Safety Study of Transcranial Static Magnetic Field Stimulation (tSMS) of the Human Cortex

A. Oliviero , M.C. Carrasco-López , M. Campolo , Y.A. Perez-Borrego , V. Soto-León , J.J. Gonzalez-Rosa , A.M. Higuero , B.A. Strange , J. Abad-Rodriguez , G. Foffani

A B S T R A C T

Background: Transcranial static magnetic field stimulation (tSMS) in humans reduces cortical excitability.
Objective: The objective of this study was to determine if prolonged tSMS (2 h) could be delivered safely in humans. Safety limits for this technique have not been described.
Methods: tSMS was applied for 2 h with a cylindric magnet on the occiput of 17 healthy subjects. We assessed tSMS-related safety aspects at tissue level by measuring levels of neuron-specific enolase (NSE, a marker of neuronal damage) and S100 (a marker of glial reactivity and damage). We also included an evaluation of cognitive side effects by using a battery of visuomotor and cognitive tests.
Results: tSMS did not induce any significant increase in NSE or S100. No cognitive alteration was detected.
Conclusions: Our data indicate that the application of tSMS is safe in healthy human subjects, at least within these parameters.

Introduction

Non-invasive brain stimulation (NIBS) techniques have made an important contribution to cognitive neuroscience and have been proposed as a treatment for neuropsychiatric disorders [1]. Repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) are commonly used for NIBS in humans and animals. Recently we described that the application of transcranial static magnetic field stimulation (tSMS) in humans reduces the output of motor cortex – tested using TMS – for a few minutes after the end of stimulation [2]. Reduced motor output after tSMS can be explained by reduced motor cortex excitability. These results have been recently replicated by a different group [3]. tSMS using small magnets may thus be a promising tool to modulate cerebral excitability in a non-invasive, painless and reversible way.

Static magnetic fields, unlike time-varying magnetic fields, are not associated with induced electric currents and have been shown to influence a variety of biological systems [4]. A number of studies suggest that static magnetic fields act primarily at the synapse and alter the function of membrane ion channels [5], and the application of static magnetic fields to different animal preparations seem to have an effect that outlasts the time of stimulation [6]. When tSMS is applied in humans, the cortex is at least 2 cm away, so most of the strength of the magnetic field will not reach the target. The recommended limits by the World Health Organization (WHO) about safe exposure to static magnet fields are "time weighted average of 200 mT during the working day for occupational

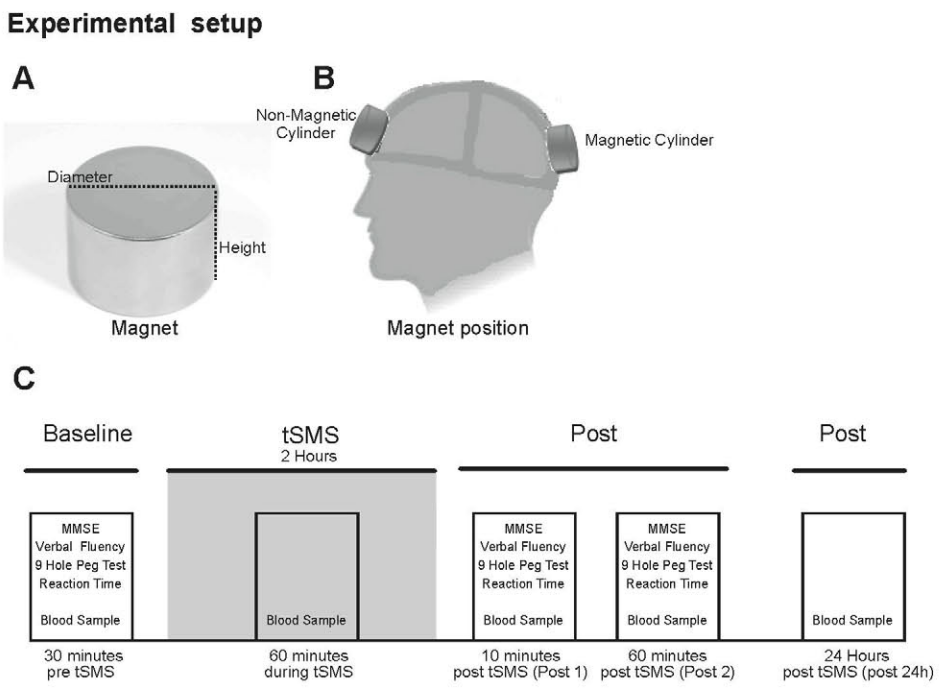

Figure 1. Schematic representation of experimental set up. A. Magnet (MAG60r, Neurek SL, Toledo, Spain). B. Magnet location over the occipital cortex (Oz location of the 10-20 EEG international system), and an additional non-magnetic cylinder over the frontal cortex (Fpz location of the 10-20 EEG international system) to counterbalance the weight of the occipital magnet. C. Time course of the experiment. The experimental protocol is divided in five time points: baseline, during tSMS, post1, post2 and 24 h.

exposure" (i.e. 8 h/day, 5 days/week) (http://www.who.int/peh-emf/publications/facts/fs299/en/index.html). With the magnets we normally use in our lab (MAG45r and MAG60r; Neurek SL, Toledo, Spain), at 2 cm from the axis (the approximate distance from the scull to the cortex) the magnetic field is around 150–200 mT [7], so within the safe limit proposed by the WHO. Moreover, many studies about safety of magnetic resonance imaging (MRI) techniques consider safe an exposure to magnetic fields >8T for experimental sessions that can last hours [8]. On the other hand, it cannot be excluded that the different gradient shape and/or the different magnetic field orientation may have different safety profiles.

The purpose of this study was to test the safety of prolonged tSMS (2 h) of the occipital cortex in healthy volunteers to establish safety guidelines for future tSMS experiments and therapeutic trials. We tested the effects of tSMS on a cellular level, by measuring serum levels of neuron-specific enolase (NSE) and protein S-100 [9] – sensitive markers for neural or glial brain damage – in healthy volunteers before, during and after tSMS. In order to provide further evidence for the safety of tSMS, a battery of neuropsychological tests were performed to exclude cognitive adverse effects. Specifically, we chose the Mini Mental State Exam (MMSE) as an evaluation of global cognitive state [10], the Nine-Hole peg test (NHPT) to evaluate a fine motor task and visuomotor coordination, a two-choice reaction time test to assay attentional levels (and again visuomotor coordination). Verbal fluency, a cognitive process considered to be primarily frontal lobe-dependent, was tested to determine cognitive function associated to a brain location distant from the stimulated area [11].

Methods

Subjects

Seventeen healthy volunteers participated in this study (10 males; mean age 34.4 ± 7.3 years; age range 24–45 years). Exclusion criteria were significant medical or psychiatric illness, pregnancy and concurrent use of neuroactive drugs. We also excluded individuals with pacemakers, brain stimulators, medication pumps or any type of metal object in the head including eyes – except for dental appliances or fillings – which might pose a physical hazard during tSMS. All subjects but one were right handed according to the Edinburgh handedness inventory [12]. The study was approved by the local ethical committee. Informed consent was obtained from all subjects.

Experimental set-up

Experimental set-up is shown schematically in Fig. 1.

tSMS procedures

To deliver tSMS we used a cylindrical Nickel-plated (Ni–Cu–Ni) NdFeB magnet of 60 mm diameter, 30 mm of thickness and a weight of 370 g (MAG60r, Neurek SL, Toledo, Spain). North magnetic field polarity was used (i.e. north pole was placed over the scalp). During the experiment, all subjects had tSMS over the occipital cortex (Oz location of the 10-20 EEG international system), and an additional non-magnetic cylinder was located over the frontal cortex (Fpz location of the 10-20 EEG international system) and remained fixed during the whole experiment to counterbalance the weight of the occipital cylinder. The non-magnetic cylinder was a steel nickel-coated cylinder, had the same size, a weight of 368 g similar to the MAG60r (MAG60s, Neurek SL, Toledo, Spain). The cylinders were held in place with a leather strapping system (MAGlet60+, Neurek SL, Toledo, Spain). The tSMS was applied for 2 h.

Blood samples for determining NSE and S-100

We measured serum concentrations of NSE and S100, as sensitive markers of neuronal damage and glial activation, respectively. Blood samples were taken in EDTA-free tubes from each subject at

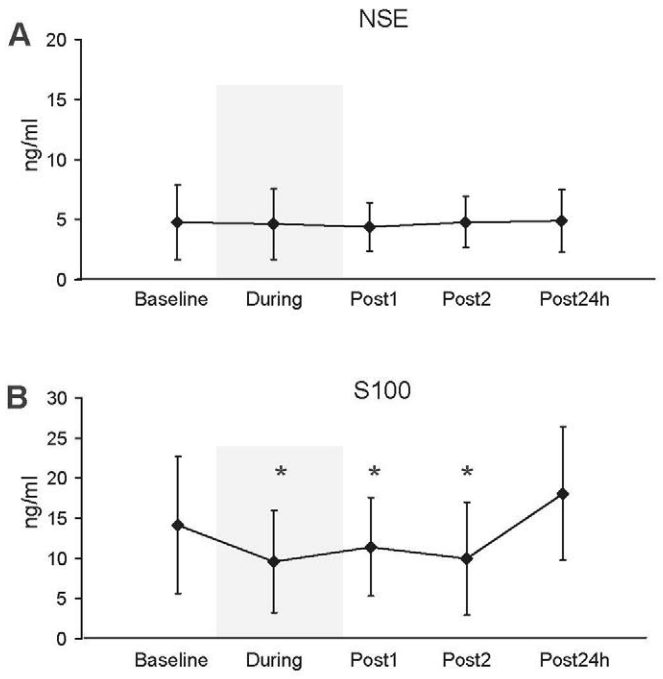

Figure 2. Time course and concentration of serum levels of neuron-specific enolase (NSE) and protein S-100. A. Time course of NSE concentration (ng/ml). B. Time course of S-100 concentration (ng/ml). * = $P < 0.05$ (paired t-test). Error bars indicate standard deviations.

baseline before the tSMS treatment was started (baseline), after 60 min during the treatment (during), immediately after (post1), 60 min after (post2), and 24 h (only 13 subjects) after the end of tSMS (post 24 h). A peripheral venous catheter in the left cubital fossa (or dorsum of the forearm) was maintained throughout the experiment to allow serial blood drawing (with the exception of the 24 h blood sample that was obtained via new venipuncture). Samples were left 1 h at room temperature and 3 more hours at 4 °C to induce clot retraction. Crude sera were obtained by low-speed centrifugation (800×g), and cleared-up by medium-speed centrifugation (5000×g). Aliquots were preserved at −20 °C until used. Serum NSE measurements were performed using the Quantikine® ELISA kit for Human Enolase 2/Neuron-specific Enolase (R&D Systems). S100 quantification was performed using the Human S100B ELISA kit (Millipore). In both cases, manufacturers' instructions were followed.

In one subject, it was not possible to obtain blood samples during tSMS and in post1. In another subject, it was not possible to obtain blood samples in post1. Two samples during tSMS were excluded from the S100 analyses as outliers. Two additional samples in post2 were excluded from the NSE analyses because of a visually evident hemolysis (these samples were included in the S100 analyses). Thus, the final serum sample size (n) was: baseline $n = 17$, during $n = 16$ ($n = 14$ for S100), post1: $n = 15$, post2: $n = 15$ ($n = 17$ for S100), 24 h: $n = 13$.

Assessment of cognitive and motor performance

To detect possible side effects, the following investigations were performed before and after the tSMS testing on the same day. The neuropsychological test battery consisted of the Folstein Mini-Mental Status Examination (MMSE) [10], and a phonemic Verbal Fluency Test [11] during which the letters F, A and S were presented with subjects required to say as many words beginning with the letter as possible during a 60 s period. Order of letter presentation was counterbalanced across subjects and conditions – pre- and post-testing. Letter fluency scores were based on the number of correct items generated across the three letter conditions. The Nine-Hole peg test (NHPT) was performed to test fine motor skills (manipulative dexterity) and complex visuomotor coordination with the right hand (this test was not performed with the left hand due to the presence of venous catheter). A two-choice Reaction Time (RT) test measured visuomotor speed. The subject was instructed to press the corresponding key to an arrow direction (pointing left or right) appearing on a screen as quickly and accurately as possible. The test consisted of three blocks, each with 120 trials. RTs and error rates were recorded, with the latter determined as a measure of accuracy to look for a possible trade-off between speed and accuracy.

Statistical analyses

Blood measures (NSE and S100) were compared to baseline with paired t-tests. Note that we purposely did not correct for multiple comparisons in order to conservatively maximize the possibility to find significant changes ($P < 0.05$). One-way ANOVA was employed to corroborate any unexpected significant findings. Cognitive measures were assessed with paired t-tests, except for data from the two-choice reaction time task, which were analyzed with a two-way repeated-measures ANOVA (with factors hand and time) to fully exploit the statistical power given by two hands. Values are given as mean ± standard deviation.

Results

The 2-h tSMS session was well tolerated by all 17 subjects. None of the subjects needed to interrupt or terminate the session due to side effects. One subject reported mild headache and numbness at the end of the tSMS, but was able to complete the study.

Blood tests

NSE concentrations were not affected by tSMS (Fig. 2A), neither during the stimulation (paired t-test, $P = 0.74$, $n = 16$), immediately after (post1, $P = 0.58$, $n = 15$), 1 h after (post2, $P = 0.98$, $n = 15$), nor 24 h after ($P = 0.92$, $n = 13$). The average NSE concentration in post2, which represents the most relevant time point from a safety perspective, was 4.79 ± 2.14 ng/ml (baseline: 4.75 ± 3.12 ng/ml).

S100 concentrations did not increase with tSMS (Fig. 2B). Interestingly, S100 concentrations were actually significantly decreased both during tSMS ($P = 0.0014$, $n = 14$), immediately after tSMS (post1, $P = 0.0166$, $n = 15$) and 1 h after (post2, $P = 0.0083$, $n = 17$), but not 24 h after ($P = 0.33$, $n = 13$). The average S100 concentration in post2 was 9.98 ± 7.05 ng/ml (baseline: 14.11 ± 8.54 ng/ml). These decrease in S100 concentrations remained significant when the data were analyzed with a more conservative one-way ANOVA, either using repeated-measures design with only the 11 subjects for which all time points are available ($F(4,40) = 5.7$, $P = 0.0009$), or an independent-measures design with all subjects and all available time points ($F(4,71) = 3.2$, $P = 0.0178$).

Cognitive tests

In the two-choice reaction time task, RTs were faster with the right hand, as expected (two-way ANOVA, hand: $F(1,16) = 24.0$, $P = 0.0002$), but tSMS did not induce any significant effect (time: $F(2,32) = 1.5$, $P = 0.24$; interaction: $F(2,32) = 1.5$, $P = 0.24$; Fig. 3A). The number of errors made by the subjects during the task was slightly higher with the right hand (hand: $F(1,16) = 4.8$, $P = 0.0440$) and again was not affected by tSMS (time: $F(2,32) = 1.3$, $P = 0.29$; interaction: $F(2,32) = 0.9$, $P = 0.41$; Fig. 3B).

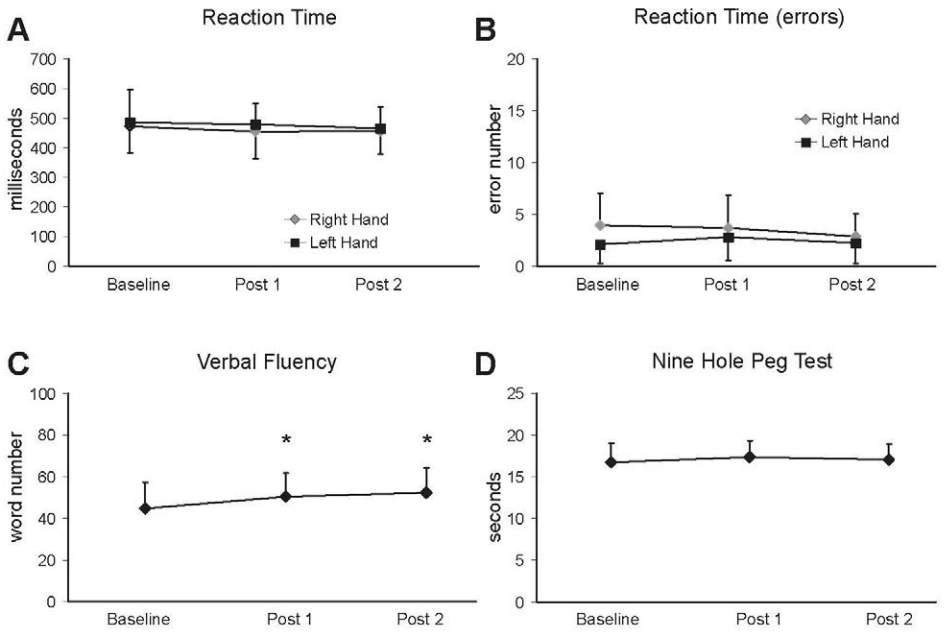

Figure 3. Time course of the cognitive assessment and motor performance. A. Time course of the Reaction Time (in milliseconds). B. Time course of the number of errors. C. Verbal Fluency Test. Time course of the total words counting. D. Time course of the Nine Hole Peg Test. * = $P < 0.05$ (paired t-test). Error bars indicate standard deviations.

Word fluency was not impaired by tSMS (Fig. 3C). There was actually an improvement at post1 (paired t-test, $P = 0.0091$) and at post2 ($P = 0.0026$), which was confirmed by a more conservative one-way ANOVA ($F(2,32) = 7.9$, $P = 0.0016$), probably reflecting a slight learning effect.

The time to perform the NHPT was not affected by tSMS ($P > 0.22$; Fig. 3D). MMSE did not change after tSMS (baseline = 29.3 ± 1.1; post1 = 29.6 ± 0.6; post2 = 29.6 ± 0.8; $P > 0.33$, data not shown in figures).

Discussion

The principal finding of this study was that 2 h of tSMS over the occipital cortex appears to be a safe procedure.

There is currently no gold standard for assessing safety for NIBS protocols. At least three types of safety studies can be found in the NIBS literature: (i) studies that evaluate safety a posteriori (similarly to phase 4 clinical trials), assessing the incidence of possible adverse effects in large cohort of patients/subjects that already received specific NIBS protocols (e.g. Refs. [13–15]); (ii) studies that evaluate safety of a known NIBS protocol for its application to a new indication (similarly to phase 1 repositioning clinical trials), assessing the incidence of possible adverse effects in a small cohort of patients (e.g. Refs. [16,17]); (iii) studies assessing safety of a recently-introduced NIBS technique in healthy subjects (e.g. Ref. [18]). Our study belongs to the third group.

The serum level of NSE is a marker of neuronal damage that has been previously used to assess NIBS safety [18–20]. No NSE changes have been reported with cathodal tDCS in 5 normal subjects [19], rTMS in 14 depressed patients [20] and tACS in 8 normal subjects [18]. We also found no changes in NSE levels either during or after tSMS in our experiments. Even though negative results always need to be considered with caution, our study was conservatively designed to achieve higher statistical power compared to previous NIBS safety studies ($n = 15$ for NSE at the most relevant time point, i.e. post2), in order to minimize the risk of type II error.

We also measured serum levels of S100, a marker of glial damage. Only one of the above NIBS safety studies assessed S100 levels, finding no changes [20]. Our *a priori* hypothesis was that if S100 increased it would have suggested a glial damage and a safety hazard [21]. Surprisingly, we found that the S100 concentration decreased during and after the tSMS. This unexpected result might intriguingly suggest reduced glial reactivity, which could reflect a certain level of tissue protection [21]. However, it needs to be stressed that this is not a definitive finding, and should be confirmed in sham-controlled experiments in order to exclude non-specific effects [22], which is beyond the scope of the present work. Even though we do not intend to make any strong claims about this unexpected S100 decrease, it does convincingly show that S100 does not increase, strongly supporting the safety of our tSMS protocol.

We observed no significant impairment in performance of visuomotor or cognitive tests. These tests are not typically included in previous NIBS safety studies, but are particularly important in our protocol given the significant behavioral effects recently reported with the application of tSMS to the visual cortex in monkeys [23]. It is important to note that we intended to exclude gross behavioral changes that would be hazardous for the everyday living. Indeed, because tSMS reduces cortical excitability, tSMS is expected to induce subtler behavioral effects associated with the execution of more complex visuomotor/cognitive tasks.

In the present study, we did not perform EEG recordings, which were instead used in previous NIBS safety studies (e.g. Ref. [18]). The rationale for using EEG recordings to assess NIBS safety is because of the increased risk of seizures with protocols that increase cortical excitability. tSMS was shown to *reduce* cortical excitability both in the sensorimotor cortex of humans [2,3,24], and in the visual cortex of cats and monkeys [23]. Decreased cortical excitability should reduce rather than increase the risk of seizures. In fact, it has been demonstrated that tSMS reduces epileptic activity in animal models [25,26]. We therefore argue that EEG recordings are not necessary to establish tSMS safety.

Overall, the purpose of this study was to establish if tSMS could be delivered safely in humans in order to provide safe stimulation patterns for future experimental and therapeutic trials. We confirmed the safety of static magnet fields within the

recommended limits reported by the WHO (http://www.who.int/peh-emf/publications/facts/fs299/en/index.html). However, it cannot be excluded that stimulation parameters different from ours (different gradient shape, magnetic field orientation, duration, etc.) might have different safety profiles.

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