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Quantifying the Effect of Repetitive Transcranial Magnetic Stimulation in the Rat Brain by μSPECT CBF Scans

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ABSTRACT

Background: Repetitive transcranial magnetic stimulation (rTMS) is used to treat neurological and psychiatric disorders such as depression and addiction amongst others. Neuro-imaging by means of SPECT is a non-invasive manner of evaluating regional cerebral blood flow (rCBF) changes, which are assumed to reflect changes in neural activity.
Objective: rCBF changes induced by rTMS are evaluated by comparing stimulation on/off in different stimulation paradigms using microSPECT of the rat brain.
Methods: Rats ($n = 6$) were injected with 10 mCi of ^{99m}Tc-HMPAO during application of two rTMS paradigms (1 Hz and 10 Hz, 1430 A at each wing of a 20 mm figure-of-eight coil) and sham. SPM- and VOI-based analysis was performed.
Results: rTMS caused widespread significant hypoperfusion throughout the entire rat brain. Differences in spatial extent and intensity of hypoperfusion were observed between both stimulation paradigms: 1 Hz caused significant hypoperfusion ($P < 0.05$) in 11.9% of rat brain volume while 10 Hz caused this in 23.5%; the minimal t-value induced by 1 Hz was −24.77 while this was −17.98 due to 10 Hz. Maximal percentage of hypoperfused volume due to 1 Hz and 10 Hz was reached at tissue experiencing 0.03–0.15 V/m.
Conclusion: High-frequency (10 Hz) stimulation causes more widespread hypoperfusion, while 1 Hz induces more pronounced hypoperfusion. The effect of rTMS is highly dependent on the electric field strength in the brain tissue induced by the TMS coil. This innovative imaging approach can be used as a fast screening tool in quantifying and evaluating the effect of various stimulation paradigms and coil designs for TMS and offers a means for research and development.

Introduction

Repetitive transcranial magnetic stimulation (rTMS) is an emerging method for the non-invasive stimulation of the human cortex through the intact skull. A rapidly changing perpendicular magnetic field is generated by the currents in a rTMS coil, but it is the induced electrical field in the conducting brain caused by this varying magnetic field (B-field) that triggers depolarization or hyperpolarization of neuronal ensembles, by forcing the shift of free charges in the intra- and extracellular space of neuronal tissue [1]. rTMS is a promising treatment for a variety of neurological and psychiatric disorders, such as depression, phantom pain and noise, ischemic stroke, neuropathic pain, migraine and Parkinson's disease [2–8]. Despite these promising results, the precise mechanism of action of rTMS and the pathways affected due to it are unknown. Furthermore, the optimal stimulation parameters and coil design are still undetermined, hampering its therapeutic potential. There is an innumerate number of degrees of freedom in terms of possible combinations of stimulation frequency, duration, intensity, coil design, stimulation pattern, brain target etc. emphasizing the need for a fast research, development and screening tool in the evaluation of rTMS' neurophysiological effect of each of these parameters. Human studies are restricted due to ethical considerations, the difficulty in gathering large and homogenous patient groups and the high costs. Therefore, to explore rTMS in a systematic, flexible and reliable manner, miniaturization of rTMS for rodent brain studies is

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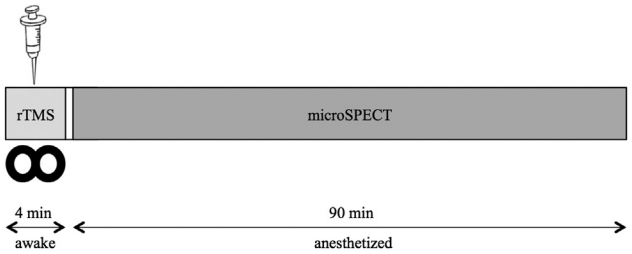

Figure 1. Protocol of one scan session. Before, during and after intravenous injection with 370 MBq ^{99m}Tc-HMPAO, rats received continuous rTMS (1 Hz or 10 Hz) or sham, while awake. Then, rats were anesthetized following uptake of the tracer and μSPECT (1.5 h) was started.

an indispensable and complementary addition to the human studies.

Moreover, neuro-imaging by means of Single Photon Emission Computed Tomography (SPECT) is a non-invasive technique to evaluate regional cerebral blood flow (rCBF) changes, which are assumed to reflect changes in neural activity [9–11]. Intravenously injected ^{99m}Tc-Hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO) distributes rapidly (<2 min) within the brain, representing perfusion at the time of injection and is assumed to reflect neuronal and interneuronal activity downstream from cell bodies and in distant input pathways [9,12]. Consequently, μSPECT is a useful tool to indicate alterations in the local (inter)neuronal activity that is provoked by rTMS and can be used to evaluate changes induced by different rTMS-paradigms and coil designs. Recently, SPECT scanners have also been successfully miniaturized to enter the preclinical arena allowing for a high spatial resolution with an acceptable sensitivity in rats and mice (μSPECT) [10,13,14].

In the current study, a voxel-of-interest (VOI)-based and statistical parametric mapping (SPM) analysis of stimulation-on versus stimulation-off (sham stimulation) μSPECT images was performed. Stimulation parameters were varied and effect on location, spatial extent and intensity of rCBF-changes were evaluated, in relation to the electrical field induced by the rTMS coil.

Methods

Animals

Male Wistar rats (250–300 g body weight; Harlan, the Netherlands) were treated according to guidelines approved by the European Ethics Committee (decree 86/609/EEC). The study protocol was approved by the Animal Experimental Ethical Committee of Ghent University Hospital (ECP 04/08 complement). The animals were kept under environmentally controlled conditions (12 h normal light/dark cycles, 20–23 °C and 50% relative humidity) with food and water *ad libitum*.

Experimental procedure

Before initiating the μSPECT scanning experiments, rats ($n = 6$) were trained during one week to accustom to awake and comfortable positioning in a silicone rat head mold (cfr. Section 3.). After the behavioral training, all rats underwent three μSPECT scans. Each μSPECT scan was separated in time by at least 48 h for sufficient radioactive decay between scans. For each rat, the three stimulation paradigms (i.e. sham, 1 Hz and 10 Hz) were presented in a randomized order. For each of the μSPECT scans, rats were first shortly (max. 10 s) and lightly anesthetized with isoflurane (2% mixture with medical O_2) to ensure accurate positioning of the head of the animal under the TMS coil in the silicone rat mold (cfr. Section 3.). Minimum 5 min was allowed between the arousal from anesthesia and the initiation of rTMS or sham. After 2 min of rTMS or sham stimulation, rats were, while awake and still under the silicone mold, intravenously injected with 370 MBq ^{99m}Tc-HMPAO (Ceretec, GE Healthcare, UK). Stimulation was not interrupted during injection and was continued during the entire uptake of the ^{99m}Tc-HMPAO tracer (for at least 2 min) following injection. After discontinuation of stimulation (or sham), rats were removed from the silicone mold and anesthetized with a mixture of isoflurane (2–5% isoflurane and O_2) and placed onto the bed of the scanner. Body temperature was kept constant with a heating resistance mat and respiration frequency was measured throughout the duration of the μSPECT scan. The experimental protocol is illustrated in Fig. 1.

Positioning of the TMS coil

Fixed position of the TMS coil in relation to the rat brain was ensured by using a custom-made silicone-mold (Belosil, Equator, Belgium), with an impression of the TMS coil at one side and an impression of the rat head at the other side (Fig. 2). The center of the TMS coil was positioned ±0.5 cm to the left, ±0.5 cm anterior to bregma and the distance between the coil and the rat's head was kept as small as possible without there being direct contact between skin and coil (±0.5 cm).

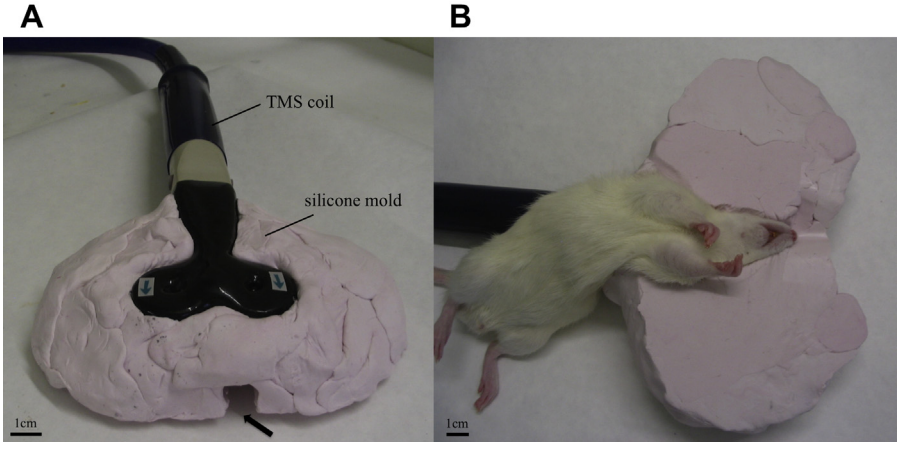

Figure 2. To ensure the exact position of the rTMS coil in relation to the rat brain, a silicone mold (pink) was used with an impression of the rTMS coil at one side (A) and an impression of the rat head at the other side (B). The animal was fully awake under the mold; therefore a breathing hole was foreseen (arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)