Although transcranial magnetic stimulation (TMS) is gaining popularity as a basic research tool in neuroscience as well as in clinical neurorehabilitation, there is a paucity of well-established small animal models with clinically relevant protocols. Given the insufficient understanding of the molecular mechanisms, clinical application of repetitive TMS (rTMS) is necessarily controversial. The vital question whether rTMS brings about long–lasting molecular changes essential for the recovery after stroke, including angiogenesis, is still unanswered.

EXPERIMENT I. Establishment of an Animal Model for Unilateral Stimulation with Clinically Relevant Protocols

The experiment I aimed to establish a small animal model for unilateral hemispheric application of rTMS with clinically relevant stimulation protocols, and to characterize rTMS–induced changes in the expression of the proteins and gene transcription in the different brain regions.

Twenty-nine adult male Sprague–Dawley rats were subjected to a single session of unilateral rTMS with low– (1 Hz, n=12), high–frequency (20 Hz, n=12), or sham (n=5) stimulation groups. Low– and high–frequency groups were further divided into subgroups of 10–, 20–, and 30–minute application. Stimulation was applied to the left hemisphere using 25 mm figure–of–8 coil with intensity set at 50% of motor threshold. We also developed a hydraulic cooling system to enable repetitive stimulation. Five minutes after applying rTMS on the left hemisphere, we obtained the brain tissue from the left cortex, striatum, and contralateral cortex, which were analyzed respectively.

Levels of phosphorylation of Akt and endothelial nitric oxide synthase (eNOS), and vascular endothelial growth factor (VEGF) were measured using Western blot. Real–time PCR was conducted on arc, fos, akt1, bdnf, angpt, ntrk, tek, vegfa and pik3cg genes. One rat underwent micro–positron emission tomography (PET) using 2–[F–18] fluoro–deoxyglucose (18FDG) to confirm unilaterality of the stimulation.

In western blot, the stimulated cortex showed greater phospho–Akt expression than the contralateral cortex in both low– (optical density ratio, 4.5±3.3 vs. 1.0±0.8, P<0.05) and high–frequency (3.3±0.9 vs. 0.7±0.5, P<0.05) groups. Low–frequency rTMS reduced phospho–eNOS expression in the contralateral cortex (0.90 ± 0.31) compared to the sham (2.23 ± 0.67, P<0.05) and high–frequency (2.66 ± 0.44, P<0.05) groups. In real–time PCR, low–frequency group showed significantly lower level of akt, ntrk, tek and vegfa transcription in the stimulated cortex than in the contralateral cortex. The micro–PET showed that the local glucose metabolism of the stimulated cortex was increased.

EXPERIMENT II. Influence of rTMS on the Stroke Recovery

Seventy-nine adult male Sprague–Dawley rats were subjected to middle cerebral artery occlusion (day 0) and subsequently treated with low– (1 Hz), high–frequency (20 Hz), or sham stimulation on their lesioned hemispheres for 2 weeks. To label proliferating cells, animals (Sham, 20 Hz, and 1 Hz; n=3, 3, and 4, respectively) were injected intraperitoneally 100 mg/kg of 5’–Bromo–2′–deoxyuridine (BrdU) on day 3 through 14. Stimulation was applied using figure–of–8 coil with intensity set at 100% of motor threshold. Neurological function was evaluated performed on day 3, 10, and 17. Infarct volume, angiogenesis, angiogenic factor expression, and angiogenesis–related gene transcription were measured by histology, immunohistochemistry, Western blot, and real–time PCR, respectively. Brain tissue was harvested in the ischemic core (IC), ischemic border zone (BZ), and contralateral homologous cortex (CH).

The results of neurological functional tests were comparable between groups. Difference in the infarct volume was insignificant between groups (P=0.34). The
number of BrdU-positive cells was also comparable (P=0.43, 0.40, and 0.11 for IC, BZ, and CH, respectively). Optical density of angiopoietin1 and synaptophysin in the IC was significantly greater in the low-frequency than in the sham group (P=0.03 and 0.03, respectively). Real-time PCR revealed that low-frequency stimulation significantly increased transcriptional activity of Tie2 (tek), a receptor of angiopoietins, in IC (1.22±0.39 vs. 0.49±0.07, 1 Hz vs. Sham, respectively; mean fold change±SD, P=0.03). In contrast to the result in IC, transcriptional level of the mRNA of VEGF-a (vegfa) in CH was significantly reduced in 1-Hz group (0.61±0.16 vs. 1.01±0.18, P=0.04).

We established a small animal model of unilateral hemispheric rTMS and confirmed its differential molecular effects on the stimulated and contralateral cortices especially in reference to the angiogenic signaling pathways.

In early subacute phase of stroke, low-frequency rTMS induces changes in regulation of angiogenic mechanisms, including Tie2, Akt, and eNOS.

Future study will be required to reveal the differential effects of rTMS on diverse cell signaling pathways and neuronal activity and to determine optimal stimulation parameters.

* Note: The text above is the abstract of the thesis.
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