European Journal of Neuroscience, Vol. 24, pp. 2961–2966, 2006 doi:10.1111/j.1460-9568.2006.05154.x

Extending lifetime of plastic changes in the human brain

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Keywords: cortical plasticity, saccades, theta burst protocol, transcranial magnetic stimulation

Abstract

The ability of the brain to adjust to changing environments and to recover from damage rests on its remarkable capacity to adapt through plastic changes of underlying neural networks. We show here with an eye movement paradigm that a lifetime of plastic changes can be extended to several hours by repeated applications of theta burst transcranial magnetic stimulation to the frontal eye field of the human cortex. The results suggest that repeated application of the same stimulation protocol consolidates short-lived plasticity into long-lasting changes.

Introduction

The role of activity-dependent synaptic plasticity in the adaptation of the brain to new environmental changes is a central issue in neuroscience. During the past 30 years, evidence for long-lasting functional plastic events of synapses, such as long-term potentiation (LTP) or long-term depression (LTD) was found in several animal species (Malenka & Bear, 2004). LTP can be defined as a long-lasting increase in synaptic strength following the delivery of a short-train of high-frequency stimulation (50–200 Hz), but many other induction protocols have been described (Tsodyks, 2002). LTP has been most extensively investigated in the hippocampus, but investigations have also been made in the neocortex (Fox, 2002). *In vitro*, the lifetime of LTP is up to several hours, whereas *in vivo* preparations LTP may last up to weeks or months (Abraham, 2003). LTD is defined as a long-lasting decrease in synaptic strength and has been demonstrated in the cerebellum, hippocampus and neocortex. Induction of LTD typically requires prolonged low-frequency stimulation (1–5 Hz) (Bliss & Collingridge, 1993).

A non-invasive approach to analyse human cerebral plasticity is transcranial magnetic stimulation (TMS). The application of a brief magnetic pulse on the scalp induces a current flow in the brain, and this flow is able to inhibit or excite restricted cortical areas. The application of trains of pulses, so-called repetitive TMS (rTMS), influences the functioning of the brain beyond the time of stimulation (Kobayashi & Pascual-Leone, 2003). LTP-like or LTD-like mechanisms have been proposed to occur after rTMS (Hallett, 2000; Maeda *et al*., 2000). Similar to stimulation protocols used in animals, rTMS can be applied in humans using low- or high-stimulation frequencies. Generally, low-frequency rTMS (1 Hz) over the motor cortex reduces neural activity, whereas high-frequency rTMS (> 5 Hz) increases neural activity (for a review, see Hallett, 2000). Repetitive TMS was also used in clinical trials to treat different neurological and psychiatric disorders (Hallett, 2000; Lisanby *et al*., 2002; Kobayashi & Pascual-Leone, 2003; Martin *et al*., 2003; Hausmann *et al*., 2004; Rossi & Rossini, 2004). The rationale was that abnormally decreased or increased levels of cortical activity might be normalized by inhibiting or exciting the brain by using rTMS. However, TMS-induced behavioural changes have only been transient, which limited the therapeutic benefit.

A new type of rTMS protocol has recently been applied to circumvent these limits and to extend the lifetime of plastic cortical changes (Huang *et al*., 2005). In animals, long-lasting LTP can be induced with theta burst stimulation, a high-frequency stimulation protocol that is spaced at a frequency that mimics the theta wave, a spontaneous 5–7 Hz neural rhythm (Abraham, 2003). In analogy, Huang and colleagues (Huang *et al*., 2005) used a modified theta burst stimulation protocol, and found that a short and intermittent application of theta burst TMS facilitated the motor-evoked potential, whereas a long, continuous application of theta burst TMS suppressed the motor-evoked potential for up to 1 h. Given the facilitatory effects of theta burst TMS when applied in a short, intermittent protocol, and the inhibitory effects when applied in a longer-lasting, continuous protocol, it was speculated that theta burst TMS may induce a mixture of LTP and LTD, respectively (Di Lazzaro *et al*., 2005; Huang *et al*., 2005).

The aim of the present study was to extend the lifetime of plastic changes in the human brain induced by theta burst TMS further. If induction of very long-lasting plastic changes was possible, this would clearly enhance the future role of TMS by increasing the therapeutic benefit (Paulus, 2005). In animal models, activity-dependent enhancement of synaptic transmission can be achieved by repeated trains of theta burst stimulations. Depending on the number of theta burst stimulation trains, the persistence of LTP *in vivo* may be prolonged up to days (Bliss & Gardner-Medwin, 1973; Barnes, 1979; Jeffery *et al*., 1990; Abraham *et al*., 1993; Abraham *et al*., 2002). We hypothesized that repeated trains of theta burst TMS should be capable of extending the lifetime of the plastic modifications in the human brain through consolidation mechanisms as proposed in the cascade model of

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Received 3 July 2006, revised 29 August 2006, accepted 31 August 2006

animals (Bailey *et al.*, 1996; Frey & Morris, 1997), and we would expect that repeated trains of theta-burst rTMS generate a disproportionately longer-lasting effect than obtained from a single train.

We used the oculomotor system as a model, as it has the advantage that quantifiable parameters such as saccade latency, amplitude and velocity can be easily and precisely assessed (Müri *et al.*, 2005). We stimulated the frontal eye field (FEF) that is critically implied in saccade triggering (Pierrot-Deseilligny *et al.*, 1995; Tehovnik *et al.*, 2000), and assessed the latencies of horizontal saccades in response to a visual target. This experimental design is suitable to investigate behavioural effects of rTMS as previously shown (Nyffeler *et al.*, 2006).

Materials and methods

Subjects

The study was approved by the ethical committee of the State of Bern and consistent with the latest Declaration of Helsinki. Prior to participation, all subjects gave informed written consent. The subjects were six healthy, right-handed males with normal or corrected-to-normal vision (age range 29–59 years). Five of them participated in the first and second experiments (one train of theta burst TMS). In the third experiment (two trains of theta burst TMS), one subject was not available and therefore substituted. In the fourth experiment, one subject was stimulated with four trains of theta burst TMS.

Saccade paradigm

Each trial started with a central fixation point. After pseudo-randomized durations between 2000 and 2900 ms, a lateral target with unpredictable amplitude between 4 ° and 16 ° from the central fixation point was shown for 80 ms. The instruction was to make a saccade to the position where the target was shown, and then to fixate again the central fixation point. Subjects were seated in total darkness with the head fixed on a chin rest to avoid head movements, 5–10 min were allowed for dark adaptation. Eye movements were measured with an infrared corneal reflection device (Iris Skalar, Delft, the Netherlands) with a spatial resolution of 0.1 ° and a sample rate of 1000 Hz. The digitized signals were stored on the computer for off-line analysis.

TMS procedure

A TMS stimulator (MagPro, Medtronic Functional Diagnostics, Skovlunde, Denmark) was used to generate repetitive biphasic magnetic pulses. Magnetic pulses were delivered with a figure-eight-coil (Magnetic Coil Transducer MC-B70, Medtronic) with an outer radius of 50 mm.

In the first experiment, one stimulus train of theta burst TMS (200 bursts, each burst consisting of 3 pulses at 30 Hz, repeated at intervals of 100 ms) was applied over the right FEF. The FEF was localized according to previously described procedures (Müri *et al.*, 1991; Ro *et al.*, 1999). In brief, stimulating the right motor cortex with single pulses determined the individual motor threshold by corresponding muscle twitching of the subject's relaxed small hand muscles. The coil was then moved anterior to the hand area, 2 cm on average. The handle of the coil pointed backwards (45 ° angle to the sagittal line). In the second experiment, which served as a control condition, the vertex was stimulated (the centre of the coil was held over the vertex and the handle pointed backwards). During stimulation, the coil was held by the examiner and subjects were asked to keep their eyes closed. Theta burst TMS was delivered at 80% of the subject's motor rest threshold. The first and second experiments (stimulation of the FEF and stimulation of the vertex) were separated by at least 1 week. After theta burst TMS application, saccade trials were tested in blocks. In each block, the recording device was calibrated initially and then 42 saccades were tested, the direction of the saccades being pseudo-randomized to obtain the same number of leftward and rightward saccades. In the first and second experiments, the blocks were performed immediately after theta burst TMS application, followed by recordings 10, 20, 30 and 60 min later. Between measurements, subjects were free to move their eyes and left the laboratory for 30–60 min. Finally, in a non-TMS condition, the same paradigm was performed without stimulation.

In the third experiment, two trains of theta burst TMS were applied over the right FEF with an interval of 15 min. The decision to choose a 15-min interval was based on the results of the first experiment and on LTP protocols used in animals, where it has been shown that trains spaced in the order of 10-min intervals generate more persistent LTP than massed delivery (Abraham, 2003). Saccades were measured 0, 10, 20, 30, 60, 80, 110, 140, 170 and 200 min after the first train. During the 30-min intervals subjects left the laboratory. There were at least 3 weeks between the first, second and third experiments.

In the fourth experiment, four trains of theta burst TMS were applied over the FEF in one subject (0, 15, 60 and 75 min). Saccades were measured 0, 10, 20, 30, 50, 80, 170, 230, 290, 350, 410, 470, 530, 590, 650 min and finally 24 h after the first stimulation. During the 30-min intervals the subject left the laboratory.

Data analysis

Mean latencies for saccades of the non-TMS condition were calculated for each subject. Latencies were then standardized to the non-TMS values, i.e. for a given individual, the percentage of increase in saccade latency was calculated for each saccade with the following formula: $100\% \times [(\text{Latency TMS}/\text{mean latency without TMS}) - 1]$. Thus, a value of 0% means no TMS effect.

In the first and second experiments, the statistical analysis was based on a repeated-measures ANOVA with the variable 'increase in latency' as the dependent variable and the categorical factors 'block' (no stimulation, 0 min, 10 min, 20 min, 30 min and 60 min), 'direction' (leftward vs. rightward saccades) and 'stimulation site' (FEF vs. vertex). Bonferroni-corrected *post-hoc* comparisons between FEF and vertex stimulation were performed with least-squares-means for blocks (Statistica 6.0, StatSoft, Tulsa, Oklahoma, USA). To evaluate whether the observed increase of saccade latency after theta burst stimulation of the FEF is an effect of reduced attention or fatigue, two additional parameters were analysed: (1) the saccade amplitude–velocity relationship; and (2) the mean peak velocity over the different blocks. Fatigue and reduced attention affect saccade parameters such as latency and velocity, in particular peak velocity of saccades (Schmidt *et al.*, 1979; Groner & Groner, 1989; Zils *et al.*, 2005). The relationship between amplitude and peak velocity of saccades is described in the so-called main sequence (Bahill *et al.*, 1975). Fatigue or reduced attention results in a breakdown of the main sequence. For saccade amplitudes smaller than 20 ° (such as those we tested), the peak velocity–amplitude relationship is linear (Bahill *et al.*, 1975; Leigh & Zee, 2006). If fatigue or reduced attention would be the reason for the increase of saccade latency then the linear regression after stimulation should be different from that without stimulation.

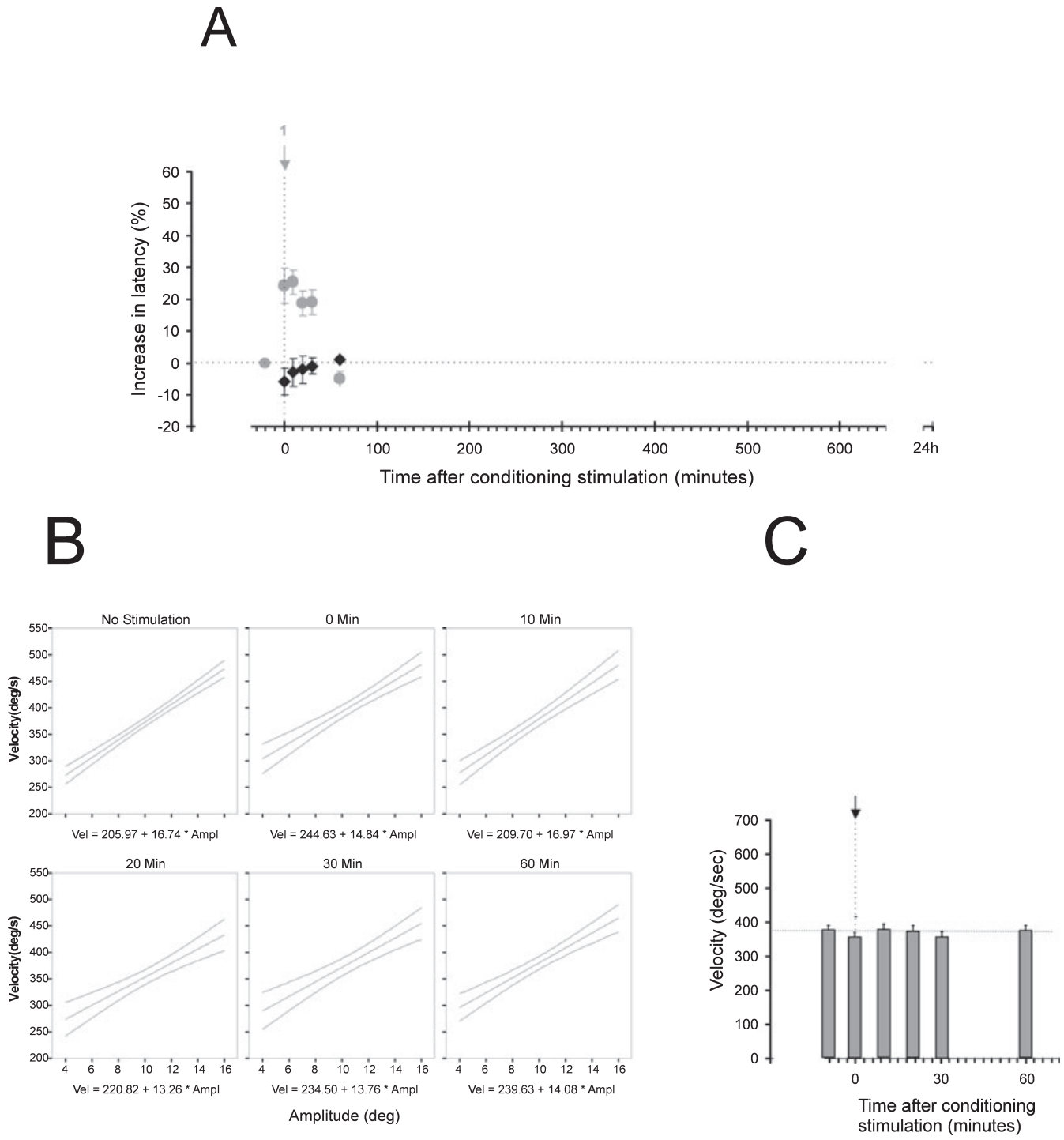

FIG. 1. The effect of a single train of theta-burst TMS over the FEF or vertex is shown. (A) Average latency increase in percent of the initial saccadic latency (error bar: SEM) induced by a single theta-burst train over the FEF (filled circles, arrow indicates timing of stimulation) and the vertex (filled diamonds). (B) Main sequence analysis (linear regression) of velocity amplitude relationship for no stimulation and the different blocks after theta burst stimulation. Stimulation did not change regression parameters significantly. (C) Mean peak saccade velocity for saccades without stimulation and after theta burst stimulation. There was no significant reduction of peak velocity during the experiment.

For the third and fourth experiments, repeated-measures ANOVA were performed with the variable 'increase in latency' and 'latency', respectively, as dependent variables and the categorical factor 'block', 'direction' (leftward vs. rightward saccades). Bonferroni-corrected *post hoc* comparisons between stimulation and no stimulation were performed with least-squares-means for blocks. For the fourth experiment, analysis of mean peak velocity was also performed.

Because none of the four experiments revealed a significant effect of direction, left- and rightward saccades were pooled together for the presentation of the results.

Results

The results of the first and second experiments suggest that the behavioural effect of theta burst TMS was inhibitory (i.e. delaying saccade triggering) and specific to the FEF region: Fig. 1A shows that immediately after a single train of theta burst TMS over the FEF, the saccade latency increased by about 25% (filled circles), i.e. the saccade triggering was delayed. Saccade latency started to decay after 10 min and returned to baseline level after the next 20 min. Vertex stimulation, which served as a control site to evaluate non-specific theta burst TMS effects, had no significant effect on saccade latency, resulting in changes of latency between −6% and 1% (Fig. 1A, filled diamonds). Statistical analysis using repeated-measures ANOVA showed significant main effects for stimulation site, i.e. FEF and vertex ($F_{1,386} = 188.31$, $P < 0.0001$) and block ($F_{4,1544} = 20.33$, $P < 0.0001$). Bonferroni-corrected *post hoc* comparisons showed significant differences for the blocks obtained at 0, 10, 20 and 30 min (for all $P < 0.0001$). Figure 1B revealed that the main sequence is not affected by the theta burst stimulation. Furthermore, Fig. 1C shows that the mean peak velocity after stimulation was not significantly reduced. Taking these two results together, fatigue or reduced attention as an explanation for the increased saccade latency is very improbable.

In the third experiment, a second conditioning stimulus train of theta burst TMS led to an additional increase in saccade latency up to 50% (Fig. 2, filled circles). In addition, this effect lasted for much longer, i.e. for over 170 min, before the latency decreased back to baseline level (repeated-measures ANOVA: significant main effect for block ($F_{12,4005} = 81.09$, $P < 0.0001$). Bonferroni-corrected *post hoc* comparisons demonstrated significant differences for the blocks obtained at 0, 10, 20, 30, 60, 80, 110, 140 (for all, $P < 0.0001$) and 170 min ($P = 0.0002$) compared with no stimulation. These results show that two pluses generate a disproportionately longer-lasting effect than obtained from a single train.

Finally, for the fourth experiment, Fig. 3A shows that four separate stimulus trains increased latency for over 650 min, while the magnitude of latency change did not increase any further (repeated-measures ANOVA: significant main effect for block ($F_{16,4856} = 78{,}18$, $P < 0.0001$). Bonferroni-corrected *post hoc* comparisons showed significant differences for the blocks obtained from 0 to 650 min ($P < 0.0001$) compared with no stimulation. The analysis of mean peak velocity (Fig. 3B) shows that the mean peak velocity after stimulation was not significantly reduced for the entire evaluation period.

Discussion

Our results provide evidence that repeated trains of theta burst TMS over the FEF generate disproportionately longer-lasting inhibitory effects on saccade triggering than obtained from a single train. Repeated stimulation apparently consolidates the plastic changes, preventing the oculomotor system to relax back to the initial state, even though the subjects were allowed to move their eyes between the measurements. It is known from animal experiments in the Xenopus retino-tectal system that spontaneous activity disrupts activity-induced synaptic modifications. This susceptibility to reversal of synaptic modification could be reduced by repeated theta burst stimulation (Zhou *et al.*, 2003). It is now generally accepted that the number of theta burst trains delivered determines how long effects will persist (Abraham, 2003). Brief forms of potentiation in the order of seconds to minutes after short delivery of theta burst stimulation are distinct from LTP and rely on transitory calcium changes in the presynaptic terminal (Zucker & Regehr, 2002). Longer forms of potentiation can be obtained with more trains of theta burst stimulation and have been divided into two mechanistically distinct phases (Frey & Morris, 1997): 'early' LTP that lasts less than 3 h; and 'late' LTP that lasts longer than 3 h. 'Early' LTP is depending on post-translational modifications such as protein phosphorylation, whereas 'late' LTP requires transcription and translational processes that may play a role in structural modifications (Bailey *et al.*, 1996; Frey & Morris, 1997). This latter consolidation phase of LTP (e.g. Hoffman *et al.*, 2002) may last tens of minutes and would correspond to the delayed peak in the

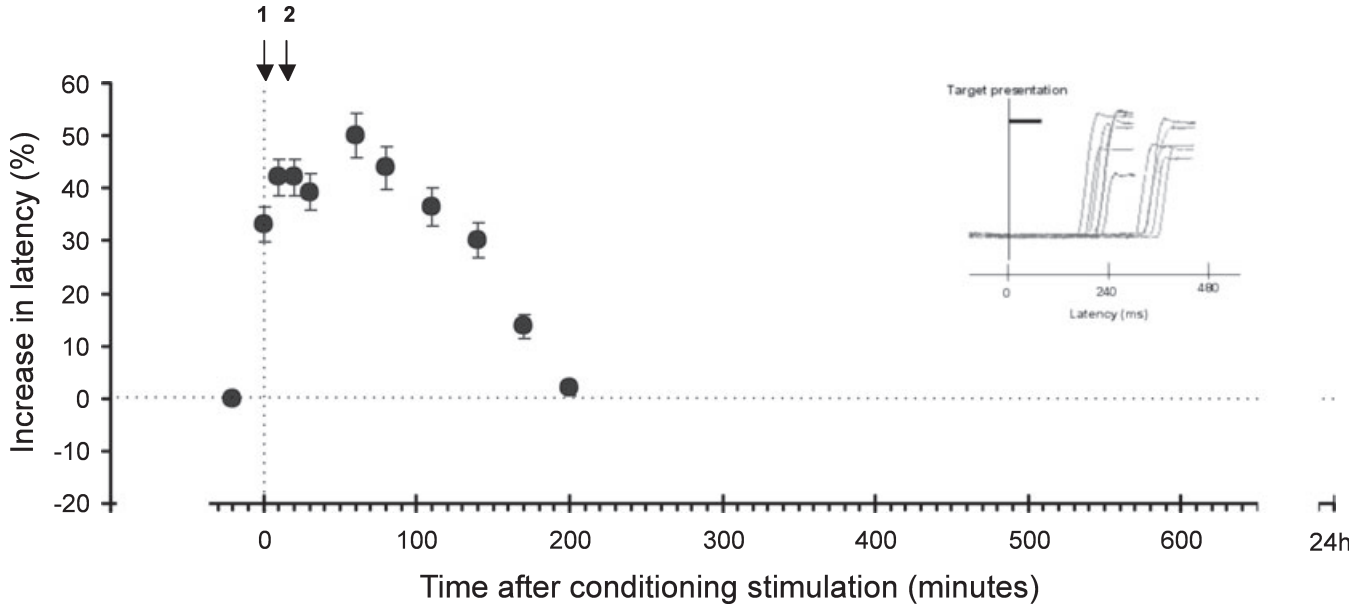

FIG. 2. The effect of two theta-burst trains over the FEF (trains applied at the time of arrow 1 and 2). Note that the application of a second train induced a disproportional prolongation by a factor five: 170 min following two trains versus 30 min following one train. The inset shows representative trials of saccades (gaze angle versus time) without stimulation (the cluster of responses before 240 ms) and after two trains of theta burst TMS (the cluster of responses after 240 ms).

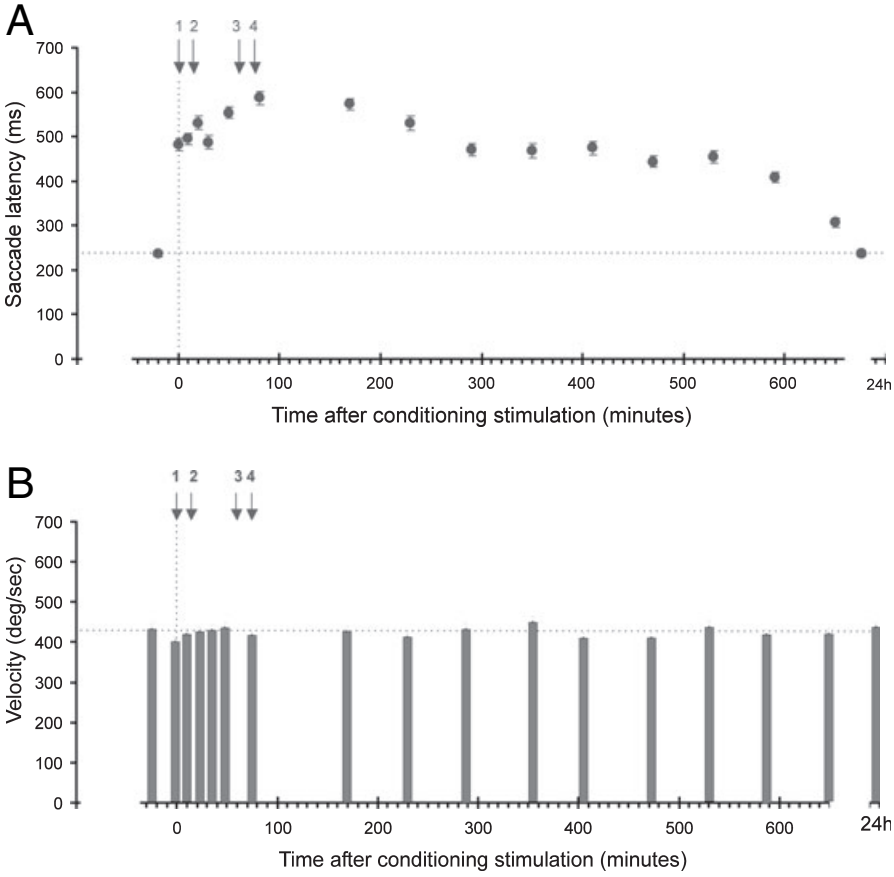

FIG. 3. The effect of four theta-burst trains over the FEF. (A) When applying four consecutive theta-burst trains (at the times of the arrows 1–4) in one subject, the significant effect of TMS was consolidated over a period of 650 min. (B) The mean peak saccade velocity was not significantly reduced during the entire experiment.

saccadic latencies roughly 30 min after the second stimulus (Fig. 2). The resemblance of our observation in the human brain to that seen in animal models is striking. Similar to results obtained in animal studies, the number of applied theta burst trains determined the lifetime of the plastic changes in the neural network of the human brain. One might therefore speculate that repeated stimulation is capable of extending the lifetime of these plastic modifications through a consolidation mechanism as proposed in the cascade model of animals.

On a behavioural level our results illustrate that repeated trains of theta burst TMS have an inhibitory effect on the oculomotor cortex, and they are in line with results of previous theta burst TMS studies that found inhibitory electrophysiological or behavioural effects in the motor or premotor cortex (Di Lazzaro *et al*., 2005; Huang *et al*., 2005; Mochizuki *et al*., 2005). Whether a consolidation mechanism of LTP, as suggested by the resemblance of our results with those observed in animal studies, might explain inhibitory behavioural effects remains speculative. In the FEF, cell populations are implied in saccade control whose activity correlates with inhibiting saccade triggering (Pierrot-Deseilligny *et al*., 1995; Tehovnik *et al*., 2000). This organization reflects the possibility of the FEF to suppress inappropriate or unwanted saccades. For instance, depending on the exact location, electrical stimulation delivered to the FEF inhibits saccade triggering both in humans (Milea *et al*., 2002) and monkeys (Azuma *et al*., 1986; Burman & Bruce, 1997; Izawa *et al*., 2004). Theoretically, LTP of excitatory synapses of FEF suppression neurons, which are implied in saccade suppression, might explain the observed increase of saccade latencies. FEF suppression neurons excite directly or indirectly via the superior colliculus, omnipause neurons in the brain stem, which tonically inhibit excitatory burst neurons implied in saccade triggering (Burman & Bruce, 1997; Izawa *et al*., 2004). However, TMS in humans is quite non-focal and it is not established whether a special TMS protocol may stimulate specifically precise neural connections. A consolidation of LTP of inhibitory synapses might also be conceivable. It is known that horizontal connections of pyramidal neurons that are mediated by glutamate (Hess *et al*., 1994) excite γ-aminobutyric acid (GABA)ergic inhibitory neurons (Keller & Asanuma, 1993). In a recent oculomotor study, it was suggested that in the FEF, GABAergic inhibitory circuits play an essential role in eye movement generation (Schiller & Tehovnik, 2003). Infusion of muscimol, a GABA agonist, in the FEF induced a decreased neuronal activity in single-cell recordings and interfered with saccadic eye movement generation.

In motor studies, it has been suggested that theta burst TMS might also induce LTD-like mechanisms (Di Lazzaro *et al*., 2005; Huang *et al*., 2005). For the oculomotor cortex, LTD of excitatory synapses of FEF fixation neurons, which are essential in the release of visual fixation (Tehovnik *et al*., 2000), or FEF burst neurons, which trigger the saccade (Tehovnik *et al*., 2000), might also explain our results. A decreased activity of these neurons would result in prolonged saccade latencies.

In conclusion, our results provide evidence that theta burst TMS on the oculomotor cortex has inhibitory behavioural effects that can be disproportionately prolonged if more than one train is applied. This suggests that repeated stimulation is capable of extending the lifetime of plastic modifications through a consolidation mechanism. This possibility to induce and consolidate long-lasting plastic changes in the human brain offers new and promising perspectives for the use of TMS. In basic research and cognitive neuroscience, a cortical area may now be stimulated and studied over a much longer time period. Furthermore, it may now be possible to considerably prolong the

therapeutic effect of TMS in patients with various neurological or psychiatric diseases.

Acknowledgements

This study was supported by Swiss National Foundation Grant no. 320000-108146-1 and Grant no. 0031-61335.00 to H.R.L. T.N. was supported by Swiss Foundation for Grants in Biology and Medicine, and Roche.

Abbreviations

FEF, frontal eye field; GABA, γ-aminobutyric acid; LTD, long-term depression; LTP, long-term potentiation; rTMS, repetitive transcranial magnetic stimulation; TMS, transcranial magnetic stimulation.

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