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The neural mechanisms of body representation: new insights to facilitate prosthesis' incorporation in upper-limb amputees

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ABSTRACT

One of the biggest challenges in prosthetics is to design artificial hands which feel like patients' own body part. Although in the last years neuroscience, technology and medicine put a great effort in developing innovative prostheses, the improvements have been almost exclusively restricted to their functional aspects. A significant proportion of patients choose not to use their prosthesis or decide to wear it only for cosmetic purposes. In most cases, the prosthesis is perceived as an extracorporeal tool and not as part of their body. Only little attention has been devoted, so far, in promoting artificial limbs' acceptance within patients' body representation. The aim of the research conducted in this doctoral program was to expand the current understanding on the mechanisms of body representation and promoting the embodiment of artificial limbs. Five studies are presented in this thesis: in three of them, an experimental paradigm for testing the plasticity of the body representation (i.e. the Rubber Hand Illusion – RHI) has been employed on groups of healthy volunteers. In another study, a multimodal approach with behavioral and neurophysiological investigations was conducted on a group of healthy participants to find the optimal posture of a hand prosthesis through which it would be easier to embody. In the remaining one, an upper limb amputee implanted with intraneural electrodes has been longitudinally tested to investigate the level of embodiment over two different neurally-interfaced prostheses. To this aim, two objective paradigms based on multisensory integration (i.e. the Visuo Tactile Integration – VTI and the Temporal Order Judgement – TOJ) have been exploited. The results suggest that the amount of practice with a prosthesis and the richness of somatosensory feedback are crucial for facilitating its integration with one's body representation. The optimal posture for a prosthesis to be embodied is the one performed with the thumb-down and other fingers-up. Furthermore, when designing a prosthesis which employ sensory substitution feedback, the richness of the somatosensory stimuli is another aspect to take into consideration to facilitate its embodiment. Finally, a new and objective way to measure the onset and the initial evolution of the sense of ownership over an artificial limb has been discovered: the Non-Specific Skin Conductance Response (NS-SCR).

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Chapter 1: General introduction

1. Body representation and embodiment of prostheses

When we look at our hands, we immediately perceive them as part of our body. This experience of owning our hands is commonly taken for granted, but the mere visual presentation of others' bodies and the feeling that our body parts belong to us are subtended by completely different neural mechanisms.

The dissociation between others- and self- identification of body parts has been first described in neurological patients affected by the syndrome of somatoparaphrenia, which gained attention towards the middle of the XX century (Gerstmann, 1942). This syndrome typically emerges after a brain injury, in the acute post-ictal phase and it is characterized by a strong sense of disownership of the patient's contralesional limbs (Romano & Maravita, 2019). The impaired limbs are not recognized as own or misattributed to someone's else body, highlighting a disruption of the patient's body representation (Vallar & Ronchi, 2009).

The concept of "body representation" can be defined as a mental representation of the body, which is relatively stable among healthy individuals. Aside from somatoparaphrenia, there is a wide number of disorders of bodily awareness characterized by neurological and/or psychiatric causes (De Vignemont, 2010). Each one of them impacts the patient's body representation in a different way such as: impairment in the recognition of own's body through vision, touch, proprioception, semantic understanding, motor behavior, emotional affect, etc., suggesting that multiple aspects of body representation exist (De Vignemont, 2010). Instead of classifying these disorders by their clinical description, for the sake of simplicity, a dual taxonomy of body representation has been postulated: one part implies the sensorimotor representations of the body that guide actions (i.e. the body schema) and the other refers to the perceptual, conceptual or emotional representations of the body (i.e. the body image) (Dijkerman & De Haan, 2007; Gallagher, 2006; Paillard, 1999; Rossetti, Rode, & Boisson, 1995).

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The study of the neural processes that allow us to perceive a body part as own is crucial when it comes to the effective incorporation of a prosthesis into the body representation of the patient. An amputation dramatically impacts the patient's life not only in terms of loss of sensorimotor abilities, but also for the alteration of their body representation. Although prostheses are specifically designed to restore the lost motor functionality, the rejection rates are very high in adult population (from 23% to 26%), and it gets even worse in pediatric population (from 35% to 45%) (Biddiss & Chau, 2007). Furthermore, amputees who choose not to use a prosthetic limb report higher level of depression, lower levels of social integration and everyday activities restriction (Murray, 2004; Williamson, Schulz, Bridges, & Behan, 1994). The failure of the integration of the prosthesis into their body representation is the main reason why these patients decide not to wear it: amputees tend to perceive the prosthesis, regardless of its level of functionality, as an "inert supplement" or an "extracorporeal structure" and not as part of their body (Scarry, 1994).

The most important tool used to disclose the complex relationship between an artificial limb and its integration in the body representation of the user is the Rubber Hand Illusion (RHI), an experimental paradigm first described by Matthew Botvinick and Jonathan Cohen, in 1998 (Botvinick & Cohen, 1998). Since then, this paradigm has been extensively used for scientific research in Cognitive Neuroscience and Neuroprosthetics.

2. The Rubber Hand Illusion as a tool for understanding the body ownership

In its original setting, the sight of brushing an artificial upper-limb while synchronously brushing a person's hand hidden from view, is sufficient to produce the feeling of ownership of the rubber hand (Fig.1) (Botvinick & Cohen, 1998). In the experimental condition, the rubber hand is placed next to the participant's hand, in a biologically-plausible position. RHI experiments are typically accompanied also by a control condition where no illusion is induced, either with an asynchronous brushing (Botvinick & Cohen, 1998) or with the rubber hand detached by the participant's body and rotated by 90° (Tsakiris & Haggard, 2005).

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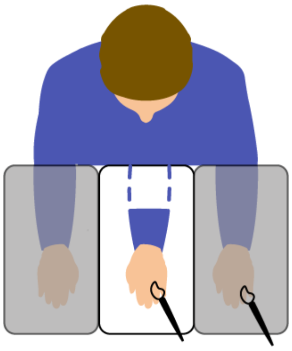

Figure 1. The Rubber Hand Illusion (RHI) original experimental setup.

Whether it is a "joke shop" rubber hand (Durgin, Evans, Dunphy, Klostermann, & Simmons, 2007; Farne, Pavani, Meneghello, & Ladavas, 2000) or a pair of stuffed gloves (Spence, Pavani, & Driver, 2004) it seems to make a modest influence on the illusory hand incorporation (Spence, 2015).

During the last 20 years, several experimental measures of incorporation have been employed in the massive body of literature that has been produced worldwide. The most commonly used is the original *questionnaire* first introduced by Botvinick and Cohen (1998) (Fig.2).

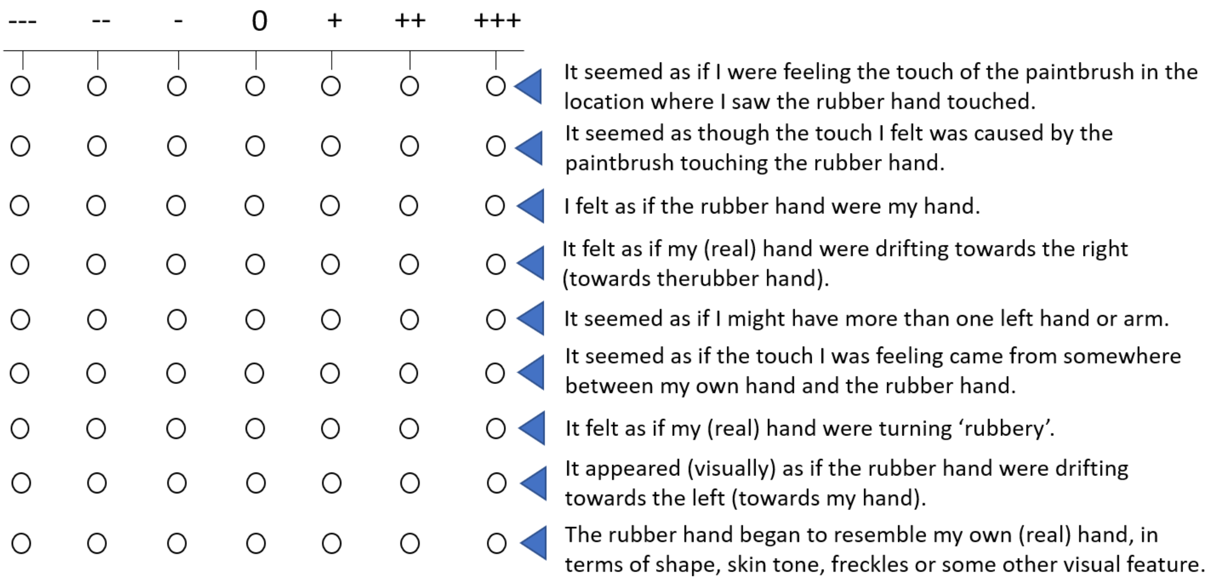

Figure 2. The Rubber Hand Illusion (RHI) questionnaire developed by Botvinick and Cohen (1998).

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The questionnaire includes 9 statements: participants have to rate each item in a 7-point Likert scale ranging from "agree strongly" (+++) to "disagree strongly" (---) according to their experience after a session of RHI. The first three statements are directly related to the feeling of ownership of the rubber hand, while the other six describe experiences typically not related with the RHI, serving as control for suggestibility.

A more behavioral and less subjective measure is the *proprioceptive drift* (Botvinick & Cohen, 1998). Before and after a session of RHI, a measuring tape above the participant's hidden hand is shown. At the question "Where do you perceive your hand?", while before the RHI session the participants generally indicate on the tape the correct location of their hidden hand, after a synchronous brushstroking session they tend to misjudge their hand's position towards the fake hand's position (Mioli, D'alonzo, Pellegrino, Formica, & Di Pino, 2018). The displacement of their perceived hand's position does not occur if participants undergo one of the control conditions of RHI. Although questionnaire's outcomes and proprioceptive drift are meant to measure the extent of illusory hand ownership, some studies have highlighted the fact that these measures do not always correlate (Crucianelli, Metcalf, Fotopoulou, & Jenkinson, 2013; Holmes, Snijders, & Spence, 2006; Rohde, Di Luca, & Ernst, 2011). Probably, these two measures investigate different aspects of the sense of body ownership that are subtended by different multisensory mechanisms (Blanke, 2012).

There are also physiological measures of RHI. The *skin conductance* represents the electrical conductivity of the skin due to the activity of the sweat glands, whose secretion can be triggered by emotional stimulation. In fact, skin conductance response results to be higher when measured in correspondence to physical threats to the embodied artificial hand after its synchronous stroking with the participants' hand (Armel & Ramachandran, 2003; Petkova & Ehrsson, 2009; Tsuji et al., 2013; Yuan & Steed, 2010). Similarly, also the *skin temperature* has also been indicated as a physiological index modulated by the entity of the sense of body ownership over an artificial limb (Moseley et al., 2008). Moseley et al. (2008) showed a decrease of the skin temperature of the real hand after a synchronous session of RHI, that has been interpreted as a disownership for the real hand in favor to an incorporation of the fake limb. In line with this interpretation of "disownership" of the participants' real hand during the induction of embodiment over a non-body object, other authors have highlighted an increased histamine reactivity on the real hand after a synchronous brushstroking session, probably a sign of rejection put into action by the immune system (Barnsley et al., 2011). However, the

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consistency and reliability of these results and interpretations is currently under debate (de Haan et al., 2017; Rohde, Wold, Karnath, & Ernst, 2013).

3. How does the sense of body ownership arise?

Several neurocognitive models have been postulated to account for the arise of the sense of body ownership. On one hand, a bottom-up neurocognitive model claims that sense of ownership over an object that does not belong to the biological body depends from the integration of information from different senses. On the other, a top-down neurocognitive model relies on the involvement of internal body maps.

The Bayesian perceptual learning theory states that an object can be considered as part of one's body if the visual and tactile information are spatiotemporally congruent (Armel & Ramachandran, 2003). For these authors, this is a necessary and sufficient condition to trick our mind and to consider that visual and tactile information come from one common event. A more complex mechanism has been postulated by Makin et al. (2008): visual input coming from the fake limb and proprioceptive information from the real hand are computed in unimodal brain areas and conveyed to multisensory brain regions where they are integrated in one coherent multisensory event (Makin, Holmes, & Ehrsson, 2008). In this way, if the artificial limb is located in an anatomically plausible position, the tactile and proprioceptive information is weighed in favor of vision. What happens to the sensory stimuli in the multisensory areas is a recalibration in hand-centered coordinates so as to refer the touch felt to the paintbrush stimulation seen on the rubber hand.

Tsakiris' (2010) neurocognitive model of sense of body ownership posits that the embodiment of an artificial limb results from the integration of different information sources into internal body maps, which are pre-existent. The strength of sense of body ownership is determined by a hierarchical series of comparator mechanisms between sensory information and different representations of the body (Tsakiris, 2010). The first of these comparators contrasts the shape of the object that is being viewed with a model of the visuo-structural properties of the participants' hand. If the observed object bears enough similarities to the pre-existing body model, the next comparator comes into play. This comparator contrasts the current postural configuration of participants' body against the postural properties of the observed object. Again, if the result is a match, the last comparison is between the visual stimulation on the

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observed object and the perceived sense of touch. At this point, the sense of body ownership over the observed object can finally arise (Braun et al., 2018; Tsakiris, 2010).

Going more into deep to the constraints to be fulfilled in order to determine the sense of body ownership over an artificial object, many authors have found several constraints that are able to modulate the strength of the incorporation effects after a RHI session. The distance between the rubber hand and participants' real hand is one of them. In fact, a distance of more than 27cm is reported to dilute the effects of RHI (Lloyd, 2007) (i.e. a virtual limit where our mind is no more being tricked). Furthermore, no illusion can be induced if the artificial hand is placed in an anatomically implausible position (Tsakiris & Haggard, 2005). The temporal proximity between the visual and tactile stimulus also turned out to be of major importance for the strength of the illusory ownership in RHI. Shimada et al. (2009) conducted an experiment with multiple RHI conditions where the visual and the tactile stimulus were delayed in steps of 100 ms, up to 600 ms. The illusion was reduced when the delays of the stimuli of the two different modalities exceeded 300 ms (Shimada, Fukuda, & Hiraki, 2009). Finally, as previously stated, even if the visual appearance of the artificial limb seems to make little difference on its incorporation (Spence, 2015), other studies claim that if its skin texture looks natural, RHI effects are more strongly experienced (Haans, IJsselsteijn, & de Kort, 2008). Also, if the skin color does not match with the participant's one, the induction time of the illusory body ownership is longer and its strength is overall diminished (Lira et al., 2017).

4. The neural basis of body ownership

Various neurophysiological methods have been employed to investigate the neural correlates of the illusory feeling of owning an artificial limb. Most of these studies attempt to correlate the outcomes obtained by means of such techniques during RHI sessions with the classical measures (subjective, behavioral and physiological ones).

Single cell recordings

This technique allows a direct recording of neural activity by means of microelectrodes inserted directly into the skull, on a brain area of interest. Due to its invasiveness and ethical issues, this recording method is limited to animal models.

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Graziano et al. (2000) performed a RHI experiment on two monkeys implanted with microelectrodes. These authors could not be sure that the false arm (i.e. a realistic artificial monkey limb) actually induced the illusion on the monkeys, but they could observe its influence on the behavior of their neurons (Graziano, Cooke, & Taylor, 2000). In fact, neurons from area 5 in the parietal lobe, fired at higher rate when the animals saw the fake arm in a congruent position with respect to their arm (Graziano et al., 2000). These responses did not occur if the fake arm was placed in an incongruent position or if it was substituted with other objects (e.g. a rectangle of white paper with the same length and width as the fake arm) (Graziano et al., 2000). Another study investigated the cortical activity of two monkeys implanted with microwire arrays in the primary motor cortex (M1) and the primary somatosensory cortex (S1) in a slightly different experimental setup (Shokur et al., 2013). Neurons of those areas, after a period of synchronous visuo-tactile stimulation, started to respond to virtual stimuli applied to an avatar arm alone (Shokur et al., 2013). Such results give us a hint that, at least in primates, primary cortical areas such as M1 and S1 and multisensory areas such as area 5 in the parietal lobe are part of a neural network which subtend the incorporation of external objects into one's body representation (Graziano et al., 2000; Shokur et al., 2013). Finally, a recent study provided a demonstration in macaque monkeys that neural signals in their premotor cortex directly express the strength of illusion of owning a fake limb (Fang et al., 2019).

Positron Emission Tomography (PET)

PET is a neuroimaging technique that can give us a picture of the cerebral metabolism by detecting the concentration of a previously injected radioactive tracer into the blood stream of the patient or the healthy participant. Once injected, the tracer concentrates in the brain areas where blood flow is transiently more pronounced, that is typically correlated with a higher neural activity.

Tsakiris et al. (2007) investigated the dynamic changes of the regional cerebral blood flow using PET in healthy participants who undergone several sessions of RHI. The synchronous brushstroking induced to the participants the illusory feeling of owning the rubber hand as measured by the proprioceptive drift, and this behavioral data positively correlated with activity in the right (ipsilateral side of paintbrush stimulation) frontal operculum and right posterior insula (Tsakiris, Hesse, Boy, Haggard, & Fink, 2007). These areas are reciprocally connected by efferent and afferent projections (Augustine, 1996). Furthermore, other studies found that

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right insular activation is implicated in the representation of an egocentric reference frame (Fink et al., 2003), in self-attribution (Farrer et al., 2003; Farrer & Frith, 2002) and self-processing (Fink et al., 1996; Vogeley et al., 2004).

Functional Magnetic Resonance Imaging (fMRI)

With the fMRI, researchers can indirectly measure the cerebral activity by measuring the hemodynamic responses which accompany the increased neuronal activity. fMRI is able to detect the BOLD (Blood Oxygenation Level Dependent) signal, that is the level of deoxyhemoglobin (Hbr) divided by the level of oxyhemoglobin (HbO_2) in the neural tissues.

Increased BOLD responses at the level of bilateral premotor cortex, especially for the ventral part (PMv), have been reported by several studies which induced the experience of body ownership for an artificial hand within a RHI experimental paradigm (Bekrater-Bodmann et al., 2014; Brozzoli, Gentile, & Ehrsson, 2012; Ehrsson, Spence, & Passingham, 2004; Gentile, Guterstam, Brozzoli, & Ehrsson, 2013; Petkova et al., 2011). Furthermore, these enhanced responses were found to correlate with the self-reported ratings of illusion's strength (Ehrsson et al., 2004; Gentile et al., 2013; Petkova et al., 2011). Primate studies suggest that the premotor cortex may contain neurons with visual and tactile receptive fields that respond either when a specific body part is being touched or a stimulus is seen approaching to that specific body region (Botvinick, 2004; Ramakonar, Franz, & Lind, 2011). It is possible that the premotor activity during RHI underlies a shift in the receptive fields initially centered on the participants' hand, becoming aligned with the ones of the visible fake hand (Botvinick, 2004).

The posterior parietal cortex (PPC) is another region that has been frequently reported to be active during RHI experiments (Brozzoli et al., 2012; Ehrsson et al., 2004; Gentile et al., 2013; Petkova & Ehrsson, 2009). This area contributes to map the position and the orientation of our limbs in space within a body-centered reference frame (Grivaz, Blanke, & Serino, 2017). PPC, along with PMv, integrate visual and somatosensory information by means of their multimodal neurons, encoding and constantly updating participants' corporeal space (Braun et al., 2018).

Likewise the previously reported investigation with PET (Tsakiris et al., 2007), also with fMRI the insula resulted to be active during illusory hand ownership. When the rubber hand was threatened after the illusion had been induced, an increased insular activity was reported (Ehrsson, Wiech, Weiskopf, Dolan, & Passingham, 2007). Additionally, the increased BOLD

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response positively correlated with the subjective level of ownership of the rubber hand (Ehrsson et al., 2007).

Another noteworthy brain region that has been associated with the illusion of owning an artificial limb is the exstrastriate body area (EBA) (Limanowski, Lutti, & Blankenburg, 2014). Limanowski et al. (2014) found enhanced brain activity in EBA during RHI which particularly reflected interindividual differences in the intensity of the illusion of owning the fake hand. This area, historically thought to selectively respond to vision of bodies and body parts (Downing, Jiang, Shuman, & Kanwisher, 2001; Pitcher, Charles, Devlin, Walsh, & Duchaine, 2009), seems to be also critically involved with illusory hand ownership (Limanowski et al., 2014).

Electroencephalography (EEG) and electrocorticography (ECoG)

EEG is a neurophysiological technique that allows a direct registration of the oscillations of the electrical activity from large populations of neurons. The signal is recorded by a series of electrodes placed on the scalp.

EEG activity in the gamma band (40-50 Hz) has been considered as a correlate of multimodal integration (Kanayama, Sato, & Ohira, 2007, 2009). In fact, positive correlations between RHI strength and the degree of synchrony of gamma band activity in the parietal lobe has been observed (Kanayama et al., 2007, 2009). These authors supported the view that gamma band activity within parietal areas may promote visuo-tactile cross-modal integration, generating the illusion by the interaction with other brain areas. Furthermore, a recent study observed that illusory hand ownership reduces the oscillatory power in the alpha band over frontocentral regions, and in the beta band over frontoparietal regions (Rao & Kayser, 2017).

Event related potentials (ERP) reflect the synchronous activity of a large group of neurons in response to the presentation of a stimulus (i.e. the mean EEG activity recorded in conjunction with the stimulus presentation). Many studies focused on ERPs resulting from brushstrokes to the artificial limb during RHI. The ownership illusion resulted to attenuate ERPs' amplitude at around 55ms on frontal and parietal electrodes (Zeller, Litvak, Friston, & Classen, 2015), at around 330ms on frontal and central electrodes (Rao & Kayser, 2017) and at around 460ms on frontal electrodes (Peled, Pressman, Geva, & Modai, 2003). The attenuation of ERPs highlighted by these studies may reflect the integration of vision, touch and proprioception

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during the illusion, which leads the participants to experience the sense of ownership over the rubber hand (Rao & Kayser, 2017).

Electrocorticography (ECoG) is a type of electrophysiological monitoring which uses electrode arrays placed directly on the exposed cortical surface. Since it requires craniotomy, ECoG is an invasive procedure. A study investigated the neural mechanisms that underlie body ownership during RHI by using ECoG in 5 patients with intractable epilepsy in preparation for surgery. The results showed an increased high-gamma (70-200 Hz) activity in premotor and intraparietal areas, which reflected the feeling of ownership of the fake hand (Guterstam et al., 2018).

Transcranial Magnetic Stimulation (TMS) and transcranial Direct Current Stimulation (tDCS)

TMS is a noninvasive form of brain stimulation. Its functioning is based on the application of a transient magnetic field on the scalp by means of a magnetic coil. The neural tissue underneath the coil is subject to a current flow which cause a local neuronal depolarization. TMS is commonly used to investigate the activation and the functioning of specific brain circuits by delivering single or paired magnetic pulses.

TMS, in its repetitive form (rTMS), allows to non-invasively modulate brain function for few minutes. By means of rTMS, researchers can interfere with neural activity of a target area. Kammers et al. (2009) administrated rTMS over the inferior parietal lobule (IPL) of a group of healthy participants shortly before a session of RHI. This application transiently attenuated the strength of RHI as measured by proprioceptive drift, suggesting that the IPL is probably involved in the perceptual re-localization of our involved limb during RHI (Kammers et al., 2009). Conversely, other authors targeted the extrastriate body area (EBA) with the same technique and obtained a more pronounced misjudgment of participants' hand location toward the rubber hand (Wold, Limanowski, Walter, & Blankenburg, 2014). In this case, rTMS over EBA may have led to an impaired visual discrimination between the rubber hand from participants' own hand (Wold et al., 2014).

tDCS is a neuromodulation technique that uses low voltage direct current delivered by electrodes on the scalp. A cathodic current is able to reduce the excitability of a target area and an anodic current enhances it up to 90 minutes post-stimulation (Ardolino, Bossi, Barbieri, & Priori, 2005; Nitsche & Paulus, 2000; Stagg & Nitsche, 2011).

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Anodic current delivered over the premotor cortex or the temporo-parietal junction before RHI induction increased the misjudgment of the real limb location towards the rubber hand (Convento, Romano, Maravita, & Bolognini, 2018). tDCS over those areas may induce a general recalibration of hand coordinates (Convento et al., 2018). Plus, the effects of anodal tDCS on the temporo-parietal junction extended to the subjective report (questionnaire) of the participants, indicating that anodic stimulation over this area modulates the RHI depending on the temporal congruency of the visual and tactile stimulation (Convento et al., 2018). Another study administered anodal, cathodal and sham tDCS in three different sessions on the posterior parietal cortex and the premotor cortex of a large group of participants (Lira, Pantaleao, de Souza Ramos, & Boggio, 2018). In this case, the researchers have reported a reduced illusion onset time and subjective experience of body ownership after an anodal application of tDCS over the posterior parietal cortex (Lira et al., 2018). This recent study highlighted the crucial role of this parietal area in the speed of visuo-tactile multisensory integration in the RHI (Lira et al., 2018).

5. Thesis rationale

The use of RHI to study the various aspects of the body representation is widespread in neuroscience and neuroprosthetics. In the series of experiments presented in this thesis I conducted research on several groups of healthy participants and on an upper-limb amputee. My major goals were i) to study the features of the visuo-tactile stimuli that facilitate the incorporation of an artificial limb into own's body representation; ii) to find new physiological and objective measures to assess the strength of embodiment; iii) to establish which prosthesis, characterized by different features, is embodied the most by an upper-limb amputee; iv) to promote the sense of ownership with TMS by actively enhancing the excitability of the key regions typically involved in a fake limb incorporation and, finally, v) to identify in which posture a hand prosthesis would have been easier to embody.

To pursue the i) goal, the experiment described in Chapter 2 has been conducted (D'Alonzo, Mioli, Formica, Vollero, & Di Pino, 2019). With the help of a virtual reality environment, it has been investigated how much the virtualization of sensory inputs affects the self-attribution of an avatar's limb. Here, visual and tactile inputs have been manipulated within different RHI experimental protocols, assessing the relative weight of the virtualization of sight and touch. Virtualization decreased embodiment of the avatar's limb, but lowest incorporation was found

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when only one sense was fully virtualized. This suggested that beside timing, spatial constraints and realism of feedback, a matched degree of virtualization of seen and felt stimuli is a further constraint in building the representation of the body.

For the ii) goal – Chapter 3, it has been tested if the modulation of body representation would have had an impact on the efferent branch of the Autonomic Nervous System (ANS), as measured by the non-specific skin conductance response during RHI (D'Alonzo, Mioli, Formica, & Di Pino, 2020). The standard deviation of non-specific skin conductance resulted to be increased by the illusion and correlated with all the typical measures of embodiment. This newfound measure is a cheap, easy and objective candidate to assess the depth of the illusion of owning an artificial hand.

The iii) goal – Chapter 4, led to a longitudinal study in which the embodiment over two different prostheses has been studied in an amputee receiving feedback through intraneural and perineural multichannel electrodes implanted in her stump (Di Pino et al., in review). Three factors – invasive (vs non-invasive) stimulation, training and anthropomorphism – have been tested, by comparing the results with healthy controls, through two multisensory integration tasks: Visuo Tactile Integration (VTI) and crossing-hand effect in Temporal Order Judgement (TOJ). Testing the participant with intraneural stimulation produced an extension of peripersonal space, sign of prosthesis embodiment. One-month training extended the peripersonal space selectively on the side wearing the prostheses. Although more and less-anthropomorphic prosthesis benefited of intraneural feedback and extended the peripersonal space, the worsening of TOJ performance following arm crossing was present only wearing the more trained, despite less anthropomorphic, prosthesis, suggesting that training is critical to achieve operative tool-like embodiment.

To achieve the iv) goal – Chapter 5, TMS has been employed to administer an excitatory brain stimulation protocol (intermittent Theta Burst Stimulation – iTBS) on a group of healthy subjects (Mioli et al., 2018). iTBS has been delivered over the ventral premotor cortex and the inferior parietal lobule to enhance embodiment of an artificial hand during RHI. Neuromodulation of both areas did not result in an enhancement of embodiment, as assessed by the results collected from a self-evaluation questionnaire for the extent of self-attribution of the rubber hand and proprioceptive drift. The absence of effects suggests testing other

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neuromodulating techniques, acting on cortical networks different from the ones sensitive to iTBS to enhance artificial hand embodiment.

Finally, for the v) goal – Chapter 6, a group of healthy volunteers underwent a series of experiments where physiological and behavioral advantages of two opposite hand pinch grips have been tested, to search for any preferential (standard) hand posture (Romano, Mioli, D'Alonzo, Maravita, Di Lazzaro, Di Pino, submitted). The pinch keeping thumb down and index-up revealed increased cortico-spinal excitability, faster movement onset and faster target reaching. Remarkably, motor excitability was also increased when thumb-down pinch was only observed, imagined or prepared, keeping the hand at rest in a neutral position.

Chapter 2: Different level of virtualization of sight and touch produces the uncanny valley of avatar's hand embodiment

1. Introduction

One of the most astonishing behavioral revolution of present everyday life, based on deep technological and social changes, is that humans are increasingly often acting through virtual or robotic substitutes of their physical body. On one side, today's robots are widespread in our everyday life; they are increasingly used for tele-operated activities, domestic tasks, for companionship and entertainment. Social robots, designed to actively interact with humans also in the emotional domain, have often hands and faces resembling the human body (Cabibihan, Pradipta, & Ge, 2011; Hara & Kobayashi, 1995).

On the other side, virtual human-like-avatars have begun to be employed as proxy of individuals to substitute their real human body and facilitate social and environmental interaction in an immersive virtual reality setting for gaming, social network and entertainment. Soon, human-like avatars will be employed in other on-line activities such as e-travel, e-commerce, banking etc.

For centuries, humans built social relations and behaved in the environment by physically taking advantage of their body; as side effect, telecommunications, internet, social networks, and remotely controlled robots can deprive human behaviours of the human body. In exchange of easiness of action, interactions became less experienced and depersonalization makes people feel less responsible of their actions.

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In any case, this behavioural revolution opens novel fascinating neuroscientific questions. Since avatars can feed back to their users only virtual or substitutive sensory information, 1) how the virtualization of senses affects how much the user perceives the avatar more real and human-like? 2) How such realism can be evaluated?

1) Indeed, the interaction through a teleoperated robot, which is real but transposed (i.e. coming from another place), and the interaction through a virtual avatar in a virtual environment can be coded to their users only through substitutive sensory feedback. Today, available technology allows to relay such information only with virtual, mostly cartoonish, visual feedback and modality-mismatched haptic feedback where vibration is delivered instead of touch. Virtualization of sensory feedback is likely to decrease the realism of the avatar.
2) Hitherto, the realism of virtual and robotic avatars has been evaluated through the acceptance of human subjects interacting with them, mainly through questionnaires (Ho & MacDorman, 2010; Schubert, Friedmann, & Regenbrecht, 2001). Anthropomorphism of the appearance (Mori, MacDorman, & Kageki, 2012), of movements (Kulic & Croft, 2007; Kupferberg et al., 2011) and behavior (Karwowski & Rahimi, 1991), and the ability to provide a close-to-natural physical interaction with people (Cabibihan et al., 2011; Controzzi et al., 2014; Di Pino, Maravita, Zollo, Guglielmelli, & Di Lazzaro, 2014), seem to determine the acceptance of humanoid robots and avatars.

However, an avatar is defined as an incarnation of a human form in an artificial or virtual image, hence the self-attribution of the avatar or part of it, would most likely be a more direct proxy of its realism.

Neural correlates of self-attribution depend on multi-sensory perception (Botvinick & Cohen, 1998; Rochat, 1998; Van Den Bos & Jeannerod, 2002); e.g. the attribution of a limb to the self depends on the integration of the afferent somatic stimuli and visual feedback from the limb. Thus, it's likely that the more the sensory inputs from the avatar resemble physiologic sensory feedback, the more its embodiment will be effective. The low-fidelity and modality-mismatch of visual and somatosensory feedback coming from an avatar are features that could make the sensory code less physiologic.

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Considering that many applications of robots for teleoperated activities and virtual avatars use especially the hands, and taking advantage of the huge body of literature on the embodiment of fake hands, we chose to employ the rubber hand illusion (RHI) paradigm to estimate individuals' self-attribution of the avatar. This paradigm tests the depth of a body ownership illusion, i.e. experiencing a rubber hand to be part of the own body, that emerges when a visible fake rubber hand and the subject's hidden hand are synchronously stroked with paintbrushes (Botvinick & Cohen, 1998).

VR and haptic technologies can be employed to easily manipulate the perceived scenario in a virtual version of the typical RHI paradigm, eliciting the so called Virtual Hand Illusion (VHI). One of the first experimental versions of VHI was performed by stroking a 2D video image of a hand projected on a table (IJsselsteijn, de Kort, & Haans, 2006). Subsequently, with the progressive improvement of this technology, the systems for virtual reality have become more immersive and realistic. Several studies employed a projection screen placed in front to the user associated with head-tracked stereo-lenses in order to reproduce the objects projected on the screen from the participant's point of view and determine the virtual image depending on the user's head direction (Padilla-Castañeda, Frisoli, Pabon, & Bergamasco, 2014; Sanchez-Vives, Spanlang, Frisoli, Bergamasco, & Slater, 2010; Slater, Pérez Marcos, Ehrsson, & Sanchez-Vives, 2008; Slater, Spanlang, Sanchez-Vives, & Blanke, 2010). More recent works employed head-mounted displays (HMD) showing real-time stereoscopic video imagery (D'Alonzo & Cipriani, 2012; Kokkinara & Slater, 2014; Ma & Hommel, 2013; Maselli & Slater, 2013; Slater, Pérez Marcos, Ehrsson, & Sanchez-Vives, 2009; Tieri, Tidoni, Pavone, & Aglioti, 2015; Yuan & Steed, 2010). Vibrotactile devices are the most used to reproduce the contact and the interaction with objects within the virtual environment in VHI paradigm (Ma & Hommel, 2013; Padilla-Castañeda et al., 2014; Slater et al., 2009) because they are usually little, inexpensive, power-efficient and can be easily fitted on clothes worn by the users. In the past, by employing VR headset in virtual environment and substituting the touch of the paintbrush with vibrators, it has been shown that the illusion survives to the virtualization of this sensory input.

In the past, by employing VR headset in virtual environment (Maselli & Slater, 2013) and substituting the touch of the paintbrush with vibrators (D'Alonzo & Cipriani, 2012), it has been shown that the illusion survives to the virtualization of this sensory input. To date, no studies directly compared the depth of the illusion induced by the typical RHI paradigm with the same

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paradigm in a virtual environment by taking in consideration both visual and tactile sensory input.

This study assessed the relative weight of progressively virtualizing visual and tactile sensory inputs in the induction of the embodiment of the hand. Being virtual something that is such in the effects, though not formally in reality, with progressive virtualization it is meant decreasing the adherence to the physical reality, while trying to reproduce the information content of the sensory inflow. Sensory substitution has important applications in Prosthetics. Thus, identifying the impact on the VHI of the virtualization of sensory feedback may suggest the sensory features (appearance and characteristic of somatosensory stimulation), which are more likely to determine the self-attribution of prosthetic limbs.

Three levels of virtualization of the visual sensory input (factor *Sight: Real*, *Robotic*, *Virtual*) and two levels of the virtualization of tactile sensory input (factor *Touch: Real*, *Virtual*) were employed to design a 3x2 matrix of six experimental conditions readapted from the RHI classic paradigm (Fig. 3).

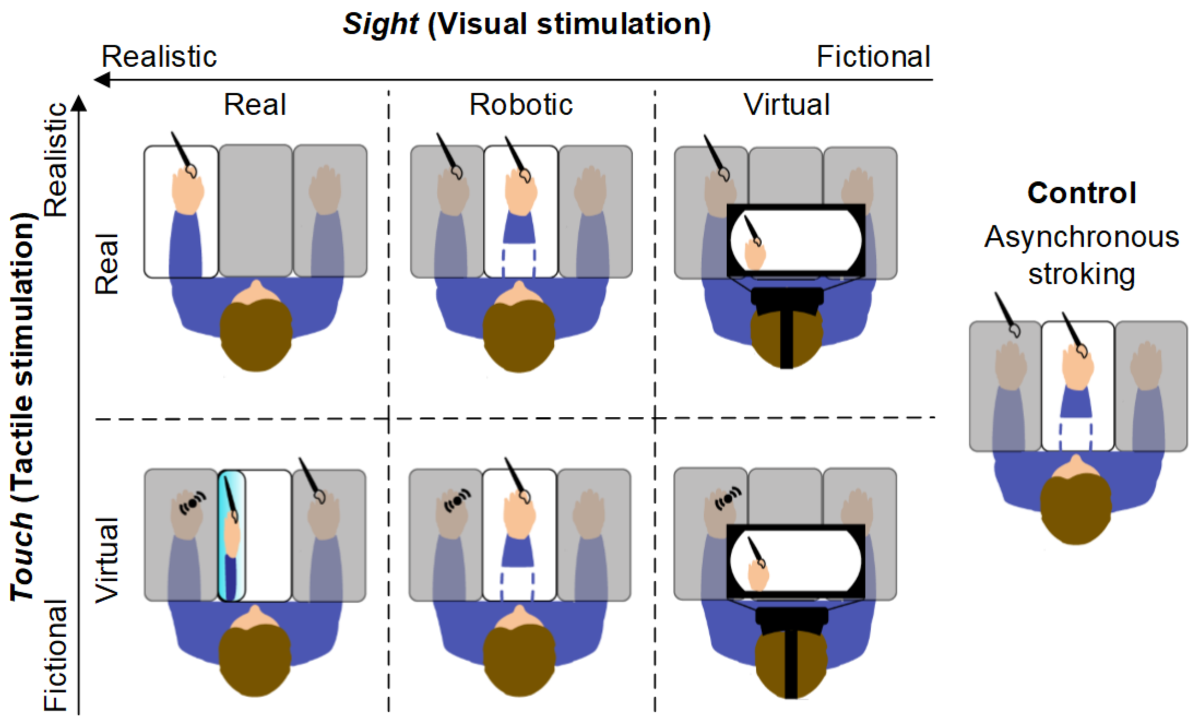

Figure 3. Schematic illustration of the six experimental conditions plus the control. Along the rows the degree of virtualization of visual stimulus increases: (Factor: Sight); along the

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coloumns the degree of virtualization of tactile stimulus increases: (Factor: Touch). In the right side: the control condition.

Subjects saw a hand stimulated by a paintbrush and synchronously felt the stimulation on their hand. In *Sight-Real*, the seen hand was their real own, in *Sight-Robotic* it was a robotic hand with a rubber cover, and in *Sight-Virtual* a virtual hand was displayed though a Head Mount Display (HMD). Participants' hand was stimulated with a real paintbrush in *Touch-Real*, and with vibrators in *Touch-Virtual.*

Additionally, an asynchronous condition (*Control*), where a small temporal delay between the stroking of the rubber hand and real hidden hand was introduced, was employed as no illusion condition. The strength of the illusion among the different conditions was quantified by a self-evaluation questionnaire and the proprioceptive drift. Three illusion outcomes were derived from the questionnaire: RHI index, vividness and prevalence (see Materials and Methods). The *Sight-Real_Touch-Real* condition was carried out to evaluate the full-scale value reachable by the employed measures of embodiment.

2. Materials and methods

Test platform

The employed test platform was a flexible hardware/software system tailored for the rapid deployment of cognitive experiments. The platform was designed to simplify the development and management of virtual setups populated with cyber-physical objects and to manage and control their interaction with the user. The platform was composed of a Head Mounted Display (HMD) (HTC Vive), an array of vibrotactile stimulation devices (Precision Microdrives Inc.) controlled by a PIC development kit (CCS Inc.), a VR software application (Unity3D) and an ad hoc physical setup where the participant was seated.

The VR application showed a scenario in first person perspective through the HMD where the participant saw a brushstroke stimulation on a virtual hand (Fig. 3). In such environment, participants were head-tracked to show the virtual scene according to the head position and orientation. The position of the hands could be tracked by using the controllers associated to the HMD.

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The VR application was capable of providing a visual and auditory stimulation to the experimenter in order to synchronize his movements to the events of contact and release of the virtual paintbrush (i.e. a metronome). Additionally, it was possible to send a series of commands by serial communication to the PIC board which controls the vibrotactile devices (two for the specific experiments); in this way the vibrotactile devices could provide a tactile stimulation on the participant's real hand with different timings with respect to the virtual brushstroke. The vibrators could be also sequentially activated by the experimenter by using a push-button connected to the PIC board. Each vibrator could be activated to operate at a pre-defined vibration frequency (165 Hz) or deactivated.

During the experiments, the participant sat up in front of an ad hoc built experimental setup. Such structure was composed of a table (dimensions [Length x Width x Height]: 120 x 60 x 70 cm) split into three compartments (dimensions: 40 x 60 x 21 cm) by two dividers. A two-way mirror was placed on top to cover the compartments. Each compartment had its own illumination system granted by a strip of LED lights, so the content of each compartment could be visible to the participant only if the experimenter turned on the relative strip of LEDs by an electrical switch. The dividers between the compartments could be removed and substituted by a mirror of the same size. Such frame was employed to perform the experimental conditions in real environment.

Participants

After signing a written informed consent including the permission for their images threatment, 26 volunteers (12 females, age = 29 ± 3 [mean ± standard error]) naïve to the RHI participated in the study. All participants were healthy and verbally reported to have normal hand sensation and normal, or corrected to normal, vision. Three of them were left-handed. Experiments were conducted according to the Declaration of Helsinki and after approval of the Ethics Committee of Università Campus Bio-Medico di Roma (EMBODY protocol).

Experimental procedure

All the experimental conditions were performed in a within-subject randomized-order design. The rows (*Sight*) and the columns (*Touch*) of the matrix in Fig. 3 represent the two independent variables of the study, and each cell of the matrix corresponds to an experimental condition:

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Sight-Real_Touch-Real, Sight-Robotic_Touch-Real, Sight-Virtual_Touch-Real, Sight-Real_Touch-Virtual, Sight-Robotic_Touch- Virtual, Sight-Virtual_Touch- Virtual.

In all conditions, participants comfortably sat on a chair in front of the experimental platform, with forearms placed inside the two compartments, palm-down oriented. Tactile stimulation was delivered at a frequency of about 1 Hz and the duration was of 0.6-0.7 s. In each condition, the experiment lasted 90 s.

The classical asynchronous RHI procedure was employed as a supplementary (seventh) control condition. It has been selected as it is the one usually employed as standard no-embodiment condition in real environment and it was used as the "lowest common denominator" to remove the baseline embodiment offset related to each participant in each condition, and to compare all the conditions among them. Moreover, it has been chosen to employ a single control in order to reduce the overall number of repetitions of the experimental paradigms. After switching the light on, the experimenter started to stroke with two identical paintbrushes the dorsal surface of the index finger of both the visible fake hand and of the hidden real hand. The fake hand was a left rubber hand matching the participant's gender and placed in the central compartment of the structure at a distance of 15 cm from the left real hand, with the same orientation. Asynchrony was achieved introducing a small temporal delay (0.5 s) between stroking the rubber hand and real hidden hand (Botvinick & Cohen, 1998) (Fig.3).

The *Sight-Robotic_Touch-Real* condition was the synchronous version of the control condition, employing as fake hand a left robotic hand with a rubber cover matching the participant's gender.

In all the *Touch_Real* conditions tactile stimulation was delivered by stroking with a paintbrush the real left hand of the participant.

In all the *Touch_Virtual* conditions tactile stimulation was delivered through two vibrators placed on the proximal and distal phalanx of the index finger (Fig.3) which delivered a vibration matched in time, duration and spatial sequence (proximal to distal) to the visual stimulation.

The *Sight-Real_Touch-Real* condition was achieved by stroking the real, and visible, left hand of the participant. This condition was representative to what extent a subject consider embodied her/his real hand, filtered by the confounding factors that the experimental setup and circumstances can add to that estimation. Despite we are aware that the *Sight-Real_Touch-Real*

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condition is not a perceptual illusion, we thought helpful to add it to the study design because it allows to estimate the maximum values of the chosen measures of embodiment, being the visible hand really part of the tested subject's body. These maximum values were employed to normalize the data of the other conditions.

The *Sight-Real_Touch-Virtual* condition was achieved by stimulating the hidden real left hand with vibrators, while showing to the subject their mirrored right hand that from their point of view seemed to be stroked with a paintbrush. None of the subjects declared that the seen hand was actually their contralateral one. 19 out of 26 subjects were tested for the *Sight-Real* conditions.

All the *Sight-Virtual* conditions were delivered through a HMD (Fig.3) which displayed an ad hoc virtual environment. In particular, during the *Touch-Real* condition the experimenter delivered, with the help of a metronome, a brushstroke stimulation synchronous to the same movement on a virtual hand presented by the system (Fig.3); in this case the system alerted the experimenter by providing a specific sound before the touch of the paintbrush in the virtual environment. Here, the experimenter was trained to deliver the touch stimulation after a fixed time-period from such sound. For the *Touch-Virtual* condition, the stimulation was directly provided by the vibrotactile array. To ensure that in all conditions the visible hand was placed 15 cm medially to the real hand the VR controller was employed to track the position of the real hand in the VR environment. Before the beginning of the experiment, the experimenter positioned the controller over the participant's hand placed still on platform and tracked the hand's position. After such calibration, the participant was asked to not move his/her hand.

Seven volunteers of the group of participants could not participate to the *Sight-Real* conditions, because these conditions were inserted in a later stage of the experiment; however, for both sub- groups of volunteers, the experimental sessions were carried out in two different days, at least a week apart from one another. In case of the sub-group of participants that performed all seven conditions, four conditions were administered in the first session and three in the second one; in case of the other sub-group three conditions were administered in the first session and two in the second one. The order of the experimental conditions was randomized across the subjects and sessions.

Embodiment measures

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The strength of illusion in the different conditions was quantified by four state of art measures (Botvinick & Cohen, 1998; Tsakiris & Haggard, 2005): three outcomes of a self-evaluation questionnaire and the proprioceptive drift (PD).

Questionnaire. Each participant filled-in a questionnaire (one after each experimental condition). The questionnaire included a *statements list* first designed by Botvinick and Cohen (1998) and translated in Italian. A part of the statements measured the illusion level (Illusion statements) and another part measured the suggestibility of the participants (Control statements). The list of the statements was slightly adapted from the traditional statement list, considering the type of visible hand in the different conditions (*Virtual* vs. *Robotic*). In case of *Sight-Robotic* and *Sight-Virtual* conditions there were six control statements. In case of *Sight-Real* conditions, three of the control statements were not applicable (i.e. the ones relative to the appearance of the hands), so, they were not presented (Table I). Participants were asked to rate the extent to which these statements did or did not apply to them by using a seven-point Likert scale (ranged from -3 and 3). The *RHI index*, defined as the difference between the mean of rates of the pooled illusion statements and of the control statements, was calculated for each condition and employed as the illusion measure in the following analyses (Abdulkarim & Ehrsson, 2016). In addition to the statements list, participants were asked to rate *vividness* and *prevalence* of self-attribution of the artificial hand (Armel & Ramachandran, 2003; Botvinick & Cohen, 1998; D'Alonzo & Cipriani, 2012). The vividness was defined as how life-like and realistic the illusion was when it was experienced (rated from 0 to 10). The prevalence rating (from 0 to 100%) reflected the amount of time in which the illusion was experienced.

Number	**Condition***	**Item**
1	Common	It seemed as if I were feeling the tactile stimulation at the location where I saw the visible hand touched
2	Common	It seemed as though the stimulation I felt was caused by the touch on the visible hand
3	Common	I felt as if the visible hand was mine
4	Robotic-Virtual	I felt as if the position of my real hand was drifting towards the alien hand

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	Real	I felt as if the position of my real hand was drifting towards the right side
5	Common	It seemed as if I had more than two hand or arm
6	Robotic-Virtual	It seemed as if the tactile stimulation I was feeling came from somewhere between my own hand and the visible one
	Real	-
7	Robotic	I felt as if my real hand were turning 'rubbery'
	Virtual	I felt as if my real hand were turning 'virtual'
	Real	-
8	Robotic-Virtual	It appeared as if the position of the visible hand was drifting towards my real hand
	Real	It appeared as if the position of the visible hand was drifting towards the left side
9	Robotic-Virtual	The alien hand began to resemble my own hand, in terms of shape, skin tone, freckles or some other visual features
	Real	-

*: Condition: *Sight-Real*: Real, *Sight-Robotic*: Robotic, *Sight-Virtual*: Virtual.

Table I. List of statements for the different conditions.

Proprioceptive drift. It was reported that, after synchronous stimulation, the perceived location of participant's hand shifts towards the rubber hand: this effect has been known as proprioceptive drift (PD). Such PD, as defined by Tsakiris and Haggard (2005), was calculated as the difference between the measurements of post- and pre-stimulation pointing task for each condition. During the pointing task measurement, the participants indicated with their right hand, while closing their eyes, the felt position of the tip of their left second digit. In all conditions, except for the *Sight_Real* one, the visible hand was set 15 cm medially to the stimulated hand, while in the *Sight_Real* conditions, real and visible hand matched. In order to make comparison across conditions, PD in the *Sight_Real* conditions was calculated as if the real hand was 15 cm away the visible hand, thus subtracting to 15 cm the distance between the

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post-stimulation pointing task measure and the actual position of the hand. In such way, a value of proprioceptive drift equal to 15 cm corresponds to correct identification of the position of the visible hand; whereas lower values indicate a discrepancy with the visible hand's actual position.

Embodiment measures analysis

Data from embodiment measures were collected and organized on the basis of the condition. The Shapiro-Wilk test ($p > 0.05$) was used to verify that the data were normally distributed.

To confirm that the recruited subjects were not suggestible or, in other words, that illusion statements would get a higher mean score than the control statements, a two tailed paired t-test, or signed rank test on basis of the data distribution, were employed (Armel & Ramachandran, 2003; Botvinick & Cohen, 1998; D'Alonzo & Cipriani, 2012; Mioli et al., 2018). Signed rank test was employed just for *Sight-Real_Touch-Real*, *Sight-Virtual_Touch-Real* and *Control* condition.

In order to confirm that each experimental condition was different from the typical no illusion control condition (asynchronous RHI), six pre-planned pairwise comparisons were performed on the extracted measures (i.e. RHI index, proprioceptive drift, and vividness and prevalence scores) by means of paired t-test or Wilcoxon signed-rank test when not normally-distributed, and Bonferroni corrected (degree of freedom of the analyses equal to 18 and 25 for Real and the other conditions, respectively). A p-value less than 0.05 was considered as statistically significant. Signed rank test was employed for the comparison of the *Control* condition with all the other ones for proprioceptive and vividness score and just for the comparison with *Sight-Real_Touch-Real* condition for RHI index and prevalence score data.

Once established that the illusion happened in all synchronous conditions, in order to remove any confounding common effect due to the experimental procedure, for all the collected measures and in each participant, the score of the asynchronous control condition was subtracted from the relative score of synchronous condition. The obtained outcomes were defined as *Δ scores.*

The Δ scores were analyzed with a linear mixed-effects model in case of RHI index and proprioceptive drift. Generalized linear mixed model was employed in case of vividness and prevalence scores. For all the analyses, participants were modelled as the random effects factor,

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and type of visual (*Sight:* Real vs. Robotic vs. Virtual) and tactile feedback (*Touch*: Real vs. Virtual) were modelled as the fixed effects factors. Therefore, the data were processed in an ANOVA-like analysis, resulting in a 3 x 2 model design. This has several advantages over the classic, repeated measures ANOVA approach: it allows to effectively fit large and unbalanced data sets (e.g. missing data of the *Sight-Real* conditions in our case) and requires less restrictive assumptions to run the analysis properly.

Nine post-hoc pre-planned pairwise comparisons were performed on Δ scores by compairing all the pairs that maintain one factor-level (e.g. *Touch-Real*) in common and significance was Bonferroni-corrected accordingly because we have no interest in evaluating the embodiment effect by crossing the level between factors.

Principal component analysis

A principal component analysis (PCA) was performed on the normalized Δ scores dataset in order to simplify the analysis and identify a single feature capable to resume the common informative content of the data. Dataset normalization was performed by using z-score transformation. Since PCA is a mean removal process, the offset, calculated as the principal component (PC) score obtained by a null Δ scores vector (i.e. no difference with *Control* condition), was added to the PCA outcomes. The more relevant offset-included principal components (i.e. the first PC of the Embodiment, 1PCE) were analyzed with an ANOVA-like linear mixed-effects model (fixed effects factors: *Sight* and *Touch*; random effects factor: participants).

The values of the 1PCE were employed to quantify the difference on illusion level for the different conditions, in particular between the condition of embodiment of the real limb (i.e. *Sight-Real_Touch-Real* condition) and the other ones.

The dimensionality reduction at the base of the PCA does not resume part of the data variance, thus further analyses based on the extracted 1PCE may not be completely adherent to the one extracted from raw data analyses. However, PCA is among the best options to resume all the collected data in a single value, making further analysis, and consequent findings, more simple and straightforward.

Nine post-hoc pre-planned pairwise comparisons were performed on 1PCE by compairing all the pairs that maintain one factor-level (e.g. *Touch-Real*) in common and significance was

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Bonferroni-corrected accordingly because we have no interest in evaluating the embodiment effect by crossing the level between factors.

A further statistical evaluation was performed in order to assess the effect of concordance between the level of virtualization of visual and somatosensory inputs. Concordance was considered when both the input modalities were physical (real or robotic) or both virtual, discordance when a modality was physical and the other virtual. The concordant conditions (*Sight-Real_Touch-Real*, *Sight-Robotic_Touch-Real*, *Sight-Virtual_Touch-Virtual*) were compared, by means of a paired t-test, to the discordant ones (*Sight-Real_Touch-Virtual*, *Sight-Robotic_Touch-Virtual*, *Sight-Virtual_Touch-Real*). Moreover, in order to exclude the strong effect on embodiment outcomes due to *Sight-Real_Touch-Real* conditions from the analysis, a two-way repeated measures ANOVA (factors: *Sight* and *Touch*) was also employed removing the *Sight-Real* conditions.

The surface of Fig.6 represents the best fit surface of the blue dots, where each single dot represents a participant in each condition. The point for each subject was identified by three coordinates in a 3D space where z-value was the 1PCE, x-value was the marginal effect on the 1PCE of *Sight* pooling together all the levels of factor *Touch* and y-value was the marginal effect of *Touch* pooling together the level of factor *Sight*. The marginal effect is the effect on the dependent variable of one factor pooling together all the levels of the other factor (e.g. the marginal effect of *Sight-Real* condition for each subject was equal to the mean 1PCE value of *Sight-Real_Touch-Real* together with *Sight-Real_Touch-Virtual* conditions for that particular subject). The data were fitted on polynomial curves (maximum tested order equal to 5). Several factors were taken into account for the selection of fit curve. The selected curve, besides having a normal distribution of the residuals, has the best trade-off between the goodness of fit (i.e. coefficient of determination R^2) and the polynomial order employed in curve model. In particular, the curve with a small number of polynomial coefficients (less than 15) and the highest value of goodness of fit was selected.

3. Results

Embodiment measures analysis

The group of 26 tested subjects were found to be not-suggestible (questionnaire illusion statements mean rate vs questionnaire control statements mean rate: $p < 0.001$). The illusion

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arised in all six experimental conditions, since all the collected measures (i.e. *RHI index*, *vividness*, *prevalence* and *proprioceptive drift*) were significantly higher than in the control condition of typical asynchronous RHI stroking, as highlighted by the pre-planned pairwise comparisons (*RHI index, vividness and prevalence*: $p < 0.001$; *proprioceptive drift*: $p < 0.05$) (Fig.4a).

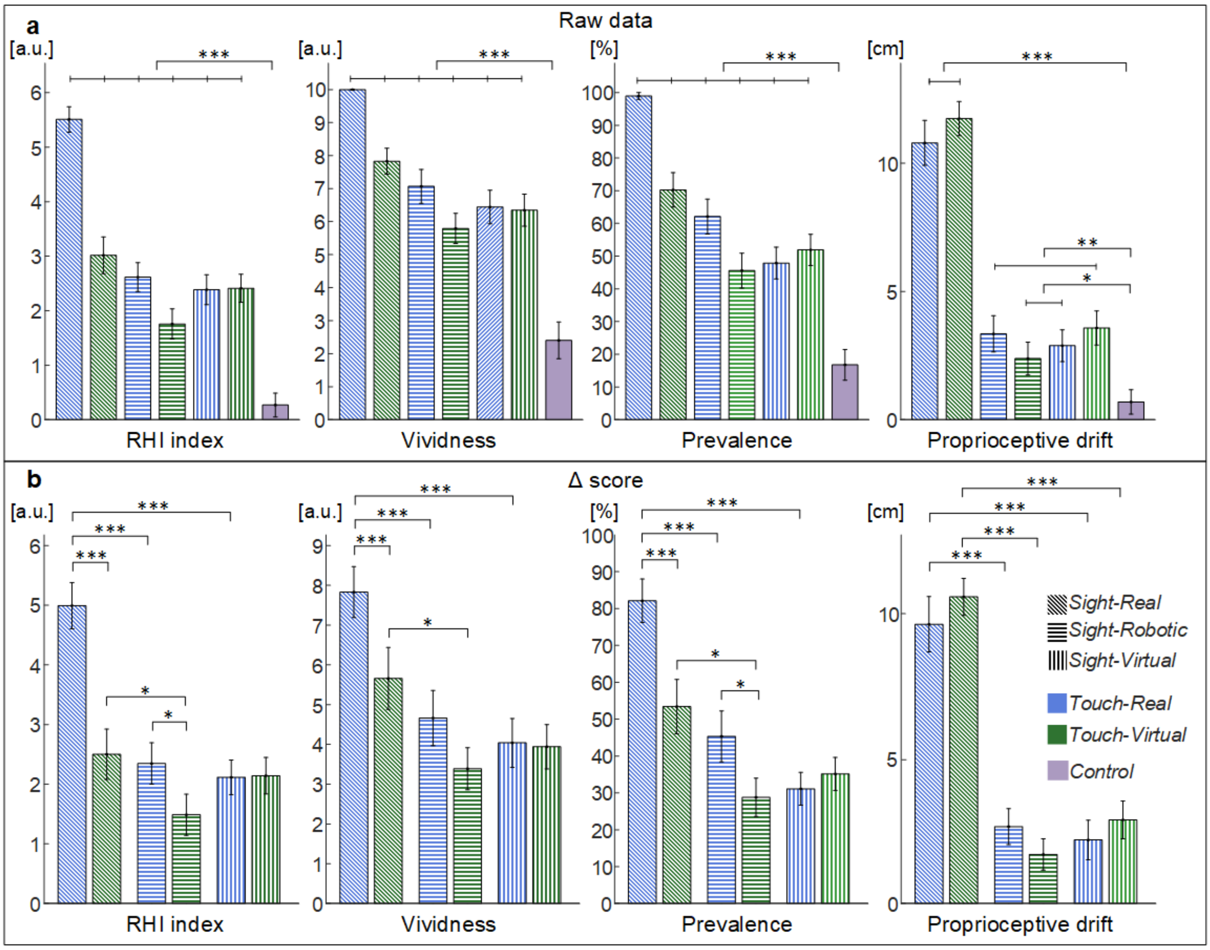

Figure 4. a) Values (mean ± sem) of the RHI index (difference between the means of pooled illusion statements and control ones), vividness, prevalence rating and proprioceptive drift for different conditions. The horizontal lines indicate a statistical difference between a synchronous condition and the asynchronous one. b) Δ scores (mean ± standard error) of the RHI index (difference between the means of pooled illusion statements and control ones), vividness, prevalence rating and proprioceptive drift for all the different conditions. Different fill patterns of the bars were employed to differentiate the degree of Sight virtualization, whereas different colors differentiate the degree of Touch virtualization. * indicates a p-value < 0.05; ** indicates a p-value < 0.01; *** indicates a p-value < 0.001.

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Both the virtualization of visual and somatosensory feedback decreased all the collected measures of the embodiment of the hand. Considering each Δ score measure (difference between condition and control in each subject) independently, there were main effects of the virtualization of sight and of touch, and the effect of their interaction, for the outcome of the *RHI index* (*Sight:* $F_{2,136}$= 27.8, $p < 0.001$; *Touch:* $F_{1,136}$= 34.1, $p < 0.001$; interaction: $F_{2,136}$= 23.2, $p < 0.001$), *vividness* (*Sight:* $F_{2,136}$= 11.5, $p < 0.001$; *Touch:* $F_{1,136}$= 12.0, $p < 0.01$; interaction: $F_{2,136}$= 6.0, $p < 0.01$) and *prevalence* of the illusion (*Sight:* $F_{2,136}$= 18.2, $p < 0.001$; *Touch:* $F_{1,136}$= 15.0, $p < 0.001$; interaction: $F_{2,136}$= 10.8, $p < 0.001$); only the main effect of the virtualization of *Sight* was identified for the *proprioceptive drift* (*Sight:* $F_{2,136}$= 55.8, $p < 0.001$; *Touch:* $F_{1,136}$= 0.3, $p = 0.58$; interaction: $F_{2,136}$= 2.1, $p = 0.07$) (Fig.4b).

Principal component analysis

The results were very consistent among the four collected dependent measures (Botvinick & Cohen, 1998; M. R. Longo, Schuur, Kammers, Tsakiris, & Haggard, 2008), so that we decided to merge those measures with a PCA algorithm for dimensionality reduction. The resulting first component (Principal Component of Embodiment: 1PCE), which explained 61% of the variance of all the four measures, was a single parameter comprehensive of self-reported and behavioural measures of the RHI (Abdi & Williams, 2010).

The 1PCE was robust so that calculating it pooling all the conditions together and calculating it in each condition separately did not significantly change its correlations with the four collected measures ($p > 0.05$) (Fig.5a) (see supplementary material).

The virtualization of both visual and somatosensory input significantly affected such parameter (*Sight:* $F_{2,136}$= 36.2, $p < 0.001$; *Touch:* $F_{1,136}$= 21.0, $p < 0.001$; interaction: $F_{2,136}$= 13.9, $p < 0.001$) (Fig.5b).

Considering the 1PCE achieved in the condition of both real inputs (*Sight-Real_Touch-Real*) as full-scale value (i.e. 100%), embodiment decreased to 50% (*Sight-Robotic_Touch-Real*) and 40% (*Sight-Virtual_Touch-Real*) with the virtualization of visual input, and from 72% (*Sight-Real_Touch-Virtual*), to 33% (*Sight-Robotic_Touch-Virtual*) and 43% (*Sight-Virtual_Touch-Virtual*) with the virtualization of touch (Fig.5c). All the tested conditions were significantly different from the condition of both real inputs (*Sight-Real_Touch-Real*) (pre-planned post-hoc analysis $p<0.001$), and one from the other, except for the difference between *Sight-*

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Virtual_Touch-Real and the two contiguous conditions (*Sight-Robotic_Touch-Real* and *Sight-Virtual_Touch-Virtual*), whereas the difference between *Sight-Robotic_Touch-Virtual* and *Sight-Virtual_Touch-Virtual* did not overrun Bonferroni correction (Fig.5b).

Surprisingly, the two main factors *Sight* and *Touch* showed a significant interaction ($F_{2,136}$= 13.9, $p < 0.001$), exhibiting a trend change; in particular, *Virtual-Touch* condition showed a significant decrease of the embodiment level in both *Sight-Robotic* and *Sight-Real* conditions; whereas in *Virtual-Sight* condition the difference between *Virtual-* and *Real-Touch* was not significant. Conversely, the mean value of embodiment when both inputs were virtual was higher than the two contiguous conditions. This suggested us to test whether the concordance between the level of virtualization of visual and the level of virtualization of somatosensory inputs had a significant impact on the avatar's embodiment. Concordance of inputs had a strong main effect considering all conditions ($p < 0.001$), and even considering all but the *Sight-Real* conditions ($p < 0.01$), to exclude that the effect would have been due only to the high level of embodiment achieved in this condition (Fig.5d).

It is worth noting that, considering only the *Sight-Robotic* and *-Virtual* conditions, there was not significant main effect of *Sight* and *Touch*, but only a strong interaction between them ($F(1,25) = 9.7$, $p < 0.01$). Similar findings were also obtained by applying such analysis to the typical illusion outcomes: there is a strong interaction for RHI index, vividness and prevalence scores. This means that the presence of a statistically significant interaction persists when considering all but the Sight-Real conditions. It suggests that the effect of the concordance in degree of virtualization is not due to the Sight-Real conditions.

Thus, the decrease of embodiment (1PCE) was due to both virtualization of inputs and discordance between the level of their virtualization. In order to highlight this phenomenon beyond the effect averaged across subjects, each participant in each condition was represented as a point in a 3D space, being the dependent variable (z-value) the 1PCE and the other two coordinates the relative contribution of each single factor (*Sight* and *Touch*) on the measured effect. The points were fitted on a polynomial curve quartic in x coordinate and cubic in y one ($R^2 = 0.80$). The fitted surface had valley shape with a decrement and a local minimum within the conditions *Sight-Robotic_Touch-Virtual* and *Sight-Virtual_Touch-Real* and an increment with a local maximum within the condition *Sight-Virtual_Touch-Virtual* (Fig.6).

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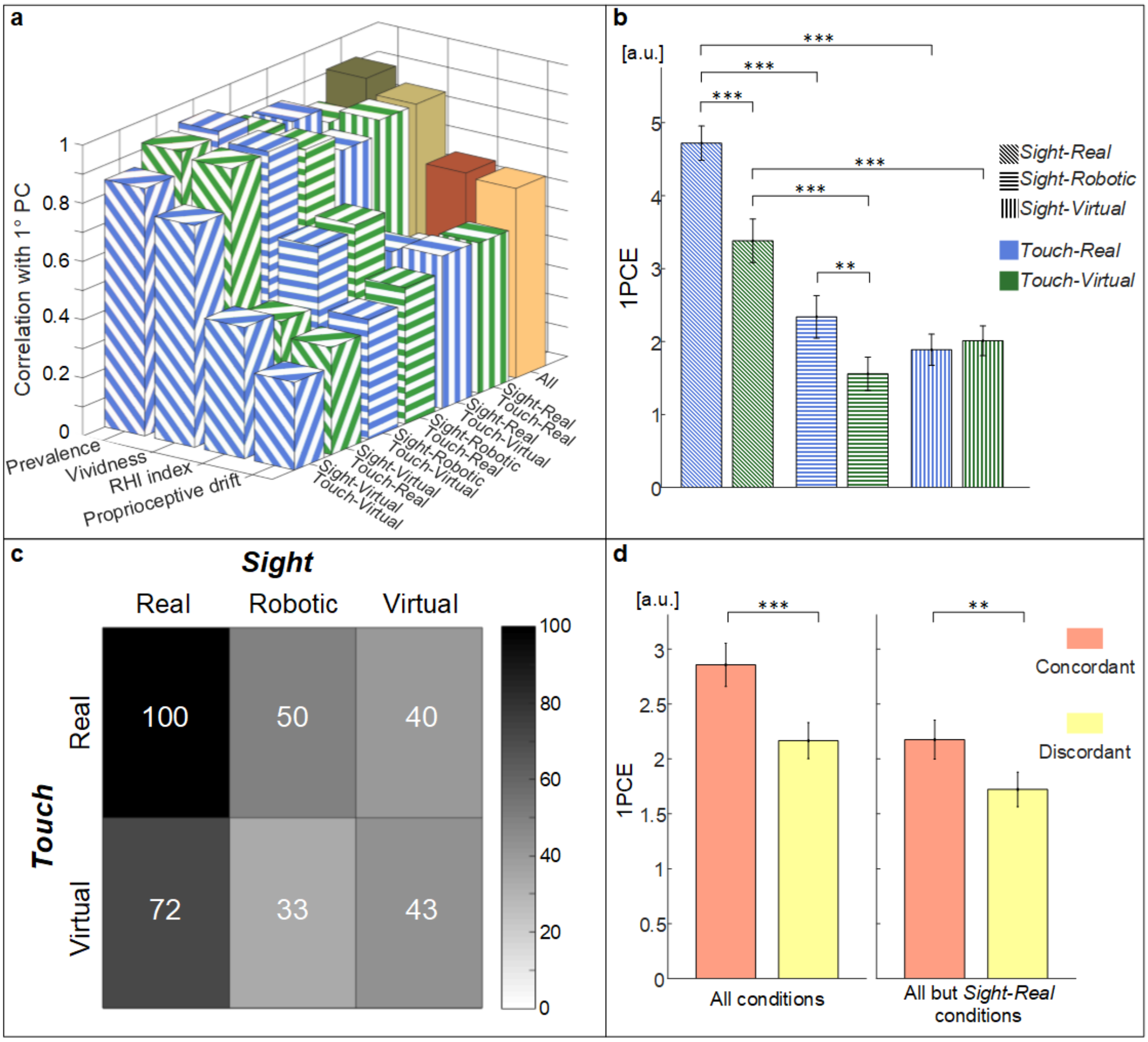

Figure 5. a) Correlation coefficients between the four measures and the first component of the PCA calculated pooling all the conditions together (solid colored bars), and independently for each condition (fill patterned bars) b) 1PCE (mean ± sem) for the different conditions. The horizontal lines indicate a statistical difference between conditions highlighted by the pre-planned pairwise comparisons.c) Mean percentage of 1PCE for different conditions with respect to the standard embodiment of a limb (Sight-Real_Touch-Real). d) 1PCE (mean ± sem) of the pooled data of concordant and discordant conditions. In the top panels (a and b) different fill patterns of the bars were employed to differentiate the degree of Sight virtualization, whereas different colors differentiate the degree of Touch virtualization. ** indicates a p-value < 0.01; *** indicates a p-value < 0.001.

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4. Discussion

This study was designed to assess the relative weight of the virtualization of visual and somatosensory inputs in the embodiment of a robotic or virtual avatar, and in particular of the hand. This may have several possible applications, such as in gaming or in the development of future prosthetic hands which employ sensory substitution feedback.

We reckon that the embodiment is a more direct mean to assess the realness and human-likeness of an avatar, and of the easiness of interaction, than simple subjective acceptance of the avatar. Having knowledge of these relative weights is fundamental in order to design easily embodiable devices and applications in virtual environment and in robotics, and to better understand humans behaving through them.

The main hypothesis of the study was that the progressive virtualization of visual and tactile stimuli, along a scale that goes from completely real to completely virtual stimuli, would have decreased avatar hand embodiment.

This is the first time that embodiment in the RHI is directly compared with embodiment in the VHI and, moreover, the first time that they are compared to the standard embodiment of an owned limb (i.e. Real vision and consistent tactile feedback: *Sight-Real_Touch-Real* condition).

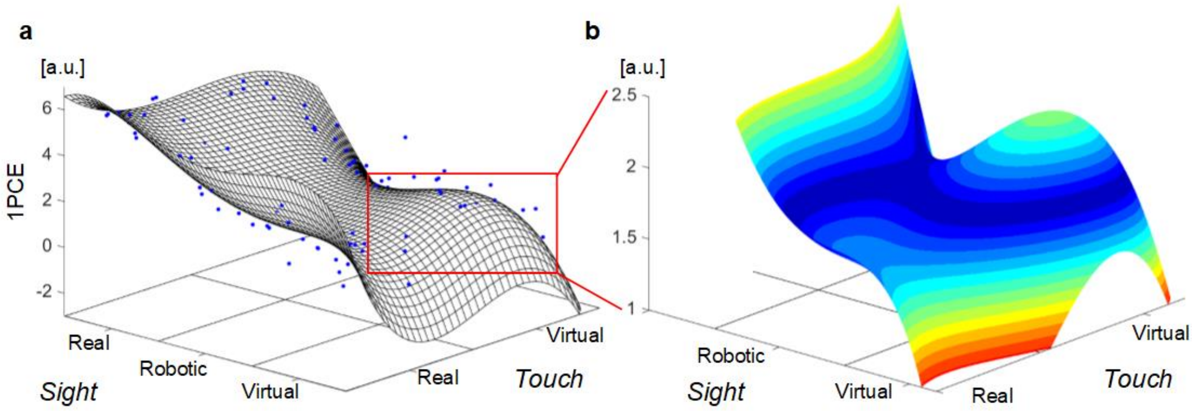

Figure 6. a) Best fit surface (R2 = 0.80) of the number of points that represent each participant in each condition. The points were identified by three coordinates in a 3D space where z-value was the 1PCE, x-value was the marginal effect on the 1PCE of Sight and y-value was the marginal effect of Touch. The marginal values were scaled as x and y coordinates to fit their distribution within the 2D spatial square (the black lines on the x-y plane) of the relative condition. b) Particular of the valley: the different colors indicate different level of 1PCE.

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A certain level of embodiment was present in all the real, robotic and virtual condition, thus our results confirm that embodiment can be induced through sensory substitution and in a virtual environment. However, the depth of embodiment differed across conditions, suggesting that the use of the VHI as an alternative to the RHI should be done with caution.

As expected, all the participants reported higher values of the four collected measures in *Sight-Real_Touch-Real* compared to all the other conditions, justifying our attempt to use this condition to derive the full-scale value for the collected measures in our experimental setup and circumstances. Indeed, the impact of experimental setup and circumstances led some participants not to report the maximum rating in some illusion statements (S1 and S2) and in *prevalence*, because they did not immediately realize that the stroked visible hand was truly their own.

Despite the four collected measures are particularly sensible to different aspects of the embodiment (i.e. intensity of sensation for vividness, continuance of the sensation for prevalence, depth of the illusion with respect to the suggestibility of the subject for RHI index and spatial update of sense of hand's position for proprioceptive drift), they manifested the same trend of change across all the conditions. Indeed, the correlation analysis between the four measures and the first component of the PCA calculated pooling all the conditions together, and independently for each condition was not different. This is in support of the robustness of the employed experimental design and of the use of the 1PCE as single derived parameter to describe the embodiment.

In the RHI, a tricky match between sight (i.e. the brushstroked rubber hand) and somatosensory feedback (i.e. perceiving the real hand brushed) co-exists with several possible mismatches, such as the one between the seen position of the rubber hand and the felt position of the real hand. The extent of the illusion depends upon how much the induced false match overwhelms the mismatches. In this study, different conditions corresponded to different modulation of the mismatch between seen and felt stimuli, so that the changes in embodiment may be interpreted in such framework.

However, our 3x2 experimental matrix was specifically designed to modulate the adherence of the sensory feedback to the physical reality, thus a more accurate framework of interpretation of our results is the one which links them to changes of virtualization of visual and somatosensory feedback, which was the main hypothesis of this work. The virtualization

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significantly decreased embodiment, both when the scores of the questionnaire, prevalence, vividness and proprioceptive drift were analysed separately or condensed in the 1PCE. Moreover, we found one unexpected result. The decrease of embodiment due to the virtualization of *Sight* and the one due to the virtualization of *Touch* are not independent one from the other, and they even seem to interact in a bimodal way, or at least their rate of decrease does. Specifically, the loss of embodiment due to the virtualization of *Sight* is tempered if also *Touch* is virtualized and *viceversa*. Concordance between the levels of virtualization of the two sensory pathway matters; indeed, this factor was found to be significant.

We attributed changes of embodiment to the progressively virtualization of visual and sensory inputs; however the assessment of virtualization we employed may have been affected by the fact that we did not assess a continuous scale of virtualization, but only six scenarios, and we cannot guarantee that they were separated by steps of equal magnitude.

Despite such limit, it can be speculated that the more a sensory modality is virtualized, the more the embodiment process is robust against virtualization of the other sensory modality, as if the subject does not notice it; in other words, the more a sensory input (e.g. the appearance of the environment and hand's aspect) is close to reality the more a mismatch between the seen and felt tactile stimulation is perceived. A similar result was previously reported with the 2 dimensional/ 3 dimensional conflict: a 2D projected image of hand brushed by a real 3D paintbrush elicited a milder illusion than a projection of a 2D video of a brushed hand (IJsselsteijn et al., 2006).

A possible explanation of the reason why the virtualization of both sight and touch (*Sight-Virtual_Touch- Virtual*) seems to show a higher level of embodiment with respect to the discordant conditions, expecially the *Sight-Robotic_Touch-Virtual* one, may be that such condition is the one commonly employed in virtual applications, thus participant may be familiar with it. However, we think this unlikely because participants' group was eterogeneous and more than half of them was naïve for immersive VR.

The bimodal trend linking virtualization of visual and somatosensory inputs with embodiment of virtual and robotic avatar hands is in line with the *uncanny valley* that has been described between appearance and subjective acceptance of robots (Mori et al., 2012) and of virtual avatar (Jerald, 2017). Indeed, when we spread the mean of 1PCE in each condition into a multitude of points each one representing a participant, and we separated the contribution of the two factors

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(Virtualization of *Sight* on the x-axis and virtualization of *Touch* on the y-axis), the best fit surface clearly shows a region of depression of the effect (i.e. a valley), located within the discordant (*Sight-Robotic_Touch-Virtual* and *Sight-Virtual_Touch-Real)* conditions, surrounded by a region with higher effect.

The concept of the uncanny valley suggests that the relationship between the degree of an object's resemblance to a human being and the emotional response to the object increases with the object's human likeness, but when humanoid objects appear almost, but not exactly, like real human beings they elicit uncanny, or strangely familiar, feelings of revulsion in observers. Recently, the valley has been also shown for subjective experience in virtual reality when haptic feedback cannot be justified by what the subject sees on the display (Berger, Gonzalez-Franco, Ofek, & Hinckley, 2018).

Therefore, this work extends the concept of the uncanny valley beyond avatar's appearance to cover the embodiment of avatars, and beyond a single sensory modality, either vision or touch, towards the concordant vs discordant interaction of the two in matching seen and felt stimuli to build the body image.

Self attribution arises from a coherent match of visual and somatosensory incoming information. Hitherto, big efforts have been spent to identify in the real world the constraints of such coherency, thus for a successful embodiment; time (Shimada et al., 2009), spatial (Tsakiris & Haggard, 2005) and appearance (Tieri et al., 2015; Tsakiris, Carpenter, James, & Fotopoulou, 2010) constraints have to be satisfied to allow the illusion to arise. Going beyond the real world in a virtual or transposed scenario, our findings for the first time candidate the match between the degree of virtualization of seen and felt stimuli as a further constraint in the embodiment process.

It can be speculated that, in order to be immersed in a virtual visuo-haptic environment, our brain changes focus of input modality and begins to increase trustiness on both visual and tactile virtual sensory inputs, while reducing its trustiness in real feedbacks or perceiving as annoying the mismatch. Thus, contrary to what hypothesized when we planned the study, the amount of realism of a visuo-tactile sensory feedback is not an absolute value in building the representation of the body, and the coherent match between the degree of virtuality needs to be taken into account as a determinant factor. Coherent virtualization of different sensory

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pathways may be crucial also for developing easily embodiable devices and for re-personalizing depersonalized behaviours.

The valence of the results of our study may have been affected by some choices made in experimental procedure:

i. We included only a single common control condition based on asynchronous tactile stimulation of the rubber hand. This has been done to reduce the length of the experimental sessions and preventing the progressive lack of novelty.

ii. We tested two conditions where the real hand was stimulated, which of course does not induce a proper perceptual illusion. Testing the real hand stimulation has been done in order to have a full scale measure of the reachable level of embodiment. To implement such conditions we had to delete three control items of the questionnaire and how we evaluated the proprioceptive drift. Moreover, when the touch was virtual we employed a mirror to show a fake brush-stroking while the real hand was instead stimulated by vibrators. It's worth to note that the type of interaction between Sight and Touch factors, that is the main finding of our work, still persists if the Sight- Real conditions were not analyzed.

iii. We employed currently-available VR system and vibrotactile stimulators. The virtualization of tactile stimulation achievable with vibrators lacks the richness of tactile details proper of brush-stroking, as well as the richness of graphic reproduction of real objects is still limited by the performance of the present technology. In the next future, the daily improving of 3D graphics rendering and haptics technology may be able to modify the richness of the virtual experience and our findings.

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Chapter 3: Modulation of body representation impacts on efferent autonomic activity

1. Introduction

Different senses, such as sight and somatosensation, inform on the state of the body. Sensory information is persistently present and updated and exploited to feed a particular perceptual status to feel part of the body as self (Blanke, 2012; Gallagher, 2000), constituting a fundamental aspect of self-awareness.

The constitution of a body representation is mediated by the correlations among coherent multimodal sensory afferences (Botvinick & Cohen, 1998; Rochat, 1998; Van Den Bos & Jeannerod, 2002); for example, the self-attribution of a visible hand is highly dependent on the match between the multimodal afferent somatic signals and the corresponding visual feedback. The Rubber Hand Illusion (RHI) is a body ownership illusion whereby congruently stroking a rubber hand and one's hidden hand while observing the artificial limb, produces the illusion that the rubber hand is part of the one's body (Botvinick & Cohen, 1998). Such paradigm, which has been broadly employed as a tool to study the sense of body ownership and brain plasticity (Di Pino, Maravita, et al., 2014), seems to be heavily modulated by interoception (Seth, 2013).

Indeed, who has worst ability to detect their heartbeat experience stronger RHI, suggesting that interoceptive sensitivity is predictive of the malleability of the body representation (Tsakiris, Tajadura-Jimenez, & Costantini, 2011). Moreover, the ownership of a virtual hand has been shown to be enhanced when it synchronously changed color with the participant's heartbeat; when experiencing the ownership of a body part, exteroceptive and interoceptive multisensory integration matters (Suzuki, Garfinkel, Critchley, & Seth, 2013).

Thus, to experience the body as self, besides exteroception and proprioception, the brain takes into account also interoception; i.e. the state of the inner organs based on cues led by an afferent pathway of the autonomic nervous system (ANS) (Craig, 2009; Tsakiris et al., 2011).

The autonomic system is structured as *afferences-CNS node-efferences* reflex loops, as it becomes particularly evident in dysautonomic disorders where it is upregulated (Reichgott, 1990).

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The application of such functional organization to the process of building the body representation, would suggest that manipulation of the body representation, due to the modulation of interoception, as well as due to the modulation of other sensory afferences as in the RHI paradigm, should in turn affect the output of the efferent pathway of ANS.

The skin conductance (SC), a physiological measure of autonomic arousal, is the expression of the electrical conductivity of the skin which depends on the moisture due to the activity of the sweat glands. Indeed, besides being induced by thermoregulation, secretion can be triggered also by emotional stimulation.

Event related skin conductance response (ER-SCR) is due to a specific eliciting stimulus, such as a sudden handclap. Physical threats to an embodied artificial hand evoke enhanced ER-SCR (Armel & Ramachandran, 2003); this procedure has been widely used to study and evaluate the strength of the RHI (Armel & Ramachandran, 2003; Petkova & Ehrsson, 2009; Tsuji et al., 2013; Yuan & Steed, 2010). However, the menace-induced ER-SCR may be biased by participants' prior experiences which give different emotional valence to different threatening stimuli (e.g. a hammer, a knife, a screwdriver etc.) (Ma & Hommel, 2013). Moreover, the menace can induce movements of the tested hand (Kilteni, Normand, Sanchez-Vives, & Slater, 2012), which may invalidate the further collection of the proprioceptive drift.

The non-specific skin conductance response (NS-SCR) is another information that can be inferred by the skin conductance; i.e. the fast fluctuation of the signal, not time-locked with an evoking stimulus, but due to a circumstance which has an influence on one's general arousal (Braithwaite, Watson, Jones, & Rowe, 2013).

Considering that body representation is influenced by the ANS, and that NS-SCR is a measure of the autonomic outflow, the hypothesis of this study is that changes of the body representation would affect the ANS efferent branch and can be revealed by alterations of NS-SCR. To make a long story short, the brain process involved in the building of body representation, tested with the RHI paradigm, would be accompanied by changes of the NS-SCR.

However, despite NS-SCR is a simple, cost-effective and easy-to-use physiological measure and although it has been found to be altered during challenging stressors such as cognitive or behavioral tasks (Dawson, Schell, & Filion, 2007), it has never been employed to evaluate the embodiment of the rubber hand.

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In the RHI, the effectiveness of the illusion depends upon the spatiotemporal congruency of the visuo-tactile stimuli. Indeed, the RHI does not occur when the rubber hand and the participant's own hand are asynchronously stroked (Botvinick & Cohen, 1998) or when the rubber hand is not placed in a position congruent with participant's first perspective (Tsakiris & Haggard, 2005). These spatiotemporal constraints are exploited to build the two most common RHI control conditions.

To test the hypothesis that embodiment could affect NS-SCR, we employed the RHI and compared NS-SCR collected during synchronous brush-stroking stimulation with NS-SCR during asynchronous stimulation (Experiment A) (Botvinick & Cohen, 1998) and with NS-SCR collected during synchronous brush-stroking, but with the fake hand placed in a incongruent position (Experiment B) (Tsakiris & Haggard, 2005). Additionally, in order to assess the persistence of the effect of the embodiment on NS-SCR across multiple presentations of the RHI paradigm, we performed two iterations of synchronous and asynchronous RHI conditions on a new naïve group of participants (Experiment C).

2. Materials and methods

Participants

Three RHI experiments were performed: ExpA (synchronous vs asynchronous brushstroke), ExpB (congruent vs incongruent position) and ExpC (two iterations of synchronous vs asynchronous brushstroke).

37 volunteers took part to ExpA (age: 29 ± 5; 18 female; 33 right-handed as by self-report); 18 volunteers took part to ExpB and ExpC (ExpB - age: 26 ± 4; 10 female; ExpC - age: 25 ± 3; 11 female; all right-handed in both experiments as by self-report). Exp A was the first to be performed and completed; ExpB was run to test the repeatability of ExpA findings with an additional control condition. ExpC was run to test the persistence of the illusion after multiple repetitions of the experimental conditions. Considered the effect size calculated on the extracted SC index from ExpA (≥ 0.49), and a medium statistical power of about 0.5 (two tailed test and alpha value equal to 0.05), we enrolled 18 participants for ExpB and ExpC.

Participants, naïve for the study, did not report to be affected by any neurological impairment and claimed to have normal hand sensation and normal, or corrected to normal vision.

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Participants were enrolled after having signed a written informed consent and experimental procedures were approved by the Ethics Committee of the Università Campus Bio-Medico di Roma (EMBODY protocol) and carried out according to the Declaration of Helsinki.

Experimental procedure

Participants were placed in front of a custom-made experimental set-up, made of three parallel compartments covered by a two-way mirror. They could see the content of each compartment only when the experimenter turned on the internal light (Mioli et al., 2018).

Then, participants were invited to place their forearms inside the two lateral compartments while their shoulders were covered by a black cloak. A left rubber hand that matched the participants' gender, was placed in the central compartment of the structure 15cm apart from the real hidden left hand of the subject. The left hand was tested because it seems the side where the RHI is easier to arise (Ocklenburg, Rüther, Peterburs, Pinnow, & Güntürkün, 2011). A well-trained experimenter used two identical paintbrushes to stroke both the second digit of the rubber hand and the corresponding digit of the real hidden hand. The tactile stimulation was delivered at a frequency of about 1Hz, the brushstroke duration was about 0.6-0.7s, and it was delivered from the proximal to the distal phalanx.

In the asynchronous control condition (ExpA and ExpC) a small temporal delay (about 0.5s) was added between the stimulus delivered on the rubber hand and the one delivered on the real hand, while in the incongruent control condition (ExpB) the rubber hand was placed at 90° with respect to the real hand's orientation and the brushstrokes were synchronously administered (Fig. 7).

For each subject, in each condition, the physiological measures of skin conductance (SC) and the electrocardiogram (ECG) were recorded during the whole period of stimulation, along with the most typical and validated measures of the illusion: the responses to the self-evaluation questionnaire and the proprioceptive drift (Botvinick & Cohen, 1998). ECG recording was added to obtain the participants' heart rate variability, that is another index related to autonomic reactivity, generally dependent to both branches of the ANS (Bradley & Lang, 2000; Kreibig, 2010). In such way, it has been attempted to disentangle the single contribution of the two branches of the autonomic system.

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In ExpA and ExpB, before the brushstroke stimulation, the participant had to verbally report a number on a measuring tape reflected on the two-way mirror that corresponded to the perceived location of his/her left index finger by maintaining the hands still and relaxed. The measuring tape had the possibility to slide so as to select a random offset before every assessment. The experimenter turned on the light in the central compartment, making the rubber hand visible, and started to stroke the participant's hand and the rubber hand with the paintbrushes. After this phase, the experimenter switched off the light of the central compartment and asked the participant to repeat the estimation of his/her hand's location. Post minus pre stimulation positive differences of the estimated position of the hand indicate a drift of the perceived location of the real hand towards the rubber hand.

Subsequently, the experimenter handed to the participant a 9-item questionnaire (Botvinick & Cohen, 1998), aimed at investigating the extent of the self-attribution of the rubber hand (Table II). The participants were asked to rate the extent to which these items did or did not apply to them, using a seven-point scale. On this scale, -3 meant "absolutely certain that it did not apply", 0 meant "uncertain whether it applied or not", and +3 meant "absolutely certain that it applied". Such questionnaire was provided with two additional items to rate the vividness (0 - 10) of the perceived illusion (i.e. how realistic the illusion was when it was experienced) and the prevalence (0 - 100%), which reflected the percentage of time that the illusion was experienced (i.e. how long with respect to the length of section the perception of the illusion was).

Questionnaire	**Item**	**Rating**
Statement 1	It seemed as if I were feeling the tactile stimulation at the location where I saw the visible hand touched	
Statement 2	It seemed as though the stimulation I felt was caused by the touch on the visible hand	-3 - +3
Statement 3	I felt as if the visible hand was mine	
Statement 4	I felt as if the position of my real hand was drifting towards the visible hand	

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Statement 5	It seemed as if I had more than two hand or arm	
Statement 6	It seemed as if the tactile stimulation I was feeling came from somewhere between my own hand and the visible one	
Statement 7	I felt as if my real hand were turning 'rubbery'	
Statement 8	It appeared as if the position of the visible hand was drifting towards my real hand	
Statement 9	The visible hand began to resemble my own hand, in terms of shape, skin tone, freckles or some other visual features	
Vividness	How realistic and life-like was the illusion that the visible hand was yours when it was experienced?	0 - 10
Prevalence	How long with respect to the length of section the perception of such illusion was?	0 - 100%

Table II: List of items of the questionnaire

SC and ECG were recorded (Biopac MP160, Biopac, Goleta, USA) all along the stroking time (90 seconds) with Ag-AgCl electrodes placed on the fingertips of the second and third digit of the right hand in case of SC signal, and near the left (active electrode) and right (reference) mid-clavicular line below the clavicles and at level of the left ankle (ground) in case of ECG. SC, which is modulated by a systemic response, was recorded from the right hand, contralateral to the tested hand, so that electrodes did not influence the testing environment.

In case of ExpA and ExpB, the order of the experimental and control conditions was randomized among participants. The overall experimental session lasted about 15 minutes, including the placement of electrodes, the time to fill in the questionnaires and a 2-minute break to relax between conditions. In case of ExpC, two consecutive iterations of the synchronous and asynchronous condition were performed on the same participant (Iteration1 – trial 1 and trial 2, and Iteration2 – trial 3 and trial 4, for a total of 4 trials). The synchronous vs asynchronous order in both iterations was full-randomized across participant. In this case, the overall experimental session lasted about 20 minutes. The ExpC was performed to assess the contribute of novelty in the effect of the RHI on NS-SCR.

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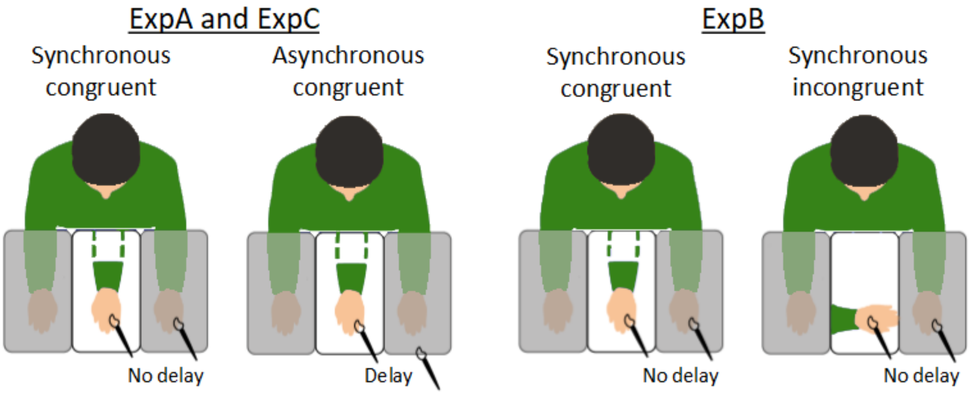

Figure 7: Schematic illustration of the experimental conditions.

Data analysis

The SC signal was band pass filtered (0.05 - 2Hz) to eliminate the signal offset and the high frequency noise (Braithwaite et al., 2013).

The standard deviation of NS-SCR was extracted from the filtered SC signal to estimate signal variability (Bach, Friston, & Dolan, 2010; Boucsein et al., 2012).

Since SC signal is highly affected by inter-individual variability and by the circumstance on which the experiment is run (e.g. the room temperature). To minimize the impact of those factors on the data, the value of the non-specific Skin Conductance Standard Deviation (SCSD) was normalized on each subject, by dividing the individual SCSD computed on the trial of interest by the standard deviation computed on his/her entire recording session (both illusion and control condition together).

The standard deviation of the inverse of the time between two contiguous R peaks of the ECG signal has been calculated as the index of heart rate variability (HRV) for each trial and each participant.

The Kolgomorov-Smirnov test ($p > 0.05$) was used to verify that the data were normally distributed. On the basis of data distribution, the more adequate statistical test was selected for the following analysis (paired t-test or Wilcoxon signed rank test).

To verify that the results of the questionnaires were not due to subjects' suggestibility, the mean score of the three items employed to measure the illusion was compared against the mean score

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of the six items that served as control for compliance, suggestibility, and ''placebo effect'', by using a two-tailed paired t-test on the basis of data distribution.

Then, the RHI index, expressed as the difference between the mean score of the illusion items and the mean score of the other ones (Abdulkarim & Ehrsson, 2016), was calculated for each condition and employed as illusion outcome in the following analyses.

All data except for the vividness and prevalence scores in ExpA and vividness scores in the second iteration of Exp C were normally-distributed.

Normally-distributed data were analyzed with paired t-tests to highlight differences between illusion conditions (synchronous congruent one) and control conditions (asynchronous or incongruent one) both for the already validated RHI outcomes (RHI index, vividness, prevalence score and proprioceptive drift) and for the hypothesized ones (SCSD and HRV).

Wilcoxon signed rank test was employed to compare the illusion condition to the control one for the data with a non-normal distribution.

For normally- distributed data the effect size (d) was calculated as Cohen's d, whereas, in the other case, effect size (r) was calculated as $z/\sqrt{n}$, where z is test statistic for signed rank test, and n is the number of observations (Rosenthal, Cooper, & Hedges, 1994).

For each subject in each condition (i.e. illusion and control conditions of both ExpA and ExpB), the SCSD calculated on the entire trial duration was correlated to the validated measures of illusion (i.e. RHI index, vividness, prevalence scores and proprioceptive drift). The results of this correlations were indicated with $\rho_{tot.}$

Additionally, to identify the optimal onset and length of the time-window suited to highlight differences between illusion and no illusion conditions, a series of statistical comparisons (paired t-test) was performed for SCSD between illusion and control conditions (pooling together data from both ExpA and ExpB), calculated for 120 different time-windows with window onset (ton) ranging between 0 and 70s (step of 5s) and window length (Boucsein et al.) ranging between 20 and 90s (step of 5s). The minimum value of window length (20s) was selected on the basis of the dynamic features proper of the SCR peaks (frequency of the NS-SCR peaks ranged between 0.05 and 0.5Hz) (Braithwaite et al., 2013). The statistical comparisons were corrected with Bonferroni adjustment. The p-values obtained for the different time-windows were employed as intuitive index of the difference between illusion and

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control condition (i.e. the lower the p-value the higher the difference). Additionally, to monitor the time-behavior of SCSD with a high time-resolution, in order to identify the most informative time-interval (lowest p-value), a statistical comparison between illusion and control conditions was performed on the values calculated on the shortest meaningful window (20s length), moving in time with a 1s step.

The correlation analysis between the SCSD and the validated measures of illusion was also implemented for each of those 120 time-windows and the time-window (onset and duration) showing the maximum correlation value (ρ_{max}) was identified for each measure.

A frequentist approach has been used to assess whether ρ_{max} and ρ_{tot} are significantly different and whether the ρ_{max} and ρ_{tot} of SCSD with RHI index, vividness, prevalence scores and proprioceptive drift are significantly different among them. The difference is considered significant if the confidence interval of the distribution computed with a percentile bootstrap does not comprise the zero value (Wilcox, 2009).

All the analyses were performed by using *Matlab* software (*R2015a*).

3. Results

Experiments A and B

In the synchronous condition of both experiments, the mean value of illusion items was higher than the mean value of the ones that served as control items ($d = 1.82$, $t(36) = 11.1$, $p < 0.001$; $r = 2.60$, $t(17) = 11{,}0$, $p < 0.001$ for ExpA and ExpB, respectively), thus the groups of participants were generally not suggestible.

The previously-validated illusion outcomes in synchronous congruent conditions were significantly higher than those ones relative to the asynchronous (ExpA - RHI index: $d = 1.22$, $t(36) = 7.41$, $p < 0.001$; vividness: $r = 0.77$, $z = 4.70$, $p < 0.001$; prevalence: $r = 0.73$, $z = 4.44$, $p < 0.001$; proprioceptive drift: $d = 0.76$, $t(36) = 4.62$, $p < 0.001$) (Fig. 8) and the incongruent conditions (ExpB - RHI index: $d = 2.15$, $t(17) = 9.14$, $p < 0.001$; vividness: $d = 2.66$, $t(17) = 11.28$, $p < 0.001$; prevalence: $d = 1.54$, $t(17) = 6.52$, $p < 0.001$; proprioceptive drift: $d = 0.84$, $t(17) = 3.58$, $p = 0.002$) (Fig. 8). This confirms that participants effectively experienced the RHI during both experiments.

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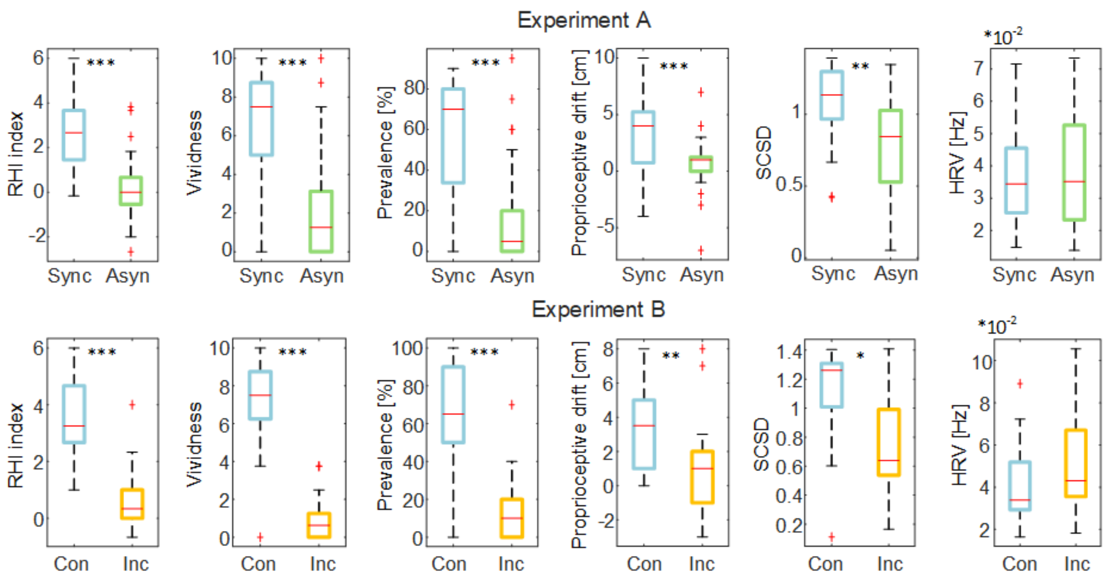

Figure 8: Box and whisker plots of the illusion outcomes (RHI index, vividness, prevalence rating and proprioceptive drift), SCSD and heart rate variability for the synchronous congruent (ExpA and ExpB) and asynchronous congruent (ExpA) and synchronous incongruent conditions (ExpB): median (red lines), 1st and 3rd quartiles (box), lowest and highest values comprised within 1.5 times the interquartile range from the 25th and 75th percentiles (whisker). * indicates a p-value < 0.05.; ** indicates a p-value < 0.01; *** indicates a p-value < 0.001.

In the two experiments, SCSD was significantly higher in the illusion condition compared to the control one (ExpA - SCSD: d = 0.52, t(36) = 3.19, p = 0.003; Fig. 8. ExpB - SCSD: d = 0.55, t(17) = 2.34, p < 0.032; Fig. 8).

On the contrary, in the case of HRV, the difference was not significant (ExpA – d = -0.13, t(36) = 0.77, p = 0.44; ExpB - d = -0.45, t(17) = 1.90, p = 0.07) (Fig. 8).

The SCSD computed along the whole duration of the experimental/control session (90s) significantly correlated with all the traditional RHI measures (all p<0.01) with correlation coefficient in the range of $0.36 < \rho_{tot} < 0.40$, except for the proprioceptive drift which was $\rho_{tot} < 0.30$ (see table III). The difference between the correlation of SCSD with proprioceptive drift and the other typical measures was significant (95% confidence intervals of all three bootstrapping distributions were comprised between -0.04 and -0.18). This shows that the correlation between SCSD and proprioceptive drift was significantly lower than the correlations of SCSD with the other embodiment measures.

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As regards the comparison of SCSD between illusion and control conditions computed for time-windows with different lengths, the lowest Bonferroni corrected p-value was obtained for the time-window with onset equal to 10s and window length equal to 45s (Bonferroni-corrected p-value equal to 0.002) (Fig. 9). In general, the time-windows that in Fig. 9 are represented by the tiles close to the one with 10s onset and 45s length showed Bonferroni-corrected p-value < 0.01.

The differences between illusion and control conditions were statistically-significant for all the analyzed windows except for the windows: i) with early onset (≤ 5s) and short lengths; ii) with 20s onset and long lengths and iii) with onset later than 20s. In particular, focusing on the analysis of the SCSD calculated with 20s moving window with 1s steps, it is possible to note that the p-values (not corrected) lower than 0.001 were obtained for 20s time windows with onset between 7 and 26s, and the lowest p-value (most informative time interval) was found for the window ranging from 14 to 34s (Fig. 9b).

SCSD correlation analysis computed for time-windows with different lengths showed that higher correlation coefficients were obtained for time windows shorter than the whole duration of the trial.

In the correlation between SCSD and the measures of embodiment, the maximum correlation coefficients (ρ_{max} equal to 0.44 and 0.46; all $p<0.001$) and the highest differences between the maximum correlation coefficients and those ones calculated on the whole duration of the trial (ρ_{max} minus ρ_{tot} equal to 0.05 and 0.08) were obtained for the correlation of SCSD with vividness score and with RHI index respectively (Table III). In particular, the correlation coefficient between RHI index and SCSD calculated in the entire 90s window (ρ_{tot}) was significantly lower than the correlation with SCSD calculated on a window interval comprised between 5 and 55s (95% confidence interval: [0.02, 0.11]).

Windows with early onset (ranged between 0 and 20s) showed higher correlation coefficients, while windows with onset later than 50s had all correlation coefficients lower than 0.1 ($p > 0.05$) (Fig. 10).

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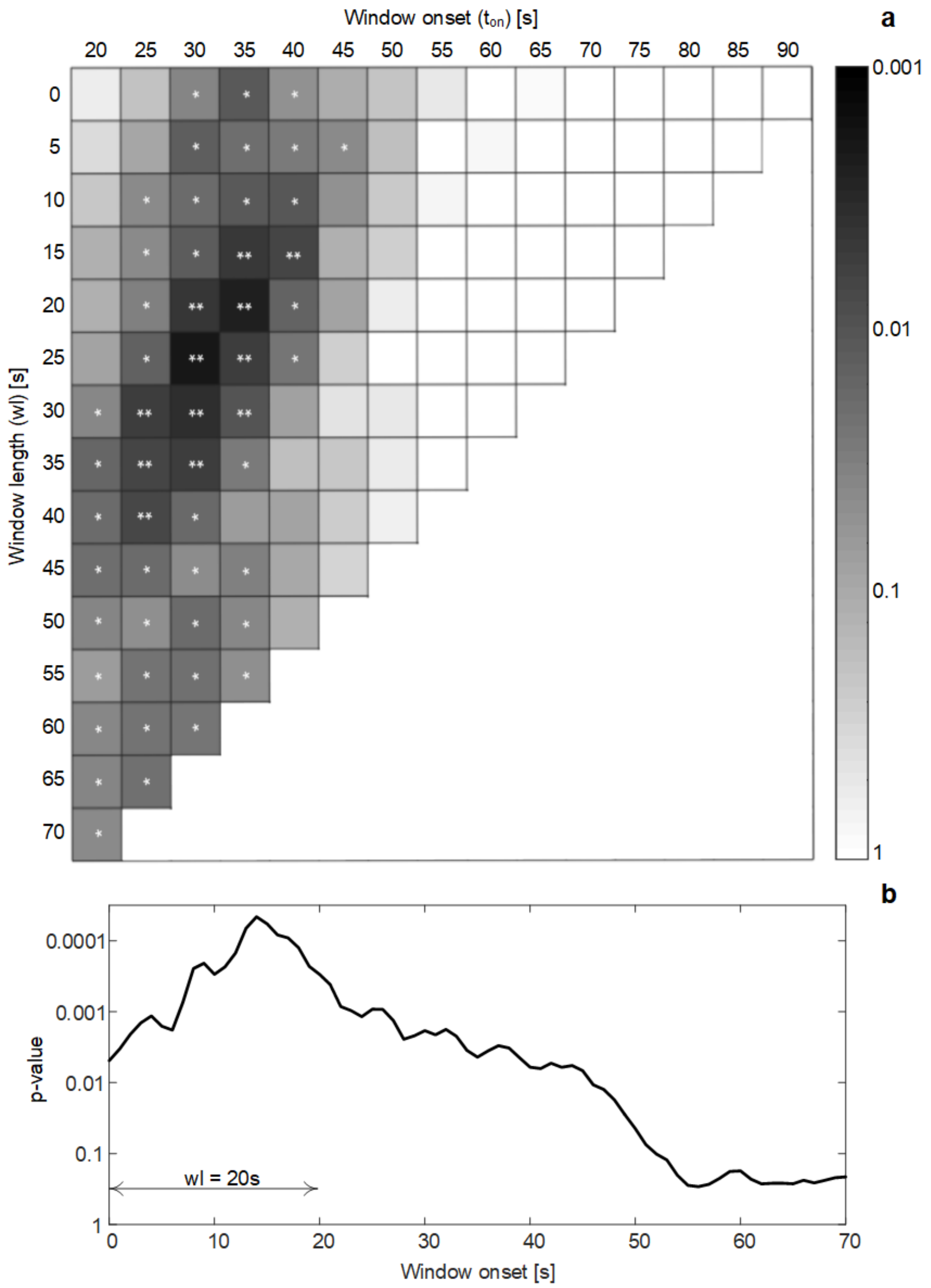

Figure 9: a) Graphic representation of the results of the SCSD comparison analysis between illusion and control conditions (pulled together) for each time window with onset (t_{on} plotted on x-axis) and duration (window length: wl – plotted on the y-axis). Darker colour corresponds to lower Bonferroni corrected p-value. * indicates a p-value < 0.05.; ** indicates a p-value < 0.01. b) The SCSD comparison analysis between illusion and control conditions calculated on 20s moving window with 1s steps, the p-values are plotted with respect to the onset of the moving window (p-values were not corrected because our interest was on their behaviour in time).

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	ρ_{tot}	p-value	ρ_{max}	p-value	$t_{on}(\rho_{max})$	$wl(\rho_{max})$
RHI index	0.38	<0.001	0.46	<0.001	5	50
Vividness	0.39	<0.001	0.44	<0.001	20	35
Prevalence	0.37	<0.001	0.41	<0.001	5	55
Prop. Drift	0.26	0.006	0.27	0.004	0	65

Table III: Summary of the data relatives to the correlation analysis between SCSD and illusion outcomes obtained for different time-windows and represented in Fig. 10. #: ρ_{tot} indicates the correlation coefficient between the illusion outcomes and the SCSD extracted from the entire length of the trial (t_{on} = 0s, wl = 90s). The coefficients were calculated pooling together each trial of the ExpA (synchronous and asynchronous conditions) and ExpB (congruent and incongruent conditions) . $t_{on}(\rho_{max})$ and $wl(\rho_{max})$ are the window onset and length relative to time-windows with the maximum correlation value (ρ_{max}), respectively.

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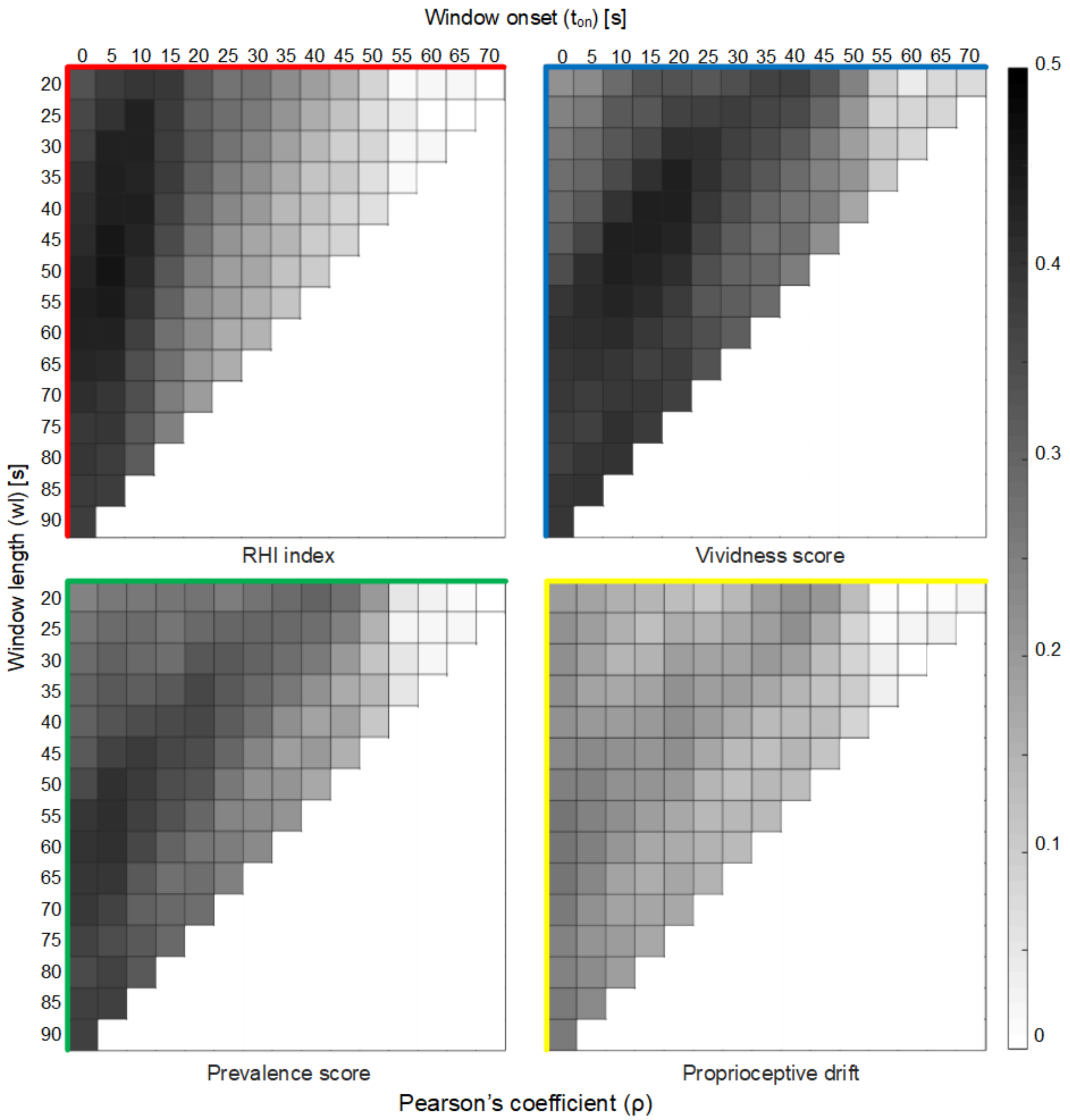

Figure 10: Graphic representation of the results of correlation analysis between the SCSD and each illusion outcome: RHI index (red graph), vividness score (blue graph), prevalence score (green graph) and proprioceptive drift (yellow graph). The SC standard deviation was calculated for each time window with onset (ton plotted on x-axis) and duration (window length: wl – plotted on the y-axis). The correlation values are summarized in Table III. Darker colour corresponds to higher correlation value.

Experiment C

In the synchronous conditions of both iterations of this experiment, the mean value of illusion items of the questionnaire was higher than the mean value of the ones that served as control

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items ($d = 2.11$, $t(17) = 8.71$, $p < 0.001$; $d = 2.11$, $t(17) = 8.70$, $p < 0.001$ for Iteration1 and Iteration2, respectively), thus participants were generally not suggestible.

The validated illusion outcomes in the synchronous conditions were significantly higher than those ones relative to the asynchronous conditions in both iterations (Iteration1 - RHI index: $d = 1.73$, $t(17) = 7.33$, $p < 0.001$; vividness: $d = 1.63$, $t(17) = 5.60$, $p < 0.001$; prevalence: $d = 1.12$, $t(17) = 4.73$, $p < 0.001$; Iteration2 - RHI index: $d = 1.66$, $t(17) = 6.92$, $p < 0.001$; vividness: $r = 0.84$, $z = 3.47$, $p < 0.001$; prevalence: $d = 1.48$, $t(17) = 6.30$, $p < 0.001$) (Fig. 11). This confirms that participants effectively experienced the RHI during the synchronous conditions of both the first and the second iterations.

SCSD, calculated for 10-55s time window, was significantly higher in the illusion condition compared to the control one for the Iteration1 (10-55 SCSD: $d = 0.63$, $t(17) = 2.69$, $p = 0.016$), but not for the Iteration2 (10-55 SCSD: $d = -0.30$, $t(17) = -1.28$, $p = 0.219$).

Furthermore, to assess the impact of novelty on the described effect of embodiment upon SCSD, an analysis on the effect of condition (synchronous vs. asynchronous) has been performed only on the first two trials of the first iteration. Since each participant performed only one condition for each trial, the analysis became unpaired and the sample half-sized. To benefit of a larger sample size, data from the first iteration of ExpC were pooled together with data from ExpA. In all those participants, the 10-55s window SCSD data of synchronous and asynchronous conditions were compared in the first and second trials of the RHI protocol.

In the two-way ANOVA performed with factors *repetition* (two level: trial 1 vs. trial 2) and *condition* (two level: synchronous vs. asynchronous), significant main effects of both factors were obtained (*repetition*: $F(1,106) = 13.25$, $p < 0.001$; *condition*: $F(1,106) = 22.86$, $p < 0.001$) and no interaction effect was found (Fig. 12). Pre-planned two-tailed unpaired Bonferroni corrected (4 comparisons) t-tests were employed to perform the comparison between SCSDs.

The synchronous 10-55s SCSD was significantly higher than the asynchronous one not only in the first trial ($d = 0.49$, $t(53) = 3.55$, $p < 0.001$), but also in the second one (trial 2: $d = 0.45$, $t(53) = 3.24$, $p = 0.008$) when participants were not anymore exposed to the novelty of the paradigm. As regards the decreasing of the effect due to repetition of the protocol, the difference was significant only for the synchronous condition ($d = 0.37$, $t(53)= 2.68$, $p = 0.040$), not for the asynchronous one ($d = 0.34$, $t(53)= 2.48$, $p > 0.05$).

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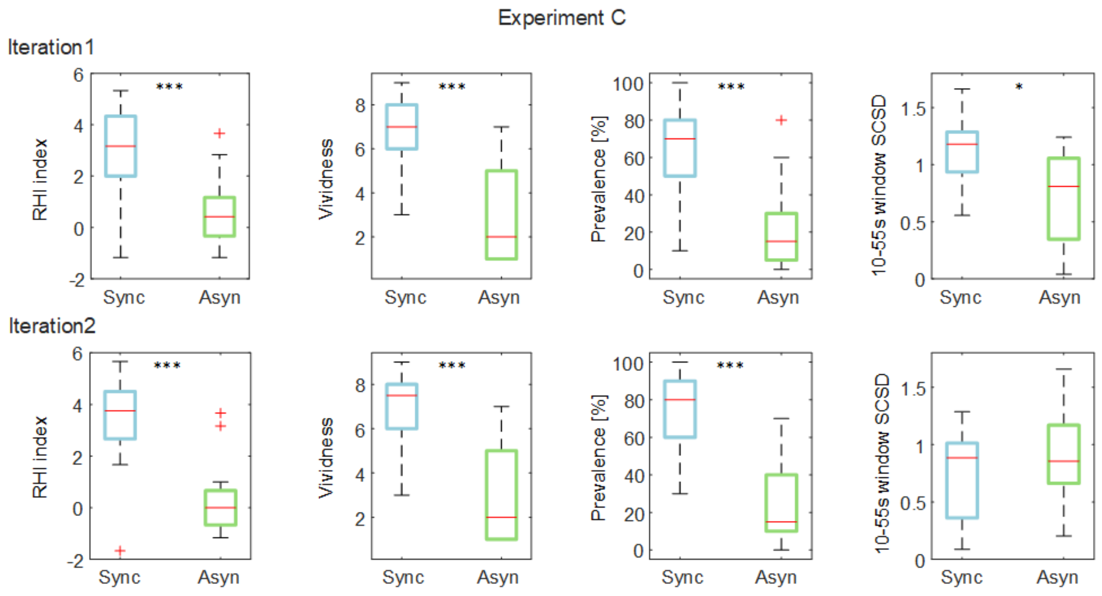

Figure 11: Box and whisker plots of the illusion outcomes (RHI index, vividness and prevalence rating) and SCSD for the synchronous and asynchronous congruent conditions for both iterations of ExpC: median (red lines), 1st and 3rd quartiles (box), lowest and highest values comprised within 1.5 times the interquartile range from the 25th and 75th percentiles (whiskers). * indicates a p-value < 0.05; *** indicates a p-value < 0.001.

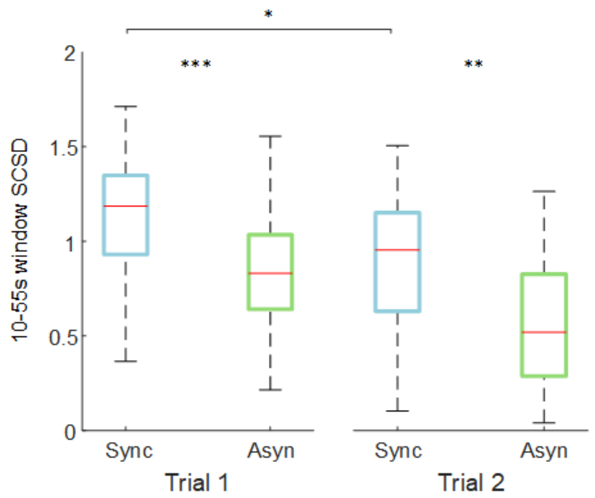

Figure 12: Box and whisker plots of the SCSD for the synchronous and asynchronous congruent conditions for ExpA and the first two trials of ExpC: median (red lines), 1st and 3rd quartiles (box), lowest and highest values comprised within 1.5 times the interquartile range from the 25th and 75th percentiles, respectively (whiskers). ** indicates a p-value < 0.01; *** indicates a p-value < 0.001.

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4. Discussion

The activity of the afferent branch of the ANS that leads to interoception, contributes to build the representation of the body, which is essential to experience the body as self. We argued that an update of such representation, even if due to the modulation of non-interoceptive sensory afferences, would have had an impact on the efferent branch of ANS.

Skin Conductance is an index of autonomic efferent activation. Event related skin conductance response (ER-SCR) to a menace has been used as a smart tool to assess how much the fake hand in the RHI is embodied because, similarly to a startle reflex, physical threats to a body part evoke a stronger autonomic reflex. However, having a higher protective response for the body does not imply that the body representation has -by itself- correlates on the efferent autonomic activity.

Indeed, conversely to ER-SCR, NS-SCR has never been employed in studies where the experience of body ownership is manipulated. We hypothesized that, in the RHI paradigm, the arising of the embodiment illusion over the fake hand would have affected the NS-SCR.

To test this hypothesis, we ran three RHI experiments in healthy participants: both ExpA and ExpB compared the NS-SCR collected during the illusion condition with NS-SCR during the control one. ExpA exploited the asynchronous brush-stroking as a control condition and in ExpB the fake hand was placed in an incongruent position. In ExpC, the NS-SCR has been evaluated across the repetition of the RHI paradigm presentation.

In Exp A and B, we found that the standard deviation of NS-SCR, employed to extract its variability, was increased by the illusion condition. This is likely due to an induction of a stronger arousal which recruited the sympathetic division of ANS and increased the fluctuations of skin conductance.

In line with our findings, highly arousing experiences such as demanding cognitive tasks (Munro, Dawson, Schell, & Sakai, 1987), states of fear and anger (Ax, 1953) or stressful social interactions (Levenson & Gottman, 1983, 1985), increase the frequency of occurrence of peaks of NS-SCR, their amplitude and their standard deviation value (Boucsein et al., 2012). It has been suggested that the heightened autonomic activation due to an effortful allocation of attentional resources for task accomplishment (Jennings, 1986) increases NS-SCR because it enhances the "energy mobilization" (Dawson et al., 2007).

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On the contrary, but in line with (Tieri, Gioia, Scandola, Pavone, & Aglioti, 2017), we did not find differences of heart rate variability (HRV) between experimental and control conditions. HRV is related to a general autonomic reactivity of both branches of the ANS (Bradley & Lang, 2000; Kreibig, 2010), while it is likely that the described effect on SC is mainly dependent on the activity of cholinergic sympathetic sudomotor fibers innervating the sweat glands (Shields, MacDowell, Fairchild, & Campbell, 1987; Wallin, 1981). The sympathetic and the parasympathetic branches of the ANS have different latent periods and different time courses on HRV; in addition to this, sympathetic effects were found to be much slower than parasympathetic ones (Guzzetti, 2005; Warner & Cox, 1962). It is possible that the dynamic of the expression of the sympathetic branch of the ANS over the cardiac dynamics are less suited to highlight the induction of body ownership over a fake limb.

Once established that embodiment impacts on NS-SCR, the neural substrate through which this happens remains to be elucidated. It may be speculated that, at a cortical level, the anterior cingulate cortex and particularly the insula, play a key role for closing the afferent-efferent ANS loop involved in the process of building our body representation: the results of anatomical, lesional, behavioural and fMRI studies converge on such structures.

Indeed, the afferent activity of vagal, glossopharyngeal, facial and spinal nerves, which codes the visceral perception, and of small-diameter (Aδ and C) primary afferent fibers, which innervate all tissues of the body, is directed to the anterior cingulate cortex and to the insula (Craig, 2003, 2008). Besides being a projection site of viscerosensory input from different modalities (Herbert & Pollatos, 2012), the insula is involved in cross-modal visual and somatosensory integration (Bottini et al., 1995; Hadjikhani & Roland, 1998) and in temperature regulation (Maihöfner, Kaltenhäuser, Neundörfer, & Lang, 2002). Accordingly, it has been widely demonstrated a link between autonomic mechanisms of temperature regulation and cognitive processes behind body representation (Llobera, Sanchez-Vives, & Slater, 2013; Tieri et al., 2017; M. Tsakiris et al., 2011). Insular cortex is involved in the modulation of the immune function (Sinha, 2014), which is tightly under control of the sympathetic branch of the ANS (Nance & Sanders, 2007). In line with that, the histamine reactivity increases in the RHI, probably as a sign of rejection of the participants' real hand (Barnsley et al., 2011). In the RHI, the ownership over a third artificial hand seems to result in a disownership and a decrease of the skin temperature of the real hand (Moseley et al., 2008). However, the consistency of such finding is still under debate (de Haan et al., 2017).

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Functional imaging reports showed that the activation of the insula positively correlates to the ownership experience after a synchronous session of RHI (Grivaz et al., 2017; Tsakiris et al., 2007), and that both insula and anterior cingulate cortex become consistently active in response to the rubber hand physical threatening (Ehrsson et al., 2007). Lesions of the insular cortex are very common in stroke patients affected by a pathological alteration of their body ownership, e.g. showing asomatognosia (i.e. experiencing their limb as not belonging to them) or somatoparaphrenia (attributing their limb to someone else) (Karnath & Baier, 2010).

The process of embodiment happening in the RHI is known to be paired with statistically-significant changes of well-validated measures of illusion; an increase in the mean value of illusion statements of the Botvinik and Cohen (1998) derived questionnaire, an increase in subjectively and explicitly-estimated vividness and prevalence of the illusion and a shift of the estimated position of the real hidden hand toward the fake one. A novel effective measure of embodiment in the RHI procedure should partly correlate with those validated measures, and the extent of specific correlation may be informative of the specific aspect of embodiment to which the measure is more sensible.

SCSD correlated significantly with all the other employed measures of the illusion, but the higher values of correlations were found for the measures designed to rate the strength of the illusion, i.e. RHI Index, the vividness and prevalence score. In particular, SCSD correlations with those subjective measures of the embodiment were significantly higher than the one with proprioceptive drift. This is in line with the knowledge that different mechanisms of multisensory integration are responsible for the feeling of ownership and for the implicit measure (the proprioceptive drift) (Rohde et al., 2011).

In relation to the evolution in time of NS-SCR, a further hypothesis can be done: if the time needed for the measure to become significant resulted to be short enough to achieve a good time sensitivity, NS-SCR would be a good candidate also to investigate the timing of arising and the dynamic of the illusion.

Thus, we analyzed shorter time-windows ($wl < 90s$) with different length and onset to highlight the time-windows which held the more pronounced difference between illusion and control condition. No information seems to be present in the first 15s of the trial and for the window with onset after 25-30s. However, once the period from 15 to 30s is included in the analysis, p-value decreases for wider windows up to 55 seconds. Indeed, the highest difference was found

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for a 45 s-long window, from 10 to 55s. The shortest time-interval (wl=20 seconds) with lowest p-value ranged from 14 to 34s (see Fig. 9).

The onset of the referral of touch sensation on the fake limb has been reported to be between 7s (Lloyd, 2007) and 11s (Ehrsson et al., 2004) from the beginning of paintbrush stroking. Considering that few seconds of delay (2-5s) should be taken into account for a SCR peak to occur after an event (Braithwaite et al., 2013), the significant increase of NS-SCR found after 15s perfectly fits with the onset of the illusion which start to update the representation of the body. Therefore, from the analysis of NS-SCR computed along time windows with different length and onset, it can be argued that NS-SCR can be exploited as a useful and objective tool to evaluate the presence of illusion and to have cues on its arising, but not on its end.

The findings obtained for the p-value analysis were indirectly confirmed by the values of correlations between SCSD values and the previous validated measures for the different time-windows. For all the measures, the maximum correlation coefficient was obtained for time-windows shorter than the whole duration of the trial. The lower correlations (namely the ones with proprioceptive drift and with prevalence) were also the ones less affected by the shortening of the window. This suggests that SCSD modulation is more tightly related with the intensity of the illusion than with its duration.

Since NS-SCR is known to be influenced by arousal, and the RHI setup is likely to induce surprise, we wanted to test how much the described effect was strong against the multiple repetition of test and control conditions. In the Exp C, the second subsequent iteration of a synchronous and an asynchronous RHI condition fails to highlight the difference in NS-SCR, suggesting that the reported effect was at least partially due to a reaction to a novelty, which decreases by repeating the paradigm.

Could this surprise be the reaction to the unexpected feeling of ownership over the artificial limb, or just could it be the reaction to the perceptual differences in stimuli presentation between experimental and control condition?

The latter hypothesis could hamper the value of our findings. However, the analysis of the results of both ExpA and ExpB makes it not likely for a number of reasons:

1) the SCSD was subject to the same modulations despite different control conditions; 2) SCSD positively correlates with all the widely-validated measures of the illusion; 3) also the control

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conditions can induce surprise in the participants (the unnatural position of the hand and the asynchrony in stimulation) in similar way to tested conditions; and 4) all the circumstantial possible confounding factors which can induce arousal (light, brushstroke, setup, etc..) were maintained as constant as possible between conditions.

Moreover, considering all the subjects that underwent the synchronous vs asynchronous paradigm (Exp A together with Exp C), the SCSD resulted to be significantly higher in the condition eliciting the rubber hand embodiment (synchronous) also when considering only the second administration of the protocol (trial 2). All the subjects that in trial 2 underwent synchronous stimulation, were already exposed to all the novelties of the experiment in the previous first administration of the protocol (i.e. performance anxiety, novelty in presentation of the setup and tactile stimulation), except to the one due to the induction of embodiment. This strongly suggests that the induction of the embodiment is the cause of the enhanced variability of the NS-SCR, and that this change is sensible to the novelty.

The dependence to the novelty of our effect is in line with other habituation processes which have been widely described in electrodermal activity (see Boucsein et al., 2012 for a thorough review). Moreover, the embodiment induced by the rubber hand illusion procedure suffers by itself of habituation (Bekrater-Bodmann, Foell, Diers, & Flor, 2012; Convento et al., 2018). The combination of the habituation of the electrodermal activity and the habituation of the induction of embodiment in the RHI may have contributed to the absence of effect in the second iteration of the ExpC.

Conclusion

In conclusion, modifications of the body representation are likely among the processes that increase arousal and sympathetic response of ANS, as showed by the increase of fluctuations of NS-SCR.

This modification goes beyond the already known enhanced autonomic reaction to a threatening stimulus and does not seem to be just due to a differently presented sensory stimulus, but more likely it seems to be the effect to the novelty due to the update of the perceptual status.

Our results unveil a further link between apparently unrelated complex cognitive activities and low-level peripheral physiologic responses by depicting a control loop probably involving

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interoception and exteroception, the building of the representation of the body and the output of the efferent autonomic system.

From an applicative point of view this link can be exploited to impact on the process that builds the representation of the body. For instance, the *Vagus* nerve invasive or non-invasive stimulation may be a good candidate to modulate interoceptive afferences and enhance embodiment.

Moreover, the index of fluctuation of NS-SCR signal, i.e. the standard deviation, is an optimal candidate to assess the extent of embodiment over an artificial body limb and seems to be specifically suited to assess the onset of the illusion and its initial development. This physiological measure suffers of habituation, thus it is not suitable when there are multiple repetitions of the process to induce the embodiment (e.g. the RHI). However, it has several strengths: it is non-invasive, cost-effective, easy to employ, and potentially free from subjective biases (such as in self-report evaluations of the illusion), its recording does not interfere with the collection of other behavioral measures (e.g. proprioceptive drift or SCR to threatening event against the fake hand) and it catches quite accurately the strength of the illusion.

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Chapter 4: Sensory- and motor-oriented embodiment of neurally-interfaced robotic hand prostheses

1. Introduction

Despite improved mechatronic features have made hand prostheses more dexterous, yet their abandonment exceeds 30% (Cordella et al., 2016).

As a multipurpose tool allowing capabilities and conveying sensory inflows, the hand is located at the human-environment frontier and defines the boundary of the body, so that its lost greatly affects how amputees perceive themselves and their body.

Somatosensory feedback plays a key role in dexterous manipulation and boosts motor learning. Today, commercial hand prostheses do not offer sensory feedback, however there is a strong research effort to develop and test prosthesis-user interfacing system that, in parallel of a better motor control, can offer a rich and pleasant feedback. Sensory feedback from hand prostheses have been delivered employing intraneural (Dhillon & Horch, 2005; Petrini et al., 2019; Rossini et al., 2010) and perineural (Ortiz-Catalan, Håkansson, & Brånemark, 2014; Tan et al., 2014) electrodes, targeted sensory reinnervation (Kuiken, Marasco, Lock, Harden, & Dewald, 2007; Paul D Marasco, Schultz, & Kuiken, 2009) or non-invasive sensory substitution (Antfolk et al., 2012). Richer sensory feedback from the prosthesis showed to improve motor ability (Valle et al., 2018; Zollo et al., 2019), object features discrimination (Raspopovic et al., 2014), and to counteract amputation-induced maladaptive brain plasticity (Di Pino, Maravita, et al., 2014; A. Serino et al., 2017). Convergent multisensory afferences build the representation of the body in the brain, which has been shown to be flexible to the point of integrating external objects not belonging to the Self (Iriki, Tanaka, & Iwamura, 1996; Maravita & Iriki, 2004). Thus a prosthesis enabling a more physiologic sensory feedback is expected to greatly improve its embodiment and acceptance.

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Few reports tested the embodiment of worn prostheses able to deliver sensory feedback in amputees with targeted sensory reinnervation (Paul D Marasco et al., 2018), perineural Flat Interface Nerve Electrodes (FINE) (Graczyk, Resnik, Schiefer, Schmitt, & Tyler, 2018; Schiefer, Tan, Sidek, & Tyler, 2015), and with intraneural electrode (Rognini et al., 2019; Valle et al., 2018). All of them employed the rubber hand illusion (RHI) paradigm (Botvinick & Cohen, 1998) or modified versions of it (Rognini et al., 2019).

However, the translation of RHI findings to prosthesis embodiment in amputees is not free from possible pitfalls (Niedernhuber, Barone, & Lenggenhager, 2018); RHI setup is very structured and artificial, the fake hand is not worn and typically cannot move, and the illusion is only temporary.

Easy to collect clues on embodiment also come from changes reported by the subject in the perceived length of the phantom limb (Graczyk et al., 2018; Rognini et al., 2019; Rossini et al., 2010; Valle et al., 2018). Still, body representation and phantom awareness are very likely different concepts, partly relying on different brain networks (Di Pino, Guglielmelli, & Rossini, 2009; Ehrsson, Holmes, & Passingham, 2005; Flor, Nikolajsen, & Jensen, 2006; Kikkert et al., 2019; Reilly & Sirigu, 2008).

In the present study, we longitudinally investigate prosthesis embodiment in a chronic amputee volunteer, who received sensory feedback from the prosthesis through intraneural and perineural multichannel electrodes implanted on her stump (Fig. 13 – upper row). After a period of training, she was able to perform grasps and dexterous manipulation thanks to a closed-loop control enabled by neural feedback (Zollo et al., 2019).

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Figure 13: Upper row, from left to right: surgical field on the median aspect of the left severed arm and identification of median and ulnar nerves (left); higher magnification of the perineural (central up) and one of the intraneural electrodes (central down) implanted in the nerves, participant during a blinded manipulation training session learning to exploit the neural sensory feedback to control the robotic prostheses (right). Lower row, from left to right: the participant involved in a Visuo Tactile Integration (VTI) experimental session (left) and in a Temporal Order Judgement (TOJ) experimental session with the arms crossed (right).

Embodiment was assessed using two different tasks: a Visuo Tactile Integration (VTI) and a Temporal Order Judgement (TOJ) tasks. Both of them are proxies of body representation and prosthesis embodiment, not merely relaying on the description of participant's subjective feelings.

In both tasks, somatosensory stimuli were delivered non-invasively through mechanical tappers placed on the intact right index fingertip and to the stump, on the skin area where the touch was referred as coming from the phantom left index fingertip.

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VTI assesses the computation of sensory stimuli in reference to the somatotopic map. The participant had to respond to a stimulus delivered through the prosthesis, while a concurrent incoming visual stimulus was delivered at different distances (Fig. 13 – lower row and Fig. 14). The closer to the body the visual stimulus is the faster the reaction time (RT), because our brain integrates faster somatosensory and visual stimuli delivered close to the body (peripersonal space - PPS) (Fogassi et al., 1996).

Since VTI tests the area of visuo-tactile integration, which is extended by an embodied extracorporeal tool, it has been employed to assess embodiment in the animal and in humans (Iriki et al., 1996; Maravita & Iriki, 2004).

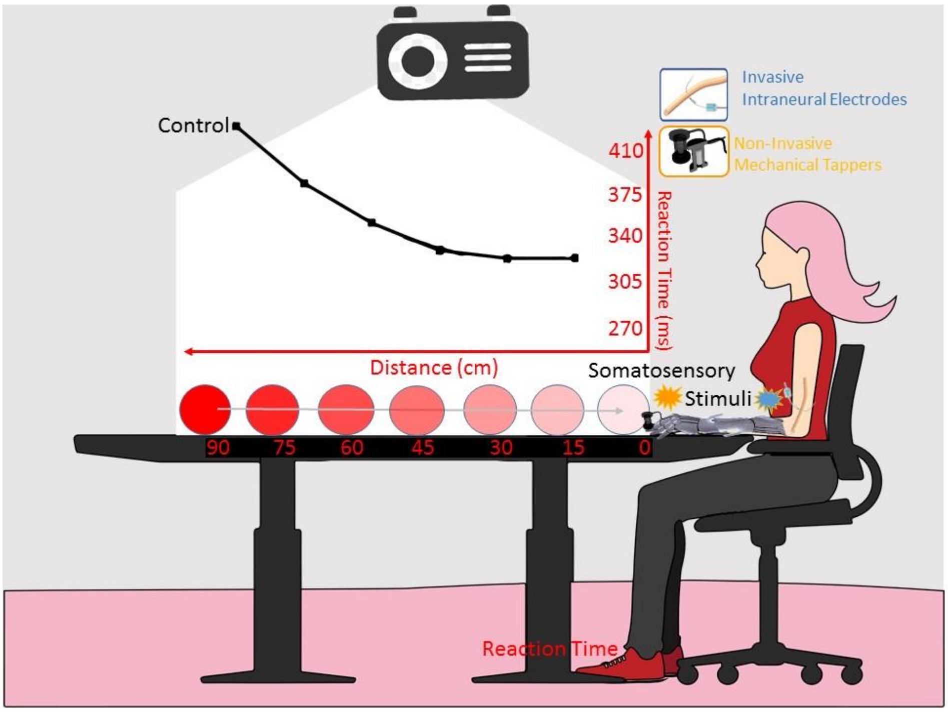

Figure 14: Visuo Tactile Integration (VTI) Experimental Setup. The somatosensory stimulation was delivered either invasively (blue) through intraneural electrodes, or non-invasively (orange) through mechanical tappers, when the visual stimulus, approaching the participant, was at six possible distances (90, 75, 60, 45, 30, 15 cm). Participant had to respond to somatosensory stimuli as fast as possible. Reaction time was taken with a pedal. The black line is the RTs/distance curve of the healthy subjects control group.

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The same approach, albeit using audio-tactile stimuli, has been recently adopted to assess the extension of PPS as a proxy of tools (Canzoneri, Magosso, & Serino, 2012) and prosthesis embodiment (Canzoneri, Marzolla, Amoresano, Verni, & Serino, 2013). In these paradigms, an overall speeding of RT expresses general better performance, while a variation of the shape of the RTs/distance curve, with a decrease of RTs only for the farther distances, is a clue of enlargement of peripersonal space, sign of embodiment (Spaccasassi, Romano, & Maravita, 2019).

The second task, namely Temporal Order Judgement (TOJ), was used to assess the computation of sensory stimuli delivered to the prosthesis in reference to the external, egocentric space (Yamamoto & Kitazawa, 2001a). The participant states which one of two tactile stimuli, delivered with variable asynchrony to her upper limbs, was perceived first (Fig. 13 – lower row and Fig. 15).

The TOJ determines two parameters (Shore, Spry, & Spence, 2002; Yamamoto & Kitazawa, 2001a) : i) the point of subjective simultaneity (PSS) - the stimulus onset asynchrony (SOA) where the two limbs have the same probability to be perceived as firstly stimulated- which is taken as laterality of the stimulation, and ii) the Esteem Accuracy (EA) – linked with the slope of the sigmoid psychometric probability/SOA function and computed through a SOA (the shorter the EA value the higher the accuracy) - which reflects the performance.

Crossing the arms puts in contrast somatotopic and external egocentric spatial coordinates and dramatically reduces TOJ performance (Yamamoto & Kitazawa, 2001a) (Fig. 15). Critically, the embodiment of tools (Yamamoto & Kitazawa, 2001b) and prostheses (Sato, Kawase, Takano, Spence, & Kansaku, 2017) are subjected to the hand crossing effect.

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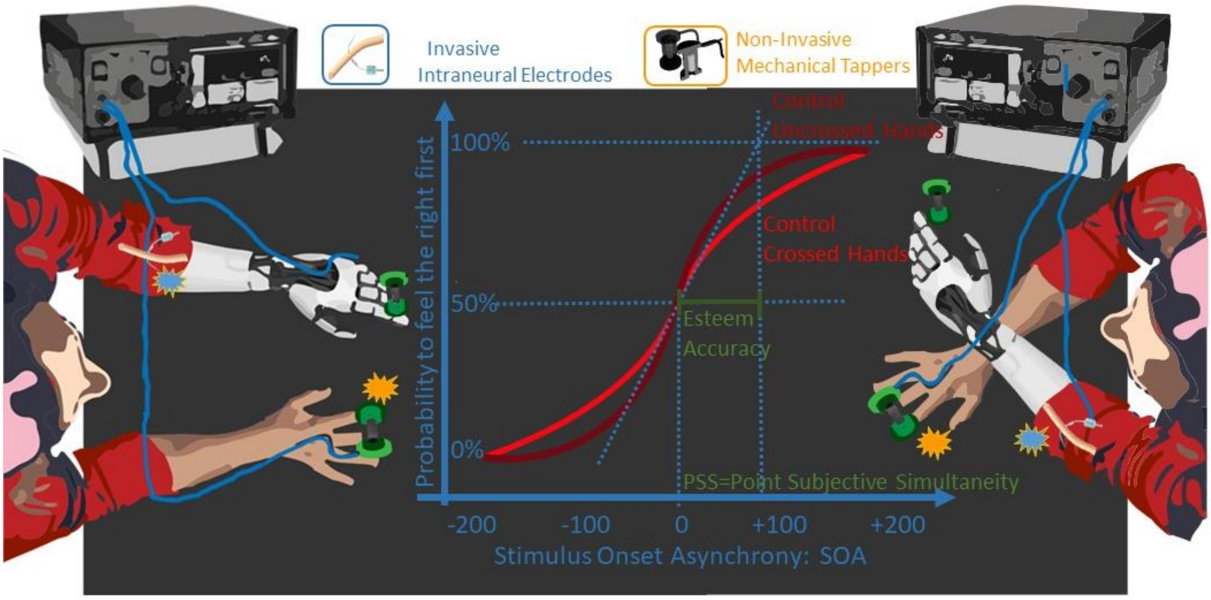

Figure 15: Temporal Order Judgement (TOJ) Experimental Setup. A pair of somatosensory stimuli were delivered non-invasively (orange) through mechanical tappers, and only in the POST_I session to the left severed limb invasively (blue) through intraneural electrodes. Stimuli were delivered, one to each limb, with a stimulus onset asynchronies (SOA) randomly assigned from 15 to 200 ms. The participant had to discern to which limb the first stimulus was delivered. The task was performed either with uncrossed and with the crossed arms. The red lines are the probability to feel the right limb stimulated first depending on the SOA, fitted with a sigmoid function, when the task was performed by a healthy subjects control group with the arms uncrossed (dark red) and crossed (light red). The Point of Subjective Simultaneity (PSS) is the SOA where both limbs have the same probability to be perceived as firstly stimulated. The decrease of the slope of the curve from uncrossed to the crossed condition represents the loss of esteem accuracy typical of the arm crossing effect. The Esteem Accuracy (EA) is computed through the SOA corresponding to half of the inverse of its derivate for PSS, thus the shorter the EA value is the higher the accuracy is.

Experiments have been designed to assess the impact of three factors on prosthesis embodiment: i) type of stimulation (intraneural invasive vs non-invasive), ii) training and iii) type of prostheses (anthropomorphic vs more trained) (Fig. 16).

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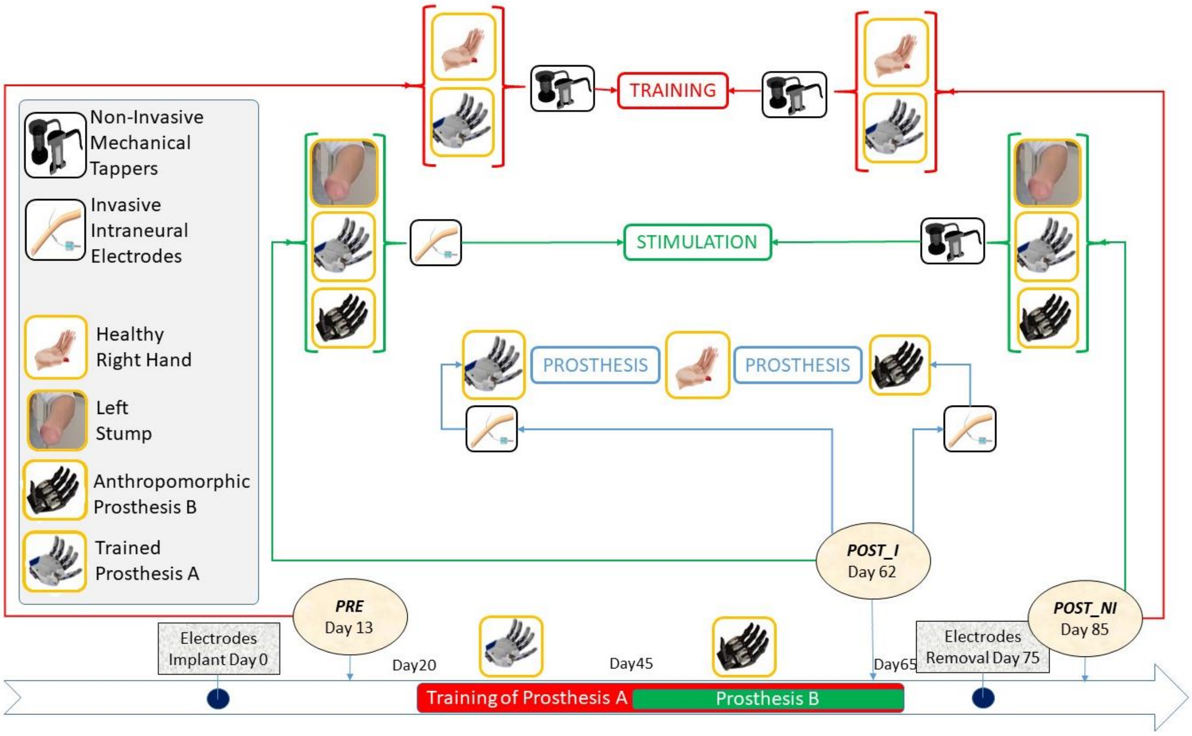

Figure 16: Timeline and protocol design for the three tested factors: i) Stimulation (green loop), ii) Training (red loop), and iii) Prosthesis (blue loop).

i) To test the impact of invasive stimulation on the achieved embodiment, the data from two sessions were compared: POST_NI collected after electrodes removal was compared with POST_I performed while the participant had still the intraneural electrodes implanted. In both sessions, the healthy limb was stimulated non-invasively. In POST_I the stimulation was delivered intraneurally to the affected left limb, using parameters that produced a sensation referred to be the closest to the one evoked by the mechanical tapper, in terms of intensity, modality and referred territory.

ii) Training has been shown to be enable the embodiment process (Farnè, Iriki, & Làdavas, 2005; Iriki et al., 1996; Maravita, Spence, Kennett, & Driver, 2002); its impact was showed also in healthy subjects taught to use a mechanical hand (Marini et al., 2014). To investigate the impact of training on embodiment of neurally-interfaced prosthesis, the participant, who was naïve for active prosthesis use, was tested non-invasively before (PRE), and after training (POST_NI).

iii) Embodiment was also assessed in relation to the anthropomorphism of the prosthesis, due to reported stronger embodiment for more human-like non corporeal

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objects (Tsakiris et al., 2010). In the invasive session (POST_I), the participant was tested while wearing two different prostheses: a less-anthropomorphic, although more trained, research prototype (Prosthesis A) and a more-anthropomorphic, although less-trained, commercial device (Prosthesis B).

To evaluate statistical significance of the results achieved with our participant in both experiments they were compared with the ones coming from a group of healthy controls.

2. Materials and methods

Amputee Participant, Electrodes and Surgery

The part of this study involving the amputee participant was conducted at Campus Bio-Medico University Hospital of Rome in accordance with the Declaration of Helsinki and following amendments, and it was approved by the local Ethics Committee and by the assigned office of the Italian Ministry of Health. The volunteer participant signed an informed consent form. She was exposed to an explosion that produced a trans-radial left upper limb amputation almost 30 years before. She is a right-handed female, 40 years old at the time of the experiment. The participant underwent a surgical procedure under general anesthesia where two intraneural multichannel electrodes (ds-FILEs) and one cuff electrode (Ardiem Medical Inc.) were implanted in the ulnar and the same in the median nerve trunks, achieving a total of 64 intraneural plus 28 perineural channels of communication. Electrodes were removed 75 days after implantation to comply with the constraints of the obtained formal approval. For full description of the surgical procedure and electrodes implanted see (Zollo et al., 2019).

Prostheses and training

The volunteer subject habitually wore a cosmetic prosthesis for her everyday life, both during working hours and in social circumstances. She was, though, naïve for active prosthesis use. For the experimental tasks, she was tested with a robotic hand research prototype (prosthesis **A**;: IH2 Azzurra, Prensilia s.r.l.) and with a more anthropomorphic commercial device (prosthesis **B**;: RoboLimb, TouchBionics s.r.l.). Shape and dimensions of prosthesis B were closer to human hand; e.g. all the digits of prosthesis A have the same length, whereas the digits of prosthesis B have proportion similar to human hand.

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During training both prostheses were controlled through surface EMG sensors (Ottobock 13E200) embedded into the socket, while forces of interaction with objects were measured with force-sensing resistors (Interlink Electronics Inc.) embedded in both prostheses' fingers, and fed back through neural interfaces.

An ad-hoc developed algorithm based on non-linear logistic regression allowed to perform power, pinch and lateral grasps, rest and to open the hand and to apply three level of force (Zollo et al., 2019).

The participant trained to exploit neural feedback to control both prostheses during grasps and manipulation, but training with prosthesis A lasted approximately 45 days, while training with prosthesis B only 20 days. Thus, prosthesis A was the less anthropomorphic and the more trained, while prosthesis B was the more anthropomorphic but it was the less-trained.

Test of multisensory integration

Visuo-somatic multisensory integration was evaluated through two tasks: Visual Tactile Integration (VTI) and Temporal Order Judgement (TOJ) in order to assess the impact of type of stimulation, training and anthropomorphism on prosthesis embodiment. In both experiments the participant was placed in a silent and slightly illuminated room and she was acoustically shielded with white noise playing headphones. Visual and tactile stimuli were presented by the open-source "OpenSesame" software v.3.1 (Mathôt, Schreij, & Theeuwes, 2012).

In order to assess the achievement of proficiency due to the training, the participant was tested at three different time points:

- (PRE) Pre-training, non-invasive stimulation: Basal level when the electrodes were already implanted but the subject did not start the training with the prosthesis. Stimulation was delivered to the stump through mechanical tappers;
- (POST_I) Post-training, invasive stimulation: 50 days after PRE and after 30 days of training with prostheses. Stimulation was delivered to the severed limb through intraneural electrodes;
- (POST_NI) Post-training, non-invasive stimulation: after electrodes removal. Stimulation was delivered to the severed limb through mechanical tappers.

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Two different groups of healthy subjects were enrolled to be control for the two experiments. 36 participants (13 males, age= 24.11 years, StDev= 4.15, range: 19–42) performed the VTI task and 11 participants (6 males, age=26.36 years, StDev= 3.34, range: 23-32) performed the TOJ. This research was approved by the local committee of the Department of Psychology, University of Milano-Bicocca.

Somatosensory stimulation

Experiments were designed to deliver sensory stimuli on the fingertips of both hands' index finger; stimuli were delivered non-invasively and invasively.

Non-invasive tactile stimuli were administered by means of solenoid tappers (magnetic rod diameter: 4 mm) controlled by an ad-hoc built relay box (Tactile Box, EMS, Bologna, Italy). Healthy participants, part of control groups, were always stimulated non-invasively. Non-invasive stimulation was also always exploited for stimulating amputee participant healthy right upper limb, and it was exploited in PRE and POST_NI to stimulate the severed left limb. This was achieved by placing the tapper right upon the area of the skin that once touched the participant reported to feel her left index fingertip to be touched (referred phantom sensations on her missing left index finger).

Intraneural invasive stimulation was exploited in POST_I to stimulate the amputee participant severed left limb, employing parameters that induced a sensation that she referred to be closer to the one evoked by the mechanical tapper, in terms of intensity, modality and referred territory. Indeed, after electrode implantation, a whole-contact psychophysical sensory mapping was performed to establish the match between stimulated contact, modality amplitude and referred territory of the evoked sensation. Moreover, sensory mapping has been tested and retested day after day before PRE to have an estimation of the day by day reliability of the evoked sensations. In both tasks, invasive stimulation was delivered in form of square pulse stimuli to the channel number 12 of the intraneural electrode (ds-FILE) positioned proximally within the median nerve (stimulus intensity 300 µA, duration 200 µs) (Intraneural Stimulator: Grass S88X, Astro-Med Inc., West Warwick, RI, USA).

VISUO-TACTILE INTEGRATION TASK

In this experiment, a visuo-tactile interaction task was carried out to assess the extent of the peripersonal space (PPS) in the different conditions and at the different time points.

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Experimental setup and procedure

The participant was seated beside the wall with her tested upper-limb resting on a table in a prone position. A PC-driven digital projector was set to a distance suited for projecting a video that covered a 100x75 cm surface on the wall. The video showed a visual stimulus approaching to the participant. Visual stimuli were looming images on a white background covering a distance of 1 meter on the bottom side of the projecting area and travelling at a constant speed of 66cm/s.

In all the three time points, the space of visuo-tactile interaction was tested for the participant's right healthy limb, the severed left limb wearing prosthesis **A** and prosthesis **B** (in *PRE* session prosthesis **B** was not available), and for the bare stump. Participant's elbow was always kept at 42 cm from the limit of the projecting area. This resulted in a distance from the projecting area of 30 cm for the endpoint of the stump, while in all the other conditions the extremity of the limb or the prosthesis was in contact with the limit of the projecting area.

Along with the visual stimulus, in 85% of the trials, a tactile stimulus was randomly delivered when the visual approaching stimulus was at one of 6 distance points (15, 30, 45, 60, 75, 90 cm) from the end of the projecting area. The participant was asked to press a pedal as soon as she perceived the tactile stimulus. The remaining 15% of the trials were "catch trials", where no tactile stimulus was administered and no response was expected.

The task lasted approximately 8 minutes, consisting in a total of 168 trials (24 repetitions per distance points and 24 catch trials) and 800 ms of inter-trial interval. A small training phase with 15 stimuli presentation preceded the experimental task.

Data analysis

Data were analyzed by addressing the three experimental questions defined in the introduction section. Analysis was implemented in order to weight different factors, while minimizing the total number of comparisons. The dependent variable which was taken into consideration was the reaction time (RT) in response to the tactile stimulus. The factor *Distance* was computed as a continuous variable.

- STIMULATION. In order to assess the relative weight of the stimulation interface (*non-invasive* vs *intraneural*), we run a *Linear Mixed Model* (*LMM*). The model was analysed with an Analysis of Variance (ANOVA) with Satterthwaite approximation for degrees

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of freedom. We included as predictors the continuous factor *Distance* and the dichotomous factor *Stimulation* (*intraneural* vs *non-invasive*). The number of trial was added as a random effect variable. The two levels of factor Stimulation have been implemented by pooling together all the conditions exploiting intraneural interface at *POST_I* (prosthesis **A** + prosthesis **B** + bare stump: level *intraneural)* tested against the same conditions recorded at *POST_NI* (level *non-invasive*). Since data from *PRE* miss prosthesis **B,** they were not included for maintaining homogeneous samples. *POST_NI* session was performed 22 days after the *POST_I* session, because the participant had to go through all the activities linked with presurgical exams, surgery for electrode removal and recovery after the surgery before to be available to perform the non-invasive stimulation session. The time spent training with the prostheses between *POST_I* and *POST_NI* was marginal, thus the impact of any additional training between the two sessions can be considered negligible.

- TRAINING. In order to assess the relative weight of the training to control the prosthesis, we used the same approach of stimulation analysis. We employed a *LMM*, analysed with an ANOVA with Satterthwaite approximation for degrees of freedom. The number of trial was added as a random effect variable. Predictors were the continuous variable *Distance* and two dichotomous variables: *Hand* (prosthesis **A** vs healthy limb) and *Time* (*PRE* vs *POST_NI*). We have chosen these time points to avoid introducing noise in the data due to different types of stimulation (at *POST_I* stimulation was delivered intraneurally). Additionally, in order to highlight the effects of training on the performance of the task, we conducted two independent ANOVA with the predictors *Distance* and *Time* (*PRE* vs *POST_NI*).
- PROSTHESIS. In order to assess the relative weight of the employed prostheses, and whether they were embodied, we again employed a *LMM*, analysed with an ANOVA with Satterthwaite approximation for degrees of freedom. The number of trial was added as a random effect variable. Predictors were *Distance* and *Hand* (three levels: prosthesis **A**, prosthesis **B**, and healthy hand). The three levels of factor *Hand* have been implemented with data recorded at *POST_I*. *POST_I* has been chosen because it was the only time point when prosthesis feedback could have been done through intraneural stimulation, and because the

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proficiency with prosthesis was already achieved. Additionally, we run an analysis to evaluate the performance in the spatial transition from peripersonal to extrapersonal space. We selected the distances of 15 (Mogk, Rogers, Murray, Perreault, & Stinear) and 45 (far) cm. When the participant was tested on the stump condition, thus not wearing any kind of prosthesis, the tip of the stump was 30cm away of the tip of the prosthesis (e.g., closer to the trunk). By doing so the same distances resulted in a 45 (Mogk et al.) and 75 (far) cm away, although the physical position of the visual stimulus was exactly the same and the physical position of the stump was also exactly the same in both conditions. We thus adopted a *LMM,* analysed with an ANOVA with Satterthwaite approximation for degrees of freedom including the number of trials as a random effect variable. The predictors were the *Distance* (near vs far) and *Hand* (prosthesis A vs stump).

TEMPORAL ORDER JUDGEMENT TASK

In this experiment, a temporal order judgement (TOJ) of two stimuli delivered on the upper limb was carried out to investigate participant's body awareness in the different conditions and at the different time points.

Experimental setup and procedure

The participant was seated in front of a table, with both her upper-limbs lying on its surface in a prone position. Two tactile stimuli were delivered in rapid succession, one to each limb, with an inter-stimulus interval randomly assigned from the following stimulus onset asynchronies (SOA): (-200, -90, -55, -30, -15, 15, 30, 55, 90, 200 ms). Negative intervals indicate that the right limb was stimulated before the left limb and *vice versa*.

Each trial started with a visual cue (100 ms red LED light) which was followed, after 300 ms, by two tactile stimuli, delivered one to each limb. Before the experiment started, two coloured stickers were applied to the patient arms (the association between colour and arms varied across the testing conditions) and they were used as a code to indicate the stimulated limb without referring to laterality tags (left/right).

Each experimental condition was tested with 8 experimental blocks, four while the subject's hands were uncrossed (with a gap of 40 cm between her hands) and the other four when her

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hands were crossed. In the crossed conditions, in half of these blocks the right limb was kept over the left limb and in the other half the left limb was kept over the right limb. In four experimental blocks, the participant was asked to verbally report whether the first of the two stimuli was administered on the right or the left limb, while in the other four, on the contrary, she had to report where the second stimulus occurred.

Testing each condition, consisting in a total of 200 trials (20 repetitions per SOA) for the uncrossed limbs and the same amount of trials for the crossed limbs, lasted approximately 35 minutes. In *POST_I*, the task was repeated in two different conditions: healthy hand vs prosthesis **A** and healthy hand vs prosthesis **B.**

Data analysis

The order judgment of the subject in each condition were plotted with the different "*stimulus onset asynchrony (SOA)*" as independent variable (x-axis) and "*the probability to judge the right limb as the one firstly stimulated*" as the dependent variable (y-axis). Then, data distribution was fitted with a psychophysics sigmoid function:

$$P(SOA; PSS, EA) = \frac{1}{1 + exp\left(-\frac{SOA - PSS}{0.5 * EA}\right)}$$

Where the two parameters PSS and EA represents:

- Point of Subjective Simultaneity (PSS):

$$PSS = SoA|_{P_{0.5}}$$

This is the SOA value on the curve where the first stimulus had the same probability (P=0.5) to be felt on right and on the left limb. It testifies the laterality stimulation bias measured in milliseconds.

- Esteem Accuracy (EA):

$$EA = \left(2 * \frac{dP}{dSOA}\bigg|_{SOA=PSS}\right)^{-1}$$

This is the SOA needed for the line tangent to the curve at (P=0.5) to reach the value P=1. It is the inverse of the slope of the curve multiplied by 0.5. The shorter it is, the more accurate the esteem is.

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Fitting TOJ data with the previous function gives back a value of PSS and EA for each tested condition. To give statistic relevance to those outputs, they were compared with the ones obtained by the control group of healthy subjects performing the same task, by means of Crawford t-tests.

- STIMULATION: Pooling together the bare stump, prosthesis A and prosthesis B, two different pair of stimulations (in *POST_I*: right limb stimulated non-invasively and left limb stimulated invasively vs in *POST_NI* both limbs stimulated non-invasively) were compared through their PSS in the uncrossed condition. The uncrossed condition is the standard situation of the TOJ task, without conflicting information about laterality, thus typically results in the highest accuracy. Therefore, this is the best condition to evaluate any laterality temporal bias which may be only due to the type of stimulation.
- TRAINING: In *PRE* session the participant was extremely inaccurate and variable in performing the crossed condition so data have not been analyzed.
- PROSTHESIS: To assess embodiment of prostheses with intraneural sensory feedback we evaluated in *POST_I* the worsening of Esteem Accuracy due to hand crossing, while participant was wearing prosthesis **A** or prosthesis **B**.

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3. Results

Stimulation

The VTI experiment, first replicated the well-known reduction of RT when the somatosensory stimulus was delivered while the visual stimulus was closer to the upper limb extremity, with a main effect of *Distance* [$F(1, 766) = 47.758$, $p<0.001$]. Critically, the participant accomplished the task differently according to the type of somatosensory stimulation, as shown by the main effect of *Stimulation* [$F(1, 760) = 5.842$, $p=0.016$]. Moreover, the type of stimulation affected the pattern of the RT/distance curve, as shown by the interaction *Distance* x *Stimulation* [$F(1, 766) = 5.544$, $p=0.019$] (Fig. 17).

When the participant was stimulated non-invasively, the presence of clearly significant *Distance x Group* interaction (*amputee stimulated non-invasively* vs *Control*) [$F(1,5077) = 28.379$, $p<0.001$] suggests that the participant behaved differently from the control healthy group. The same remarkable difference was not observed when the participant was stimulated invasively (*Distance x Group* - *amputee stimulated invasively* vs *Control*: [$F(1,5033) = 3.3954$, $p=0.066$], showing a behavior resembling the control group (Fig. 17). Having a similar RT/Distance pattern than healthy control is in favor of facilitation of prosthesis embodiment when this was tested with intraneural stimulation.

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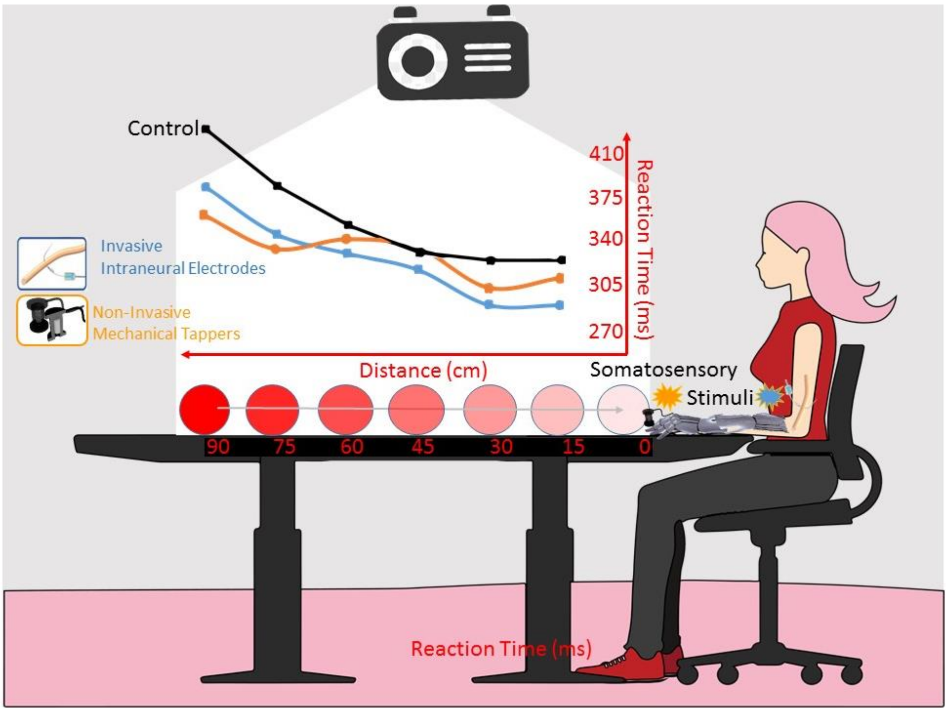

Figure 17: Different RT/Distance patterns in the VTI experiment of Invasive Intraneural Stimulation (blue) and Non-Invasive Stimulation (orange) compared to the pattern of the control group (black). The absence of interaction between Intraneural and Control [F(1,5033) = 3.3954, p=0.066], compared to the presence of interaction between Non-Invasive and Control [F(1,5077) = 28.379, p<0.001], supports a facilitation of prosthesis embodiment when this was tested with intraneural stimulation.

In the TOJ with uncrossed limbs, when right limb was stimulated non-invasively and the left invasively there was a significant right laterality bias, so that the participant perceived intraneural left stimulation with about 30ms delay compared to the healthy limb [Point of Subjective Simultaneity (PSS) = +28.9, CI: (+14.8ms, +42.5ms)]. PSS was significantly different than the one of the control group only when left limb was stimulated invasively (non-invasive/intraneural vs control: t(10)= 2.582, p=0.027; non-invasive/non-invasive vs control: t(10)=.946, p=0.336).

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When left limb was stimulated intraneurally and the right limb non-invasively the performance of the task was not worse than when both limbs were stimulated non-invasively, despite the asymmetric stimulation. Indeed, both conditions had Esteem Accuracy (EA) not different than the one of the control group (non-invasive/intraneural vs control: t=1.011, p=0.336; non-invasive/non-invasive vs control: t=-0.383, p=0.71) (Fig. 18).

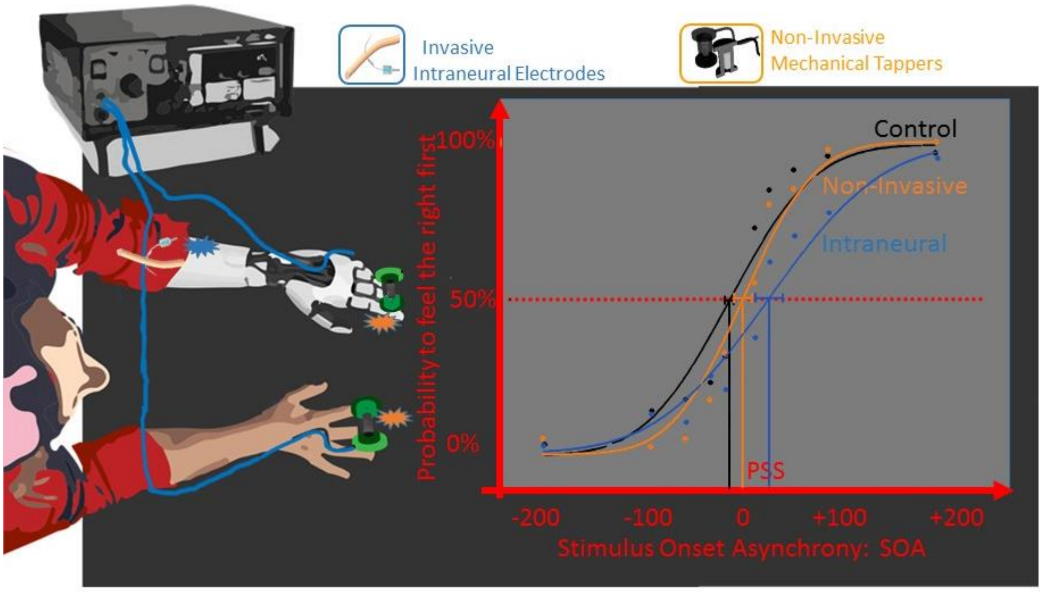

Figure 18: Plot of uncrossed hand order judgment with the different SOAs as independent variable (x-axis) and the probability to judge the right limb as the one stimulated first, as the dependent variable (y-axis). Then, data distribution was fitted with a sigmoid function, and the SOA value in the curve where the first stimulus had the same probability (P=0.5) to be felt on right and on the left limb was defined as Point of Subjective Simultaneity (PSS) and it testifies the laterality stimulation bias. Blue: Invasive vs Non-Invasive; Orange: Non-invasive vs Non-Invasive; Black: Control.

Training

Training, changed the way the participant accomplishes the task depending on the hand tested; this was suggested by the presence of significant Hand x Time [F(1, 537) = 8.166, p=0.004)], Distance x Time [F(1, 549) = 5.965, p=0.015)] and Distance x Hand x Time [F(1, 550) = 5.990, p=0.015)] interactions. In the healthy limb training induced a general speeding of the task with RTs reduced for all distances, as suggested by the presence of main effect of Time [F(1, 271) = 5.523, p=0.019)], but it did not change the pattern of the RT/distance; absence of Distance x

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Time interaction [F(1, 277) = 0.011, p=0.916]. Conversely, testing the prosthesis, training changed the pattern of the RT/distance curve as shown by the presence of interaction Distance x Time [F(1, 277) = 10.764, p=0.001], besides the main effects of Distance [F(1, 277) = 8.780, p=0.003] and Time [F(1,277) = 4.392, p=0.037]. Change of pattern, with a decrease of RTs in far space, going from 60 to 90 cm, suggested an extension of the PPS and it is in favor of a positive effect of training on the embodiment of the prosthesis (Fig. 19).

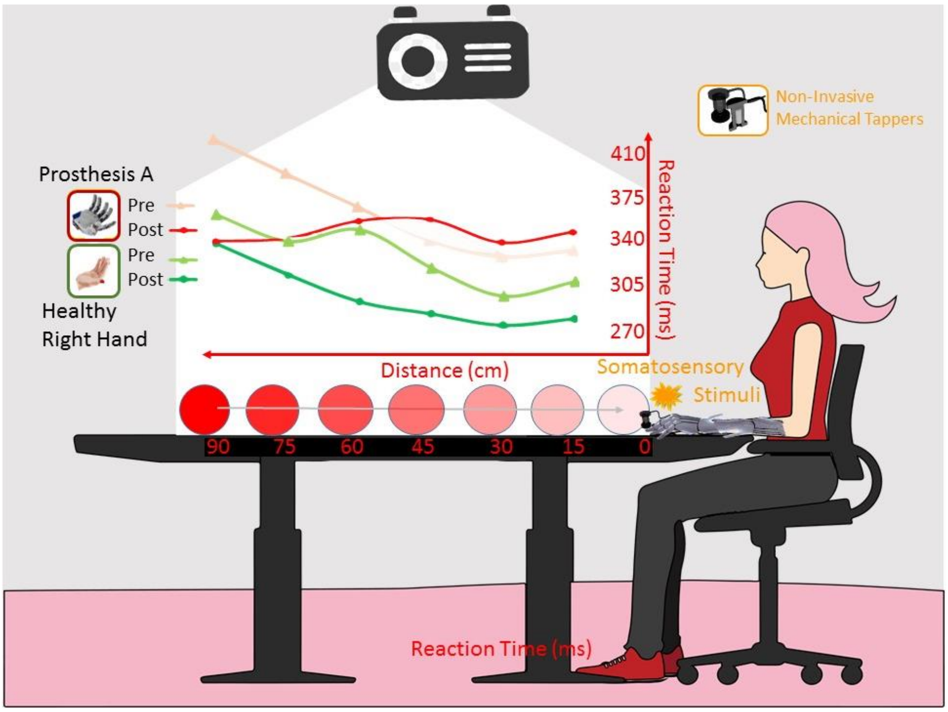

Figure 19: Effect of training on VTI when the participant was tested with the healthy right limb (green) and with the more trained prosthesis (red) in the PRE session (lighter colors) and in the POST_NI session (darker colors). Somatosensory stimulation was always delivered non-invasively. The healthy hand underwent a general speeding of RTs for all the tested distances but maintaining a similar RT/Distance pattern, while the prosthesis changed the pattern of the RT/distance with a decrease of RTs in far space, suggesting an extension of the PPS induced by the embodiment of the prosthesis.

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The TOJ task did not give additional information on the effect of training since, in the PRE, the participant was extremely inaccurate and variable in performing the crossed hand condition (EA: uncrossed SOA=70±15 vs crossed SOA=220 ± 140) and data have not been analyzed.

Prosthesis

In the VTI task, the healthy limb was in general more rapid than both the tested prostheses, as suggested by the presence of a main effect of *Hand* [$F(2, 338) = 4.136$, $p=0.017$]. The direct comparison between the levels of the factor *Hand* showed that the healthy limb was different from both the prosthesis A, ($p<0.001$) and the prosthesis B, ($p<0.001$), while prosthesis A did not differ from prosthesis B, ($p=0.428$). Despite the different average RT, the two prosthesis and the healthy limb did not show a statistically different pattern of response across the distances (*Distance* x *Hand* interaction: $F(2, 351) = 1.180$, $p=0.309$) suggesting that the stimuli approaching the prosthesis were processed similarly to those approaching the healthy limb. This is in favor of an embodiment of both prostheses (Fig. 20).

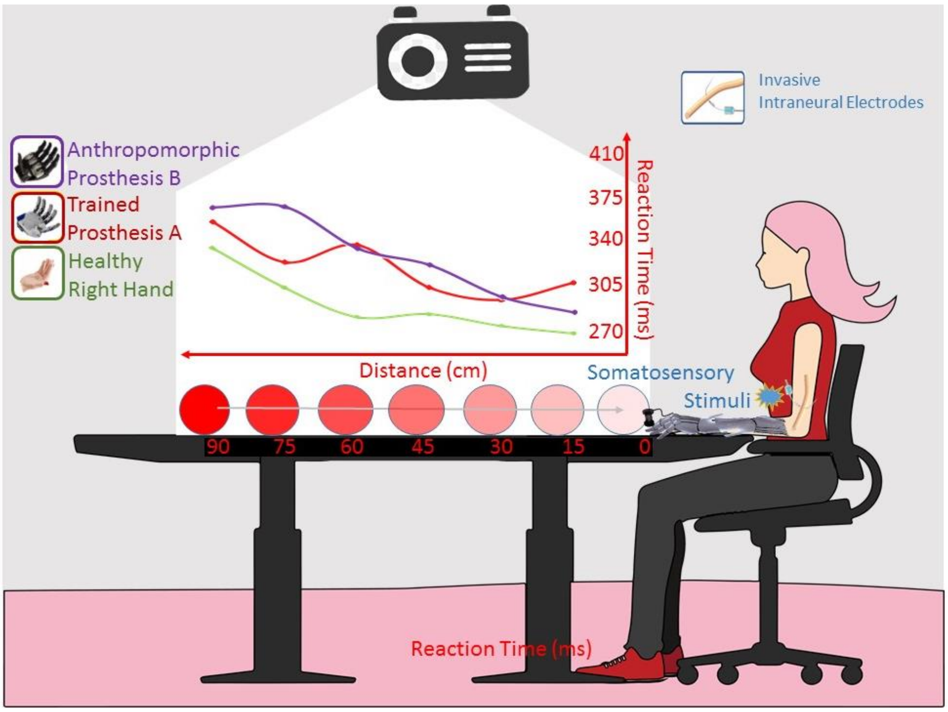

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Figure 20: Effect of different prostheses on VTI. In the POST_I session when the participant was tested with both the prostheses she was slower for all the tested distances than when she was tested with the right healthy limb (green), however the RT/Distance pattern was not different (Distance x Hand interaction p=0.309), which is in favor of an embodiment of both prostheses.

Moreover, VTI gave an additional cue of prosthesis embodiment. The 1st and 3rd distances, i.e. 15cm and 45cm from the prosthesis respectively, actually corresponded to 45cm and 75cm from the stump, because the tip of the stump was 30cm shorter than the tip of the prosthesis. Considering the bare stump, within these distances (45-75cm range) would likely fall the shift from PPS to extrapersonal space (Serino et al., 2015), so that when the stump was tested there was an important decrease of RTs from the 3rd=368 to 1st=314ms. An embodied prosthesis would shift the boundary of peripersonal space, so that both 1st and 3rd distances would fall within PPS, because they correspond to 15cm and 45cm from the prosthesis (Fig. 21). Indeed, when prostheses were tested there was almost no RT difference (from 3rd=335 to 1st=330ms). Prosthesis embodiment was statistically confirmed by the presence of a significant Distance (1st vs 3rd) x Hand (stump vs prosthesis) interaction [$F(1, 256.92) = 8.3077$, $p=0.004$].

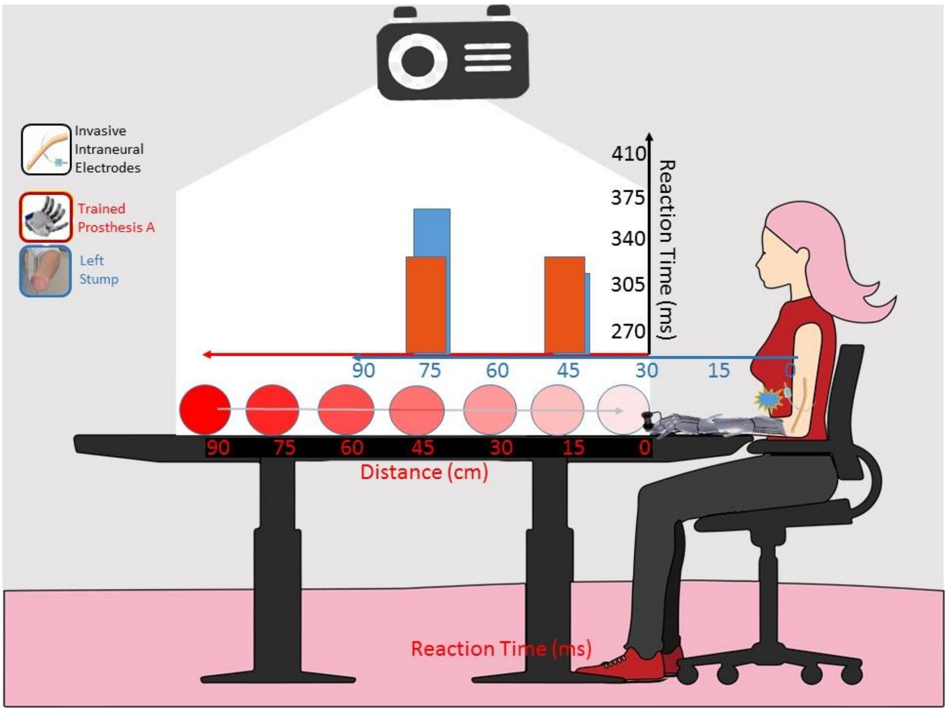

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Figure 21: Comparison of RTs for the 1st and 3rd distance tested with the more trained prosthesis (red) and with the bare stump (blue) in the POST_I session stimulating invasively through the intraneural interface. The passage between peripersonal to extrapersonal space, which seems to be between those distance, appears to be shifted forward when the participant was tested with the prosthesis.

So far VTI task showed that both prostheses tested with intraneural stimulation behaved as the healthy limb, thus were embodied, however TOJ gave contrasting results.

In the TOJ, the decrease of esteem accuracy going from the uncrossed to crossed hands, typical of healthy subjects (EA control group: 66.3 vs. 97.2ms), was present only for the less-anthropomorphic more-trained prosthesis A (EA: 55.9 vs. 114.1ms). Indeed, esteem accuracy with this prosthesis was not significantly different with the ones of the healthy subject control group, both in crossed ($t(10)=.480$, $p=0.642$) and uncrossed hand ($t(10)=.432$, $p=0.675$) conditions. By contrast, the crossed/uncrossed difference was absent for the more-anthropomorphic less-trained prosthesis B (EA: 121.7 vs. 113.5ms), where esteem accuracy was significantly different from controls in the uncrossed hand condition ($t(10)= 2.298$, $p=0.044$) and not in the crossed hand condition ($t(10)=.463$, $p=0.653$) (Fig. 22).

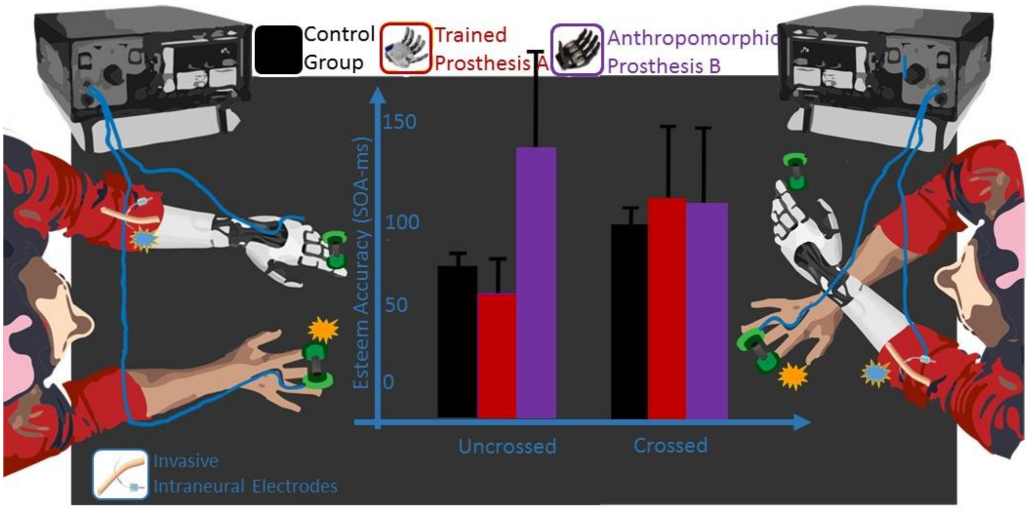

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Figure 22: Changes of Esteem Accuracy between uncrossed and crossed-arm TOJ in the POST_I session. EA is computed through the SOA corresponding to half of the inverse of its derivate for PSS, thus the shorter the SOA is the higher the accuracy is. The decrease of esteem accuracy going from the uncrossed to crossed hands, typical of healthy subjects (black), was present only for the more-trained prosthesis (red) and absent for the more anthropomorphic (violet) prosthesis. TOJ crossing hand effect is in favor of the embodiment of only the more trained prosthesis.

4. Discussion

This study was designed to investigate the embodiment of prosthesis achieved by practicing with a closed sensorimotor control loop enabled by a more natural and rich sensory feedback delivered through multichannel neural interface, in a context of ecologic continuative use of a worn and neurally-interfaced prosthesis.

It has peculiar features compared to the previous studies approaching prosthesis embodiment. We had the opportunity to test our subject longitudinally, before and after a one-month period when she achieved proficiency in controlling the prosthesis. Moreover, the studied subject had a stable chronic amputation, thus at the time of the study, she was not going through any spontaneous recovery plasticity, and she had not used active prostheses before the study, allowing us to have a clean measure of her baseline prosthesis embodiment at the beginning of the experiments.

Patients having intraneural electrodes implantation to receive prosthesis sensory feedback are still rare, thus single-case studies are worth to be run to gather clues on the embodiment of neurally-interfaced prostheses. To minimize possible biases linked with studying single cases, we decided not to rely on subjective and explicit statements, but to employ two implicit and objective paradigms, to test-retest the subject, and to statistically compare the results from the volunteer with those from a healthy subject control group.

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The paradigms investigated prosthesis embodiment through the study of multisensory integration and spatial remapping; they were developed in healthy subjects (Gray & Tan, 2002; Shore et al., 2002; Yamamoto & Kitazawa, 2001a) and demonstrated to be sensible to the embodiment of tools (Canzoneri, Ubaldi, et al., 2013; Yamamoto & Kitazawa, 2001b) and were already validated in amputees (Canzoneri, Marzolla, et al., 2013; Sato et al., 2017).

During the period of training the participant used both the prostheses, always receiving a rich tactile feedback related to manipulation activities made with the prosthesis through intraneural and perineural multichannel electrodes. However, when the participant was tested in the multisensory integration tasks, somatosensory stimuli were delivered in different sessions either with non-invasive mechanical tappers or with invasive intraneural stimulation to highlight possible advantages of the latter on embodiment.

Intraneural stimulation did not speed VTI RT, and when TOJ was tested by stimulating the right limb non-invasively and the left limb invasively there was a laterality bias toward the healthy right limb, so that intraneural stimulation had to be delivered about 30ms before the contralateral to have the same probability to be felt as the first. Since electrodes are implanted in the median third of the arm and since intraneural stimulation does not need mechanoreceptor transduction, the longer duration of the tapper stimulation (40ms vs 200µs) has likely hidden the perception of the intraneural one.

Being the electrode implanted only in the left limb, we could not test TOJ performed with both sides stimulated intraneurally and demonstrate any improvement of performance due to intraneural stimulation, but we could show that when stimulation was delivered non-invasively on the right and invasively on the left limb, performance in the TOJ did not decrease, despite the asymmetry of stimulation.

Even more importantly, we found in VTI that when the relation between multisensory integration and distance from the body, expressed by the RT/Distance pattern, was tested with intraneural stimulation the amputee wearing the prosthesis performed the task more like the control group of healthy subjects. This is in favor of a selective advantage of intraneural stimulation on prosthesis embodiment.

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To the best of our knowledge, this is the first study that demonstrated in amputee that training the control of the prosthesis favors the process of embodiment by testing-retesting the participant before and after the period of training. To avoid any bias due to different stimulation, the effect of training was tested always with the same stimulation, i.e. non-invasively.

During the month of training the participant was often involved in performing skilled bimanual tasks, thus both limbs were trained. When the effect of training on PPS expansion was assessed through the VTI task, we found a differential effect depending on the tested limb. The healthy limb showed an overall increase of performance, boosting up the effect of incoming visual stimuli on touch detection, thus decreasing RTs at all distances of the visual approaching stimulus. Notably, the affected limb showed a modification of the RT/Distance pattern, with a clear extension of the PPS, a strong clue to embodiment of the extracorporeal device (Maravita & Iriki, 2004). We could not gather additional information on the effect of training from the TOJ task because the esteem accuracy in the crossed hand condition was extremely low and variable, probably due to the rarity of exploiting the stump to act and explore the contralateral side of the space.

In the VTI, prostheses recalibrated PPS around the subject, as shown by the shift of the PPS/Extrapersonal boundary which was between 30 and 60cm with the bare stump and beyond 60cm from the stump when the subject was tested wearing the prosthesis. This was in line with the previously demonstrated partial recovery of PPS shrank by the amputation (Canzoneri, Marzolla, et al., 2013). Moreover, the participant behaved as she did with her right healthy limb when wearing both prostheses, suggesting that they were similarly able to expand visuo-tactile integration in the PPS.

Conversely, only wearing the more trained, despite less anthropomorphic, prosthesis the participant experienced worsening of TOJ performance following arm crossing, comparably to the control group.

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Recently, it has been shown that even prostheses unable to give any sensory feedback were able to induce the crossing hand effect typical of healthy limbs (Sato et al., 2017). It is worth noting that in that study, the three amputees were tested with their own, daily used (thus hyper trained) prosthesis. This suggests that using the prosthesis to act in external space, provides sufficient clues to allow the remapping of the cortical representation of the prosthesis in external space, inducing the relocation of somatosensory stimuli in the contralateral side, thus the detrimental effect on TOJ on arm crossing.

Notably, previous studies that reported the hand crossing effect on TOJ, always stimulated the tip of the hand, drum stick or prosthesis, which could be relocated in the contralateral space quite far from the midsagittal plane on limb/tool crossing. By contrast, intraneural stimulation was delivered to our participant through electrodes placed on the median portion of the arm, which even in the crossed condition remained in the same side of the space, as if electrodes were uncrossed. Thus, in our case, we had a dissociation between the side were the stimulation was physically delivered and the side where it was referred. Performance worsening occurred with uncrossed real stimulation which was perceived as crossed only because of the sensation remapping, reinforcing the link between prosthesis embodiment and hand crossing effect.

Both VTI and TOJ are based upon multisensory integration. Somatosensory feedbacks, such as touch, proprioception and the efference copy are coded in an egocentric reference frame, where they are compared to "where I am", while environmental feedbacks, such as sight and hearing, are coded in an allocentric reference frame, i.e. where the information is compared to the rest of the environment. To integrate information that do not share the same reference frame, a process of remapping is needed.

In sensory remapping, a sensory modality is not just recoded in the frame of another modality. Instead, it is recoded in a new space that mixes the spaces of the two modalities using weights in line with a policy based on Bayesian integration (Heed, Buchholz, Engel, & Röder, 2015; Miyazaki, Yamamoto, Uchida, & Kitazawa, 2006) or a Kalman filter-like noise optimization of sensory fusion (Deneve, Duhamel, & Pouget, 2007). However, for simplicity we will treat it as a simple sequential transformation in the following discussion.

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To achieve an effective hand-environment interaction, it is needed the knowledge of where the environment is in relation to the hand and what the hand movement will affect. Thus, a close sensorimotor control loop involves a double transformation: along the afferent branch, environmental information must be re-referenced in a bodily frame, while in the efferent branch, the knowledge of the body coordinates has to be re-referenced in the allocentric frame of the environment.

In VTI and related acoustic-tactile integration tasks, environmental inputs collected by the sight or hearing, assume different valence, and are able to enhance the effect of touch, depending on where they are in respect to the body (Serino, 2019). This process subtends a re-referencing of environmental information in a bodily frame, thus VTI tests the remapping typical of the afferent branch.

PPS is the space around the body where external events are considered more relevant. It has been suggested that we have a motor-based PPS that has the higher possibility to be directly acted upon by the body, and a defense-based PPS where external stimuli may be more effective upon the body (Cléry, Guipponi, Wardak, & Hamed, 2015; de Vignemont & Iannetti, 2015).

In our VTI task we tested fast approaching stimuli. Looming stimuli are potentially more dangerous than static, so that they induce a protection response with enhanced tactile sensitivity in their predicted time and site of impact (Cléry et al., 2015; Gray & Tan, 2002). It's likely that we tested the extension of a safe margin around the body, since training was less relevant than sensory feedback in determining VTI outcome.

In the uncrossed hand TOJ, the right hand is in the right side of the environment, egocentric and allocentric reference frames are concordant, and the judgment of which hands was stimulated first can rely only on somatosensory stimuli analysed in their original bodily-referenced frame. On the contrary, when the TOJ is tested with the hands crossed, the delivered somatosensory stimuli assume different value depending on where they are in the environment, thus the judgment subtends a remapping of the origin of tactile stimuli in external world coordinates (Schicke & Röder, 2006).

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The typical increase of reaction time is a proof of such remapping which becomes more complex and requires longer computational time, being the hands not crossed in the body default representation (Yamamoto & Kitazawa, 2001a), or because time is needed for the resolution of conflict between sensory modalities (Shore et al., 2002) or for their integration (Heed et al., 2015). Accordingly, we think that the TOJ hand crossing effect tests the remapping typical of the efferent branch, which is more aimed at acting.

Despite TOJ is a tactile task, several cues are in favor of a motor-based origin of the transformation it tests, because external spatial coordinates allow the movement toward the tactile event. Indeed, TOJ is modulated by hand movements, which are able to compress time interval (Tomassini, Gori, Baud-Bovy, Sandini, & Morrone, 2014). The crossing effect is also present behind the body where the space can be only coded by movement (Gillmeister & Forster, 2012), and may be due to the efference copy since it is present when the hands are uncrossed but a crossing movement is planned (Hermosillo, Ritterband-Rosenbaum, & van Donkelaar, 2011). Moreover, the crossing effect sticks with the part where the motor operational ability is focused, while the position of the rest of the arm or of the tool is irrelevant, as showed by the absence of the effect when double crossing with drumstick (Yamamoto & Kitazawa, 2001b) and its presence with L-shape stick (Yamamoto, Moizumi, & Kitazawa, 2005).

The amputee we tested controlled prostheses with an ad hoc developed EMG control and received tactile feedback from both prostheses related to dexterous manipulation and slippage trough invasive nerve stimulation (Zollo et al., 2019). Hence, the control loop of both prostheses benefited of invasive feedback, and this may explain why both prostheses were embodied accordingly to the VTI. Both prostheses behaved as her right real hand because they were both able to trigger the remapping of visual stimuli into bodily coordinates.

The time our volunteer spent in training skilled manipulation with each of two prosthesis was very different [45 days for prosthesis A vs 20 days for prosthesis B] and this may be the reason of a different embodiment of the two prostheses according to the TOJ. Only the more trained prosthesis, despite being less anthropomorphic, was able to trigger the bodily-into-environment remapping and to induce the hand crossing effect.

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Training is needed by the plastic processes at the base of remapping and neurally-interfaced hand prostheses have shown a strong ability to foster such plasticity. This has been widely demonstrated in primary sensorimotor cortices (Rossini et al., 2010) and in their interplay (Di Pino et al., 2012; Ferreri et al., 2014), but it failed to be shown in the fronto-parioetal network. In targeted muscle and sensory reinnervated patients, which benefit of high effective bidirectional interface with the prosthesis, M1 and S1 activity and connectivity were almost normal, but the interplay with the frontal and parietal areas was highly impaired (Serino et al., 2017). Is the induction of plasticity on that network still beyond the ability of highly-interacting prostheses? The present study offers a behavioral demonstration of plasticity of the frontoparietal network induced by neurally-interfaced prostheses.

Indeed, TOJ has been ascribed to the activity of parietal and prefrontal cortices and the crossing hand effect to their combination with multisensory perisylvian cortices coding the representation of motion (Takahashi, Kansaku, Wada, Shibuya, & Kitazawa, 2012). The multisensory integration at the base of VTI has been widely ascribed to fronto-parietal interplay (di Pellegrino & Làdavas, 2015) and TMS entrainment and disruption studies highlighted the importance of posterior parietal cortex in frame remapping (Bolognini & Maravita, 2007; Konen & Haggard, 2012; Ruzzoli & Soto-Faraco, 2014).

In monkey, two partly separated networks with bimodal visuotactile neurons are responsible for multisensory integration in PPS: the VIP-F4 more involved in coding a defense PPS around the vulnerable parts, especially hand and face (Graziano & Cooke, 2006), which is sensible to emotional and social aspects (Cléry et al., 2015) and the areas7b and AIP-F5, in charge of the visuomotor transformation needed for grasping objects in the environment (Rizzolatti & Luppino, 2001), that codes the motor PPS. We may speculate that, prosthesis embodiment revealed by VTI relies more on the former and has been achieved in our subject with both prostheses, while embodiment revealed by TOJ relies more on the latter, and it has been achieved only with the more trained prosthesis.

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Not only a continuative use of multichannel and multi-nerve intraneural stimulation, able of a richer and more pleasant sensory feedback, showed to induce prosthesis embodiment, but also the acute employment of such feedback signals during the test induced an even deeper embodiment compared to non-invasive tactile substitution. However, a comprehensive analysis of both experiments suggests that sensory- and action- oriented embodiment may not always completely-match, and if the quality of sensory feedback and the degree of human-like appearance of the prosthesis are key factors to achieve the former, an operative tool-like embodiment is only achieved through a learning process that leads to the achievement of proficiency.

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Chapter 5: Intermittent theta burst stimulation over ventral premotor cortex or inferior parietal lobule does not enhance the rubber hand illusion

1. Introduction

Among the cognitive functions the establishment of the representation of the body is one of the most investigated while, in bionics, the cognitive aspects of prosthetics are attracting great attention and funds. Hand amputation distorts body representation so that an enhanced sense of prosthesis ownership may be the key for a successful treatment. Embodiment, the process of feeling not-owned limbs as part of our body, rises from the activation of premotor and multisensory associative areas within the frontoparietal network (Ehrsson et al., 2004), thus inducing plastic changes in this network should impact on embodiment. Excitatory protocols of repetitive transcranial magnetic stimulation (rTMS) are effective in enhancing plasticity and have behavioral effects in healthy and impaired subjects (Di Pino, Pellegrino, et al., 2014), but they have never been used to boost prosthesis embodiment so far (Di Pino, Maravita, et al., 2014).

A reliable way to induce and test embodiment of an artificial hand is by mean of the rubber hand illusion (RHI) paradigm (Botvinick & Cohen, 1998). A rubber hand is located directly in front of the participant, parallel to their real hand. The latter is hidden from the participant's sight. The illusion is induced when the hidden real hand is brush-stroked synchronously (in time) and congruently (in space) with the visible rubber hand.

A bayesian bottom-up integration process of convergent multisensory inputs enables a sense of ownership for the rubber hand, by integrating it into the participant's body schema (Armel & Ramachandran, 2003). Moreover, a distortion of proprioception also emerges, because the participant tends to estimate the position of their real hand closer to the rubber hand than it actually is (proprioceptive drift) (Botvinick & Cohen, 1998).

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Electroencephalography (EEG) (Rao & Kayser, 2017; Zeller et al., 2015) and functional magnetic resonance imaging (fMRI) studies (Brozzoli et al., 2012; Ehrsson et al., 2004; Gentile et al., 2013) link the neural correlates of the RHI induction to a frontoparietal network where premotor and intraparietal sulcus areas tightly interact. These areas contain multimodal neurons that are able to integrate visual and somatosensory information, probably underpinning the representation of our corporeal space (Braun et al., 2018).

In this study in healthy subjects, intermittent theta burst stimulation (iTBS), a facilitatory rTMS protocol, has been used to enhance the excitability of the right ventral premotor cortex (rPMv) or inferior parietal lobule (rIPL). The leading hypothesis was that a facilitatory neuromodulation of those areas would have enhanced the embodiment of the rubber hand. We formulated this hypothesis because we thought that the administration of iTBS over those areas would have changed their excitability and interplay, resulting in an enhancement of body ownership over the artificial limb. In this case, our approach would have been useful in the future to enhance prosthesis embodiment in amputees. TBS has been chosen because of its average strong efficacy and its favorable ratio between time need to neuromodulate (2-3 minutes) and length of the effect (20 to 30 minutes) (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005; Suppa et al., 2016).

Previously, amputees have been reported able to experience the RHI with an enhancement of embodiment over the fake hand (assessed through a self-evaluation questionnaire of the body-ownership index) and drift score of about 50% compared to the control condition (Ehrsson et al., 2008). Thus, in order to achieve a valuable change of embodiment, we targeted half of the reported mean shift, i.e. a 25% increase of the body-ownership index assessed by the self-evaluation questionnaire, which was chosen as the main expected outcome of the study.

2. Materials and methods

Twenty-eight participants (sex:16F, 12M; age: 26.68, SD: 4.66, range: 21-39. Four of the participants were left-handed as assessed with the Edinburgh Handedness Inventory), after signing a written informed consent, took part in the study. The number of enrolled subjects was determined considering that: i) based on the RHI Index data distribution from (Abdulkarim & Ehrsson, 2016) (Mean: 2.1, SD: 1.15), to show a 25% mean shift, achieving an effect size of

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about 0.5 and a power of about 0.5, 25 subjects were needed, ii) two subjects were excluded from the study because they did not complete all the experimental sessions and another one for being unable to follow experimenter's instructions.

Participants underwent three sessions of neuromodulation (iTBS over rPMv; iTBS over IPL and SHAM) in a random order. Each session consisted of neuronavigation, neuromodulation (i.e. iTBS), synchronous and asynchronous (control condition) RHI (Fig. 1). In each session synchronous and asynchronous RHI order was randomized and both delivered in a range of 10-20 min when the effect of iTBS was reported to be at his peak (Huang et al., 2005).

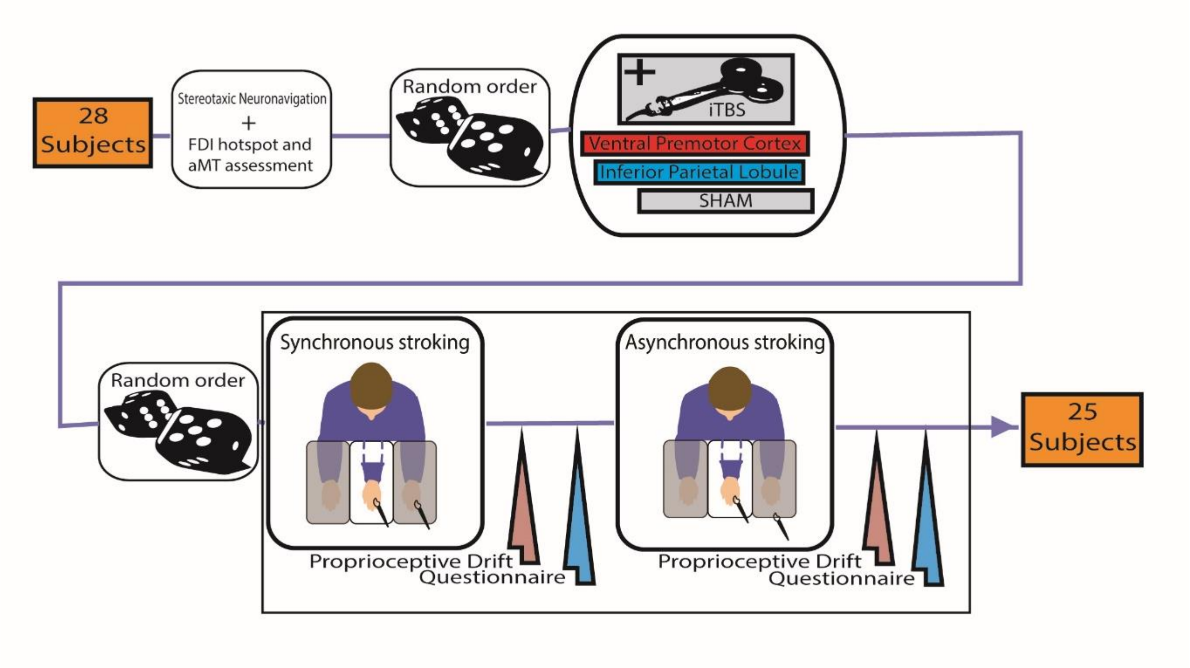

Figure 23: Schema of the experimental procedure of the study. Intermittent theta burst stimulation (iTBS) was administered before Synchronous or Asynchronous stroking (in a random order) using paintbrushes. In the latter, the temporal mismatch of the stimuli was about 0.5s.

Participants were placed in front of a purposely-assembled structure with three separated compartments below a two-way mirror. Each compartment had its own illumination system, so that the experimenter could choose whether or not the participant could see its content.

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The participant's forearms were placed inside the two lateral compartments while shoulders were covered by a black cloak. The rubber hand was placed in the central compartment 15 cm medially to the participant's left hand, and it was made visible by turning on the central compartment light. Once the rubber hand was visible, the experimenter started to synchronously or asynchronously (depending on the current condition) strike the participant's hand and the rubber hand with paintbrushes for 90s.

A 9-item questionnaire and the proprioceptive drift (Botvinick & Cohen, 1998) were collected to measure the effectiveness of RHI induction. The questionnaire required the participants to rate the strength of their agreement or disagreement with nine statements by using a 7-point Likert scale. Three of the statements (i.e. illusion statements) referred to the extent of self-attribution of the rubber hand during the trial. The other six statements (i.e. control statements) served as controls for compliance, suggestibility, and ''placebo effect''. Questionnaire outcome was evaluated through the RHI Index (mean of the three illusion questions minus mean of the six control questions) (Abdulkarim & Ehrsson, 2016). For the proprioceptive drift assessment, participants had to report a number on a measuring tape reflected on the two-way mirror that corresponded to the perceived location of their left index finger. The proprioceptive drift was calculated by subtracting the score obtained before each RHI procedure from the score collected right after it, where positive values indicate a drift towards the rubber hand in participants' sense of the hand position.

Magnetic stimulation was performed with a biphasic magnetic stimulator (Duomag XT-100, Deymed, Hronov, CZ) and a 9 cm figure-of-eight coil. Active motor threshold (aMT) was determined as the minimum single-pulse intensity required to produce at least five out of 10 MEPs greater than 200 μV in the left first dorsal interosseous (FDI), while the subject was maintaining a voluntary contraction of about 20% of maximum force. iTBS pattern was produced with three pulses of stimulation given at 50 Hz, repeated every 200 ms for 2s, then a pause of 8s for a total of 600 pulses and 190s (Huang et al., 2005). The stimulus intensity was set at 80% of aMT.

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Real stimulation was delivered either over PMv or over IPL on the right hemisphere and the RHI tested on the contralateral left hand, since right hemisphere is prevalent in the RHI task and in the establishment of body ownership (Ehrsson et al., 2005; Meador, Loring, Feinberg, Lee, & Nichols, 2000; Ocklenburg et al., 2011). Stimulation points on the scalp were found at the beginning of the first session referring to Talairach coordinates, corresponding to rPMv (x=52, y=10, z=24) and rIPL (x=56, y=-27, z=37), with the help of an optoelectronic neuronavigation system (SofTaxic 2.0, EMS, Bologna, Italy). Sham iTBS was done over the vertex with the coil placed perpendicular to the scalp. Sessions differed at least 48 hours from each other.

The three-session, single-blind, sham-controlled, counterbalanced cross-over experimental protocol was conducted in accordance to the ethical standards of the Declaration of Helsinki and was approved by the relevant Ethics Committee.

3 X 2 repeated measures ANOVA was employed separately for RHI Index and proprioceptive drift, with the factors Neuromodulation (Sham vs. rPMv vs. rIPL) and Synchrony (Synchronous vs. Asynchronous).

3. Results

Data were normally distributed (Shapiro-Wilks, $p>0.05$). For both RHI Index and proprioceptive drift there was the main effect of Synchrony (Fig. 24) [RHI Index: $F(1, 24) = 62.738$, $p<0.001$); proprioceptive drift: $F(1, 24) = 24.554$, $p<0.001$)], while there was not effect of Neuromodulation [RHI Index: $F(2, 48) = 0.527$, $p=0.594$); proprioceptive drift: $F(2, 48) = 0.243$, $p=0.785$)] or interaction Synchrony x Neuromodulation [RHI Index: $F(2, 48) = 0.323$, $p=0.726$); proprioceptive drift: $F(2, 48) = 0.227$, $p=0.798$)]. Planned comparisons with Holm corrected t-tests between synchronous and asynchronous condition were significant in all neuromodulating conditions (RHI Index: all $p<0.001$; proprioceptive drift: all $p<0.021$).

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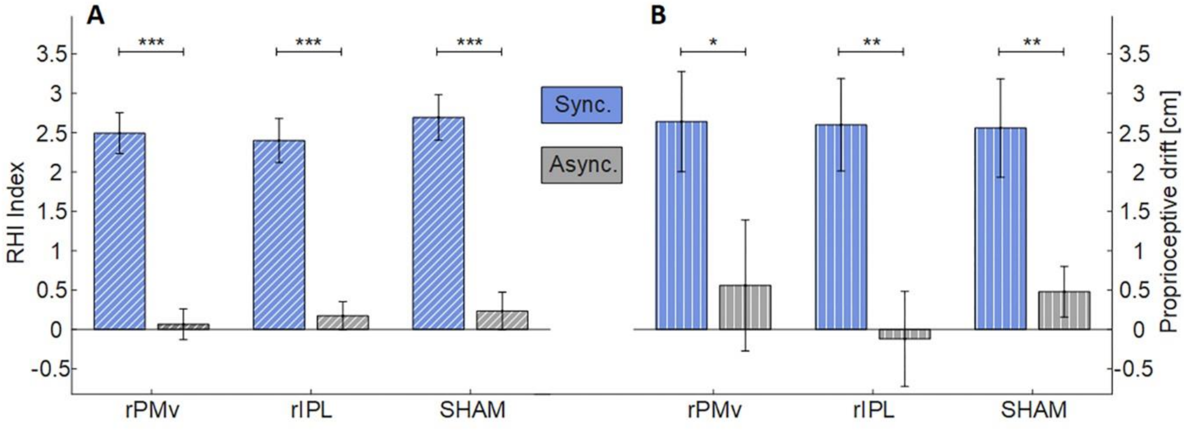

Figure 24: Mean and standard error of RHI Index (A) and Proprioceptive drift (B) in each neuromodulating condition. (*: p<0.05, **: p<0.01; ***: p<0.001). rPMV = right ventral premotor cortex; rIPL = right inferior parietal lobule.

4. Discussion

This is the first study where a facilitatory rTMS protocol has been employed to foster artificial hand embodiment. Hitherto, rTMS has been exploited only with plasticity-decreasing inhibitory protocols to induce virtual lesions. Indeed, 1Hz rTMS over IPL has been reported to reduce proprioceptive drift (Kammers et al., 2009), while over the primary motor cortex (M1) to reduce the real hand ownership, making participants more prone to embody the rubber hand (Fossataro, Bruno, Giurgola, Bolognini, & Garbarini, 2018). The real hand disembodiment was due to the down-regulation of its motor pathway after the "virtual lesion" of M1. This finding was not suited to be exploited for our aim because we target to increase a behavior (embodiment), thus we have chosen a facilitatory protocol, and because our final aim is the enhancement of prosthesis embodiment in amputees who have not their real own hand to disembody.

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Despite a large sample size and a well-controlled within-subject design, iTBS over rPMv or rIPL did not result in an enhancement of embodiment. No significant effects of the stimulation were found on two different investigated measures: the readout of the questionnaire, which is more informative of changes in ownership, the proprioceptive drift which is more specific for a spatial update of the sense of hand's position (Rohde et al., 2011). The presence of the well-known difference of induced embodiment between synchrony and asynchrony in stroking confirms that the illusion was successfully induced in all cases.

The interpretation of these results can be twofold. Embodiment of a fake limb cannot be increased with non-invasive brain stimulation techniques due to a possible ceiling effect. However, the recent reports of a slight increase of proprioceptive drift and subjective experience of body ownership with transcranial direct current stimulation (Convento et al., 2018; Lira et al., 2018) may suggest that iTBS could be not the better suited neuromodulating protocol for this purpose. iTBS was chosen because it is short and effective, but differences in the individual network sensitivity to the magnetic pulse (Hamada, Murase, Hasan, Balaratnam, & Rothwell, 2012) and in individual functional connectivity (Nettekoven et al., 2015) make iTBS efficacy highly variable across subjects and, especially, across brain regions other than primary motor cortex (Suppa et al., 2016).

Supported by the methodological strengths of the study, we can conclude for an absence of effect of iTBS, over the main areas responsible of embodiment induction. We tested only typical iTBS parameters and right hemisphere. However, what we see as more promising to enhance hand prosthesis embodiment and prosthesis users' quality of life is to test neuromodulation techniques acting on cortical circuitry other than the one sensitive to iTBS (e.g. Paired Associative Stimulation).

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Chapter 6: A standard posture of the hand for action

1. Introduction

We all use our hands daily for a multitude of scopes, from object manipulation to interpersonal contacts, thus experiencing every possible posture. During hand actions, we are extremely quick and precise in choosing and shaping grips and assuming any posture. We are also able to localise precisely sensory stimuli delivered to the hand in the space, and couple them with spatial visual information. Such abilities suggest that our brain owns a precise, dynamic spatial representation of our body, the so-called Body Representation (BR). BR could be continuously built by sensory feedback, as an image in a TV screen is built on the information flow, and cease to exist when the stream is interrupted. Alternatively, BR could exist as an a- priori internal representation and be only, but still continuously, modulated by sensory afference. In this latter case, there should be a "standard" posture in which the body is represented.

The possibility that the brain represents the body with a 'standard' posture is longstanding (Nobuyuki Inui, Masumoto, Ueda, & Ide, 2012; Longo & Haggard, 2012; Melzack & Bromage, 1973), but finding ways to test this hypothesis has been challenging.

Movements, especially rapid and ballistic ones, cannot be accomplished only relying on the feedback of the action since the sensory delay would lead to instability of control. Two main control algorithms have been theorized to solve this issue: i) a forward model, built on efference copies of motor commands, that makes a prediction of action-outcome that is readily available and minimizes the impact of sensory delay; ii) an inverse model that implements a feedforward controller algorithm which estimates the motor command required to achieve the desired outcome (Kawato, 1999; Wolpert & Ghahramani, 2000). Such algorithms need to start from an internal model to estimate the desired outcome properly. However, whether internal models hold a standard spatial representation of body posture is not known.

Consistent hand posture-related advantage of sensory processing has been found: tactile stimuli are discriminated faster and more accurately when delivered to a thumb held in a relatively low elevation or to any other finger occupying a relatively high position (Romano, Marini, & Maravita, 2017; Romano et al., 2019). These data suggest that a standard internal model of the

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hand exists for sensory processing (Romano et al., 2017).

The motor counterpart would be that if the body is represented in a standard posture, planning to perform a grip that matches with that posture would require lower computational cost. The motor system would be more prone and faster to perform a grasping action requiring the standard grip posture, than an equally difficult action that requires a posture not matching the standard one. We sought for the prototypical orientation that the hand would have for a pinch action. Being thumb and the index finger crucial for both power and precision grips (MacKenzie & Iberall, 1994; Napier, 1962), we decided to compare the pinch grip performed in the thumb-down posture with the opposite position of thumb-up pinch.

We tested the facilitation of the motor system to performing actions with those two postures at multiple levels, with four experiments in healthy people.

In *experiment 1*, corticospinal excitability (MEP) while holding the two postures was tested through transcranial magnetic stimulation of the motor cortex.

In *experiment 2*, we implemented a behavioural task to measure movement-computation costs and any functional advantage of them.

In *experiment 3* we tested if the motor facilitation for one posture is independent of the actual motor output (i.e., precede the movement). MEPs were measured with the hand at rest while: i) observing; ii) imagining and iii) preparing the pinch grips.

In *experiment 4*, the short-latency afferent inhibition (SAI) protocol, based on coupling motor cortex stimulation with peripheral nerve stimulation, was employed to test if the sensory facilitation for tactile processing (D. Romano et al., 2017) and motor postural advantages are independent, so that a multimodal representation of body posture is likely supra-ordinated.

2. Materials and methods

Participants

Sixteen subjects participated in Experiments 1, 3, and 4 of the study (7 female; mean age: 27.1 years, range: 21-36). The study was approved by the local ethics committee (EMBODY

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protocol). All participants were right-handed (self-report) and they expressed their informed consent to the experiment. The entire experimental session took around 3 hours including the breaks between the three consecutive tasks.

The data from experiment 4 (SAI) of two participants were excluded from the analysis due to excessive EMG artifacts, so that the final sample of experiment 4 is 14.

40 additional participants were enrolled for the experiment 2 and randomly divided into two different groups depending on the starting position of the right hand used to complete the task: prone for 20 participants (10M; 10F; mean age: 25.45; SD: 2.95) and supinated for the other 20 (10M; 10F; mean age: 29.90; SD: 5.08).

Experiment 1

Participants were sitting on a comfortable chair. The elbow was resting on the armrest. MEPs were collected while the right hand was held in two different postures in independent blocks. In one block, the hand was held with the thumb in a lower position opposed to all the other fingers that were occupying an upper elevation modeling a c-shape configuration, as for preparing a pinch grip. This posture was found to induce faster and more accurate tactile discrimination (Romano et al., 2017). In the other block the hand was held upside-down thus with the thumb above all the other fingers (Fig. 25). Participants were asked to maintain this position with the thumb-index pinch-like open (about 8cm of separation between the two fingers), but not stretched.

Twenty MEPs have been collected on each posture and the order of tested posture was counterbalanced across participants.

Electromyography recording

We collected Motor Evoked Potentials (MEP), by measuring electromyographic (EMG) response to a single TMS pulse delivered to the M1 cortex. EMG was recorded from the First Dorsal Interosseous (FDI) and Abductor Pollicis Brevis (APB) (Interelectrode distance 4 cm, active electrode proximally) of the right hand to monitor hand-intrinsic muscles primarily controlling the index finger and the thumb movements.

Resting motor threshold (rMT) was determined as the minimum single-pulse intensity required

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to produce at least five out of 10 MEPs greater than 50 µV in the left first dorsal interosseous (FDI), while subjects kept their arm on the armrest in a resting position.

MEPs were obtained by stimulating the participants in the spot individuated with rMT procedure with the coil with a posteroanterior orientation, setting the intensity to 120% to the resting motor threshold.

EMG responses were processed following this procedure:

a) The Root Mean Square (RMS) of activity in the time window of 1800ms before the TMS pulse was taken as a measure of the muscular tonic activation. 50ms pre-stimulus were not computed.

b) MEPs were calculated extracting the peak-to-peak response evoked by the TMS pulse in a time window of 10 to 40ms post-stimulus. MEPs that did not evoke an amplitude of at least 0.2mV were rejected, during the conditions where the posture was actively maintained.

c) RMSs and MEPs responses were then standardized by dividing the responses in one posture by the responses in the other posture. Specifically, we divided the thumb-down by the thumb-up posture ($EMG_{\text{thumb-down}} / EMG_{\text{thumb-up}}$).

By doing so, MEPs and RMSs become directly comparable to being expressed as a proportion of change in respect to the posture held, they were computed as ratio between their values recorded in the thumb-down by the thumb-up posture. Values were then log-transformed to avoid asymmetric distribution typical of ratios. Values bigger than 0 indicate thumb-down posture facilitation, while values smaller than 0 suggests facilitation induced by the thumb-up posture.

Analysis

Inferential statistics were performed through linear mixed models (LMM) with the statistical software R (R Core Team, 2017), using the package lme4. Standardized EMG responses were analyzed as dependent variable. Participants were added to the model as random effect variable. The model was analyzed with an Analysis of Variance (ANOVA) with Satterthwaite approximation for degrees of freedom.

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In experiment 1 we entered, as fixed-effect model, a 2X (TMS: MEP/RMS) 2X (Muscle: FDI/APB) full factorial design.

To investigate significant effects we calculated the 95% Confidence Intervals (CI) of the diverse conditions. The facilitation for a specific posture is supported by the data if the CI does not include the value 0.

Experiment 2

Three magneto-inertial sensors (Delsys Trigno, Natick, MA, USA) were placed on the participant's right arm: one on the dorsum of the hand at the level of the FDI, one on the forearm at the level of the flexor digitorum superficialis and the last one at the level of the biceps. The participant sat on a chair, lying their right arm on an armrest. The experimental task consisted of 50 randomized grasping actions, 25 were done with a thumb-down posture, the remaining 25 with a thumb-up position. In each trial participants had to grasp a target object (wooden cube, side: 4cm) in front of them at 50cm of distance, after a visual go-signal delivered through a PC monitor presented by a computerized software (OpenSesame). The go-signal consisted in a coloured circle (diameter: 24cm) on a black background presented at the centre of the screen for 500ms, preceded by a ready-signal: an empty circle with white borders which randomly lasted from 500ms to 1200ms. The inter-trial interval was 7000ms, during which the participant had to bring his arm back to the resting position. The color of the go-signal (yellow or blue) indicated the participant to grasp the target object with one or the other posture (thumb-down or thumb-up). The target object had mounted a force-sensitive resistor (FSR) sensor on both its upper and lower surface, to detect the exact time of touch by the participant.

The initial movement of the body segments (i.e. hand, forearm, and arm) was detected using the modulus of the 3D accelerometer (sampling rate: 148 Hz). The threshold for movement-detection was calculated as the mean measurement of the signal recorded 200ms before the go-signal (resting phase) + 5*SD (Wentink, Schut, Prinsen, Rietman, & Veltink, 2014).

As regards the detection of touch on the target object, the FSR signal (sampling rate: 512 Hz) was filtered by a notch filter at 50 Hz and a fixed threshold was employed to identify the time of touch. The selected threshold corresponded to 200 mN.

Analysis

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Inferential statistics were performed through linear mixed models (LMM) with the statistical software R(R Core Team, 2017) using the package lme4 as in experiment 1. We used the reaction time (RT) to initiate the action recorded by the accelerometers as dependent variable. Participants were added to the model as random effect variable. The model was analyzed with an ANOVA with Satterthwaite approximation for degrees of freedom as in experiment 1. We entered, as fixed-effect model, a 2X (Posture: Thumb- Down/Thumb-Up) 3X (Sensor position: hand/forearm/arm) 2X (forearm orientation: prone/supine) full factorial design.

Additionally, we measured the reaching component of the action by calculating the delta between the onset of action and the first contact with the cube on a trial-by-trial basis (delta RT). This method gives an index of the behavioral advantage for a posture that is independent of the eventual difference of the initial computation of the action. We entered the delta RT as dependent variable of an independent LMM. Participants were modeled as random effect variable. The model was analyzed with an ANOVA with Satterthwaite approximation for degrees of freedom. The fixed-effect model, included a 2X (Posture: Thumb-Down/Thumb-Up) 2X (forearm orientation: prone/supine) full factorial design.

Experiment 3

Participants were asked to keep the right arm resting on the armrest in a comfortable relaxed position with the hand in a resting neutral position with the palm down and all the fingers relaxed and aligned on the horizontal plane of the armrest.

Participants were involved in three pre-motor representation of action tasks, during which MEPs were collected: a) observation, b) imagination or c) preparation. All these three conditions featured the same two hand postures of experiment 1, and MEPs were collected following experiment 1 procedure except that in this case the hand was in a resting position so that MEPs that did not evoke an amplitude of at least 0.05mV were rejected as non-evoked responses.

The action to observe, imagine, or prepare was a pinch grip towards a cube block that could have been done with the thumb-up or thumb-down posture. The height of the cube was adjusted is such a way that participants judged the action equally easy with both hands configurations.

a) *Observation.* For the observation task, we recorded two videos of a right hand

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performing a precision grip action toward a wooden cube. The cube side was of 4cm and it was attached to a wooden panel. By doing so, the action resulted completely isolated from any context or background. Participants only saw a right hand entering the scene from the right side, already oriented in the required posture, grasping the cube with one of the two postures. TMS pulse was delivered 1000ms before the first touch of the hand with the block when the grip posture was very clear. Video clips were recorded in 720p definition with 30 frames/second with a camera (GoPro HERO4 Silver, GoPro, San Mateo, CA, USA). Video-clips were recorded and edited in such a way to last 2000ms, and the grasping action was simultaneous in the clips with the two postures.

b) *Imagination.* In the imagination condition, we asked participants to imagine the precision grip action towards the wooden block. A pure sound lasting 300ms was used as go-signal. The instruction was to imagine the grip with one of the two postures and to start the imagery movement only after the sound. TMS pulse was synchronized with the go-signal delivering the stimulation 300ms after the sound. The Inter Trial Interval was of 3500ms. We recorded 20 imagery actions asking to imagine the thumb-up and 20 the thumb-down posture in a block design, counterbalancing which posture has to be imagined first, across the participants.

c) *Preparation.* In the preparation condition, we asked participants to respond to two different pure sounds separated by 1200ms. The first sound indicated to the participant to prepare the action, while the second sound indicated to release the action and perform it. The actions were the same of observation and imagination conditions. TMS pulse was given during the preparation phase 400ms after the first sound.

Analysis

The same statistical approach of experiment 1 and 2 was adopted, namely a linear mixed model (LMM) with the statistical software R (R Core Team, 2017) using the package lme4. The dependent variable was the standardized EMG responses. Participants were added to the model as random effect variable. The model was analyzed with an Analysis of Variance (ANOVA) with Satterthwaite approximation for degrees of freedom.

Here we entered, as fixed-effect model, a 2X (TMS: MEP/RMS) 2X (Muscle: FDI/APB) 3X

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(pre-motor task: observation/ imagination/ preparation) full factorial design.

Additionally, the three tasks were analyzed independently in a sequence of three independent 2X (TMS: MEP/RMS) 2X (Muscle: FDI/APB) factorial designs.

To explore the meaning of significant effects and interactions we calculated the 95% CIs. The same interpretation of experiment 1 is valid: values bigger than 0 suggest facilitation for the thumb-down posture, values smaller than 0 indicate facilitation for the thumb-up posture.

Experiment 4

The short-latency afferent inhibition (SAI) TMS protocol, likely measuring cholinergic cortical inhibition (Di Lazzaro et al., 2000), was employed to assess whether the cortico-spinal facilitation of one posture could be due to the different sensory afference, in line with a sensory-motor interaction hypothesis. SAI responses were collected in the same two hand-postures adopted in experiment 1: thumb-down and thumb-up. The first posture to hold was counterbalanced across the participants. Each SAI procedure included 50 MEPs recordings (see below).

At first, the precise timing of individual N20 component elicited by the stimulation of the median nerve was identified. The N20 component is considered the first cortical component evoked by somatosensory stimulation and it is located in primary somatosensory cortex (S1). To individuate the N20 we adopted an electrical stimulator (Digitimer DS7A, Hertfordshire, UK) delivering stimuli to the median nerve of the right arm at the wrist level at a frequency of 4.9 Hz. N20 was individuated following 500 stimulations. Resulting in a procedure of fewer than 3 minutes.

TMS intensity and hotspot was determined as in experiment 1, except that in this case, we set the intensity at the 110% of the resting Motor Threshold.

The SAI follows a fixed procedure where standard MEPs are mixed with conditioned MEPs. The protocol that we followed was composed of fifty TMS stimulations. The first 10 trials were collected without a concurrent median nerve electrical stimulation as well as the last 10 (i.e., non-conditioned MEP trials). The remaining 30 trials are conditioned by concurrent electrical stimulation of the median nerve. The median nerve stimulation was synchronized with the TMS

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pulse in such a way that the TMS followed the median nerve stimulation by the latency of the individual N20 +2ms (10 trials), +3ms (10 trials), or +4ms (10 trials). The effect of the SAI is calculated subtracting 1 to the ratio of the MEPs evoked during the conditioned trials by the non-conditioned trials and multiplied by 100. By doing so, the decrease of the amplitude is expressed in percentage of the non-conditioned trials (Di Lazzaro et al., 2007; Fischer & Orth, 2011). For example a value of -30% indicates that the conditioned response is 30% less of the non-conditioned response. Following this procedure, values smaller than 0 indicate that the median nerve stimulation reduced the amplitude of the MEPs (i.e., the SAI procedure was effective), thus it has inhibited the cortico-spinal excitability.

For each participant, the SAI procedures were run twice: first while holding the thumb-up posture and second with the thumb-down posture.

Analysis

Since the outcome of SAI is a single data-point per participant LMM could not be used as in experiments 1, 2 and 3. Therefore, we adopted a more classic repeated measure Analysis of Variance design, calculated with the software JASP 0.9.1.

The percentage of decreased response, calculated as described above, was the dependent variable. We tested a 2X (Hand posture: ThumbDown/ ThumbUp) 2X (Muscle: FDI/APB) full factorial design. Once again, significant effects have been explored by using the 95% CIs.

3. Results

Experiment 1. Increased cortico-spinal excitability holding hand postures

In experiment 1, MEPs change significantly in the two postures, while RMS did not (main effect of factor TMS ($F(1,1261)=9.857$, $p<.01$). Moreover, this effect was different depending on the studied muscle (TMS x Muscle interaction $F(1,1261)=8.177$, $p<.01$). The facilitation for a specific posture appeared only in the FDI muscle for the MEP as showed by the inspection of 95% Confidence Interval (CI) that did not include the value 0 only for the FDI muscle (CIs: FDI = .519, 1.272; ABP = -.044, 1.007). Crucially, basal muscle activity (RMS) was not affected by posture neither for FDI (CI= -.243, .927) nor for APB (CI= -.874, .989), suggesting

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that the facilitation of the corticospinal excitability was independent of basal tonic activity (Fig. 25).

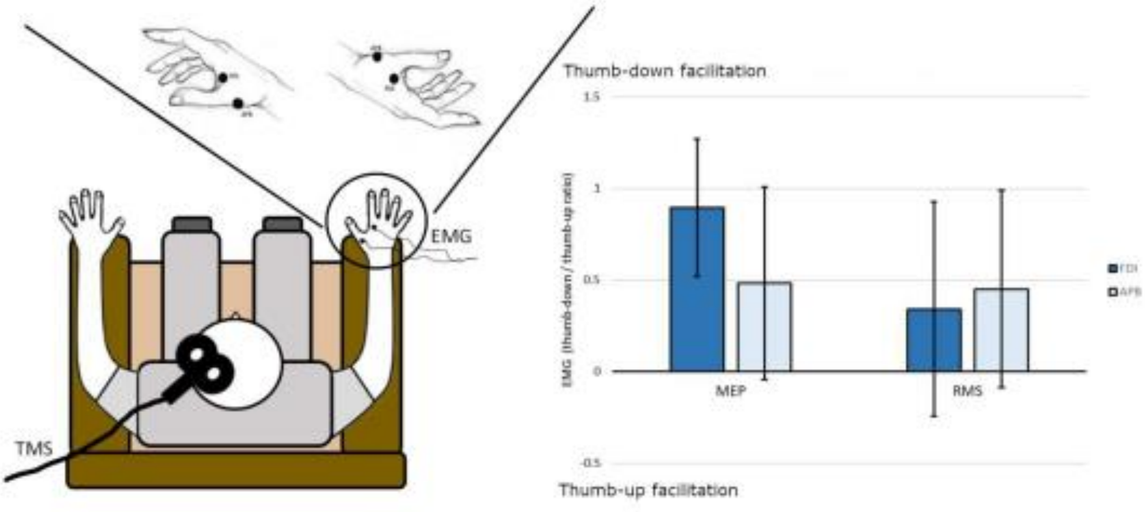

Figure 25. Experiment 1 setup and Results Left panel: schematic representation of Experiment 1setup. The hand postures (thumb-down or thumb-up) are depicted in the upper section. Each black dot shows the position of the pair of electrodes used for the FDI and APB EMG recording. Right panel: Experiment 1 results. Bars represent the average standardized EMG activity (EMG thumb-down / EMG thumb-up). Error lines indicate 95% Confidence Intervals (CI). MEP=EMG evoked by TMS; RMS= root mean square of the tonic basal EMG activity, computed 1.8sec before TMS stimulus. CIs above 0 indicate motor facilitation for the thumb-down posture (FDI-MEP). When the CIs cross the axis, there is no clear evidence for the facilitation of one of the two postures (APB-MEP and both RMS).

Experiment 2. Behavioural advantage of hand posture in action

In experiment 2 we measured the onset of a pinch action with the thumb up, or down; we also measured the time to actually perform the entire action. We found that the pinch started earlier when it was done with the thumb-down configuration showing a behavioural advantage in the computation of the action plan (main effect of factor hand posture ($F(1,5498.1)=127.460$, $p<.001$; interaction hand posture * forearm orientation ($F(1,5498.1)=15.694$, $p<.001$)). The fastening effect for the thumb-down pinch was stronger if the participants had to start the pinch with a prone orientation of the forearm (prone forearm CIs: thumb-down= 292ms, 342ms;

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thumb-up= 319ms, 367ms). Nonetheless, the temporal advantage was detectable also when starting the action with a reversed orientation of the arm (supine forearm hand posture CIs: thumb-down= 324ms, 372ms; thumb-up= 337ms, 385ms), suggesting that the computational advantage for the hand posture is not determined by the synergic advantage of the position of the whole arm (Fig. 26).

Moreover, the advantage of the thumb-down pinch was not limited to the initial computation of the action program. We isolated the reaching component subtracting the time to initiate the action from the time to pinch the wooden cube. The reaching component of the action was faster when it was done with the thumb-down posture (main effect of factor hand posture ($F(1,1839.01)=64.062$, $p<.001$), no matter the starting orientation of the arm forearm (Prone forearm CIs: thumb-down= 502ms, 626ms; thumb-up= 528ms, 652ms. Supine forearm CIs: thumb-down= 501, 627ms; thumb-up= 538ms, 662ms) (Fig. 26).

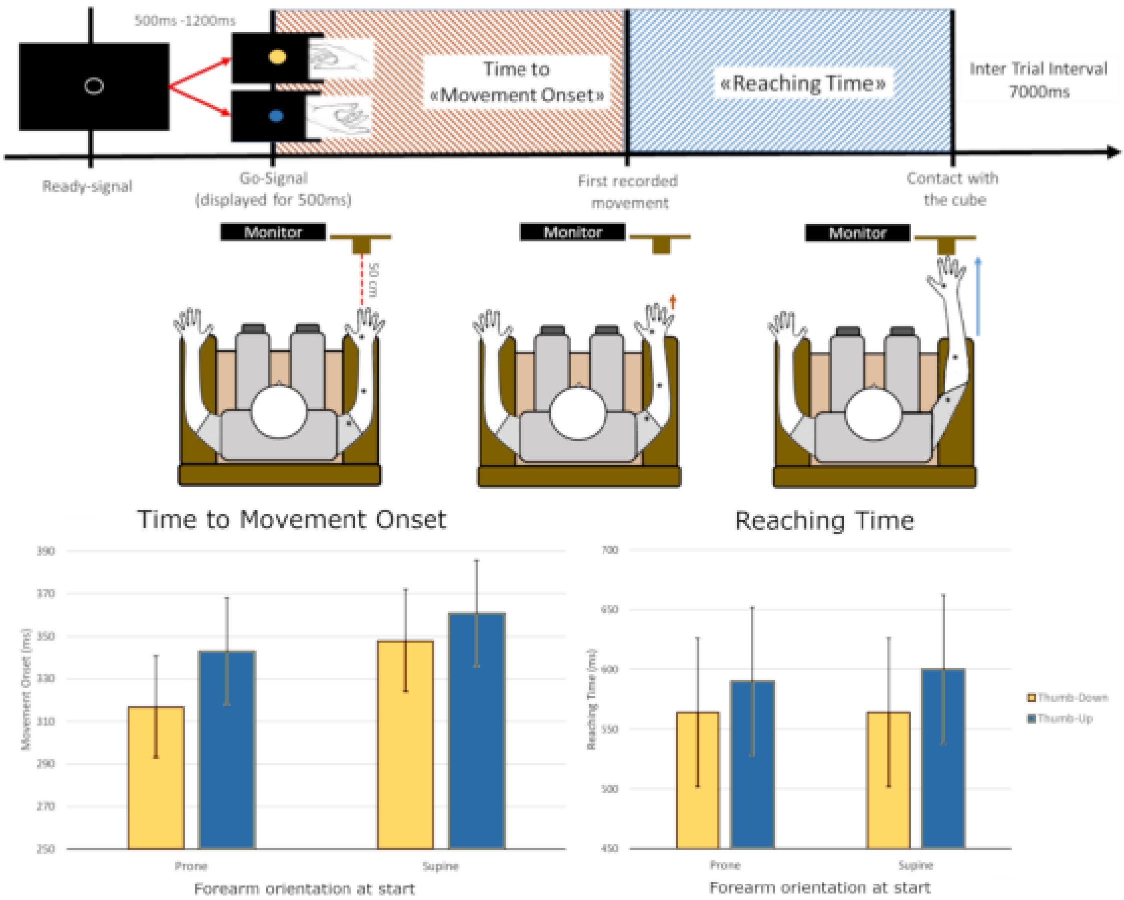

Figure 26. Experiment 2 setup and Results. The upper panels schematically show the

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Experiment 2 setup and procedure. Movement onset: the time from the go-signal to the very first movement recorded by the three sensors (black dots). Reaching time: the time that separates the movement onset from the first contact with the target. The lower panels show Experiment 2 results split according to the initial orientation of the arm. The time recorded by the sensors is averaged because the factor Sensor did not interact with the forearm orientation and the hand posture. Error lines indicate the 95% Confidence Intervals. The thumb-down pinch starts earlier than thumb-up both when the forearm starting position was prone and supine (left graph: movement onset). The thumb-down pinch had faster reaching time independently from the forearm starting orientation and the movement onset (right graph: reaching time).

Experiment 3. Increased cortico-spinal excitability representing hand postures

In experiment 3, compared to experiment 1, we added a third predictor with three levels specifying for the task condition: Observation, Imagination, and Preparation of the action (main factor: pre-motor task). Notably, in experiment 3, the actual posture of the hand was at rest in a neutral position: relaxed on the armrest, open, with the fingers aligned on the vertical plane (i.e., no relative up or down position for one finger than the others).

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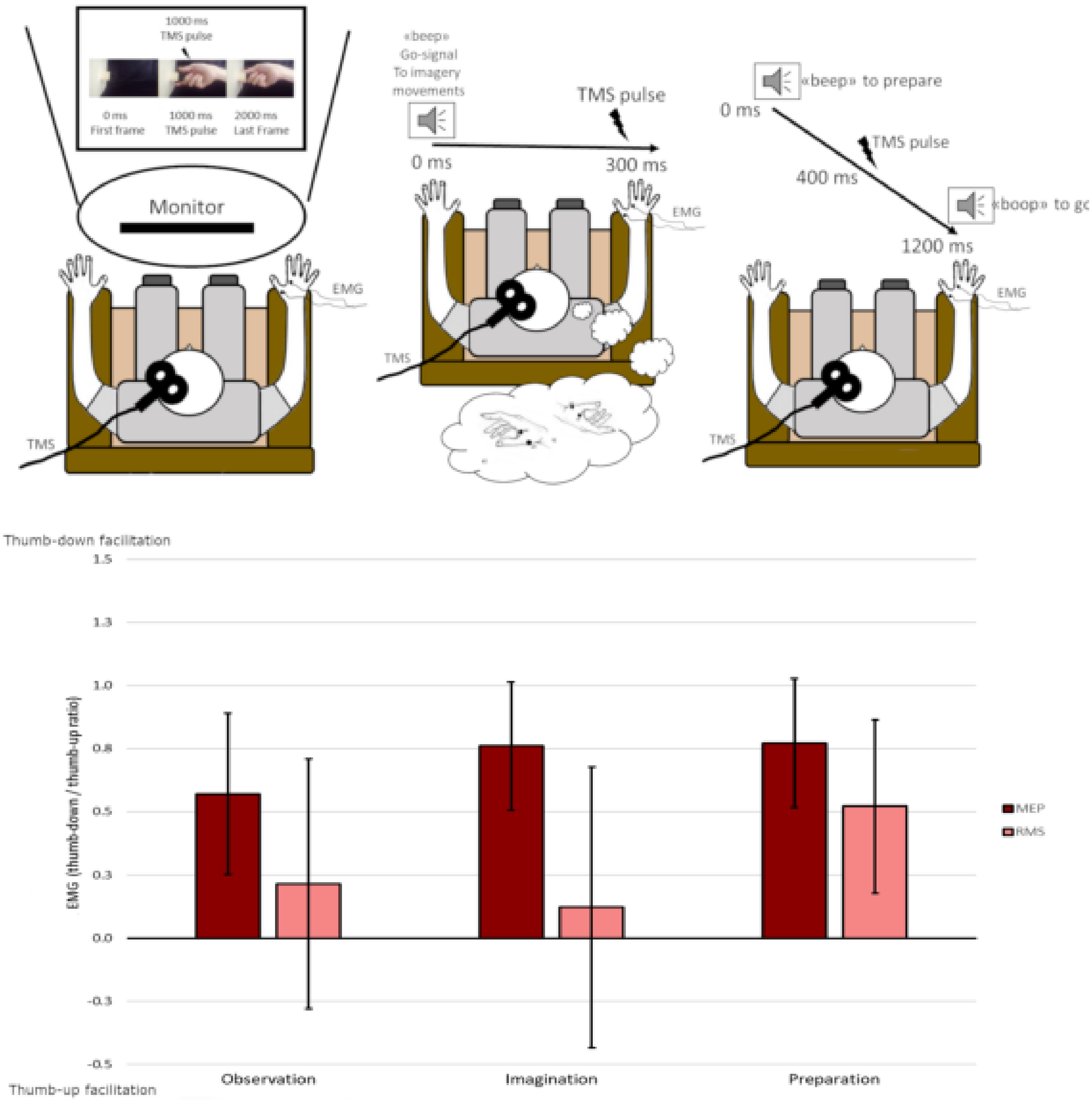

Figure 27. Experiment 3 setup and Results. The upper panels show the pre-motor task conditions. Observation (left): the action was observed in short video-clips of pinch grips on a monitor. Imagination (middle): the thumb-down/-up pinches had to be imagined. Preparation (right): the action was prepared at first and released only after a go-signal. The lower panel shows the results. Bars represent the average standardized EMG activity (EMG thumb-down / EMG thumb-up). Error lines indicate 95% CIs. When the CIs are above the x-axis, there is evidence that thumb-down posture is facilitated (e.g., MEPs in all the three tasks). When the CIs cross the axis, there is no facilitation for one of the two postures.

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The MEPs change significantly in the two postures, while the RMS did not (main effect of factor TMS (F(1,3658.9)=23.021, p<.001). Crucially the MEPs were larger in the thumb-down pinch (CI = .428, .978), while the RMSs were not (CI = -.144, .746), suggesting that also when the thumb-down posture is just mentally-represented, it induces the facilitation of the cortico-spinal excitability independently from basal tonic activity (Fig.27). This effect did not interact with the factor Muscle, and more importantly, it did not interact with the factor pre-motor task (all ps>.23), suggesting that the effect found is independent also from the level of motor representation. Additionally, the main effect pre-motor task was significant (F(1,3658.9)=3.130, p=.045) showing that EMG is larger during motor preparation condition (.362, .945) than the other two: motor imagery (.142, .839), motor observation (.021, .795). Crucially to the aim of the study, this effect is independent from the hand posture.

Indeed, the facilitation for the thumb-down posture was confirmed by the results of the independent analysis of the three pre-motor tasks. In all the three tasks we found that MEPs were larger than 1 (MEPs CI: Observation=.252, .889; Imagination=.506, 1.015; Preparation=.518, 1.026), supporting the facilitation for the actions mentally represented with the thumb-down (Fig.27). In agreement with experiment 1, the MEPs were significantly different than RMS (main effect of factor TMS: Observation, F(1,1195.1)=6.557, p=.01; Imagination, F(1,1201.7)=8.118, p<.01; Preparation, F(1,1213.2)=23.352, p<.001), strengthening the idea that the motor facilitation is independent from the pre-TMS tonic activation.

Experiment 4. No posture-dependent sensory-motor interaction modulation

In experiment 4, the effect of SAI is expressed as the percentage change of conditioned average MEP compared to average unconditioned MEP, negative values mean that conditioned MEP is inhibited. As expected, SAI was effective in modulating the motor cortex excitability in all postures and muscles studied (CI: Thumb-Up / FDI= -46.45%, -21.0%; Thumb-Down / FDI = -40.5%, -23.7%, Thumb-Up / APB = - 36.7%, -10.3%, Thumb-Down / APB = -35.3%, -13.3%). APB was slightly less inhibited (-36.0%, -11.8%) than FDI (-43.4%, -22.4%) (significant main effect Muscle: F(1,13)=4.911, p=.045), probably because TMS was delivered on the FDI hotspot and it has been demonstrated that SAI is maximal at the cortical hotspot of the tested muscle (Dubbioso, Raffin, Karabanov, Thielscher, & Siebner, 2017).

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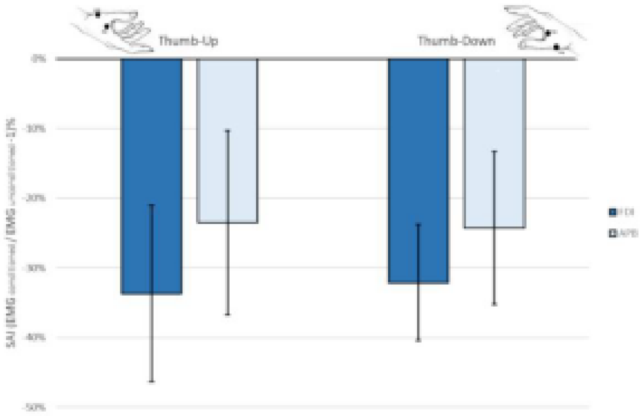

Figure 28. Experiment 4 Results. Bars represent the average SAI effect calculated as a percentage of change of the conditioned trials to the unconditioned trials (SAI = ($MEP_{conditioned}$ / $MEP_{unconditioned}$ -1)*100). Error lines indicate the 95% CIs. The afferent median nerve stimulation inhibited motor cortex excitability, but this was independent from the hand posture (i.e., thumb-up and thumb-down inhibition is comparable).

Crucially, the effect of SAI was not different in the two postures ($F(1,13)=.005$, $p=.945$), neither postures did interact with the muscle studied ($F(1,13)=.101$, $p=.756$) (Fig.28). This suggests that the facilitation induced by the thumb-down posture, found in Experiment 1 and 3, is independent of contingent sensory afferences.

4. Discussion

We investigated the hypothesis that the motor representation of the human body in the brain has a standard posture that results in facilitation of motor output, operationalized in an increased motor cortex excitability, and a behavioral advantage performing an action with the hand in the preferred posture.

We matched two very similar grips, equally difficult and equally used in the everyday-life activity. We found strong and consistent facilitation of the motor system for the pinch grip posture with the thumb-down. The facilitation was not only strong, but it spread to different domains, tested with various experiments, which all gave concordant results in favour of the thumb-down pinch.

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Experiment 1 showed that, while holding the thumb-down pinch posture, the excitability of the primary motor cortex was enhanced. The enhancement was not due to different ongoing muscular and spinal loop activity, because its increase was significantly higher than any increase seen in the tonic activity of the muscle recorded before the TMS stimulus.

Strengthening these results, we also found a congruent behavioural advantage. By doing so we excluded that the results of experiment 1 were limited to the technique employed.

Experiment 2 demonstrated a behavioural advantage of the thumb-down pinch in two aspects. i) Neural computation is faster when thumb-down pinch grip is performed, as shown by the shorter interval from the cue signal to the onset of muscle activation. This advantage was independent of the phase of the grip as measured through sensors placed in the hand, forearm and arm, monitoring the reaching, shaping and grasping phases. ii) The thumb-down pinch grip was also faster to reach the target, independently from the shorter movement onset. Of note, thumb-down shorter time to onset and time to target was independent of the starting posture (supine or prone) of the forearm.

Experiment 3 showed that facilitation for the same hand posture can be observed when no actual movement was required. The hand was kept stationary in a resting neutral position, and hand posture was only representational. Motor facilitation was found when the hand action was either observed, imagined or planned. Notably, the facilitation was found both when those three levels were modeled altogether and when each of them was modeled independently. This strongly suggests that the effect of motor facilitation relies on central mechanisms, possibly related to deeply rooted motor representation quite early before the level of motor output.

Noteworthy, when the posture was actually held stationary, as in experiment 1, the advantage was significant only for the FDI muscle. Conversely, when the action was performed, even only mentally as in experiment 3, the action involved also the shaping phase of the pinch, for which APB is needed. In the latter case, the advantage for the thumb-down configuration become significant for both APB and FDI.

From a more general perspective, we can affirm that when the hand has a C-shape, with the thumb-down and all other fingers-up, the neural processes in charge of motor representation improve, as if that posture serves as an a-priori standard for hand action.

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Two-hypotheses could explain its relationship with the one previously found for the somatosensory system (Romano et al., 2017; Romano et al., 2019). On one side, the motor advantage could be a balancing of concurrent afferent inhibition, exerted by sensory-motor gating mechanisms. In this case, one should find that the postural advantage for the motor system has the opposite configuration than the one found for sensory tasks. However, the pinch posture we found facilitated for action was the same of somatosensory stimuli. Moreover, in experiment 4, the SAI was equally effective in inhibiting motor cortex excitability in both postures, ruling out the possibility that standard posture motor facilitation is due to lower sensory inhibition.

On the other hand, the consistency between the favoured postures in sensory and motor systems and the absence of a different sensory-driven inhibition in the thumb-up and opposite posture supports an alternative, and more intriguing, hypothesis: the standard posture is a representation of the hand supra-ordinate to any sensory or motor processing.

The present results fit with the idea that the brain contains a representation of the body holding a standard posture that works as a Bayesian prior for perception and action. This standard representation would feature a stable baseline spatial configuration of its constituents, a link between hand segments and spatial positions, functional to guide sensory and motor interactions.

Previous theories suggested that the brain may code defaults representations of body posture and, in the 1970s, Melzack and Bromage found that after deafferentation of the brachial plexus or subarachnoid anesthesia participants felt 'phantom' body parts holding few stereotyped postures (Bromage & Melzack, 1974; Melzack & Bromage, 1973). However, those seminal results have been recently challenged when other authors failed to replicate their findings (Inui, Walsh, Taylor, & Gandevia, 2011); the limited evidence left the question unsolved and the hypothesis relatively abandoned.

In healthy adults, body posture may affect the spatial processing of sensory stimuli (Azañón & Soto-Faraco, 2008; Parsons, 1987). For example, in the classic hand laterality task when participants discriminate if a displayed hand is left or right, reaction times increase if participants' hand posture and the displayed hand do not match (Parsons, 1987), suggesting that multisensory matching facilitates motor imagery. Our findings suggest that similar facilitation

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can be obtained by aligning the sensory-motor information coming from the hand with the internal representation of its standard posture.

An important aspect of our study is that the preferred posture was not determined by action goals or object affordance, that may facilitate a given hand configuration for grasping (Jeannerod; Sartori, Straulino, & Castiello, 2011), given that the tasks used were neutral to these aspects. We observed that, in the absence of an environmental trigger, humans motor system still hold a preferred grip posture. Indeed, both hand configurations tested are equally easy to adopt in humans. Specific actions may require one or the other; for example, texting on a smartphone requires typically the thumb above the other fingers, while eating a sandwich requires the thumb below the other fingers. Although there is no clear evidence that one posture is used more frequently than the other, it is possible that the structure of the hand or the more frequent use of a specific posture plays a role developing the preferential posture. However, this cannot explain entirely our results in any case, as we found motor facilitation also when the hand was held at rest in a neutral position and action was only imagined.

Why this specific configuration? Is the standard posture innate, or learned? These are crucial and still open questions.

At present, since the standard posture is not associable with a prototypical grasping for all everyday actions, its sensorimotor advantages in the interaction with the environment may have an evolutional explanation.

In the full-hand power grip, the side of the pinch where the 2^{nd} to 5^{th} fingers are placed is stronger than the side where the thumb is; this would be functional to fight against a force directed down-up. In evolutional terms, what we call standard posture may have given advantages to primates bouncing from branch to branch during arboreal locomotion (C. Fang, Jiang, & Yuan, 2014). On the contrary, when humans started to live on the ground most of the forces were directed up-down, as when lifting objects. In the standard posture only the thumb antagonizes up-down forces, a disadvantage in activities requiring great force. However, being the 2^{nd} to 5^{th} fingers not busy to fight against loads they were free to perform fine and dexterous movements, from sewing to piano playing. Thus, the sensorimotor advantage of standard posture may have contributed to the development of human manipulation, a crucial functional advantage for human evolution.

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Conclusions

We started the present study by questioning the existence of a standard posture for hand actions. We can conclude that thumb-down grip favours central computation, increases motor cortex excitability, gives behavioural advantages and that this posture is the same that facilitates tactile discrimination. These findings overall suggest the existence of a generalized standard representation of hand posture for perception and action, a Bayesian prior that impacts any body-space interaction.

The discovery of this standard hand configuration is key for interpreting the evolutionary shaping of the representation of the body in the brain and may have tremendous applicative impact. For instance, knowing the detailed features of standard body posture may help the understanding of pathological motor control of the hand, e.g., following stroke, and guide its rehabilitation (Di Pino, Maravita, et al., 2014). It may also inform the development of dexterous hand prostheses, where the implementation of a low-level internal control loop that complies with standard posture facilitation (Di Pino, Maravita, et al., 2014; Zollo et al., 2019) would result in more functional and possibly embodiable prostheses.

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Chapter 7: General discussion

Summary of the results of the PhD program

Neuroscience, in the last years, has made great strides uncovering the neurophysiological mechanisms of body ownership. However, the incorporation of prosthetic limbs into amputees' body representation still faces big challenges in clinical reality (Niedernhuber et al., 2018). In order to allow patients to facilitate the activities of daily living, which are being hampered by their amputation, tremendous effort has been put in the past for the development of innovative technological prostheses with the aim of enhancing their functionality (Jang et al., 2011; Wijk & Carlsson, 2015). The majority of research in medical engineering is thus focused on improving prostheses' functionality (Fougner, Stavdahl, Kyberd, Losier, & Parker, 2012; Geethanjali, 2016), but little attention has been devoted to promote their acceptance into patients' body representation. It is well known that the larger part of patients do not wear or use the prosthesis that has been assigned to them, regardless of its functional purposes (Biddiss & Chau, 2007; Jang et al., 2011). The prosthesis is still largely considered as an extracorporeal tool and technological advances are not sufficient to promote self-attribution to the patient's body (Spence, 2015).

The aim of the work presented in this thesis was to take one step forward the current understanding of the processes of artificial limbs' incorporation, with special attention to translational applications for upper-limb amputees.

The RHI is by far is the experimental paradigm that has been most extensively exploited to attribute an artificial hand to one's body. While it is an excellent tool to study the neural underpinnings of body ownership over artificial limbs in healthy volunteers, the same cannot be said for upper-limb amputees. To date, few studies employed RHI to induce a transitory incorporation of artificial hands in amputees (Ehrsson et al., 2008; Marasco, Kim, Colgate, Peshkin, & Kuiken, 2011; Rosen et al., 2009). Furthermore, only one of them (Ehrsson et al., 2008) tested this paradigm on a relatively large sample of patients (n=18) showing that, on average, they are less susceptible to the illusion when the investigated side was the one affected by the amputation. On the contrary, RHI employed on the healthy side shows comparable results with ones obtained with control individuals (Ehrsson et al., 2008). The main challenge in these amputees is to deliver touch stimulation on particular skin areas of their stump while

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synchronously stimulating the related area on the prosthesis. Some amputees may show a rudimentary topographic phantom map in their stump. In fact, when touched on their stump, they may refer tactile sensations on their fingers of their phantom hand (Knecht et al., 1998). However, in so doing, these experimental conditions significantly differ from the ideal ones under which the illusion of owning the artificial hand within the RHI paradigm emerge. The rough nature of the stump map could hamper the correct delivery of spatially congruent touch, preventing the illusion to arise (Niedernhuber et al., 2018). Another essential contingency for the RHI is the alignment between the real hand (or the phantom hand) and the rubber hand (Tsakiris & Haggard, 2005). Amputees, though, have a phantom limb that not always is the exact proprioceptive copy of a hand: some of them are retracted, immobilized or less vivid (Schott, 2013). Finally, limb loss could trigger cortical changes that, in the long term, may prevent the illusion to arise: in the study of Ehrsson et al. (2008), patients with recent amputations tend to exhibit stronger RHI than others.

These reasons induced us to investigate the mechanisms of body ownership using the RHI paradigm on groups of healthy individuals and other experimental paradigms on a group of healthy participants and on an upper-limb amputee who volunteered for our study.

In the first experiment (D'Alonzo et al., 2019) – Chapter 2 – the relative weight of the virtualization of visual and somatosensory stimuli in the embodiment of an artificial or virtual hand has been assessed. Results show that the progressive virtualization of these sensory inputs decreased avatar hand embodiment. However, the lowest embodiment level was found when one sense was virtual and the other was not. It can be argued that the more a sensory input is close to reality the more a mismatch between the seen and felt tactile stimulation is perceived. Coherence of virtualization of different senses seems to be an additional constraint that must be taken into account in the development of future prosthetic hands which employ sensory substitution feedback.

The second study (D'Alonzo et al., 2020) – Chapter 3 – presented here shows how NS-SCR can be employed as a physiological and, thus, objective measure to assess the presence of the embodiment over an artificial hand. From a neurophysiological point of view, the establishment of the sense of body ownership is accompanied by a stronger arousal, which recruits the sympathetic division of ANS and increases the fluctuation of SC. Whilst NS-SCR is an optimal tool to evaluate the arising and the presence of the embodiment, the same can't be said for its

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evolution in the long run. In fact, it is known that electrodermal activity suffers from a habituation effect after a few dozens of seconds (Boucsein et al., 2012), therefore, after a while, we cannot be sure if individuals just lose the sense of ownership over the fake hand or NS-SCR stops being informative. In any case, from an applicative point of view, it could be used with upper-limb amputees to assess the onset of a prosthesis' embodiment and its initial development. The strength of this newfound measure is that it is noninvasive, cost-effective, easy to employ, it does not interfere with the collection of other behavioral measures and it is free from subjective biases such as in self-report evaluations.

The experiment presented in chapter 4 (Di Pino et al., in review) explored the embodiment of two neurally-interfaced prostheses on an upper-limb amputee. One prosthesis was more trained but less anthropomorphic in its aspect and the other one was less trained but more anthropomorphic. The patient could move the prostheses with an ad hoc developed EMG control and received the sensory feedback by an intraneural electrodes implantation. She has been longitudinally tested in a context of ecologic continuative use of the prostheses. Two objective paradigms (VTI and TOJ) based on multisensory integration have been employed to test-retest the subject and to statistically compare the results from the volunteer with those from a control group of healthy subjects. Results suggest that in this volunteer, training the control of the prosthesis is a determinant factor that favors the process of embodiment. Furthermore, the employment of richer sensory feedback through multichannel and multi-nerve intraneural stimulation is able to induce a deeper embodiment compared to non-invasive tactile substitution.

The study of chapter 5 (Mioli et al., 2018) aimed at employing iTBS (a facilitatory rTMS protocol) to foster artificial hand embodiment. Despite the methodological strengths of the study, iTBS over one of the two brain regions (PMv and IPL) that are believed to subtend the sense of body ownership over an artificial hand did not result in an enhancement of embodiment. To the best of my knowledge, until now rTMS has been exploited only with plasticity-decreasing inhibitory protocols to induce virtual lesions and this was the first study to employ a facilitatory protocol. Considering that other studies effectively report a (slight) increase of embodiment using tDCS (Convento et al., 2018; Lira et al., 2018), iTBS might not be the better suited neuromodulating protocol for this purpose.

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Finally, with the study in chapter 6 (Romano et al., submitted) the aim was to identify in which posture a hand prosthesis would have been easier to embody. With a multimodal approach, it has been demonstrated that a grip performed with the thumb-down and other fingers-up has a behavioral advantage, a neurophysiological advantage (higher cortical excitability) and faster computational time compared to its inverse, either if the grip is performed or even if it is only mentally represented. Since such posture of the hand would be a bias favoring any sensation and action, a hand prosthesis postured in this configuration would be easier to embody.

Conclusions

The results presented in this thesis bring one step forward the previous knowledge on body representation and embodiment of prostheses. The acquired knowledge gives the basis to a new set of features and aspects that must be taken into account in the development of innovative, easy-to-embody prostheses. The achieved results suggest that the richness of somatosensory feedback, such as the one delivered by neurally-interfaced prostheses through an intraneural electrode implantation, facilitates the embodiment of artificial hands. Another essential element is the amount of practice: the prosthesis' user should use it as much as possible in his/her every-day environment. Furthermore, in virtual reality applications, it is extremely important to make the level of virtualization of visual and somatosensory inputs related to the avatar's limb as more congruent as possible. As regards the posture in which a hand prosthesis is easier to embody, it has been identified a specific configuration: thumb-down and all the other fingers-up.

In the attempt of unveiling the mechanisms underlying the incorporation of a prosthesis into the user's body representation, I presented a completely new and objective way to assess the onset and the initial evolution of the embodiment over an artificial hand: the NS-SCR. Before the study performed during this doctoral program, the skin conductance has been employed only after a physical threat to the embodied artificial hand to measure its response. However, in this way, the menace can induce movements of the participants' tested limb and invalidate the further collection of other measures. Lastly, it should be noted how iTBS resulted to be an ineffective stimulating protocol to enhance embodiment over an artificial hand in healthy individuals. Other neuromodulating protocols (e.g., Paired Associative Stimulation) or

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techniques (e.g. tDCS) may be further investigated in the future to promote the sense of ownership of prostheses.

Future directions

The next big challenge is represented by the integration, in a new upper limb prosthesis prototype, of all the pro-embodiment features suggested by the results of the studies presented in this thesis. This prototype should be based on a commercially-available hand prosthesis to exploit the recent developments in terms of functionality and performance. At the same time, new effective means of neurostimulating techniques which act at the brain level or at the level of the peripheral nerves should be employed to enhance the embodiment of the prosthesis. Finally, a large group of upper-limb amputees should be recruited and provided with this prototype to verify if they exhibit, as expected, a significant enhancement in the incorporation of the prosthesis into their body representation.

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References

Abdi, H., & Williams, L. J. (2010). Principal component analysis. *Wiley interdisciplinary reviews: computational statistics, 2*(4), 433-459.

Abdulkarim, Z., & Ehrsson, H. H. (2016). No causal link between changes in hand position sense and feeling of limb ownership in the rubber hand illusion. *Atten Percept Psychophys, 78*(2), 707-720. doi:10.3758/s13414-015-1016-0

Antfolk, C., D'Alonzo, M., Controzzi, M., Lundborg, G., Rosén, B., Sebelius, F., & Cipriani, C. (2012). Artificial redirection of sensation from prosthetic fingers to the phantom hand map on transradial amputees: vibrotactile versus mechanotactile sensory feedback. *IEEE transactions on neural systems and rehabilitation engineering, 21*(1), 112-120.

Ardolino, G., Bossi, B., Barbieri, S., & Priori, A. (2005). Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *The Journal of physiology, 568*(2), 653-663.

Armel, K. C., & Ramachandran, V. S. (2003). Projecting sensations to external objects: evidence from skin conductance response. *Proc Biol Sci, 270*(1523), 1499-1506. doi:10.1098/rspb.2003.2364

Augustine, J. R. (1996). Circuitry and functional aspects of the insular lobe in primates including humans. *Brain research reviews, 22*(3), 229-244.

Ax, A. F. (1953). The physiological differentiation between fear and anger in humans. *Psychosomatic medicine, 15*(5), 433-442.

Azañón, E., & Soto-Faraco, S. (2008). Changing reference frames during the encoding of tactile events. *Current Biology, 18*(14), 1044-1049.

Bach, D. R., Friston, K. J., & Dolan, R. J. (2010). Analytic measures for quantification of arousal from spontaneous skin conductance fluctuations. *Int J Psychophysiol, 76*(1), 52-55. doi:10.1016/j.ijpsycho.2010.01.011

Barnsley, N., McAuley, J. H., Mohan, R., Dey, A., Thomas, P., & Moseley, G. L. (2011). The rubber hand illusion increases histamine reactivity in the real arm. *Curr Biol, 21*(23), R945-946. doi:10.1016/j.cub.2011.10.039

Bekrater-Bodmann, R., Foell, J., Diers, M., & Flor, H. (2012). The perceptual and neuronal stability of the rubber hand illusion across contexts and over time. *Brain research, 1452*, 130-139.

Bekrater-Bodmann, R., Foell, J., Diers, M., Kamping, S., Rance, M., Kirsch, P., . . . Flor, H. (2014). The importance of synchrony and temporal order of visual and tactile input for illusory limb ownership experiences - an FMRI study applying virtual reality. *PLoS One, 9*(1), e87013. doi:10.1371/journal.pone.0087013

Berger, C. C., Gonzalez-Franco, M., Ofek, E., & Hinckley, K. (2018). The uncanny valley of haptics. *Science Robotics, 3*(17), Art. No. eaar7010.

Biddiss, E. A., & Chau, T. T. (2007). Upper limb prosthesis use and abandonment: a survey of the last 25 years. *Prosthetics and orthotics international, 31*(3), 236-257.

Blanke, O. (2012). Multisensory brain mechanisms of bodily self-consciousness. *Nature reviews neuroscience, 13*(8), 556.

Blanke, O. (2012). Multisensory brain mechanisms of bodily self-consciousness. *Nat Rev Neurosci, 13*(8), 556-571. doi:10.1038/nrn3292

Bolognini, N., & Maravita, A. (2007). Proprioceptive alignment of visual and somatosensory maps in the posterior parietal cortex. *Current Biology, 17*(21), 1890-1895.

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a condizione che ne venga citata la fonte.

Bottini, G., Paulesu, E., Sterzi, R., Warburton, E., Wise, R., Vallar, G., . . . Frith, C. D. (1995). Modulation of conscious experience by peripheral sensory stimuli. *Nature, 376*(6543), 778.

Botvinick, M. (2004). Probing the neural basis of body ownership. *Science, 305*(5685), 782-783.

Botvinick, M., & Cohen, J. (1998). Rubber hands' feel'touch that eyes see. *Nature, 391*(6669), 756-756.

Boucsein, W., Fowles, D. C., Grimnes, S., Ben-Shakhar, G., Roth, W. T., Dawson, M. E., & Filion, D. L. (2012). Publication recommendations for electrodermal measurements. *Psychophysiology*, *49*(8), 1017-1034.

Bradley, M. M., & Lang, P. J. (2000). Measuring emotion: Behavior, feeling, and physiology. *Cognitive neuroscience of emotion*, *25*, 49-59.

Braithwaite, J. J., Watson, D. G., Jones, R., & Rowe, M. (2013). A guide for analysing electrodermal activity (EDA) & skin conductance responses (SCRs) for psychological experiments. *Psychophysiology*, *49*(1), 1017-1034.

Braun, N., Debener, S., Spychala, N., Bongartz, E., Soros, P., Muller, H. H. O., & Philipsen, A. (2018). The Senses of Agency and Ownership: A Review. *Front Psychol, 9*, 535. doi:10.3389/fpsyg.2018.00535

Bromage, P. R., & Melzack, R. (1974). Phantom limbs and the body schema. *Canadian Anaesthetists' Society Journal, 21*(3), 267-274.

Brozzoli, C., Gentile, G., & Ehrsson, H. H. (2012). That's near my hand! Parietal and premotor coding of hand-centered space contributes to localization and self-attribution of the hand. *J Neurosci*, *32*(42), 14573-14582. doi:10.1523/JNEUROSCI.2660-12.2012

Cabibihan, J.-J., Pradipta, R., & Ge, S. S. (2011). Prosthetic finger phalanges with lifelike skin compliance for low-force social touching interactions. *Journal of neuroengineering and rehabilitation*, *8*(1), 16.

Canzoneri, E., Magosso, E., & Serino, A. (2012). Dynamic sounds capture the boundaries of peripersonal space representation in humans. *PLoS One*, *7*(9), e44306.

Canzoneri, E., Marzolla, M., Amoresano, A., Verni, G., & Serino, A. (2013). Amputation and prosthesis implantation shape body and peripersonal space representations. *Scientific Reports*, *3*(1). doi:10.1038/srep02844

Canzoneri, E., Ubaldi, S., Rastelli, V., Finisguerra, A., Bassolino, M., & Serino, A. (2013). Tool-use reshapes the boundaries of body and peripersonal space representations. *Experimental Brain Research, 228*(1), 25-42.

Cléry, J., Guipponi, O., Wardak, C., & Hamed, S. B. (2015). Neuronal bases of peripersonal and extrapersonal spaces, their plasticity and their dynamics: knowns and unknowns. *Neuropsychologia*, *70*, 313-326.

Controzzi, M., D'Alonzo, M., Peccia, C., Oddo, C. M., Carrozza, M. C., & Cipriani, C. (2014). Bioinspired fingertip for anthropomorphic robotic hands. *Applied Bionics and Biomechanics*, *11*(1-2), 25-38.

Convento, S., Romano, D., Maravita, A., & Bolognini, N. (2018). Roles of the right temporo-parietal and premotor cortices in self-location and body ownership. *Eur J Neurosci*. doi:10.1111/ejn.13937

Cordella, F., Ciancio, A. L., Sacchetti, R., Davalli, A., Cutti, A. G., Guglielmelli, E., & Zollo, L. (2016). Literature review on needs of upper limb prosthesis users. *Frontiers in Neuroscience*, *10*, 209.

Craig, A. (2003). Interoception: the sense of the physiological condition of the body. *Current opinion in neurobiology*, *13*(4), 500-505.

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Craig, A. (2008). Interoception and emotion: a neuroanatomical perspective. *Handbook of emotions, 3*(602), 272-288.

Craig, A. (2009). How do you feel--now? The anterior insula and human awareness. *Nature reviews neuroscience, 10*(1).

Crucianelli, L., Metcalf, N. K., Fotopoulou, A. K., & Jenkinson, P. M. (2013). Bodily pleasure matters: velocity of touch modulates body ownership during the rubber hand illusion. *Frontiers in psychology, 4*, 703.

D'Alonzo, M., Mioli, A., Formica, D., & Pino, G. D. (2020). Modulation of Body Representation Impacts on Efferent Autonomic Activity. *Journal of cognitive neuroscience, 0*(0), 1-13. doi:10.1162/jocn_a_01532 %M 31951156

D'Alonzo, M., & Cipriani, C. (2012). Vibrotactile sensory substitution elicits feeling of ownership of an alien hand. *PLoS One, 7*(11), e50756.

D'Alonzo, M., Mioli, A., Formica, D., Vollero, L., & Di Pino, G. (2019). Different level of virtualization of sight and touch produces the uncanny valley of avatar's hand embodiment. *Scientific Reports, 9*(1), 1-11.

Data, A. L. (2008). A practical introduction to statistics using R. *Cambridge UP: Cambridge*.

Dawson, M. E., Schell, A. M., & Filion, D. L. (2007). The electrodermal system. *Handbook of psychophysiology, 2,* 200-223.

de Haan, A. M., Van Stralen, H. E., Smit, M., Keizer, A., Van der Stigchel, S., & Dijkerman, H. C. (2017). No consistent cooling of the real hand in the rubber hand illusion. *Acta psychologica, 179*, 68-77.

De Vignemont, F. (2010). Body schema and body image—Pros and cons. *Neuropsychologia, 48*(3), 669-680.

de Vignemont, F., & Iannetti, G. (2015). How many peripersonal spaces? *Neuropsychologia, 70*, 327-334.

Deneve, S., Duhamel, J.-R., & Pouget, A. (2007). Optimal sensorimotor integration in recurrent cortical networks: a neural implementation of Kalman filters. *Journal of Neuroscience, 27*(21), 5744-5756.

Dhillon, G. S., & Horch, K. W. (2005). Direct neural sensory feedback and control of a prosthetic arm. *IEEE transactions on neural systems and rehabilitation engineering, 13*(4), 468-472.

Di Lazzaro, V., Oliviero, A., Profice, P., Pennisi, M., Di Giovanni, S., Zito, G., . . . Rothwell, J. (2000). Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. *Experimental Brain Research, 135*(4), 455-461.

Di Lazzaro, V., Pilato, F., Dileone, M., Profice, P., Ranieri, F., Ricci, V., . . . Ziemann, U. (2007). Segregating two inhibitory circuits in human motor cortex at the level of GABAA receptor subtypes: a TMS study. *Clinical neurophysiology, 118*(10), 2207-2214.

di Pellegrino, G., & Làdavas, E. (2015). Peripersonal space in the brain. *Neuropsychologia, 66*, 126-133.

Di Pino, G., Guglielmelli, E., & Rossini, P. M. (2009). Neuroplasticity in amputees: main implications on bidirectional interfacing of cybernetic hand prostheses. *Prog Neurobiol, 88*(2), 114-126. doi:10.1016/j.pneurobio.2009.03.001

Di Pino, G., Maravita, A., Zollo, L., Guglielmelli, E., & Di Lazzaro, V. (2014). Augmentation-related brain plasticity. *Front Syst Neurosci, 8,* 109. doi:10.3389/fnsys.2014.00109

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a condizione che ne venga citata la fonte.

Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., . . . Di Lazzaro, V. (2014). Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat Rev Neurol, 10*(10), 597-608. doi:10.1038/nrneurol.2014.162

Di Pino, G., Porcaro, C., Tombini, M., Assenza, G., Pellegrino, G., Tecchio, F., & Rossini, P. M. (2012). A neurally-interfaced hand prosthesis tuned inter-hemispheric communication. *Restor Neurol Neurosci, 30*(5), 407-418. doi:10.3233/RNN-2012-120224

Di Pino, G., Romano, D., Spaccasassi, C., Mioli, A., D'Alonzo, M., … Maravita, A., (in review). Sensory- and Motor-Oriented Embodiment of Neurally-Interfaced Robotic Hand Prostheses. *Frontiers in Neuroscience.*

Dijkerman, H. C., & De Haan, E. H. (2007). Somatosensory processing subserving perception and action: Dissociations, interactions, and integration. *Behavioral and brain sciences, 30*(2), 224-230.

Downing, P. E., Jiang, Y., Shuman, M., & Kanwisher, N. (2001). A cortical area selective for visual processing of the human body. *Science, 293*(5539), 2470-2473.

Dubbioso, R., Raffin, E., Karabanov, A., Thielscher, A., & Siebner, H. R. (2017). Centre-surround organization of fast sensorimotor integration in human motor hand area. *Neuroimage, 158*, 37-47.

Durgin, F. H., Evans, L., Dunphy, N., Klostermann, S., & Simmons, K. (2007). Rubber hands feel the touch of light. *Psychological Science, 18*(2), 152-157.

Ehrsson, H. H., Holmes, N. P., & Passingham, R. E. (2005). Touching a rubber hand: feeling of body ownership is associated with activity in multisensory brain areas. *J Neurosci, 25*(45), 10564-10573. doi:10.1523/JNEUROSCI.0800-05.2005

Ehrsson, H. H., Rosen, B., Stockselius, A., Ragno, C., Kohler, P., & Lundborg, G. (2008). Upper limb amputees can be induced to experience a rubber hand as their own. *Brain, 131*(Pt 12), 3443-3452. doi:10.1093/brain/awn297

Ehrsson, H. H., Spence, C., & Passingham, R. E. (2004). That's my hand! Activity in premotor cortex reflects feeling of ownership of a limb. *Science, 305*(5685), 875-877.

Ehrsson, H. H., Wiech, K., Weiskopf, N., Dolan, R. J., & Passingham, R. E. (2007). Threatening a rubber hand that you feel is yours elicits a cortical anxiety response. *Proceedings of the National Academy of Sciences, 104*(23), 9828-9833.

Fang, C., Jiang, T., & Yuan, X. (2014). Human Bipedalism, Evolved from Arboreal Locomotion of Two-arm Brachiation. *arXiv preprint arXiv:1411.0295*.

Fang, W., Li, J., Qi, G., Li, S., Sigman, M., & Wang, L. (2019). Statistical inference of body representation in the macaque brain. *Proceedings of the National Academy of Sciences, 116*(40), 20151-20157.

Farnè, A., Iriki, A., & Làdavas, E. (2005). Shaping multisensory action–space with tools: evidence from patients with cross-modal extinction. *Neuropsychologia, 43*(2), 238-248.

Farne, A., Pavani, F., Meneghello, F., & Ladavas, E. (2000). Left tactile extinction following visual stimulation of a rubber hand. *Brain, 123*(11), 2350-2360.

Farrer, C., Franck, N., Georgieff, N., Frith, C. D., Decety, J., & Jeannerod, M. (2003). Modulating the experience of agency: a positron emission tomography study. *Neuroimage, 18*(2), 324-333.

Farrer, C., & Frith, C. D. (2002). Experiencing oneself vs another person as being the cause of an action: the neural correlates of the experience of agency. *Neuroimage, 15*(3), 596-603.

Ferreri, F., Ponzo, D., Vollero, L., Guerra, A., Di Pino, G., Petrichella, S., . . . Rossini, P. M. (2014). Does an intraneural interface short-term implant for robotic hand control

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
discussa presso l'Università Campus Bio-Medico di Roma in data 9/07/2020.
La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

modulate sensorimotor cortical integration? An EEG-TMS co-registration study on a human amputee. *Restor Neurol Neurosci, 32*(2), 281-292. doi:10.3233/RNN-130347

Fink, G. R., Markowitsch, H. J., Reinkemeier, M., Bruckbauer, T., Kessler, J., & Heiss, W.-D. (1996). Cerebral representation of one's own past: neural networks involved in autobiographical memory. *Journal of Neuroscience, 16*(13), 4275-4282.

Fink, G. R., Marshall, J. C., Weiss, P. H., Stephan, T., Grefkes, C., Shah, N. J., . . . Dieterich, M. (2003). Performing allocentric visuospatial judgments with induced distortion of the egocentric reference frame: an fMRI study with clinical implications. *Neuroimage, 20*(3), 1505-1517.

Fischer, M., & Orth, M. (2011). Short-latency sensory afferent inhibition: conditioning stimulus intensity, recording site, and effects of 1 Hz repetitive TMS. *Brain stimulation, 4*(4), 202-209.

Flor, H., Nikolajsen, L., & Jensen, T. S. (2006). Phantom limb pain: a case of maladaptive CNS plasticity? *Nature reviews neuroscience, 7*(11), 873.

Fogassi, L., Gallese, V., Fadiga, L., Luppino, G., Matelli, M., & Rizzolatti, G. (1996). Coding of peripersonal space in inferior premotor cortex (area F4). *Journal of neurophysiology, 76*(1), 141-157.

Fossataro, C., Bruno, V., Giurgola, S., Bolognini, N., & Garbarini, F. (2018). Losing my hand. Body ownership attenuation after virtual lesion of the primary motor cortex. *European Journal of Neuroscience*.

Fougner, A., Stavdahl, Ø., Kyberd, P. J., Losier, Y. G., & Parker, P. A. (2012). Control of upper limb prostheses: Terminology and proportional myoelectric control—A review. *IEEE transactions on neural systems and rehabilitation engineering, 20*(5), 663-677.

Gallagher, S. (2000). Philosophical conceptions of the self: implications for cognitive science. *Trends in cognitive sciences, 4*(1), 14-21.

Gallagher, S. (2006). *How the body shapes the mind*: Clarendon Press.

Geethanjali, P. (2016). Myoelectric control of prosthetic hands: state-of-the-art review. *Medical Devices (Auckland, NZ), 9*, 247.

Gentile, G., Guterstam, A., Brozzoli, C., & Ehrsson, H. H. (2013). Disintegration of multisensory signals from the real hand reduces default limb self-attribution: an fMRI study. *J Neurosci, 33*(33), 13350-13366. doi:10.1523/JNEUROSCI.1363-13.2013

Gerstmann, J. (1942). Problem of imperception of disease and of impaired body territories with organic lesions: Relation to body scheme and its disorders. *Archives of Neurology & Psychiatry, 48*(6), 890-913.

Gillmeister, H., & Forster, B. (2012). Hands behind your back: effects of arm posture on tactile attention in the space behind the body. *Experimental Brain Research, 216*(4), 489-497.

Graczyk, E. L., Resnik, L., Schiefer, M. A., Schmitt, M. S., & Tyler, D. J. (2018). Home Use of a Neural-connected Sensory Prosthesis Provides the Functional and Psychosocial Experience of Having a Hand Again. *Sci Rep, 8*(1), 9866. doi:10.1038/s41598-018-26952-x

Gray, R., & Tan, H. Z. (2002). Dynamic and predictive links between touch and vision. *Experimental Brain Research, 145*(1), 50-55.

Graziano, M. S., & Cooke, D. F. (2006). Parieto-frontal interactions, personal space, and defensive behavior. *Neuropsychologia, 44*(6), 845-859.

Graziano, M. S., Cooke, D. F., & Taylor, C. S. (2000). Coding the location of the arm by sight. *Science, 290*(5497), 1782-1786.

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
discussa presso l'Università Campus Bio-Medico di Roma in data 9/07/2020.
La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

Grivaz, P., Blanke, O., & Serino, A. (2017). Common and distinct brain regions processing multisensory bodily signals for peripersonal space and body ownership. *Neuroimage, 147*, 602-618.

Guterstam, A., Collins, K. L., Cronin, J. A., Zeberg, H., Darvas, F., Weaver, K. E., . . . Ehrsson, H. H. (2018). Direct Electrophysiological Correlates of Body Ownership in Human Cerebral Cortex. *Cereb Cortex*. doi:10.1093/cercor/bhy285

Guzzetti. (2005). Symbolic dynamics of heart rate variability: A probe to investigate cardiac autonomic modulation (vol 112, pg 465, 2005). *CIRCULATION, 112*(9), E122-E122.

Haans, A., IJsselsteijn, W. A., & de Kort, Y. A. (2008). The effect of similarities in skin texture and hand shape on perceived ownership of a fake limb. *Body Image, 5*(4), 389-394.

Hadjikhani, N., & Roland, P. E. (1998). Cross-modal transfer of information between the tactile and the visual representations in the human brain: a positron emission tomographic study. *Journal of Neuroscience, 18*(3), 1072-1084.

Hamada, M., Murase, N., Hasan, A., Balaratnam, M., & Rothwell, J. C. (2012). The role of interneuron networks in driving human motor cortical plasticity. *Cerebral cortex, 23*(7), 1593-1605.

Hara, F., & Kobayashi, H. (1995). *Use of face robot for human-computer communication.* Paper presented at the 1995 IEEE international conference on systems, man and cybernetics. intelligent systems for the 21st century.

Heed, T., Buchholz, V. N., Engel, A. K., & Röder, B. (2015). Tactile remapping: from coordinate transformation to integration in sensorimotor processing. *Trends in cognitive sciences, 19*(5), 251-258.

Herbert, B. M., & Pollatos, O. (2012). The body in the mind: on the relationship between interoception and embodiment. *Topics in cognitive science, 4*(4), 692-704.

Hermosillo, R., Ritterband-Rosenbaum, A., & van Donkelaar, P. (2011). Predicting future sensorimotor states influences current temporal decision making. *Journal of Neuroscience, 31*(27), 10019-10022.

Ho, C.-C., & MacDorman, K. F. (2010). Revisiting the uncanny valley theory: Developing and validating an alternative to the Godspeed indices. *Computers in Human Behavior, 26*(6), 1508-1518.

Holmes, N. P., Snijders, H. J., & Spence, C. (2006). Reaching with alien limbs: Visual exposure to prosthetic hands in a mirror biases proprioception without accompanying illusions of ownership. *Perception & psychophysics, 68*(4), 685-701.

Huang, Y.-Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron, 45*(2), 201-206.

IJsselsteijn, W. A., de Kort, Y. A. W., & Haans, A. (2006). Is this my hand I see before me? The rubber hand illusion in reality, virtual reality, and mixed reality. *Presence: Teleoperators and Virtual Environments, 15*(4), 455-464.

Inui, N., Masumoto, J., Ueda, Y., & Ide, K. (2012). Systematic changes in the perceived posture of the wrist and elbow during formation of a phantom hand and arm. *Experimental Brain Research, 218*(3), 487-494.

Inui, N., Walsh, L., Taylor, J., & Gandevia, S. (2011). Dynamic changes in the perceived posture of the hand during ischaemic anaesthesia of the arm. *The Journal of physiology, 589*(23), 5775-5784.

Iriki, A., Tanaka, M., & Iwamura, Y. (1996). Coding of modified body schema during tool use by macaque postcentral neurones. *Neuroreport, 7*(14), 2325-2330.

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
discussa presso l'Università Campus Bio-Medico di Roma in data 9/07/2020.
La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

Jang, C. H., Yang, H. S., Yang, H. E., Lee, S. Y., Kwon, J. W., Yun, B. D., . . . Jeong, H. W. (2011). A survey on activities of daily living and occupations of upper extremity amputees. *Annals of rehabilitation medicine, 35*(6), 907.

Jeannerod, M. The neural and behavioral organization of goal-directed movements. 1988. In: Clarendon Press, Oxford.

Jennings, J. R. (1986). Bodily changes during attending. *Psychophysiology: Systems, processes, and applications*, 268-289.

Jerald, J. (2017). *Human-centered design for immersive interactions.* Paper presented at the 2017 IEEE Virtual Reality (VR).

Kammers, M. P., Verhagen, L., Dijkerman, H. C., Hogendoorn, H., De Vignemont, F., & Schutter, D. J. (2009). Is this hand for real? Attenuation of the rubber hand illusion by transcranial magnetic stimulation over the inferior parietal lobule. *Journal of cognitive neuroscience, 21*(7), 1311-1320.

Kanayama, N., Sato, A., & Ohira, H. (2007). Crossmodal effect with rubber hand illusion and gamma-band activity. *Psychophysiology, 44*(3), 392-402. doi:10.1111/j.1469-8986.2007.00511.x

Kanayama, N., Sato, A., & Ohira, H. (2009). The role of gamma band oscillations and synchrony on rubber hand illusion and crossmodal integration. *Brain Cogn, 69*(1), 19-29. doi:10.1016/j.bandc.2008.05.001

Karnath, H. O., & Baier, B. (2010). Right insula for our sense of limb ownership and self-awareness of actions. *Brain Struct Funct, 214*(5-6), 411-417. doi:10.1007/s00429-010-0250-4

Karwowski, W., & Rahimi, M. (1991). Worker selection of safe speed and idle condition in simulated monitoring of two industrial robots. *Ergonomics, 34*(5), 531-546.

Kawato, M. (1999). Internal models for motor control and trajectory planning. *Current opinion in neurobiology, 9*(6), 718-727.

Kikkert, S., Mezue, M., O'shea, J., Henderson Slater, D., Johansen-Berg, H., Tracey, I., & Makin, T. R. (2019). Neural basis of induced phantom limb pain relief. *Annals of neurology, 85*(1), 59-73.

Kilteni, K., Normand, J.-M., Sanchez-Vives, M. V., & Slater, M. (2012). Extending body space in immersive virtual reality: a very long arm illusion. *PLoS One, 7*(7), e40867.

Knecht, S., Henningsen, H., Höhling, C., Elbert, T., Flor, H., Pantev, C., & Taub, E. (1998). Plasticity of plasticity? Changes in the pattern of perceptual correlates of reorganization after amputation. *Brain: a journal of neurology, 121*(4), 717-724.

Kokkinara, E., & Slater, M. (2014). Measuring the effects through time of the influence of visuomotor and visuotactile synchronous stimulation on a virtual body ownership illusion. *Perception, 43*(1), 43-58.

Konen, C. S., & Haggard, P. (2012). Multisensory parietal cortex contributes to visual enhancement of touch in humans: a single-pulse TMS study. *Cerebral cortex, 24*(2), 501-507.

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: A review. *Biological psychology, 84*(3), 394-421.

Kuiken, T. A., Marasco, P. D., Lock, B. A., Harden, R. N., & Dewald, J. P. (2007). Redirection of cutaneous sensation from the hand to the chest skin of human amputees with targeted reinnervation. *Proceedings of the National Academy of Sciences, 104*(50), 20061-20066.

Kulic, D., & Croft, E. A. (2007). Affective state estimation for human–robot interaction. *IEEE Transactions on Robotics, 23*(5), 991-1000.

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
discussa presso l'Università Campus Bio-Medico di Roma in data 9/07/2020.
La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

Kupferberg, A., Glasauer, S., Huber, M., Rickert, M., Knoll, A., & Brandt, T. (2011). Biological movement increases acceptance of humanoid robots as human partners in motor interaction. *AI & society, 26*(4), 339-345.

Levenson, R. W., & Gottman, J. M. (1983). Marital interaction: physiological linkage and affective exchange. *Journal of personality and social psychology, 45*(3), 587.

Levenson, R. W., & Gottman, J. M. (1985). Physiological and affective predictors of change in relationship satisfaction. *Journal of personality and social psychology, 49*(1), 85.

Limanowski, J., Lutti, A., & Blankenburg, F. (2014). The extrastriate body area is involved in illusory limb ownership. *Neuroimage, 86*, 514-524.

Lira, M., Egito, J. H., Dall'Agnol, P. A., Amodio, D. M., Gonçalves, Ó. F., & Boggio, P. S. (2017). The influence of skin colour on the experience of ownership in the rubber hand illusion. *Scientific Reports, 7*(1), 15745.

Lira, M., Pantaleao, F. N., de Souza Ramos, C. G., & Boggio, P. S. (2018). Anodal transcranial direct current stimulation over the posterior parietal cortex reduces the onset time to the rubber hand illusion and increases the body ownership. *Exp Brain Res*. doi:10.1007/s00221-018-5353-9

Llobera, J., Sanchez-Vives, M. V., & Slater, M. (2013). The relationship between virtual body ownership and temperature sensitivity. *Journal of the Royal Society Interface, 10*(85), 20130300.

Lloyd, D. M. (2007). Spatial limits on referred touch to an alien limb may reflect boundaries of visuo-tactile peripersonal space surrounding the hand. *Brain and cognition, 64*(1), 104-109.

Longo, M. R., & Haggard, P. (2012). Implicit body representations and the conscious body image. *Acta psychologica, 141*(2), 164-168.

Longo, M. R., Schuur, F., Kammers, M. P., Tsakiris, M., & Haggard, P. (2008). What is embodiment? A psychometric approach. *Cognition, 107*(3), 978-998. doi:10.1016/j.cognition.2007.12.004

Ma, K., & Hommel, B. (2013). The virtual-hand illusion: effects of impact and threat on perceived ownership and affective resonance. *Frontiers in psychology, 4*, 604.

MacKenzie, C. L., & Iberall, T. (1994). *The grasping hand*: Elsevier.

Maihöfner, C., Kaltenhäuser, M., Neundörfer, B., & Lang, E. (2002). Temporo-spatial analysis of cortical activation by phasic innocuous and noxious cold stimuli–a magnetoencephalographic study. *Pain, 100*(3), 281-290.

Makin, T. R., Holmes, N. P., & Ehrsson, H. H. (2008). On the other hand: dummy hands and peripersonal space. *Behav Brain Res, 191*(1), 1-10. doi:10.1016/j.bbr.2008.02.041

Marasco, P. D., Hebert, J. S., Sensinger, J. W., Shell, C. E., Schofield, J. S., Thumser, Z. C., . . . Blustein, D. H. (2018). Illusory movement perception improves motor control for prosthetic hands. *Science translational medicine, 10*(432), eaao6990.

Marasco, P. D., Kim, K., Colgate, J. E., Peshkin, M. A., & Kuiken, T. A. (2011). Robotic touch shifts perception of embodiment to a prosthesis in targeted reinnervation amputees. *Brain, 134*(Pt 3), 747-758. doi:10.1093/brain/awq361

Marasco, P. D., Schultz, A. E., & Kuiken, T. A. (2009). Sensory capacity of reinnervated skin after redirection of amputated upper limb nerves to the chest. *Brain, 132*(6), 1441-1448.

Maravita, A., & Iriki, A. (2004). Tools for the body (schema). *Trends in cognitive sciences, 8*(2), 79-86.

Maravita, A., Spence, C., Kennett, S., & Driver, J. (2002). Tool-use changes multimodal spatial interactions between vision and touch in normal humans. *Cognition, 83*(2), B25-B34.

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
discussa presso l'Università Campus Bio-Medico di Roma in data 9/07/2020.
La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

Marini, F., Tagliabue, C. F., Sposito, A. V., Hernandez-Arieta, A., Brugger, P., Estevez, N., & Maravita, A. (2014). Crossmodal representation of a functional robotic hand arises after extensive training in healthy participants. *Neuropsychologia, 53*, 178-186. doi:10.1016/j.neuropsychologia.2013.11.017

Maselli, A., & Slater, M. (2013). The building blocks of the full body ownership illusion. *Frontiers in human neuroscience, 7*, 83.

Mathôt, S., Schreij, D., & Theeuwes, J. (2012). OpenSesame: An open-source, graphical experiment builder for the social sciences. *Behavior research methods, 44*(2), 314-324.

Meador, K. J., Loring, D., Feinberg, T., Lee, G. P., & Nichols, M. (2000). Anosognosia and asomatognosia during intracarotid amobarbital inactivation. *Neurology, 55*(6), 816-820.

Melzack, R., & Bromage, P. (1973). Experimental phantom limbs. *Experimental neurology, 39*(2), 261-269.

Mioli, A., D'alonzo, M., Pellegrino, G., Formica, D., & Di Pino, G. (2018). Intermittent theta burst stimulation over ventral premotor cortex or inferior parietal lobule does not enhance the rubber hand illusion. *Frontiers in Neuroscience, 12*, 870.

Miyazaki, M., Yamamoto, S., Uchida, S., & Kitazawa, S. (2006). Bayesian calibration of simultaneity in tactile temporal order judgment. *Nature neuroscience, 9*(7), 875.

Mogk, J. P., Rogers, L. M., Murray, W. M., Perreault, E. J., & Stinear, J. W. (2014). Corticomotor excitability of arm muscles modulates according to static position and orientation of the upper limb. *Clin Neurophysiol, 125*(10), 2046-2054. doi:10.1016/j.clinph.2014.02.007

Mori, M., MacDorman, K. F., & Kageki, N. (2012). The uncanny valley [from the field]. *IEEE Robotics & Automation Magazine, 19*(2), 98-100.

Moseley, G. L., Olthof, N., Venema, A., Don, S., Wijers, M., Gallace, A., & Spence, C. (2008). Psychologically induced cooling of a specific body part caused by the illusory ownership of an artificial counterpart. *Proc Natl Acad Sci U S A, 105*(35), 13169-13173. doi:10.1073/pnas.0803768105

Munro, L. L., Dawson, M. E., Schell, A. M., & Sakai, L. M. (1987). Electrodermal lability and rapid vigilance decrement in a degraded stimulus continuous performance task. *Journal of Psychophysiology*.

Murray, C. D. (2004). An interpretative phenomenological analysis of the embodiment of artificial limbs. *Disabil Rehabil, 26*(16), 963-973. doi:10.1080/09638280410001696764

Nance, D. M., & Sanders, V. M. (2007). Autonomic innervation and regulation of the immune system (1987–2007). *Brain, behavior, and immunity, 21*(6), 736-745.

Napier, J. (1962). The evolution of the hand. *Scientific American, 207*(6), 56-65.

Nettekoven, C., Volz, L. J., Leimbach, M., Pool, E.-M., Rehme, A. K., Eickhoff, S. B., . . . Grefkes, C. (2015). Inter-individual variability in cortical excitability and motor network connectivity following multiple blocks of rTMS. *Neuroimage, 118*, 209-218.

Niedernhuber, M., Barone, D. G., & Lenggenhager, B. (2018). Prostheses as extensions of the body: progress and challenges. *Neuroscience & Biobehavioral Reviews*.

Nitsche, M. A., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of physiology, 527*(3), 633-639.

Ocklenburg, S., Rüther, N., Peterburs, J., Pinnow, M., & Güntürkün, O. (2011). Laterality in the rubber hand illusion. *Laterality, 16*(2), 174-187.

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
discussa presso l'Università Campus Bio-Medico di Roma in data 9/07/2020.
La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

Ortiz-Catalan, M., Håkansson, B., & Brånemark, R. (2014). An osseointegrated human-machine gateway for long-term sensory feedback and motor control of artificial limbs. *Science translational medicine, 6*(257), 257re256-257re256.

Padilla-Castañeda, M. A., Frisoli, A., Pabon, S., & Bergamasco, M. (2014). The modulation of ownership and agency in the virtual hand illusion under visuotactile and visuomotor sensory feedback. *Presence: Teleoperators and Virtual Environments*, *23*(2), 209-225.

Paillard, J. (1999). Body Schema and body image-a double dissociation. *Motor control, today and tomorrow*, 197-214.

Parsons, L. M. (1987). Imagined spatial transformations of one's hands and feet. *Cognitive psychology, 19*(2), 178-241.

Peled, A., Pressman, A., Geva, A. B., & Modai, I. (2003). Somatosensory evoked potentials during a rubber-hand illusion in schizophrenia. *Schizophrenia Research, 64*(2-3), 157-163. doi:10.1016/s0920-9964(03)00057-4

Petkova, & Ehrsson, H. (2009). When right feels left: referral of touch and ownership between the hands. *PLoS One, 4*(9), e6933.

Petkova, V., Bjornsdotter, M., Gentile, G., Jonsson, T., Li, T. Q., & Ehrsson, H. H. (2011). From part- to whole-body ownership in the multisensory brain. *Curr Biol, 21*(13), 1118-1122. doi:10.1016/j.cub.2011.05.022

Petrini, F. M., Valle, G., Strauss, I., Granata, G., Di Iorio, R., d'Anna, E., . . . Clemente, F. (2019). Six-month assessment of a hand prosthesis with intraneural tactile feedback. *Ann. Neurol, 85*(1), 137-154.

Pitcher, D., Charles, L., Devlin, J. T., Walsh, V., & Duchaine, B. (2009). Triple dissociation of faces, bodies, and objects in extrastriate cortex. *Current Biology, 19*(4), 319-324.

Ramakonar, H., Franz, E. A., & Lind, C. R. (2011). The rubber hand illusion and its application to clinical neuroscience. *J Clin Neurosci, 18*(12), 1596-1601. doi:10.1016/j.jocn.2011.05.008

Rao, I. S., & Kayser, C. (2017). Neurophysiological Correlates of the Rubber Hand Illusion in Late Evoked and Alpha/Beta Band Activity. *Front Hum Neurosci, 11*, 377. doi:10.3389/fnhum.2017.00377

Raspopovic, S., Capogrosso, M., Petrini, F. M., Bonizzato, M., Rigosa, J., Di Pino, G., . . . Micera, S. (2014). Restoring natural sensory feedback in real-time bidirectional hand prostheses. *Sci Transl Med, 6*(222), 222ra219. doi:10.1126/scitranslmed.3006820

Reichgott, M. J. (1990). Clinical evidence of dysautonomia. In *Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition*: Butterworths.

Reilly, K. T., & Sirigu, A. (2008). The motor cortex and its role in phantom limb phenomena. *Neuroscientist, 14*(2), 195-202. doi:10.1177/1073858407309466

Rizzolatti, G., & Luppino, G. (2001). The cortical motor system. *Neuron, 31*(6), 889-901.

Rochat, P. (1998). Self-perception and action in infancy. *Experimental Brain Research, 123*(1-2), 102-109.

Rognini, G., Petrini, F. M., Raspopovic, S., Valle, G., Granata, G., Strauss, I., . . . Mange, R. (2019). Multisensory bionic limb to achieve prosthesis embodiment and reduce distorted phantom limb perceptions. *Journal of Neurology, Neurosurgery & Psychiatry, 90*(7), 833-836.

Rohde, M., Di Luca, M., & Ernst, M. O. (2011). The Rubber Hand Illusion: feeling of ownership and proprioceptive drift do not go hand in hand. *PLoS One, 6*(6), e21659. doi:10.1371/journal.pone.0021659

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
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a condizione che ne venga citata la fonte.

Rohde, M., Wold, A., Karnath, H. O., & Ernst, M. O. (2013). The human touch: skin temperature during the rubber hand illusion in manual and automated stroking procedures. *PLoS One, 8*(11), e80688. doi:10.1371/journal.pone.0080688

Romano, D., Mioli, A., D'Alonzo, M., Maravita, A., Di Lazzaro, V., Di Pino, G. (submitted). A standard posture of the hand for action. *The Journal of Neuroscience.*

Romano, D., & Maravita, A. (2019). The dynamic nature of the sense of ownership after brain injury. Clues from asomatognosia and somatoparaphrenia. *Neuropsychologia*, 107119.

Romano, D., Marini, F., & Maravita, A. (2017). Standard body-space relationships: Fingers hold spatial information. *Cognition, 165*, 105-112. doi:10.1016/j.cognition.2017.05.014

Romano, D., Tamè, L., Amoruso, E., Azañón, E., Maravita, A., & Longo, M. R. (2019). The standard posture of the hand. *Journal of Experimental Psychology: Human Perception and Performance, 45*(9), 1164.

Rosen, B., Ehrsson, H. H., Antfolk, C., Cipriani, C., Sebelius, F., & Lundborg, G. (2009). Referral of sensation to an advanced humanoid robotic hand prosthesis. *Scand J Plast Reconstr Surg Hand Surg, 43*(5), 260-266. doi:10.3109/02844310903113107

Rosenthal, R., Cooper, H., & Hedges, L. (1994). Parametric measures of effect size. *The handbook of research synthesis, 621*, 231-244.

Rossetti, Y., Rode, G., & Boisson, D. (1995). Implicit processing of somaesthetic information: a dissociation between where and how? *Neuroreport: An International Journal for the Rapid Communication of Research in Neuroscience*.

Rossini, P. M., Micera, S., Benvenuto, A., Carpaneto, J., Cavallo, G., Citi, L., . . . Dario, P. (2010). Double nerve intraneural interface implant on a human amputee for robotic hand control. *Clin Neurophysiol, 121*(5), 777-783. doi:10.1016/j.clinph.2010.01.001

Ruzzoli, M., & Soto-Faraco, S. (2014). Alpha stimulation of the human parietal cortex attunes tactile perception to external space. *Current Biology, 24*(3), 329-332.

Sanchez-Vives, M. V., Spanlang, B., Frisoli, A., Bergamasco, M., & Slater, M. (2010). Virtual hand illusion induced by visuomotor correlations. *PLoS One, 5*(4), e10381.

Sartori, L., Straulino, E., & Castiello, U. (2011). How objects are grasped: the interplay between affordances and end-goals. *PLoS One, 6*(9).

Sato, Y., Kawase, T., Takano, K., Spence, C., & Kansaku, K. (2017). Incorporation of prosthetic limbs into the body representation of amputees: Evidence from the crossed hands temporal order illusion. *Prog Brain Res, 236*, 225-241. doi:10.1016/bs.pbr.2017.08.003

Scarry, E. (1994). The merging of bodies and artifacts in the social contract. *Culture on the brink: ideologies of technology*, 85-97.

Schicke, T., & Röder, B. (2006). Spatial remapping of touch: confusion of perceived stimulus order across hand and foot. *Proceedings of the National Academy of Sciences, 103*(31), 11808-11813.

Schiefer, M., Tan, D., Sidek, S. M., & Tyler, D. J. (2015). Sensory feedback by peripheral nerve stimulation improves task performance in individuals with upper limb loss using a myoelectric prosthesis. *Journal of neural engineering, 13*(1), 016001.

Schott, G. (2013). Revealing the invisible: the paradox of picturing a phantom limb. *Brain, 137*(3), 960-969.

Schubert, T., Friedmann, F., & Regenbrecht, H. (2001). The experience of presence: Factor analytic insights. *Presence: Teleoperators & Virtual Environments, 10*(3), 266-281.

Serino, A. (2019). Peripersonal space (PPS) as a multisensory interface between the individual and the environment, defining the space of the self. *Neuroscience & Biobehavioral Reviews*.

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
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La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

Serino, A., Akselrod, M., Salomon, R., Martuzzi, R., Blefari, M. L., Canzoneri, E., . . . Blanke, O. (2017). Upper limb cortical maps in amputees with targeted muscle and sensory reinnervation. *Brain*, *140*(11), 2993-3011. doi:10.1093/brain/awx242

Serino, A., Noel, J. P., Galli, G., Canzoneri, E., Marmaroli, P., Lissek, H., & Blanke, O. (2015). Body part-centered and full body-centered peripersonal space representations. *Sci Rep, 5*, 18603. doi:10.1038/srep18603

Seth, A. K. (2013). Interoceptive inference, emotion, and the embodied self. *Trends in cognitive sciences, 17*(11), 565-573.

Shields, S. A., MacDowell, K. A., Fairchild, S. B., & Campbell, M. L. (1987). Is mediation of sweating cholinergic, adrenergic, or both? A comment on the literature. *Psychophysiology, 24*(3), 312-319.

Shimada, S., Fukuda, K., & Hiraki, K. (2009). Rubber hand illusion under delayed visual feedback. *PLoS One, 4*(7), e6185. doi:10.1371/journal.pone.0006185

Shokur, S., O'Doherty, J. E., Winans, J. A., Bleuler, H., Lebedev, M. A., & Nicolelis, M. A. (2013). Expanding the primate body schema in sensorimotor cortex by virtual touches of an avatar. *Proc Natl Acad Sci U S A, 110*(37), 15121-15126. doi:10.1073/pnas.1308459110

Shore, D. I., Spry, E., & Spence, C. (2002). Confusing the mind by crossing the hands. *Cognitive Brain Research, 14*(1), 153-163.

Sinha, R. (2014). Disgust, insula, immune signaling, and addiction.

Slater, M., Pérez Marcos, D., Ehrsson, H., & Sanchez-Vives, M. V. (2008). Towards a digital body: the virtual arm illusion. *Frontiers in human neuroscience, 2*, 6.

Slater, M., Pérez Marcos, D., Ehrsson, H., & Sanchez-Vives, M. V. (2009). Inducing illusory ownership of a virtual body. *Frontiers in Neuroscience, 3*, 29.

Slater, M., Spanlang, B., Sanchez-Vives, M. V., & Blanke, O. (2010). First person experience of body transfer in virtual reality. *PLoS One, 5*(5), e10564.

Spaccasassi, C., Romano, D., & Maravita, A. (2019). Everything is worth when it is close to my body: How spatial proximity and stimulus valence affect visuo-tactile integration. *Acta psychologica, 192*, 42-51.

Spence, C. (2015). The Cognitive Neuroscience of Incorporation: Body Image Adjustment and Neuroprosthetics. In K. Kansaku, L. G. Cohen, & N. Birbaumer (Eds.), *Clinical Systems Neuroscience* (pp. 151-168). Tokyo: Springer Japan.

Spence, C., Pavani, F., & Driver, J. (2004). Spatial constraints on visual-tactile cross-modal distractor congruency effects. *Cognitive, Affective, & Behavioral Neuroscience, 4*(2), 148-169.

Stagg, C. J., & Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *The Neuroscientist, 17*(1), 37-53.

Suppa, A., Huang, Y. Z., Funke, K., Ridding, M. C., Cheeran, B., Di Lazzaro, V., . . . Rothwell, J. C. (2016). Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects. *Brain stimulation, 9*(3), 323-335. doi:10.1016/j.brs.2016.01.006

Suzuki, K., Garfinkel, S. N., Critchley, H. D., & Seth, A. K. (2013). Multisensory integration across exteroceptive and interoceptive domains modulates self-experience in the rubber-hand illusion. *Neuropsychologia, 51*(13), 2909-2917. doi:10.1016/j.neuropsychologia.2013.08.014

Takahashi, T., Kansaku, K., Wada, M., Shibuya, S., & Kitazawa, S. (2012). Neural correlates of tactile temporal-order judgment in humans: an fMRI study. *Cerebral cortex, 23*(8), 1952-1964.

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
discussa presso l'Università Campus Bio-Medico di Roma in data 9/07/2020.
La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

Tan, D. W., Schiefer, M. A., Keith, M. W., Anderson, J. R., Tyler, J., & Tyler, D. J. (2014). A neural interface provides long-term stable natural touch perception. *Science translational medicine, 6*(257), 257ra138-257ra138.

Tieri, G., Gioia, A., Scandola, M., Pavone, E. F., & Aglioti, S. M. (2017). Visual appearance of a virtual upper limb modulates the temperature of the real hand: a thermal imaging study in Immersive Virtual Reality. *European Journal of Neuroscience, 45*(9), 1141-1151.

Tieri, G., Tidoni, E., Pavone, E. F., & Aglioti, S. M. (2015). Mere observation of body discontinuity affects perceived ownership and vicarious agency over a virtual hand. *Experimental Brain Research, 233*(4), 1247-1259.

Tomassini, A., Gori, M., Baud-Bovy, G., Sandini, G., & Morrone, M. C. (2014). Motor commands induce time compression for tactile stimuli. *Journal of Neuroscience, 34*(27), 9164-9172.

Tsakiris, M. (2010). My body in the brain: a neurocognitive model of body-ownership. *Neuropsychologia, 48*(3), 703-712.

Tsakiris, M., Carpenter, L., James, D., & Fotopoulou, A. (2010). Hands only illusion: multisensory integration elicits sense of ownership for body parts but not for non-corporeal objects. *Experimental Brain Research, 204*(3), 343-352.

Tsakiris, M., & Haggard, P. (2005). The rubber hand illusion revisited: visuotactile integration and self-attribution. *J Exp Psychol Hum Percept Perform, 31*(1), 80-91. doi:10.1037/0096-1523.31.1.80

Tsakiris, M., Hesse, M. D., Boy, C., Haggard, P., & Fink, G. R. (2007). Neural signatures of body ownership: a sensory network for bodily self-consciousness. *Cereb Cortex, 17*(10), 2235-2244. doi:10.1093/cercor/bhl131

Tsakiris, M., Tajadura-Jimenez, A., & Costantini, M. (2011). Just a heartbeat away from one's body: interoceptive sensitivity predicts malleability of body-representations. *Proc Biol Sci, 278*(1717), 2470-2476. doi:10.1098/rspb.2010.2547

Tsuji, T., Yamakawa, H., Yamashita, A., Takakusaki, K., Maeda, T., Kato, M., . . . Asama, H. (2013). *Analysis of electromyography and skin conductance response during rubber hand illusion.* Paper presented at the 2013 IEEE Workshop on Advanced Robotics and its Social Impacts.

Vallar, G., & Ronchi, R. (2009). Somatoparaphrenia: a body delusion. A review of the neuropsychological literature. *Experimental Brain Research, 192*(3), 533-551.

Valle, G., Mazzoni, A., Iberite, F., D'Anna, E., Strauss, I., Granata, G., . . . Micera, S. (2018). Biomimetic Intraneural Sensory Feedback Enhances Sensation Naturalness, Tactile Sensitivity, and Manual Dexterity in a Bidirectional Prosthesis. *Neuron*. doi:10.1016/j.neuron.2018.08.033

Van Den Bos, E., & Jeannerod, M. (2002). Sense of body and sense of action both contribute to self-recognition. *Cognition, 85*(2), 177-187.

Vogeley, K., May, M., Ritzl, A., Falkai, P., Zilles, K., & Fink, G. R. (2004). Neural correlates of first-person perspective as one constituent of human self-consciousness. *Journal of cognitive neuroscience, 16*(5), 817-827.

Wallin, B. G. (1981). Sympathetic nerve activity underlying electrodermal and cardiovascular reactions in man. *Psychophysiology, 18*(4), 470-476.

Warner, H. R., & Cox, A. (1962). A mathematical model of heart rate control by sympathetic and vagus efferent information. *Journal of applied physiology, 17*(2), 349-355.

Tesi di dottorato in Scienze biomediche integrate e bioetica, di Alessandro Mioli,
discussa presso l'Università Campus Bio-Medico di Roma in data 9/07/2020.
La disseminazione e la riproduzione di questo documento sono consentite per scopi di didattica e ricerca,
a condizione che ne venga citata la fonte.

Wentink, E., Schut, V., Prinsen, E., Rietman, J., & Veltink, P. (2014). Detection of the onset of gait initiation using kinematic sensors and EMG in transfemoral amputees. *Gait & posture*, *39*(1), 391-396.

Wijk, U., & Carlsson, I. (2015). Forearm amputees' views of prosthesis use and sensory feedback. *Journal of Hand Therapy*, *28*(3), 269-278.

Wilcox, R. R. (2009). Comparing Pearson correlations: Dealing with heteroscedasticity and nonnormality. *Communications in Statistics-Simulation and Computation*, *38*(10), 2220-2234.

Williamson, G. M., Schulz, R., Bridges, M. W., & Behan, A. M. (1994). Social and psychological factors in adjustment to limb amputation. *Journal of Social Behavior and Personality*, *9*(5), 249.

Wold, A., Limanowski, J., Walter, H., & Blankenburg, F. (2014). Proprioceptive drift in the rubber hand illusion is intensified following 1 Hz TMS of the left EBA. *Front Hum Neurosci*, *8*, 390. doi:10.3389/fnhum.2014.00390

Wolpert, D. M., & Ghahramani, Z. (2000). Computational principles of movement neuroscience. *Nature neuroscience*, *3*(11), 1212-1217.

Yamamoto, S., & Kitazawa, S. (2001a). Reversal of subjective temporal order due to arm crossing. *Nature neuroscience*, *4*(7), 759.

Yamamoto, S., & Kitazawa, S. (2001b). Sensation at the tips of invisible tools. *Nature neuroscience*, *4*(10), 979.

Yamamoto, S., Moizumi, S., & Kitazawa, S. (2005). Referral of tactile sensation to the tips of L-shaped sticks. *Journal of neurophysiology*, *93*(5), 2856-2863.

Yuan, Y., & Steed, A. (2010). *Is the rubber hand illusion induced by immersive virtual reality?* Paper presented at the 2010 IEEE Virtual Reality Conference (VR).

Zeller, D., Litvak, V., Friston, K. J., & Classen, J. (2015). Sensory processing and the rubber hand illusion--an evoked potentials study. *J Cogn Neurosci*, *27*(3), 573-582. doi:10.1162/jocn_a_00705

Zollo, L., Di Pino, G., Ciancio, A. L., Ranieri, F., Cordella, F., Gentile, C., . . . Guglielmelli, E. (2019). Restoring tactile sensations via neural interfaces for real-time force-and-slippage closed-loop control of bionic hands. *Science Robotics*, *4*(27), eaau9924. doi:10.1126/scirobotics.aau9924