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Cumulative sessions of repetitive transcranial magnetic stimulation (rTMS) build up facilitation to subsequent TMS-mediated behavioural disruptions

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Abstract

A single session of repetitive transcranial magnetic stimulation (rTMS) can induce behavioural effects that outlast the duration of the stimulation train itself (off-line effects). Series of rTMS sessions on consecutive days are being used for therapeutic applications in a variety of disorders and are assumed to lead to the build-up of cumulative effects. However, no studies have carefully assessed this notion. In the present study we applied 30 daily sessions of 1 Hz rTMS (continuous train of 20 min) to repeatedly modulate activity in the posterior parietal cortex and associated neural systems in two intact cats. We assessed the effect on visuospatial orientation before and after each stimulation session. Cumulative sessions of rTMS progressively induced visuospatial neglect-like 'after-effects' of greater magnitude (from 5–10% to 40–50% error levels) and increasing spatial extent (from 90–75° to 45–30° eccentricity locations), affecting the visual hemifield contralateral to the stimulated hemisphere. Nonetheless, 60 min after each TMS session, visual detection–localization abilities repeatedly returned to baseline levels. Furthermore, no lasting behavioural effect could be demonstrated at any time across the study, when subjects were tested 1 or 24 h post-rTMS. We conclude that the past history of periodically cumulative rTMS sessions builds up a lasting 'memory', resulting in increased facilitation to subsequent TMS-induced disruptions. Such a phenomenon allows a behavioural effect of progressively higher magnitude, but equal duration, in response to individual TMS interventions.

Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive neuromodulation technique able to modulate activity in the targeted brain region not only during (on-line) but also beyond the duration of the stimulation train itself (off-line; Robertson *et al*., 2003). Such long-lasting effects have been demonstrated using, among others, electrophysiological (Pascual-Leone *et al*., 1994b; Moliadze *et al*., 2003; Valero-Cabré & Pascual-Leone, 2005; Aydin-Abidin *et al*., 2006), metabolic (Valero-Cabré *et al*., 2005a, 2007) and haemodynamic (Paus *et al*., 1998; Lee *et al*., 2003; Rounis *et al*., 2005) methods, as well as in related behavioural paradigms (Pascual-Leone *et al*., 1994a; Hilgetag *et al*., 2001; Thut *et al*., 2005; Valero-Cabré & Payne, 2006; Valero-Cabré *et al*., 2006). Even though the duration of the off-line effects is frequently thought to be relatively short-lived, lasting minutes to hours depending on the duration and stimulation pattern of the rTMS train, there are suggestions of longer-lasting effects. A second train of rTMS applied even 24 h after a first one to the motor cortex has been shown to have a more robust effect on corticospinal excitability (Maeda *et al*., 2000, 2002; Baumer *et al*., 2003). However, such studies have only assessed the effect of two rTMS sessions, and a more extensive and systematic exploration of the effects of multiple daily sessions in humans is understandingly limited by concerns of possible long-lasting deleterious effects (May *et al*., 2007). Nonetheless, daily sessions of rTMS on sequential days are employed in trials exploring its therapeutic potential in a variety of neurological and psychiatric conditions, under the implicit hypothesis that cumulative effects of the repeated rTMS sessions do develop.

In the current study we used a well-known feline model of visuospatial assessment to test the hypothesis that the accumulation of daily rTMS sessions to visuoparietal (VP) cortical areas progressively results in changes in the magnitude, extent and duration of the 'off-line' behavioural after-effects. We also hypothesize that systematic perturbations of intact visuospatial neural networks by rTMS might produce enduring modulation in subject's baseline performance. The feline VP region is a crucial node of an extended cortico-subcortical network in charge of driving correct visually guided orienting responses towards visual stimuli (Payne *et al*., 1996b; Payne & Rushmore, 2004). Unilateral lesions (Sprague, 1966; Payne *et al*., 2003) or reversible cooling deactivation (Payne *et al*., 1996a; Rushmore *et al*., 2006) of the VP region result in a florid hemispatial

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neglect affecting the visual field contralateral to the manipulated region. This experimental model provides many advantages. First, the structural and functional anatomy underlying the visuoparietal cortex and its involvement in the orienting response has been well studied (Tusa *et al*., 1981; Updyke, 1981; Payne, 1994; Payne & Lomber, 2003; Payne & Rushmore, 2004). Secondly, permanent or transient visual behaviour alteration as induced by localized injuries or reversible manipulations, such as those provided by invasive cooling probes and noninvasive TMS modulation (Lomber & Payne, 1996; Payne *et al*., 1996a, 2003; Valero-Cabré *et al*., 2006), have been previously tested and measured. Third, the metabolic activity patterns resulting from such interventions have been thoroughly explored and correlated with the underlying anatomical connectivity (Vanduffel *et al*., 1997; Valero-Cabré & Pascual-Leone, 2005; Rushmore *et al*., 2006; Valero-Cabré *et al*., 2007). Fourth, cortically restricted and visuotopically specific on-line and off-line visuospatial disruptions have already been achieved by using high- and low-frequency rTMS patterns with an acceptable TMS coil : brain size ratio (Valero-Cabre *et al*., 2005a; Valero-Cabré *et al*., 2007). Fifth, the use of animals kept under highly controlled and consistent conditions (e.g. food intake, physical environment, duration of light cycle, social contact to other congeners, and the daily pattern of activities and events providing sensory and motor experiences, etc.) allows a much better control of factors likely to interact with or modulate the effects of TMS, particularly across long follow-up periods.

We contemplate three prospective outcomes: (i) the magnitude and extent of the neglect-like effect does not significantly covary with the number of accrued sessions; (ii) increasing the number of accumulated stimulation sessions produces progressive decreases in the magnitude and spatial visual span of the effect due to the emergence of cortically mediated behavioural compensations (Lee *et al*., 2003); or, in contrast (iii) a progressively larger effect of rTMS emerges with session accrual, an observation compatible with processes of facilitation 'build-up' occurring at network level.

Materials and methods

Two adult female cats (2.7 and 2.4 kg) were obtained from a commercial research animal vendor (Liberty Laboratories) for use in this study. All procedures were performed in accord with federal, state and institutional guidelines, including the US Public Health Service's Policy on *Humane Care and Use of Laboratory Animals*, the *Guide for the Care and Use of Laboratory Animals* (the Guide) and the NIH *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (1996), in compliance of the official Policy on the Use of Animals in Neuroscience Research by the Society for Neuroscience, and with the final approval of the Institutional Animal Care and Use Committee (IACUC) at Boston University School of Medicine.

Visuospatial training and testing

Animals were trained over the course of 3.5 months in a task designed to test detection, localization and head orienting responses to punctate static light-emitting diode (LED) stimuli (3 mm LED, MCL-934HD, Bright Red, 0.4 mCd/m^2; SPC Technology, Chicago, IL, USA) introduced at several eccentricities throughout the visual field (Valero-Cabré *et al*., 2006). Cats were trained to fixate on a central (0°) illuminated LED stimulus. The fixation light was then turned off and a peripheral LED was illuminated. Peripheral LEDs were spaced at 15° intervals from the central fixation point spanning from left 90° to right 90° visual angles. All targets were located at a radial distance of 54 cm from the cat (Fig. 1a). The arena background illumination level was set at the beginning of each session (0.27 cd/m^2) with a high-precision

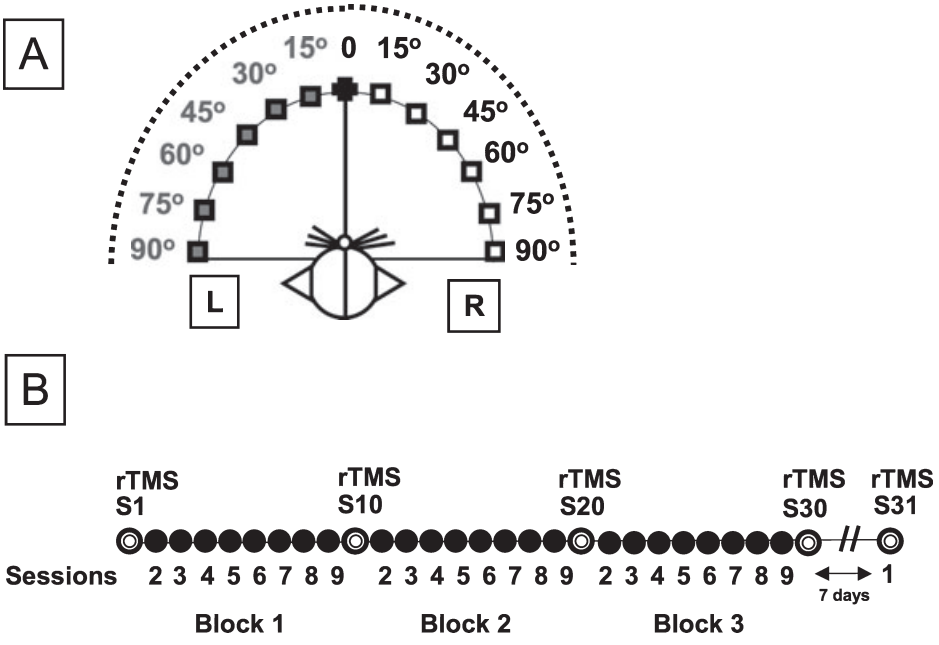

FIG. 1. (A) Schematic drawing displaying how awake cats were tested to evaluate their visuospatial abilities. Cats had their eyes aligned with a 90° horizontal line and fixated on the illuminated 0° target. In 84 different trials divided into three runs, LED lights were displayed pseudo-randomly at different eccentricities located ipsilateral or contralateral to the stimulated VP region and located on both sides (L, left; and R, right) of a fixation point. An orienting response was considered correct when the subjects were able to detect and precisely orient and move in a straight trajectory to a specific target. (B) Schematic drawing displaying the distribution of rTMS sessions. Each of the subjects underwent a daily session of 20 min rTMS (1 Hz, 1200 pulses) for 30 consecutive days (S1–S30). For eccentricity analysis purposes the sessions were divided into three blocks of 10 sessions (block 1, block 2 and block 3). After a period of 7 days free of rTMS stimulation, animals were stimulated again for an additional session (31st).

light meter (Mavolux 5032C, Gossen, Nuremberg, Germany). Cats were trained to orient and approach the illuminated peripheral stimulus and were rewarded with a morsel of soft cat food. Incorrect orienting or localizations were not rewarded. The order of peripheral presentation was pseudorandom and balanced across positions and hemifields. The responses of the cat were monitored using a video system positioned above the perimetry arena. Cats were trained until they achieved plateau performance at < 10% errors (~ 3.5 months) and they were subsequently evaluated for an additional month to ensure consistent and stable performance at plateau levels (after ~ 12 000 trials per animal). During this time, cats were also acclimated to receiving single TMS pulses at increasing intensities of stimulation to several scalp regions.

Repetitive rTMS stimulation delivery and study design

Repetitive TMS was delivered through a 50-mm outer-diameter circular coil (Magstim Inc., UK) held by hand tangential to the skull over the VP cortex. This method provides the most focal form of TMS (Amassian *et al*., 1990; Roth *et al*., 1991). The contact surface was kept 10 mm lateral to the midline and 15 mm rostral from the cat's inion, at an identical position and orientation as the one used in previous experiments (Valero-Cabré *et al*., 2005b, 2006, 2007). According to past and present post-mortem studies, those coordinates coincide with the location of cat VP cortex at the posterior suprasylvian region (Reinoso-Suarez, 1961; Fig. 2). Cats were trained to tolerate rTMS without distress during a 2-week-long familiarization period, in which only single TMS pulses were delivered at increasing intensity of stimulation. Cats were first tested for ~ 15 min, performing 84 trials in which each peripheral eccentricity was tested six times with 12 trials presented at 0° cynosure for fixation control purposes. On the basis of our own prior experience in the matter, TMS coil hand-held stimulation, in which a coil sustained and guided by an operator moves along with the head, was preferred over head-restrained preparations combined with the use of a mechanical arm.

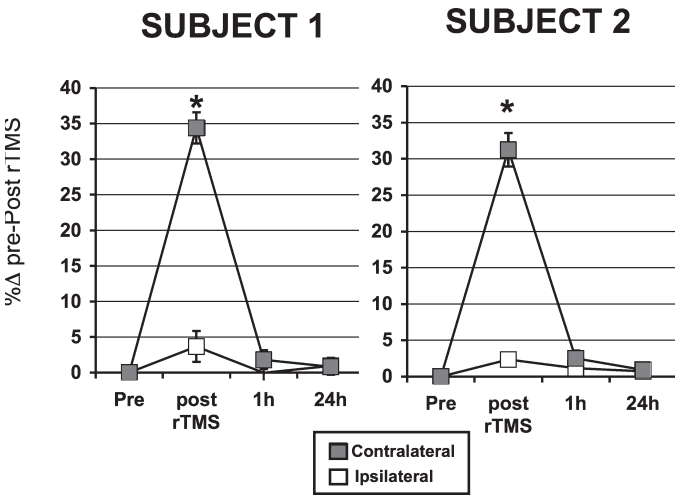

FIG. 3. Percentage difference (%Δ post- vs. pre-rTMS) in detection errors for punctate static targets (LEDs) appearing in the visual hemifield ipsilateral (white squares) or contralateral (grey squares) to the rTMS-targeted cortex. Data correspond to a period before (pre-rTMS), immediately after (post-rTMS) and 1 and 24 h (1 h, 24 h) after the end of the 20-min of unilateral 1 Hz rTMS stimulation on the VP cortex. Data are the mean ± SEM percentage difference from the 30 sessions undergone by each subject (1 and 2). Notice the dramatic but reversible increase in the percentage of errors detecting, localizing and orienting towards contralateral targets after real rTMS. Sham rTMS (not shown in the figure) yielded no significant changes in performance. $*P < 0.01$ vs. before real rTMS.

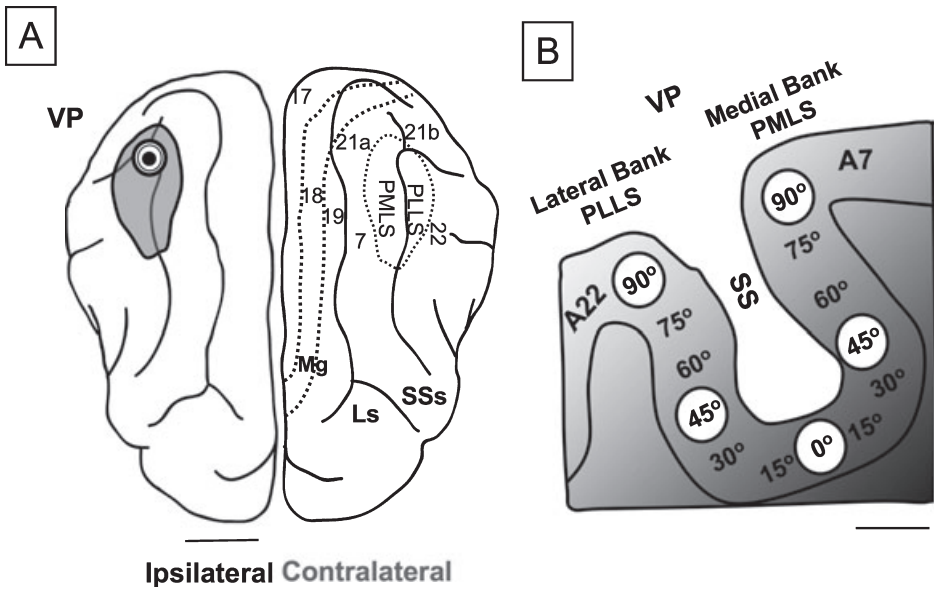

FIG. 2. (A) Dorsal view of the cat's brain. The location of the right visuoparietal (VP) cortex is identified (shading) with the target area of rTMS stimulation (concentric circles). The medial (PMLS) and lateral (PLLS) banks of the suprasylvian sulcus (SSs) are labelled. The edge of a 50-mm circular coil was placed tangential to the scalp area on top of the VP region according to pre-established coordinates (Valero-Cabré *et al*., 2006). The coil was held tangential to the skull location above the VP region, but tilted down 35–40°. Additionally, the axis of the coil was held in a posterior-to-anterior direction with an angle of ~ 35–45° with respect to the midline. (B) Coronal section of the left VP area, showing (left) the PMLS and PLLS banks on the sides of the suprasylvian sulcus (SS). This visuotopically organised representation (data from Palmer *et al*., 1978) shows how the peripheral contralateral visual space is represented in the superficial portions of both the medial and lateral banks across the suprasylvian sulcus (~ 90–75° of eccentricity). As the location of the target moves towards the centre of the visual space, the corresponding map progresses inferiorly on both banks (60, 45, 30 and 15°), until it reaches the depth of the sulcus (~ 0°). Scale bars, 10 mm (A), 2 mm (B).

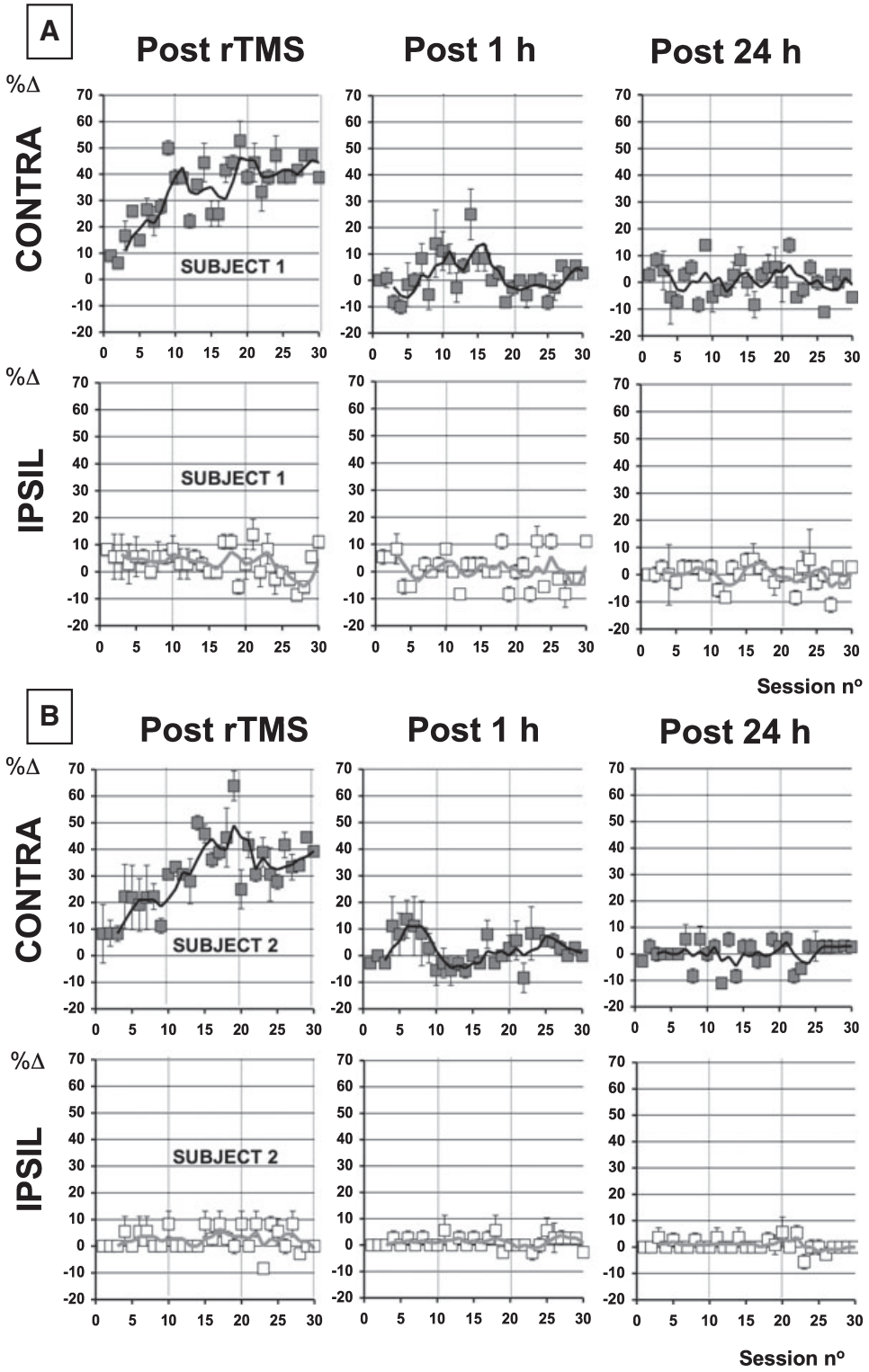

FIG. 4. Average percentage difference (%Δ post- vs. pre-rTMS performance) in detection errors for static visual LED targets across each of the 30 consecutive rTMS sessions for subjects 1 and 2 (A and B, respectively). Data are plotted separately for contralateral (grey squares, top row), and ipsilateral (white squares, bottom row) visual targets with respect to the stimulated VP cortex. Each data point is the mean ± SEM for the subject across assessment blocks. Incorrect performance (% incorrect/total trials) levels across daily sessions is presented in different graphs for assessments carried out immediately (post-rTMS), 1 h (Post 1 h) and 24 h (Post 24 h) after the end of rTMS (i.e. the following day). A moving average ($n = 3$; grey or black line) has been overlapped to highlight the direction of the cumulative change. Notice the dramatic increase in the magnitude of errors detecting, localizing and orienting towards contralateral but not ipsilateral targets occurring across accrued sessions. Assessment of performance errors to ipsilateral or contralateral targets 1 h or 24 h after rTMS remained relatively similar across sessions in both animals.

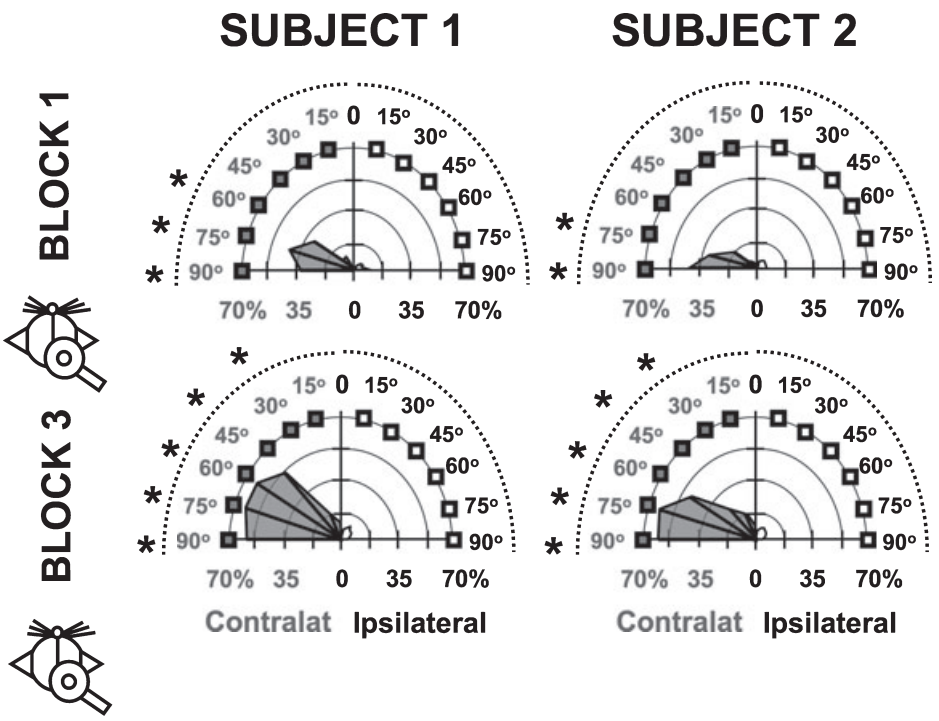

FIG. 5. Visuotopic regions of incorrect detection performance in response to LED stimuli appearing at specific ipsilateral and contralateral eccentricities, for each individual subject (subjects 1 and 2). The perimetry detection error maps display the average of the first 10 (block 1, upper row) and the last 10 (block 3, bottom row) sessions for each cat. The length of each bold line in the perimetry graph corresponds to the percentage difference in errors (% Δ pre-rTMS − immediately post-rTMS) for targets presented at each specific eccentricity. The overlaying grey semitransparent area labels the extent of the region and the intensity of significant contralateral neglect-like symptoms generated by 'off-line' rTMS trains. Note the increase in the extent of detection errors when comparing blocks 1 (average across sessions 1–10) and block 3 (average across sessions 20–30), within each animal. The area of significant rTMS-induced neglect-like effects extended from peripheral locations (90–75°) to mid-pericentral targets 60–45° (*$P < 0.05$ vs. before real rTMS).

When performed consistently, following a period of training, the former, which are frequently used for human clinical applications, clearly eliminate distress and provide direct control and online correction of slight head motion.

Data were collected in three runs of 28 targets with an equal number of samples per eccentricity. Testing blocks were carried out without any specific break and took 5 min each. Twenty minutes of continuous 1 Hz rTMS (1200 pulses) were then applied unilaterally over the VP cortex (Fig. 2a). Similar parameters of stimulation have been successfully used to induce behavioural impairment in a visual attention task (Hilgetag *et al*., 2001; Thut *et al*., 2005) or lasting modulation of cortical excitability in the primary motor cortex in awake humans (Gangitano *et al*., 2002). Stimulation intensity was set for both animals at 40% of the maximal machine output, a level corresponding to 120% of the subject's individual motor thresholds (Valero-Cabré *et al*., 2005b; Valero-Cabré & Payne, 2006). Animals were then tested for 15 min in 84 additional trials immediately after the stimulation, and for an identical number of trials 60 min and 24 h thereafter. Subjects underwent a total of 30 consecutive rTMS daily sessions, i.e. every 24 h, followed by an additional session (31st) separated 7 days from the last (30th session; Fig. 1b). For analysis purposes, rTMS sessions and pre- and post-evaluations were divided into three consecutive blocks of 10 sessions each (Fig. 1b). A series of 10 sham TMS experiments were carried prior to the initiation of the first real TMS session to rule out potential confounding effects of TMS clicking noise and scalp tapping sensation. Sham rTMS was provided with the same coil oriented at 90°, accompanied by manual finger tapping at a 1 Hz pace for 20 min over the same VP region.

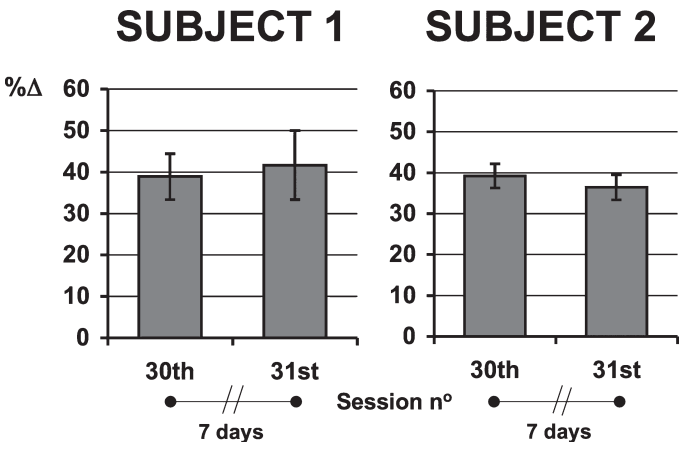

FIG. 6. Average percentage difference (%Δ post- vs. pre-rTMS performance) in detection errors in the contrastimulated hemifield as assessed immediately after stimulation, comparing the last consecutive daily session (30th) and those of an additional session (31st) carried after a 7-day rTMS-free interval. Notice that the magnitude of the induced neglect-like effect showed no statistically significant differences between the two sessions ($P > 0.05$), thus suggesting that the facilitation to rTMS induction might remain active for a period of time in the absence of the delivery of daily rTMS.

Data presentation and statistical analysis

Data are displayed as average ± SEM. The pre- and post-rTMS percentage difference in error levels (%Δ pre- vs. post-rTMS errors) was calculated as 100 × [(number of errors post rTMS − number of

errors pre rTMS)/total number of trials] and displayed individually across the 30 consecutive sessions. Pearson's correlation coefficient (r) was computed between rTMS session number and magnitude of effect for targets ipsi- and contralateral to stimulation. Percentage differences in error levels were statistically compared within the same animal across conditions (Sham vs. real rTMS or pre-rTMS vs. immediate, 1 h or 24 h post-rTMS) by means of a t-test for paired data. The significance level was set at $P < 0.05$.

Results

Following a training period of 3 months ($\sim$ 10 000 trials), both cats achieved plateau performance levels in the detection–localization task, with average error levels of 7 ± 4% and 5 ± 2% for subjects 1 and 2, respectively. Raw performance levels were not significantly different when comparing two consecutive training blocks of five sessions (block 1, 6 ± 4 and 7 ± 6% vs. block 2, 5 ± 2 and 4 ± 2% for subjects 1 and 2, respectively; $P > 0.05$). As found in prior studies, a series of 10 consecutive sham rTMS sessions preceding the real sessions yielded no significant changes in visuospatial orientation to either ipsilateral targets (1.4 ± 2 and −2.0 ± 2%; $P > 0.05$) or contralateral targets (+1.3 ± 1 and +2.4 ± 3% for subjects 1 and 2, respectively; $P > 0.05$ vs. pre-sham rTMS values). However, the average of 30 daily sessions of real low-frequency (1 Hz) rTMS resulted in significant lasting disruptions in the detection–localization abilities for targets presented in the contralateral visual space (+34 ± 2 and +31 ± 2%, increases in errors for each animal; $P < 0.01$). This was accompanied by nonsignificant patterns of disruption for targets presented in the visual hemifield ipsilateral to the TMS coil location (+4 ± 3% and +2 ± 1%; $P > 0.05$) (Fig. 3). Neither cat showed clinically noticeable side-effects following any of the daily rTMS sessions (e.g. seizures, personality changes, decrease in appetite or weight loss).

The magnitude of the effect increased with increasing number of rTMS daily sessions. Significant positive correlations were found between the magnitude of the disruptive effects of the TMS in the visual field contralateral to the stimulated parietal cortex and the number of rTMS sessions ($r = +0.71$ and $r = +0.64$ for subjects 1 and 2; $P < 0.001$). A peak in the maximum effect was reached in both subjects after the 18th or 19th daily session (+35 ± 2 and +41 ± 1%; subject 1, Fig. 4a; and subject 2, Fig. 4b). No specific pattern or significant correlations were found between performance and accumulation of TMS session at the 1 h ($r = +0.01$ and $r = -0.03$) or 24 h ($r = -0.08$ and $r = +0.09$) assessment time points, across the 30 days of follow-up. Raw incorrect baseline performance as assessed before each rTMS session remained relatively stable over time and did not significantly improve or worsen with accumulation of stimulation sessions ($r = -0.12$ and $r = +0.02$; $P > 0.01$).

Detection and localization performance towards ipsilateral targets showed, immediately after rTMS, a nonsignificant pattern towards progressive improvement (subject 1, $r = -0.16$; $P > 0.01$) or no change across sessions (subject 2, $r = -0.05$; $P > 0.01$). No significant lasting modulation across the sessions was observed in any of the evaluations carried out 1 h ($r = +0.01$ and $r = -0.03$; $P > 0.01$) or 24 h post-rTMS ($r = -0.08$ and $r = +0.09$; $P > 0.01$; Fig. 4).

Repetitive TMS preferentially affected peripheral targets corresponding to the monocular portion of the visual field, rather than those presented in pericentral locations. A detailed analysis of the visual field portions involved in such lasting modulation, comparing the first (block 1) and the third (block 3) of 10 sessions, indicates that the magnitude and span of the contralateral neglect-like area progressed from peripheral (90 and 75°) to include more pericentral targets (60, 45 and 30°; $P < 0.05$, block 1 vs. block 3; Fig. 5).

Finally, an additional session (31st) carried out 7 days after the last of the rTMS sessions showed levels of contralateral disruption which were not significantly different from those detected after the 30th rTMS session (subject 1, 39 ± 6 vs. 41 ± 8%; and subject 2, 39 ± 4 vs. 36 ± 3%; Fig. 6).

Discussion

Transcranial magnetic stimulation holds enormous promise as a tool for manipulating brain network plastic properties and modulating cognitive abilities. Previous feline experiments have shown constancy in the magnitude of the effect of rTMS when the sessions are separated by at least 2 days. The current experiment provides direct evidence in an intact system that when several rTMS sessions were separated by a shorter interval ($\sim$ 24 h), the effects of a given session were not independent of the preceding ones. Indeed, the magnitude of the rTMS-induced visuospatial effects progressively increased as daily rTMS sessions accrued. In addition, the extent of the visual field affected by rTMS also changed with increasing stimulation sessions: rTMS-induced errors in the initial sessions were restricted to the far peripheral eccentricities but, with increasing sessions, errors also became prevalent in more pericentral eccentricities represented in deeper cortical regions. Interestingly, this accrual effect of rTMS session was limited to the immediate off-line period, and no lasting changes in behaviour were noted 1 h or 24 h after stimulation throughout the study. The current observations have important implications for the use of rTMS and other neuromodulation tools in regimes to modify (improve or disrupt) cognitive performance and re-program brain functions in intact individuals or neuropsychiatric patients.

Unilateral rTMS stimulation of visuoparietal systems: patterns of effect

Isolated and short-lasting cortical perturbations, such as those generated in our study through rTMS, induce unilateral decreases in metabolic activity in the posterior areas of the feline parietal cortex. Such effects result in the observed neglect or neglect-like symptoms affecting the contralateral visual field (Lomber & Payne, 1996; Payne *et al*., 1996a, 2003; Valero-Cabré *et al*., 2006). Prior studies have shown that local deactivation patterns translate through anatomical pathways into outlasting metabolic decreases within the VP region (anatomically referred to as posterior suprasylvian cortex), and to the superficial layers of the ipsilateral superior colliculus, subnuclei of the posterior thalamus, and richly interconnected primary visual regions (Vanduffel *et al*., 1997; Valero-Cabré & Pascual-Leone, 2005; Valero-Cabré *et al*., 2007). Thus, the effects of rTMS result in distributed patterns of deactivation, which interfere with the normal signalling activities between nodes, conveyed through short- and long-range connectivity. In particular, rivalrous (inhibitory) interactions between cortical and collicular regions in both hemispheres and paired midbrain structures seem to be among the elements which are key to understanding the underlying dynamics of such circuitry and the effects of its manipulation (Payne & Rushmore, 2004; Rushmore *et al*., 2006). Individual sessions of unilateral rTMS-mediated VP deactivations induce short-lasting interhemispheric imbalances during rest and result in a partial suppression of mutually inhibitory circuits, enhancing collicular asymmetry in response to visual activation.

Under such circumstances, the threshold level for triggering head-orienting responses cannot be surmounted, detection and orienting fail and crossed visuospatial neglect-like effects arise (Payne & Rushmore, 2004). Prior studies have proven that the magnitude of such visuospatial effects is commensurate with the level of cortical deactivation and the richness and sign of the anatomical connectivity (Vanduffel *et al*., 1997; Valero-Cabré & Pascual-Leone, 2005) whereas their duration over time is related to the temporal persistence of its metabolic effect (Rushmore *et al*., 2006; Valero-Cabré *et al*., 2007). The magnitude and consistency of the behavioural effects reported in the current feline study (with an average of ∼32–35% increase in errors) is very significant, given that, normally, rTMS based interventions in humans result in very moderate lasting behavioural effects (Hilgetag *et al*., 2001; Thut *et al*., 2005), or might paradoxically terminate in significant haemodynamic (Lee *et al*., 2003) and/or electrophysiological modifications (Huang *et al*., 2005) which do not translate into measurable behavioural disruptions. The absolute or relative refractoriness to stimulation of certain neural systems seems to be based in their inherent robustness or in their ability, once altered, to recruit local or associated areas to prevent or attenuate a behavioural 'default' (Lee *et al*., 2003). Thus, cortical modulation combined with emerging neural compensations can be held responsible for behavioural changes (in magnitude, spatial extent and temporal duration) such as the ones recorded in our study, and for their modification across accrued sessions.

Modelling (Hilgetag *et al*., 1999) and experimental data have suggested that during lesion- or TMS-induced unilateral parietal lasting deactivations (Kinsbourne, 1977; Hilgetag *et al*., 2001) the threshold for triggering detection and orienting responses to ipsilateral targets is decreased by a release of transcallosal inhibition exerted from altered regions onto intact posterior parietal areas. As a result, 'paradoxical' behavioural improvements in ipsilateral detection are to be expected. However, our own data, both past (Valero-Cabré *et al*., 2006) and present, have failed to display such ipsilateral patterns of amelioration (Valero-Cabré *et al*., 2006) after TMS. Studies documenting short-lasting paradoxical ipsilateral improvements in intact humans (Hilgetag *et al*., 2001; Thut *et al*., 2005) were carried out in a virtually untrained task, with baseline performance levels of ∼50–60% correct trials. In contrast, in our studies we opted for an intensively overtrained task providing consistent performance at much higher accuracy levels (∼90–95% correct). In such conditions, our ability to detect ipsilateral ameliorations was clearly undermined by such close-to-ceiling baseline levels of correct performance. In fact, recent cat experiments performed in our lab using a more challenging version of the same paradigm (70–75% accuracy baseline level) demonstrated that difficulty titration is a fundamental variable that might determine the magnitude and sign of the rTMS-induced changes in ipsilateral detection–localization performance (Anthony Jedd, MSc thesis, GMS Boston University Medical School, 2007).

Cumulative neuromodulatory effects: the effect of daily rTMS

In order to become credible in the service of behavioural neuroscience and neurology as research or therapeutic tools, neuromodulation techniques might need to demonstrate their ability to provide long-lasting effects far beyond the duration of the stimulation. Prior experiments in the cat have indicated that the accrual of deactivation sessions, interspersed by at least 48 h, does not modify (i.e. does not attenuate or increase) the magnitude, span or duration of the rTMS-mediated effect on visuospatial abilities with respect to prior sessions (Lomber & Payne, 1996; Payne *et al*., 1996a, 2003; Valero-Cabre *et al*., 2006). In the current study, however, we demonstrated dramatic cumulative effects reaching maximal plateau levels after the accrual of ∼20 consecutive rTMS sessions (out of a total of 30) carried out every 24 h. Our baseline data provide robust evidence that pre-rTMS performance did not significantly covary with increasing number of sessions, discarding any change in the cat's intrinsic ability to perform the task across days. Furthermore, the effect was always lateralized to the visual field contralateral to that stimulated, thus ruling out the confounding effects of progressive fatigue or motivation loss within and across sessions which, in intact cats, should equally affect targets on both visual hemifields.

In agreement with prior studies, rTMS preferentially affected performance when targets were in far peripheral (90–75°) locations (Valero-Cabré *et al*., 2006). As we hypothesized elsewhere, such findings might be a direct consequence of the limited penetration capacity of the effect of rTMS (Valero-Cabré *et al*., 2005b; Valero-Cabré *et al*., 2007), and the fact that visual receptive field mapping data in the cat show that far peripheral visual eccentricities are represented in neurons located at the most dorsal and deeper aspects of the VP cortex (Palmer *et al*., 1978). This concordance allows for the suggestion that rTMS might have produced a temporary depression of rather superficial sulcal regions representing far peripheral targets, and this neural deactivation manifested as the appearance of behavioural errors specifically in these visual locations (see Fig. 2A and B for anatomical details). Interestingly, our data indicate that the deeper representations of more pericentral eccentricities might also be affected if the number of accrued rTMS sessions is increased and local inhibitory effects are potentiated. The more central locations (i.e. 45–30°) are found within the depth of the suprasylvian sulcus and, as a result, the errors in targets presented in these locations must represent an extension of the deactivation effect through the sulcus. As rTMS parameters were kept constant across sessions, this disruption of neurons located deeper in the sulcus is likely to arise from a facilitation of the transmission of the deactivating effect within the VP region from the neurons at the top. Such an effect, correlated with the accrual of stimulation sessions, could be mediated through rich intrinsic connections between neurons within the VP region (Norita *et al*., 1996). This idea is compatible with the notion that behavioural effects following focal deactivation are induced by both local and connectivity-mediated changes throughout associated networks (Vanduffel *et al*., 1997; Valero-Cabré & Pascual-Leone, 2005; Valero-Cabré *et al*., 2007). Further evidence supporting these hypotheses could be provided by means of electrophysiological, haemodynamic or metabolic mapping experiments of the same cat brain region.

Finally, the magnitude of the behavioural effects increased linearly during blocks 1 and 2, reaching and remaining at plateau levels after the accrual of ∼20 daily sessions of rTMS. Such incomplete ceiling of maximal effects, achieved at ∼40–45% induced errors (i.e. with ample leverage for further change), it is very likely to be caused by the above-mentioned TMS difficulties in penetrating deeper portions of the sulcus. Alternatively, the dramatic inflexion in the magnitude of the effect with rTMS session accrual could be explained by the emergence of local homeostatic plasticity phenomena, directed at avoiding a destabilization of neural activity and triggered beyond certain levels of activity-dependent plasticity (Sejnowski, 1977; Abbott & Nelson, 2000). The role of such a mechanism in attenuating or even inverting the phase of the effects induced by a given neuromodulatory intervention (by high- or low-frequency rTMS) when closely (∼10 min or less) preceded by the preconditioning of the same systems (by cathodal or anodal transcranial direct current

stimulation) has been invoked previously in the intact human motor cortex (Lang *et al*., 2004; Siebner *et al*., 2004). Notwithstanding, the hypothesis that compensatory homeostatic plasticity in parietal systems could be triggered by the accrual of multiple preconditioning identical sessions separated by much longer intervals (∼24 h) remains to be demonstrated.

Memory traces left by rTMS sessions: building up 'facilitation' to further TMS mediated disruptions?

The current behavioural results indicate that the history of prior rTMS sessions plays a role in dictating the response of neural circuits to subsequent sessions. As a consequence, the behavioural outcome of a given effect of rTMS becomes greater in quantity and might change in quality (for example in spatial span) when multiple sessions are accrued within a given interval. Surprisingly, as shown by the lack of effect in any of our later post-rTMS assessments, the daily regime of rTMS did not seem to be capable of affecting permanently or beyond each session the basal levels of visuospatial performance. This observation indicates that, in intact cerebral systems, the build-up in the level of behavioural disruption observed across an accrual of sessions might not be made evident as lasting changes in behaviour but in terms of the ease by which a behavioural effect can be induced by a subsequent neuromodulatory events. In other words, the history of accumulated rTMS sessions might render the targeted region or its connected neural network more prone to modify its activity levels when subsequently re-exposed to rTMS. This explanation is in agreement with similar observations in freely moving rodents demonstrating enhanced inhibitory postsynaptic potential effects after accrual of periodic sessions of long-term depression (900 pulses, 1 Hz) patterns in the somatosensory cortex, by means of intracortical electrical stimulation (Froc *et al*., 2000; Monfils & Teskey, 2004).

In a first attempt to estimate the duration of such distributed 'memory', both subjects were tested with a single additional session (31st), 7 days after the very last one at the end of the 30-day rTMS regime. The results indicate that, after a 7-day-long rTMS-free interval, the magnitude of the effect achieved by a low-frequency rTMS session was not significantly different from that measured at the height of the facilitation. This would push back the duration of long-lasting modulation far beyond the 22–24 h revealed by electrophysiological studies in the human motor system (Maeda *et al*., 2000, 2002; Baumer *et al*., 2003). The significantly higher number of daily sessions accrued in our study, besides differences in the outcome measures or the specifics of the targeted region, might easily account for such disagreement. Unfortunately, in the current experiment, our ability to test further intervals was limited by the fact that every new rTMS evaluation constitutes in itself an intervention, capable of maintaining or potentiating the level of previously achieved facilitation. Thus, specific experiments using shorter regimes will need to be developed to determine the relation between the durability of the effect and the number and frequency of accrued rTMS sessions.

Unfortunately, the current behavioural results only allow us to speculate on the specific underlying mechanisms explaining such build-up of facilitation, without long-lasting effects, and its consolidation over time. We hypothesize that the constancy of TMS-mediated daily suppression exerted in the same cortical area might trigger Hebbian-type modifications in synaptic strength within the targeted region and its associated neural systems, similar to those described by experience- or activity-dependent plasticity in adults. In parallel and in order to avoid the destabilization of neural activity, homeostatic plasticity mechanisms might dynamically re-adjust synaptic strengths and promote local and network stability (Sejnowski, 1977; Abbott & Nelson, 2000). Our data demonstrate the cumulative and long-lasting nature of TMS-mediated modulation. It is thus possible that both short- and longer-term molecular changes [e.g. in the subtypes of postsynaptic (NMDA or AMPA) receptors, expression of neurotrophins such as NGF or BDNF, and late long-term potentiation and long-term depression plasticity-related proteins] and cellular modifications in local neurons and interneurons (e.g. the remodelling of the number and activity patterns of axonal projections, dendritic processes and spines) might account for the induction and stabilization of such changes (Zito & Svoboda, 2002; Karmarkar & Dan, 2006). Interestingly, the underlying mechanisms would not constantly modify (either increase or decrease) normal synaptic function in the absence of the specific type of modulation that triggered it. Stored as a functional 'memory', they could be rapidly retrieved to set up a specific program of action, providing equal or higher levels of output, every time the modulating agent (in the current case TMS) renewed its effect. The current study provides a description of behavioural phenomena as induced by multisession regimes of neuromodulation. Further effort is, however, required to improve the poor understanding we currently have of their molecular and cellular underpinnings.

Concluding remarks: relevance for rTMS studies

The current study suggests that, for human studies seeking to efficiently manipulate the behavioural contribution of cerebral areas, complete plateau deactivation levels can be achieved after a long conditioning phase involving periodic stimulation sessions at intervals no longer than 24 h. Moreover, cerebral regions difficult to reach due to their deep anatomical location might be affected if the local and distributed neuromodulatory effects are potentiated through the accrual of rTMS sessions. Finally, the observed lack of behavioural effects beyond the period immediately following each stimulation session does not necessarily cast doubt on the ability of rTMS to produce long-lasting and therapeutically meaningful changes in neural activity. In fact, as suggested by the clinical success of relatively short rTMS regimes on spared regions of damaged motor, language or visuospatial systems, injured brains might be more likely than intact systems to benefit from rTMS-mediated improvements, to achieve punctual and short-lasting recovery (Oliveri *et al*., 1999; Oliveri *et al*., 2001; Mansur *et al*., 2005), maintain relatively stable beneficial clinical outcomes or even keep improving after discontinuation of rTMS (Naeser *et al*., 2005; Valero-Cabré *et al*., 2005a; Shindo *et al*., 2006). A systematic comparison of the neuromodulation potential and the associated risks of classical and novel types of cumulative regimes, in particular TMS theta burst (Huang *et al*., 2005) and TMS preceded by TMS (Iyer *et al*., 2003) or transcranial direct current stimulation (Lang *et al*., 2004; Siebner *et al*., 2004) preconditioning patterns remains, in both intact and injured systems, an essential avenue for future investigations.

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Abbreviations

LED, light-emitting diode; rTMS, repetitive transcranial magnetic stimulation; VP, visuoparietal.

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