Effects of high-frequency motor cortex repetitive transcranial magnetic stimulation (rTMS) on hand representation in primary somatosensory cortex. A source modelling study of somatosensory evoked potentials.

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Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a powerful tool that allows non-invasive stimulation of the cortex. It has been shown to have physiological effects (modulation of cortical excitability, oscillatory activity and of the network of brain regions involved in a specific task), sometimes -but not always- associated to behavioural consequences (see Ziemann et al., 2008 for a review on motor cortex rTMS-evoked plasticity). Since its after-effects can outlast the stimulation itself (Siebner & Rothwell, 2003; Thut & Pascual-Leone, 2010), rTMS is being actively tested as a potential non-invasive therapeutic tool for neurological and psychiatric disorders (Ridding & Rothwell, 2007). In the treatment of pharmaco-resistant neuropathic pain (i.e. pain resulting from a lesion or a disease of the somatosensory system, Treede et al., 2008), rTMS might become a non-invasive alternative to motor cortex epidural stimulation (MCS). Although its effects are weaker and of shorter duration than those of MCS, rTMS of the motor cortex M1 has demonstrated a significant potential for pain relief (André-Obadia et al., 2006; André-Obadia et al., 2008; Hosomi et al., 2008; Lefaucheur, 2006; Lefaucheur, 2011; Leo & Latif, 2007). This effect is reached only when rTMS is delivered to the motor cortex at high frequencies (5 to 20 Hz), rather than low frequencies (0.1 to 1 Hz) (André-Obadia et al., 2006; Lefaucheur, 2011).

How high-frequency rTMS pain relief is brought about remains speculative. In any rTMS protocol, stimulation is directed at one given cortical area. However, there is evidence that the effects can spread to other connected brain regions and affect distant sites (Bestmann et al., 2003; Gerschlager et al., 2001; Münchau et al., 2002; Sack et al., 2007). Of the many areas connected to the motor cortex (Fox et al., 1997), somatosensory cortex S1 has been shown to display somatotopic plasticity that correlates with neuropathic pain development (Flor et al., 1995; Wrigley et al., 2009). This “maladaptive plasticity” seems to be reversed by successful treatment of the pain (Birbaumer et al., 1997; Huse et al., 2001; Lotze et al., 1999; Napadow et al., 2007). Effective treatment of neuropathic pain by rTMS of the motor cortex might therefore be correlated to S1 map plasticity. This hypothesis is further supported by studies showing that rTMS can indeed induce changes in cortical sensori-motor representations (Lee et al., 2003; Siebner & Rothwell, 2003; Tegenthoff et al., 2005). However, somatotopic reorganisation of S1 following high-frequency motor cortex rTMS has not yet been formally explored. The main aim of this study is to investigate this question in healthy subjects, as a previous step to a more comprehensive study addressed to patients with drug-resistant neuropathic pain.

As current rTMS protocols are approaching a limit, especially in their clinical applications, more and more efforts are allocated to increasing both size and duration of effects (Thut & Pascual-
Leone, 2010). Among the possible parameters to be modified, duration of stimulation, number of TMS pulses and their timing have been investigated. Unfortunately, the relationship between these temporal parameters and the actual after-effects is far from simple. For example, Gamboa et al., (2010) showed that the after-effects of theta-burst stimulation (a variation of classical rTMS protocols) could be reversed just by doubling the duration of stimulation. This suggests that at some point during the stimulation, a boundary was reached, that determined the direction of after-effects (increase or decrease of excitability). Whether or not such a boundary exists and has been reached -or even exceeded- in other rTMS protocols is an open question. In support of the existence of temporal discontinuities in the establishment of rTMS effects within a session, Gerschlager et al., (2001) described effects of 1Hz rTMS over pre-motor cortex as being maximised after 3/5 of the total stimulation. However, such studies are scarce and more information is needed about the time-course of the effects of rTMS during stimulation. This is a challenging task: data acquisition is limited in time by the parameters of the stimulation and the effects to detect are much smaller in magnitude than the change observed from before to after the stimulation. Taking into account this double difficulty, the second aim of this study is to evaluate the possibility of uncovering the time-course of putative S1 somatotopic plasticity induced by high-frequency rTMS of the motor cortex in healthy subjects. Optimisation of the methods will be pursued to come as close as possible to this goal.

In this study, we asked two novel questions: (a) Can a session of high-frequency rTMS to the motor cortex induce plasticity in somatosensory representations in healthy subjects? (b) If so, is it possible to determine a time-course for this plasticity during the session? To answer these questions, we used a 128-electrode cap to record N20 activity in response to ulnar nerve and thumb stimulation. The cortical representation of these two points is spatially separated, the ulnar border of the hand being posterior and superior (dorso-caudal) relative to the position of the thumb in human S1. Modelling the cortical sources of these two hand regions allowed us to assess the extent of hand representation in somatosensory primary cortex (S1) and to study its evolution following 20Hz rTMS of the hand motor cortex.
Materials and methods

What methods and why?

To ensure the relevance of this work both to fundamental and to clinical research, we chose to evaluate the effects of a high-frequency (20Hz) rTMS protocol which is currently used to treat patients with neuropathic pain (Hosomi et al., 2008; Lefaucheur, 2011; Leo & Latif, 2007). High-frequency stimulation of motor cortex is also studied in other contexts (Azila Noh & Fuggetta, 2011; Cosentino et al., 2012). Stimulation was delivered to the hand motor cortex for several reasons. First, an important number of patients treated by rTMS for neuropathic pain receive stimulation to the superior limbs or to the face motor areas. Indeed, the motor representation of the lower half of the body is difficult to stimulate because it is deeper located in inter-hemispheric fold. Second, of the superior limbs and face somatosensory representations, hand representation in S1 has been the most studied. It displays a robust somatotopic organisation of the fingers, with the thumb located significantly more ventral than the fifth digit within area S1. Third, this somatosensory representation is susceptible to plasticity, and changes in its somatotopic organisation have been described after amputation or deafferentation (Flor et al., 2006), anaesthesia (Rossini et al., 1994; Waberski et al., 2003) but also attention (Iguchi et al., 2001), or behavioural experience (Schwenkreis et al., 2007; Weibull, et al., 2011; Xerri, 2008).

Finger representation in S1 has been assessed by functional magnetic resonance imaging (Kurth et al., 1998; Maldjian et al., 1999; van Westen et al., 2004), magnetoencephalography (Baumgartner et al., 1991; Hari et al., 1993; Nakamura et al., 1998) and electroencephalography (Baumgartner et al., 1991; Baumgartner et al., 1993; Schaefer, et al., 2002a). Of all these techniques, we chose electroencephalography because it is the most flexible to use in combination with TMS, especially in the event of studying effects during stimulation (Siebner et al., 2009). Indeed, MEG imaging does not allow concurrent rTMS delivery. fMRI would, but it requires special a-magnetic equipment (carbon electrodes, special coil...) and does not allow adjustments to the position of the coil in case the subject moves. In contrast, EEG is easily interleaved with TMS (free coil placement, semi-specific equipment...), can be repositioned online (in real time) in case of head movements, and can be used to successfully uncover finger somatotopy by modelling the cortical sources of early somatosensory evoked potentials (SEPs) (Buchner et al., 1995; Houzé, et al., 2011). SEPs are an electrical activity recorded at the scalp that results from the nervous system in response to peripheral stimulation. Summing the EEG activity over numerous stimulations allows the specific response of the nervous system to emerge from other ongoing EEG activity (« noise »). Like other event-related potentials, SEPs can be broken down into discrete « components », which
are identified by their polarity (N for negative and P for positive) and their latency relative to the stimulation, as well as their distribution on the scalp (scalp topography). Non-pathological SEPs can be described as follows (Mauguière, 1995). A first diffuse positivity, P14, generated at a subthalamic level is followed by a diffuse negativity, N18, that peaks 18ms after stimulation, but persists and decays slowly. These subcortical components are followed by N20/P20, a parietal negativity and a frontal positivity contralateral to the stimulus occurring 20ms after stimulation. N20/P20 is the first cortical component; there is strong evidence that it arises from area 3b of somatosensory cortex (Allison et al., 1989; Papadelis et al., 2011). It might sometimes overlap with P22, a radial activity whose origin is still debated (pre-central area 4 or post central area 1). Following components include P25/N25, P27, and P45, which have a post-central origin (subdivisions of area S1). Up until 50ms, activity seems to be restricted to primary sensory-motor cortices (Allison et al., 1989).

When a patch of cortex (a “source”) is active and its resulting scalp activity is recorded, it is sometimes possible to estimate the location of the source by a modelling approach. Typically, non-moving equivalent current dipoles are created to explain activity during a given time-window, often ideally encompassing one component (BESA® 5.3; Scherg, 1990; Scherg & Picton, 1991). The location, orientation and magnitude of activity of each dipole are allowed to vary. These parameters are iteratively adjusted by the software in order to minimise the discrepancy between modelled and recorded activity, as reflected by the residual variance (RV). The resulting dipoles give an estimation of the barycentre of the modelled activity. As a consequence, components that result from the simultaneous activity of many different cortical areas are often difficult to model. By studying early SEPs and focusing on the first 50ms following stimulation, one can study primary somatosensory cortex in the absence of concurrent activity. Among the early SEP components described above, N20/P20 (henceforth simply called “N20”) has been shown to yield the most reliable cortical representation of the hand from stimulating the thumb and ulnar nerve (rather than the fifth digit) (Houzé et al., 2011). Its source lies close to the cortical surface and tangential to the scalp, which is the configuration best amenable to EEG modelling. To model N20 accurately, an appropriate number of electrodes should be used (128 electrodes or more) in order to increase spatial sampling and therefore spatial resolution. Overlap from other components such as P14/N18 and P22 must be reduced as much as possible (Buchner et al., 1995), which can be achieved by using multiple dipoles over the first 50ms. The full details of these methods will now be presented.

**Subjects**

Thirteen subjects (9 women, 22 to 63 years old, mean age: 32.2 years, 10 right-handed) gave their written informed consent to take part in this study and were remunerated for their...
participation. They had no previous neurological or psychiatric history. The study was approved by the local ethics committee (CPP Léon Bérard-Lyon; 2008-A01437-48). Ten subjects took part in the main experiment; three of them underwent the sham experiment. Three new subjects undertook the sham experiment only. As three subjects (2 from the main group and one from the sham group) did not yield data of appropriate quality for modelling, they were removed from analysis. Therefore, main experiment analysis was conducted on 8 subjects and sham experiment analysis on 5 subjects.

**Experiment time-course**

At the beginning of a session, subjects were fitted with a 128-electrode EEG cap. To evaluate the cortical representation of the hand before rTMS, SEPs were recorded in response to electrical stimulation of the first digit and ulnar nerve of the left hand. For each of the two peripheral stimulation sites, two runs of ~1000 stimuli (ie 2000 stimuli per site) were applied at 3Hz (Figure 1A). SEP acquisition lasted approximately 20 minutes.

The subject's left hand motor “hotspot” and motor threshold were then determined by neuronavigated (i.e. MRI-guided) single-pulse TMS over the right primary motor cortex (Figure 1B). The rTMS session itself consisted of 20 consecutive trains of 80 stimulations, delivered at 20Hz (Figure 1C). These brief 4 seconds-long stimulation trains were separated from one another by 84s inter-train intervals.

**Figure 1: Experiment time-course: interleaved SEPs and TMS.** A) The experiment started with the recording of somatosensory evoked potentials (SEPs), elicited by left hand ulnar nerve and thumb electrical stimulation. For each peripheral stimulation site, 2 consecutive runs of ~1000 stimuli each, delivered at 3Hz, were recorded. Once the pre-rTMS SEP recordings were done, B) hand motor hotspot and threshold intensity were established using single pulses of TMS to the right motor cortex. C) 20 Hz repetitive TMS was then applied for 20 minutes to the left-hand motor hotspot, at a 90% threshold intensity. rTMS pulses were delivered in short 4-seconds trains, followed by inter-train intervals of 84s. D) Inter-train intervals were used to record short runs of SEPs, interleaved to the motor cortex stimulation. An SEP run consisted of ~200 stimulations; peripheral stimulation sites (U: ulnar nerve, T: thumb) alternated at each new run. Altogether, ten short runs for each stimulation site were recorded. These SEPs recorded during rTMS were followed by E) post-rTMS SEPs using a procedure identical to the pre-rTMS SEP session.
In order to assess hand representation plasticity during rTMS, twenty very short SEP runs (150-200 stimuli) were recorded interleaved to the rTMS stimulation, during the 84s inter-train intervals (Figure 1D). This procedure yielded ten measurements for the first digit, alternating with ten measurements for the ulnar nerve, across the entire rTMS session. Left hand somatotopy was again assessed shortly after rTMS (Figure 1E) (2 runs of ~1000 stimulations x 2 peripheral sites; approximately 20 minutes). A typical session would last at least 3 hours.

**Spatially guided rTMS**

rTMS was delivered through a figure-of-eight coil (Cool-B70 butterfly coil, MagVenture®) connected to a MagVenture stimulator (Alpine Biomed®) generating biphasic repetitive magnetic pulses. Coil position relative to the subject's brain anatomy was monitored and guided by an MRI-based neuronavigation system (VISOR, ANT®) in which the subject's individual 3D-MRI was loaded.

Placement of the coil over the left-hand motor cortex was done as follows. First, with the help of the neuronavigating system, the coil was centered over the hand “omega” (Yousry et al., 1997) with an antero-posterior orientation, i.e., orthogonally to the central sulcus. Single pulses of TMS were then applied with varying intensity and position, while the left hand muscle responses were recorded by bipolar electrodes placed over the muscle *abductor digiti minimi*. The cortical point allowing best motor responses with lowest TMS intensity was defined as the motor ‘hot spot’. The rTMS session was then performed over the hot spot, at intensity equal to 90% of the motor threshold, itself defined as the TMS intensity eliciting 5/10 motor responses with at least 50µV amplitude at rest (Rossini et al., 1994). 1600 stimulations were delivered at 20Hz over 20 minutes.

**Somatosensory evoked potentials (SEP)**

**Optimising the methods : choice criteria**

When modelling early SEP components that obey only a few sources (such as the N20 component studied here), the quality of the processed data is determinant for the quality of the modelling results. Indeed, these components are generated by sources of small volume, whose activity recorded at the scalp is of very small amplitude. This makes them extremely sensitive to an increase in the background noise: if the signal-to-noise ratio is too low, the signal will be drowned in the noise and it may be impossible to model the desired activity. Conversely, a change in the number of dipoles, the interval of interest and other parameters intrinsic to the modelling process should lead to very similar solutions in terms of localisation and timing.
In most instances where source localisation of scalp signals is attempted, the problem is finding a broad localisation of the most probable source, and therefore solutions providing millimetric uncertainty may be largely sufficient. The questions asked in this study require, on the contrary, as little incertitude as possible, as changes in cortical hand representation due to experimental manipulations are likely to amount to some millimetres only, i.e. barely exceeding the intrinsic localisation incertitude of the technique. Taking into account the above constraints, a substantial part of this work was devoted to optimise data processing and model, which would yield robust, accurate and well fitting solutions.

a) In order to evaluate robustness, five different time-intervals were chosen on which to fit the N20 dipole. They varied in total duration and latency on the ascending slope of the N20 component, but could all have reasonably been chosen by an experimenter. A robust model should resist to an operator-dependant choice of time-window, it should therefore display little variability in response to this manipulation.

b) Accuracy was evaluated according to physiological and anatomical knowledge: the cortical representation of the thumb should be located anterior and inferior to that of the ulnar nerve and all dipoles should be posterior (or close to) the central sulcus.

c) Finally, residual variance indicated how well the model fitted the data. Albeit a minimal residual variance (RV) on the N20 time-window was sought, the ability of the data and model to uncover sources of other SEP components within S1 was also taken into account, and a situation where one far-field (brainstem), one tangential (area 3b) and one radial components (areas 1-2) could be modelled was preferred, as this reflected more accurately the underlying physiology (Allison et al., 1989). To ensure a high signal-to-noise ratio, all tests on robustness and accuracy were done on grand-average data (i.e. averaged across all participants).

**SEP recording**

Subjects were comfortably seated in a quiet, semi-darkened room. They were instructed to relax; they could close their eyes but were asked to stay awake. First, the sensory threshold was determined by applying stimulations of increasing intensity to the first digit (ring electrode) and to the ulnar nerve, close to the left wrist (bipolar electrode) until subjects mentioned they felt them. Then, series of stimuli (0.2ms square waves; 3Hz) were delivered at an intensity of threefold the sensory threshold, via an IRES-600 isolated, constant-current stimulator (Micromed®). Stimulation was applied at only one site at a time.

A high-density 128-channel EEG cap (WaveGuard® Cap, ANT®) was used to record
cortical electrical activity. Sensors in the cap being ‘floating electrodes’, the skin-electrode impedance was kept below 5kOhm using a conductive gel (Electro-Cap®). The left mastoid (M1), ipsilateral to peripheral stimulation was chosen as reference (Cruccu et al., 2008)(Cruccu et al, 2008). The ground electrode was incorporated in the cap between Fz and Afz. An ASA amplifier and ASA software (ANT®) were used to record the signal at a sampling rate of 2048 Hz, and with a 0.2-1024 Hz band pass filter.

**SEP analysis**

Electrophysiological data was analysed under BrainVision Analyzer software (BrainProducts®). A 50Hz notch filter was applied to the raw EEG signal in order to minimise spurious activity from the electrical network. The continuous signal was then segmented in 120ms-long epochs, composed of 20ms pre-stimulus and 100ms post-stimulus periods. Each epoch was detrended in order to minimise the influence of slow linear trends (using the first and the last 10ms of the segment). A baseline correction was applied using a slightly shortened pre-stimulus interval (-20 to -3ms), in order to exclude the edges of the stimulation artefact from the calculation of the baseline. Semi-manual artefact rejection was performed, so as to discard any segment with activity exceeding ±150μV. This resulted in the exclusion of ~10% of epochs. The segments were then averaged. The resulting average was band-pass filtered between 15 and 350 Hz (Butterworth filters, 24 dB/oct). Since source localisation heavily relies on a good signal-to-noise ratio at all electrode sites, the signal from electrodes that remained of poor quality after the processing steps described above (detrending, baseline correction and filtering) was interpolated using spherical spline functions. This method allows to reconstruct the signal at one electrode site by taking into account the signal from the 128 electrodes of the whole cap (Perrin et al., 1989). The final product could then be averaged across time (combination of two following stimulation runs) and/or averaged across all subjects, resulting in a grand-average.

**Base-line correction and physiological constraints**

While the quality of the data was made as high as possible by the use of a high-density electrode cap (128 electrodes) and by classical SEP processing, a difficulty specifically linked to the modality of SEPs was encountered in this study. The electrical stimulation of the peripheral nerves used to trigger the SEP was recorded at the scalp, resulting in a big stimulation artefact which disrupted the previously stable base-line (Figure 2A). Given that, when using a cephalic reference, no physiological activity should be recorded at the scalp before 14ms post-stimulation (Mauguière, 1995), the situation called for a re-definition of the base-line. A last processing step was therefore
taken: base-line correction using a post-stimulus interval (5 to 13ms) was applied to segments before source reconstruction.

SEP source reconstruction

Models of early SEPs described in the literature typically used between one to three dipoles, describing N20 activity, and sometimes P14 or P22 activities (Baumgartner et al., 1993; Buchner et al., 1995; Pleger et al., 2001). Other studies have suggested modelling the noise in order to remove it from the physiological model (Godinho et al., 2006; Houzé et al., 2011). In an attempt to reflect this diversity, three different types of models were fitted to the data. Four dipoles were used to describe SEP activity over the first 50ms: (1) a dipole accounting for subcortical activity previous to N20, (2) a dipole accounting for cortical N20 activity, (3) a dipole accounting for later activity (up to 50ms) and (4) a noise dipole explaining noise at the same time as the N20 dipole. Model M1 included dipoles n°1 and n°2; model M2 complemented model M1 by the addition of dipole n°3; finally, model M3 comprised all four dipoles.

Global Field Power (GFP) profile, in combination to recorded data, was used to determine the time-windows during which dipoles should be reconstructed (see Figure 4, top for an illustration). GFP corresponds to the spatial standard deviation in a series of maps of potentials (Skrandies, 1990); a peak of GFP over time corresponds to a series of fields with high similarity. It helps to determine the latency of evoked-potentials components (Hamburger & Van der Burgt, 1991). P14 and N20 dipoles were fitted over intervals of varying lengths that covered the ascending phase and/or the peak of the GFP curve at their respective latencies. The third dipole was fitted from the end of the N20 dipole time-window to the end of the epoch. It accounted mostly for the radial activity elicited by P22, N30 and P45 components. The noise dipole was added last.

Solutions with a residual variance (RV) less than 10% and compatible with physiological knowledge were considered reliable. The model was first created on grand-average data (averaged across participants). It was then adjusted to individual data; the time-window, dipole number and dipole order could be modified if justified (artefact in the middle of a time-window, difference in latency compared to the grand-average...). A four-shell ellipsoidal head-model was used (default adult parameters values); regularisation constant was set at 1% (default value).

Coordinates, measures and projection on anatomical image

Dipole position was defined by its coordinates in the Talairach system of reference, whose origin is the anterior commissure, with X for the lateral medial axis, with X=0 being the coordinate of the sagittal inter-hemispheric plane; Y for the rostro-caudal (anterior-posterior) axis, Y=0 being
the coordinate of the vertical anterior commissure (VAC) plane and Z for the inferior-superior axis, Z=0 being the coordinate of the horizontal AC-PC plane orthogonal to the mid-sagittal vertical plane and passing through the anterior and posterior commissures. According to their respective Y and Z coordinates, dipoles were located on the surface of an individual Talairach-normalised brain (MRIcro) and 2-D euclidean distances between dipoles pre- and post-rTMS were calculated. Coordinates and distances are expressed in millimetres.

Sham stimulation

Six subjects (3 of the main group, 3 new participants) received “sham” rTMS, i.e. they underwent the entire standard experimental procedure, but unbeknownst to them, no actual rTMS was delivered. After measuring the individual hand motor hotspot and motor threshold, the stimulating coil was switched with a sham coil (MCF-P-B65 coil, MagVenture®) identical in shape, size and noise produced by an active coil, but not delivering any significant magnetic field. This allowed to control for any confounding effects such as placebo, noise of the coil during stimulation, or repeated finger stimulation for the recording of SEPs.

Statistical analysis

A Kolmogorov-Smirnov test was used to make sure that the data were normally distributed. Paired t-tests were applied to the coordinates of thumb and ulnar nerve dipoles. Two-way repeated measures ANOVAs, with “time” (pre- or post- stimulation) as within factor and “type of stimulation” (active rTMS or sham) as between factor were run on the 2-D Euclidean distance between thumb and ulnar nerve dipoles. Post-hoc paired t-tests were then applied with a Bonferroni correction. The level for significance was set to p<0.05.
Results

A. Searching for the best possible parameters for source modelling.

In order to investigate the somatotopic representation of the hand in S1 and its plasticity with a maximal precision, we sought to optimise modelling conditions. The results of the optimisation process described in the Methods section above is presented below.

I. Data pre-processing

Changing the base-line from a pre-stimulus interval (-20 to -3ms) to a post-stimulus interval (5 to 13ms) considerably modified scalp topography of early potentials and GFP (Global Field Power) profiles. This allowed a better identification and differentiation of the early sub-cortical SEP component P14 (Figure 2A, (1)).

![Figure 2A](image)

**Figure 2: Change of base-line from pre- to post-stimulus.** A) Overlay of electrical signal from all 128 electrodes following stimulation of the ulnar nerve, prior to rTMS. The signal was base-line corrected with a classical pre-stimulus interval (-20 to -17ms, top), or with a post-stimulus interval (5 to 13ms, bottom). Changing the base-line from pre- to post-stimulus decreased the non-physiological scatter of activity following stimulation (red arrows) and modified early SEP components P14 (1) and N20 (2) as well as following activity. Grand-average data. B) Projection of N20 dipoles onto the lateral convexity of a Talairach-normalised brain. The data presented in A) was analysed with a four-dipole model (see methods), using 5 possible time-windows for fitting the N20 dipole (groups of points of one symbol and one colour). All N20 dipoles were located posterior to the central sulcus.

Modelling of thumb and ulnar nerve sources before rTMS yielded dipoles that were all posterior to the central sulcus, with the thumb always anterior and inferior to the ulnar nerve dipole (Figure 2B); RV was no more than 4%. Also, dipoles obtained for different time-intervals were less scattered when a post-stimulus base-line was used, indicating that using the post-stimulus period as baseline enhanced the robustness of the model. Moreover, the P14 component that was difficult to
model accurately in a pre-stimulus base-line condition became available to modelling when the base-line was post-stimulus. For these reasons, a post-stimulus baseline was preferred for all subsequent calculations.

II. Type of model

The three modelling variants described in the Methods (section SEP modelling) were compared in terms of residual variance and agreement to human physiology. Figure 3 shows that model M1 (two dipoles: one describing P14, the other N20 activity) successfully described more than 90% of the variance of the N20 time-window (8.8% of residual variance). The thumb was positioned anterior to the ulnar nerve, according to physiology. However, vertical differentiation of the two dipoles was not possible. Model M2 (model M1 plus a dipole describing activity after N20) partly solved this issue and marginally reduced the residual variance (RV). Further improvement, both in physiology and RV values was achieved with M3 (model M2 plus a dipole accounting for noise). Positions of thumb and ulnar nerve sources were separable, physiologically positioned, located posterior to the CS and only 3.5% of variance remains unexplained by the model. The use of model M3 was therefore preferred for the rest of the study.

![Figure 3: Testing three types of models of increasing complexity. Projection on a Talairach-normalised brain of ulnar and thumb N20 dipoles, based on three different models. Model M1 comprised two dipoles: one accounting for P14 activity and the other one for N20 activity. Model M2 consisted of M1 plus a dipole characterising activity from the end of the N20 up to 50ms. M3 complemented M2 with an additional dipole fitted at the same time as N20 that could account for noise. Mean residual variance (RV) describes the percentage of data that remained unexplained by the model. Data was a grand-average; it was base-line corrected based on a post-stimulus interval.](image-url)
III. Analysis time-window

Because it selects the activity to be analysed, the choice of the analysis time-window could potentially influence the results, to an unknown extent. Latency is often determined by the ascending phase of the N20 component (either in the raw data or in the GFP waveform, Figure 4, top). A later time-window might capture P22 activity. In choosing the length of the time-window, there is a trade-off between residual variance -which gives an estimate of how well the model fits the data- and how much representative activity is captured. A short fit interval will yield a small residual variance but will be very sensitive to any artefact within that fit interval. Stability of source location with changes in time window was one of the parameters used to estimate robustness of the model.

Figure 4: Influence of the N20 analysis time-window.
Five time-windows were chosen around the N20 peak of the GFP (Global Field Power) curve: a long (4ms), a short (2ms) and three 3ms-long intervals whose latency varied (top). An N20 dipole (model M3) was determined based on these different time-windows (bottom). Thumb and ulnar grand-average data were post-stimulus base-line corrected. The dotted line on the GFP curve replaces the profile of the stimulation artefact to increase readability.
**Figure 4** (bottom) shows that ulnar nerve dipole position was particularly robustly determined, as changing the time-window did not lead to important changes in dipole position. Thumb dipoles, however, displayed more scatter, especially following the Z axis. This slightly increased incertitude in thumb dipole position could be explained by the quality of the signal. Indeed, nerve trunk stimulation (ulnar nerve) as opposed to stimulation of cutaneous fibres (thumb) activates a much greater number of peripheral axons, hence yielding signals of greater amplitude. However, in both cases, the scatter was relatively limited and did not prevent the two dipoles from being easily separated. The choice of the time-window was therefore not considered crucial, as long as it was on the ascending slope of the N20.

**B. Source modelling of SEPs**

**I. Estimating hand cortical representation before rTMS**

Having determined the optimal conditions for modelling as per the results above, we investigated whether or not 20 minutes of 20 Hz rTMS delivered to the hand motor cortex could induce a plastic reorganisation of somatosensory S1 hand representation.

a) *Grand-averaged data.* When modelling N20 component using different fitting time-windows, all dipoles were obtained with no more than 4% residual variance. When projected onto a standard Talairach-based MRI, all groups of dipoles located posterior to the central sulcus (**Figure 5**). Changing the N20 analysis time-window did not affect positions much. With this reliable modelling, the positions of ulnar and thumb dipoles were clearly different and dissociable: the thumb was anterior and inferior to the ulnar nerve (**Figure 5**, blue symbols).

**Figure 5: Modelling of grand-average data, before and after rTMS.** Projection of N20 dipoles on a Talairach-normalised MRI. Ulnar nerve and thumb N20 sources have been modelled both before (blue) and after 20 minutes of 20Hz rTMS (red). The five points for each group (same symbol, same colour) correspond to different N20 analysis time-windows tested successively on the same data.
b) Individual data. The model obtained in the best possible conditions (grand-average, maximal signal-to-noise ratio) was used as an adjustable template to describe individual data obtained from an active stimulation experiment as well as from a sham (placebo) control experiment. For two subjects out of ten from the active group and one out of six from the sham group, modelling was unsuccessful according to our rather strict criteria (i.e. RV was >10% and positions of dipoles were non-realistic, with presence of clear artefacts and/or exit from the model ellipsoid). These three subjects were removed from later analysis as well as from the grand-average analysis. Residual variance in another subject from the control group was slightly superior to 10% but he was kept since localisation of sources was consistent with that of the other subjects. Position of individual dipoles reproduced in each subject the findings described above for the grand average: i.e. all dipoles respected finger somatotopy, and were located posterior or only slightly overlapping the central sulcus. Mean RV for ulnar nerve and thumb before rTMS was small (Table 1), and the modelling was therefore considered reliable.

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Table 1: Talairach coordinates of N20 dipoles and residual variance. Mean data, with standard error of the mean, for active rTMS stimulation (N=8) and for sham rTMS (N=5). Residual variance is expressed as a percentage.

Previous to rTMS, the position of the ulnar nerve dipoles and thumb dipoles was significantly different on the Z axis (t(4) = 3, p<0.05), but not on the Y axis (t(4) = 0.6, p>0.05) for sham stimulation. For active rTMS stimulation, the position of ulnar nerve dipoles (Table 1) was significantly different from that of the thumb dipoles, both on the Y axis (paired-t-test : t(7) = 3.56, p<0.01) and on the Z axis (paired t-test : t(7) = 3.65, p<0.01). Thus, the method used allowed to separate significantly ulnar nerve and thumb representations and therefore to determine a cortical
representation of the hand before any manipulation—a necessary step to validate the rest of our analyses (Figure 6A, blue symbols).

II. Changes in source localisation after rTMS

a) Grand-averaged data. Preliminary observation of this reliable modelling showed a movement of dipoles after rTMS, relative to their position before rTMS, both for thumb and for ulnar nerve. As both dipoles moved away from each other, the distance between ulnar nerve and thumb on the grand average increased (from 15 to 18 mm, Δ = 3 mm) (Figure 5, red symbols).

a) Active high-frequency rTMS. This was confirmed by modelling of individual data. Twenty minutes of 20 Hz rTMS applied on the motor cortex modified the hand representation: the mean ulnar nerve dipole (average position across all subjects) displayed a posterior and superior movement, while the mean thumb dipole showed an anterior and inferior displacement (Figure 6A, red symbols).

Figure 6: Modelling of individual data, before and after rTMS. A) Mean position of individual N20 dipoles, for the ulnar nerve and the thumb, both before (blue) and after 20 minutes of 20Hz rTMS (red). Error bars denote the SEM (standard error of the mean); N=8. Projection on a Talairach-normalised MRI. B) Individual change in dipole position from pre- to post-rTMS in a sagittal Talairach plane. Black numbers represent individuals. Red lines correspond to the individual difference in position of N20 dipole after rTMS compared to before rTMS. All movements have been represented relative to the mean position of dipoles before rTMS (blue symbols) in order to improve readability.
This proved to be significant at group level, (see below paragraph c, and Figure 6B), even though a few subjects followed only one of the trends described above. For instance, one subject’s ulnar nerve dipole moved upwards (subject 4), but towards the central sulcus; his thumb dipole moved mainly downwards. More generally, thumb dipoles moved mainly along one axis, while the movement of ulnar nerve dipoles often involved both Y and Z axes. Inter-individual differences were important, for example displacement for the ulnar nerve dipole ranged from 2 to 17mm. These modifications of dipole position in opposite directions resulted in an important enlargement of the hand representation (Δ = 10.5 mm, SEM = 3.5 mm, N = 8) (see Table 2 for sizes of hand representation).

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Table 2: Mean 2D euclidean distance between ulnar nerve and thumb dipoles for sham and active rTMS and elements of analysis (ANOVA). Distances are expressed in millimetres.

b) Sham (placebo) high-frequency rTMS. A control experiment with sham stimulation was run to ensure that neither a placebo effect, nor the noise of the coil during stimulation, nor the peripheral stimulation used to record SEPs were responsible for the findings described above. Changes in location of dipoles after sham stimulation were much smaller and disorganised in space relative to those obtained after active rTMS, (ulnar nerve dipole range : 2 to 6 mm) resulting in a mean position of dipoles barely modified after placebo stimulation (Δ = 0.8 mm, SEM = 2 mm; N = 5, Table 2) (Figure 7A).

c) Comparison between active and sham rTMS. The effect of active and sham stimulation on the distance between ulnar nerve and thumb dipoles was quantified using repeated-measures ANOVA, with “time” (pre- or post-stimulation) as within factor and “stimulation type” (active or sham) as between factor. The ANOVA showed no main effect of stimulation type (F(1,11) = 0.42, p = 0.5), a main effect of time (F(1,11) = 5.64, p = 0.04), and a strong trend for interaction (F(1,11) = 4.19, p = 0.06).
Figure 7: Sham stimulation compared to active rTMS. A) Mean dipolar position of individual N20 dipoles after sham stimulation (light blue symbols, N=5) or after active rTMS (red symbols, N=8). The change in position following sham rTMS has been reported relative to the mean position before active rTMS (dark blue symbols). Error bars denote SEM. Projection on a Talairach-normalised MRI. B) Mean 2D-euclidean distance between ulnar nerve and thumb N20 dipoles, before and after rTMS, either active or sham. Error bars represent SEM, ns : non significant, ** : p<0.01.

Post-hoc Bonferroni tests showed that the distance between ulnar nerve and thumb dipoles was significantly increased after active rTMS ($\Delta = 10.5$ mm; 95% CI : 3 to 18 mm; p<0.01), but not after sham stimulation ($\Delta = 0.8$ mm; 95% CI : -9 to 10 mm; p>0.05) (Figure 7B). Thus, real 20 Hz rTMS delivered to the hand motor cortex did modify S1 somatotopy, enlarging the cortical representation of the hand, whereas sham stimulation failed to do so.

III. Source modelling of PES during rTMS

High-frequency 20 Hz rTMS to the hand motor cortex enlarged the representation of the hand in S1. Is it possible to study the time-course of this plasticity? To answer this question, we investigated the evolution of dipole localisation estimated from short SEP runs obtained sequentially during the administration of rTMS (see Figure 1). Thus, short runs (~1500-200 stimuli) of peripheral stimulations were “interleaved” between rTMS bursts, and applied during the 84 seconds that separated consecutive rTMS trains. The analysis was performed on grand-averaged data from all subjects. Twenty small SEPs recordings spread across the rTMS session allowed the reconstruction of 8 “consecutive” dipoles for each stimulation site, reflecting the putative evolution of dipole position during the progression of the rTMS session (the first and last SEP recordings for each stimulation site were discarded because they were not consistently recorded in all subjects).

Although the dipoles were obtained with acceptable RVs and all but one located posterior to
the central sulcus, not all pairs of dipoles for a given time point respected somatotopic arrangement (Figure 8A).

**Figure 8: Modelling of grand-average data during stimulation.** A) N20 dipole position for small time-slots during stimulation (2 to 9, spread out across the 20 minutes of stimulation, cf Figure 1), for the ulnar nerve (U) or the thumb (T). Grand-average model of pre- and post-rTMS shows the “starting” and “finishing” points for the cortical representation. The light green ellipsoid shows the area of presence of ulnar nerve dipoles, the dark green one of thumb dipoles. B) N20 dipole position for data that has been averaged across subjects (grand-average) and across time (first 5 time-slots and 5 last time-slots of the rTMS session), for the ulnar nerve (U) and the thumb (T). Projection on a Talairach-normalised MRI.

Thumb dipoles were more scattered than ulnar nerve dipoles, and no coherent progression with time could be seen during the rTMS session, especially when comparing to dipole positions before and after rTMS. An attempt at fitting a regression curve to the data was highly unsuccessful for a simple linear regression model ($r^2<0.005$) (Figure 9).

**Figure 9: Evolution of 2D inter-dipole distance during rTMS.** 2D euclidean distance between ulnar nerve and thumb N20 dipoles was computed. A simple linear regression was applied. As it did not describe the data well ($r^2<0.005$), no best-fit curve was displayed. Modelling of grand-average data (N=8).
In view of these negative result, and in order to increase the signal-to-noise ratio, half the grand-averages were combined together; this yielded two measurement points, one summarising all recordings obtained during the first half of the stimulating session, the other summarising the other half. These data were again modelled with acceptable RV. One dipole was markedly anterior to the central sulcus, while in a pair of dipoles the thumb was posterior to the ulnar nerve (Figure 8B). All dipoles modelled during the rTMS session located superior to dipoles modelled before and after rTMS. These results did not allow us to draw any conclusions about the time-course of plasticity in S1 induced by motor cortex 20 Hz rTMS.
Discussion

In this study we assessed, in healthy subjects and using somatosensory evoked potentials, the ability of high-frequency rTMS over the motor cortex to induce plastic changes in the cortical somatotopic representation of the hand. The results of source localisation showed the following:

d) A session of high-frequency rTMS delivered to the motor cortex did alter S1 somatotopic maps: in our subjects, S1 hand representation was enlarged by 10.5 ±3.5 mm following 20 minutes of high-frequency rTMS.

e) A sham rTMS session with identical physical characteristics but absence of magnetic field failed to induce any significant variation of the S1 hand representation.

f) The methodology used to derive the above results was not powerful enough to describe the details of the time-course of S1 plastic changes, when using ultra-short SEP runs interleaved between the rTMS trains.

In what follows, each of these relevant results obtained will be discussed, but first some preliminary comments on the general methodology followed in this work appear necessary.

Modelling methods

Alteration of somatosensory maps was uncovered using dipole modelling of SEPs. This method is most accurate when sources of activity lie close to the cortical convexity and their activity is recorded by a high-density array of electrodes (Scherg, 1990). Here, the conditions were optimal, since we studied responses originating in area 3b, which is close to the parietal convexity, using a 128 electrodes EEG cap. Of notice, in patients with neural lesions previous descriptions of plastic changes in cortical representations via EEG modelling used different modes of stimulation (electrical, tactile or air-puff) and stimulation sites (digits, nerve trunks, cutaneous territories), as well as a substantially smaller number of electrodes as compared with the present study, ranging from 32 to 61 (Birbaumer et al., 1997; Grüsser et al., 2001; Karl, et al., 2001; Montoya et al., 1998; Schaefer et al., 2002a; Schwenkreis et al., 2001), which may explain some disparity of results. Work performed in our laboratory previous to this report evaluated the impact of electrode density (64 vs 128 electrodes) and territory stimulated (four territories of the hand) on the quality of modelling data. This work strongly suggested the need of high-density electrode arrays (128 electrodes) and stimulation of ulnar nerve vs thumb to optimise the estimation of hand cortical representation using potentials from the primary sensory cortex (Houzé et al., 2011). The methodology used in the present study benefited from these previous results, and can be considered on theoretical grounds as superior to most of previously documented work in this area.
Our methodology was however not optimal in absolute terms: for instance, localisation accuracy could have been improved by using realistic head models with co-registration of the electrode positions to the individual subject MRI (Brinkmann et al., 1998). As well, position relative to central sulcus could have been assessed according to individual anatomy, even if the main object of this study was not the exact anatomical position of the thumb and ulnar nerve dipoles but their relative distance. Under those conditions, localisation of sources in 3b has been shown to be highly reliable and reproducible when tested several times in the same subject, both at short and longer intervals (Schaefer et al., 2002a, 2002b). However, to our knowledge no systematic study has evaluated the real improvement of modelling solutions by direct contrast of models calculated with and without coregistration of electrode positions with MRI. In the absence of such data, the actual benefit of coregistration on the assessment of dipole position within the cortex, although highly probable, remains hypothetical.

Two other parameters that could have improved the efficacy of source modelling would have been increasing the number of EEG sensors (e.g. to 250 electrodes or more) and the number of stimuli delivered. The optimal number of sensors to resolve spatially scalp EEG signals remains a matter of debate. The discrete sampling of the brain’s electrical field at the scalp surface with individual recording sensors is subject to the same sampling error as the discrete sampling of the time series with analog-to-digital conversion. However, since the skull acts as a low-pass spatial filter of the brain electrical field, high spatial frequency information in the EEG is attenuated or suppressed, and it may be intrinsically useless to enhance the number of electrodes beyond such resolution capacities. Previous work in the laboratory (Houzé et al., 2011) as well as theoretical considerations (Srinivasan et al., 1998) suggests that adequately sampling the human EEG requires a minimum of 128 sensors; however, determining whether additional increases in sampling will improve the spatial resolution of the scalp EEG remains debatable, since it largely depends on accurate estimation of skull resistivity—the main factor determining signal smearing. Recent reports have suggested that the skull may be less resistive than previously estimated (Oostendorp et al., 2000), and this may allow higher spatial frequency of the human EEG than previously believed. Should this be confirmed, greater spatial sampling than offered by 128-channel arrays should further improve the quality and accuracy of cortical representation estimates, and some reports have suggested that interelectrode distances of 1 cm—corresponding to about 500 channels—may be required to fully represent the spatial frequency of the scalp EEG (Freeman et al., 2003; Ryynänen et al., 2005).

Concerning the number of stimuli delivered, there is little doubt that this is a crucial factor allowing to improve the signal-to-noise ratio and enhance model accuracy. In our case, we were
limited by the total time of the experiment (almost 4 hours) and the need to avoid as much as possible the subject’s fatigue (itself a source of artefacts and degrading of the data). However, retrospectively it is clear that modelling was easier, more robust and optimally physiological in subjects in whom a greater number of artefact-free SEP samples could be recorded, and that 2000 stimuli is just a ‘lower limit’ to model correctly signals of low amplitude, not exceeding 0.5 to 2 μV, as is the case of parietal N20.

As per the above considerations, efforts will be devoted in the future to (a) increase the number of stimuli, for example by increasing the stimulus rate to 5-6 Hz, which is compatible with stability of the N20 (Garcia Larrea et al., 1992); (b) use realistic head models with coregistration of electrodes on the subjects’MRI, so as to ensure that the orthogonal projection of sensors over the brain surface is not mis-estimated by the head model, and (c) enhance the number of sensors to 250, at least when discrimination of high spatial sampling is needed.

Finally, we presented a methodology for testing the robustness of different models. Fitting the model to different time-intervals allowed discriminating between situations where concomitant time-intervals produced very similar solutions and others where more disparities were noted. This allows a fast and useful quality check of the model and data set regarding the non-dispersion of measures of a same activity of interest. This quality check could become a systematic first step to future modelling studies undertaken in the laboratory.

Mechanisms of rTMS-evoked plasticity

Somatosensory cortex is thought to have a somatotopic organisation. That is, somatosensory stimuli from one part of the body are detected by the receptive fields of a dedicated group of neurons, thus creating a cortical representation of the whole body. These so-called “somatotopic maps” are thought to be dynamically maintained entities, that are shaped by a neuronal network and the information relayed through it (Xerri, 2008). In this study, we found that high-frequency rTMS over the hand motor cortex was able to modify such somatotopic maps in the neighbouring sensory cortex, hence triggering motor-to-sensory plasticity. This suggests that the “information” contained in the 20 Hz rTMS trains was relayed from the motor cortex to the somatosensory cortex, possibly in a somatotopic way.

The anatomical pathways mediating S1 plasticity might have been direct, as direct reciprocal connections between sensory and motor areas in primate brain exist (Krubitzer & Kaas, 1990). Instead, or as well, plasticity could have been mediated by more indirect cortico-thalamo-cortical connections. Indeed, there is evidence for cortico-thalamic connections from motor cortex M1 to ventral lateral (VL) and ventroposterolateral (VPLo) nuclei of the thalamus in primates (Kultas-
Ilinsky et al., 2003; Rouiller & Welker, 2000). Cortico-thalamic projections from M1 could modulate ascending sensory signals, as can be seen in rodents (Lee et al., 2008). In support of this hypothesis, functional imaging studies have demonstrated greater metabolic changes in the lateral thalamus than in sensorimotor cortex following subthreshold motor cortex stimulation, either transcranial or epidural (Bestmann et al., 2003; Garcia-Larrea & Peyron, 2007).

It should be noted that a spread of stimulation from the motor to the somatosensory cortex might have also theoretically contributed to S1 plasticity. Indeed, it has been claimed that the focality of TMS stimulation may have been overestimated in early models of the spread of stimulation (Wagner et al., 2009). It appears that even though the peak of the magnetic field might be placed over a given brain region, other regions can be affected; the geometry of this field of influence is complex and still under investigation (Toschi et al., 2008). In the present work, however, several steps were taken to ensure that the motor cortex, and not the somatosensory cortex, would be directly stimulated in our study. First, magnetic stimulation was performed under the guidance of a neuronavigation system, thus ensuring the correct positioning of the coil over the precentral cortex (Lefaucheur, 2010). Second, the site of stimulation was always anterior to the central sulcus on the subject's own 3D-MRI. Finally, suprathreshold stimulation always triggered localised hand electromyographic responses and never induced paresthesiae or other somatosensory sensations. The reasons for such care in avoiding any direct stimulation of the sensory cortex by rTMS lie in the potentially adverse effects of S1 stimulation on pain perception, which will be discussed later.

The plasticity observed here took place over a very short amount of time (20 minutes). While its duration was not measured, other studies suggest that the effects of one session of rTMS are detectable a few tens of minutes after stimulation (Eisenegger et al., 2008; Gerschlager et al., 2001). The cellular mechanisms involved are very likely to be transient modifications of excitation and inhibition through a change of activity in existing structures rather than structural modifications per se (Xerri, 2008). The details of representational plasticity are however far from being understood and extensive research in many domains is needed to draw a better picture of its mechanisms and consequences (Siebner & Rothwell, 2003; Thut & Pascual-Leone, 2010; Xerri, 2008).

**Functional relevance of S1 plasticity**

It is currently admitted that somatosensory maps are continuously modified by a subject's experiences (for a review, see Xerri, 2008); this plasticity can lead to enlargement or shrinkage of the cortical representation of a body part. For instance, some situations, such as intensive training
simple allocation of attention to a tactile stimulus (Buchner et al., 2000; Iguchi et al., 2001; Noppeney et al., 1999) or high-frequency rTMS of S1 (Tegenthoff et al., 2005) can lead to an expansion of a body part cortical representation. On the contrary, a diminution of body part representation was shown to occur in some populations of patients, including those suffering amputation and deafferentation (Flor et al., 1995), or focal dystonia (Mogilner et al., 1993). In our study, the possibility of plasticity being caused by an attentional shift, or by simple tactile activation was controlled for by a sham control condition, in which subjects displayed no plasticity. We found an enlargement of hand somatosensory representation after active high-frequency motor cortex rTMS, a procedure known to relieve neuropathic pain in patients (for a review, see Lefaucheur, 2011). Other studies found that a number of pain-relieving procedures different from rTMS were also followed by enhancement (i.e. normalisation) of previously altered somatotopic maps in patients suffering from phantom-limb pain (Birbaumer et al., 1997; Huse et al., 2001; Lotze et al., 1999). Moreover, a parallel project in our laboratory (Houzé et al., submitted) performed quantitative sensory testing of the subjects before and after rTMS. Nociceptive thresholds were increased after the rTMS session, therefore indicating a state of hypo-algesia following motor cortex rTMS. Some association may exist, therefore, between the expansion of S1 hand representation following motor cortex rTMS, and a modification in pain sensitivity towards hypoalgesia in both healthy subjects and pain patients.

However, as tempting as it might be to consider that the above findings indicate a causal link between S1 somatotopic plasticity and pain relief, no such relationship has been formally proven and it can be doubted on several grounds. First, a similar S1 hand representation expansion can be evidenced after high-frequency rTMS to the somatosensory, rather than motor cortex (Tegenthoff et al., 2005) are far from being antalgic. For instance, attention directed to a limb enhances pain perception on that limb (Garcia-Larrea et al., 1997; Miron et al., 1989). rTMS over S1 for the treatment of pain has been shown to be at the best ineffective (Hirayama et al., 2006) and may even enhance pain (Tsubokawa et al., 1991, 1993). Second, it is likely that primary somatosensory cortex is not a crucial region for the processing of nociceptive afferents. Indeed, less than 4% of spinothalamic system projections may reach S1, while more than 70% of them project to the opercular and posterior insular cortices (Dum et al., 2009). In accordance, selective lesions of S1 rarely entail deficits in pain perception in humans (Kim, 2007), while operculo-insular injury consistently attenuates nociception (Garcia-Larrea et al., 2010; Greenspan et al., 1999). Finally, a recent study of somato-motor cortex reorganisation in amputees reported somatosensory reorganisation regardless of development of phantom limb pain (Simoes et al., 2012). Therefore, the relationship between map plasticity and lowered pain threshold is less likely to be mechanistic than
to reflect two parallel processes elicited by motor cortex rTMS. Functional changes within S1 might then be viewed as a *marker* of the ability of rTMS to exert influences over cortical networks.

The size of S1 hand representation could however be related to tactile acuity. Indeed, enlargement of hand representation in S1 by various manipulations can sometimes be correlated with an improvement in tactile performance (Pleger et al., 2001; Tegenthoff et al., 2005). On the contrary, a reduced hand representation seems associated with impaired tactile performance (Sterr et al., 1998). There is also evidence for improved tactile performance in patients treated for neuropathic pain by epidural motor cortex stimulation (Drouot et al., 2002). This suggests that high-frequency rTMS of the motor cortex has -at least- two types of consequences. One is the modification of S1 somatotopy, which might impact on tactile acuity. The other is pain relief, that could be brought about via functional changes at more distant sites. Such sites include the parasyylvian operculo-insular cortex (S2, insula) which receives direct projections from the motor cortex (Leichnetz, 1986) and probably cortico-thalamo-cortical loops (see above). Future investigation will therefore be oriented towards evaluating high-frequency motor cortex rTMS-evoked plasticity in the parasyylvian operculo-insular cortex. Promising technological developments might also allow to magnetically stimulate this region and study its impact on pain perception.

**Lack of on-line assessment of ongoing plasticity**

The methods described above allowed us to establish that plasticity in S1 occurred after 20 Hz rTMS over the motor cortex. However, this same methodology did not allow us to unravel the time-course of this plasticity (see Results, section B III).

The main difference between the successful and unsuccessful situations seems to lie in the signal-to-noise ratio of the modelled SEP, the principal factor of which -as explained above- is very likely the number of stimulations averaged in each run. Indeed, we managed to separate ulnar nerve and thumb dipoles and to assess their movement after rTMS, when using ~2000 peripheral stimulations at each site. On the contrary, it was impossible to model adequately SEPs obtained from 200 stimulations. Even combining several short runs to reach 1000 stimulations did not allow appropriate modelling of SEPs, suggesting that the limits of the modelling method were reached in terms of how few stimulations could be used to reconstruct sources.

Few options are available to increase signal quality through number of stimulations. Because the SEP recording time is conditioned by the rhythm of rTMS delivery, it does not seem possible to increase recording time. Indeed, recording during 20 Hz rTMS stimulation would require special equipment, introduce enormous artefacts (Siebner et al., 2009) and would not bring much more information (gain of 15 stimulations over 5 seconds). The only other option would be to increase the rate of delivery of peripheral stimuli. It has been shown that N20 is unhindered by an increase in
stimulation rate up to 5 Hz (Garcia Larrea et al., 1992). However, combination of 3 short runs of SEPs would still be required to reach more than 1000 stimuli, thus limiting temporal resolution. A few other minor adjustments could be made to increase the signal-to-noise ratio. For example, subjects could be given a pharmacological product that reduces muscle artefacts but does not affect somatosensory N20 (Loughnan et al., 1987), such as diazepam (Buchner et al., 1995). However, this procedure is quite invasive and requires considerable organisation for the subject’s safety. Blink artefacts could be modelled and removed from the model instead of rejecting the contaminated epochs (Lins et al., 1993). Finally, even though every possible care was taken to reduce electromagnetic noise, data could be acquired in an electrically-shielded room. But it is likely that these last few adjustments would not impact the results as much as the number of stimulations. The limitations found here therefore drastically reduce the interest of using this method to study the time-course of rTMS-induced plasticity.

Even if accurate modelling of sources had been achieved during rTMS stimulation, there is no guarantee that a coherent time-course could have been unmasked. Indeed, inter-individual differences were marked: among our 8 participants, some showed very little plasticity while others presented a large cortical reorganisation (Figure 6B). Important inter-individual variability in susceptibility to plastic reorganisation of the cortex can be attributed to many factors, such as age, attentional state, circadian rhythm, level of cortical excitability previous to stimulation (Ridding & Ziemann, 2010; Sale et al., 2010), and maybe also genetic factors (Cheeran et al., 2008; Missitzi et al., 2011). These differences were not problematic in determining the existence of a plasticity. But if differences did exist not only in the extent and direction but also in the time-course of the plasticity, any temporal discontinuities would have been masked in the grand-average data. This stresses the importance of individual modelling. A solution to this issue could be to study thoroughly a small number of subjects in whom plastic changes are high, or to investigate a large enough number of subjects that could allow grouping into sub-categories. However, it is important to note that the number of subjects participating in this study was large enough to detect rTMS-induced plasticity, and is definitely within the range used in studies of somatosensory representation by a dipole modelling approach (Buchner et al., 2000; Elbert et al., 1995; Noppeney et al., 1999; Sterr et al., 1998; Ziemus et al., 2000).
Conclusion

High-frequency repetitive transcranial magnetic stimulation (HF-rTMS) of the motor cortex is one of the few procedures that can bring relief to patients suffering from drug-resistant neuropathic pain. In an effort to understand its basic mechanisms better, we investigated the effects of a session of HF-rTMS of the motor cortex in healthy subjects, using source modelling of somatosensory evoked potentials (SEPs). We asked whether or not somatotopic hand representation in primary somatosensory cortex S1 would display plasticity, and if so, whether or not its time-course could be studied using the same methods. We found that 20 minutes of HF-rTMS over the hand motor cortex significantly increased the size of hand representation in S1. A session of sham (placebo) stimulation failed to do so. The methods developed, although optimised for this protocol, did not allow us to study the time-course of such plasticity. Plasticity of the somatosensory cortex following a treatment inducing pain relief in patients has sometimes been taken as an indication that pain relief was mediated by S1 plasticity. However, our results were obtained in healthy subjects and extrapolation to patients suffering from chronic spontaneous pain should be done with caution. Moreover, no causal link between S1 plasticity and pain relief was established. Rather, it is likely that both phenomena are parallel consequences of HF-rTMS of the motor cortex. S1 plasticity might be more strongly linked to tactile acuity, while pain relief could be mediated by structures such as the insula-opercular cortex. Future efforts will therefore be devoted to investigating plasticity of operculo-insular cortex following HF-rTMS to the motor cortex.
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