Comparison of BOLD and CBV-weighted resting state connectivity to an anatomical 'gold standard' in the motor network of the squirrel monkey brain

Yurui Gao^{1,2}, Feng Wang^{2,3}, Iwona Stepniewska⁴, Ann S Choe^{1,2}, Kurt G Schilling^{1,2}, Landman A Bennett^{2,5}, Adam W Anderson^{1,2}, Zhaohua Ding^{2,3}, Limin Chen^{2,3}, and John C Gore^{2,3}

¹Department of Biomedical Engeneering, Vanderbilt University, Nashville, Tennessee, United States, ²Institute of Imaging Science, Vanderbilt University, Nashville, Tennessee, United States, ³Department of Radiology and Radiological Science, Vanderbilt University, Nashville, Tennessee, United States, ⁴Department of Psychology, Vanderbilt University, Tennessee, United States, ⁵Department of Electrical Engeneering, Vanderbilt University, Nashville, Tennessee, United States

TARGET AUDIENCE This work is intended for researchers interested in the anatomical basis of functional connectivity. **PURPOSE**

This study aims to compare functional connectivity, estimated using resting state fMRI, against anatomical connectivity, revealed by histological analysis of the distribution of a neuronal tracer injected in the primary motor cortex. In addition to traditional blood-oxygenation level dependent (BOLD) connectivity, we also obtained cerebral blood volume (CBV)-weighted functional connectivity using a monocrystalline iron oxide nanoparticle (MION) contrast agent to increase sensitivity to functional fluctuations.

MATERIALS AND METHODS

Anatomical connectivity and parcellation: Anatomical connectivity was measured in a single animal. The bidirectional neuronal tracer BDA (biotinylated dextran amine) was injected into eight cortical sites covering the forelimb representation of the left primary motor cortex (M1). The sites were identified by intracoritcal microstimulation. Two weeks later, the brain was extracted and sectioned at 50µm thickness on a freezing microtome. The tissue blockface was photographed after every three sections to construct an intermediate space for registration from microscopy space to MRI atlas space. Every sixth section was reacted to reveal BDA and each adjacent section was stained for Nissl substance. All the stained sections were photomicrographed. To quantify the anatomical connectivity, we identified and counted the BDA-stained fibers crossing the white-gray matter (WGM) interface on high resolution micrographs (~2µm/pixel) and created a series of density distribution maps (DDM, ~150µm/pixel) in micrograph space. In the value of each voxel in the DDM is the number of BDA fibers crossing the interface in that voxel. The borders of nine pairs of symmetric cortical parcels (i.e., PF-prefrontal cortex; PM-premotor cortex; M1-primary motor cortex; PA-anterior parietal cortex; PP-posterior parietal cortex; SMA-supplementary motor area; AC-anterior cingulate cortex; PVR-parietal ventral and rostral areas) were identified based on architectonic features of Nissl-stained neurons^{2,3} in micrograph space.

Atlas space: Before sectioning, the extracted brain was scanned at 9.4T for whole-brain structure (spin-echo multi-slice, TR/TE=5s/26ms, voxel size=0.3mm isotropic). This dataset was used as a reference, i.e., the MRI atlas space. The histological DDMs and the cortical parcels were transformed to MRI atlas space using deformation fields calculated via a multi-step registration procedure from light microscopy to MRI atlas space^{1,3-5}.

Functional connectivity: Anaesthetized monkeys were scanned at 9.4T for whole-brain structure (gradient-echo multi-slice, TR/TE=300/6ms, voxel size=0.5x0.5x1mm³), BOLD (2 shot gradient-echo EPI, TR/TE=750/16ms, voxel size=1x1x1mm³, number of volumes per run=300, number of runs=3) and CBV-weighted function (10 minutes following a slow i.v. bolus of 12mg/kg MION, TE=10ms, same sequence as BOLD). Raw functional data were realigned, slice-timing corrected and smoothed (FWHM=1mm) using SPM12⁶. The preprocessed data were coregistered to structural images using FLIRT⁷. Then the timecourse of each voxel was separately detrended and filtered (pass-band: 0.01-0.1Hz). The injection region of the neuronal tracer (transformed from the microscopy space) was used as the seed region. Pearson's correlation coefficients (rs*CC* and *p* value) between the average timecourse in the seed region and the timecourse of every other voxel in the brain were calculated. The functional connectivity measures were transformed into the atlas space using FLIRT.

Comparison: The functional connectivity measures (i.e. rsCC with *p* value) and histological DDM were rendered on the WGM interface in atlas space for visual comparison. To quantitatively assess the relationship between functional connectivity and anatomical ground truth, we calculated correlations (Pearson's and Spearman's rank) between local rs*CC* of BOLD (or CBV-weighted) data and the number of local BDA fibers across the 18 identified parcels. To calculate the Pearson's correlation, the numbers of BDA fibers were resampled and rescaled into a Gaussian distribution with mean of 0.5 and standard deviation of 0.1 as described by Honey et al⁸ (Spearman's rank correlation is not affected by the transformation to a Gaussian distribution).

RESULTS

Figure 1A shows the BDA injection region (in pink) and 18 histological parcels with other colors, rendered on the silver WGM interface of the monkey brain in atlas space. Fig.1B-D shows the DDM of BDA, significance (displayed as $-log_{10}(p)$) of BOLD connectivity, and significance (displayed as $-log_{10}(p)$) of CBV-weighted connectivity rendered on the same surface. Fig.2 illustrates the relationship between the resampled number of BDA fibers and functional connectivity, rsCC, across all 18 cortical parcels.

A ACSMA B C D

CONCLUSION AND DISCUSSION

The histological fiber density map (Fig.1B) reflects strong anatomical connections from M1 to the ipsilateral parietal area, contralateral M1 and SMA, while the significance of CBV-weighted timecourse correlation (Fig.1D) shows the highest functional connectivity to the contralateral M1, parietal area and SMA. According to Fig. 2, there is good correspondence ($r_s{=}0.82$ for CBV) between the strength of anatomical and functional connections to M1. Differences between the maps (e.g., cPP region, pointed by red arrows in Fig.1B,D, has few BDA fibers connected to M1 but significant functional connectivity) may indicate indirect functional connections between regions, since BDA reveals only direct (single-neuron) connections. In any case, underlying anatomical connectivity is not necessary for observation of strong functional connectivity $^{8,9}_{\circ}$. In addition, BOLD connectivity maps were noisier than CBV-weighted connectivity maps (Fig1.C,D). In other words, CBV-weighted fMRI exhibits higher sensitivity and spatial specificity to low-frequency functional fluctuations $^{10}_{\circ}$.

This work provides a framework to investigate anatomy-function relationships based on histological data, which reveal true anatomical connectivity and so are expected to be more accurate than diffusion MRI-derived connectivity. Functional data also show subcortical (e.g., putamen and thalamus) connectivity to M1 during the resting state, whereas, BDA-stained fibers reaching these subcortical regions are more difficult to localize accurately.

REFERENCES 1. Gao, PlosONE, 2013. 2. Stepniewska, J Comp Neurol, 1993. 3. Choe, Magn Reson Imaging, 2011. 4. Rohde, IEEE Trans Med Imaging, 2003. 5. Gao, ISMRM, 2013. 6. www.fil. ion.ucl.ac.uk/spm. 7. fsl.fmrib.ox.ac.uk/fsl/fslwikl/FLIRT. 8. Honey, PNAS, 2008. 9. Koch, Neuroimage, 2002. 10. Kim, NMR in Biomedicine, 2012.

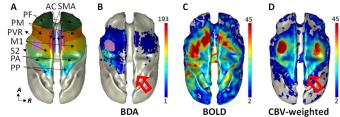


Fig.1. Dorsal view of BDA injection region (pink) together with 18 histological parcels **(A)**, anatomical fiber density **(B)**, significance $-\log_{10}(p)$ of BOLD connectivity **(C)** and CBV-weighted connectivity **(D)**. (Silver color-WGM interface).

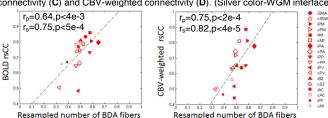


Fig 2. Relationships between resampled numbers of BDA fibers and rsCC of BOLD or CBV-weighted connectivity across 18 cortical parcels. r_p is the Pearson's correlation coefficient calculated from resampled data and r_s is the Spearman's rank correlation coefficient.