MRI of bilateral sensorimotor network activation by direct intracortical stimulation in rats after unilateral stroke

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Introduction

Functional MRI (fMRI) is increasingly applied to study changes in activation patterns after stroke. Both patient and animal fMRI studies have provided important insights in rearrangement of ipsi- and contralesional functional brain fields during recovery from stroke[1,2]. fMRI protocols in patients and animal stroke models typically employ a peripheral somatosensory stimulation paradigm or, in patients, execution of a task. In this study we applied BOLD and CBV-weighted fMRI during direct intracortical stimulation (DICS) of the primary motor cortex (M1) in rats. This approach may provide distinctive additional insights into (changes in) central functional connectivity within the bilateral sensorimotor network. In particular, by stimulation of cortical neurons in layer III, the corticocortical and transcallosal connections may be evaluated more explicitly than with peripheral stimulation. In the present study we assessed excitability of ipsi- and contralateral sensorimotor network regions following DICS of contralesional M1 after unilateral stroke as compared to controls.

Methods

Experimental stroke was induced by permanent intraluminal occlusion of the right middle cerebral artery (pMCAO) in male rats, weighing 250-270 grams. MRI was done at 24 hours after stroke (n=3). Eight rats served as controls. Animals were anesthetized with 1-2% isoflurane in air/O_2 (2:1). MRI-compatible custom-made bipolar insulated gold electrodes with a 200 µm diameter exposed tip were carefully positioned in two burr holes of the skull at 2.5 mm lateral and 0.5 and 3 mm anterior to bregma. Tips of the electrodes were placed in the left M1 at a depth of 1.7 mm in order to primarily stimulate layer III of the cortex. MRI was conducted on a 4.7 T horizontal bore Varian MR system, and included T_2-weighted fast spin echo MRI (TR/TE = 2913/36 ms; ETL = 8; ESP =18.2 ms; matrix size = 256 × 256; 19 1-mm slices) for coregistration purposes, and T_2-weighted (TR/TE = 3600/15 ms; ETL = 12; matrix size = 128 × 128; 19 1-mm slices) and diffusion-weighted MRI (eight-shot EPI; TR/TE = 3000/31 ms; b = 1014 s/mm^2 with diffusion-weighting in 6 directions; matrix size = 128 × 128; 19 1-mm slices) to determine ischemic lesion size and location. For BOLD and CBV-weighted contrast-enhanced MRI[3] we used T_2*-weighted EPI (50° flip angle, TR/TE =19/1000 ms; matrix size = 64 × 64; 13 1.5-mm slices). CBV-weighted MRI was performed after intravenous injection of ultrasmall superparamagnetic particles of iron oxide (Sinerem® (Guerbet); 20 mg/ml, 14.4 mg/kg). All MRI experiments were conducted with a field-of-view of 32 × 32 mm^2. For DICS, we used a stimulation paradigm that was largely similar to Austin et al.[4], which involved 5 blocks of 3-s periods of stimulation (1.5 ± 0.1 mA amplitude and 0.3 ms duration at 300 Hz in 50 ms trains repeated five times per second), followed by 60 s rest. To prevent motion during DICS we intravenously administered pancuronium bromide (0.67 mg/kg bolus, followed by continuous infusion at 0.67 mg/kg/h). After MRI, rats were euthanized and brain sections were stained with triphenyltetrazolium chloride (TTC) to evaluate potential electrolytic damage in the stimulated M1. For image analysis, all anatomical images were coregistered to a control T_2-weighted rat brain MRI template that was coregistered to the Paxinos rat brain atlas[5], using in-house developed software. Motion-corrected, smoothed BOLD signal intensity and relative CBV (rCBV) time series were coregistered to the template. Statistical activation maps were calculated using FEAT, the FMRIB Easy Analysis Tool (www.fmrib.ox.ac.uk), with a convoluted gamma function model fitted to the 5 stimulation blocks. Z statistic images were thresholded at a Z-value of 2.3 (uncorrected). This thresholded value equated to a corrected statistical significance of P = 0.05. Regions-of-interest (ROIs) were selected based on Paxinos' rat brain atlas and included ipsi- and contralateral primary motor cortex (M1), secondary motor cortex (M2), primary somatosensory cortex of the forelimb (S1fl), secondary somatosensory cortex (S2), caudate putamen (Cpu) and thalamus (Th). Activation responses were measured by calculating the area under the BOLD signal intensity and rCBV time curves (averaged across four on-off periods) for each ROI. Unpaired t-testing was done for statistical comparison of each ROI between groups. P < 0.05 was considered significant.

Results

None of the rats showed severe cortical damage on the TTC-stained brain sections at the site of electrode stimulation. BOLD and CBV-dependent fMRI activation maps of a control rat and a rat at 24 h after stroke are shown in figure 1, represented as a color-coded Z-statistic overlaid onto the control rat brain template. Z scores are given from 2.3 - 25.5 for each activation map. All cortical (M1, M2, S1fl and S2) and subcortical (Cpu and Th) sensorimotor ROIs ipsilateral to the stimulation site (=left) showed significant BOLD and rCBV activation after stimulation of M1 in the healthy control animals. Contralateral to the stimulation site (=right) M1, M2, S1fl and Cpu displayed clear activation responses (figure 1a and 1c). At 24 hours after stroke, the unilateral ischemic lesion on T_2- and diffusion-weighted MR images included S2, Cpu, Th and partially S1fl. DICS-induced activation responses were drastically reduced in ipsilesional M1, M2 and S1fl, however, in some animals increased activation was observed in the contralesional (=left) S2 and subcortical areas of the sensorimotor network (i.e. ipsilateral to the stimulation site) (figure 1b and 1d). Figure 2 shows the mean area under the BOLD signal intensity and rCBV time curves (averaged across four on-off periods) in different ROIs. At 24 hours after pMCAO, activation responses in intact ipsilesional M1 and M2 were strongly diminished. We found that the averaged area under the curve of the BOLD responses in contralesional Cpu, Th and S2 were somewhat increased, however, this trend was not clear for the rCBV responses.

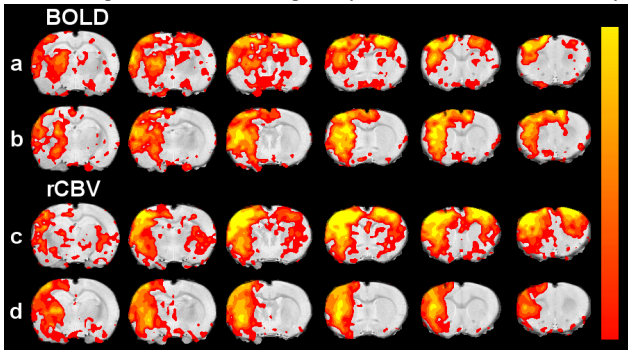

Figure 1. Maps of BOLD (a,b) and rCBV (c,d) activation in response to DICS of left (contralesional) M1 in control (a,c) and 24h post–stroke brain (b,d).

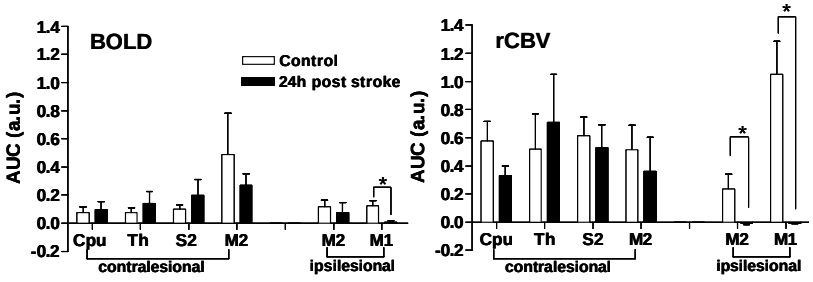

Figure 2. Mean area under the curve ± SEM of ipsi- (right) and contralesional (left) BOLD and rCBV responses to left M1 stimulation in control (n=8) and 24h post stroke.

Discussion

This study demonstrates that fMRI during DICS allows assessment of activation responses in bilateral functional networks in control rat brain and after stroke induction. Our preliminary data point toward loss of ipsilesional activation and, in some cases, increased excitability of contralesional brain regions at 24 h after pMCAO, which is in agreement with previous fMRI studies using a peripheral forelimb stimulation paradigm.[2] Differences between BOLD and rCBV changes may be related to differences in neurovascular mechanisms, but could also be caused by regionally dependent changes in excitability due to the recurrent stimulations.

References

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