Analysis of changes in functional connectivity patterns with serial resting state fMRI after transient ischemic stroke in rat brain

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Introduction

Resting state fMRI analysis allows assessment of spatial organization of brain function without the need of stimulating a particular functional system[1]. Synchronization of spontaneous neural activity across the brain, observed in the low-frequency spectrum of the BOLD fMRI signal, is considered indicative of functional connectivity. Because resting state fMRI does not require specific peripheral stimulation or active task execution, it provides an ideal tool to study, under relatively stable conditions, the dynamics of changes in functional organization of different neuronal networks during development or after brain injury. In order to evaluate this potential, we applied serial resting state fMRI to assess changes within the sensorimotor network in a well-controlled rat model of ischemic stroke.

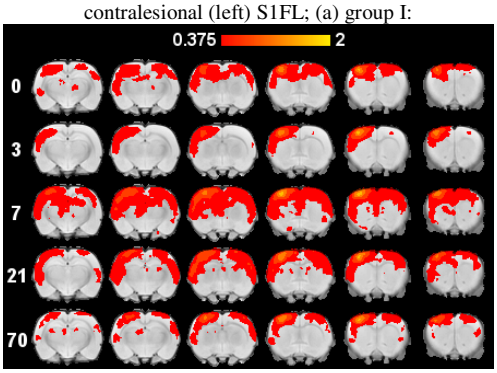
Methods

Experimental stroke was induced by transient (90 minutes) intraluminal occlusion of the right middle cerebral artery (tMCA-O) in male Wistar rats (n=17), weighing 250-280 grams[2]. Sensorimotor function was measured longitudinally by scoring neurological deficiency (NDS) and adhesive removal (in seconds) from the affected forelimb[2]. Structural MRI and resting state fMRI measurements were acquired before and at 3, 7, 21, and 70 days after tMCA-O. Rats were mechanically ventilated with 1-2% isoflurane in air/O2 (2:1) during MRI. MRI measurements were performed on a 4.7 T horizontal bore Varian MR system with use of a Helmholtz volume coil (90-mm diameter) and an inductively coupled surface coil (35-mm diameter) for signal excitation and detection, respectively. Blood oxygen saturation and heart rate were monitored during MRI, and body temperature was maintained at 37.0 ± 0.5 °C. Multi-echo multi-slice T2-weighted MRI (TR/TE=3000/17.5 ms; echo train length=8; 128×128 matrix; 0.25×0.25×1.0 mm^3 voxels) and multi-slice, diffusion-weighted 8-shot EPI (TR/TE=3000/32 ms; b-values=303 and 1213 s/mm^2 in 6 directions; 128×128 matrix; 0.25×0.25×1.0 mm^3 voxels) were performed to determine ischemic lesion size and location. Then, for at least 15 minutes end-tidal isoflurane was reduced to 1%. Subsequently, 10 minutes of resting state fMRI was performed using a T2*-weighted gradient echo EPI sequence (50° flip angle, TR/TE=1000/19 ms, 128×128 matrix; 0.5×0.5×1.0 mm^3 voxels, 600 BOLD images).

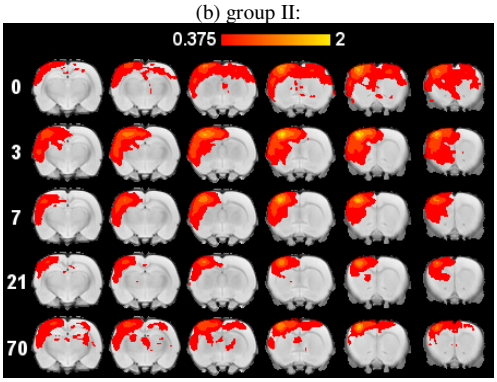
Inclusion and group assignments were based on NDS and adhesive removal scores, measured at 3 days after tMCA-O, and size and location of ischemic lesions, respectively. Animals with NDS below 2 or with adhesive removal times within 10 seconds at day 1 post-stroke were excluded. Group I animals had only subcortical lesions (n=5); group II animals had both cortical and subcortical lesions (n=9).

After motion-correction with FLIRT (FMRIB FSL, www.fmrib.ox.ac.uk/fsl) and spatial smoothing, low frequency BOLD fluctuations were obtained from the functional time series with a band-pass filter ($0.01 < f < 0.08$ Hz) using AFNI (R.W. Cox, http://afni.nimh.nih.gov/afni). For functional connectivity (FC) analysis the first and last 50 seconds of BOLD acquisition were discarded. T2-weighted images were registered non-rigidly to a reference image, which was matched to a 3D model of a rat brain atlas[3]. Bilateral regions-of-interest (ROIs) were selected within the sensorimotor network, i.e. the primary and secondary motor cortex (M1, M2), primary somatosensory cortex of the forelimb (S1FL), secondary somatosensory cortex (S2), caudate putamen (CPu) and thalamus (Th). The ROIs were projected from the atlas onto the functional time series. Lesioned tissue was identified with a fuzzy C-means clustering algorithm[4] on the T2 images, and subsequently excluded from the analysis. Functional connectivity was measured as the correlation coefficient r and Fisher-transformed according to $z' = \ln((1+r) / (1-r)) / 2$. Interhemispheric FC between bilaterally homologous ROIs was calculated as the correlation coefficient on the mean signals of each ROI. FC maps were obtained by calculating the voxel-wise correlation with the mean signal of a seed ROI. Functional integrity within the ROI was assessed as the average z'-value of ROI voxel signals and the mean. Averaging across subjects yielded group mean FC maps. A correlation coefficient $r = 0.2$ ($z' > 0.375$, $P < 10^{-4}$) was considered significant and thresholded connectivity maps were overlaid on an anatomical template image.

Figure 1 Functional connectivity maps from contralesional (left) S1FL; (a) group I:



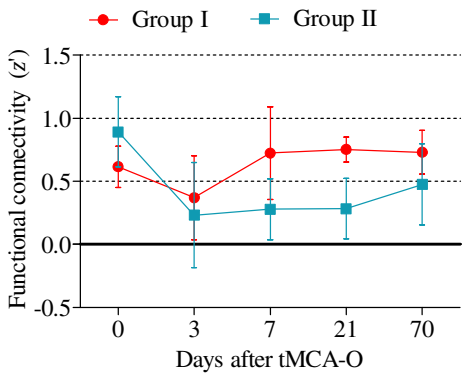
(b) group II:



Results

Mean interhemispheric FC maps with contralesional (left) S1FL as seed ROI are shown for both groups in figure 1. Figure 2 shows the temporal pattern of mean FC between contralesional and ipsilesional S1FL. The FC maps before stroke induction display significant correlation with bilateral sensorimotor network ROIs, M1, M2, S1FL, S2 and CPu. At 3 days after stroke, FC with ipsilesional sensorimotor regions was reduced in both groups. Group I animals demonstrated recovery of interhemispheric FC at 7 days after tMCA-O in correspondence with a decrease in NDS and adhesive removal time. In group II animals, however, interhemispheric FC remained reduced at 7 days, in parallel with lower sensorimotor function scores as compared to group I animals. Partial recovery of interhemispheric FC in group II was observed after 10 weeks, which was accompanied with improved behavioral scores.

Figure 2 Mean ± SD functional connectivity from contralesional (left) S1FL:



Discussion

Our study shows that resting state fMRI can be applied to study changes in functional connectivity after experimental stroke in a rat model. We detected a decrease in FC between the contralesional S1FL and ipsilesional sensorimotor regions acutely after stroke, despite the absence of clear structural ischemic damage in these areas. Animals with only subcortical ischemic lesions showed restoration of interhemispheric FC at 7 days after tMCA-O, whereas recovery of interhemispheric FC was delayed in animals with both cortical and subcortical lesions. The temporal correspondence between the evolution of FC within the sensorimotor network and changes in sensorimotor function scores emphasizes the potential of resting state fMRI to assess spatio-temporal characteristics of functional brain reorganization in relation to recovery after stroke.

References

[1]Biswal et al., *MRM* 34 (1995): 537-541. [2]van der Zijden et al., *J. Cereb. Blood Flow Metab.* 28 (2008): 832-840. [3]Paxinos and Watson, *The rat brain in stereotaxic coordinates* (Elsevier Academic Press, 2005, 5th edition). [4]Cai et al., *Pattern Recognition* 40 (2007): 825-838.