Optical Imaging of Functional Connectivity in the Mouse Brain

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Introduction

Functional neuroimaging (e.g., with fMRI) has been difficult to perform in mice, making it challenging to translate between human fMRI studies and molecular and genetic mechanisms. A method to easily perform large-scale functional neuroimaging in mice would enable the discovery of functional correlates of genetic manipulations and bridge with mouse models of disease. To satisfy this need, we combined resting-state functional connectivity mapping with optical intrinsic signal imaging (fcOIS). We demonstrate functional connectivity in mice through highly detailed fcOIS mapping of resting-state networks across most of the cerebral cortex. Synthesis of multiple network connectivity patterns through iterative parcellation and clustering provides a comprehensive map of the functional neuroarchitecture and demonstrates identification of the major functional regions of the mouse cerebral cortex. The method relies on simple and relatively inexpensive camera-based equipment, does not require exogenous contrast agents and involves only reflection of the scalp making it minimally invasive. In principle, fcOIS allows new paradigms linking human neuroscience with the power of molecular/genetic manipulations in mouse models.

Methods

Male Swiss Webster mice 6-10 months of age were used for experimentation. All mice were anesthetized with ketamine-xylazine. Prior to imaging, a midline incision was made along the top of the head and the scalp was reflected, exposing approximately 1 cm^2 of the intact skull (the majority of the convexity of the cerebral cortex). Light emitting diodes (LEDs) operating at four wavelengths illuminated the skull, and diffuse, reflected light was detected by an EMCCD camera. In each pixel of the image, light intensity at each wavelength was interpreted using the Beer-Lambert law, and converted to differential changes in oxy- and deoxy-hemoglobin at each time point. Data were filtered to the functional connectivity band (0.009-0.08 Hz) [1]. Time traces of all pixels defined as brain were averaged to create a global brain signal for each mouse. This global signal was regressed from each pixel's time trace to remove global sources of variance.

Results

To investigate the strength of network correlations, we focused on bilateral connectivity between homotopic areas in contralateral hemispheres: the left and right, frontal, cingulate, motor, somatosensory, retrosplenial and visual cortices. Seeds placement was guided by the Paxinos atlas [2]. Seed time-traces were calculated by averaging the time traces within 0.25 mm of the seed locus. Functional connectivity analysis was performed by correlating these time courses against the time course of every pixel in the brain (**Figure 1**). With the goal of regenerating atlas divisions in a data-driven manner, we parcellated the brain into functional regions using the resting-state brain signals and an iterative strategy (top row, left, **Figure 2**). An initial assumption about the organization of the neuroarchitecture can be refined with a method consisting of two steps: (1) updated time traces are found for each parcel by averaging over all pixels in each parcel, (2) updated spatial arrangements are found for each parcel by calculating the correlation values between the time traces for each pixel and each parcel (constructing a cross correlation matrix), and then assigning every pixel to the parcel with which it had the highest correlation coefficient. New averaged time traces are calculated for each updated parcel spatial arrangement (a return to Step 1). This cycle is repeated until no pixel changes regions from one cycle to the next. Once we had stable parcellations, we investigated the network membership of the obtained regions using a clustering algorithm (bottom row, **Figure 2**)

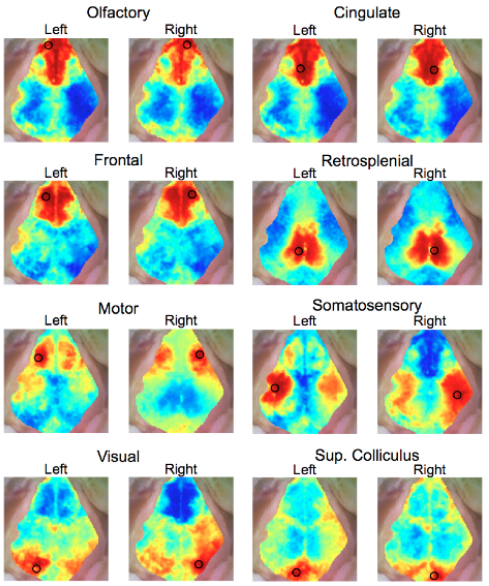

Figure 1 Correlation maps for seeds chosen using the expected cortical positions of various functional areas. Seed positions and sizes are shown with black circles. The scale for all correlation maps is from r = -1 to 1. Maps are shown overlaid on a white light image of the brain viewed through the intact skull. Field of view approximately 1 cm x 1 cm.

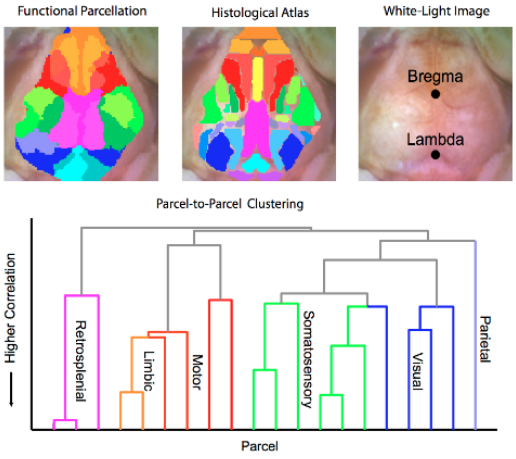

Figure 2 The results of iterative parecellation. We see clear delineation of a frontal/olfactory/cingulate (limbic) network (oranges), a motor network (reds), a somatosensory network (greens), a visual network (blue), the retrosplenial cortex (magenta), and the superior colliculus (light blues). To the right are a histological atlas applied to this mouse brain and a white-light image for comparison. Below is a dendrogram showing clustering of the parcels based on their correlations. Each terminal branch is a parcel (color-coded for visualization); parcels that share similar correlation maps have branches that meet lower on the tree.

Summary

We have demonstrated functional connectivity mapping with OIS in mice. Because we have determined that fcOIS is able to map both functional regions and their connections, this methodology should be a powerful tool for detecting when functional connectivity networks are disrupted (either in the distribution of the neuroarchitecture or in the pattern of connections). Thus, one could examine the functional consequences of disease models including genetic and surgical disruptions. Imaging the development of neurodegenerative disease (e.g., Alzheimer's and Huntington's) in the mouse brain could provide a less circumstantial link between the molecular mechanisms of disease and the tendency for disease to target specific cortical networks; providing better insight into both pathophysiology and therapeutic targets. We expect that fcOIS could be a useful tool to connect the intriguing neuroimaging results of human disease obtained through fcMRI studies with the powerful tool of the mouse model

References

1. Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, et al. (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. PNAS 102: 9673-9678.

2. Franklin KBJ, Paxinos G (2008) The Mouse Brain in Stereotactic Coordinates. New York: Academic Press.