Official Title: Mechanisms of Non-Invasive Neuromodulation Interventions: Influence on Human Neurochemistry and Functional Connectivity

NCT Number: NCT02677740

Date: June 1st, 2019

Protocol procedures

Experimental Design, safety and confidentiality

Adult, healthy, right-handed males were recruited for the study, following the criteria listed in the relevant section of the protocol registered on clinical-trial.gov. The protocol was approved by the Institutional Review Board: Human Subjects Committee of the University of Minnesota in accordance with the recommendations of The Code of Federal Regulations and the Declaration of Helsinki. A written informed consent was provided by each subject right before the beginning of the study.

The study consisted of one study visit during which resting state fMRI and MRS data were collected before and after the rTMS intervention. The subject first underwent the pre-rTMS MRI session, then was transferred to another room for the rTMS intervention, and finally was returned to the MR scanner room for the post-rTMS MRI session. For both pre- and post-rTMS MRI sessions, data were acquired in the following order: anatomical MRI, MRS from left then from right motor cortex and finally rsfMRI.

Each participant was evaluated for any adverse event throughout the study. A medical director was present throughout the rTMS session and the second imaging session. The medical director reviewed all participant outcomes including responses to report of symptoms questionnaires after the MRI and rTMS sessions, along with the vital signs (blood pressure before rTMS, blood oxygenation during the MRI scan), and approved continuation of the study after each session. A designated medical monitor was assigned to review any adverse event that occurred.

The records of this study are kept private and only available to the PI. All experimental data related to the study are de-identified prior to being stored on PC or emails. Hard copies of protected health information (PHI) are locked in a file cabinet in the PI's office.

rTMS intervention

TMS was delivered using a 70-mm figure-eight TMS coil connected to a Magstim 200 machine. The coil was positioned over the hand region of the left motor cortex (approximately 5 cm lateral to each individual vertex of the head), contralateral to the dominant hand in all studied participants. TMS-induced motor evoked potentials (MEPs) were recorded by placing surface EMG electrodes over the first dorsal interosseous muscle of the participant's dominant hand, contralateral to the side of TMS. The position of the coil was moved systematically to find the location that evoked the largest and most reliable MEP ("hotspot"). The resting motor threshold (RMT) was then measured as the minimum TMS intensity required to produce a 50 μV MEP while the participant was at rest. Immediately after completion of the

RMT assessment, the rTMS intervention began. Participants received either 1-Hz or 5-Hz rTMS, applied over the motor cortex hotspot contralateral to the dominant arm for 20 minutes. Corticospinal excitability was finally re-tested within no more than 10 minutes after the rTMS session.

MRI/MRS acquisitions

The imaging sessions were performed using a 7-T/90-cm magnet (Agilent/Magnex Scientific, UK) interfaced to a Siemens Syngo console. A single channel transmit/32-channel receive head (NOVA Medical, Wilmington, MA). Head motion was minimized using a molded foam head cushion and padding placed snugly around the participant's head. Participants were asked to close the eyes but remain awake during both the resting state fMRI and MRS acquisitions.

High-resolution 3D-MPRAGE images were first obtained to visualize the anatomical structure of the motor cortices. Then automatic B_0 field mapping and adjustment of 1st- and 2nd-order shims were achieved by FASTMAP (Gruetter and Tkac, 2000). Spectroscopy voxels of interest (24 x 22 x 17 mm^3) were selected in left and right motor cortices based on anatomical landmarks. ^{1}H-MRS data were acquired using the semi-LASER localization (Bednarik et al., 2015; Oz and Tkac, 2011). Unsuppressed water signal was also acquired for eddy current correction and as an internal reference for metabolite quantification.

Resting state fMRI was performed with a 2D single-shot gradient echo EPI sequence to collect blood oxygenation level dependent (BOLD) data. A total of 280 volumes were acquired, corresponding to an acquisition time of approximately 6 min.

MRI/MRS data processing

Single-scan MRS data were corrected for small frequency and phase variations, summed and finally corrected for the residual eddy current effects using unsuppressed water signal (Klose, 1990). Brain metabolites were quantified by LCModel (Pfeuffer et al., 1999; Provencher, 1993; Provencher, 2001; Tkac et al., 2009). Only metabolite concentrations quantified with Cramèr-Rao lower bounds (CRLB) below 50% were included in further analysis. rTMS-induced changes in GABA concentrations, separately in left and right motor cortices, were the primary MRS outcomes.

Resting-state data were preprocessed with the HCP "minimal-preprocessing pipeline" (Glasser et al., 2013), denoised with the FMRIB's independent component analysis (ICA)-based X-noiseifier (FIX) (Salimi-Khorshidi et al., 2014), and smoothed with an isotropic Gaussian kernel at 4 FWHM. Resting-state activity was then quantified by examining the fractional amplitude of low-frequency fluctuations

(fALFF) (Zou et al., 2008), which is thought to reflect the intensity of spontaneous brain activity. fALFF was calculated via AFNI function 3dRSFC (Cox, 1996) as the ratio of the power spectrum within the frequency range of interest ($0.008 < f < 0.09$ Hz) to that of the entire spectrum. fALFF was obtained for each voxel in the brain and then averaged within the left and right motor cortices, separately. These two regions of interest (ROIs) were identified in the upper limb region of the precentral gyrus, as defined in the Brainnetome atlas (Fan et al., 2016).

Statistical analyses

Pre- vs post-rTMS comparisons of metabolite concentrations and functional connectivity metrics were carried out with 2-sided paired t-test separately for left and right motor cortices. Given the nature of the study as a pilot investigation of safety and feasibility, multiple comparison corrections were not applied.

REFERENCES

Bednarik, P., Tkac, I., Giove, F., DiNuzzo, M., Deelchand, D. K., Emir, U. E., et al. (2015). Neurochemical and BOLD responses during neuronal activation measured in the human visual cortex at 7 Tesla. *J Cereb Blood Flow Metab.* 35, 601-610. doi: 10.1038/jcbfm.2014.233.

Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res.* 29, 162-173.

Fan, L., Li, H., Zhuo, J., Zhang, Y., Wang, J., Chen, L., et al. (2016). The Human Brainnetome Atlas: A New Brain Atlas Based on Connectional Architecture. *Cereb Cortex.* 26, 3508-3526. doi: 10.1093/cercor/bhw157.

Glasser, M. F., Sotiropoulos, S. N., Wilson, J. A., Coalson, T. S., Fischl, B., Andersson, J. L., et al. (2013). The minimal preprocessing pipelines for the Human Connectome Project. *Neuroimage.* 80, 105-124. doi: 10.1016/j.neuroimage.2013.04.127.

Gruetter, R., and Tkac, I. (2000). Field mapping without reference scan using asymmetric echo-planar techniques. *Magn Reson Med.* 43, 319-323.

Klose, U. (1990). In vivo proton spectroscopy in presence of eddy currents. *Magn Reson Med.* 14, 26-30.

Oz, G., and Tkac, I. (2011). Short-echo, single-shot, full-intensity proton magnetic resonance spectroscopy for neurochemical profiling at 4 T: validation in the cerebellum and brainstem. *Magn Reson Med.* 65, 901-910. doi: 10.1002/mrm.22708.

Pfeuffer, J., Tkac, I., Provencher, S. W., and Gruetter, R. (1999). Toward an in vivo neurochemical profile: quantification of 18 metabolites in short-echo-time (1)H NMR spectra of the rat brain. *J Magn Reson.* 141, 104-120. doi: 10.1006/jmre.1999.1895.

Provencher, S. W. (2001). Automatic quantitation of localized in vivo 1H spectra with LCModel. *NMR Biomed.* 14, 260-264.

Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med.* 30, 672-679.

Salimi-Khorshidi, G., Douaud, G., Beckmann, C. F., Glasser, M. F., Griffanti, L., and Smith, S. M. (2014). Automatic denoising of functional MRI data: combining independent component analysis and hierarchical fusion of classifiers. *Neuroimage.* 90, 449-468. doi: 10.1016/j.neuroimage.2013.11.046.

Tkac, I., Oz, G., Adriany, G., Ugurbil, K., and Gruetter, R. (2009). In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4T vs. 7T. *Magn Reson Med.* 62, 868-879. doi: 10.1002/mrm.22086.

Zou, Q. H., Zhu, C. Z., Yang, Y., Zuo, X. N., Long, X. Y., Cao, Q. J., et al. (2008). An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. *J Neurosci Methods.* 172, 137-141. doi: 10.1016/j.jneumeth.2008.04.012.