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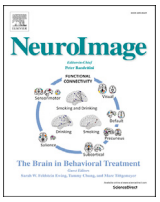

# Long-term limb immobilization modulates inhibition-related electrophysiological brain activity

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ABSTRACT

The effect of long-term immobilization on the motor system has been described during motor preparation, imagination or execution, when the movement has to be performed. But, what happens when the movement has to be suppressed? Does long-term limb immobilization modulate physiological responses underlying motor inhibition? Event-related potentials (ERPs) were recorded in healthy participants performing a Go/Nogo task, either with both hands free to respond (T1/T4: before/after the immobilization) or when left-hand movements were prevented by a cast (T2: as soon as the cast was positioned; T3: after one week of immobilization). In the right (control) side, N140, N2, and P3 components showed the expected greater amplitude in Nogo than in Go trials, irrespective of the timepoint. On the contrary, in the left (manipulated) side, each component of the ERP responses to Nogo trials showed specific differences across timepoints, suggesting that the inhibition-related EEG activity is significantly reduced by the presence of the cast and the duration of the immobilization. Furthermore, inhibition-related theta band activity to Nogo stimuli decreased at post-immobilization blocked session (T3-blocked). Altogether these findings can be interpreted as a consequence of the plastic changes induced by the immobilization, as also demonstrated by the cast-related corticospinal excitability modulation (investigated by using TMS) and by the decreased beta band in response to Go and Nogo trials. Thus, only if we are free to move, then inhibitory responses are fully implemented. After one week of immobilization, the amount of inhibition necessary to block the movement is lower and, consequently, inhibitory-related responses are reduced.

## 1. Introduction

In our everyday life, the ability to reset and inhibit motor performance is crucial, enabling humans to rapidly cancel the motor activity. However, how movements' inhibition interacts with other aspects of the motor system is far from being clear and it can be an interesting issue in the field of motor cognition. In principle, if we are free to move, then we have to implement inhibitory responses in order to avoid unwanted actions. But what happens during conditions in which our movements are prevented, as during limb immobilization? In the present study, we asked whether long-term limb immobilization modulates physiological responses underlying motor inhibition.

The effect of mechanical limb immobilization on the motor system has been extensively investigated. Several studies, focusing on the brain plasticity induced by the immobilization, showed that physiological measures of corticospinal excitability, such as resting motor threshold (rMT) or motor evoked potential (MEPs) amplitude, as well as force parameters, were significantly decreased after immobilization (Avanzino et al., 2014, 2011; Burianova et al., 2016; Facchini et al., 2002; Huber et al., 2006; Ngomo et al., 2012; Opie et al., 2016; Rosenkranz et al., 2014). Coherently, functional magnetic resonance imaging (fMRI) studies showed that the activity of the primary motor cortex contralateral to the immobilized limb was significantly reduced (Avanzino et al., 2011; Garbarini et al., 2019; Huber et al., 2006; Langer et al., 2012). Furthermore, other studies on both immobilization procedure in healthy subjects (Avanzino et al., 2011) and constraint-induced movement therapy

*Abbreviations:* EEG, electroencephalography; ERPs, event-related potentials; TMS, Transcranial Magnetic Stimulation; rMT, resting motor threshold; MEPs, motor evoked potentials; MVC, maximum voluntary contraction; FR, force recruitment; T1, timepoint 1; T2, timepoint 2; T3, timepoint 3; T4, timepoint 4.

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(CIMT) in brain-damaged patients (Wittenberg and Schaechter, 2009) showed increased activity of the hemisphere ipsilateral to the immobilized limb due to hyper-use of the other side.

Interestingly, the effect of immobilization has been investigated even on more cognitive aspects of motor behavior, such as motor imagery (e.g. Bassolino et al., 2014; Burianova et al., 2016) and motor awareness (e.g. Bozzacchi et al., 2012; Garbarini et al., 2019). A recent study (Burianova et al., 2016), by means of different physiological techniques [i.e. fMRI, magnetoencephalography (MEG) and transcranial magnetic stimulation (TMS)], showed that 24 h of hand immobilization led to a significant decrease in the sensorimotor areas contralateral to the immobilized hand and a faster resynchronization in the beta frequency band (i.e. beta rebound) during motor imagery of the constrained hand. These results demonstrate a rapid effect of immobilization on motor imagery processes of the constrained hand, suggesting that limb nonuse affects not only motor execution, but also motor imagery (Burianova et al., 2016). Another TMS study (Bassolino et al., 2014) investigated motor imagery during short-term immobilization (i.e. 10 h of upper limb nonuse). The authors asked whether motor imagery and action observation, due to their physiological similarities with motor execution (Jeannerod, 2001), could prevent the corticomotor depression typically following the nonuse of a limb, by activating the motor system even during limb inactivity. They found that only action observation prevents the cortical effects induced by immobilization (i.e. when immobilization was combined with observation, no decrease of motor cortex excitability was found, as well as no reduced hand cortical representation). This facilitation was not related to motor imagery during immobilization, suggesting that motor simulation had no beneficial effects on short-term cortical plasticity (Bassolino et al., 2014). The lack of this facilitation during immobilization could be explained by the less efficiency of motor imagery in activating the motor cortex, which, on the contrary, has to be suppressed during simulation in order to prevent overt movements (on the role of motor inhibition in motor imagery see also Bruno et al., 2018).

With respect to the motor awareness, an fMRI study (Garbarini et al., 2019) showed that, when participants were asked to move, but no movement was performed due to the immobilization, a motor monitoring related cortical activity was increased. In particular, when the movement was precluded by the immobilization, higher activation of the ventral premotor cortex (vPMC) contralateral to the immobilized hand was found, together with enhanced functional connectivity between the vPMC and the primary somatosensory area. It has been suggested that the increased vPMC activity during impossible movements is related to its motor monitoring function, i.e. the conscious detection of the mismatch between movement planning and (no) movement execution (Fornia et al., 2020; Garbarini et al., 2019). Another study (Bozzacchi et al., 2012), by using electrophysiological measures, focused on the physiological processes underlying impossible movements. Healthy participants were asked to perform a possible action (i.e. to grasp a cup) or an impossible action, in which grasping was hindered by closing the fingers with a band. In the impossible conditions, specific prefrontal activity was observed during motor preparation, and it has been interpreted as a marker of no-movement awareness (i.e. the conscious monitoring of the impossibility to perform the requested action) (Bozzacchi et al., 2012).

The above-mentioned studies often reported heterogeneous methodologies and specific aims, being focused on the effects of limb immobilization within different aspects of the motor action (i.e. preparation, imagination or execution). Still, in all the previously mentioned studies, programming or actually performing a movement is always required. For this reason, a modulation induced by the immobilization on the behavioral/physiological parameters underlying the motor performance is easily expected. In the present study, we tried to go a step further: can the immobilization induce a modulation on behavioral and physiological parameters in a motor task in which a movement, instead of being performed, has to be suppressed? And if so, can the duration of the immobilization induce plastic changes? To the best of our knowledge, no previous studies investigated the effect of long-term limb immobilization on motor inhibition. Motor inhibition can be defined as the ability to suppress, withhold, delay or interrupt ongoing or planned actions (Galdo-Alvarez et al., 2016) and the most commonly used paradigms to investigate it are Go/Nogo and stop-signal tasks. During the Go/Nogo task, participants are required to respond to frequent imperative stimuli, but they must withhold the response to other infrequent alternatives (Donders, 1969). Several electrophysiological studies on this task described enhanced frontocentral negativity occurring around 140–300 ms, as well as an enhanced central positivity occurring around 300–600 ms, following the presentation of a Nogo stimulus (Falkenstein et al., 2000, 1995; Pfefferbaum et al., 1985; Veen and Carter, 2002). These peaks are referred to as the N2 and P3 respectively, and they have been interpreted as indexes of response inhibitory process in the frontal lobe (Bokura et al., 2001; Kok, 1986; Smith et al., 2008).

The purpose of the present study was to investigate whether and to what extent plastic changes, induced by long-term limb immobilization, can modulate the electrophysiological processes underlying action inhibition. To this aim, we recorded event-related potentials (ERPs) during a Go/Nogo task, by contrasting conditions in which participants were free to perform the Go/Nogo task using both hands (i.e. they had to respond by pressing a button either with the left or with the right hand according to the imperative stimulus) against conditions in which the execution of the left hand movements was precluded by a cast (i.e. participants had to respond with the free right hand and to try to respond with the blocked left hand). We replicated the experimental time-line employed in the previously described fMRI study (Garbarini et al., 2019) investigating the effect of long-term immobilization on motor awareness. ERP responses to Go and Nogo trials were collected, in separate days, just before (T1-free) and immediately after (T2-blocked) the left hand was immobilized (day 1: first day of EEG sessions) and after one week of immobilization, just before (T3-blocked) and immediately after (T4-free) the cast was removed (day 2: second day of EEG sessions, 7 days later than the first EEG recording). Analyses in the frequency domain were performed too, focusing on post-stimulus neural oscillatory activity within the theta band, since it has been described as a marker of response inhibition condition (Cohen, 2014; de Vega et al., 2016; Harper et al., 2016, 2014; Huster et al., 2013; Kirmizi-Alsan et al., 2006; Nigbur et al., 2011) and on the beta band, since it has been described to be modulated by limb immobilization (Burianova et al., 2016; Fortuna et al., 2013; Manaia et al., 2013). In addition, the same participants underwent a TMS experiment to control that long-lasting immobilization actually induced plastic changes on the corticospinal excitability and force parameters. See Materials and methods and Fig. 1. In the left (manipulated) side, an inhibition-related modulation on the EEG activity should be expected as a consequence of the presence/absence of the cast (in T2-blocked and T3-blocked compared to T1-free and T4-free) and the duration of the immobilization (in T3-blocked and T4-free compared to T2-blocked and T1-free). Indeed, if the long-lasting immobilization of the left arm were able to affect the motor system, by decreasing its activity as previously described, we should expect not only a plasticity-dependent modulation on corticospinal excitability and force parameters, but also on motor-inhibition responses. Additionally, since the frequency of presentation of Go and Nogo stimuli was different (i.e. 75% of Go stimuli, 25% of Nogo stimuli), and since the P3 component has been described not only in the domain of inhibition, but also in response to infrequent stimuli (i.e. novelty P3 [for a review see Friedman et al., 2001]), a *Control experiment* was carried out in a different group of participants in which the same visual elements of the Go/Nogo task, with the same frequency of occurrence, were presented during an EEG session. This *Control experiment* was devised to disentangle between ERP modualtions simply related to stimulus novelty induced by infrequent stimuli and those especially driven by motor inhibition, such as during our Go/Nogo task (see Materials and methods).

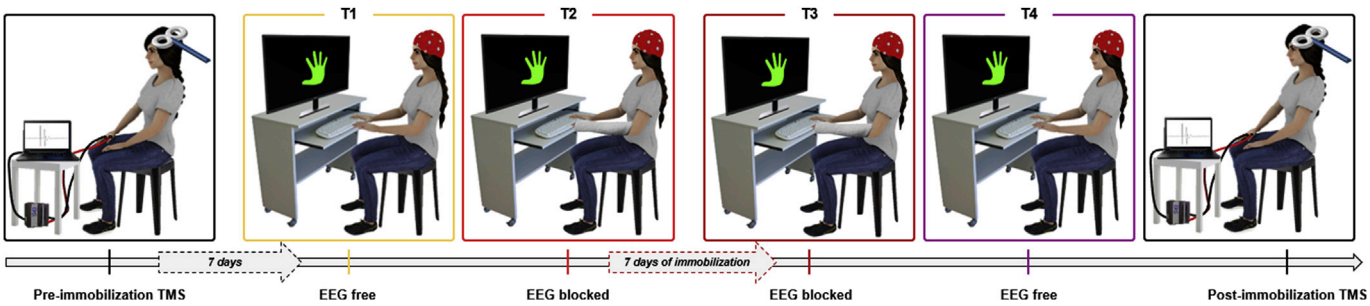


**Fig. 1. Schematic representation of study timeline.** Four EEG sessions (i.e. T1, T2, T3 and T4) in two different days (i.e. day 1: T1 and T2; day 2: T3 and T4) separated by 7 days of immobilization. EEG free: Go/Nogo task with both hands free; EEG blocked: Go/Nogo task with left hand blocked and right hand free. Between T1 and T2 the left hand was blocked with the cast; between T3 and T4, the cast was removed from left hand. Participants performed the TMS experiment one week before T1 (Pre-immobilization TMS) and immediately after T4 (Post-immobilization TMS).

## 2. Materials and methods

### 2.1. Participants

Thirty-two neurologically healthy volunteers participated in the study (8 men; mean age ± sd: 23.9 ± 2.5 years; mean educational level ± sd: 16.6 ± 1.5 years). Sixteen participants (3 men; mean age ± sd: 23.8 ± 2.9 years; mean educational level ± sd: 17.1 ± 1.4 years) performed the *Main experiment* and sixteen different participants were recruited for the *Control experiment* (5 men; mean age ± sd: 24.06 ± 2.01 years; mean educational level ± sd: 16.18 ± 1.42 years). All participants were right-handed, according to the Standard Handedness Inventory (Oldfield, 1971), with normal or corrected-to-normal visual acuity. None of them had a history of neurological, major medical, or psychiatric disorders, and they were free from any contraindication to TMS (Bruno et al., 2017b; Rossi et al., 2009). All participants were naïve to the purpose of the study and gave written informed consent according to the declaration of Helsinki. The Ethical Committee of the University of Turin approved the project (prot. n. 125055, 12/07/16).

### 2.2. Experimental design

In the *Main experiment*, the first day (i.e. day 1), participants underwent an EEG recording session while they performed a Go/Nogo task (see details in the paragraph 2.3) with both hands free (T1). At the end of the task, participants' left hand was immobilized with a thermoplastic cast and, after the cast positioning, they were asked to perform (or try to perform with the immobilized left hand) the same Go/Nogo task with the left hand blocked and the right hand free (T2). In order to investigate possible modulations due to the long-term immobilization on electrophysiological parameters, after one week of immobilization, participants were called back (i.e. day 2) and they underwent another EEG recording session while they were performing the same Go/Nogo task with the left hand blocked and the right hand free (T3). At the end of the task, the cast was removed from the left hand and the task was performed as in T1 with both hands free (T4). It is important to highlight that participants performed a training phase before starting the experiment to familiarize themselves with the task, and they were strongly encouraged to try to perform the task during the blocked sessions (i.e. T2 and T3), without employing other motor strategies (e.g. motor imagery). One week before T1 (pre-immobilization) and immediately after T4 (post-immobilization), all participants underwent one session of TMS to investigate plasticity effects on the corticospinal system induced by the immobilization (Fig. 1).

In the *Control experiment*, participants underwent a single EEG recording session while they were simply looking at visual stimuli representing different colored hands (see details in the paragraph 3.2) occurring on a monitor.

### 2.3. Experimental task and procedure

In the *Main experiment*, we adopted the same Go/Nogo task used in a previous study (Bruno et al., 2019), a similar version of the task used by the study of Cojan and colleagues, aiming at comparing motor inhibition mechanisms responsible for paralysis during hypnosis and those recruited by voluntary inhibition (Cojan et al., 2013). Participants were seated on a chair in front of a 21-inches Sony CRT screen placed at a distance of 55 cm, in a dimly illuminated room. The visual stimuli were presented on the computer screen and represented the dorsal view of a left or a right hand, which could be of three different colors: grey, green, or red. Each trial started with a fixation cross (jittered interval 6000–8000 ms), followed by a preparation cue (i.e. Preparation) which represented a grey hand, either left or right (jittered interval 1000–1200 ms). The grey hand instructed the participant to prepare to press a key on the keyboard with the corresponding hand. Then, the grey hand could turn either green (i.e. Go stimulus) or red (i.e. Nogo stimulus) (fixed duration of 750 ms). Participants had to press the key as quickly as possible when the hand turned green (70%), and to withhold the prepared response if the hand turned red (30%). After each imperative stimulus (i.e. Go or Nogo), the fixation cross re-appeared (see Bruno et al., 2019 for a schematic representation of the task and the visual stimuli). The order of stimuli presentation was pseudorandomized, in a way that more than two sequential Nogo stimuli never appeared. Stimulus display and behavioral response recording (i.e. accuracy and response times, RT) were controlled by E-prime v.2 (Psychology Software Tools, http://www.pstnet.com). Six blocks of 40 trials (half right hand, half left hand) were performed per session, resulting in a total of 240 stimuli: 90 Go left, 90 Go right, 30 Nogo left and 30 Nogo right. Each block lasted about 6 min.

The *Control experiment* was carried out in a different group of participants with the very same set of visual stimuli of the Go/Nogo task described above, with the only difference that instead of pressing a key in response to Go and to withhold the motor response after Nogo stimuli, participants only had to look at them, without performing any other task. The visual task was performed in one session only. The same probability of stimulus presentation was employed: 75% of green hands (high-probability stimuli) and 25% of red hands (low-probability stimuli). The *Control experiment* was carried out in order to disentangle between ERPs components evoked by a motor inhibition mechanism, in which the red hands represent Nogo stimuli (*Main experiment*), and ERPs evoked by stimulus-novelty, in which the red hands represent low-probability stimuli without any other cognitive-related meanings (*Control experiment*).

### 2.4. Electroencephalogram recording and processing

All the participants were seated in a comfortable chair in a silent, temperature-controlled room. They were asked to focus on the task, keep their eyes open, and try to avoid blinking when stimuli appeared.

Continuous EEG activity was acquired from 32 channels (HandyEEG, SystemPlus Evolution, Micromed, Treviso, Italy) by using tin electrodes mounted in an elastic cap according to the International 10–20 system and referenced to the nose. Eye movements (electrooculogram, EOG) were recorded from two surface electrodes, one placed over the right lower eyelid, and the other placed lateral to the outer canthus of the right eye. Electrode impedances were kept below 5 kΩ. Signal was digitized at a sampling rate of 1024 Hz (SD32; Micromed, Treviso, Italy). Data were continuously streamed to a laptop connected to a second computer generating the stimuli. These two computers interfaced via a serial port for precise synchronization.

### 2.5. Immobilization procedure

In the *Main experiment*, we replicated the same immobilization method adopted by previous studies (Burin et al., 2017; Garbarini et al., 2019). The immobilization of the metacarpophalangeal (MCP) joints, proximal interphalangeal (PIP) and distal interphalangeal (DIP) joints was performed with a palmar thermoplastic splinting. The wrist joint was in 30–45 degrees of extension, the MCP joints in 60–70 degrees of flexion, the PIP and DIP joints were extended, and the thumb was abducted. No movement could be performed with the fingers, neither with the upper part of the phalanxes.

### 2.6. Transcranial magnetic stimulation and electromyographic activity

In the *Main experiment*, TMS was performed using a figure-of-eight coil connected to a Magstim Rapid$^2$ stimulator (Magstim, Whitlan, Dyfed, Wales, UK). The coil was held over the left or right motor cortices, in an anterior-posterior orientation tangential to the skull, 45° from the midline. This orientation is optimal for trans-synaptic activation of the corticospinal pathway (Brasil-Neto et al., 1992; Mills et al., 1992). A functional hot-spot method was used to localize the primary motor cortex (M1). First, we used the International 10–20 EEG system for electrodes placements to localize the vertex, then the coil was positioned about 5 cm laterally (Herbsman et al., 2009) (either left or right) over M1. Then, the coil was moved in steps of 0.5 cm over M1 to determine the individual optimal position from which maximal motor evoked potentials (MEPs) amplitude was elicited in first dorsal interosseous (FDI), and then directly marked on the scalp with a pen and used for the rest of the experiment. The resting motor threshold (rMT) was defined as the lowest stimulator output intensity capable of inducing electromyographic (EMG) responses with a magnitude of at least 50 μV of the peak-to-peak MEPs amplitude in the FDI for a minimum of five of the ten trials (Groppa et al., 2012; Rossini et al., 1994). RMT was measured at the beginning of the experimental session, following the international standards (Rossi et al., 2009). After the determination of the rMT, the stimulator intensity was kept at 120% of the rMT (as in e.g. Burin et al., 2017) and 10 MEPs with an amplitude of at least 50 μV were recorded. The order of the rMT determination (and the following MEPs recording) was counterbalanced between subjects: half participants started with the left hemisphere, the other half with the right. The rMT and MEPs were recorded before and after immobilization: in the pre-immobilization session, rMT and MEPs were recorded one week before T1, in order to avoid any possible effect of TMS on EEG parameters. In the post-immobilization session, rMT and MEPs were recorded immediately after T4, thus the same day of the EEG recording, after the session with both hands free. MEPs were recorded from participants' FDI muscles (either left or right). EMG activity was recorded by pairs of Ag–AgCl surface pre-gelled electrodes (24 mm diameter), one on muscle belly and the other on the metacarpophalangeal joint of the index finger, following standard skin preparation. The electrodes were connected to a Biopac MP-150 electromyograph (Biopac Systems Inc., Santa Barbara, CA). The EMG signal was acquired according to the method used by previous studies (Bruno et al., 2018, 2017a; Bucchioni et al., 2016; Fossataro et al., 2018b, 2018a). MEPs were analyzed off-line using AcqKnowledge (version 4.1) software. All MEPs with an activity of at least 50 μV before the TMS pulse were not considered for analyses (i.e. 1.25% of the MEPs collected in the whole sample). In addition, to investigate cast-related modulation on force parameters related to EMG activity, while seated on the chair, each participant was instructed to maintain a thumb/index finger opposition for 2.5 s. The activity of FDI was recorded and the Maximum Voluntary Contraction (MVC) level in each subject was determined with EMG-MVC, represented as a root-mean-square (RMS) value. As in the method used by Burin et al. (2017), we considered two values of the force parameters, as they can be selectively affected by immobilization: force recruitment (FR, from 0 to 0.5 s) corresponding to the very first phase of the movement preparation, and MVC (from 0.5 to 2.5 s), more related to the movement execution (Burin et al., 2017).

All the data of the present study are available upon request.

## 3. Data analysis

In the present work, we were interested in the *comparison between free conditions* (i.e. T1 vs T4), the *comparison between blocked conditions* (i.e. T2 vs T3) and the *comparison between free and blocked conditions* (i.e. T1 vs T2, T1 vs T3, T2 vs T4, T3 vs T4). For this reason, we simplified the data analysis by using for each variable of interest (behavioral data, TMS data, force parameters, and EEG data) the very same statistical approach, i.e. a direct comparison between conditions by means of T tests (Bonferroni corrected). However, according to an anonymous reviewer's suggestion, we tested in additional analyses more complex Anova models, one for each variable of interest (see Supplementary materials).

### 3.1. Behavioral data

In the *Main experiment*, statistical analyses were performed using Statistica software (StatSoft, release 8). The statistical analyses were performed on mean accuracy in response to both Go and Nogo stimuli, and on mean RTs (transformed using a natural logarithm for normalization purpose) in response to Go stimuli, calculated for each participant. The normality of the residual distribution was verified by Shapiro-Wilk test ($p < 0.05$). With respect to RTs, analyses were performed only on correct responses to Go stimuli (i.e. incorrect responses corresponded to a wrong key press or a delayed response were excluded). Trials with RTs faster than 150 ms or deviating more than 2 standard deviations from individual mean RT were also excluded (Ronga et al., 2018). The analyses were performed on all the accuracy and RTs recorded in response to right hand stimuli and on accuracy and RTs recorded in response to left hand stimuli recorded during free sessions (i.e. T1- and T4-free), because during blocked sessions (i.e. T2- and T3-blocked) no accuracy nor RTs were collected due to left hand immobilization. Separately for accuracy and RTs, with respect to the right (control) side, six identical two-tailed paired T tests (Bonferroni corrected) were performed to compare all the timepoints (i.e. T1 vs T2, T1 vs T3, T1 vs T4, T2 vs T3, T3 vs T4). In a similar way, separately for accuracy and RTs, with respect to the left (manipulated) side, two-tailed paired T tests were performed by comparing T1- and T4-free. Statistical significance was set at 0.05.

### 3.2. Transcranial magnetic stimulation data and force parameters

With respect to TMS and force parameters, the rMT (expressed as the percentage of the stimulator output), MEPs peak-to-peak amplitude, RMS-EMG of both FR and MVC were considered as dependent variables. For all the dependent variables, two-tailed paired T tests (Bonferroni corrected) were conducted separately for each side, comparing pre- vs post-immobilization values.

### 3.3. Electrophysiological data

Event-related potentials were pre-processed and analyzed offline using Letswave v.6 (http://www.nocions.org/letswave/, Mouraux and

Iannetti, 2008). In both experiments (i.e. *Main* and *Control*), for Nogo/low-probability and Go/high-probability trials (stimulus-locked), epochs were selected from 500 ms before onset to 1000 ms after the onset of the imperative stimulus (total epoch duration: 1500 ms). All the epochs were band-pass filtered (0.5–35 Hz) using a fast Fourier transform filter (Cojan et al., 2013). Each epoch was baseline corrected using the 100 ms pre-stimulus recording period as reference (Cojan et al., 2013). The nose reference was maintained for analyses. Artifacts due to eye blinks or eye movements were rejected using a validated method based on an Independent Component Analysis (Jung et al., 2000). Blinks were found to be the most frequent cause of rejection.

#### 3.3.1. Main experiment

With respect to the *Main experiment*, only correct responses to Go and Nogo imperative stimuli were analyzed (on average, for each condition, the following trials per subject were included: Go right T1: 88.13 ± 3.77; Go right T2: 89.44 ± 0.89; Go right T3: 88.5 ± 3.77; Go right T4: 89.69 ± 0.6; Go left T1: 88.56 ± 3.97; Go left T2: 90 ± 0; Go left T3: 90 ± 0; Go left T4: 89.69 ± 0.6; Nogo right T1: 29 ± 1.46; Nogo right T2: 29.44 ± 1.5; Nogo right T3: 28.81 ± 1.91; Nogo right T4: 28.69 ± 2.36; Nogo left T1: 28.93 ± 2.08; Nogo left T2: 30 ± 0; Nogo left T3: 30 ± 0; Nogo left T4: 29.25 ± 1). Epochs belonging to the same experimental condition were averaged time-locked to the onset of the stimulus. Thus, separately for each hand (left hand; right hand) and for each timepoint (T1, T2, T3, T4), 8 average waveforms (left T1-free, left T2-blocked, left T3-blocked, left T4-free, right T1, right T2, right T3, right T4) both for Go and Nogo trials were obtained for each subject.

Separately for each side (i.e. left hand; right hand), mean ERP waveforms were analyzed by means of point-by-point two-tailed paired T tests, performing one T test for each single point in a curve. Significant intervals were then corrected for multiple comparisons across different points in time by using cluster-based permutation testing approach (1000 random permutations testing on all the 32 channels; cluster threshold was set at the 95th percentile of the cluster magnitude distribution; i.e. $p < 0.05$ (Maris and Oostenveld, 2007). For a similar methodological approach, please refer to (Harris et al., 2018; Novembre et al., 2018). More specifically, paired T tests were performed separately for each hand and for each timepoint (i.e. T1, T2, T3, T4) to investigate differences between Go and Nogo trials (i.e. Go vs Nogo in left T1-free; Go vs Nogo in left T2-blocked; Go vs Nogo in left T3-blocked; Go vs Nogo in left T4-free; Go vs Nogo in right T1; Go vs Nogo in right T2; Go vs Nogo in right T3; Go vs Nogo in right T4). Since more than one T test was performed, Bonferroni's correction was applied to alpha level ($0.05/4 = 0.0125$). Then, since the main interest of the present study was motor inhibition, we compared, separately for each side, the Nogo trials among each timepoint. Specifically, we performed *comparisons between free conditions* (i.e. T1 vs T4), *comparisons between blocked conditions* (i.e. T2 vs T3) and *comparisons between free and blocked conditions* (i.e. T1 vs T2, T1 vs T3, T2 vs T4, T3 vs T4). Bonferroni's correction was applied to alpha level ($0.05/6 = 0.008$).

Analyses in the frequency domain were applied to both Go and Nogo trials for each side (left; right) and each timepoint. The 500 ms pre-stimulus was cropped by each subject's single trials, to obtain epochs selectively containing post-stimulus activity. Furthermore, we applied a Fast Fourier Transform (FFT) to such epochs, in order to quantify the power in the theta (4–7 Hz, which is typically described in Go/Nogo tasks, e.g. Harper et al., 2014; Kirmizi-Alsan et al., 2006; Pscherer et al., 2019; van de Vijver et al., 2018) and the beta (13–30 Hz, which is involved in motor activity and sensorial factors related to limb-immobilization, e.g. Burianova et al., 2016; Fortuna et al., 2013; Manaia et al., 2013) frequency bands, for each experimental condition. Power values were calculated extracting power peaks from each single subject, for each frequency band. Values belonging to the same experimental conditions were then averaged, thus yielding eight different measurements (i.e. left T1-free, left T2-blocked, left T3-blocked, left T4-free, right T1, right T2, right T3, right T4) for each frequency band. We performed paired T tests between Go and Nogo trials separately for each hand and for each timepoint (i.e. Go vs Nogo in left T1-free; Go vs Nogo in left T2-blocked; Go vs Nogo in left T3-blocked; Go vs Nogo in left T4-free; Go vs Nogo in right T1; Go vs Nogo in right T2; Go vs Nogo in right T3; Go vs Nogo in right T4) with power values as the dependent variable. Based on the Go/Nogo differential effect of the inhibition-related theta power, as in the ERP analysis, we then performed T tests on the theta power values of the Nogo trials between timepoints, with *comparisons between free conditions* (i.e. T1 vs T4), *comparisons between blocked conditions* (i.e. T2 vs T3) and *comparisons between free and blocked conditions* (i.e. T1 vs T2, T1 vs T3, T2 vs T4, T3 vs T4). Since in the beta power we did not found an inhibition-related effect in Nogo trials, we performed additional T tests on the beta power to separately compare both Go and Nogo trials between timepoints, with *comparisons between free conditions* (i.e. T1 vs T4), *comparisons between blocked conditions* (i.e. T2 vs T3) and *comparisons between free and blocked conditions* (i.e. T1 vs T2, T1 vs T3, T2 vs T4, T3 vs T4). Statistical threshold was set at $p < 0.05$.

Additionally, analyses in the time-frequency domain are presented in Supplementary materials.

#### 3.3.2. Control experiment

With respect to the *Control experiment*, separately for each hand (i.e. left hand; right hand) and each probability of occurrence (i.e. High-probability; Low-probability), 4 waveforms (i.e. left High-probability; right High-probability; left Low-probability; right Low-probability) were obtained for each subject. Separately for each side (i.e. left hand; right hand), mean ERP waveforms were compared between High probability and Low probability with point-by-point two-tailed paired T tests, by means of cluster-based permutation testing approach (1000 random permutations testing on all the 32 channels) to correct for multiple comparisons across different time points (cluster threshold was set at the 95th percentile of the cluster magnitude distribution; i.e. $p < 0.05$ (Maris and Oostenveld, 2007).

#### 3.3.3. Main experiment vs control experiment

In addition, to disentangle between inhibition-related and novelty-related ERP components, we computed an inhibition related index for the *Main experiment*, expressed as a delta between Nogo and Go waveforms (Nogo minus Go; i.e. Δ Nogo-Go) of the T1 (when participants performed the task for the first time with both hands free) and a novelty related index for the *Control experiment*, expressed as a delta between Low- and High-probability waveforms (i.e. Low probability minus High probability; Δ Low-High probability). Separately for each hand, we compared the Δ Nogo-Go of the *Main experiment* at T1 with the Δ Low-High probability of the *Control experiment*. The analysis was performed employing point-by-point unpaired T tests, with cluster-based permutation testing approach (1000 random permutations testing on all the 32 channels) to correct for multiple comparisons across different time points (cluster threshold was set at the 95th percentile of the cluster magnitude distribution; i.e. $p < 0.05$, Maris and Oostenveld, 2007).

## 4. Results

### 4.1. Main experiment

#### 4.1.1. Behavioral results

With respect to the accuracy, no significant effects were found for the left (manipulated) hand or for the right (control) hand in response to Go and Nogo stimuli. Indeed, our participants responded with high level of accuracy with both the left and right hand across all the timepoints (mean accuracy expressed as a percentage, Go right T1: 98.96% ± 1.59; Go right T2: 99.37% ± 0.99; Go right T3: 99.37% ± 1.28; Go right T4: 99.65% ± 0.67; Nogo right T1: 97.71% ± 3.38; Nogo right T2: 98.12% ± 5.01; Nogo right T3: 97.08% ± 5.42; Nogo right T4: 96.58% ± 6.14; Go left T1: 99.43% ± 0.92; Go left T4: 99.24% ± 1.12; Nogo left T1: 97.5% ± 6.02; Nogo left T4: 97.5% ± 3.33).

Paired T tests on the on right (control) hand RTs (log-transformed) showed significant faster RTs at T4 with respect to both T1 ($p < 0.001$) and T2 ($p < 0.008$), and with significant faster RTs at T3 with respect to T1 ($p < 0.008$) suggesting that the second day, participants were faster in performing the Go/Nogo task with the right (control) hand (Fig. 2B).On the contrary, for the left (manipulated) hand, the paired T test on mean RTs revealed no significant difference between T1-free and T4-free (Fig. 2A).

See Supplementary materials for the additional results in an Anova model.

#### 4.1.2. Transcranial magnetic stimulation results

Paired T tests on rMT recorded before and after one week of immobilization revealed a significant difference ($T_{15} = 6.82$; $p = 0.000005$) only with respect to the hemisphere contralateral to the left (immobilized) hand (i.e. right hemisphere), with a significant increase of rMT after one week of immobilization ($p < 0.001$), (mean rMT±sd; right hemisphere: pre-immobilization = 54.69 ± 7.24; post-immobilization = 60.19 ± 8.87; left hemisphere: pre-immobilization = 53.88 ± 7.05; post-immobilization = 54.06 ± 7.78) (Fig. 2C). Paired T tests on MEPs amplitude did not find any significant effects, confirming that, for the right hemisphere, different rMT between pre- and post-immobilization produced comparable MEPs. With respect to the force parameters, the paired T tests revealed a significant difference between pre- and post-immobilization only with respect to the left (immobilized) hand in FR ($T_{15} = 3.6$; $p = 0.002$), suggesting a significant decrease of FR in the left (immobilized) hand with respect to the right (control) hand after the immobilization (mean FR ± sd; left hand: pre-immobilization = 0.039 ± 0.014; post-immobilization = 0.024 ± 0.011; right hand: pre-immobilization = 0.033 ± 0.012; post-immobilization = 0.032 ± 0.016) (Fig. 2D). No significant effects were found on MVC parameter. See Supplementary materials for the additional results of in Anova model.

#### 4.1.3. Electrophysiological results - ERPs

Grandaverage waveforms are depicted in Fig. 3. To note, ERPs evoked by Nogo stimuli presented the typical neurophysiological markers of motor inhibition responses, replicating previous studies using similar paradigms (e.g. Bokura et al., 2001; Bruno et al., 2019; Cojan et al., 2013; Falkenstein et al., 2000; Kok, 1986).

*4.1.3.1. Right (control) hand.* As expected, when comparing Go and Nogo trials in each timepoint, a significant difference was found over fronto-centro-parietal electrodes (see Fig. 3A for significant channels), with a significantly greater amplitude in response to Nogo than to Go trials in the following time intervals: 119–242 ms [coinciding with the latency of both the N140 (p always <0.001) and N2 (p always <0.001)] and 264–491 ms [coinciding with the latency of both P3 (p always <0.0005) and the negative shoulder following the P3 wave (p always <0.001) waves] (Fig. 3A). When comparing the Nogo trials across the four timepoints, no significant differences emerged suggesting that, when no experimental manipulation was performed (i.e. the right hand was always free to respond during the task), no sequence effect was found on the inhibition-related EEG activity, even if the task was repeated two times in two different days (Fig. 3B).

*4.1.3.2. Left (manipulated) hand.* When comparing Go and Nogo trials in each timepoint, only at T1-free, comparable effects with respect to the right (control) hand was found; i.e. a significant Go/Nogo differential response in all three ERPs components (N140, N2, P3). Crucially, during (T2-blocked, T3-blocked) and after (T4-free) immobilization, specific effects was found for each component. See Fig. 3C.

More specifically, while in the time interval 273–477 ms (coinciding with the latency of the P3) we found a significantly greater amplitude in response to Nogo than to Go trials (p always <0.001) in all four timepoints, in the time interval 189–256 ms (coinciding with the latency of

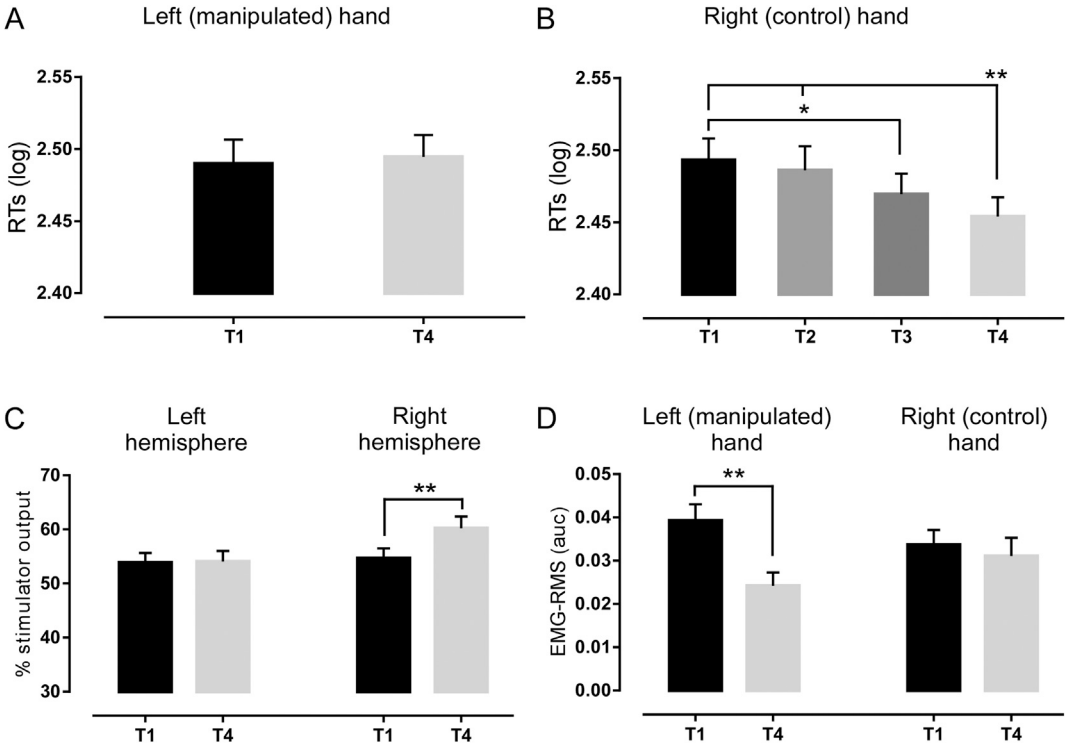


**Fig. 2. A) RTs to left stimuli.** Mean reaction times transformed using a natural logarithm, in response to left Go stimuli recorded at T1 and T4. **B) RTs to right stimuli.** Mean reaction times transformed using a natural logarithm, in response to right Go stimuli recorded at T1, T2, T3 and T4. **C) rMT results.** Mean values of rMT of both the right hemisphere (contralateral to the manipulated left hand) and the left hemisphere (contralateral to the control right hand) expressed as percentage of the stimulator output, recorded one week prior to T1 and immediately after T4. **D) FR results.** Mean values of FR of both the manipulated left hand and the control right hand, expressed as EMG-RMS, recorded one week prior to T1 and immediately after T4. *$p < 0.05$, **$p < 0.01$. Error bars represent standard error of the mean.

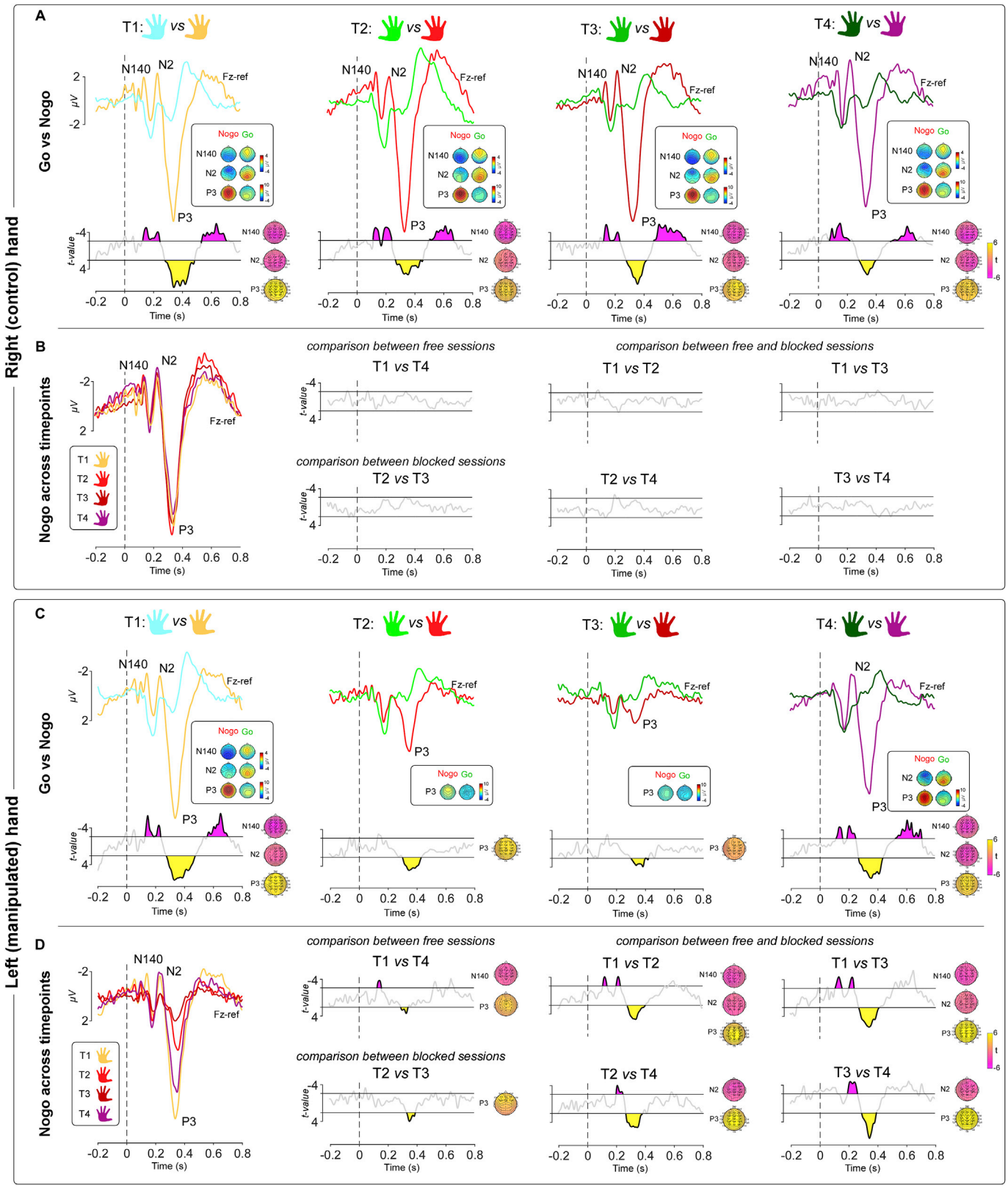


**Fig. 3. ERPs results of the Main experiment. A.** Mean ERPs in response to right Go and Nogo trials, and paired T tests between Go and Nogo trials for each timepoint (i.e. T1, T2, T3 and T4). **B.** Mean ERPs in response to right Nogo trials, and paired T tests across timepoints (i.e. T1 vs T2, T1 vs T3, T1 vs T4, T2 vs T3, T2 vs T4, T3 vs T4). **C.** Mean ERPs in response to left Go and Nogo trials, and paired T tests between Go and Nogo trials for each timepoint (i.e. T1, T2, T3 and T4). **D.** Mean ERPs in response to left Nogo trials, and paired T tests across timepoints (i.e. T1 vs T2, T1 vs T3, T1 vs T4, T2 vs T3, T2 vs T4, T3 vs T4). In **A) B) C)** and **D)** data are displayed in microvolts as a function of time post-cue onset (represented by the dashed line), for Fz electrode (referenced to the nose). Point-by-point T values for each comparison are represented below ERPs waveforms. Significant channels are presented on the topographical maps of the effect. Time intervals during which the ERPs were significantly different in the Go and Nogo conditions are colored in yellow and pink.

the N2), a significantly greater amplitude in response to Nogo than to Go trials was found only in free conditions (i.e. T1- and T4-free). Finally, in the time interval 117–186 ms (coinciding with the latency of the N140), a significant difference between Nogo and Go trials (p always <0.001) was found only at T1-free (Fig. 3C). Note that all the reported effects were found mainly over fronto-centro-parietal electrodes (see Fig. 3C for significant channels).

Interestingly, when comparing the Nogo trials across the four timepoints, significant differences emerged in all three components (specific latency of each effect is shown in Fig. 3D). In particular, when a cast-related effect was investigated, with *comparisons between free and blocked conditions* (i.e. T1 vs T2, T3 vs T4) in each day (i.e. day 1 and day 2), a significant modulation was found in both N2 and P3. In day 1, the N2 and P3 amplitudes were greater (p always <0.0001) at T1-free, when participants were free to move, than at T2-blocked, as soon as the cast was placed over the left arm. On day 2, the N2 and P3 amplitude were greater (p always <0.00001) at T4-free, when participants were free to move after a week of immobilization, than at T3-blocked, when participants still had the cast which they have been wearing for a whole week. Note that the reported cast-related effects in both day 1 and day 2 were found over fronto-centro-parietal electrodes (see Fig. 3D for significant channels). Importantly, in P3 component, a significant modulation was also found when the effect of the duration of the immobilization was investigated, by comparing pre- and post-immobilization sessions, in both blocked and free sessions. In particular, in the *comparison between blocked conditions* (i.e. T2 vs T3), the P3 amplitude over frontal electrodes (see Fig. 3D for significant channels) was lower (p always <0.00001) at T3-blocked, after a week of immobilization, than at T2-blocked, as soon as the cast was placed (Fig. 3D). In the *comparison between free conditions* (i.e. T1 vs T4), the P3 amplitude over Fcz and Cz was lower (p always <0.001) at T4-free, when the subjects were again free to move after a week of immobilization, than at T1-free, before the manipulation. Finally, the N140 amplitude over fronto-central electrodes (i.e. see Fig. 3D for significant channels) was greater (p always <0.001) at T1-free than in all the other timepoints, suggesting that this component is modulated by both the presence of the cast and the duration of the immobilization.

See Supplementary materials for the additional results in an Anova model.

#### 4.1.4. *Electrophysiological results – analyses on post-stimulus neural oscillatory activity*

With respect to the analyses in the frequency domain, the results are presented below (Fig. 4). As for the ERP analyses, when comparing Go and Nogo trials in each timepoint and across timepoints, significant differences were found.

*4.1.4.1. Right (control) hand.* With respect to theta band, when comparing Go and Nogo trials in each timepoint, significant differences were found in all the timepoints over frontal electrodes (i.e. Fz, F3, F4, Fcz, Fc3) (Fig. 4A). In particular, theta power was always higher in response to Nogo than to Go stimuli in each timepoint (p always <0.05), confirming the inhibition-specific effect of theta band oscillations. When comparing Nogo trials across timepoints, no significant differences emerged, suggesting that no sequence effect was expressed in the theta band, even if the task was repeated four times in two different days (Fig. 4A).

With respect to beta band, when comparing Go and Nogo trials in each timepoint no significant differences were found, confirming that beta band oscillatory activity is not inhibition-specific (Fig. 4B). When comparing either Go or Nogo trials across timepoints, no differences were found, suggesting that, also in the beta band, no sequence effect was found (Fig. 4B).

*4.1.4.2. Left (manipulated) hand.* With respect to theta band, when comparing Go and Nogo trials in each timepoint, significant differences were found in all the timepoints over frontocentral electrodes (i.e. Fz, F3, F4, Fcz, Fc3, Fc4, Cz) (p always <0.05), confirming, as for the right (control) hand, the inhibition-specific effect on theta band oscillatory activity (Fig. 4E). Crucially, when Nogo trials were compared across

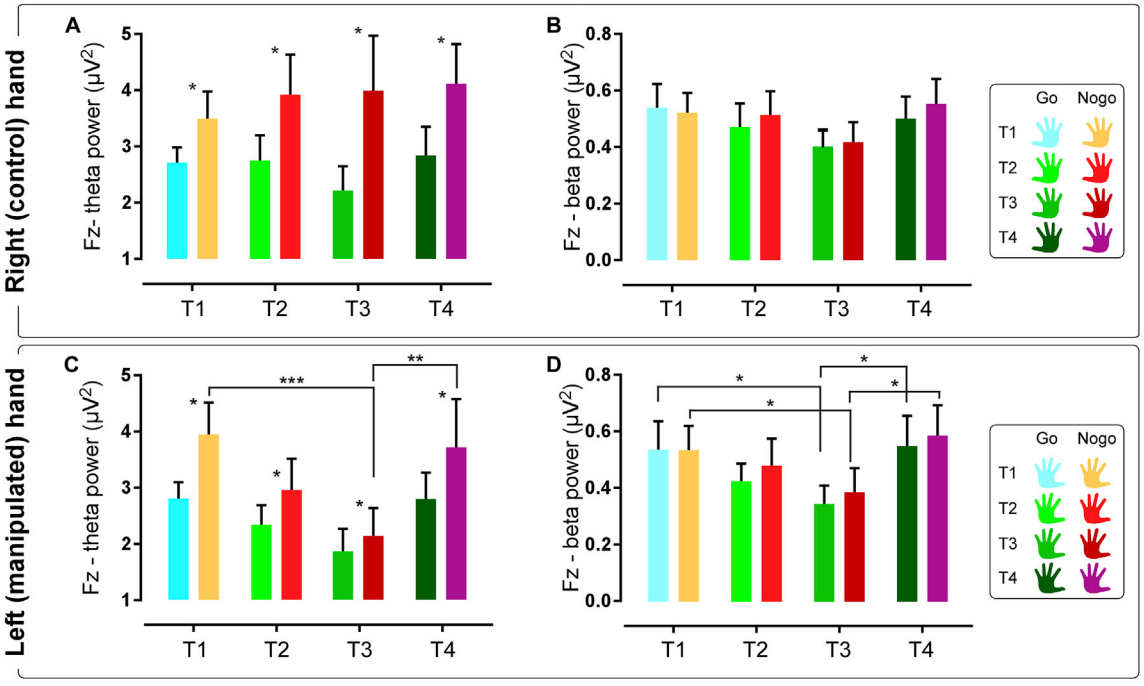


**Fig. 4. Theta and beta band oscillatory activity of the Main experiment. A.** Mean theta power activity at Fz electrode in response to right Go and Nogo trials for each timepoint. **B.** Mean beta power activity at Fz electrode in response to right Go and Nogo trials for each timepoint. **C.** Mean theta power activity at Fz electrode in response to left Go and Nogo trials for each timepoint. **D.** Mean beta power activity at Fz electrode in response to left Go and Nogo trials for each timepoint. *p < 0.05, **p < 0.01, ***p < 0.001. Error bars represent standard error of the mean.

timepoints, significant differences (p always <0.01) emerged in the *comparisons between free and blocked conditions*, and specifically between both the free sessions (i.e. T1- and T4-free) and T3-blocked over fronto-central electrodes (i.e. Fz, F3, F4, Fcz, Fc3, Cz, C3, C4) (Fig. 4C). In particular, after a week of immobilization, when participants were still blocked by the cast (i.e. T3-blocked), lower theta power was found with respect to both free sessions, suggesting an inhibition related modulation induced by the immobilization.

With respect to beta band, when comparing Go and Nogo trials in each timepoint no significant differences were found, confirming, as for the right (control) hand, that beta band oscillatory activity does not specifically codify for motor inhibition effects (Fig. 4D). When comparing both Go and Nogo trials across timepoints, significant differences were found (p always <0.05) (Fig. 4H). In particular, significant differences in beta power emerged between the free sessions (i.e. T1- and T4-free) and T3-blocked over fronto-central electrodes (i.e. Fz, F4, Fcz, Fc4, Cz, C4), with lower beta power at T3-blocked, suggesting that beta power in both Go and Nogo trials was specifically modulated by the long-lasting immobilization.

See Supplementary materials for the additional results in an Anova model. In addition, results in the time-frequency domain, which partially mirrored both the ERP analyses and the analyses on post-stimulus neural oscillatory activity, are presented in Supplementary materials.

### 4.2. Control experiment

With respect to both right and left hand, paired T tests comparing high- and low-probability stimuli revealed significant differences (Fig. 5A). In particular, with respect to the right hand, a significant effect was found over fronto-central-parietal electrodes in the time interval between 330 and 410 ms [coinciding with the latency of P3 ($p < 0.01$)]; with respect to the left hand, a significant effect was found over fronto-

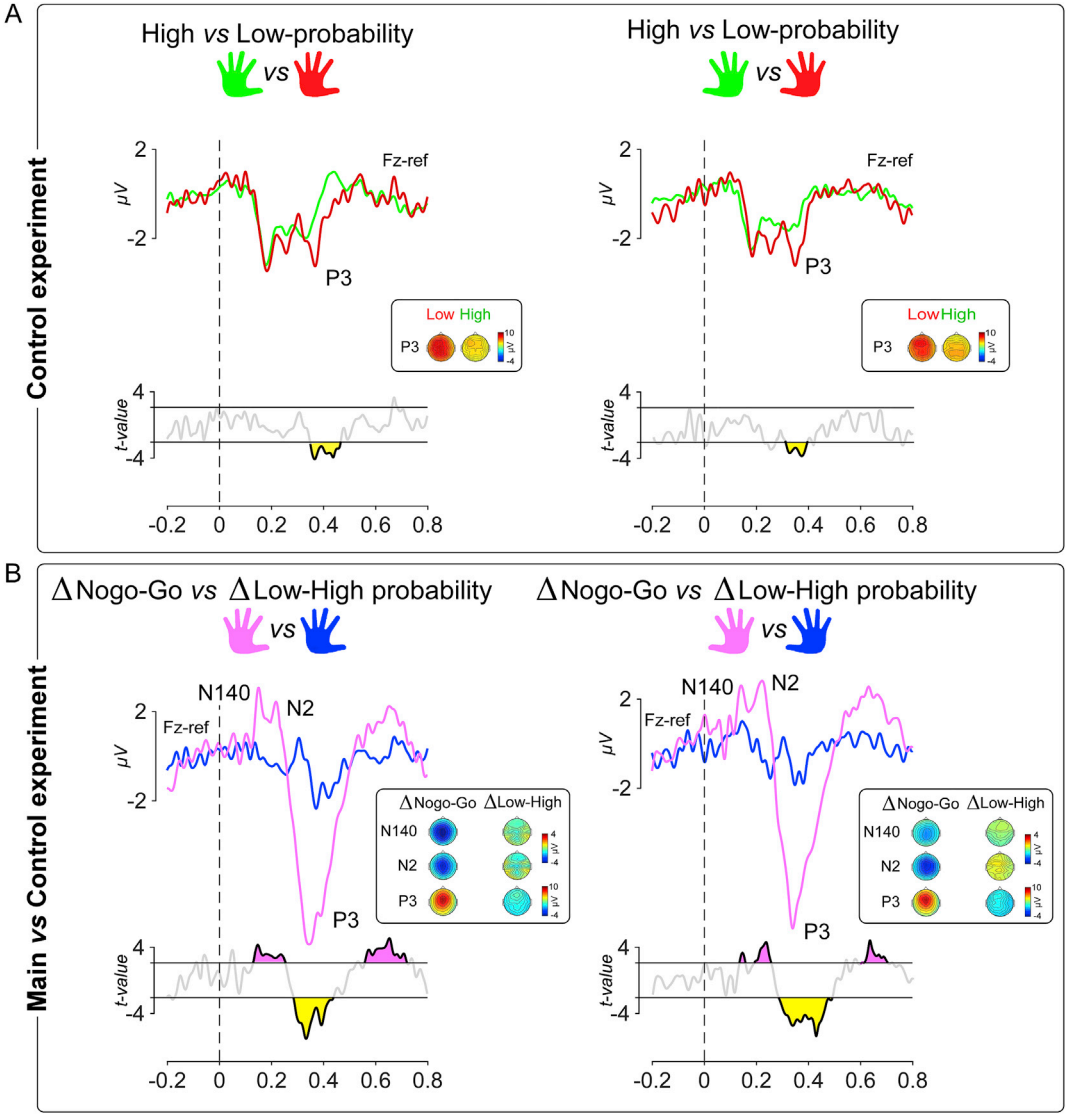


**Fig. 5. A. ERPs results of the Control experiment. A.** Mean ERPs in response to left High-probability and left Low-probability trials (left panel), and paired T tests between High- and Low-probability trials. Mean ERPs in response to right High-probability and right Low-probability trials (right panel) and paired T tests between High- and Low-probability trials. **B. ERPs results of the comparison between Main and Control experiments. A.** Mean ERP in response to the left Δ Nogo-Go of the Main Experiment at T1-free and to the left Δ Low-High probability of the Control experiment, and paired T tests between left Δ Nogo-Go and left Δ Low-High probability trials (left panel). Mean ERP in response to the right Δ Nogo-Go of the Main Experiment at T1-free and to the right Δ Low-High probability of the Control experiment, and paired T tests between right Δ Nogo-Go and right Δ Low-High probability trials (right panel). In **A)** and **B)** data are displayed in microvolts as a function of time post-cue onset (represented by the dashed line), for Fz electrode (referenced to the nose). Point-by-point T values for each comparison are represented below ERPs waveforms. Time intervals during which the ERPs were significantly different in the Go and Nogo conditions are colored in yellow and pink.

central-parietal electrodes in the time interval between 355 and 450 ms [coinciding with the latency of P3 ($p < 0.01$)], revealing, as expected, greater P3 in low- than in high-probability stimuli with respect to both sides.

### 4.3. Main vs control experiments

Crucially, when comparing the Δ Nogo-Go *Main Experiment* at T1-free (when participants performed the task for the first time with both hands free) with the Δ Low-High probability of the *Control experiment*, significant differences were found with respect to both right and left hand (Fig. 5B). In particular, in the right hand, a significant effect was found over fronto-central-electrodes in the time interval between 124 and 260 ms [coinciding with the latency of both N140 (p always <0.01) and N2 (p always <0.01)], between 290 and 460 ms [coinciding with the latency of P3 (p always <0.001)] and 595–690 [coinciding with the latency of P3 negative shoulder (p always <0.01)]. In the left hand, a significant effect was found over fronto-central-electrodes in the time interval between 128 and 265 ms [coinciding with the latency of both N140 (p always <0.01) and N2 (p always <0.01)], between 280 and 460 ms [coinciding with the latency of P3 (p always <0.001)] and 580–720 [coinciding with the latency of P3 negative shoulder (p always <0.001)]. These findings suggested that, although the probability of occurrence of Nogo stimuli in the *Main experiment* was lower with respect to Go stimuli, the P3 modulation observed in the *Main experiment* is not (only) explained by the novelty of the stimulus, but rather they clearly reflect a motor inhibition process.

## 5. Discussion

The present study deals with an important aspect of motor control, i.e. movement inhibition. The inhibition of inappropriate responses is an important part of goal-oriented behavior and different aspect of motor inhibition have been investigated in cognitive neuroscience. In the present study, we asked whether long-term limb immobilization modulates physiological mechanisms underlying inhibitory motor responses. To this aim, we recorded ERPs in healthy participants performing a Go/Nogo task, by contrasting conditions in which participants were free to respond with both hands against two "blocked" conditions in which left hand movements were prevented by a cast. During free conditions, EEG was acquired immediately before the positioning of the cast (T1-free) and as soon as it was removed (T4-free) one week later; during blocked conditions, EEG was acquired as soon as the cast was positioned on the left arm (T2-blocked) and immediately before the cast removal (T3-blocked) one week later. Our results showed that ERP responses to both Go and Nogo trials were differentially modulated by the presence of the cast *per se* (i.e. in the *comparisons between free and blocked conditions*) and by the duration of the immobilization (i.e. by comparing in both free and blocked conditions post- and pre-immobilization sessions). Furthermore, in the *comparison between free and blocked conditions*, the analysis on neural oscillatory activity showed that inhibition-related theta band activity in response to Nogo stimuli was significantly decreased at T3-blocked condition, after one week of immobilization, compared to both free sessions (T1 and T4). Interestingly, results in the time-frequency domain partially mirrored the ERPs and neural oscillatory activity results, not only with significant differences in the *comparisons between free and blocked conditions*, but also in the *comparison between free conditions* (see Supplementary materials). Overall, these findings can result from plastic changes induced by the long-term limb immobilization, as also demonstrated by the cast-related corticospinal excitability modulation and by decreased beta band oscillatory activity in response to Go and Nogo trials.

### 5.1. Immobilization after-effects: behavioral and physiological parameters

First of all, it is important to note that our immobilization procedure was effective in modulating behavioral and physiological motor parameters. We did not find any modulations on response accuracy since our participants performed only a few errors overall, however, interesting results were obtained with respect to RTs. Concerning the right (control) side, behavioral data showed faster RTs in both T3 and T4 (after one week of left side immobilization) with respect to both T1 and T2 (see Fig. 2B). Note that this behavioral effect might be related to the long-term immobilization and not to a learning effect, since, for the right (control) side, no sequence effect was found between T1 (in which subjects were free to perform the task with both hands) and T2 (as soon as the cast was positioned on the left hand). Even if we cannot exclude that, in our task, a learning effect could emerge only starting from the third repetition, a faster performance of the right hand after immobilization can be compatible with improved performance due to hyper-use of the contra-immobilization side, as suggested by previous evidence on immobilization procedure in healthy subjects (Avanzino et al., 2011) and by constraint-induced movement therapy in brain-damaged patients (Wittenberg and Schaechter, 2009). However, in our data, a putative hyper-use effect is present only at a behavioral level, since no corticospinal excitability modulation was found for the left M1 contralateral to the right (control) hand and no modulation of the force parameters was present for the right (control) hand (see Fig. 2C).

Concerning the left (manipulated) side, no behavioral difference was found between T1-free and T4-free (i.e. the only timepoints in which the left hand was free to move and RTs can be recorded). However, in agreement with previous literature (Bassolino et al., 2014; Burianova et al., 2016; Burin et al., 2017; Garbarini et al., 2019), we found that the rMT of right M1 contralateral to the left (immobilized) hand was significantly increased in post- with respect to pre-immobilization (see Fig. 2C). This means that the right M1 corticospinal excitability was significantly inhibited after long-term immobilization. Accordingly, we also found that the force recruitment phase (recorded as the first 500 ms of the EMG activity during voluntary finger-thumb opposition movements) was significantly decreased in post- with respect to pre-immobilization (see Fig. 2D), replicating previous studies which investigated force strength after immobilization (Burin et al., 2017; Kaneko et al., 2003; Lundbye-Jensen and Nielsen, 2008; Weibull et al., 2011). Taken together, these physiological results showed that our immobilization procedure was effective in modifying the activity of the motor system, supporting the view that our EEG results in left (manipulated) side can be interpreted as a consequence of plastic changes induced by the immobilization.

### 5.2. ERP modulations pre- and post-immobilization

As expected, in the right (control) side, we found the classic ERP pattern showing, for N140, N2 and P3 components, a greater amplitude in response to Nogo than to Go trials, irrespective of the timepoint. See Fig. 3A. As discussed above, even if the immobilization of one limb may induce bilateral changes (Avanzino et al., 2011), in our study, the hyper-use of the right hand is not mirrored in the ERP components related to the Go/Nogo task. It is important to note that, in the Nogo-related ERPs, we did not find differences among the four timepoints (see Fig. 3B), suggesting that no sequence effect occurred, even if in our experimental design the time points order was always the same (i.e. T1, T2, T3, T4) and in T1 and T3 the task was preceded by rest, while in T2 and T4 was preceded by the task execution.

More interestingly, in the left (manipulated) side, only P3 component was always able to discriminate between Go and Nogo trials in each timepoint, even if the Nogo-P3 amplitude was significantly modulated across timepoints by the presence of the cast (always greater in free than in blocked conditions) and by the duration of the immobilization (always lower in post- with respect to pre-immobilization sessions). See Fig. 4C and D. While the N2 component was modulated by the presence of the cast *per se* – i.e. the Go/Nogo difference in this component was present only in free (T1- and T4-free) and not in blocked (T2- and T3-blocked) conditions –, the N140 was modulated by both the presence of the cast and the duration of the immobilization – i.e. the Go/Nogo difference in

this component was present only in the free condition before immobilization (T1-free). Coherently, in Nogo trials, both N140 and N2 showed a significant modulation across timepoints. While the N2 amplitude was modulated only by the presence of the cast, being greater in free (T1- and T4-free) than in blocked (T2- and T3-blocked) conditions, the N140 amplitude was modulated by both the presence of the cast and the duration of the immobilization, being greater at T1-free with respect to all the other timepoints.

With respect to N2 and P3, several studies on Go/Nogo task showed that these two ERP components are typically associated with motor inhibition and that they are generally elicited by Nogo stimuli (Bokura et al., 2001; Cojan et al., 2013; Falkenstein et al., 1995). Some authors suggested that N2 modulation in response to Nogo stimuli may reflect the first stage of inhibition, or a recognition of the need for inhibition (Smith et al., 2008). This seems to be compatible with our data, since in free conditions (T1 and T4), where a significant difference between Go and Nogo was present, the need for inhibition is certainly greater compared to blocked conditions (T2 and T3), where the Go/Nogo difference was not significant. By contrast, P3 has been related to the actual inhibition mechanism. Several studies showed that, when comparing Go and Nogo stimuli, this component in response to Nogo stimuli has a larger amplitude than that elicited by the Go stimuli (Bruin et al., 2001; Cojan et al., 2013; Nakata et al., 2010; Schmajuk et al., 2006) and it has a more anterior distribution (Barry et al., 2010; Smith et al., 2008). This anterior distribution has been linked to the inhibition of a motor response when a Nogo stimulus is presented, a sort of index of an active inhibitory process [e.g. Randall and Smith, 2011; Rockstroh et al., 1992; Smith et al., 2008; Woodward et al., 1991]. Conversely, other authors suggested that different P3 amplitude between Go and Nogo stimuli does not reflect any specific inhibitory process, but instead is influenced by the partial overlapping of movement-related potentials in Go stimuli (Salisbury et al., 2004, 2001). The authors stated that the typical frontocentral Go/Nogo effect may be produced by a general reduction in Go-P3 due to movement-related potentials at these sites, rather than to an amplitude increase in Nogo trials (Salisbury et al., 2001) and, therefore, that the difference between Go and Nogo effect may be produced not by inhibitory potentials on NoGo trials, but rather by movement-related negativity on Go (press) trials (Salisbury et al., 2001). Overall, explanations of the mechanism underlying Nogo- and Go-P3 amplitude modulations appear still controversial. In our study, the results observed for the left (manipulated) side might provide a further contribution to this debate. In the P3 component, a significant difference between Go and Nogo trials was found in all four time-points, suggesting that, even though during blocked conditions (T2 and T3) the subjects could only try to perform the task with the immobilized hand and no overt key press occurred, the EEG responses were still able to disentangle between Go and Nogo trials. This finding is crucial for two reasons. First, it rules out the possibility that, during block conditions, the subjects were not actually performing the task with the left immobilized hand. Second, it suggests that the general reduction in Go-P3 amplitude in blocked conditions cannot be selectively ascribed to the overlap with movement-related potentials, since movements did not occur in these time points. Thus, our results seem to confirm the presence of a "genuine" inhibitory process, directly modulating the amplitude of Nogo-P3.

N1 component is commonly evoked by visual and auditory stimulation, however, the emergence of N140 has been described both during visual (Sasaki et al., 2010) and somatosensory Go/No-go paradigms (Nakata et al., 2015, 2010; 2006a, 2006b; 2005; Sakamoto et al., 2015). In a MEG study (Sasaki et al., 1993), using a visual Go/Nogo task, it has been shown that cortical activities peaking at 135 ms were predominant following Nogo trials rather than Go trials (Sasaki et al., 2010). In another EEG and MEG study (Nakata et al., 2005) on a Go/Nogo task involving somatosensory stimuli, ERP data revealed that the amplitude of the Nogo-N140 component, which peaked at about 155 ms from frontocentral electrodes, was significantly more negative than that of Go-N140. MEG data revealed that a long-latency response peaking at approximately 160 ms, termed Nogo-M140 and corresponding to Nogo-N140, was recorded in Nogo trials only (Nakata et al., 2005). The authors suggested that both Nogo-N140 and Nogo-M140 evoked by somatosensory stimuli in a Go/Nogo task, generated from the prefrontal cortex, should be related to the neural activity of response inhibition. It has also been reported that Nogo-N140 component is enhanced when a greater amount of muscle force has to be implemented to respond to Go stimuli, suggesting that, in Nogo trials, a stronger inhibitory process is required to suppress an increased preparatory motor activity (Nakata et al., 2006a). Furthermore, the authors reported that the enhanced Nogo activity in the prefrontal cortex exerts a stronger inhibitory influence on the primary motor cortex, via the premotor cortex and/or the supplementary motor area, so that, with increased muscle force, MEP amplitude was significantly reduced in Nogo trials. It is possible therefore to assume that there are important relations between the activity of the primary motor area and Nogo-N140 component. In light of this, we can interpret the effect we found in the Nogo-N140 as related to the decreased activity of the motor system after immobilization (see Physiological rMT and FR results). At day 1, when motor parameters were not altered, a significant difference between Nogo- and Go-N140 was found for the left hand as for the right hand. On the contrary, only with respect to the left (manipulated hand), in blocked conditions (T2- and T3-blocked) and in the post-immobilization free condition (T4-free), when motor parameters were weakened by the long-term immobilization, the lack of difference between Nogo- and Go-N140 can be explained by the reduced amount of prefrontal inhibitory activity necessary to suppress the (weaker) movement. Note also that the modulation of the Nogo ERP components across timepoints was mainly found at frontal locations, suggesting a specific effect of long-term limb immobilization on the physiological mechanisms subserving motor inhibition exerted by frontal areas on the motor system.

Overall, these results suggest that not only the cast *per se* is able to induce changes in ERPs related to motor inhibition, but also the duration of the immobilization, which, as a consequence of plastic changes, affects the quantitative of motor inhibition needed to suppress a movement.

### 5.3. Theta and beta band oscillatory activity modulations pre- and post-immobilization

The results on the frequency domain partially mirror the results on the time domain. A wide literature on frequency analysis of the EEG signal usually obtains power enhancement in the theta band (4–7 Hz) over frontocentral sites for the Nogo condition (Cohen, 2014; de Vega et al., 2016; Harper et al., 2016, 2014; Huster et al., 2013; Kirmizi-Alsan et al., 2006; Nigbur et al., 2011) probably indexing inhibition (Harper et al., 2014; Huster et al., 2013). For this reason, we analyzed theta band oscillations in our group of participants during the Go/Nogo task to control for possible modulations induced by the cast and by the long-lasting immobilization. First of all, in both right (control) and left (manipulated) hand, our data confirmed an inhibition-related effect in the theta band, showing greater power in Nogo than in Go trials in all four timepoints, i.e. both in free (i.e. T1- and T4-free) and blocked (T2- and T3-blocked) sessions. Importantly, in the right (control) side, the Nogo theta power was not modulated across timepoints, suggesting that no sequence effect occurred. More interestingly, in the left (manipulated) hand, the theta power was modulated by both the presence of the cast and the duration of the immobilization, mirroring the ERPs results. Indeed, when considering only Nogo trials, we found a decrease in theta band oscillations at T3, which is one week after the immobilization, with respect to both free conditions (i.e. T1- and T4-free) (Fig. 4C), suggesting that the long-lasting immobilization affected the motor inhibition performance.

In the frequency domain, we also analyzed the beta band oscillatory activity, since it has been described in various studies with quantitative EEG (Fortuna et al., 2013; Manaia et al., 2013) and MEG (Burianova et al., 2016) a beta band modulation when comparing the neural activity

before and during limb immobilization. According to previous studies, we found a decrease in beta band oscillations at fronto-central electrodes (central and, coherently, contralateral to the immobilized hand) pertaining only to the left immobilized hand. Importantly, this effect in the beta band was not related to motor inhibition, being present in both Go and Nogo trials, where a significant decrease was found at T3-blocked with respect to both the free sessions (Fig. 4D). As for the ERPs, this result could be interpreted as a consequence of the plasticity changes induced by the immobilization, in line with the results obtained in the TMS on corticospinal activity and force parameters.

### 5.4. Motor inhibition or stimulus novelty?

Since the *Main experiment* was composed by frequent Go stimuli (75% of occurrence) and infrequent Nogo stimuli (25% of occurrence), it is possible to argue that the P3 found in the *Main experiment* could be evoked not (only) by motor inhibitory processes, but rather by the fact that the occurrence of Nogo stimuli was lower with respect to Go stimuli. Indeed, the literature largely described the novelty P3 component [for a review see Friedman et al., 2001], which is elicited in response to infrequent stimuli. To control this possibility, a different group of healthy participants was recruited, and they underwent an EEG session in which the same visual elements and the same probability of occurrence of the stimuli of the Go/Nogo task of the *Main experiment* were maintained. In the *Control experiment*, participants were asked to simply watch at the visual stimuli. We acknowledge that performing a control experiment in different subjects presents some limitations, by adding possible interfering variables, such as the inter-individual variability in task performance and cortical activity, however, it is important to note that we deliberately used a different group of participants in order to have subjects naïve to the meaning of visual stimuli (i.e. green = Go; red = Nogo). As expected, the results of the *Control Experiment* showed that, irrespectively of the side (i.e. left, right), Low-probability stimuli (i.e. red hands) elicited larger P3 than High-probability stimuli (i.e. green hands). This result is in line with all the literature on oddball paradigms (Donchin et al., 2014; Pritchard, 1981), in which infrequent stimuli represent the target in a background of frequent standard stimuli and the subject is instructed to respond mentally or physically to the target stimulus and not respond otherwise. The targets typically elicit a large P3 component over the midline electrodes (Fz, Cz, Pz), which typically increases in magnitude from the frontal to parietal electrode sites (Polich, 2007). However, when we compared the P3 evoked by low-probability stimuli of the *Control experiment* with the P3 evoked by motor inhibition mechanisms of the *Main experiment* at T1, we found a significant difference between them. Crucially, the P3 evoked by the Δ Nogo-Go was significantly greater than the Δ Low-High probability stimuli in the time interval of the P3 component, suggesting that, even if the occurrence of the Nogo stimuli of the Main experiment was lower than the occurrence of Go stimuli, the P3 modulation we found could not only be ascribed to the stimulus novelty. Even if we cannot exclude that the low-probability of Nogo stimuli occurrence empowered the P3 effect, we can affirm that this effect is due to an additional cognitive mechanism, which is the motor inhibition. Finally, it is important to state that the use of these percentages of stimuli occurrence (i.e. frequent Go vs infrequent Nogo) in Go/Nogo tasks is not only largely shared in literature [e.g. Benikos et al., 2013; Cojan et al., 2013, Gow et al., 2012; Moyle et al., 2006; Harper et al., 2014; Nguyen et al., 2016, Young et al., 2018] but also recommended (Wessel, 2017).

### 5.5. Limitations

We acknowledge that the present study is not free from limitations. With respect to the right (control) hand, we recognize that avoiding to monitor the everyday motor activity of the not-immobilized hand is a limitation since it does not allow us to disentangle whether immobilization prevented learning, or whether instead, the improvement in RTs observed for the right hand was due to a compensatory overuse of the not restricted hand. On the same line, we did not monitor left-hand motor parameters (e.g. employing EMG recordings during the Go/Nogo task or through a self-report of subjects' performance) in blocked conditions (i.e. T2 and T3) during the Go/Nogo task, and this makes problematic the *comparisons between free and blocked conditions*. Indeed, even if, during the training phase, our participants were strongly encouraged to "try to perform" the task during blocked conditions, the absence of EMG data does not allow us to exclude that participants were employing other motor strategies, such as motor imagery that has been shown to modulate actual motor parameters (Piedimonte et al., 2014) and to be modulated by the immobilization (Burianova et al., 2016). However, it seems possible to exclude that during blocked conditions our participants were passively looking at the monitor without performing any activity, since, in the left (manipulated) hand, there are significant differences between Go and Nogo trials among all timepoints (i.e. T1-free, T2-blocked, T3-blocked and T4-free), in both ERP (i.e. P3 component) and neural oscillatory activity (i.e. theta frequency) analyses.

## 6. Conclusions

Altogether, our electrophysiological, behavioral, and TMS results seem to indicate that when the M1 excitability is reduced, frontal areas exert, via premotor cortex and/or supplementary motor area, a lower inhibitory control over M1. Thus, only if we are free to move, then inhibitory responses are fully implemented. After one week of immobilization, when cast-related plastic changes weakened the motor system activity, the amount of inhibition necessary to block the movement was lower and, consequently, the inhibitory-related ERP components appeared reduced. These findings shed new light on the understanding of motor plasticity, suggesting that the limb non-use modifies the activity of the motor system *in toto*, not only when movements need to be programmed or performed (as already suggested by previous studies, e.g. Avanzino et al., 2011; Burianova et al., 2016; Garbarini et al., 2019; Kaneko et al., 2003; Lundbye-Jensen and Nielsen, 2008; Weibull et al., 2011), but also when movements are needed to be suppressed. Furthermore, from a clinical perspective, the present paradigm can be used in pathological conditions showing motor impairments, in order to establish a measure of overall preserved motor control. The electrophysiological components evoked by motor inhibition tasks may serve as a predictor for positive (or negative) outcome in patients with motor deficits (e.g. after stroke) during rehabilitation programs (Ehlers et al., 2015), similarly to other electrophysiological markers providing reliable diagnostic tools in different domains as provided by other studies in different domains (Daltrozzo et al., 2007; Morlet and Fischer, 2014).

## Declaration of competing interest

The authors declare no conflict of interest.

## CRediT authorship contribution statement

**Valentina Bruno:** Conceptualization, Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Irene Ronga:** Data curation, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Carlotta Fossataro:** Data curation, Writing - review & editing. **Mattia Galigani:** Data curation, Formal analysis, Writing - review & editing. **Katiuscia Sacco:** Conceptualization, Writing - review & editing. **Francesca Garbarini:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

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## Appendix A. Supplementary data

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## References


Avanzino, L., Bassolino, M., Pozzo, T., Bove, M., 2011. Use-dependent hemispheric balance. J. Neurosci. 31, 3423–3428. https://doi.org/10.1523/jneurosci.4893-10.2011.

Avanzino, L., Pelosin, E., Abbruzzese, G., Bassolino, M., Pozzo, T., Bove, M., 2014. Shaping motor cortex plasticity through proprioception. Cerebr. Cortex 24, 2807–2814. https://doi.org/10.1093/cercor/bht139.

Barry, R.J., De Blasio, F., Rushby, J.A., Clarke, A.R., 2010. Brain dynamics in the auditory Go/NoGo task as a function of EEG frequency. Int. J. Psychophysiol. 78, 115–128. https://doi.org/10.1016/J.IJPSYCHO.2010.06.357.

Bassolino, M., Campanella, M., Bove, M., Pozzo, T., Fadiga, L., 2014. Training the motor cortex by observing the actions of others during immobilization. Cerebr. Cortex 24, 3268–3276. https://doi.org/10.1093/cercor/bht190.

Benikos, N., Johnstone, S.J., Roodenrys, S.J., 2013. Varying task difficulty in the Go/Nogo task: the effects of inhibitory control, arousal, and perceived effort on ERP components. Int. J. Psychophysiol. 87, 262–272. https://doi.org/10.1016/j.ijpsycho.2012.08.005.

Bokura, H., Yamaguchi, S., Kobayashi, S., 2001. Electrophysiological correlates for response inhibition in a Go/NoGo task. Clin. Neurophysiol. 112, 2224–2232. https://doi.org/10.1016/S1388-2457(01)00691-5.

Bozzacchi, C., Giusti, M.A., Pitzalis, S., Spinelli, D., Di Russo, F., 2012. Awareness affects motor planning for goal-oriented actions. Biol. Psychol. 89, 503–514. https://doi.org/10.1016/j.biopsycho.2011.12.020.

Brasil-Neto, J.P., Cohen, L.G., Panizza, M., Nilsson, J., Roth, B.J., Hallett, M., 1992. Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. J. Clin. Neurophysiol. 9, 132–136.

Bruin, K.J., Wijers, A.A., Van Staveren, A.S.J., 2001. Response priming in a go/nogo task: do we have to explain the go/nogo N2 effect in terms of response activation instead of inhibition? Clin. Neurophysiol. 112, 1660–1671. https://doi.org/10.1016/S1388-2457(01)00601-0.

Bruno, V., Fossataro, C., Bolognini, N., Zigiotto, L., Vallar, G., Berti, A., Garbarini, F., 2017a. The role of premotor and parietal cortex during monitoring of involuntary movement: a combined TMS and tDCS study. Cortex 96, 83–94. https://doi.org/10.1016/j.cortex.2017.09.001.

Bruno, V., Fossataro, C., Garbarini, F., 2018. Inhibition or facilitation? Modulation of corticospinal excitability during motor imagery. Neuropsychologia 111, 360–368. https://doi.org/10.1016/j.neuropsychologia.2018.02.020.

Bruno, V., Fossataro, C., Garbarini, F., 2017b. Report of seizure induced by 10 Hz rTMS over M1. Brain Stimul 11, 454–455. https://doi.org/10.1016/j.brs.2017.11.003.

Bruno, V., Ronga, I., Fossataro, C., Capozzi, F., Garbarini, F., 2019. Suppressing movements with phantom limbs and existing limbs evokes comparable electrophysiological inhibitory responses. Cortex 117, 64–76. https://doi.org/10.1016/j.cortex.2019.02.024.

Bucchioni, G., Fossataro, C., Cavallo, A., Mouras, H., Neppi-Modona, M., Garbarini, F., 2016. Empathy or ownership? Evidence from corticospinal excitability during pain observation. J. Cognit. Neurosci. 28, 1760–1771. https://doi.org/10.1162/jocn_a_01003.

Burianova, H., Sowman, P.F., Marstaller, L., Rich, A.N., Williams, M.A., Savage, G., Al-Janabi, S., De Lissa, P., Johnson, B.W., 2016. Adaptive motor imagery: a multimodal study of immobilization-induced brain plasticity. Cerebr. Cortex 26, 1072–1080. https://doi.org/10.1093/cercor/bhu287.

Burin, D., Garbarini, F., Bruno, V., Fossataro, C., Destefanis, C., Berti, A., Pia, L., 2017. Movements and body ownership: evidence from the rubber hand illusion after mechanical limb immobilization. Neuropsychologia 107, 41–47. https://doi.org/10.1016/j.neuropsychologia.2017.11.004.

Cohen, M.X., 2014. A neural microcircuit for cognitive conflict detection and signaling. Trends Neurosci. 37, 480–490. https://doi.org/10.1016/j.tins.2014.06.004.

Cojan, Y., Archimi, A., Cheseaux, N., Waber, L., Vuilleumier, P., 2013. Time-course of motor inhibition during hypnotic paralysis: EEG topographical and source analysis. Cortex 49, 423–436. https://doi.org/10.1016/j.cortex.2012.09.013.

Daltrozzo, J., Wioland, N., Mutschler, V., Kotchoubey, B., 2007. Predicting coma and other low responsive patients outcome using event-related brain potentials: a meta-analysis. Clin. Neurophysiol. 118, 606–614. https://doi.org/10.1016/j.clinph.2006.11.019.

de Vega, M., Morera, Y., León, I., Beltrán, D., Casado, P., Martín-Loeches, M., Vega, M. de, Morera, Y., León, I., Beltrán, D., Casado, P., Martín-Loeches, M., 2016. Sentential negation might share neurophysiological mechanisms with action inhibition. Evid. Front. Theta Rhythm 36, 6002–6010.

Donchin, E., Ritter, W., McCallum, W.C., 2014. Cognitive psychophysiology: the endogenous components of the ERP. In: Event-Related Brain Potentials in Man. Elsevier, pp. 349–411. https://doi.org/10.1016/b978-0-12-155150-6.50019-5.

Donders, F.C., 1969. On the speed of mental processes. Acta Psychol. (Amst). 30, 412–431. https://doi.org/10.1016/0001-6918(69)90065-1.

Ehlers, M.R., López Herrero, C., Kastrup, A., Hildebrandt, H., 2015. The P300 in middle cerebral artery strokes or hemorrhages: outcome predictions and source localization. Clin. Neurophysiol. 126, 1532–1538. https://doi.org/10.1016/j.clinph.2014.10.151.

Facchini, S., Romani, M., Tinazzi, M., Aglioti, S.M., 2002. Time-related changes of excitability of the human motor system contingent upon immobilisation of the ring and little fingers. Clin. Neurophysiol. 113, 367–375. https://doi.org/10.1016/S1388-2457(02)00009-3.

Falkenstein, M., Hoormann, J., Christ, S., Hohnsbein, J., 2000. ERP components on reaction errors and their functional significance: a tutorial. Biol. Psychol. 51, 87–107. https://doi.org/10.1016/S0301-0511(99)00031-9.

Falkenstein, M., Koshlykova, N.A., Kiroj, V.N., Hoormann, J., Hohnsbein, J., 1995. Late ERP components in visual and auditory Go/Nogo tasks. Electroencephalogr. Clin. Neurophysiol. Evoked Potentials 96, 36–43. https://doi.org/10.1016/0013-4694(94)00182-K.

Fornia, L., Puglisi, G., Leonetti, A., Bello, L., Berti, A., Cerri, G., Garbarini, F., 2020. Direct electrical stimulation of the premotor cortex shuts down awareness of voluntary actions. Nat. Commun. 11, 705. https://doi.org/10.1038/s41467-020-14517-4.

Fortuna, M., Teixeira, S., Machado, S., Velasques, B., Bittencourt, J., Peressutti, C., Budde, H., Cagy, M., Nardi, A.E., Piedade, R., Ribeiro, P., Arias-Carrión, O., 2013. Cortical reorganization after hand immobilization: the beta qEEG spectral coherence evidences. PloS One 8, e79912. https://doi.org/10.1371/journal.pone.0079912.

Fossataro, C., Bruno, V., Giurgola, S., Bolognini, N., Garbarini, F., Bolognini, Nadia, Garbarini, F., 2018a. Losing my hand. Body ownership attenuation after virtual lesion of the primary motor cortex. Eur. J. Neurosci. 48, 2272–2287. https://doi.org/10.1111/ejn.14116.

Fossataro, C., Bucchioni, G., D'Agata, F., Bruno, V., Morese, R., Krystkowiak, P., Garbarini, F., 2018b. Anxiety-dependent modulation of motor responses to pain expectancy. Soc. Cognit. Affect Neurosci. 13, 321–330. https://doi.org/10.1093/scan/nsx146.

Friedman, D., Cycowicz, Y.M., Gaeta, H., 2001. The novelty P3: an event-related brain potential (ERP) sign of the brain's evaluation of novelty. Neurosci. Biobehav. Rev. https://doi.org/10.1016/S0149-7634(01)00019-7.

Galdo-Alvarez, S., Bonilla, F.M., González-Villar, A.J., Carrillo-de-la-Peña, M.T., 2016. Functional equivalence of imagined vs. Real performance of an inhibitory task: an EEG/ERP study. Front. Hum. Neurosci. 10, 1–12. https://doi.org/10.3389/fnhum.2016.00467.

Garbarini, F., Cecchetti, L., Bruno, V., Mastropasqua, A., Fossataro, C., Massazza, G., Sacco, K., Valentini, M.C., Ricciardi, E., Berti, A., 2019. To move or not to move? Functional role of ventral premotor cortex in motor monitoring during limb immobilization. Cerebr. Cortex 29, 273–282. https://doi.org/10.1093/cercor/bhy134.

Gow, R.V., Rubia, K., Taylor, E., Vallée-Tourangeau, F., Matsudaira, T., Ibrahimovic, A., Sumich, A., 2012. Abnormal centroparietal ERP response in predominantly medication-naive adolescent boys with ADHD during both response inhibition and execution. J. Clin. Neurophysiol. 29, 181–189. https://doi.org/10.1097/WNP.0b013e31824e1025.

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L.G., Mall, V., Kaelin-Lang, A., Mima, T., Rossi, S., Thickbroom, G.W., Rossini, P.M., Ziemann, U., Valls-Solé, J., Siebner, H.R., 2012. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. Clin. Neurophysiol. 123, 858–882. https://doi.org/10.1016/j.clinph.2012.01.010.

Harper, J., Malone, S.M., Bachman, M.D., Bernat, E.M., 2016. Stimulus sequence context differentially modulates inhibition-related theta and delta band activity in a go/no-go task. Psychophysiology 53. https://doi.org/10.1111/psyp.12604 n/a-n/a.

Harper, J., Malone, S.M., Bernat, E.M., 2014. Theta and delta band activity explain N2 and P3 ERP component activity in a go/no-go task. Clin. Neurophysiol. 125, 124–132. https://doi.org/10.1016/j.clinph.2013.06.025.

Harris, A.M., Dux, P.E., Mattingley, J.B., 2018. Detecting unattended stimuli depends on the phase of prestimulus neural oscillations. J. Neurosci. 38, 3092–3101. https://doi.org/10.1523/JNEUROSCI.3006-17.2018.

Herbsman, T., Forster, L., Molnar, C., Dougherty, R., Christie, D., Koola, J., Ramsey, D., Morgan, P.S., Bohning, D.E., George, M.S., Nahas, Z., 2009. Motor threshold in transcranial magnetic stimulation: the impact of white matter fiber orientation and skull-to-cortex distance. Hum. Brain Mapp. 30, 2044–2055. https://doi.org/10.1002/hbm.20649.

Huber, R., Ghilardi, M.F., Massimini, M., Ferrarelli, F., Riedner, B.A., Peterson, M.J., Tononi, G., 2006. Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat. Neurosci. 9, 1169–1176. https://doi.org/10.1038/nn1758.

Huster, R.J., Enriquez-Geppert, S., Lavallee, C.F., Falkenstein, M., Herrmann, C.S., 2013. Electroencephalography of response inhibition tasks: functional networks and cognitive contributions. Int. J. Psychophysiol. 87, 217–233. https://doi.org/10.1016/j.ijpsycho.2012.08.001.

Jeannerod, M., 2001. Neural simulation of action: a unifying mechanism for motor cognition. Neuroimage 14, S103–S109. https://doi.org/10.1006/nimg.2001.0832.

Jung, T.P., Makeig, S., Humphries, C., Lee, T.W., McKeown, M.J., Iragui, V., Sejnowski, T.J., 2000. Removing electroencephalographic artifacts by blind source separation. Psychophysiology 37, 163–178.

Kaneko, F., Murakami, T., Onari, K., Kurumadani, H., Kawaguchi, K., 2003. Decreased cortical excitability during motor imagery after disuse of an upper limb in humans. Clin. Neurophysiol. 114, 2397–2403. https://doi.org/10.1016/S1388-2457(03)00245-1.

Kirmizi-Alsan, E., Bayraktaroglu, Z., Gurvit, H., Keskin, Y.H., Emre, M., Demiralp, T., 2006. Comparative analysis of event-related potentials during Go/NoGo and CPT:

decomposition of electrophysiological markers of response inhibition and sustained attention. Brain Res. 1104, 114–128. https://doi.org/10.1016/J.BRAINRES.2006.03.010.

Kok, A., 1986. Effects of degradation of visual stimuli on components of the event-related potential (ERP) in go/nogo reaction tasks. Biol. Psychol. 23, 21–38. https://doi.org/10.1016/0301-0511(86)90087-6.

Langer, N., Hänggi, J., Müller, N.A., Simmen, H.P., Jäncke, L., 2012. Effects of limb immobilization on brain plasticity. Neurology 78, 182–188. https://doi.org/10.1212/WNL.0b013e31823fcd9c.

Lundbye-Jensen, J., Nielsen, J.B., 2008. Central nervous adaptations following 1 wk of wrist and hand immobilization. J. Appl. Physiol. 105, 139–151. https://doi.org/10.1152/japplphysiol.00687.2007.

Manaia, F., Teixeira, S., Velasques, B., Bittencourt, J., Salles, J.I., Arias-Carrión, O., Basile, L.F., Peressutti, C., de Carvalho, M.R., Cagy, M., Piedade, R., Ribeiro, P., Machado, S., 2013. Does immobilization of dependent hand promote adaptative changes in cerebral cortex? An analysis through qEEG asymmetry. Neurosci. Lett. 538, 20–25. https://doi.org/10.1016/j.neulet.2012.12.030.

Maris, E., Oostenveld, R., 2007. Nonparametric statistical testing of EEG- and MEG-data. J. Neurosci. Methods 164, 177–190. https://doi.org/10.1016/J.JNEUMETH.2007.03.024.

Mills, K.R., Boniface, S.J., Schubert, M., 1992. Magnetic brain stimulation with a double coil: the importance of coil orientation. Electroencephalogr. Clin. Neurophysiol. 85, 17–21.

Morlet, D., Fischer, C., 2014. MMN and novelty P3 in coma and other altered states of consciousness: a review. Brain Topogr. https://doi.org/10.1007/s10548-013-0335-5.

Mouraux, A., Iannetti, G.D., 2008. Across-trial averaging of event-related EEG responses and beyond. Magn. Reson. Imaging 26, 1041–1054. https://doi.org/10.1016/j.mri.2008.01.011.

Moyle, J.J., Fox, A.M., Bynevelt, M., Arthur, M., Burnett, J.R., 2006. Event-related potentials elicited during a visual Go-Nogo task in adults with phenylketonuria. Clin. Neurophysiol. 117, 2154–2160. https://doi.org/10.1016/j.clinph.2006.05.027.

Nakata, H., Inui, K., Wasaka, T., Akatsuka, K., Kakigi, R., 2005. Somato-motor inhibitory processing in humans: a study with MEG and ERP. Eur. J. Neurosci. 22, 1784–1792. https://doi.org/10.1111/j.1460-9568.2005.04368.x.

Nakata, H., Inui, K., Wasaka, T., Tamura, Y., Akatsuka, K., Kida, T., Kakigi, R., 2006a. Higher anticipated force required a stronger inhibitory process in go/nogo tasks. Clin. Neurophysiol. 117, 1669–1676. https://doi.org/10.1016/j.clinph.2006.03.032.

Nakata, H., Inui, K., Wasaka, T., Tamura, Y., Kida, T., Kakigi, R., 2006b. The characteristics of the nogo-N140 component in somatosensory go/nogo tasks. Neurosci. Lett. 397, 318–322. https://doi.org/10.1016/j.neulet.2005.12.041.

Nakata, H., Sakamoto, K., Honda, Y., Kakigi, R., 2015. Temporal dynamics of neural activity in motor execution and inhibition processing. Eur. J. Neurosci. 41, 1448–1458. https://doi.org/10.1111/ejn.12889.

Nakata, H., Sakamoto, K., Kakigi, R., 2010. Characteristics of No-go-P300 component during somatosensory Go/No-go paradigms. Neurosci. Lett. 478, 124–127. https://doi.org/10.1016/j.neulet.2010.05.002.

Ngomo, S., Leonard, G., Mercier, C., 2012. Influence of the amount of use on hand motor cortex representation: effects of immobilization and motor training. Neuroscience 220, 208–214. https://doi.org/10.1016/j.neuroscience.2012.06.018.

Nguyen, A.T., Moyle, J.J., Fox, A.M., 2016. N2 and P3 modulation during partial inhibition in a modified go/nogo task. Int. J. Psychophysiol. 107, 63–71. https://doi.org/10.1016/j.ijpsycho.2016.07.002.

Nigbur, R., Ivanova, G., Stürmer, B., 2011. Theta power as a marker for cognitive interference. Clin. Neurophysiol. 122, 2185–2194. https://doi.org/10.1016/j.clinph.2011.03.030.

Novembre, G., Pawar, V., Bufacchi, R., Kilintari, M., Srinivasan, M., Rothwell, J., Haggard, P., Iannetti, G., 2018. Saliency detection as a reactive process: sensory events evoke cortico-muscular coupling. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.2474-17.2017 (under Revis. 10.

Oldfield, R.C., 1971. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9, 97–113.

Opie, G.M., Evans, A., Ridding, M.C., Semmler, J.G., 2016. Short-term immobilisation influences use-dependent cortical plasticity and fine motor performance. Neuroscience 330, 247–256. https://doi.org/10.1016/j.neuroscience.2016.06.002.

Pfefferbaum, A., Ford, J.M., Weller, B.J., Kopell, B.S., 1985. ERPs to response production and inhibition. Electroencephalogr. Clin. Neurophysiol. 60, 423–434. https://doi.org/10.1016/0013-4694(85)91017-X.

Piedimonte, A., Garbarini, F., Rabuffetti, M., Pia, L., Berti, A., 2014. Executed and imagined bimanual movements: a study across different ages. Dev. Psychol. 50, 1073–1080. https://doi.org/10.1037/a0034482.

Polich, J., 2007. Updating P300: an integrative theory of P3a and P3b. Clin. Neurophysiol. https://doi.org/10.1016/j.clinph.2007.04.019.

Pritchard, W.S., 1981. Psychophysiology of P300. Psychol. Bull. 89, 506–540.

Pscherer, C., Mückschel, M., Summerer, L., Bluschke, A., Beste, C., 2019. On the relevance of EEG resting theta activity for the neurophysiological dynamics underlying motor inhibitory control. Hum. Brain Mapp. https://doi.org/10.1002/hbm.24699.

Randall, W.M., Smith, J.L., 2011. Conflict and inhibition in the cued-Go/NoGo task. Clin. Neurophysiol. 122, 2400–2407. https://doi.org/10.1016/j.clinph.2011.05.012.

Rockstroh, B., Muller, M., Cohen, R., Elbert, T., 1992. Probing the functional brain state during P300-evocation. J. Psychophysiol. 6, 175–184.

Ronga, I., Sarasso, P., Fossataro, C., Salatino, A., Garbarini, F., Ricci, R., Neppi-Modona, M., 2018. Everything is illuminated: prismatic adaptation lowers visual detection threshold in normal subjects. J. Exp. Psychol. Hum. Percept. Perform. 44, 1619–1628. https://doi.org/10.1037/xhp0000559.

Rosenkranz, K., Seibel, J., Kacar, A., Rothwell, J., 2014. Sensorimotor deprivation induces interdependent changes in excitability and plasticity of the human hand motor cortex. J. Neurosci. 34, 7375–7382. https://doi.org/10.1523/JNEUROSCI.5139-13.2014.

Rossi, S., Hallett, M., Rossini, P.M., Pascual-Leone, A., 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin. Neurophysiol. 120 https://doi.org/10.1016/j.clinph.2009.08.016, 2008–39.

Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M.D., Caruso, G., Cracco, R.Q., Dimitrijević, M.R., Hallett, M., Katayama, Y., Lücking, C.H., 1994. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr. Clin. Neurophysiol. 91, 79–92.

Sakamoto, K., Nakata, H., Yumoto, M., Sadato, N., Kakigi, R., 2015. Mastication accelerates Go/No-go decisional processing: an event-related potential study. Clin. Neurophysiol. 126, 2099–2107. https://doi.org/10.1016/j.clinph.2014.12.034.

Salisbury, D.F., Griggs, C.B., Shenton, M.E., McCarley, R.W., 2004. The NoGo P300 "anteriorization" effect and response inhibition. Clin. Neurophysiol. 115, 1550–1558. https://doi.org/10.1016/j.clinph.2004.01.028.

Salisbury, D.F., Rutherford, B., Shenton, M.E., McCarley, R.W., 2001. Button-pressing affects P300 amplitude and scalp topography. Clin. Neurophysiol. 112, 1676–1684. https://doi.org/10.1016/S1388-2457(01)00607-1.

Sasaki, K., Gemba, H., Nambu, A., Matsuzaki, R., 1993. No-go activity in the frontal association cortex of human subjects. Neurosci. Res. 18, 249–252. https://doi.org/10.1016/0168-0102(93)90062-U.

Sasaki, M., Yanagihara, T., Naitoh, N., Hata, T., 2010. Four-dimensional sonographic assessment of inter-twin contact late in the first trimester. Int. J. Gynecol. Obstet. 108, 104–107. https://doi.org/10.1016/j.ijgo.2009.09.025.

Schmajuk, M., Liotti, M., Busse, L., Woldorff, M.G., 2006. Electrophysiological activity underlying inhibitory control processes in normal adults. Neuropsychologia 44, 384–395. https://doi.org/10.1016/j.neuropsychologia.2005.06.005.

Smith, J.L., Johnstone, S.J., Barry, R.J., 2008. Movement-related potentials in the Go/NoGo task: the P3 reflects both cognitive and motor inhibition. Clin. Neurophysiol. 119, 704–714. https://doi.org/10.1016/j.clinph.2007.11.042.

van de Vijver, I., van Schie, H.T., Veling, H., van Dooren, R., Holland, R.W., 2018. Go/no-go training affects frontal midline theta and mu oscillations to passively observed food stimuli. Neuropsychologia 119, 280–291. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2018.08.023.

Veen, V. Van, Carter, C.S., 2002. The anterior cingulated as a conflict monitor: fMRI and ERP. Physiol. Behav. 77, 477–482. https://doi.org/10.1016/S0031-9384(02)00930-7.

Weibull, A., Flondell, M., Rosén, B., Björkman, A., 2011. Cerebral and clinical effects of short-term hand immobilisation. Eur. J. Neurosci. 33, 699–704. https://doi.org/10.1111/j.1460-9568.2010.07551.x.

Wessel, J.R., 2017. Prepotent motor activity and inhibitory control demands in different variants of the go/no-go paradigm. Psychophysiology. https://doi.org/10.1111/psyp.12871.

Wittenberg, G.F., Schaechter, J.D., 2009. The neural basis of constraint-induced movement therapy. Curr. Opin. Neurol. https://doi.org/10.1097/WCO.0b013e3283320229.

Woodward, S.H., Brown, W.S., Marsh, J.T., Dawson, M.E., 1991. Probing the time-course of the auditory oddball P3 with secondary reaction time. Psychophysiology 28, 609–618. https://doi.org/10.1111/j.1469-8986.1991.tb01003.x.

Young, M.E., Sutherland, S.C., McCoy, A.W., 2018. Optimal go/no-go ratios to maximize false alarms. Behav. Res. Methods 50, 1020–1029. https://doi.org/10.3758/s13428-017-0923-5.