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Movements and body ownership: evidence from the rubber hand illusion after mechanical limb immobilization

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Abstract

There is no consensus on whether, and to what extent, actions contribute to constructing awareness of one's own body. Here we investigated at both physiological and behavioral level whether a prolonged limb immobilization affects body ownership. We tested a group of healthy participants, whose left-hand movements were prevented by a cast for one week, and a control group without any movement restriction. In both groups, we measured the strength of the rubber hand illusion (i.e., proprioceptive shift and questionnaire on ownership) and the physiological parameters known to be modulated by short-term arm immobilization (i.e., resting motor threshold, motor evoked potentials and force parameters) before and after the week of immobilization. Our results showed stronger illusory effects on the immobilized hand on both behavioral indexes and weaker illusory effects on the non-immobilized hand on the questionnaire. Additionally, the increased proprioceptive shift was positively correlated to the motor threshold of the contralateral hemisphere. Our findings show at both behavioral and physiological level that altering those movement-related signals which constantly stem from our own body parts, modulates the experience of those body parts as mine. This, in turn, supports the view of a direct role of actions in the developing and maintaining a coherent body ownership.

1. Introduction

Converging evidence in cognitive neuroscience shows that the sense of body ownership (i.e., the conscious experience of the body as one's own; Blanke, Slater, & Serino, 2015; Gallagher, 2000) is a plastic neurocognitive construct. Indeed, it can be selectively altered by stroke-induced brain damages (Bottini, et al., 2009; Fossataro, Gindri, Mezzanato, Pia, & Garbarini, 2016; Gandola, et al., 2012; Jenkinson, Haggard, Ferreira, & Fotopoulou, 2013; Pia, Cavallo, & Garbarini, 2014; Pia, Garbarini, Fossataro, Burin, & Berti, 2016; Pia, Spinazzola, et al., 2014; Piedimonte, Garbarini, Pia, Mezzanato, & Berti, 2016; Vallar & Ronchi, 2009) or by ad-hoc experimental manipulations in healthy participants (Burin, Pyasik, Salatino, & Pia, 2017; Costantini & Haggard, 2007; Lenggenhager, Tadi, Metzinger, & Blanke, 2007; Longo, Schuur, Kammers, Tsakiris, & Haggard, 2008; Slater, Spanlang, Sanchez-Vives, & Blanke, 2010; Tsakiris, Tajadura-Jimenez, & Costantini, 2011). In this latter case, a well-established paradigm is the Rubber Hand Illusion (hereinafter RHI) first reported by Botvinick and Cohen (Botvinick & Cohen, 1998). In this experimental manipulation, the participants' left (or right) hand is positioned on a table, out of view. Then, a rubber hand is located medially to the participant's hand. When the experimenter strokes for a few minutes both hands in synchrony (but not in asynchrony), participants experience a feeling of ownership over the rubber hand. Such phenomenon is demonstrated both behaviorally (i.e., mislocalization of the own hand toward the fake hand and/or subjective rating of ownership) and physiologically (e.g., skin conductance responses: Armel & Ramachandran, 2003; Ehrsson, Wiech, Weiskopf, Dolan, & Passingham, 2007; homeostatic regulation: Moseley, et al., 2008; motor evoked potentials: Della Gatta, et al., 2016).

The RHI is thought to arise because the initial conflict between vision of the rubber hand and tactile/proprioceptive representation of the own hand is resolved by the embodiment of the rubber hand within the participant's own body representation (Botvinick, 2004; Makin, Holmes, & Ehrsson, 2008). This, in turn, suggests that a key property for the emergence of a coherent sense of body ownership is the integration of multisensory signals that constantly reach the human body (i.e.

visual, tactile and proprioceptive stimuli). Nonetheless, multisensory integration occurs not only in static conditions but also during actions. Indeed, we experience that our body belongs to us also when we move ("I know that this moving hand is mine"). Under these circumstances, further signals add to touch, vision and proprioception such as those coming from skin/joint receptors and muscles spindles. Moreover, during willed actions also centrally generated motor commands (i.e., efferent signals) are produced which, in turn, give rise to a forward model of sensory predictions. In order to examine whether these extra signals contribute to the construction of the feeling of body ownership, some studies modified the original RHI paradigm comparing passive and/or active movements conditions with static conditions (Dummer, Picot-Annand, Neal, & Moore, 2009; Kalckert & Ehrsson, 2012, 2014; Kammers, de Vignemont, Verhagen, & Dijkerman, 2009; Longo & Haggard, 2009; Riemer, Kleinbohl, Holzl, & Trojan, 2013; Tsakiris, Prabhu, & Haggard, 2006; Walsh, Moseley, Taylor, & Gandevia, 2011). Overall, this literature reported contrasting findings with respect to whether or not movement-related signals modulate the sense of body ownership. Indeed, some of them demonstrated that body ownership decreases when we move (Walsh, et al., 2011), others found no differences in terms of body ownership between movement and no-movement conditions (Kalckert & Ehrsson, 2012, 2014), and others more that it increases (Dummer, et al., 2009; Riemer, et al., 2013; Tsakiris, et al., 2006).

At present, whether and to what extent actions contribute to the emergence of body ownership is still a matter of debate. In order to answer this question, here we investigated at both behavioral and physiological level whether body ownership is affected by preventing the possibility of making movements. Specifically, we examined whether a prolonged immobilization of one arm, obtained by means of a cast, alters the RHI. First, we checked whether mere seeing the cast and/or just experiencing a sudden movement restriction affects body ownership. Hence, we compared RHI measures before (hereinafter T0) and immediately after (hereinafter T1) having positioned the cast. In the same first session (at T0), we also recorded motor-related physiological measures (resting motor threshold, motor evoked potentials and force parameters) known to represent plastic reactions to immobilization (Avanzino, Bassolino, Pozzo, & Bove, 2011; Avanzino, et al., 2014; Burianova, et al., 2016; Facchini, Romani, Tinazzi, & Aglioti, 2002; Furlan, Conforto, Cohen, & Sterr, 2016; Kaneko, et al., 2003; Zanette, Manganotti, Fiaschi, & Tamburin, 2004). Secondly, RHI measures obtained immediately after the immobilization (T1) were compared with those after one week of complete immobilization, as well as physiological parameters (hereinafter T2). We hypothesized that if motor-related signals contribute to the emergence of body ownership, the immobilized hand should display an increased strength of the RHI as well as a correlation with physiological parameters subserving immobilization. Additionally, since it is known that unilateral motor deficits can induce an overuse of the healthy arm in order to keep on accomplishing habitual actions (Sato, Honda, Iwamoto, Kanoko, & Satoh, 2005; Sato, Kaji, Tsuru, & Oizumi, 1999) and since this increases corticospinal excitability (Avanzino, et al., 2011), we hypothesized a decreased strength of the RHI effects (i.e., a more rigid sense of body ownership) for the non-immobilized hand.

2. Materials and Methods

2.1 Participants

Forty right-handed (Oldfield, 1971) healthy participants (25 females, mean age= 22.4 years, SD= 2.9 years; mean educational level= 16.4 years, SD= 2.1 years) with no previous neurological/orthopedic diseases gave their written informed consent to participate in the study approved by the Bioethical Committee of University of Turin. In addition, we were able to assess a subset of fourteen participants for physiological measures (see below). None of them had a history of neurological, psychiatric, other relevant medical problems or any contraindications to noninvasive brain stimulation, which was applied as indicated by international safety guidelines (Rossi, Hallett, Rossini, Pascual-Leone, & Safety of, 2009).

2.2 Procedures

A general overview is shown in Figure 1.

At T0, the RHI was administered for both hands and to both the Experimental (n=20) and the Control (n=20) groups (they did not differ in terms of age and educational level at independent samples t-test, p>.05). Additionally, seven subjects from the Experimental and seven from the Control group underwent also TMS and EMG recording to measure resting motor threshold (rMT), motor evoked potentials (MEPs) and force parameters (see Physiological measures for details).

At T1, conducted twenty-four hours later (to avoid learning effects due to the repetition of the RHI procedures), the participants' left (but not right) hand was immobilized by a cast. The cast consisted in a palmar thermoplastic splinting applied on the wrist (extended for 30-40 degrees), connecting the metacarpophalangeal joint (60-70 degrees flexed), the proximal interphalangeal, the distal interphalangeal (extended) and the thumb (abducted): the position of the cast was able to prevent all finger movements and wrist rotation (the elbow was free to move). Then, they underwent the RHI procedure once more on both hands. As soon as T1 was finished, the cast on participants of the Control group was removed, while subjects from the Experimental group kept it continuously (24 hours a day) for the following week.

At T2, seven days after T1, the RHI was administered to the Experimental Group while they still had the cast on the left hand (to avoid alterations due to even small movements), whereas the Control Group performed the RHI putting again a cast on the left wrist before the procedure. Finally, in all participants the cast was removed. Then, the same fourteen participants, who at T0 were tested with TMS and EMG, underwent again TMS and EMG recording, approximately two hours after the cast was removed, to register again their rMT, MEPs and force parameters.

2.3 Rubber hand illusion

We employed a black wooden box (60 cm x 40 cm x 20 cm) divided in two equal parts (30 cm x 30 cm x 20 cm each), of which one was hidden from the view. By means of two square holes (12 cm x 12 cm) on both the horizontal sides of the box, the participants' arm (left or right) was placed within the hidden part (palm was facing down and fingers pointing forward), whereas the rubber hand (left or right) was paced in the visible part (parallel and in the correspondence of the participants' shoulder). After familiarization with procedures and ratings, the experimenter placed a panel on the open part of the box in order to cover both hands. Then, he/she had to verbally report (six trials) the perceived position of the own index finger (pre-proprioceptive judgment) according to the numbers on a ruler, positioned on the top of the box (the ruler was randomly moved at each trial in order to avoid cues). After this, the panel was removed and participants were asked to always look at the index fake finger. Then the experimenter started to stroke the participants' index finger and the rubber hand index finger with two identical small brushes for 180 sec. In the synchronous condition, the two hands (i.e., hidden own hand and the visible fake hand), were stimulated simultaneously with the brush moving in the same direction, whereas in the asynchronous condition the stimulations were temporally and directionally incongruent.

After the stimulation, participants were asked to report the perceived horizontal position (again six trials) of the own index finger (post-proprioceptive judgment) and filling out a questionnaire about the subjective experience of the illusion (revisited from Botvinick & Cohen, 1998; Burin et al., 2015) composed of three statements capturing different aspects of the illusion and three related control statements (see **Appendix**). Participants were asked to rate their agreement/disagreement on a seven-point Likert scale with a range from "+3" (agree very strongly) to "−3" (disagree very strongly) where "0" corresponded to neither agreeing nor disagreeing. The very same procedure was replicated for both hands. This whole procedure was induced once per hand (left and right) in each session (T0, T1, T2). Variables stimulations (synchronous and a synchronous) and hand (left and right) were counterbalanced between subjects.

2.4 Physiological measures

Participants seated on a chair, with both hands on a pillow. They were instructed to rest and try to achieve full muscular relaxation. Transcranial Magnetic Stimulation (TMS) pulses were administered using a Magstim Rapid^2 stimulator (Magstim, Whitlan, Dyfed, Wales, UK) connected to a 70-mm figure-of-eight coil positioned over the left and right M1. The coil was held tangentially to the scalp with the handle pointing backwards and laterally 45° away from the mid-sagittal line, such that the flow induced by the second most effective phase of the biphasic pulse moved in a posterior anterior direction (Di Lazzaro, et al., 2001; Kammer, Beck, Thielscher, Laubis-Herrmann, & Topka, 2001). This orientation permits the lowest motor threshold, optimizing the stimulation (Brasil-Neto, Pascual-Leone, Valls-Sole, Cohen, & Hallett, 1992). The rMT was defined as the lowest stimulus intensity capable of evoking 5 out of 10 MEPs with at least 50 µV peak-to-peak amplitude (Rossini, et al., 2015). The rMT was measured at the beginning of session T0 and at the end of T2. During the recording session, the stimulation intensity was set at 120% of the rMT. MEPs were recorded from the first dorsal interosseous (FDI) muscle of participants' right and left hands. Electromyographic (EMG) activity was recorded by pairs of Ag–AgCl surface pre-gelled electrodes (11 mm diameter) (EL503) connected to a Biopac MP-150 electromyograph (Biopac Systems Inc., Santa Barbara, CA). They were placed in a classical belly-tendon montage: the active electrode over the muscle belly and the reference electrode over the associated joint or tendon. The ground was placed over the participants' left elbow. The EMG signals were acquired at 10 kHz sampling rate, amplified, filtered with a band-pass (10–500 Hz) and a notch (50 Hz) filter and stored on a PC for offline analysis. In addition, while seated on the chair, each participant was instructed to maintain a thumb/index finger opposition for 2.5 seconds. 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. We considered two values of the force parameters because they can be selectively affected by immobilization: Force recruitment (FR, from 0 to 0.5 seconds) corresponding to the very first phase of the movement preparation, and MVC (from 0.5 to 2.5 seconds), more related to the movement execution.

3. Statistical analysis

3.1 Rubber Hand Illusion

Pre-proprioceptive judgment was subtracted from post-proprioceptive judgment and referred as proprioceptive drift (Tsakiris & Haggard, 2005). Then, in order to obtain the pure effect of the main synchronous stimulation, proprioceptive drift in the asynchronous condition was subtracted from proprioceptive drift in the synchronous condition and referred as proprioceptive shift (Abdulkarim & Ehrsson, 2016). Positive scores indicate higher values in synchronous condition, and so a relative displacement toward the rubber hand. For the questionnaire, we separately averaged ratings of first three "target" questions (statements that actually reveal the presence of the illusion) and ratings of the second three "control" statements (Kalckert & Ehrsson, 2012). In order to obtain the main effect of subjective experience of owning the fake hand, we subtracted the average of control ratings from the average of target ones.

The normality of the distribution was evaluated by a Shapiro-Wilk test. Accordingly, proprioceptive shift was analyzed by means of a 3x2x2 repeated measures ANOVA with TIME (T0, T1, T2) and HAND (left, right) as within subject factors, and GROUP (Experimental, Control) as between-subjects factor. All necessary post-hoc comparisons were conducted with Duncan test. The questionnaire was analyzed with Wilcoxon matched-pair test (within-subjects comparisons) and a Mann-Whitney test (between-groups' comparisons) where the significance level was set at $p < 0.05$. It is worth noticing that the T0 vs T1 comparison allowed examining the effects of the mere presence of the cast, whereas the T1 vs T2 comparison was run to analyze the effects of a prolonged period of immobilization.

3.2 Physiological Measures

In order to prevent contaminations of MEP by background EMG, trials with any background activity greater than 50 μV in the 100 ms window preceding the TMS pulse were excluded from the MEPs analysis (less than 5% of the trials was excluded). EMG data were collected for 200 ms after the TMS pulse. Data were analyzed offline using AcqKnowlege software (Biopac Systems, Inc., Santa Barbara, CA). We considered averaged peak-to-peak amplitudes of MEPs recorded from FDI.

MEPs amplitudes deviating more than two standard deviations from the mean for each condition and trials contaminated by muscular pre-activation were excluded as outliers (maximum one trial outlier per person was found). According to the normality of the residuals distribution (Shapiro-Wilk test) a 2x2 repeated measures ANOVA on MEPs amplitude with HAND (Left and Right) and TIME (T0 and T2) as within-subjects factors was run. With respect to rMT, expressed as percentage of the maximum stimulator output, we first subtracted rMT of T2 from rMT of T0 for each brain hemisphere (i.e. Left and Right hemisphere), in order to obtain an index of rMT for each participant's hemisphere. According to the non-normal distribution (Shapiro-Wilk test), the non-parametric Mann-Whitney test was used to analyze differences between the rMT of each hemisphere of the seven participants from the Experimental Group and the seven of the Control Group. With respect to force parameters (both for FR and MVC), we computed the same subtraction used for rMT. Therefore, we subtracted for each force parameter (FR and MVC) for each hand (left, right) the RMS-EMG value acquired at T2 from the RMS-EMG acquired at T0, in order to obtain an index of FR and MVC for each participant for each hand. According to the normal residuals distribution (Shapiro-Wilk test), we then performed a paired sample t-test between the two hands (left and right) to analyze differences.

3.3 Relationship between RHI and physiological measures

For the Experimental group, in order to examine the relationship between the RHI behavioral responses and the physiological measures obtained in T0 and T2, MEP, proprioceptive shift and questionnaire values (average of target questions minus average of control questions) were normalized in z-scores (Della Gatta, et al., 2016; Romano, Bottini, & Maravita, 2013). Because rMT and force parameters (FR and MVC) are single measurements, recorded once before and once after the immobilization, they were not normalized in z-scores. We performed an ANCOVA models in which physiological values were used to predict behavioral responses also controlling for time effect (Garbarini, et al., 2014). For each hand, we separately performed an ANCOVA with Time as categorical predictor (with two levels: T0, T2), one of the physiological values as continuous predictor variable (MEPs, rMT, FR, MVC) and one of the RHI behavioral responses as dependent variable (proprioceptive shift and questionnaire values).

4. Results

4.1 Rubber Hand Illusion

For proprioceptive shift (Figure 2), the ANOVA yielded a significant three-ways interaction (F (2, 76) = 4.92, p=0.01). Post-hoc comparisons showed that in the Experimental group at T2, the crucial condition for our purposes, the proprioceptive shift for the Left Hand (mean±sd: 2.56±2.33), that is the one immobilized for one week, was significantly higher with respect to both its own baseline at T0 (mean±sd: 1.30±3.05) and at T1 (mean±sd: 0.65±2.66) (p always < 0.05). By contrast, at T2, the proprioceptive shift for the Right (not immobilized) Hand (mean±sd: 0.37±2.35) did not differ from its own baseline at T0 (mean±sd: 0.27±2.87) and T1 (mean±sd: 1.26±2.36). Also the difference between hands is significant (p=0.02) at T2: the Left Hand still in the Experimental group is higher than the Right one. No differences along the three time points were found in the Control group.

Between groups comparisons revealed that in the Experimental group in the crucial condition T2 the proprioceptive shift for the Left (immobilized) Hand (mean±sd: 2.56±2.33) is significantly higher (p=0.04) with respect to the Control group (mean±sd: 0.24±2.38), while contrary to our predictions, for the Right (not immobilized) Hand (mean±sd: 0.37±2.35) the proprioceptive shift did not differ (p>0.5) with respect to the Control group (mean±sd: 0.55±1.52). Figure 2.

For questionnaire (Figure 3), we found results in line with those on proprioceptive shift: inside the Experimental group the Left Hand significantly increased (p=0.04) from T0 (mean±sd: 2.01±1.69) to T2 (mean±sd: 2.65±1.88), but not comparing T1 (mean±sd: 2.33±2.02) and T2 (p=0.17), nor T0 and T1 (p=0.51). However, in this case, we also found the opposite pattern for the Right Hand: ratings from T0 (mean±sd: 2.48±1.79) to T2 (mean±sd: 1.55±1.73) significantly decrease (p=0.01), but not comparing T1 (mean±sd: 1.88±2.00) with T2 (p=0.74), nor T0 with T1 (p=0.51). Here again, the difference between hands is significant (p=0.01) at T2: the Left Hand still in the Experimental group is higher than the Right one. No differences at all were found in the Control group. A detailed analysis on Q3 ("I felt as if the rubber hand were my hand") that's the item directly related to the feeling of ownership over the fake hand (Longo, et al., 2008), revealed the same pattern: in the Experimental group, the Left Hand increased (p=0.016) from T0 (mean±sd: 2.20±1.85) to T2 (mean±sd: 3.30±2.13), but it does not change (p=0.158) between T0 and T1 (mean±sd: 2.60±2.28) and not between T1 and T2 (p=0.497). We found the opposite pattern for the Right Hand, coherently with previous analysis: results decrease (p=0.001) from T0 (mean±sd: 3.10±2.19) to T2 (mean±sd: 1.50±2.21), but it does not change (p=0.07) from T0 to T1 (mean±sd: 2.00±2.79) nor from T1 to T2 (p=0.134). No differences were found in the Control Group respect to Q3.

Concerning between groups comparisons, the Mann-Whitney test revealed that at T2 in the Experimental group the score for the Left Hand (mean±sd: 2.65±1.88) was significantly (p=0.03) higher with respect to the one of the Control group (mean±sd: 1.31±1.54). Figure 2. Summarizing these results, we found that the RHI's proprioceptive shift (but not the questionnaire) increased after a period of immobilization, revealing a weaker sense of body ownership because of the absence of movements; by contrast, we found that both proprioceptive shift and questionnaire decreased after a hyper-utilization of the non-blocked hand, revealing a stronger sense of body ownership.

4.2 Physiological measures

With respect to the rMT, a significant difference (p=0.04) in the right rMT index was found between the Experimental (mean±sd: 3.57±4.50) and the Control group (mean±sd: 0±0.58), suggesting that, only in the Experimental group, the right rMT, i.e. the motor cortex contralateral to the left (immobilized) hand, significantly increased after the immobilization. No difference between the Experimental (mean±sd: 1.43±2.76) and the Control group (mean±sd: 0.71±2.75) was found in the left rMT index. With respect to the force parameters indexes, the paired t test revealed a significant difference ($p=0.05$) only in FR between left and right hand, suggesting a significant decrease of FR in left hand (mean±sd: -0.022±0.019) with respect to right hand (mean±sd: -0.005±0.020) after the immobilization. With respect to the other dependent variables, MVC and MEP amplitude, no significant effects were found. In summary, these results on increased rMT and decreased FR confirmed the corticospinal inhibition after limb immobilization (Burianová et al., 2016; Granert et al., 2011). Summarizing these results, we found that rMT and FR (but not in MEPs) actually showed a modulation due to the absence of movements.

4.3 Relationship between RHI and physiological measures

With respect to the right hand, the ANCOVA model did not show any significant effect between physiological measures and each behavioral responses in the RHI. As regards the left hand, no significant effect was found between physiological measures and questionnaire values. However, when considering the proprioceptive shift as dependent variable and the right rMT as covariate, the ANCOVA model revealed a significant effect of the covariate ($F(1,11)=5,22$, $p=0.04$). This, in turn, suggests that the rMT of the right hemisphere significantly predicted ($r^2=0.51$, $p=0.02$) the proprioceptive shift of the Left (immobilized) hand.

5. Discussion

The aim of the present study was to investigate the effect of arm immobilization in building up a coherent sense of body ownership as indexed by the rubber hand illusion paradigm.

As first, we examined whether the mere vision of the cast and/or the sudden experience of being blocked affects body ownership (T0 vs. T1 comparison). Indeed, a plaster cast surrounding the hand is both a strong visual cue. Additionally, the cast immediately prevents movements. Consistently, previous literature reported an quick functional reorganization within dorsal premotor cortex and cerebellum immediately after immobilization (Kühn, Werner, Lindenberger, & Verrel, 2014). This, in principle, might be sufficient to alter the representation of one's own body. However, our results showed that the mere presence of the cast does not affect the RHI strength. Indeed, both behavioral measures of the illusory effects (i.e., proprioceptive shift and questionnaire on ownership) did not change.

Secondly, we analyzed whether a prolonged absence of movements affects body ownership (T0 vs. T2 comparison). With respect to the immobilized hand, we found that both proprioceptive shift and questionnaire on ownership increased after one week of immobilization. This datum might be explained as follows. A prolonged immobilization of the hand significantly decreases the amount of movement-related signals and, consequently, progressively disrupts the integration of efferent and afferent information normally subserving its representation. Therefore, ownership of the arm would become weaker and, hence, the hand would be more susceptible to the RHI. As regards the non-immobilized hand, we found that the questionnaire on ownership decreased after one week of immobilization. We might attempt to explain these findings as follows. In everyday-life fully unimanual activities are quite rare (Guiard, 1987). On the contrary, actions requiring at least a certain degree of interaction between hands are much more common. Hence, being unable to use one arm might induce a sort of compensatory overuse of the other in order to maintain the motor efficiency. Such mechanism it has been clearly demonstrated within the context of stroke-induced unilateral motor deficits (Sato, et al., 2005; Sato, et al., 1999). This condition dramatically increases both afferent and efferent body-related signals as well as their integration. Therefore, ownership of that body part arm would strengthened (i.e., the body part would be less susceptible to the RHI). In summary, an asymmetrical distribution of the available signals would affect body ownership in opposite directions (see Burin, et al., 2015 for a similar argument).

Thirdly, we investigated the relationship between illusion strength and physiological changes following immobilization (T0 vs. T2 comparison). Capitalizing on previous literature showing that immobilization does affect motor-related signals (Avanzino, et al., 2011; Avanzino, et al., 2014; Burianova, et al., 2016), we recorded physiological measures representing the brain plasticity related to post-immobilization corticospinal excitability (i.e., rMT, MEP amplitude and force parameters). It is worth noticing that previous studies reported decreased MEP following short-term immobilization (Avanzino, et al., 2011; Avanzino, et al., 2014; Facchini, et al., 2002; Huber, et al., 2006; Ngomo, Leonard, & Mercier, 2012; Opie, Evans, Ridding, & Semmler, 2016; Rosenkranz, Seibel, Kacar, & Rothwell, 2014). In the present study, immobilization increased rMT but did not affect MEP amplitude. This, in turn, confirms that, for the hemisphere contralateral to the immobilized hand (i.e., the right hemisphere), different rMT between pre and post immobilization produced comparable MEPs (Burianova, et al., 2016). Therefore, our results on corticospinal excitability are consistent with findings on short-term immobilization. Contrary to some previous studies (Avanzino, et al., 2011; Hummel & Cohen, 2006; Liepert, et al., 2000), however, we did not find a modulation of the same parameters related to the non-immobilized overused hand. In addition, our result on EMG signal, and in particular on force parameters is in agreement with previous evidence that immobilization caused a reduction of different electromyographic parameters, such as MVC (Kaneko, et al., 2003). Crucially, we found that the rMT of the right hemisphere predicts the scores of the RHI proprioceptive shift of the contralateral immobilized hand. It is important to emphasize that proprioceptive shift is an implicit measure of the illusion related to the integration of incoming multisensory signals. Immobilization induces a prolonged reduction of the amount of incoming signals and, consequently, it decreases the efficiency of multisensory integration. It is known, for instance, that a short-term limb immobilization decreases proprioception and as well as the contralateral M1 excitability (Avanzino, et al., 2014). The correlation between shift and rMT increases for the hemisphere contralateral to the immobilized hand supports the view of a link between movements and body ownership even at physiological level. Interestingly, this seems to be consistent with the fact that during the rubber hand illusion (i.e., when we experience also the disembodiment of our own hand), decreased motor evoked potentials in primary motor cortex are reported (Della Gatta, et al., 2016).

In summary, our findings support the view that actions have a direct role in shaping the subjective experience of the body. It is worth noting that this is consistent with the results obtained in clinical populations with different movement disorders. Indeed, impairments of body ownership have been found in hemiplegia (Burin et al., 2015), spinal cord injury (Tidoni, Grisoni, Liuzza, & Aglioti, 2014), focal hand dystonia (Fiorio et al., 2011). This hypothesis is also consistent with the fact that the physical body allows us to interact with the world mainly through actions (Gallese & Sinigaglia, 2010), translating desires and goals into effects in the external word (Head & Holmes, 1911). It is important to emphasize that during actions, different categories of signals are present: visual, tactile, kinesthetic information, sensory predictions and so on. The specific role of these different categories of signals in shaping our awareness of the body is still an open question. Future studies should investigate the specific contributions of all these signals and how they interact with motor execution in modulating the feeling of body ownership.

Running head. Movements are necessary for normal body ownership

Figure legends

Figure 1. General procedure of the experiment: at T0 all forty subjects experienced the RHI on left and right hand. Then, at T2 fourteen participants first underwent physiological measurements' baseline recording; then in all participants the left hand was blocked by a plaster. Immediately after that, all participants again underwent the RHI of the left (blocked) hand and on right (free) hand. As soon as the session was finished, the cast, kept in the Experimental group, was instead removed in the Control group. At T2, one week later, we again administered the RHI on left (blocked) hand that experienced the period of immobilization, and on the right (free) hand. At the end of the session, the cast was removed in all participants. Lastly, the same fourteen participants as in T0, performed again physiological measurements.

Figure 2. Results of RHI proprioceptive shift in the Experimental and the Control group, comparing conditions before (T0 and T1) and after (T2) period of immobilization.

Error bars represents Standard Error (SE).

Figure3. Results of RHI questionnaire on ownership (target and control statements) in the Experimental and the Control group, comparing conditions before (T0 and T1) and after (T2) period of immobilization. Error bars represents Standard Error (SE).

Appendix

Target questionnaire Items

Q1. It felt as if I was feeling the stroking touch in the location where I saw the rubber hand touched

Q2. It seemed as though the touch I felt was caused by the paintbrush touching the rubber hand.

Q3. I felt as if the rubber hand were my hand

Control questionnaire items

Q4. It felt as if my hand were drifting towards the left/right (towards the rubber hand)

Q5. It seemed as if the touch I was feeling came from somewhere between my own hand and the rubber hand

Q6. It felt as if my hand were turning ‘rubbery’

Running head. Movements are necessary for normal body ownership

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Running head. Movements are necessary for normal body ownership

Figure 1

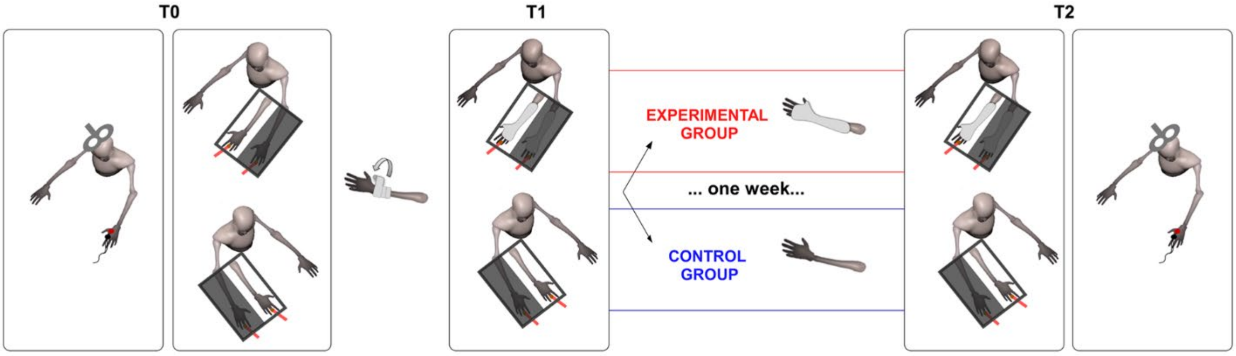


Figure 2

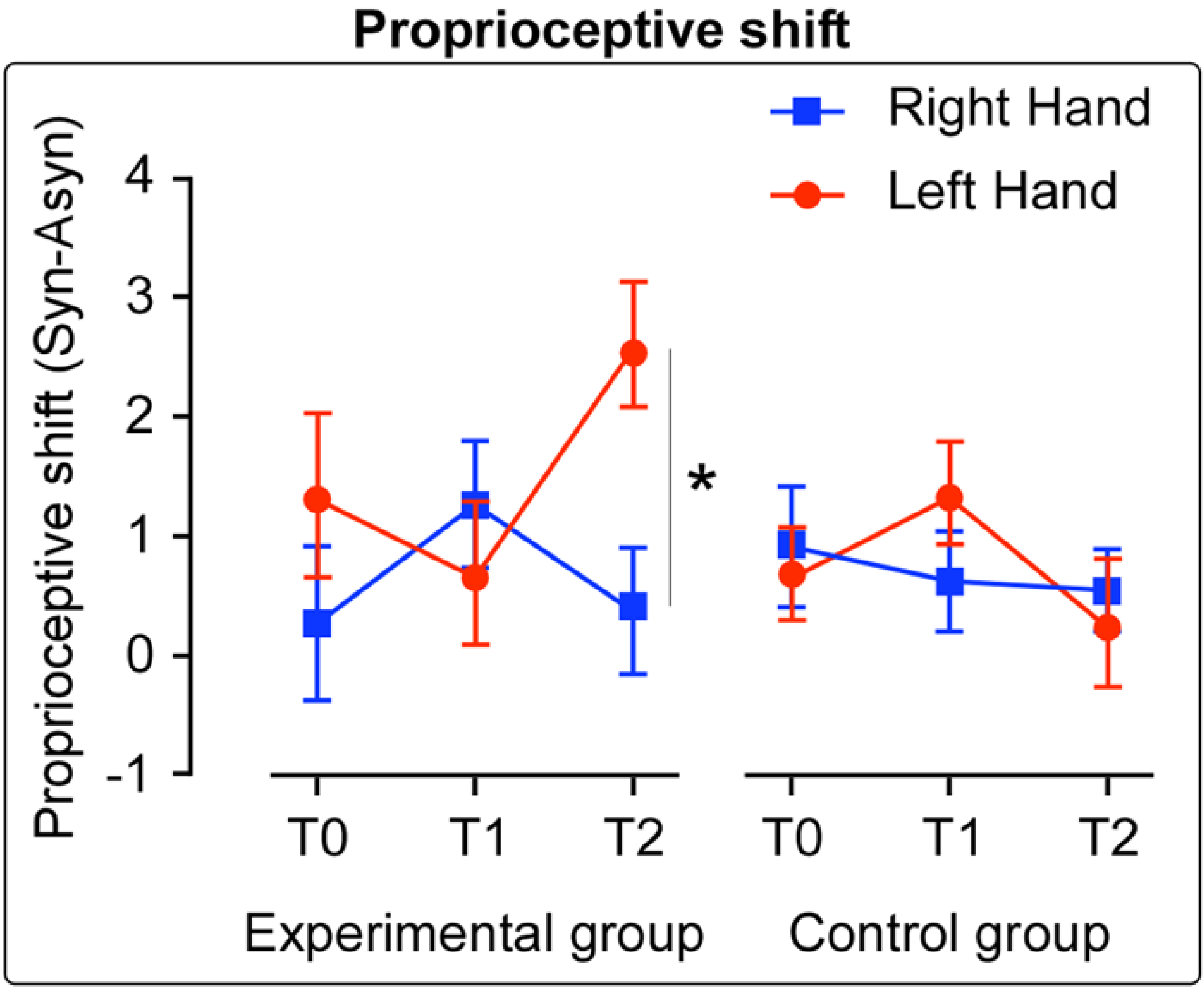
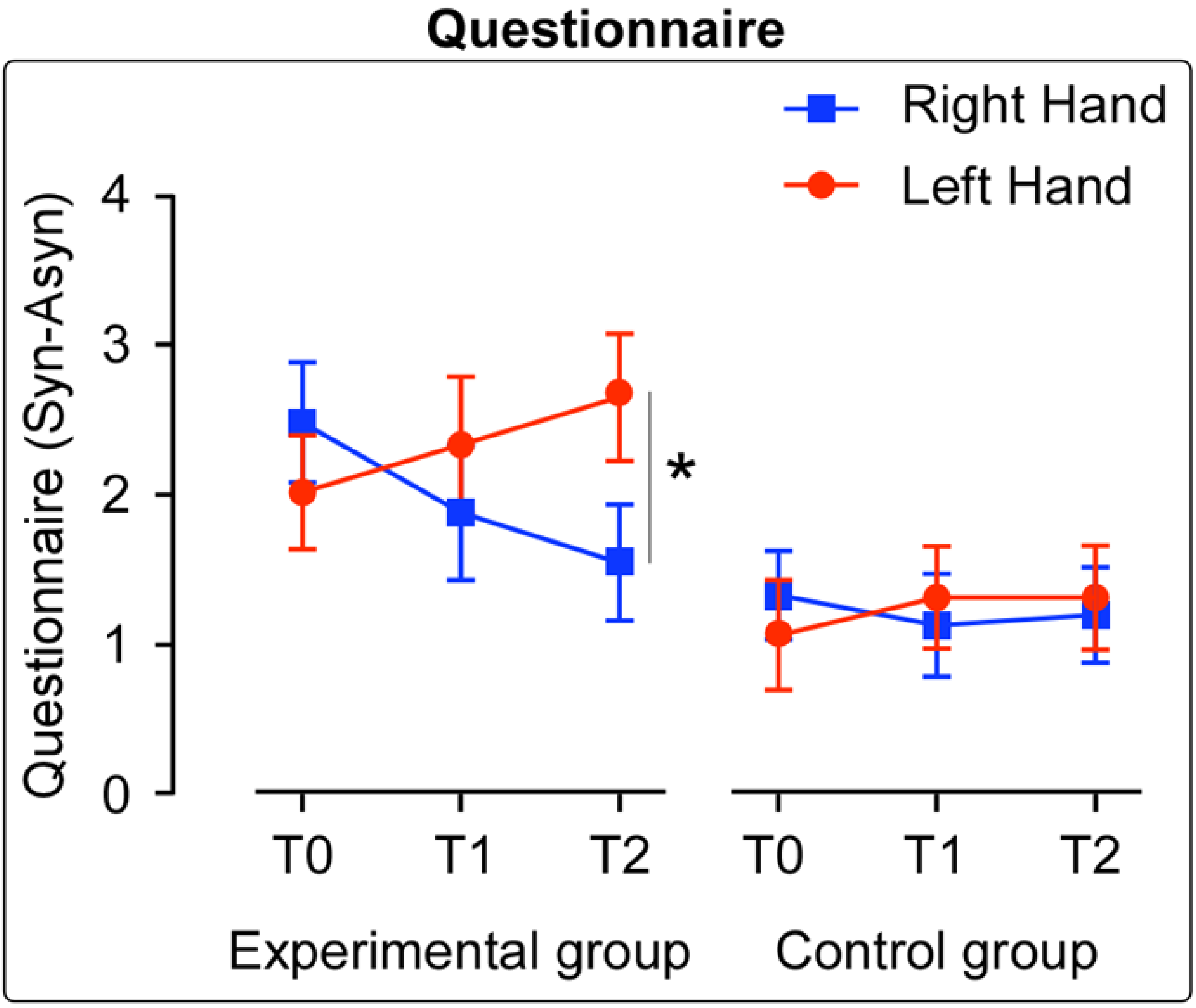

Proprioceptive shift
Right Hand
Left Hand
Proprioceptive shift (Syn-Asyn)
4
3
2
1
0
-1
*
T0
T1
T2
T0
T1
T2
Experimental group
Control group

Figure 3


Questionnaire
Right Hand
Left Hand
Questionnaire (Syn-Asyn)
4
3
2
1
0
*
T0
T1
T2
T0
T1
T2
Experimental group
Control group

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