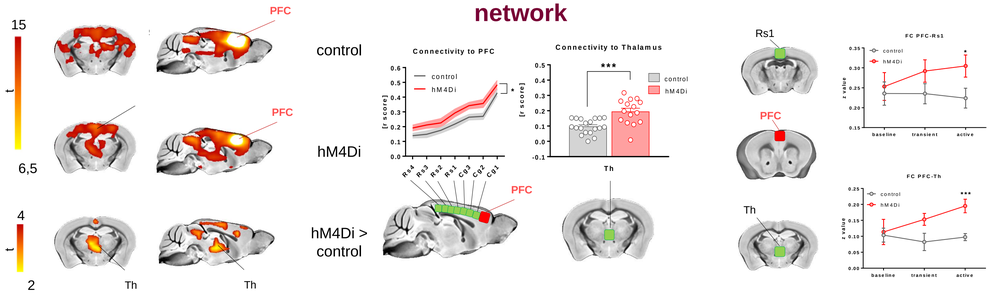


Chemogenetic silencing of resting-state activity in the PFC

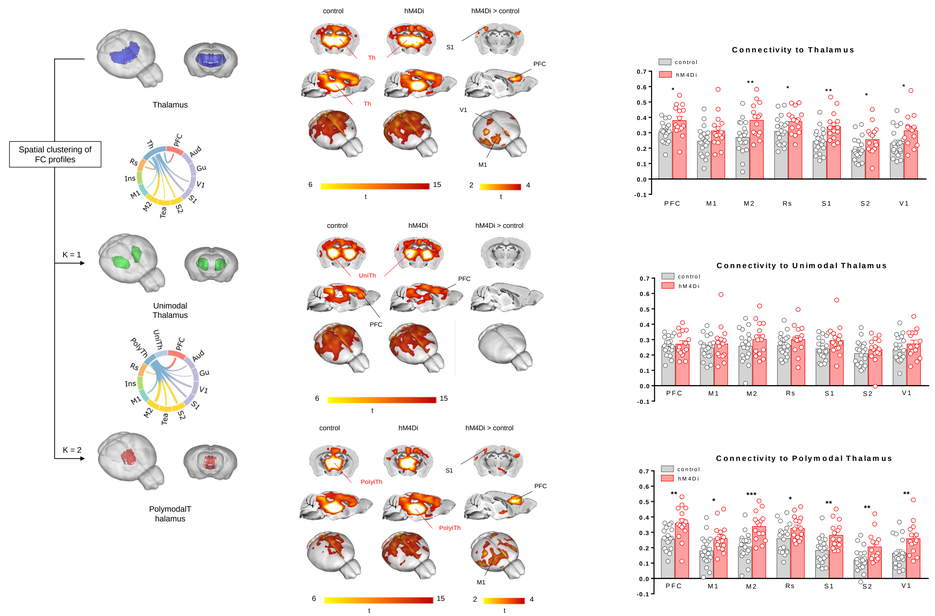




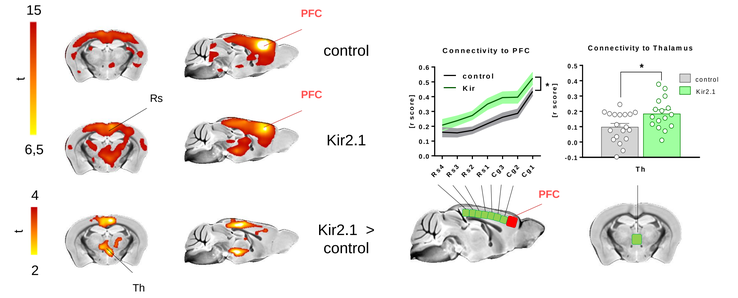
Chemogenetic silencing of the PFC induces rsfMRI over-connectivity in the default mode network



Mapping of thalamo-cortical functional connectivity



Chronic inhibition reproduces over-connectivity



We finally corroborate previous results, by showing that similar patterns of rsfMRI overconnectivity can be obtained by chronically silencing prefrontal activity via overexpression of an inward potassium channel.

Conclusions

Chemogenetic silencing of the PFC results in brain-wide rsfMRI overconnectivity with prominent involvement of posterior cingulate and thalamic areas. The observed dissociation between cortical output and fMRI synchronization challenges prevailing interpretations of functional connectivity as a proxy for brain wiring and reciprocal interregional communication.

References

[1] Van den Huevel et al., *Neuropsy* 2010; [2] Giorgi et al, *Cell reports* 2017*;* [3] Bertero et al., *Brain* 2018; [4] Sforazzini et al, *NeuroImage* 2014; [5] Schleifer et al., *J.Neurosci* 2019; [6] Betliski et al., *J.Neurosci* 2008;