ARTICLE IN PRESS

Brain Stimulation ■■ (2016) ■■–■■

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

Repetitive Transcranial Magnetic Stimulation Educes Frequency-Specific Causal Relationships in the Motor Network

Felipe S. Salinas [a,*], Crystal Franklin [a], Shalini Narayana [b], C. Ákos Szabó [c], Peter T. Fox [a]

[a] Research Imaging Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States
[b] Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN, United States
[c] Department of Neurology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States

ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form 13 January 2016
Accepted 6 February 2016
Available online

Keywords:
TMS
PET
Effective connectivity
Motor cortex
Rate
Network

ABSTRACT

Background: Repetitive transcranial magnetic stimulation (rTMS) has the potential to treat brain disorders by modulating the activity of disease-specific brain networks, yet the rTMS frequencies used are delivered in a binary fashion – excitatory (>1 Hz) and inhibitory (≤1 Hz).
Objective: To assess the effective connectivity of the motor network at different rTMS stimulation rates during positron-emission tomography (PET) and confirm that not all excitatory rTMS frequencies act on the motor network in the same manner.
Methods: We delivered image-guided, supra-threshold rTMS at 3 Hz, 5 Hz, 10 Hz, 15 Hz and rest (in separate randomized sessions) to the primary motor cortex (M1) of the lightly anesthetized baboon during PET imaging. Each rTMS/PET session was analyzed using normalized cerebral blood flow (CBF) measurements. Path analysis – using structural equation modeling (SEM) – was employed to determine the effective connectivity of the motor network at all rTMS frequencies. Once determined, the final model of the motor network was used to assess any differences in effective connectivity at each rTMS frequency.
Results: The exploratory SEM produced a very well fitting final network model ($\chi^2 = 18.04$, $df = 21$, RMSEA = 0.000, $p = 0.647$, TLI = 1.12) using seven nodes of the motor network. 5 Hz rTMS produced the strongest path coefficients in four of the seven connections, suggesting that this frequency is the optimal rTMS frequency for stimulation the motor network (as a whole); however, the premotor → cerebellum connection was optimally stimulated at 10 Hz rTMS and the supplementary motor area → caudate connection was optimally driven at 15 Hz rTMS.
Conclusion(s): We have demonstrated that 1) 5 Hz rTMS revealed the strongest path coefficients (i.e. causal influence) on the nodes of the motor network, 2) stimulation at "excitatory" rTMS frequencies did not produce increased CBF in all nodes of the motor network, 3) specific rTMS frequencies may be used to target specific none-to-node interactions in the stimulated brain network, and 4) more research needs to be performed to determine the optimum frequency for each brain circuit and/or node.

Introduction

Repetitive transcranial magnetic stimulation (rTMS) has the potential to treat brain disorders by modulating the activity of disease-specific brain networks. A prime example of this approach may be seen in the rTMS treatments of the fronto-limbic network of major depressive disorder [1–3], in which rTMS is delivered to the dorsolateral prefrontal cortex to indirectly modulate the activity levels of the subgenual cingulate, which is too deep for standard rTMS coils to reach. Traditionally, rTMS rate has been applied in rTMS treatment protocols in either an inhibitory (≤1 Hz) or excitatory (>1 Hz) fashion [4,5], where it is assumed that these inhibitory or excitatory rTMS treatments affect the targeted brain networks in the same linear way – i.e. excitatory brain activity at the target site produces excitatory brain activity at all of the remote sites of the network [6,7].

In our previous study [8], we reported that a unimodal relationship (peaking at 5 Hz rTMS) existed between rTMS frequency and the cerebral blood flow (CBF) of the motor network during concurrent positron emission tomographic (PET) imaging. We also found that some of the nodes in the motor network were maximally inhibited at 5 Hz rTMS. Therefore, it is not appropriate to assume: 1)

* Corresponding author. Tel.: (210) 567-8214; fax: (210) 567-8152.
E-mail address: salinasf@uthscsa.edu (F.S. Salinas).

1935-861X/
http://dx.doi.org/10.1016/j.brs.2016.02.006

that increasing rTMS frequency will result in higher levels of brain activity or 2) that high rTMS frequencies (e.g. > 1 Hz) will produce excitatory brain activity in all nodes of the targeted brain network. In this study, we extend our previous results [8,9] by investigating the effective connectivity of the baboon's motor network – at different rTMS frequencies – to determine if 5 Hz rTMS is the optimal frequency for stimulation of all of the nodes within the motor network.

Materials & methods

Animal preparation

Five healthy, adult baboons (*Papio hamadryas anubis*; 4 females; age = 11.61 ± 2.92 years (mean ± SEM); body weight = 17.50 ± 5.42 kg) were studied in accordance with the policies of the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio; this study fully complied with U.S. Public Health Service's *Guide for the Care and Use of Laboratory Animals* [10] and the *Animal Welfare Act* [11]. The data acquired in these five animals were used in prior publications [8,9]; the results from these prior publications were re-analyzed in this study to assess the effective connectivity of the baboon's motor network. Each animal was pre-screened – using electroencephalography [12] – to ensure that only animals with no neurological deficits were enrolled in the study. The optimized anesthetized animal preparation has been described in previous studies [9,13]. Each animal received an injection of intramuscular ketamine (5 mg/kg) and atropine (0.3 mg) before each MRI and PET imaging procedure. We maintained sedation during each imaging session with continuous *i.v.* administration of ketamine (5–6 mg/kg/hr) and a paralytic (vecuronium; 0.25 mg/kg/hr). Upon conclusion of the imaging session, we administered atropine (0.6–1.2 mg, *i.v.*) and neostigmine (0.5–2.0 mg, *i.v.*) to reverse muscle paralysis. During the entire procedure, the animals' respiration, heart rate and oxygenation were monitored.

Magnetic resonance imaging

All MRIs were performed on a Siemens TIM-Trio 3T clinical scanner using a body radiofrequency (RF) transmission coil with a 12-channel head RF receiver coil (Siemens, Erlangen, Germany). We obtained high-resolution anatomical images using an MP-RAGE sequence (TR/TE/flip angle = 2300 ms/3.66 ms/13°) with slice-select inversion recovery pulses (TI = 751 ms), FOV = 128 mm × 128 mm × 80 mm, and 0.5 mm isotropic spatial resolution. We used the anatomical MRIs for co-registration between imaging modalities (MRI and PET) in order to register each animal's $H_2{}^{15}O$ PET images to their native MRI then warp them to a representative baboon's MRI brain space.

Blood-oxygen-level dependent (BOLD) fMRI was acquired using gradient-echo, echo-planar imaging (EPI; TR/TE = 2.5 s/30 ms), FOV = 150 mm × 150 mm × 48 mm, and spatial resolutions of 1.5 mm × 1.5 mm × 4 mm. Somatosensory stimulation was applied to the animal's right hand via a custom-made pneumatic-driven vibrotactile stimulator [13]; vibrotactile stimulations were applied – at a stimulation frequency of 5 Hz – using a 50 second on/off block design. We processed the fMRI data using the FEAT toolbox [14] in the FMRIB's Software Library (FSL) [15]. The resulting fMRIs were utilized to determine the location of each baboon's primary sensory cortex representation of the right hand ($S1_{hand}$). The $S1_{hand}$ and primary motor cortex representation of the hand ($M1_{hand}$) representations lie directly across the central sulcus from one another [16]. Therefore, we determined each animal's $M1_{hand}$ location (i.e. target location) to be the site in the precentral gyrus, which is adjacent to that animal's $S1_{hand}$ fMRI activation. We validated this approach in our previous baboon TMS/PET study [9].

rTMS

All TMS delivery was image-guided, using high-resolution structural and functional magnetic resonance images (fMRIs) using previously described techniques [9,13]. We used a MagPro CoolB65 figure-of-eight rTMS coil connected to a MagPro R30 Magnetic Stimulation Unit (MagVenture A/S, Farum, Denmark) for each rTMS procedure. The TMS coil's site of maximal electric field (E-field) induction (i.e. "hot spot") was determined using methods developed by Salinas et al. [17,18]. Using each animal's fMRI map of the $S1_{hand}$ and the corresponding target locations for $M1_{hand}$, we determined the scalp location closest to the $M1_{hand}$ site, then measured the distances from this scalp location to specific anatomical landmarks (nasion, inion, earholes). Finally, we stereotactically positioned the TMS coil over each animal's left primary motor cortex ($M1_{hand}$), while lying supine in the PET scanner, so that the location of the TMS coil's maximum induced E-field coincided with the targeted M1 location. Once positioned, the orientation of the TMS coil – i.e. the E-field and current direction – was adjusted to be perpendicular to the animal's central sulcus (with the E-field directed antero-medially, toward the animal's snout); this approach is consistent with the cortical column cosine (C3) aiming theory proposed by Fox et al. [19]. We applied single pulses of TMS to each baboon's left $M1_{hand}$ to visually establish each animal's resting motor threshold (rMT) at the first dorsal interosseous (FDI) muscle of the contralateral hand; the rMT was defined as the minimum intensity of stimulation capable of producing FDI muscle contractions in at least 5 out of 10 trials. Once each baboon's rMT was found, a one-time bolus injection of vecuronium was given to eliminate movement throughout the rTMS/PET session.

Each baboon underwent rTMS at stimulation frequencies of 3 Hz, 5 Hz, 10 Hz and 15 Hz. rTMS pulses were delivered to $M1_{hand}$ at 120% rMT during concurrent $H_2{}^{15}O$ PET scans. Each rTMS frequency was delivered at least 30 seconds prior to the injection of ^{15}O-labeled water and continued until 60 seconds after the injection. The number of rTMS pulses delivered at each stimulation frequency was held constant at 450 pulses (e.g. train duration varied across frequencies); this was done to decrease any possible dose effects – which may alter the excitability state of the motor cortex [20–22]. The 3 Hz rTMS frequency was continuously applied 90 seconds prior to radiotracer injection and continued for 60 seconds afterward, for a total 3 Hz rTMS duration of 150 seconds. The 5 Hz rTMS frequency was also continuously applied, but began only 30 seconds prior to radiotracer injection and continued for 60 seconds afterward, for a total of 5 Hz rTMS duration of 90 seconds. The 10 Hz rTMS frequency was applied intermittently in 5 second trains with 5 second inter-train intervals, whereas the 15 Hz rTMS frequency was applied intermittently in 5 second trains with 10 second inter-train intervals; the 10 Hz and 15 Hz rTMS pulse trains began 30 seconds prior to radiotracer injection and continued for 60 seconds, for a total rTMS duration of 90 seconds (e.g. 450 pulses for each rTMS frequency). The order of stimulation frequencies was randomized and a rest condition was used to represent the baseline scan.

Electroencephalography

We performed EEG recordings throughout each rTMS/PET session to monitor the level of sedation and any possible onset of seizure activity. The EEG unit used in this study was not TMS-compatible, therefore we did not analyze the resulting EEG waveforms for real-time TMS-induced effects. After the baboons were sedated, we positioned eight cephalic electrodes for each rTMS session (FP1, FP2,