Influence of repetitive transcranial magnetic stimulation on functional connectivity and hemodynamics in the rat brain

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Synopsis

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation technique with the ability to change cortical excitability, however its precise mechanism of action is not completely understood. Therefore, by acquiring resting-state fMRI and perfusion MRI data we assessed the influence of unilateral low-frequency (inhibitory) rTMS on functional connectivity and hemodynamics in stimulated cortical tissue in rats. After four consecutive days of rTMS we measured reduced interhemispheric functional connectivity between homotopic sensorimotor regions, while cerebral blood flow remained largely unaffected. This reduction in interhemispheric functional connectivity may be due to the inhibitory effect of low-frequency rTMS on cortical excitability.

Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive neuromodulation technique that has the ability to alter cortical excitability and plasticity[1]. Depending on the stimulation protocol used, the neuromodulatory effects of rTMS may outlast the period of stimulation through the induction of plastic changes within the cortical network[2]. This makes rTMS a favorable therapeutic approach to modulate brain activity in several neurological disorders associated with alterations in cortical excitability and functional connectivity. Although promising treatment results have been reported, knowledge about the precise mechanism of action of rTMS, including its effect on structural and functional brain parameters, is incomplete. Thus, to further explore these mechanisms, the aim of our study was to assess the effects of low-frequency (inhibitory) rTMS on hemodynamics and functional connectivity in stimulated cortical tissue.

Methods

Animal procedures were conducted according to the guidelines of the European Communities Council Directive and approved by our institution's Ethical Committee on Animal Experiments. Four naive male Sprague Dawley rats (380-400 g) were subjected to 20 minutes of low frequency (1 Hz, 1200 pulses) rTMS for 4 consecutive days, while under isoflurane (1%) anesthesia. Animals were stimulated with a Neuro-MS/D stimulator (Neurosoft Ltd., Ivanovo, Russia) using a 25 mm figure-of-eight coil (inner diameter, 18 mm; outer diameter, 25 mm) that was positioned lateral to the midline, with the center of the coil positioned over the right sensorimotor cortex.

Prior to and following the application of rTMS, we acquired structural, functional and perfusion MRI data at 9.4 T (Varian Instruments), whilst animals were anesthetized with isoflurane (1.5%). Resting-state (rs-fMRI) images were executed with a 3D gradient echo, echo planar imaging sequence (800 images, TR/TE=26.1/15 ms, flip angle=13▯, resolution=600 µm isotropic). For perfusion MRI we applied dynamic susceptibility contrast-enhanced MRI with a 2D gradient echo, echo planar imaging sequence (800 images, TR/TE=330/11 ms, flip angle=40▯▯ resolution=764x764x1 µm) combined with an intravenous bolus injection of Gadobutrol (Gadovist®, Bayer Schering Pharma AG, Berlin, Germany) (0.35 mmol/kg bodyweight).

Inter- and intrahemispheric functional connectivity [Fisher-transformed correlation (z') of low-frequency BOLD fluctuations ($0.01<f<0.1$ Hz) derived from rs-fMRI] were calculated between the primary (M1) and secondary (M2) motor cortices, the forelimb region of the somatosensory cortex (S1FL), the secondary somatosensory cortex (S2), thalamus (Th) and caudate putamen (CPu)[3]. Relative cerebral blood flow (CBF) in the stimulated sensorimotor cortex was calculated from perfusion MRI data using tracer arrival time-insensitive deconvolution[4]. Contralateral sensorimotor cortex was used as a control. rTMS-induced changes in perfusion and functional connectivity were evaluated using a one-way repeated measures ANOVA (SPSS, IBM SPSS Statistics version 23).

Results

Four days of 1 Hz (inhibitory) rTMS resulted in reduced interhemispheric functional connectivity between homotopic regions of interest (M1, M2, S1FL, S2, Th and CPu) (Fig. 1). The loss of interhemispheric connectivity after rTMS is clearly displayed in the functional connectivity maps of the right M1 (Fig. 2) and S1FL (Fig. 3). There was a trend towards reduced intrahemispheric functional connectivity between certain stimulated regions of interest (e.g. between M1 and M2), but this was not uniformly observed (Fig. 4A-C).

Relative cerebral blood flow (CBF) in the stimulated sensorimotor cortex slightly dropped from 110±7% (pre-stimulation) to 106±16% (post-stimulation), however this was not statistically significant.

Conclusion

Our preliminary data in rats show that repetitive, low-frequency TMS reduces functional connectivity in the stimulated hemisphere, which may be due to its inhibitory effect on cortical excitability. Information about the degree to which brain activity can be modulated by rTMS may prove helpful in the development of treatment options[5], however currently little is known about its influence on neural networks in health and disease.

References:

1. Siebner HR and Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res. 2003; 148:1-16.
2. Stagg CJ, O'Shea J and Johansen-Berg H. Imaging the effects of rTMS-induced cortical plasticity. Restor Neurol Neurosci 2010; 28(4):425-36.
3. van Meer MPA, van der Marel K, Wang K, et al. Recovery of sensorimotor function after experimental stroke correlates with restoration of resting-state interhemispheric functional connectivity. J Neurosci 2010; 30:3964-3972
4. Bouts MJRJ, Tiebosch IACW, van der Toorn A, et al. Early identification of potentially salvageable tissue with MRI-based predictive algorithms after experimental ischemic stroke. J Cereb Blood Flow Metab. 2013; 33:1075-82
5. van der Werf YD, Sanz-Arigita EJ, Menning S, et al. Modulating spontaneous brain activity using repetitive transcranial magnetic stimulation. BMC Neurosci 2010; 11:145

Figures

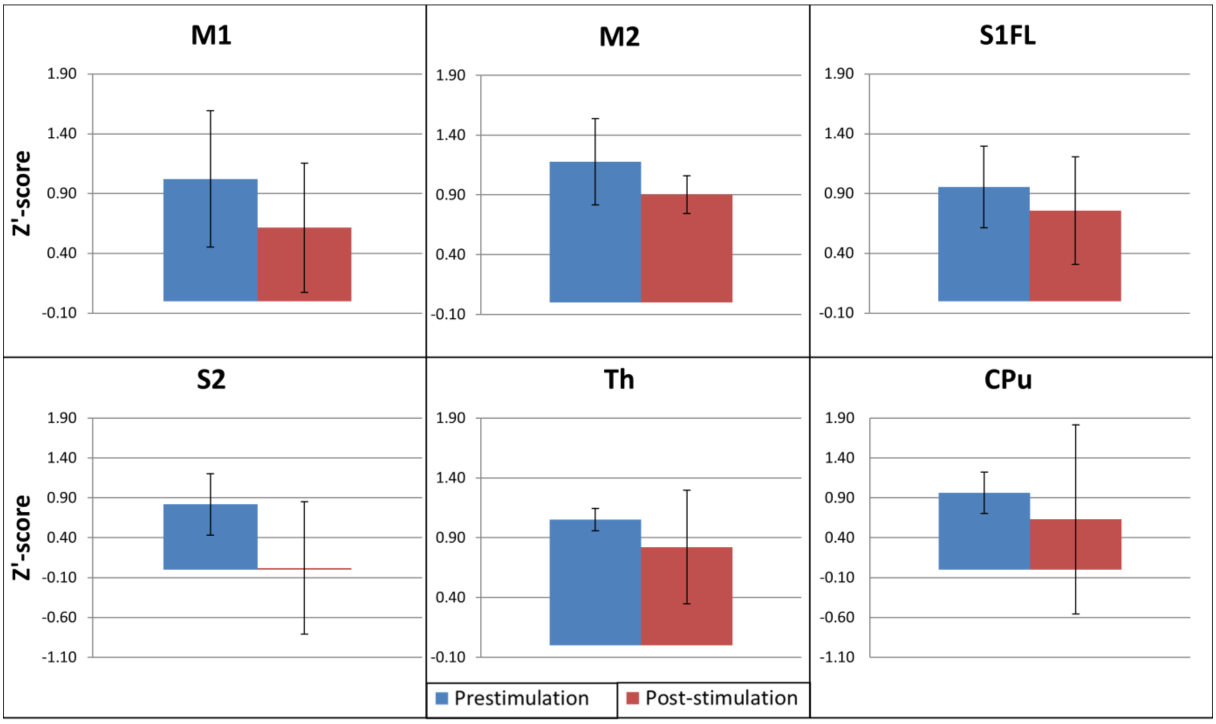

Figure 1: Interhemispheric functional connectivity (z') between homotopic regions of interest pre- and post-rTMS. The bars represent mean functional connectivity values +/- standard deviation.

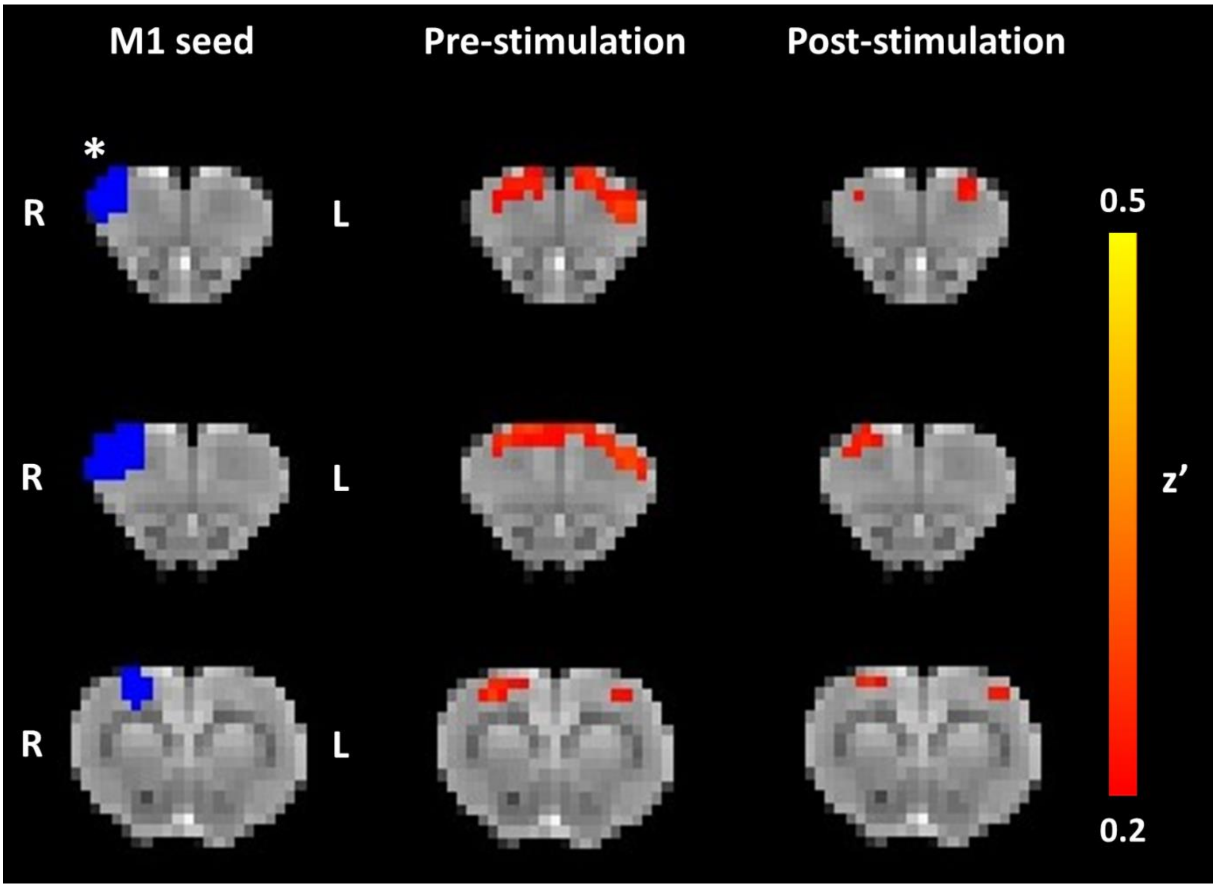

Figure 2: Average functional connectivity maps, using the right, stimulated (*) primary motor cortex (M1) as a seed region, pre- and post-rTMS. R, right; L, left.

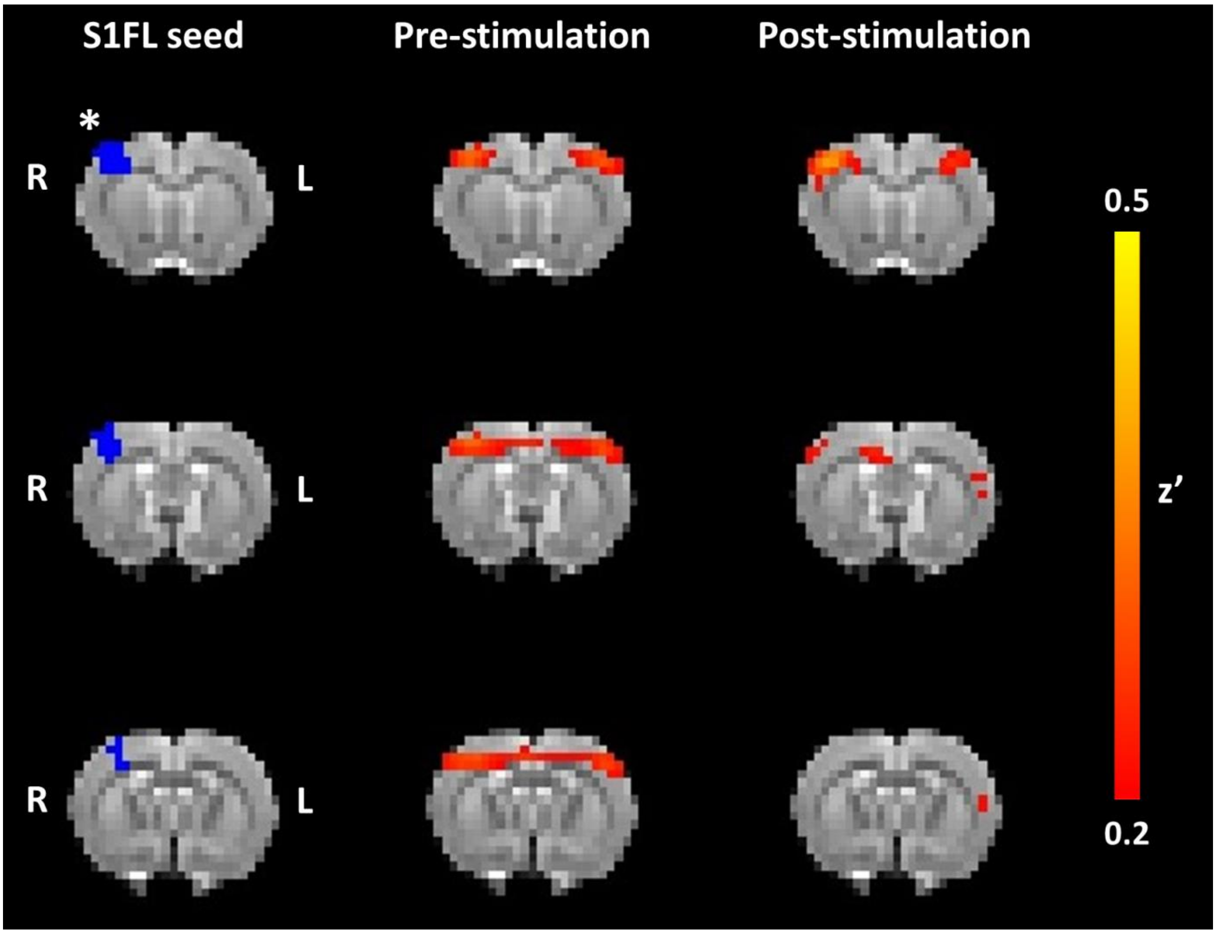

Figure 3: Average functional connectivity maps, using the right, stimulated (*) forelimb region of the somatosensory cortex (S1FL) as a seed region, pre- and post-rTMS. R, right; L, left.

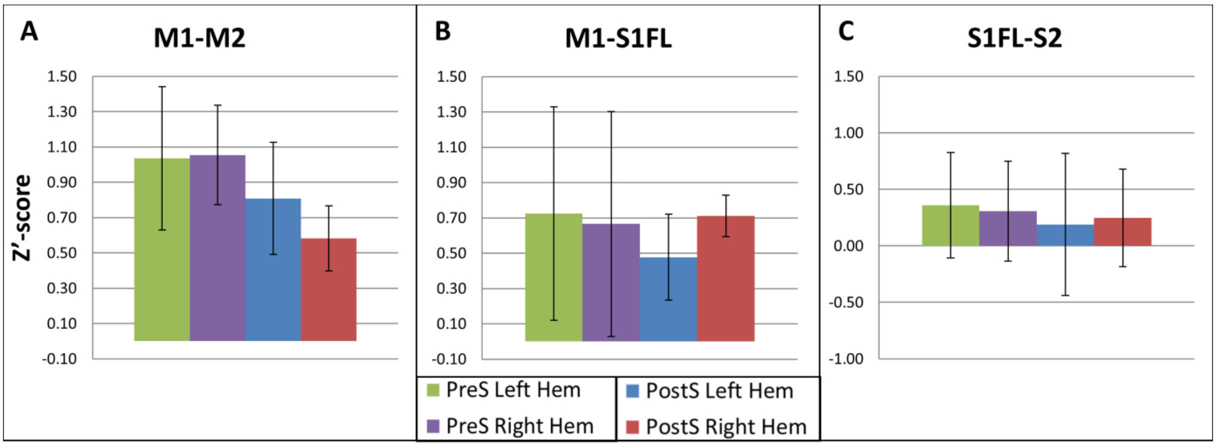

Figure 4: Intrahemispheric functional connectivity (z') between regions of interest pre- and post-rTMS. The bars represent mean functional connectivity values +/- standard deviation.