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07 January 2023

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Valentina Bruno[a], Irene Ronga[a], Carlotta Fossataro[a], Francesca Capozzi[b], Francesca Garbarini[a*]

[a] MANIBUS Lab, Department of Psychology, University of Turin, Via Po 14, 10123, Turin Italy

[b] Department of Psychology, McGill University, 2001 McGill College Ave, Montreal, Canada

*Corresponding author:

Francesca Garbarini

Psychology Department, University of Turin

Via Po 14, 10123 Turin, Italy

Phone: +39 011 6703044

Fax: +39 011 8159039

E-mail: francesca.garbarini@unito.it

Abstract

Phantom limb is a common sensation in amputees, who often report vivid experiences of voluntarily moving their phantom. Previous studies showed that phantom movement can be functionally disentangled from imagined movement comparable to the actual movement of an intact limb. How and to what extent phantom movement and real movement share similar physiological mechanisms? Here, we focused on a specific aspect of motor control, the motor inhibition, and we asked whether inhibitory physiological responses are implemented when a phantom movement has to be suppressed. Sixteen two-handed controls and two left upper-limb amputees (with and without phantom movement) underwent a Go/Nogo paradigm, while event-related potentials (ERPs) were recorded. The task was performed with both the right (intact) and the left (phantom) hand, either in real or imagery conditions. Opposite results between the moving-phantom case and the static-phantom case were found. In the real condition, moving-phantom case showed the classical motor-inhibition related ERP pattern, with large P300 inhibitory wave when the movements of both (right) intact and (left) phantom limbs have to be suppressed. This inhibitory response was not different from that found in controls (who performed the task with an existing hand; real condition), but, crucially, it was significantly different from the imagery condition of controls. Contrariwise, in the static-phantom case, the ERP responses to Nogo trials during real condition were different from the real condition in controls but were not different from their imagery conditions. Importantly, in real condition, Nogo-ERP responses were significantly different between the two phantom cases. Taken together, these findings provide compelling evidence that phantom movements share the same neurophysiological correlates of real movements, not only when an action has to be executed, but also when it should be inhibited.

Keywords: phantom movement; motor inhibition; ERPs; phantom limb; motor imagery

1. Introduction

People with a body amputation commonly continue to perceive the presence of their missing limb, as if it was still present (Herta Flor, Nikolajsen, & Staehelin Jensen, 2006). Amputees report that their phantom limb has certain sensory properties like touch (Melzack, 2006) and up to 80% of these phantom sensations are experienced as painful (Kikkert et al., 2017; Weeks, Anderson-Barnes, & Tsao, 2010). Interestingly, some patients with phantom limbs claim they can voluntary move their phantom (Garbarini, Bisio, Biggio, Pia, & Bove, 2018; Raffin, Giraux, & Reilly, 2012; Raffin, Mattout, Reilly, & Giraux, 2012; Ramachandran & Hirstein, 1998). They experience sensations of reaching out to grab an object, making a fist, or moving their fingers individually (Ramachandran & Hirstein, 1998). It has been described that phantom movement involves some aspects of "real" motor execution. For example, voluntary movements of the phantom hand trigger characteristic patterns of distal movements in the residual limb muscle activity, which differed from the activity recorded in the same muscle groups during proximal movements, suggesting that the brain's motor areas can be differentially activated according to the movement the patients intend to perform with their phantom (Reilly, Mercier, Schieber, & Sirigu, 2006). Furthermore, an ischemic nerve block applied to the residual limb abolished not only electromyographic (EMG) signal of the residual limb, but also the ability to voluntarily move the phantom (Reilly et al., 2006).

Many neuroimaging studies, examining the cerebral activity associated with phantom limb movements (Hugdahl et al., 2001; Lotze, Flor, Grodd, Larbig, & Birbaumer, 2001; Roux et al., 2003), showed a maintained representation of the missing hand in the primary sensorimotor missing hand cortex (Kikkert, Johansen-Berg, Tracey, & Makin, 2018; Makin et al., 2013) and an increased activity in motor areas, such as the contralateral primary motor cortex (M1) and the supplementary motor area (SMA), in a similar way as real/actual movements do (Raffin, Giraux, et al., 2012; Reilly et al., 2006). Even using a different neuroimaging technique, such as the electroencephalography (EEG) time-frequency analysis, it

has been shown that, in a case with congenital absence of arm, similar sensorimotor rhythms were present in both real movements and phantom movements (Walsh, Long, & Haggard, 2015). These neuroimaging findings suggest that, when amputees "perform" voluntary movements with their phantom limb, they recruit a similar neurophysiological brain activity as that employed during the actual movements of an existing limb.

Importantly, amputees, when asked to perform real or imagined movements with their phantom, report different perceptions and sensations during each task. Some studies tried to disentangle motor imagery from motor execution both with behavioral (Garbarini et al., 2018; Raffin, Giraux, et al., 2012) and neuroimaging measures (Hugdahl et al., 2001; Lotze et al., 2001; Raffin, Mattout, et al., 2012; Roux et al., 2003). From a behavioral point of view, it has been observed that motor execution with the phantom is slower than with the intact limb, whereas the time to imagine a movement is similar for both the phantom and intact limbs, suggesting that amputees limb loss differentially affects these two tasks (Raffin, Giraux, et al., 2012). In a recent study (Garbarini et al., 2018), the intermanual transfer of sequence learning, occurring when one hand motor skills improve after training with the other hand, was used to dissociate motor execution from motor imagery with a phantom limb. It has been reported that only after an active training with the phantom limb, and not with an imagery training, the moving-phantom case showed a faster performance of the intact hand, as in two-handed controls actually performing the training with an existing hand (Garbarini et al., 2018). Neuroimaging studies on real and imagery phantom movements showed that two distinct brain networks are activated during motor execution (i.e. hand area of the contralateral M1, hand area of the contralateral primary somatosensory cortex – S1, contralateral dorsal premotor areas, bilateral medial premotor areas, ipsilateral anteromedial part of the cerebellum) and motor imagery (i.e. contralateral dorsal premotor areas, bilateral medial premotor areas, bilateral inferior areas) (Raffin, Mattout, et al., 2012). Interestingly, these two networks partially overlap (i.e. parts of SMA, the dorsal premotor cortex, the posterior parietal cortex and the

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48 cerebellum as well as M1), and they are similar to those activated during imagination and execution with

49 the intact limb (Hugdahl et al., 2001; Lotze et al., 2001; Raffin, Mattout, et al., 2012; Roux et al., 2003).

50 However, when considering effective connectivity between brain areas, it has been shown that, as in

51 healthy controls (Bruno, Fossataro, & Garbarini, 2018; Kasess et al., 2008; Solodkin, Hlustik, Chen, &

52 Small, 2004; Tak, Kempny, Friston, Leff, & Penny, 2015), real and imagery tasks with the phantom limb

53 had an opposite effect on the SMA–M1 network: while during motor execution, SMA exerts an

54 excitatory effect on M1, since the movement has to be performed; during motor imagery, SMA exerts an

55 inhibitory effect on M1, since the movement has to be suppressed (Raffin, Giraux, et al., 2012).

56 Taken together, these previous findings suggest that, when a movement has to be executed, real and

57 phantom limbs share common behavioral and physiological mechanisms. But, what happens when a

58 phantom movement has to be suppressed? Are inhibitory physiological responses fully implemented, as

59 during the suppression of a real movement? The ability to suppress, withhold, delay or interrupt ongoing

60 or planned actions is a fundamental aspect of motor control in everyday life. Thus, if controlling phantom

61 and real movements rely on common mechanisms, we should expect that suppressing movements with

62 existing and phantom limbs evokes comparable inhibitory responses. The most common task employed

63 in the study of motor inhibition is the Go/Nogo task. During this task, participants are required to respond

64 to frequent imperative stimuli and they must withhold the response to other infrequent alternatives

65 (Donders, 1969). Several electrophysiological studies on the Go/Nogo task described an enhanced

66 frontocentral negativity occurring around 140–300 ms, as well as an enhanced central positivity

67 occurring around 300–600 ms, following the presentation of a Nogo stimulus (Falkenstein, Hoormann,

68 Christ, & Hohnsbein, 2000; Falkenstein, Koshlykova, Kiroj, Hoormann, & Hohnsbein, 1995;

69 Pfefferbaum, Ford, Weller, & Kopell, 1985; Veen & Carter, 2002). These peaks are referred to as N200

70 and P300 respectively, and they have been interpreted as indexes of response inhibitory process in the

71 frontal lobe (Bokura, Yamaguchi, & Kobayashi, 2001; Cojan, Archimi, Cheseaux, Waber, &

72 Vuilleumier, 2013; Kok, 1986; Smith, Johnstone, & Barry, 2008).

73 The purpose of the present study was to investigate whether and to what extent phantom limbs share

74 similar physiological mechanisms with actual limbs, not only when a movement has to be performed,

75 but also when it has to be inhibited. To this aim, we recorded event-related potentials (ERPs) during a

76 Go/Nogo task in two upper-limb amputees who have already participated in a previous study (Garbarini

77 et al., 2018). Both the amputees reported vivid sensations on their phantom, but in different ways: one

78 reported the ability to move her phantom (moving-phantom case), the other reported vivid phantom limb

79 sensation without phantom movement, which, in turn, he perceived as paralyzed (static-phantom case).

80 See details in section 2.1. The ERPs collected in amputees were compared to those collected in two-

81 handed controls. All participants underwent two different conditions of the task: the real condition,

82 during which a real key press in response to Go trials was requested (either with the intact or with the

83 phantom hand) and an imagery condition, during which a mental simulation of the key press in response

84 to Go trials was requested (either with the intact or with the phantom hand). If a/the? phantom movement

85 has similar physiological properties as the real movement, we should expect that, during the Go/Nogo

86 task, the ERP pattern of the moving-phantom case will be comparable to that found in two-handed

87 controls, with the typical inhibitory ERP response associated to Nogo trials. In two-handed controls, the

88 inhibitory ERP response was expected to be greater in real condition than in imagery condition and,

89 coherently, a similar pattern was expected in the moving-phantom case. On the contrary, the static-

90 phantom case, who did not experience kinematical sensations with his phantom, was expected to use

91 motor imagery to perform the real Go/Nogo task with his phantom limb, thus showing a similar ERP

92 pattern as controls during the imagery task.

93

94 **2. Materials and methods**

2.1 Participants

Two left upper-limb amputees took part in this study. Both of them reported phantom limb sensations, but in different ways. The first phantom case was a 31 years old female, with 18 years of education. Her left upper limb was amputated under the shoulder as a consequence of arm paralysis due to brachial plexus injury after a car accident 11 years ago. Her phantom limb sensation started immediately after the accident and continues to persist, although it may vary daily depending on various factors (e.g., stress). At the time of testing, on a visual analog scale (VAS) aiming at assessing pain intensity on her phantom limb ranging from 0 (absolutely not painful) to 10 (absolutely painful), she reported a score of 0, indicating that she did not perceive painful sensation on her phantom (even if she reported phantom pain in the past, for about two years after the accident). She reported to be able to move her phantom at will, and her perceived movement control is limited to relatively simple movements (e.g., grasping, pressing, pointing). We named her "moving-phantom case". The second phantom case was a 53 years old male, with 13 years of education. He got the amputation of the left upper limb after an accident at work when he was 35. The amputation was made above the left elbow and he was not using any prosthesis. At the time of testing, he did not report painful sensations on his phantom (even if he reported phantom pain in the past), but sometimes he felt it as ticklish. He reported a score of 0 on the VAS about pain intensity on his phantom limb. He did not report the ability to move his phantom, which, in turn, described as rigid and contracted. We named him "static-phantom case". Sixteen two-handed volunteers (6 men; mean age ± sd: 23.9 ± 1.3 years; mean of educational years ± sd: 17.6 ± 0.8) were recruited as controls. All participants (i.e. the two phantom cases and controls) were right-handed, according to the Standard Handedness Inventory (Oldfield, 1971). Neither amputees nor controls had suffered a brain lesion or had a history of neurological or psychiatric illness. 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 gave approval to the project (prot. n. 125055, 12/07/16).

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2.2 Experimental design

Each participant underwent an EEG recording session while she/he performed a Go/Nogo task (see details in the next paragraph). During the same session, participants were asked to perform the task in both *real* and *imagery* condition. The only difference between the two conditions was that during the *imagery* condition, the subjects had to imagine the hands' movements (i.e. key press, see details in the next paragraph) with a kinesthetic motor imagery, (Bisio et al., 2017; Bruno et al., 2018; Jeannerod, 1995; Piedimonte, Garbarini, Rabuffetti, Pia, & Berti, 2014) instead of moving the hand as in *real* conditions.

2.3 Go/Nogo task and procedure

A similar version of the Go/Nogo task used by Cojan and colleagues, aiming at comparing motor inhibition mechanisms responsible for paralysis during hypnosis and those recruited by voluntary inhibition (Cojan et al., 2013) was used. 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. Visual stimuli were presented on the computer screen. Each stimulus represented the dorsal view of a hand (left or right) and it could be colored in 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). During the real condition, participants had to press the key as quickly as possible when the hand turned green (75%), and to withhold the prepared response if the hand turned red (25%). During the imagery condition, participants had to imagine to press the key when the hand turned green and to withhold the prepared (imagined) response if the hand turned red. After

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each imperative stimulus (Go or Nogo), the fixation cross re-appeared (Figure 1A). It is important to note that the task, both in real and imagery condition, required that both phantom-limb cases performed it with their left/phantom and their right/intact hand. While the moving-phantom case was able to perform the task with her phantom, the static-phantom case was asked to "try to perform" the task with his phantom. The presentation of stimuli was pseudorandomized, in a way that more than two sequential Nogo stimuli never appeared. Stimuli display and reaction times (RTs) collection were controlled by E-prime v.2 (Psychology Software Tools, http://www.pstnet.com). Before starting the experiment, all the participants (i.e. the two phantom-cases and controls) performed a short 10 trials practice to familiarize with the task. Six blocks of 40 trials (half right hand, half left hand) were performed per condition (i.e. real; imagery), resulting in a total of 480 stimuli: 90 real Go left, 90 real Go right, 30 real Nogo left, 30 real Nogo right, 90 imagery Go left, 90 imagery Go right, 30 imagery Nogo left, 30 imagery Nogo right. All the participants (i.e. the two cases and the two-handed controls) performed alternately a block of real and a block of imagery condition. Half of the controls started with a real block, the other half with an imagery one. The two phantom-cases started both with a real block (Figure 1B). Each block lasted about 6 minutes. A 2 minutes break was performed between each block.

--- Figure 1 about here ---

2.4 Electroencephalogram recording and processing

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

167 electrodes, one placed over the right lower eyelid and the other placed lateral to the outer canthus of the

168 right eye. Electrode impedances were kept below 5 kΩ. Signal was digitized at a sampling rate of 1,024

169 Hz (SD32; Micromed, Treviso, Italy). Data were continuously streamed to a laptop connected to a second

170 computer generating the stimuli. These two computers interfaced via a serial port for precise

171 synchronization.

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173 **3. Data analysis**

174 **Electrophysiological data**

175 ERPs were pre-processed and analyzed offline using Letswave v.6 (https://www.letswave.org/, Mouraux

176 & Iannetti, 2008). The analyses were performed on both Go and Nogo trials (stimulus-locked). Epochs

177 were selected from 500 ms prior to onset to 1000 ms after the onset of the imperative stimulus (total

178 epoch duration: 1500 ms). All the epochs were band-pass filtered (1–30 Hz) using a fast Fourier

179 transform filter. Each epoch was baseline corrected using the 500 ms pre-stimulus recording period as

180 reference. Artifacts due to eye blinks or eye movements were subtracted using a validated method based

181 on an Independent Component Analysis (Jung et al., 2000). Only correct responses to Go and Nogo

182 imperative stimuli were analyzed. Blinks were found to be the most frequent cause of rejection. Epochs

183 belonging to the same experimental condition were averaged time-locked to the onset of the stimulus.

184 Thus, separately for each hand (left/phantom hand, right/intact hand), for each condition (Go, Nogo) and

185 for each task (real, imagery), 8 average waveforms (Go real left/phantom, Go real left/phantom, Go

186 imagery left/phantom, Go imagery left/phantom, Nogo real left/phantom, Nogo real left/phantom, Nogo

187 imagery left/phantom, Nogo imagery left/phantom) were obtained for each subject.

188

189 *3.1. Group analysis*

We firstly performed a group analysis on the two-handed controls to investigate: i) if our task was able to reproduce the classical electrophysiological pattern associated with motor inhibition (Bokura et al., 2001); ii) if there were any differences in ERPs between real and imagery conditions. Mean ERP responses to Go and Nogo stimuli were analyzed by a point-by-point repeated-measure ANOVA with three within-subject factors: Condition (two levels: Go; Nogo), Side (two levels: left; right) and Task (two levels: real; imagery). Cluster-based permutation testing approach (1000 random permutations testing across all 32 channels) was employed 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 & Oostenveld, 2007). This yielded seven distinct waveforms, representing the significance of the main effect of each of the three factors across time, as well as their first order and second-order interactions. To further explore the interactions, we then performed for each contrast of interest two-tailed paired T-tests, by means of cluster-based permutation testing approach (1000 random permutations testing across all 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).

3.2 Single-subject analyses

Based on the results of the group-analysis in two-handed controls, showing a crucial difference between real condition and imagery condition in the amplitude of the Nogo-P300 wave, we focused our single-subject analyses of the phantom limb cases on this component. In each participant (i.e. including amputees and two-handed controls), the mean amplitude of Nogo-P300 peak at Fz was measured for each Nogo stimulus of the left/phantom and right/intact hand, during both real and imagery condition. P300 wave was defined as the most positive deflection following the onset of Nogo stimulus. In particular, the amplitude of P300 was computed for each individual subject in a 100 ms time window

centered on her/his average peak in Nogo trials (P300 range: 280–380 ms). Then, Nogo-P300 peaks were normalized in z-scores, calculated within-subject across all conditions (i.e. real, imagery, left, right). Z-scores peaks were used as dependent variables.

By means of SingleBayes_ES.EXE program (http://homepages.abdn.ac.uk/j.crawford/pages/dept/Single_Case_Effect_Sizes.htm) we compared, in separated analyses, P300 amplitudes of both experimental cases (moving-phantom and static-phantom) to the P300 amplitudes recorded in the control group. The program is specifically devised to test whether an individual's score is significantly different from a control or normative sample (Crawford, Garthwaite, and Porter 2010). It provides a point estimate of the effect size for the difference between the case and controls (Z-CC) with an accompanying 95% credible interval and it provides a point and interval estimate of the abnormality of the case's score (i.e. it estimates the percentage of the population that would obtain a lower score, together with a 95% credible interval on this percentage) (Crawford, Garthwaite, and Porter 2010). This test was used to perform four comparisons, in which the amplitude of the left Nogo-P300 of each phantom limb case, during the real task, was compared with that of controls, either during the real task or the imagery task. The α value was set at 5%, and Bonferroni correction for multiple comparisons was applied (p-value: 0.05/4=0.0125).

The above described Crawford's test was used also to investigate the presence of both a side effect and a task effect in the two cases. With respect to the side effect, to analyze potential differences between left (phantom) and right (intact) hand during Nogo trials of the real task, we computed for all the participants a delta (right minus left) where positive values indicate a greater P300 amplitude in response to right than left stimuli. The resulting delta of each phantom-case was compared with the same delta of controls. The α value was set at 5%, and Bonferroni correction for multiple comparisons was applied (p value: 0.05/2=0.025). With respect to the task effect, to analyze potential differences between real and imagery conditions in the left/phantom side, we computed for all the participants a delta (real minus imagery)

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where positive values indicate greater P300 amplitude in real ~~condition~~ than in imagery condition. The

resulting delta of each case was compared with the same delta of two-handed controls. The α value was

set at 5%, and Bonferroni correction for multiple comparisons was applied (p-value: 0.05/2=0.025).

Finally, the performance of the two cases was directly compared by means of the C_CTC.exe program

(http://homepages.abdn.ac.uk/j.crawford/pages/dept/Compare_Two_Cases.htm). This program uses

Bayesian statistical methods to draw inferences concerning the difference between the test scores of two

single cases by referring their scores to a control sample. The p-values for this test are used to determine

whether we can reject the null hypothesis that the difference between the two cases is an observation

from the distribution of differences between pairs of controls. Point and interval estimates of the effect

size for the difference (Z-PCC) between the two cases are reported (Crawford, Garthwaite, & Wood,

2010). This test was used to perform four comparisons, in which the two cases was compared on the

following variables: left Nogo-P300 real, left Nogo-P300 imagery, Nogo-P300 delta right-left, Nogo-

P300 delta real-imagery. It is to note that these programs are all implemented Bayesian statistics, which

is more adequately suited for supporting null results. See Supplementary materials for other analyses

including both Go and Nogo trials and both N200 and P300 waves.

Behavioral data

The statistical analyses on behavioral data were performed on mean RTs in response to Go stimuli. The

analyses were performed on all the RTs collected in response to right/intact hand stimuli during the real

task, because i) no behavioral data were collected for the two phantom-cases with respect to left/phantom

hand stimuli during the real task; ii) no behavioral data were collected during the imagery task. Analyses

were performed only on correct responses (i.e. incorrect responses corresponded to a wrong key press or

a delayed response). Trials with RTs faster than 150 ms or deviating more than 2 standard deviations

from individual mean RT were also excluded. As for electrophysiological data, Crawford's tests were used to compare each phantom-case with controls and the two phantom-cases between each other.

4. Results

Electrophysiological data

4.1 Group analysis

Grand average waveforms are depicted in Figure 2 and 3. To note, ERPs evoked by Nogo stimuli in real condition presented the typical neurophysiological markers of motor inhibition responses, with the emerge of N200/P300 complex, replicating previous studies using similar paradigms (Bokura et al., 2001). The point-by-point ANOVA revealed different significant effects. As expected, a significant main effect of Condition was found. At Fz, this factor was a significant source of variance within three different time intervals: 165-256 ms [coinciding with the latency of N200 ($F_{1,15}=49.8$, $p<0.0000038$)]; 294-415 ms [coinciding with the latency of P300 ($F_{1,15}=31.9$, $p<0.0005$)]; and 487-650 ms [coinciding with the latency of the negative shoulder following P300 wave ($F_{1,15}=28.3$, $p<0.0001$)], with greater responses after Nogo than in Go stimuli (Figure 2A). Importantly, although a similar pattern of activity was present during imagery condition, the point-by-point ANOVA showed a significant main effect of Task at Fz within these intervals: 258-345 ms [coinciding with the latency of P300 ($F_{1,15}=39.3$ $p=0.000028$)], 443-523 ms [coinciding with the latency of the negative shoulder of P300 ($F1,15=11.4$, $p=0.004$)], with significant lower amplitudes during imagery with respect to real task (Figure 2B). Furthermore, the ANOVA found a significant Condition*Task interaction. At Fz, this factor was a significant source of variance within two different time intervals: 126-215 ms [coinciding with the latency of N200 wave ($F_{1,15}=34.2$, $p<0.00032$)]; and 254-359 ms [coinciding with the latency of P300 wave ($F_{1,15}=40.1$, $p=0.000013$)]. Two-tailed paired T-tests revealed that larger Nogo-N200 - and Nogo-P300 were elicited in real with respect to the imagery task (p always <0.001) (Figure 3).

285   --- Figure 2 about here ---

286   --- Figure 3 about here ---

287

288   *4.2 Single-subject analyses*

289   Our main goal was to examine electrophysiological activity associated with the inhibition of motor action

290   in phantom limb syndrome with and without phantom movement. Voluntary motor inhibition was probed

291   by correct responses to Nogo stimuli in the normal condition, which required an interruption of the

292   prepared action. The waveforms in response to right/intact and left/phantom Nogo stimuli of the moving-

293   phantom case, controls, and static-phantom case are depicted in Figure 4A.

294   The moving-phantom case showed the typical neurophysiological pattern of motor inhibition (Bokura et

295   al., 2001), with the emerge of the classical ERP pattern not only in response to right/intact stimuli but,

296   crucially, also in response to left/phantom Nogo stimuli, in a way similar to two-handed controls. In

297   particular, when the amplitude of the left Nogo-P300 of the moving-phantom case, during the real task,

298   was compared with the left Nogo-P300 amplitude of the two-handed controls performing the real task,

299   Crawford's test did not find any significant differences (p=0.3) (Figure 4B). However, when it was

300   compared with the left Nogo-P300 amplitude of the two-handed controls performing the imagery task, a

301   significant difference was found (Z-CC=2.880; p=0.006); i.e. the Nogo-P300 amplitude was significantly

302   greater when the moving-phantom case performed the real task than when two-handed controls

303   performed the imagery task (see Figure 4B).

304   On the contrary, the static-phantom case showed the classical ERP pattern in response to Nogo stimuli

305   only when performing the task with the right/intact hand. In particular, when the amplitude of the left

306   Nogo-P300 of the static-phantom case, during the real task, was compared with the left Nogo-P300

307   amplitude of the controls performing the real task, Crawford's test found a significant difference (Z-

308   CC=-2.654; p=0.010), with significant greater Nogo-P300 amplitudes in two-handed controls than in the

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static-phantom case (Figure 4B). However, when it was compared with the left Nogo-P300 amplitude of the two-handed controls performing the imagery task, no significant difference was found (see Figure 4B), suggesting a similar performance between the imagery task of the two-handed controls and the real task of the static-phantom case (see Figure 4B). Coherently, when directly comparing P300 amplitude in response to left/phantom Nogo stimuli during the real task of the two cases, a significant difference was found (Z-PCC=2.488; p=0.0125), corresponding to a greater P300 in moving- than in static-phantom case (Figure 4B).

With respect to the side effect in the real task, the delta right-left did not differ between the moving-phantom case and controls (p=0.3), suggesting that similar P300 amplitudes were evoked irrespective of the side (Figure 4C). Conversely, the delta right-left of the static-phantom case was significantly different with respect to both controls (Z-CC=2.889; p=0.006) and moving-phantom case (Z-PCC=-2.553; p=0.011), with significantly higher delta values for the static-phantom case, suggesting a lower P300 amplitude in response to left than right Nogo stimuli (Figure 4C).

With respect to the task effect in the left/phantom side, we found that the delta real-imagery did not differ between the moving-phantom case and controls (p=0.3), indicating that they showed similarly different ERP patterns between the real than in the imagery tasks (Figure 4D). Contrariwise, the delta real-imagery of the static-phantom case was significantly different with respect to both controls (Z-CC=-2-211; p=0.024) and moving-phantom case (Z-PCC=2.084; p=0.027), with significant lower values for the static-phantom case, indicating a similar P300 amplitude evoked in the real and in the imagery task (Figure 4D) (P300 mean amplitude z-scores; *moving-phantom case*: real left/phantom= 0.43; delta real right/intact-left= -0.08; delta left/phantom real-imagery= 0.86; *controls*: real left/phantom= 0.29±*0.16*; imagery left/phantom= -0.29±0.25; delta real right/intact-left= 0.05±0.18; delta left/phantom real-imagery= 0.58±0.38; *static-phantom case*: real left/phantom=-0.14; delta real right/intact-left= 0.57; delta left/phantom real-imagery= -0.26).

333   See Supplementary materials for the results on the differential response between Nogo and Go stimuli

334   with respect to both N200 and P300.

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336   --- Figure 4 about here ---

337

338   **Behavioral data**

339   The analysis on RTs relative to the right/intact hand in response to Go trials showed no difference (p

340   always > 0.4) between each of the two cases with respect to controls (RTs; mean±sd: *moving-phantom*

341   *case*: 361.57; *controls*: 317.32±46.17), and no difference between the two cases (p=0.93), suggesting that

342   potential differences (e.g. in age or skills) between the two cases and controls did not affect the behavioral

343   performance during the Go/Nogo task. See Supplementary Figure 4 in Supplementary materials.

344

345   **5. Discussion**

346   In the present study, the phantom limb syndrome was investigated from an electrophysiological

347   perspective, aiming at describing a neural counterpart of the vivid experience, sometimes reported by

348   amputees, of being able to perform voluntary movements with their phantom. In particular, we focused

349   on a specific aspect of the motor control, the movement inhibition (i.e. the ability to suppress, withhold,

350   delay or interrupt ongoing or planned actions) and we asked what happens when a phantom movement

351   has to be suppressed. To this aim, we recorded ERPs during a Go/Nogo tasks, where, according to a

352   consolidated literature in healthy subjects (e.g. Kok 1986; Bokura et al. 2001; Smith et al. 2008), larger

353   inhibition-related ERP components were expected in EEG responses to Nogo stimuli, as compared to

354   EEG responses to Go stimuli. In our experimental design, the effects of "actual" (real key press during

355   Go trials) and mental (imagery key press) Go/Nogo task were investigated in two phantom cases (one

356   with and one without phantom movement) and in 16 healthy controls. First of all, we found that, in

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healthy controls, suppressing real and imagined movements produces a discriminative EEG pattern, with significantly greater inhibitory ERP responses in the former task relative to the latter. More crucial for the present study, in the moving-phantom case, suppressing "real" movements with the existing limb and the phantom limb evoked comparable inhibitory ERP responses to Nogo stimuli, which were clearly distinguishable from those evoked by imagined movements. On the contrary, in the static-phantom case, who did not experience kinematical sensations with his phantom, inhibitory ERP responses to Nogo stimuli during real and imagery tasks were indistinguishable.

Disentangling inhibitory responses during the suppression of real and imagined movements in two-handed controls

As expected, a main effect of Condition showed the classical EEG pattern for a Go/Nogo task, with greater amplitude of N200 and P300 components in Nogo trials than in Go trials, irrespective of Side (left/right) and Task (real-imagery). See Figure 2A. Furthermore, a Task effect suggested that, irrespective of Side and Condition (Go/Nogo), the amplitude of P300 component was greater in real than in imagery task. See Figure 2B. More interestingly, a Condition by Task interaction suggested that, irrespective of Side, the Nogo-Go differential response in N200 and P300 components was significantly greater in real than imagery task. See Figure 3.

With respect to N200 and P300, 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 N200 Nogo effect may reflect a non-motoric stage of inhibition, or recognition of the need for inhibition, while the Nogo P300 may overlap with a positive movement-related potential occurring specifically on trials where overt motor responses must be inhibited (Smith et al. 2008). More specifically, when comparing Go and Nogo stimuli, it has been reported that P300 component in response to Nogo stimuli has a larger amplitude

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(Bruin et al. 2001; Schmajuk et al. 2006; Nakata et al. 2010; Cojan et al. 2013) and a more anterior distribution (Smith et al. 2008; Barry et al. 2010) than that elicited by Go stimuli. 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. Woodward et al. 1991; Rockstroh et al. 1992; Smith et al. 2008; Randall and Smith 2011).

When ERP responses to Nogo stimuli were considered individually (as in the post hoc comparisons of the Task by Condition interaction), the amplitude of both N200 and P300 components was significantly larger in real than in imagery task (Figure 4, lower part). However, in the Task effect, the overall difference between real and imagery tasks was found only in P300 component (Figure 2B), suggesting that this component is more relevant in disentangling when a real or imagined movement has to be suppressed. In particular, as mentioned above spazio Go-P300 has been described as a motoric stage of inhibition, while Nogo-N200 as a non-motoric motoric stage that is more related to a general need for inhibition (e.g. Smith et al. 2008). Thus, the stronger result on P300 component suggest that real, more than imagined movements, reflect the motoric stage of inhibition; the weaker results on N200 component suggest that the more cognitive stage of inhibition can be less relevant to discriminate between real and imagined movements.

Our Task effect is in agreement with previous data on motor imagery (Burle, Vidal, & Bonnet, 2004; Galdo-Alvarez, Bonilla, González-Villar, & Carrillo-de-la-Peña, 2016; González-Villar, Bonilla, & Carrillo-de-la-Peña, 2016), showing that the overt and covert execution of the task seems to be sufficient to produce a discriminative pattern in the EEG activity. Although these previous studies reported that the imagined performance of a stop-signal task produces a pattern of brain electrical activity with diminished amplitude with respect to that associated with real performance of the task, they focused more on the similarities between real and imagery EEG pattern rather than on their differences, supporting the view of a functional equivalence of imagined and real performances (Jeannerod, 2001). On the one hand, our

results confirm this functional equivalence, showing a similar effectiveness of real and imagery task in inducing an ERP modulation during Go/Nogo paradigm; on the other hand, they clearly show that the EEG discriminative patter is greater during actual than covered execution of the task, with respect to P300 component.

Suppressing phantom movements induces inhibition-related ERP responses comparable to those induced by suppressing real movements and different from those induced by suppressing imagined movements

Crucially for the purpose of the present study, we compared inhibitory motor responses in the two phantom limb cases, with and without phantom movements. Based on the results we found in two-handed controls, we focused on the Nogo-P300 component, which is crucial in the distinction between real *vs.* imagined movement suppression. We found that, when the moving-phantom case has to "actually" perform the task, she showed the classical ERP pattern associated with motor inhibition, comparable to that found in healthy controls, who performed the task with two existing hands. On the contrary, the static-phantom case, who did not experience kinematical sensations with his phantom, showed this ERP response only for the (right) intact hand, while, for the (left) phantom hand, it was not present. Furthermore, in our results, phantom movement can be functionally disentangled from motor imagery, so that, as in healthy controls, ERP responses were dramatically different when the moving-phantom case was asked to "actually" perform the Go/Nogo task (i.e. to "press" the key with her phantom) or to imagine doing it.

Interestingly, single-subject analyses revealed a strong modulation on the P300 component, in which opposite results were found in the two cases. In moving-phantom case, the P300 modulation during real condition was comparable to that found in controls during the same condition, but, crucially, it was significantly different from that found during their imagery condition (see Figure 4B). Contrariwise, in the static-phantom case, the amplitude of the P300 component during real condition was significantly

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different from real condition of controls and it was comparable to their imagery conditions (see Figure 4B). Importantly, a significant difference was found when the P300 was compared between the two phantom cases during the real task (see Figure 4B). In addition, when comparing the differential ERP response to Nogo stimuli with the right/intact hand and the left/phantom hand during the real task, no difference emerged between the moving-phantom case and controls, suggesting that the moving-phantom case showed a comparable response when the movement has to be suppressed with her intact (right) hand and with her phantom (left) hand, similarly as two-handed controls (Figure 4C). On the contrary, the static-phantom case showed a significant difference with respect to both controls and moving-phantom case, with greater differential P300 between the right/intact hand and the left/phantom hand during the real task, suggesting the presence of genuinely motoric inhibition only when the movement has to be suppressed with his intact (right) hand and not when it has to be suppressed with his phantom (left) hand (Figure 4C). Interestingly, single-subject analyses on the delta real-imagery response show that, while the moving-phantom case shares with controls similar ERP pattern, the static-phantom case presents significant differences with respect to both controls and moving-phantom case (see Figure 4D). When the movement has to be suppressed with his phantom, the static-phantom case showed no difference during the real and the imagery task contrary to what showed by controls and moving-phantom case, which presented a clearly distinct ERP pattern between real execution and motor imagery with their left/phantom hand. We can speculate that, since the static-phantom case cannot "move" his phantom limb, then he could only imagine to move it, resulting therefore in a similar ERP pattern during the real and the imagery task.

According to previous behavioral (Garbarini et al., 2018; Raffin, Giraux, et al., 2012) and fMRI (Raffin, Mattout, et al., 2012) studies, these results strongly support the view that phantom movements and imagery movement are functionally disentangled, also extending this concept to the context of motor inhibition. Indeed, the present results suggest that a similar amount of inhibitory cerebral activity is

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needed to suppress real and phantom movements, while the amount of inhibition necessary to block the imagined movement is lower and, consequently, the inhibitory-related ERP components appear reduced. Thus, only in moving-phantom case, who experienced the ability to voluntarily move her phantom, inhibitory responses are fully implemented during the real task, when frontal areas exert, via premotor cortex and/or SMA, an inhibitory control over the primary motor cortex. These results can contribute to the debate about cortical reorganization (H. Flor et al., 1998; Herta Flor et al., 2006; Lotze et al., 2001; Ramachandran, Rogers-Ramachandran, & Stewart, 1992) and maintained cortical representation (Kikkert et al., 2016, 2018; Makin et al., 2013) in phantom limb syndrome. The cortical reorganization model (H. Flor et al., 1998; Herta Flor et al., 2006; Lotze et al., 2001; Ramachandran et al., 1992) suggests that the deprivation of sensory inputs in the S1 missing hand cortex leads to cortical reorganization, where the deprived cortex becomes responsive to inputs from its cortical neighbors. Specifically, it has been showed that referred sensations in the phantom (that is, painful and non-painful phantom sensations that can be elicited by stimulating body areas adjacent to but also far from the amputated limb) are a perceptual correlate of reorganizational processes in the S1 cortex (Ramachandran et al., 1992). However, other studies showed instead that multiple factors interact to maintain local structural and functional representations (Makin et al., 2013), with a maintained representation of the phantom hand in the S1 cortex following amputation (Bogdanov, Smith, & Frey, 2012; Raffin, Mattout, et al., 2012) and comparable patterns of movement-related activity when amputees moved their phantom hand compared with two-handed controls moving their non-dominant hand (Makin et al., 2013). Our results seem to support the maintained cortical organization hypothesis, at least in the moving-phantom case, who showed an ERP pattern of motor inhibition with her phantom limb comparable to that found in two-handed controls. In particular, in agreement with previous studies, our results suggest that the representation of the amputated limb is functionally active, just like a real hand.

476 Although this study was performed only on two phantom limb cases, these findings provide a compelling

477 evidence that phantom movements share the same neurophysiological correlates of real movements, not

478 only when an action has to be executed, but also when it should be inhibited.

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480 **Acknowledgments**

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484 **Declarations of interest**: none.

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Figure 1. A. Experimental task. Stimuli were visual, and they represented the dorsal view of a right or a left hand. Gray hands: preparation cue; green hands: Go signal; red hands: Nogo signal. **B. Experimental procedure.** Each participant (i.e. controls; moving-phantom case and static-phantom case) underwent an EEG session during which she/he performed the Go/Nogo task. Each EEG session comprised both blocks of real and imagery task.

Figure 2. Group analysis results: main effects of Condition and Task. A) Main effect of Condition. Mean ERPs in response to Go and Nogo signals, irrespective of the side (left, right) and of the task (real; imagery). Data are displayed in microvolts as a function of time post-cue onset, for Fz electrode (referenced to the nose). The green waveform represents Go stimuli and the red waveform represents Nogo stimuli. Point-by-point F value is represented below. Time intervals where the difference between conditions was significant are highlighted in gray and the corresponding scalp topographies are shown. **B) Main effect of Task.** Mean ERPs in response to real and imagery task, irrespective of the side (left, right) and the condition (Go; Nogo). Data are displayed in microvolts as a function of time post-cue onset, for Fz electrode (referenced to the nose). The solid gray waveform represents ERPs in response to the real task and the dashed gray waveform represents ERPs in response to the imagery task. Point-by-point F value is represented below. Time intervals where the difference between the tasks was significant are highlighted in gray and the corresponding scalp topographies are shown.

Figure 3. Group analysis results: Condition*Task interaction. Mean ERPs in response to Go real, Go imagery, Nogo real and Nogo imagery, irrespective of the side (left, right). Data are displayed in microvolts as a function of time post-cue onset, for Fz electrode (referenced to the nose). The solid green waveform represents Go stimuli during the real task, the dashed green waveform represents Go stimuli

during the imagery task, the solid red waveform represents Nogo stimuli during the real task, the dashed red waveform represents Nogo stimuli during the imagery task. Point-by-point F value is represented below. Time intervals where the difference between conditions was significant are highlighted in gray and the corresponding scalp topographies are shown. Two-tailed paired T tests results of the contrast of interest (Nogo real vs Go real; Nogo imagery vs Nogo imagery; Nogo real vs Nogo imagery; Go real vs Go imagery) are displayed below the F value. Time intervals during which the contrasts of interest were significantly different are colored in blue and pink, such as the topographical distribution of T values reflecting the statistical comparison.

Figure 4. A. ERP waveforms of the moving-phantom case, controls and the static-phantom case. Mean ERPs of the moving-phantom case (left), controls (center) and static-phantom case (right) Nogo stimuli, both with the left/phantom (upper part) and with the right/intact (lower part) limb, during the real task (solid lines) and during imagery task (dashed lines). Data are displayed in microvolts as a function of time post-cue onset, for Fz electrode (referenced to the nose). **B. Single-subject analyses results.** Mean z-scores of the P300 amplitude in response to Nogo stimuli of the moving-phantom case (left), controls (center) and the static-phantom case (right). **C. Side effect:** Mean z-scores of the P300 amplitude of the delta right/intact-left/phantom of the moving-phantom case (left), controls (center) and the static-phantom case (right) performing the real task. **D. Task effect:** Mean z-scores of the P300 amplitude of the delta real-imagery of the moving-phantom case (left), controls (center) and the static-phantom case (right) in response to Nogo stimuli requiring the suppression of left/phantom movements. - *$p \leq 0.05$, **$p<0.01$, ***$p<0.001$. Error bars represent standard error of the mean of two-handed controls.