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Benefit of multiple sessions of perilesional repetitive transcranial magnetic stimulation for an effective rehabilitation of visuospatial function

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Abstract

Noninvasive neurostimulation techniques have been used alone or in conjunction with rehabilitation therapy to treat the neurological sequelae of brain damage with rather variable therapeutic outcomes. One potential factor limiting a consistent success for such techniques may be the limited number of sessions carried out in patients, despite reports that their accrual may play a key role in alleviating neurological deficits long-term. In this study, we tested the effects of seventy consecutive sessions of perilesional high-frequency (10 Hz) repetitive transcranial magnetic stimulation (rTMS) in the treatment of chronic neglect deficits in a well-established feline model of visuospatial neglect. Under identical rTMS parameters and visuospatial testing regimes, half of the subjects improved in visuospatial orienting performance. The other half experienced either none or extremely moderate ameliorations in the neglected hemispace and displayed transient patterns of maladaptive visuospatial behavior. Detailed analyses suggest that lesion location and extent did not account for the behavioral differences observed between these two groups of animals. We conclude that multi-session perilesional rTMS regimes have the potential to induce functional ameliorations following focal chronic brain injury, and that behavioral performance prior to the onset of the rTMS treatment is the factor that best predicts positive outcomes for noninvasive neurostimulation treatments in visuospatial neglect.

Introduction

Brain injury results in a loss of function tied to the processing of the damaged area and network-connected regions. Clinical recovery relies on intrinsic neuroplastic mechanisms, which induce functional and structural modifications in the remaining circuits to circumvent the effects of lesion, reprogram lost function in spared locations, and limit neurological impairment. With an understanding of brain injury and the spontaneous repair mechanisms that ensue, many rehabilitation strategies attempt to build on intrinsic neuroplasticity to improve clinical recovery. Nonetheless, many patients still endure permanent impairments and in search of longer-lasting and more effective interventions, rehabilitation strategies have recently been supplemented with neurostimulation.

Approaches of neurostimulation therapy are shaped on inter-hemispheric rivalry principles aimed at reducing a transcallosally-induced state of hyperexcitability in the contralesional hemisphere or directly enhancing the activity of lesion-adjacent regions to overcome a state of suppression induced by the lesion and the excess of transcallosal inhibition of the opposite hemisphere. Two commonly used noninvasive neurostimulation techniques utilized in clinical trials to attenuate neurological deficits from brain damage, and especially stroke, are repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS). Clinical improvements have been achieved by applying inhibitory rTMS patterns to either the unaffected hemisphere (Oliveri *et al.*, 2001; Brighina *et al.*, 2003; Mansur *et al.*, 2005; Fregni *et al.*, 2005; Shindo *et al.*, 2006; Takeuchi *et al.*, 2005, 2008) or excitatory rTMS patterns to the injured hemisphere (Khedr *et al.*, 2005; Kim *et al.*, 2006; Yozbatiran *et al.*, 2009). Transcranial DCS has also provided evidence of recovery in several neurological conditions using similar principles (Boggio *et al.*, 2007; Hesse *et al.*, 2007; Reis *et al.*, 2009; Sparing *et al.*, 2009). The insights provided by rTMS and tDCS studies have unequivocally elevated the scientific and clinical drive to alleviate functional impairments in

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the brain-injured population. However, despite promising results obtained in small-scale studies, limitations in clinical outcomes remain, and neurostimulation has often been considered inconsistent in delivering significant and long-lasting ameliorations when applied to larger populations of patients.

Factors such as lesion size, degree of spontaneous recovery, lesion chronicity, and influence of tissue characteristics are among the variables thought to contribute to behavioral discrepancies in large patient populations receiving neurostimulation treatment (Wagner *et al.*, 2007; Plow *et al.*, 2009). Furthermore, in order to preserve patient safety, the number of consecutive TMS sessions are restricted, yet research performed in healthy subjects has demonstrated that the accumulation of sessions might be key to enhancing rTMS efficacy (Maeda *et al.*, 2002; Bäumer *et al.*, 2003; Valero-Cabré *et al.*, 2008). Suppressive rTMS sessions not exceeding ten applications on the intact hemisphere have yielded enhancements in function which are probably still present weeks after the end of the treatment (Avenanti *et al.*, 2012; Koch *et al.*, 2012). However, the therapeutic potential of high-frequency perilesional rTMS in repeated sessions has yet to be consistently assessed in detail. We hereby hypothesized that a very high number of consecutive rTMS sessions applied to lesion-adjacent cortex could maximize functional recovery well beyond spontaneous recovery levels in the chronic phase following focal brain damage.

To freely address our hypothesis, we turned to a well-established animal model of visuospatial disorders. We induced focal unilateral lesions in a subregion of the feline posterior parietal cortex, specifically known as the posterior middle suprasylvian area (pMS), leading to enduring visuospatial deficits in the contralesional hemispace (Huxlin & Pasternak, 2004; Rushmore *et al.*, 2010; Das *et al.*, 2012). Subjects were followed for ~2.5 months post-lesion, which was the time required to consistently reach plateau levels of spontaneous recovery. Animals were then treated for 3.5 months with a daily regime of high-frequency real or sham rTMS over an anatomically related perilesional site known as the anterior middle suprasylvian area (aMS), previously found to adopt lost function after pMS damage (Fig. 1; also Lomber *et al.*, 2006). Subjects were evaluated at regular intervals and assessed in terms of hemispace and eccentricity-specific recovery. Task specificity and stability of recovery over time in the absence of active rTMS treatment was also addressed.

Material and methods

Visuospatial evaluation

A group of adult cats (n = 15, 13 females, 2 males) were used in this study. Animals were acquired from a USDA-approved licensed breeder (Liberty Laboratories, Waverly, NY, USA). Cats were maintained on a 12:12-h light : dark cycle, were group-housed in an enriched environment and had free access to water. Food intake was regulated to daily testing sessions and to a period at the end of the day when cats were fed dry food. All procedures were conducted with approval from the Institutional Animal Care and Use Committee (IACUC) at the Boston University School of Medicine, and were in compliance with the policies outlined by the National Research Council *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (2003).

In this study, a battery of three visuospatial detection tasks performed in real space were used to probe potential rTMS-driven improvements in behavioral performance. All paradigms were tested by placing subjects in the center of an 88-cm-diameter semicircular perimetry arena (Schweid *et al.*, 2008). Animals first fixated on a midline stimulus at 0° for a variable period of time (between 1 and 3 s). This event was followed by a peripheral stimulus randomly appearing at 15, 30, 45, 60, 75 or 90° of visual angles in either the left or right hemifield at the level of the horizontal meridian. Animals were trained to acknowledge the appearance of the target by orienting head and eyes to the exact target eccentricity in a single motion and then move forward in a straight trajectory to the stimulus and retrieve a high-incentive food reward ('wet' food). When the presence of a peripheral target was not acknowledged (or neglected) animals were trained to provide the 'default' response of advancing forward to the 0° midline fixation to receive a low-incentive food reward ('dry' food). Once a trial was completed, animals were trained to quickly return to the starting point, re-establish central fixation and prepare for a new trial. Correct animal head and eye positions and the trajectory of the response were monitored

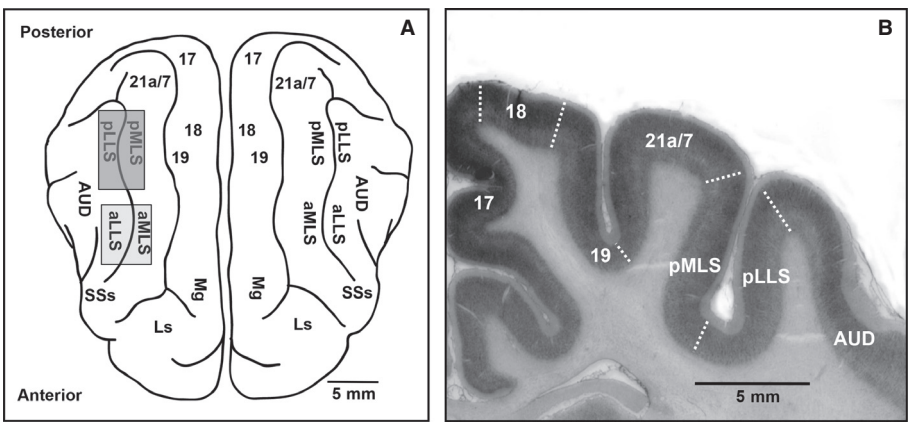

FIG. 1. (A) Dorsal view of the cat brain. Schematic drawing of the different areas of the feline posterior parietal cortex involved in the orienting of spatial attention. The anterior and posterior middle suprasylvian cortices (aMS and pMS) are comprised of medial (aMLS or pMLS) and lateral (aLLS or pLLS) banks located on each side of the suprasylvian sulcus (SSs). On the right hemisphere the dark gray shaded rectangle denotes the injured right pMS cortex. The light grey rectangle represents the intact right aMS where rTMS was applied. Other significant anatomical areas surrounding the pMS, such as areas 21a/7, visual areas 17, 18 and 19, and primary auditory cortex (AUD), are labeled. Finally, the marginal gyrus (Mg) contains the primary visual cortex (areas 17 and 18) and is separated from the pMS region by the lateral sulcus (Ls). (B) Coronal section of a cat brain. High-resolution image of a coronal section stained for Nissl substance at approximately Horsley–Clarke coordinate A9. Dotted white lines represent approximate boundaries between the different visual areas at this particular stereotaxic coordinate.

online through a closed video-camera system that provided a magnified high-resolution view of the animals' head and eyes. Targets were presented in pseudorandom order in blocks of 28 trials with an equivalent number of stimuli displayed in the two hemifields. In addition, up to 10 catch trials, which consisted of the presentation of the midline stimulus alone, were interleaved within each block to ensure correct execution of the paradigms. Each block of testing was performed in ~5 min (~8 s per trial). A trial was marked correct if subjects demonstrated a quick and direct head orienting response to the exact location of the peripheral target (Valero-Cabré *et al.*, 2006, 2008).

Subjects were trained for ~4 months in a series of tasks in order to achieve plateau performance levels before undergoing surgery. Three main paradigms were used to assess visuospatial orienting in the horizontal meridian of the visual field in real space. The Moving 1 task consisted in the presentation of a high contrast moving target (2 cm wide), a dark thin scoop, which contained on its tip a patch of high-incentive food reward (Rushmore *et al.*, 2006, 2010). Visuospatial responses to motion were tested at phototopic ambient light levels (43 cd/m^2). The Static task required animals to detect and orient to the illumination of high-contrast static light emitting diodes (LEDs; 3 mm diameter) as described in previous studies (Lomber *et al.*, 2006; Schweid *et al.*, 2008; Valero-Cabré *et al.*, 2008). The Moving 2 task was a motion version of the Static paradigm, in which the stimulus was a moving laser (3 mm diameter) light spot rather than a static LED. All other parameters, such as stimulus size and illumination between the Static and Moving 2 task, were similar and tested in low ambient light levels (0.3 cd/m^2). In contrast with the Moving 1 task, with these two tasks the rewards differed in time with regards to the presentation of the stimulus.

Typically animals reached plateau levels of performance after ~200 trials for the Moving 1 task, which was the first and less challenging task to learn. The Moving 2 and Static tasks were learned simultaneously and required ~1200–1500 and 3000 trials respectively to reach consistent plateau levels. The learning period invested in training the animals to effectively perform these three tasks required ~3.5–4 months of rigorous daily training.

Brain lesion procedures

The day prior to surgery, animals were sedated with ketamine (10 mg/kg i.m.), a venous catheter was inserted, and dexamethasone (Samuel Perkins Inc., Quincy, MA, USA; 1 mg/kg i.m.) and the antibiotic cefazolin (20 mg/kg, i.v.) were both administered. On the next morning anesthesia was induced with sodium pentobarbital (Henry Schein, Melville, NY, USA; 25 mg/kg, i.v.), and then dexamethasone (1 mg/kg i.m.) and atropine sulfate (Samuel Perkins Inc., 0.03 mg/kg s.c.) were given to reduce inflammation and mucous secretions, respectively. An endotracheal tube, EKG electrodes and a rectal probe were placed in order to monitor heartbeat and respiration rate, and to measure core body temperature. These variables were monitored and recorded every 10–15 min. Once the physiological parameters were stable, the head was secured in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) and centered in Horsley-Clarke coordinates (Reinoso-Suarez, 1961). The brain was then exposed and a 10-μl Hamilton syringe was used to inject 1μl of sterile ibotenic acid (10 μg/μl; Sigma-Aldrich Inc.) at multiple sites along the lateral and medial banks of a region of the right posterior parietal cortex known as the pMS cortex, as described in prior studies (Huxlin & Pasternak, 2001, 2004; Huxlin *et al.*, 2008; Rushmore *et al.*, 2010; Das *et al.*, 2012). An acrylic plug was placed on the skull overlying the anterior portion of the posterior parietal cortex known as the aMS, in the contralesional hemisphere, to properly pinpoint this area and direct the TMS coil placement during the ensuing rTMS regime (Fig. 1). After completing the injections and placing the acrylic plug, the dura mater was repositioned, the bone piece was replaced and the muscles and skin were sutured. Immediately after surgery animals were given dexamethasone (1 mg/kg i.m.) for 5 days in decreasing doses and cefazolin (20 mg/kg i.m.) for 10 days following surgery. Analgesics (buprenorphine; 0.01 mg/kg, s.c., Henry Schein) were administered twice a day for 2–3 days post-surgery. Sutures were removed ~10 days after surgery. Veterinary staff from the Laboratory Animal Science Center at Boston University School of Medicine supervised the recovery. Prior evidence from our lab demonstrated that this type of lesion provides, after a period of limited spontaneous recovery, enduring signs of contralateral visuospatial deficits even 2 months after damage (Rushmore *et al.*, 2010).

rTMS

rTMS was applied using Magstim Super Rapid^2 equipment (The Magstim Company Ltd, Withland, UK). Pulses were delivered with a 50-mm diameter circular coil (Magstim Company Ltd), which is one of the most focal approaches for efficiently administering rTMS in felines (Amassian *et al.*, 1990; Valero-Cabré *et al.*, 2005). The right aMS cortex was identified by palpating the location of the acrylic plug located under the dermis overlying the aMS of the intact (left) hemisphere and translating it onto the corresponding position in the ipsilesional hemiscalp. During the stimulation, a marked 1-cm^2 region on the outer perimeter of the coil, where the magnetic field of a round coil is strongest, was placed on the ipsilesional aMS region and kept tangential to the surface of the skull by tilting it down 35–45° while keeping the coil handle angled 20° rostrally (Valero-Cabré & Pascual-Leone, 2005; Valero-Cabré *et al.*, 2005, 2006, 2007, 2008). High-frequency 10-Hz rTMS (n = 12) was delivered for 20 min at a fixed intensity of 40% of the machine maximal output (~120% of the animal's motor threshold; Moliadze *et al.*, 2003), in 10-pulse trains interleaved with 5-s intertrain intervals, amounting to 2400 pulses per stimulation session. This stimulation frequency was ultimately chosen given its known excitatory effects in the human (Bohotin *et al.*, 2002; Fierro *et al.*, 2005; Fumal *et al.*, 2006) and feline (Aydin-Abidin *et al.*, 2006) visual cortex. Furthermore, preliminary experiments in our lab have shown that awake animals are highly tolerant to 10 Hz, whereas excitatory patterns using higher frequencies (15–20 Hz) may cause stress and discomfort. Sham rTMS stimulation (n = 3) followed the exact same procedure described above, except the coil surface was held at 90° perpendicular to the surface of the scalp to direct the magnetic field away from the skull. Animals received a total of seven rounds of real or sham rTMS [Round (R)1 to R7], which were defined each as a total of 10 days of stimulation (5 days on, 2 days off, repeated once more before the next rTMS round started) delivered across 2 weeks.

During the 2 weeks prior to rTMS procedures, all felines were acclimated to the sound of rTMS pulses and accustomed to remain in a veterinary bag to ensure no distress during stimulation. No signs of abnormal behavior (e.g., aggression, anxiety, stress, reductions in agility or increases in reclusiveness) were noted during or after the stimulation.

Experimental design and follow-up

The study follow-up was divided into five phases: pre-lesion (Phase I), immediate post-lesion (days 1–2 post injury; Phase II),

spontaneous recovery (days 2–70 post-injury; Phase III), rTMS treatment (R1 to R7); (Phase IV), and Post-rTMS treatment follow-up of at least 6 weeks (Phase V). Visuospatial performances assessed at the end of those five phases were taken as milestones to define the status of the animals' behavioral recovery. The day of the surgically induced focal brain injury served as a zero-point time reference. The peak of spontaneous recovery level right before the onset of the rTMS therapy (i.e., before the first rTMS session of R1) is referred as pre-rTMS performance. Measurements gauged at the end of the seven rounds of rTMS are titled 'rTMS R7 performance'. Finally, measurements recorded after the discontinuation of the treatment are termed 'post-rTMS performance'.

Throughout the rTMS phase each daily session of stimulation was immediately followed by a 15-min testing session composed of a single block of trials for each of the three above-mentioned visuospatial tasks (Static, Moving 2 and Moving 1). Every 7 days and prior to the next rTMS stimulation session, animals received three blocks of trials for each of the three above-mentioned paradigms. In total, animals completed a total of seven rounds of rTMS, i.e., seventy daily sessions of stimulation, across a total of 14 weeks of treatment. At the end of the rTMS phase, the durability of the rTMS effects in the absence of treatment was assessed in all animals for 6 weeks following the last stimulation session. This was done through weekly evaluations identical to those performed during the rTMS treatment phase (Valero-Cabré *et al.*, 2005, 2006).

The evaluation of the rTMS effects was made against the backdrop of results from our laboratory (Rushmore *et al.*, 2010) and from other studies (Huxlin & Pasternak, 2001, 2004; Sherk & Fowler, 2002; Das *et al.*, 2012) which show that unilateral ibotenic acid lesion-induced deficits are consistent and robust, and spontaneous recovery is observed only if intensive specific training is instituted. Moreover, previous studies from our laboratory demonstrate that sham rTMS, delivered in single or accrued sessions, failed to induce visuospatial effects (Valero-Cabré *et al.*, 2006, 2008) and were therefore unlikely to produce recovery. We followed three animals with sham stimulation to control for this possibility. While it is possible that with more animals we might have seen some events of a delayed natural recovery, the weight of the above mentioned evidence makes this possibility unlikely.

Tissue processing

After the rTMS regime was concluded, animals were overdosed with sodium pentobarbital (120 mg/kg, i.v.) and their vascular system perfused with a flushing solution (15% sucrose in 0.1 M phosphate buffer, pH 7.4) for 1 min followed by a fixative solution (15% sucrose with 2% paraformaldehyde in flushing solution, pH 7.4) for 5 min. Brains were quickly removed, immersed in albumin and frozen at −30°C in 2-methylbutane for 30 min and then kept frozen at −80°C. Both hemispheres were sectioned into 23 μm-thick slices yielding ~200 serial sections per animal with collected sections spaced ~100 μm apart. Sections were then digitized and uploaded using imaging software (MCID, Imaging Research, Ste. Catherines, Ontario, Canada). Every fifth section was reacted for Nissl substance and used to verify the lesion borders by marking signs of gliosis and neuron loss.

Lesion analysis

Areas of damage were assessed with a series of Nissl stained slides for each animal. The pMS area was traced from stereotaxic coordinates P2 to A8 and the aMS cortex was traced from coordinates A9 to A14 according to previous reports (Palmer *et al.*, 1978). Lesioned cortex was characterized as a focal disruption of the cortical lamination characterized by a loss of large neuronal elements and a high density of small cell bodies consistent with gliosis (see Supporting Information Fig. S3). The lesion was quantified by outlining any intact cortical tissue within the established boundaries, and expressed at each stereotaxic location as a percentage of total spared cortex [100 × area of ipsilesional bank/sum area of contralesional bank]. These data were compared across groups using a repeated-measures ANOVA with stereotaxic (A-P position) coordinate as the independent variable.

Data presentation and statistical analysis

Behavioral data are presented in the text and figures as the group averages and SEM for correct (%) performance levels. Visual hemifield and eccentricity specific individual and group values at major follow-up time phases (pre-lesion, post-lesion, spontaneous recovery phase, rTMS recovery phase and post-rTMS recovery) were calculated as the mean of three blocks of data for each of the three tasks tested. Summary data corresponding to the end of each specific follow-up phase were calculated by averaging the last three blocks of data in each task (Valero-Cabré *et al.*, 2005, 2006). Animals treated with real rTMS were divided into 'Responders' and 'Non-responders' on the basis of the behavioral progress shown in the Static task for contralesional visual hemifield targets (see Results section for further details). Responders had to meet two pre-established criteria: (i) show statistically significant increases for detection performance of at least one additional eccentricity at the end of the rTMS treatment (with regards to their performance at the end of the spontaneous recovery phase); and (ii) display significant performance improvements for the overall contralesional hemifield. If either one or both of these two criteria were not met then the subject was assigned to the Non-responder group.

A repeated-measures ANOVA was initially used to determine whether spontaneous recovery or rTMS treatment yielded statistically significant ameliorations over the course of treatment for the active 10-Hz rTMS group. These analyses were done for performance levels (% correct detection) as a dependent variable, and follow-up phase (spontaneous recovery, rTMS treatment, post-rTMS phase), visuospatial task (Static, Moving 2 tasks), and visual hemispace (ipsilesional, contralesional) as independent factors. The *F*-statistic from the repeated-measure ANOVA is reported in the format $F_{\text{df factor, df error}}$. We also conducted *a-priori* planned pair-wise comparisons using a Student's *t*-test of the critical time points in the study (pre-lesion, post-lesion, pre-rTMS and post-rTMS). For lesion analysis, the percentage of spared cortex was determined with the above-mentioned calculation, and percentages of spared cortex were then averaged for each group. Repeated-measures ANOVA was first conducted between groups using stereotaxic coordinates (A-P coordinates) as factors to determine whether significance in lesion size was present throughout the visual areas. Student's *t*-tests were used to compare the total area of lesion between groups. Statistical significance was set to $P < 0.05$ for all parametric analyses used in this study.

Results

Impact of parietal damage and ensuing visuospatial spontaneous recovery

In accordance with prior studies, lesions targeting both banks of the feline right posterior parietal cortex (known as pMS) induced a

complete contralesional visuospatial orienting deficit in all tasks. These deficits were present immediately after the lesion (only 24 h post-injury) and started to improve spontaneously shortly thereafter. The basis of this improvement is likely to be a combination of network modulation vicariation (Rushmore *et al.*, 2010) and reduction in acute effects such as inflammation, lesion-induced depolarization and cortical spreading depression events (see reviews by Cramer, 2008; Nudo, 2011).

For the high-contrast moving task (Moving 1), subjects regained function in the contralesional visual hemispace within 5–10 days, and exhibited complete and stable recovery 30 days thereafter (Moving 1, 30 days post-injury 93 ± 4% vs. 98 ± 1% pre-lesion, $P = 0.05$; data not shown in figure form) which remained unaltered across the follow-up period. In contrast, recovery for static or laser-based moving targets (Day 70: Static pre-rTMS, 39 ± 7% vs. pre-lesion, 82 ± 3%; $P = 0.00$; and Moving 2 pre-rTMS, 62 ± 9% vs. pre-lesion 88 ± 3%; $P = 0.02$ correct performance) operated at a slower pace and reached plateau levels of incomplete recovery between 40 and 60 days after the injury (see Fig. 2).

Unilateral lesions not only induced the expected pattern of contralesional visuospatial defects, but significantly affected detection performance for visual targets presented in the ipsilesional hemispace. Such effects were particularly noticeable for the Static detection task (Static: drop from 72 ± 2% to 58 ± 3%; $P = 0.00$). The drop in ipsilesional performance was significant in Moving 2 task but negligible for Moving 1 (Moving 2, from 78 ± 4% to 70 ± 4%, $P = 0.01$; see Fig. 2; and Moving 1, from 98 ± 1% pre-lesion to 93 ± 5% Day 70, $P = 0.05$; data not shown in figure form) and remained unaltered across the follow-up.

Effects of the rTMS regime on visuospatial recovery

Once plateau levels of pre-rTMS were achieved, animals started a daily rTMS regime consisting of a total of 70 consecutive sessions delivered across 14 weeks of treatment. In agreement with published observations (Rushmore *et al.*, 2010), the sham group demonstrated a complete absence of improvement, and those effects endured beyond pre-rTMS levels for both the Static (from 20 ± 9% to post-sham rTMS 22 ± 12% correct performance; $P = 0.68$) and Moving 1 tasks (from pre-TMS 77 ± 20% to post-sham rTMS 70 ± 13%, $P = 0.55$; data not shown in figure form).

As for the 12 subjects assigned to sessions of real 10-Hz rTMS, a significant three-way interaction between follow-up phase, task, and visual hemispace was found ($F_{13,130}$, $P = 0.01$). As a group improvements reached statistical significance over time for the Static task (pre-rTMS, 39 ± 7% to post-rTMS, 53 ± 7%; $P = 0.00$; Fig. 2). Overall, results accounted for variable levels of contralesional correct performance ranging from improvements of +67% to losses of -15% with respect to individual subject's pre-rTMS treatment levels.

According to statistical criteria for minimal neglect recovery (see Material and Methods section), the groups of active rTMS-treated animals were classified into the categories of Responders ($n = 6$) and Non-responders ($n = 6$). Overall the rTMS regime generated two groups of equally treated animals, which thus far had performed equivalently in the Static task (Pre-rTMS: Responders, 36 ± 6% vs. Non-responders, 42 ± 14% correct performance; $P = 0.89$). An initial decrease in performance characterized the Non-responders in the Static task, and in any case active rTMS treatment failed to

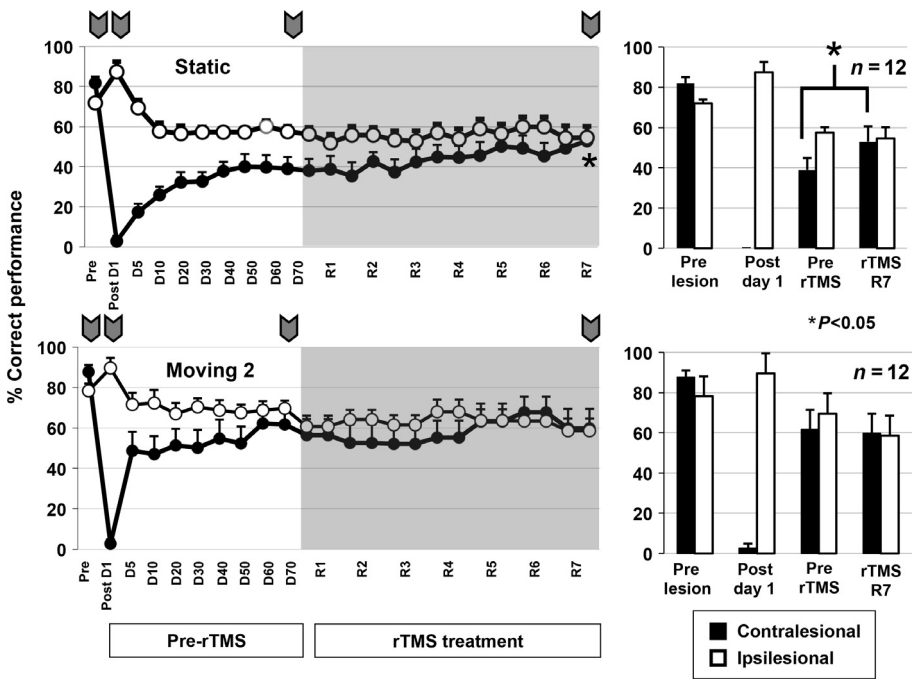

FIG. 2. General group visuospatial orienting performance levels for static and motion tasks. Group average levels of correct visual detection performance ($n = 12$) for static (upper panel, STATIC) and or moving LED targets (bottom panel, MOVING 2) presented in the ipsilesional (white circles) or the contralesional (black circles) visual hemispace respectively, across the three follow-up phases (pre-lesion, post-lesion (from days 1 to 70 post-injury; D1–D70) and rTMS treatment phase (from R1 to R7). Error bars represent the SE of the group mean. Gray shaded portion represents the rTMS treatment phase across rTMS rounds (from R1 to R7). Gray arrows placed above each panel highlight key time periods of the follow-up which are summarized in the histogram panel on the right (Prelesion, Post-lesion Day 1, Pre-rTMS treatment, post-rTMS-R7). *$P < 0.05$ for values at rTMS R7 vs. pre-rTMS levels. Notice the effects of the lesion on contralesional visual detection leading to hemispatial neglect, its spontaneous recovery across the 70 days that follow damage and subsequent mild rTMS-induced improvements for contralesional detection, particularly for static visual targets.

influence correct performance levels (rTMS R7, 38 ± 12% vs. pre-rTMS, 40 ± 14%; $P = 0.70$). In contrast, within the contralesional hemispace Responders exhibited progressive increases in visuospatial orienting with the accrual of active rTMS sessions, and reached their performance peak after seven rounds of rTMS (rTMS R7, 68 ± 4% vs. pre-rTMS, 42 ± 6%; $P = 0.01$; Fig. 3). This recovery appeared, however, to be task-dependent and accordingly Responders experienced little improvements with respect to pre-rTMS treatment levels in the Moving 2 task (pre-rTMS, 70 ± 13% vs. post-rTMS, 79 ± 6%; $P = 0.67$; Fig. 3).

For the Static task, the rTMS regime did not significantly alter performance in the Responders group for ipsilesional targets (Pre-rTMS, 60 ± 3% vs. rTMS R7, 67 ± 8%; $P = 0.45$; Fig. 4). Interestingly, in the Non-responders group, while rTMS treatment failed to positively influence contralesional detection it did produce decreases in correct performance for ipsilesional targets (Static task pre-rTMS, 58 ± 5% vs. rTMS R7, 43 ± 2%; $P = 0.03$). Similar effects were observed for the Moving 2 task (Pre-rTMS, 68 ± 6% vs. rTMS R7, 47 ± 3%; $P = 0.01$; Fig. 4). Taken together, these data strongly suggest that in a specific subpopulation of participants the rTMS treatment could have modulated cortical function in an unexpected manner, impairing an ipsilateral function which should had remained otherwise unaffected.

Eccentricity analysis

Prior to lesion all subjects displayed nearly complete correct performance for the detection of static contralesional pericentral targets corresponding to the binocular portions (15–45°) of the visual field (Static 15°, 98 ± 1%; 30°, 96 ± 2%; 45°, 93 ± 4% correct detection performance). In contrast, peripheral targets presented at monocular visual field eccentricities (60–90°) were detected at more moderate performance rates (Fig. 5; Static 60°, 82 ± 7%; 75°, 69 ± 8%; 90°, 42 ± 10%). A gradient evolving from pericentral to periphery and extending to the contralesional 15°, 30°, and 45° eccentric locations characterized the spontaneous recovery phase for all visuospatial paradigms (Static 15°, 83 ± 8%; 30°, 58 ± 10%; and 45°, 44 ± 11%). Ipsilesionally, a paradoxical expansion of the visuospatial attention span towards the periphery (60°, from 78 ± 6% to 96 ± 0%; 75°, from 45 ± 8% to 83 ± 0%; and 90°, from 14 ± 4% to 75 ± 0%) was followed by a progressive return to pre-injury correct performance levels (60°, 52 ± 10%; 75°, 19 ± 8%; and 90°, 12 ± 5%) by the end of the spontaneous recovery period (Fig. 5). Very similar findings were also obtained for the Moving 2 task (data not shown in figure form).

Our analysis shows that, prior to rTMS, the spontaneous recovery patterns for Static contralesional targets were not significantly different between Responders and Non-responders. This occurred regardless of the contralesional visual space in either binocular (15°, Responders 97 ± 2% vs. Non-responders 70 ± 13%, $P = 0.10$; 30°, 68 ± 10 vs. 48 ± 18%, $P = 0.40$; 45°, 42 ± 1% vs. 47 ± 19%, $P = 0.73$) or monocular (60°, 17 ± 11% vs. 40 ± 18%, $P = 0.18$; 75°, 20 ± 16% vs. 17 ± 11%, $P = 0.89$; 90°, 10 ± 8% vs. 13 ± 13%, $P = 0.58$; Fig. 6) vision. Very similar findings were also observed for the Moving 2 task (Fig. 7).

After seventy sessions of rTMS treatment significant differences between the two subgroups of rTMS-treated animals emerged. Responders showed significant gains in the detection of Static targets presented in pericentral locations (Static: 45°, pre-rTMS 42 ± 11% vs. rTMS R7 92 ± 4%, $P = 0.01$; and 60°, 17 ± 11 vs. 61 ± 10% correct detections, $P = 0.04$), but not for eccentricities in

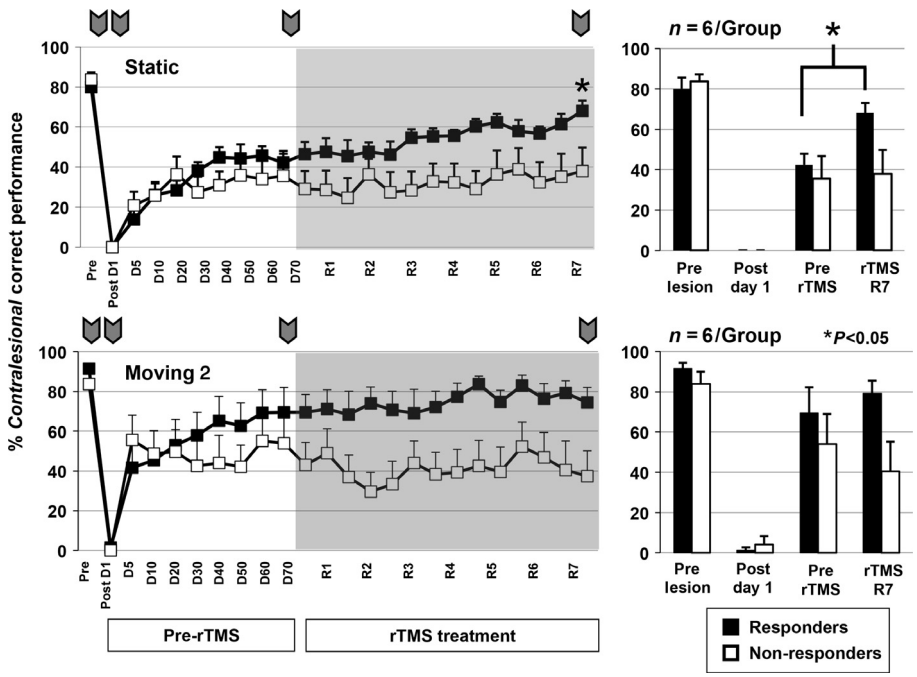

FIG. 3. Contralesional visuospatial orienting performance for Responders and Non-responders in static and motion tasks. Group average levels of correct contralesional detection performance for the Static (upper panel) and Moving 2 (lower panel) tasks for the Responders (black squares, $n = 6$) and Non-responders (white squares, $n = 6$), throughout the three-phase follow-up. Error bars represent the SE of the group mean. The gray shaded portion represents the rTMS treatment phase from R1 to R7. Gray arrows placed above each panel highlight key time periods which are summarized in the histogram panel on the right. *$P < 0.05$ for values at rTMS R7 vs. pre-rTMS levels. Notice in Responders the rTMS-induced improvements in contralesional detection performance levels, particularly for the Static task. In contrast, Non-responders showed a stabilization of performance for these two tasks during the same period of time. Similar patterns, although not reaching significance, were found in contralesional and ipsilesional performance for the Moving 2 task.

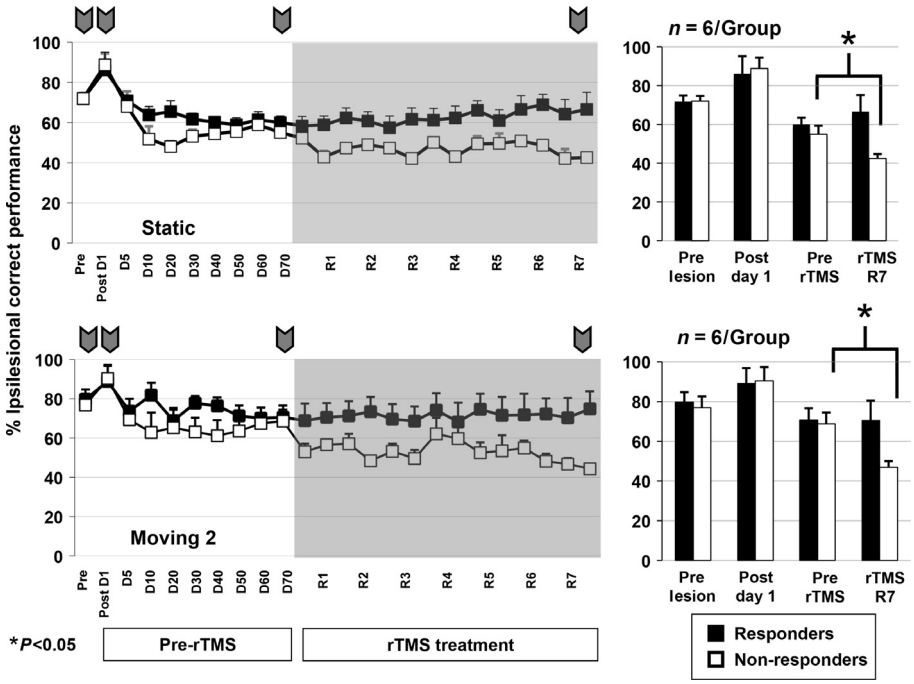

FIG. 4. Ipsilesional visuospatial orienting performance for Responders and Non-responders in static and motion tasks. Average levels of correct ipsilesional detection performance for the Static (upper panel) and Moving 2 (lower panel) tasks for the Responders (black squares) and Non-responders (white squares). Error bars represent SE of the group mean. The gray shaded portion represents the rTMS treatment phase from R1 to R7. Gray arrows placed above each panel highlight key time periods of the follow-up, which are summarized in the histogram panel on the right. *$P < 0.05$ for values at rTMS R7 vs. pre-rTMS levels. Notice Responders' ipsilesional performance showed no differences between the rTMS pre and post-treatment. In contrast, Non-responders displayed maladaptive response to rTMS treatment that significantly reduced their ability to detect and orient to ipsilesional targets in both tasks.

the periphery (Fig. 6). Similar patterns of eccentricity-dependent ameliorations, mainly involving binocular visual locations in the Moving 2 task were also found, although they failed to reach statistical significance (Moving 2: 15°, pre-rTMS 94 ± 3% vs. rTMS R7 100%, $P = 0.09$; 30°, 82 ± 11% vs. 97 ± 3%, $P = 0.20$; 45°, 73 ± 16% vs. 89 ± 7%, $P = 0.39$; 60°, 70 ± 18% vs. 83 ± 8%, $P = 0.37$; Fig. 7). In contrast, in the Non-responders group the rTMS treatment resulted in a pattern of degraded performance for monocular targets (Static: 60°, pre-rTMS 40 ± 18% vs. rTMS R7 28 ± 16%, $P = 0.06$; 75°, 17 ± 11 vs. 7 ± 5%, $P = 0.25$; 90°, 13 ± 13% vs. 0%, $P = 0.36$; Moving 2: 45°, pre-rTMS 66 ± 20% vs. rTMS R7 50 ± 18%, $P = 0.37$; 60°, 64 ± 19% vs. 43 ± 19%, $P = 0.14$; 75°, 44 ± 17% vs. 27 ± 16%, $P = 0.37$; 90°, 18 ± 8% vs. 4 ± 4%, $P = 0.14$).

Interestingly, Responders and Non-responders also showed different patterns for ipsilesional performance. More precisely, with rTMS Non-responders exhibited a reduction in performance for the detection of targets at monocular eccentricities with significance only found at Static 45° and some Moving 2 targets (Static: 90°, pre-rTMS 17 ± 7% vs. rTMS R7 0%, $P = 0.05$; 75°, 23 ± 11% vs. 6 ± 6%, $P = 0.09$; 60°, 39 ± 14 vs. 21 ± 14%, $P = 0.41$; 45°, 94 ± 3% vs. 68 ± 8%, $P = 0.04$; Moving 2: 90°, pre-rTMS 19 ± 9% vs. rTMS R7 0%, $P = 0.01$; 75°, 45 ± 17% vs. 0%, $P = 0.04$; 60°, 68 ± 14% vs. 9 ± 4%, $P = 0.09$).

Behavioral predictors of rTMS-induced recovery

The behavioral data derived from this study indicate that rTMS significantly improved contralesional performance in a subset of animals. Interestingly, the single most contributing predictor of positive rTMS-induced recovery for the whole group was found to be the plateau levels of spontaneous recovery achieved prior to the onset of neurostimulation. In other words, the greater the levels of spontaneous levels an animal exhibited the greater the potential rTMS-induced recovery (correlation coefficient of $r = 0.74$, $P = 0.03$). Finally, the eccentricities of the contralesional visual hemispace that appeared most highly correlated with final recovery levels were the 15° ($r = 0.85$, $P = 0.00$), 30° ($r = 0.72$, $P = 0.00$), and 45° ($r = 0.60$, $P = 0.04$) visual targets.

Durability of achieved recovery in absence of rTMS treatment

Six weeks after the discontinuation of the rTMS regime, recovery rates for contralesional detection in the Responders group remained at similar levels to those reached after the last round of treatment (Static: rTMS R7 68 ± 5% vs. post-rTMS 65 ± 5% correct performance, $P = 0.21$) and this long-lasting performance was most apparent in the mid-periphery targets (Fig. 8). Interestingly, for Non-responders the discontinuation of rTMS sessions induced significant gains in performance, which had progressively degraded during the neurostimulation phase. Those effects were particularly significant for the detection of motion targets (Moving 2: ipsilesional targets, post-rTMS R7 46 ± 3% vs. rTMS R7 58 ± 3%, $P = 0.01$; contralesional targets, 41 ± 15% vs. 65 ± 10%, $P = 0.01$) whereas it did not influence the detection of static targets (Static ipsilesional targets R7, 42 ± 5% vs. post-rTMS 48 ± 3%, $P = 0.10$; and contralesional post- rTMS R7, 38 ± 3% vs. post-rTMS 45 ± 12%, $P = 0.56$). These effects reverted to pre-rTMS values particularly for mid-central ipsilesional eccentricities (Moving 2: 45°, post-rTMS 50 ± 18% vs. rTMS R7 81 ± 19%, $P = 0.24$; 60°, 43 ± 19% vs. 67 ± 23%, $P = 0.26$; Fig. 8). Overall, the restoration of performance in Non-responders proved to be reversible once the rTMS regime ended,

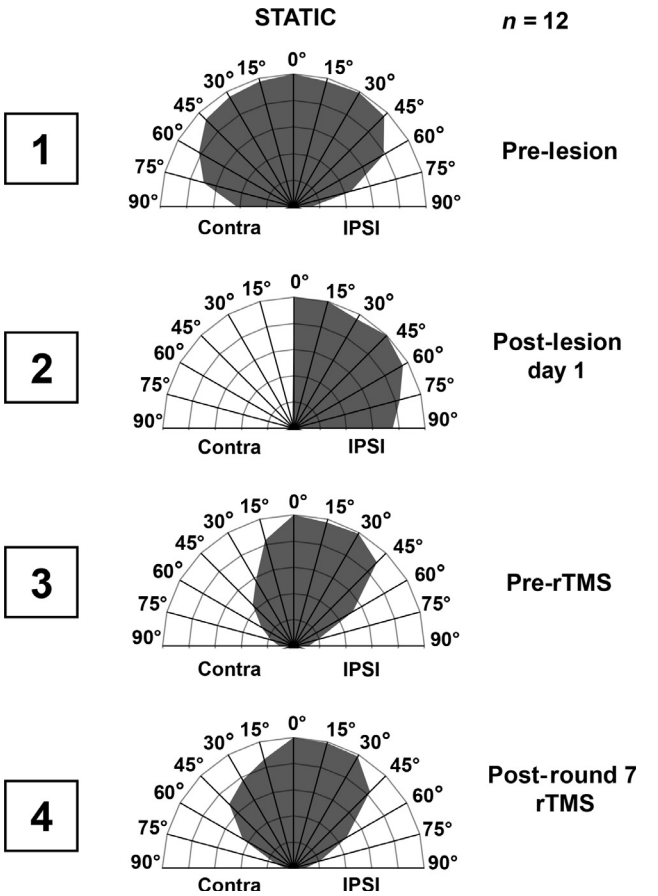

FIG. 5. General group eccentricity-specific recovery effects of orienting to static visual targets. Radial maps indicating percentage correct performance in static target detection at each visual eccentricity for the entire population (n = 12) of participants. Bar length represent the correct performance for the ipsilesional (right) or contralesional (left) visual hemispace. Data are shown (top to bottom) for [1] pre-lesion, [2] postlesion day 1, [3] end of spontaneous recovery phase or pre-rTMS phase and [4] at the end of the seventh round of rTMS treatment. Concentric half circles represent steps of 20% performance, from the inner (15°) to the outermost (90°) eccentricity. Gray shaded portions display the space area of correct visual detection and orienting performance. Note that felines performed better for pericentral and midperipheral than for far peripheral eccentricities pre-lesion, and that unilateral parietal damage mainly affected orienting response towards targets in the contralesional visual hemispace. Spontaneous recovery of function progressed from pericentral to mid peripheral locations (0–60°) and rTMS driven recovery increased performance levels for some of those same locations (30–60°).

which further supports the role of neurostimulation as being responsible for the maladaptive effects observed in this subset of animals.

Lesion analysis and anatomical–behavioral correlations

The intention of the experiment was to damage the homologue of the human posterior parietal cortex, known as pMS, and to later apply rTMS on the rostrally adjoining aMS cortex, which is known for its ability to adequately compensate lost function after lesion (see Fig. 1 for details on the anatomy). A comprehensive lesion analysis indicated that, for all animals, the majority of the injured cortical area was removed. Nonetheless, areas of incomplete damage were found extending 1–3 mm rostrally in some subjects (n = 3 in Responders and n = 3 in Non-responders), impinging into the aMS cortex (stereotaxic levels A9–A11) or 1 mm caudally into the ventral posterior suprasylvian and the dorsal posterior suprasylvian regions (stereotaxic level P3; n = 2 in Responders and n = 3 in Non-responders). In addition, all 12 subjects showed very minor collateral damage to the pMS-adjacent visual areas such as primary visual area A19 and the splenial visual area, due to a minor but unpreventable diffusion of the neurotoxin. This spread appears to be consistent with other studies using the same methods (also see Rudolph & Pasternak, 1996; Huxlin *et al.*, 2008; Rushmore *et al.*, 2010; Das *et al.*, 2012; Supporting Information Figs S1 and S2).

Quantification of injured area (mm^2) showed no significant differences in the amount of lesion between groups, either for the medial (pMLS) or the lateral (pLLS) bank of the posterior parietal (pMS) cortex along the length of both pMS and aMS visual areas. Overall, the amount of spared tissue between Responders and Non-responders in both the injured pMS cortex (pMLS: 21 ± 8% vs. 14 ± 6%, P = 0.2; pLLS: 18 ± 6% vs. 15 ± 6%, P = 0.60) and the rTMS-stimulated aMS cortex (aMLS, 79 ± 7% vs. 58 ± 13%, P = 0.10 and aLLS, 79 ± 7% vs. 64 ± 13%, P = 0.10; data not shown in figure form) was not statistically different across groups. Responders and Non-responders also did not show significant differences in spared cortex at any specific coordinates across the rostral–caudal extent from pMS through aMS (medial bank, $F_{4,32}$, P = 0.32; lateral bank, $F_{4,32}$, P = 0.60). The final step was to determine whether the amount of spared tissue correlated to behavioral measures in the pMS cortex, and we found no relationship of anatomy with spontaneous (r = 0.35, P = 0.24) or rTMS-induced recovery (r = 0.15, P > 0.05). Overall, this observation suggests that lesion size was not the main determinant of the observed discrepancies between Responders and Non-responders.

Discussion

In the current study, we aimed at maximizing our chances of driving significant recovery by accruing 70 sessions of excitatory rTMS on a well-determined perilesional area shown to adopt lost visuospatial function after parietal injuries in felines (Lomber *et al.*, 2006). Our rTMS regime generated significant improvements in visuospatial orienting deficits in approximately half of our subjects, while the other half experienced maladaptive effects for the detection of static or motion stimuli displayed mainly in the ipsilesional visual hemispace. Furthermore, our data indicate that, while ameliorations outlasted the discontinuation of the rTMS regime, maladaptive ipsilesional visuospatial phenomena tended to regress as soon as the rTMS regime ceased. Our data provide new insights into the advantages and disadvantages of stimulating patients afflicted by different severities of hemispatial neglect, and sheds light on the potential and limitations of noninvasive neurostimulation approaches applied on perilesional cortex to rehabilitate visuospatial attentional orienting.

Clinical impact of rTMS in visuospatial recovery

In agreement with the initial hypothesis of this paper, the accrual of a high number of rTMS sessions proved to be a key factor in the achievement of significant levels of recovery (Valero-Cabré *et al.*, 2008), as enhancements in performance emerged only after ~30–40 sessions of stimulation. If, similarly to most clinical studies, we had limited our rTMS regime to 2 weeks or less of treatment we would not have observed functional recovery. Therefore, our findings strongly emphasize the role of the accumulation of a high number of perilesional rTMS sessions to induce significant and long-lasting clinical ameliorations, particularly in chronic brain-damage patients.

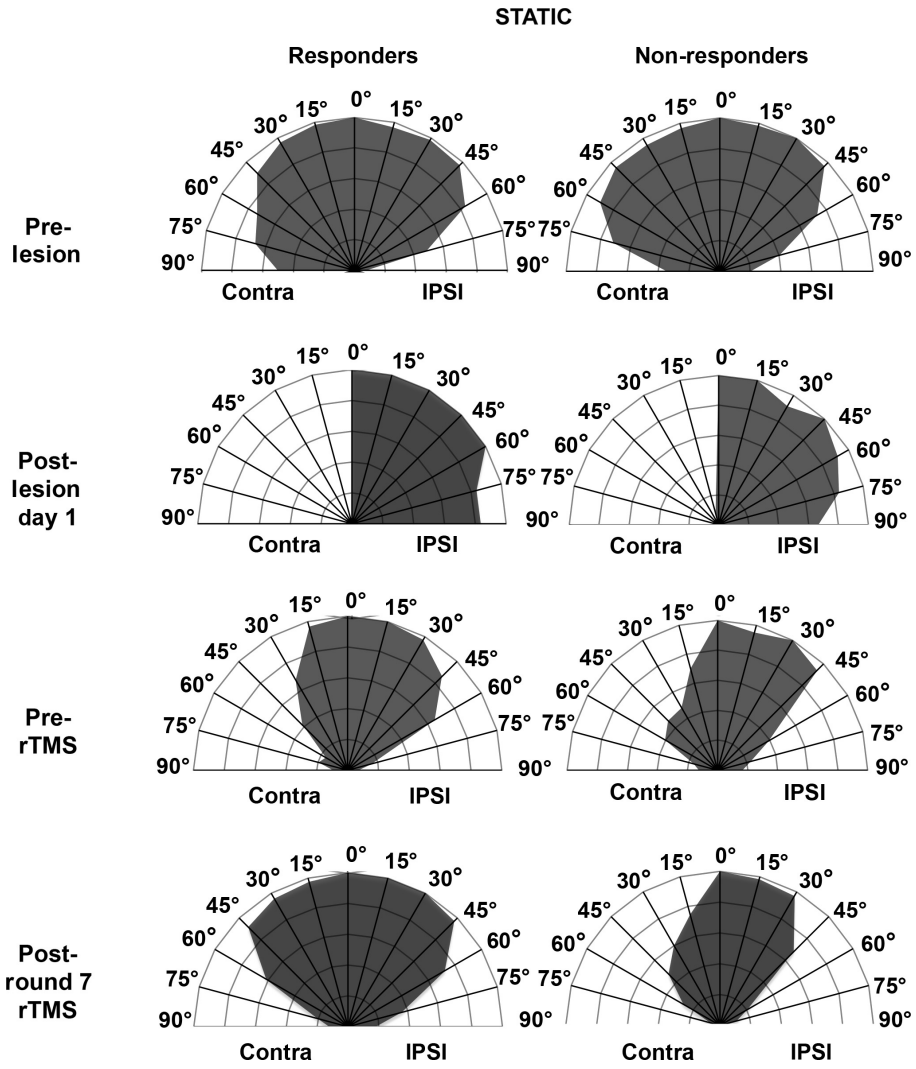

FIG. 6. Eccentricity-specific recovery of orienting to static visual targets for Responders and Non-responders. Radial maps displaying the percentage correct performance in static target detection at each visual eccentricity for the subsets of Responders (n = 6) and Non-responders (n = 6) in the Static task. Data are shown (top to bottom) for four stages of the study. Bar length represents the correct performance for the ipsilesional (right) or contralesional (left) visual hemispace. Concentric half circles each represent steps of 20% performance, from the most pericentral (15°) to the most peripheral (90°) eccentricity. Gray shaded portions represent the area of correct visual detection and orienting performance. Notice that in both groups of subjects, Responders and Non-responders, post-lesion spontaneous recovery progressed at the contralesional space from 0 to 45°, at uneven levels of performance, while suffering ipsilesional peripheral losses (90–60°). Only Responders showed rTMS-driven ameliorations, which extended to the further contralesional periphery (60°), while increasing performance levels at the prior eccentricities. In contrast, Non-responders showed paradoxical losses in mid peripheral ipsilesional eccentricities (45°).

It is critical to point out that during the rTMS phase no negative behavioral effects of the stimulation were noted. Animals displayed normal motor and sensory behavior during the execution of the tasks and exhibited normal behavior outside of the testing arena, indicating the safety of such an extensive rTMS regime.

Conventionally, functional recovery aims to restore the imbalance of interhemispheric inhibition by treating an overexcited contralesional hemisphere (Oliveri *et al.*, 2001; Brighina *et al.*, 2003; Shindo *et al.*, 2006). The latter approach might have the advantage of acting on a structurally intact cortex, and the effect of magnetically induced electric current fields can be better predicted (Wagner *et al.*, 2007). Moreover, seizures would be less likely, particularly due to the use of suppressive instead of excitatory stimulatory patterns (Rossi *et al.*, 2009). In spite of some hypothesized shortcomings related to the unpredictability of intracortical current patterns tied to the risks of excitatory neurostimulation on a sensitive region, single sessions of direct perilesional neurostimulation have previously demonstrated beneficial outcomes in the rehabilitation of stroke sequels (Hummel *et al.*, 2005; Khedr *et al.*, 2005; Kim *et al.*, 2006; Talelli *et al.*, 2007; Sparing *et al.*, 2009). Moreover, anatomical and functional evidence supports the notion that perilesional tissue is a key component for reorganization and plasticity, leading to behavioral improvements after focal brain damage (Nudo, 1999, 2006; Mittmann & Eysel, 2001; Werhahn *et al.*, 2003). Accordingly, we tested the hypothesis that a direct manipulation of perilesional tissue activity in multiple sessions would maximize the magnitude and duration of the pursued therapeutic outcomes.

Indeed, our findings confirm that in spite of inter-individual variability, high-frequency perilesional rTMS stimulation is capable of recovering real-space visuospatial function in chronically brain-damaged individuals. Nonetheless, the discussion on which factors might best account for such variability remains open. Results reveal that

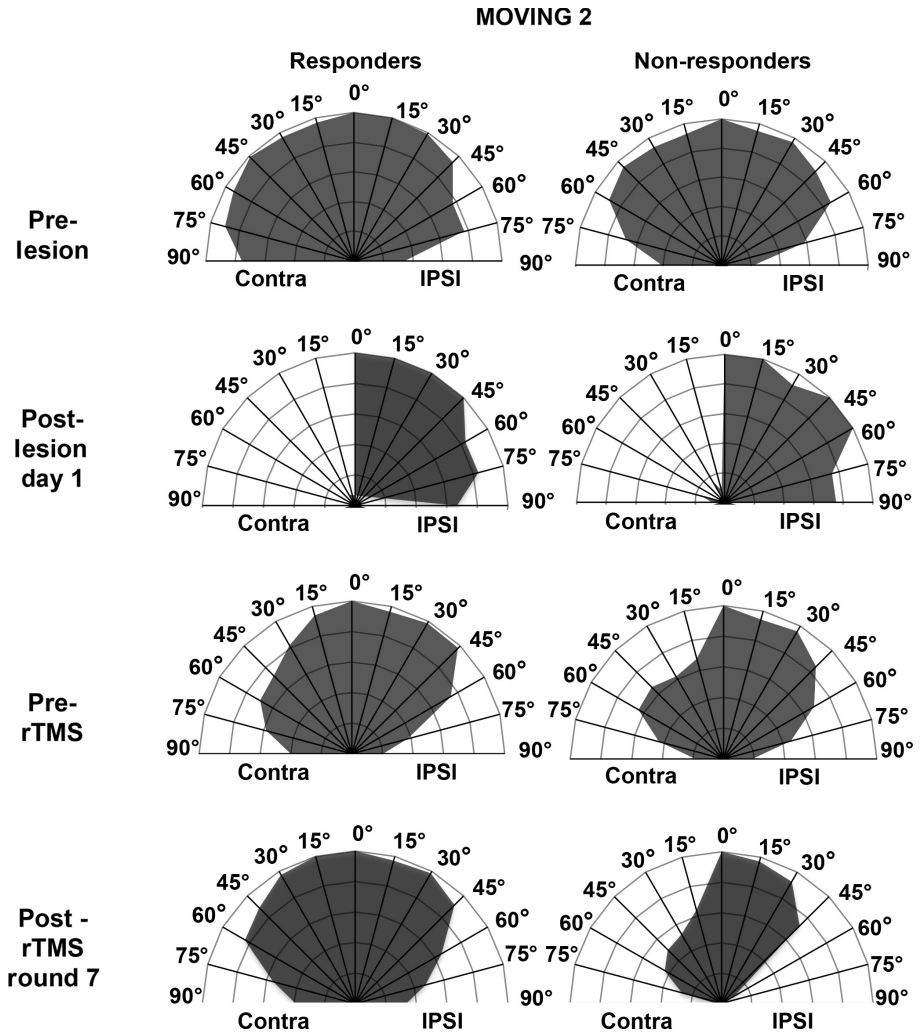

FIG. 7. Eccentricity-specific recovery of moving stimuli for Responders and Non-responders. Radial maps displaying the percentage correct performance in motion target detection at each visual eccentricity for the groups of Responders (n = 6) and Non-responders (n = 6) for the Moving 2 task. Data are shown (top to bottom) for four stages of the study. Bar length represent the percentage correct performance for the ipsilesional (right) or contralesional (left) visual hemispace. Concentric half circles represent steps of 20% correct performance, from the inner (15°) to the outermost (90°) eccentricity. Gray shaded portions represent the area of correct visual detection and orienting performance. Notice that mainly Responders and, at lower rates, also Non-responders, recovered contralesional orienting spontaneously in a pericentral to mid-peripheral gradient (from 0 to 45°). Both groups had ipsilesional peripheral losses (from 90 to 75°). Responders improved performance from pericentral to far peripheral eccentricities (30–75°). Non-responders showed paradoxical losses in specific ipsilesional eccentricities (75–45°).

the level of spontaneous recovery seems to be the only significant predictor of positive rTMS improvements. More specifically, low spontaneous recovery levels were associated with little benefit from rTMS rehabilitation in our group of Non-responders, while those with moderate spontaneous recovery prior to rTMS, within the group of Responders, benefited the most from stimulation. A closer inspection of eccentricity-specific recovery values shows that ameliorations progressed from pericentral to peripheral eccentricities. Furthermore, Non-responders as opposed to Responders failed to fully recover spontaneously the ability to orient to targets presented at the most pericentral contralesional eccentricity, separated only 15° from fixation. This result suggests that a consistent and complete recovery of this specific spatial location might be essential to recover orienting in further peripheral eccentricities during the spontaneous recovery phase and to show further improvements under neurostimulation. Regardless of where the processing and recovery of 15° took place, it appears that these early-recovered pericentral eccentricities served as a critical visual cue acting as a stepping-stone to facilitate awareness to progressively more eccentric locations within the neglected visual hemispace, increasing overall recovery.

Inter-individual variability in the clinical effects of the rTMS regime

Without a doubt, one of the most intriguing aspects of the current study is the existence of contrasting behavioral effects in equally treated animals. Several studies have demonstrated that it is not uncommon to find large levels of inter-individual variability in electrophysiological and behavioral responses of healthy humans to rTMS (Maeda *et al.*, 2000; Maeda & Pascual-Leone, 2003; Gangitano *et al.*, 2002; Bäumer *et al.*, 2003). Such phenomena could become even more apparent in brain-damaged populations, as differences in lesion size and/or location, shifts in cortical areas, or diaschisis-mediated alterations in activity patterns confound the predicted focality, depth and magnitude of the induced electrical

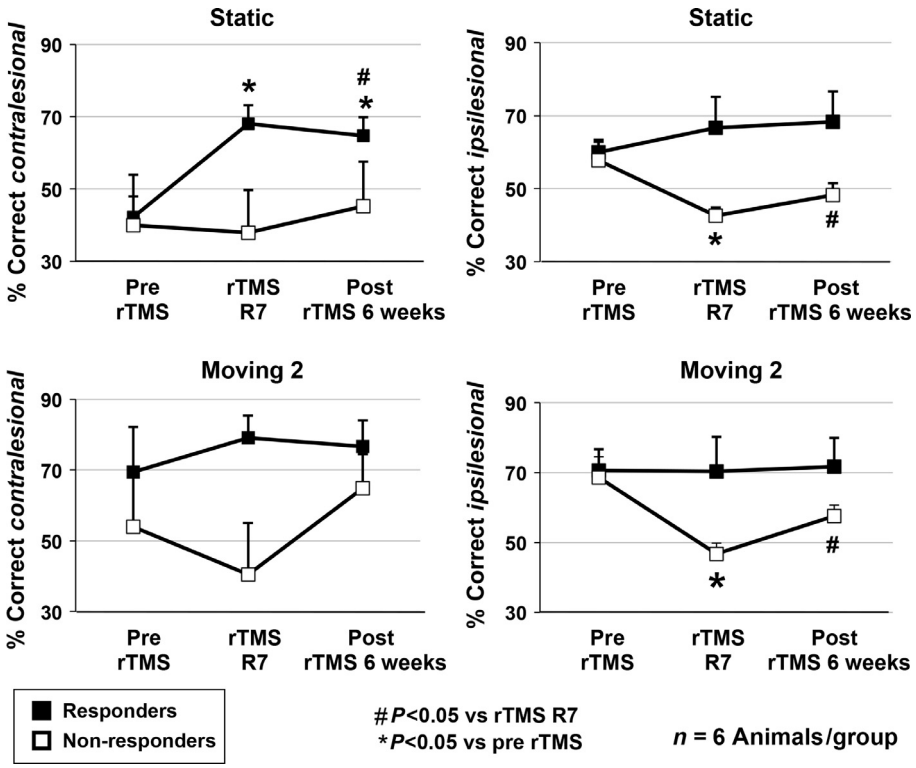

FIG. 8. Durability of rTMS-induced effects on visuospatial performance. Summary graphs of post-lesion correct performance recovery for contralesional (left column) or ipsilesional (right column) visuospatial detection in the Static (upper panel) and Moving 2 (bottom panel) tasks. Data recorded pre-rTMS (end of the spontaneous recovery phase), post-rTMS R7, and 6 weeks post-rTMS are compared to demonstrate the durability of the adaptive and maladaptive rTMS-driven effects in our population of Responders (black squares) and Non-responders (white squares). Error bars represent SEM. $^{*}P < 0.05$ for values vs. pre-rTMS treatment; $^{\#}P < 0.05$ for 6 weeks post-rTMS vs. rTMS R7 levels. Notice that the rTMS recovery achieved by Responders in the Static task remained and did not show any signs of wearing off 6 weeks after the discontinuation of the rTMS regime. Interestingly, the unexpected and maladaptive ipsilesional (and more moderately also contralesional) decreases in performance in Non-responders wore off once the rTMS was discontinued. The latter effects proved particularly significant for the detection of static and laser-based motion stimuli presented in the ipsilesional hemispace.

currents (Wagner *et al.*, 2007). Nonetheless, the lack of significant discrepancies in lesion location and size between our two subgroups of individuals would in principle rule out damage extent as a major factor probably influencing the outcome of our rTMS regime. Hence, a possibility that remains to be demonstrated is that variability could emerge from the interaction of the 10 Hz rTMS regime, with different levels or patterns of ongoing local parietal activity at the time of stimulation, which could be directly or indirectly related to the degree of recovery achieved spontaneously (Silvanto *et al.*, 2007a,b).

Considering interhemispheric rivalry principles, we inferred that the perilesional aMS cortex had a reduced excitability state. Given this, our data suggest that, in at least half of our subjects, excitatory rTMS patterns should have increased perilesional activity levels and caused visuospatial progress beyond spontaneous recovery levels. The lack of amelioration seen in the remaining subjects could have been caused by a state-dependent reduction in the likelihood of rTMS to induce further local perilesional excitation, more prone to yield insufficient regional modulations (Silvanto *et al.*, 2007a) or even reverse the direction of such local effects (Siebner *et al.*, 2004). Considering state-dependent principles as a factor explaining response differences to rTMS, and given that variability in local baseline activity in intact areas of the spared hemisphere might be less than on lesional and perilesional tissue, it is reasonable to hypothesize that the stimulation of the spared contralesional parietal regions with low-frequency rTMS could have led this same cohort of animals to respond more consistently. In the absence of further data, this hypothesis remains speculative and future studies combining rTMS with neuroimaging techniques will have to demonstrate its likelihood.

Long-term effects of rTMS and neural plasticity mechanisms

The long duration of the recovery achieved in the group of Responders, spanning at least 6 weeks beyond the end of the rTMS regime, strongly supports the notion that the beneficial rTMS-driven effects on visuospatial neglect reach a level of stability over time well beyond what has been demonstrated thus far in human patients (Shindo *et al.*, 2006; Koch *et al.*, 2012). Furthermore, our data indicate that, in contrast with the latter effects, ipsilesional orienting losses also generated by the stimulation regime in some subjects regressed as soon as the treatment was discontinued. In other words, stability was reached and maintained for adaptive but not for maladaptive outcomes. Events of synaptic plasticity such as LTP- and LTD-like processes or local and/or network-wide connectivity rearrangements through the unmasking of silenced collaterals or axonal sprouting could underlie both phenomena. Nonetheless, the techniques employed in this study allow us only to speculate with regards to the mechanisms involved and the ability of the network to facilitate behavioral recovery and its stability over time. There are, however, grounds for arguing that the periodicity of the rTMS-mediated daily excitation exerted on the perilesional region may generate Hebbian-type modifications in the synaptic strength of specific connections within postsynaptic targets (such as the ipsilateral

superior colliculus or the contralateral posterior parietal regions), similar to those elicited by experience- or activity-dependent plasticity in the adult visuospatial system during task learning or consolidation. In particular, in the current study, excitatory rTMS might have helped perilesional neurons overcome a state of low activity caused by input losses from damaged ipsilesional homotopic sites. Such rearrangements would cause visual inputs access to the system and allow two crucial events: first, a more balanced attentional deployment in space and, second, the subsequent triggering of head- and eye-orienting activity towards static targets which were formerly neglected.

Our data clearly show that such adaptive processes were consolidated on a step-by-step basis with the accrual of rTMS sessions. Hence these effects could probably be mediated through homeostatic plasticity mechanisms, which might dynamically readjust synaptic strengths and promote local and network stability (Sejnowski, 1977; Abbott & Nelson, 2000). The characteristic features of the rTMS-mediated effects described in this paper, with a slow building process followed by a self-sustained stability, is also compatible with the two-step plasticity hypothesis, predicting that the acquisition of skills by the brain would first operate through the reinforcement of pre-established circuits and then by the formation of new pathways, the former being a necessary requirement for the latter to occur (Pascual-Leone *et al.*, 2005).

At a more cellular level, short- and longer-term molecular modifications such as changes in the subtypes of postsynaptic NMDA or AMPA receptors (Redecker *et al.*, 2002) and expression of neurotrophins (which mainly operate on synaptic plasticity mechanisms, modifying the efficiency of functional connectivity patterns within existing networks) could be held responsible for the initial induction of events by unmasking of existing circuits. This process may be then followed by more energy-costly processes based on collateral sprouting and other structural modifications in local neurons and interneurons, which would remodel the anatomical and functional pathways underlying the behavioral task and lead to a stability of rewired changes (Zito & Svoboda, 2002; Karmarkar & Dan, 2006). It is well known that, during development for example, task-specific training has the ability to shape the structural organization of connection patterns (Sur & Rubenstein, 2005). Similarly, in post-injury adults, the mere execution of task-specific training on a periodic basis would also help consolidate a labile system and maintain newly formed synapses while ensuring that new collaterals remain active (Rossini *et al.*, 2003). Indeed, the reversibility of maladaptive events observed in our population of Non-responders speaks in favor of the crucial role played by task-related sensory inputs, intracerebral processing, and motor outputs into those newly remodeled systems. Once the extrinsic source of plasticity, i.e. the rTMS periodical stimulation regime, was discontinued in those animals, the absence of a coherent source of intrinsic input signals could have prevented the maintenance of maladaptive gains and those would have consequently worn off quickly. This is in contrast to adaptive improvements that use newly reorganized input pathways to convey, information and signals serving as a basis for further refinement and stability. Although all these hypotheses could be considered plausible, our study provides an incomplete picture of the underlying basis for behavioral modulatory phenomena induced by multi-session neurostimulation regimes. Further effort is required to take advantage of animal models to expand the current understanding of the plasticity mechanisms underlying recovery, its consolidation or reversibility over time, and the specific cause of maladaptive effects.

Conclusions

In recent years, noninvasive neurostimulation has been used to treat brain-damaged patients with promising but often controversial results. We have shown that the accrual of multiple sessions of rTMS applied to areas adjacent to a lesion can provide high levels of lasting improvements for the symptoms of visuospatial neglect. This finding suggests rTMS therapeutic potential might have been underestimated due to the short duration of the stimulation regimes normally used, for cautionary reasons, in human patients. Nonetheless, our therapeutically relevant effects were restricted to a special cluster of animals that did not experience significant recovery but were rather prone to maladaptive outcomes. In that regard, our results emphasize the need to customize the clinical indications of perilesional neurostimulation and to develop tools to anticipate the potential maladaptive effects of such treatments.

Supporting Information

Additional supporting information can be found in the online version of this article:

Fig. S1. Lesion reconstructions for the animals of the 'Responders' group.

Fig. S2. Lesion reconstructions for the animals of the 'Non-Responders' group.

Fig. S3. Coronal section of lesioned pMS cortex.

Acknowledgements

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Abbreviations

AMS, anterior middle suprasylvian cortex; pMS, posterior middle suprasylvian cortex; R1, Round 1 (of real or sham rTMS); rTMS, repetitive transcranial magnetic stimulation; tDCS, transcranial direct current stimulation.

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