# TMS-INDUCED CONTROL OF BODILY SELF-CONSCIOUSNESS BY COMBINING NON-INVASIVE BRAIN STIMULATION AND IMMERSIVE VIRTUAL REALITY

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# Abstract

Our brain constantly receives and integrates a flow of sensory and motor information that shapes the way our body is represented. Several experimental approaches have been proposed to alter the body representation, by manipulating exteroceptive or interoceptive bodily signals. During my thesis, I studied the contribution of central, rather than peripheral, motor brain mechanisms underlying bodily self-consciousness (BSC). For this purpose, I attempted to manipulate BSC, specifically inducing embodiment for a fake hand in healthy participants, by using a novel method that combines non-invasive transcranial magnetic stimulation (TMS) with immersive virtual reality (VR).

First, we managed to induce embodiment for a virtual hand utilizing the spatio-temporal coherence between an artificial TMS-induced activation of the motor system and movement-related visual feedback in VR (**Study 1**). I observed that embodiment for a virtual hand was induced only when the provided stimulation is supra-threshold but not when TMS is delivered sub-threshold. I argue that such TMS-induced illusory embodiment is due to neuro-visual integration between TMS-induced primary motor cortex (M1) activation and hand twitches with visual VR feedback.

In a second study (**Study 2**), by refining the parameters of our TMS-VR protocol we investigated the different peripheral components elicited by TMS with a special focus on hand perception. Our data revealed the main features of TMS-evoked hand perceptions (in terms of threshold and evoked subjective perceptions) and show that these are discernible from MEPs and TMS-evoked movements associated with single-pulse TMS over M1.

Finally, we combined the TMS-VR setup (**Study 1**) and our stimulation protocol (**Study 2**) to investigate whether the awareness of peripheral bodily stimuli is necessary to induce changes in BSC (**Study 3**). Our findings reveal a prominent role of conscious bodily perceptions in triggering illusory embodiment, even in the absence of peripheral stimuli.

Furthermore, I have been involved in other projects investigating the plasticity of body and space representation in healthy young and elderly participants (listed in appendix 1 & 2) and the robotically controlled induction of presence hallucinations (appendix 3).

The present work provides important findings and promising tools regarding the role of non-invasive brain stimulation and virtual technology in the study of BSC. In particular, I showed that it is possible to manipulate BSC by directly stimulating the M1 cortical representation of the hand inducing embodiment for a virtual hand. Furthermore, I investigated the role of the TMS-evoked hand sensations and their role in the embodiment showing that the hand ownership is mainly driven by the detection of TMS stimuli on the hand.

# Sommario

Il nostro cervello riceve de integra costantemente un flusso di informazioni sensoriali e motorie che modellano il modo in cui il nostro corpo è rappresentato. Numerosi approcci sperimentali sono stati proposti per alterare la rappresentazione corporea, manipolando segnali corporei esterocettivi o intericettivi. Durante la mia tesi, ho studiato il contributo dei meccanismi cerebrali centrali, piuttosto che periferici, alla base dell'autocoscienza corporea (BSC). A tale scopo, ho tentato di manipolare la BSC, inducendo specificamente l'embodiment di una mano falsa in partecipanti sani, usando un nuovo metodo che combina la stimolazione magnetica transcranica non invasiva (TMS) con la realtà virtuale immersiva (VR).

Innanzitutto, siamo riusciti a indurre l'embodiment di una mano virtuale utilizzando la coerenza spazio-temporale tra un'attivazione artificiale indotta da TMS del sistema motorio e il feedback visivo relativo al movimento in VR (**Studio 1**). Ho osservato che l'embodiment per una mano virtuale è indotta solo quando la stimolazione è sopra la soglia, ma non quando la TMS viene erogata al di sotto della soglia. Sostengo che tale forma di realizzazione illusoria indotta da TMS sia dovuta all'integrazione neuro-visiva tra l'attivazione della corteccia motoria primaria (M1) indotta da TMS e le contrazioni della mano con feedback VR visivo.

In un secondo studio (**Studio 2**), perfezionando i parametri del nostro protocollo TMS-VR abbiamo studiato i diversi componenti periferici suscitati dal TMS con un focus speciale sulla percezione della mano. I nostri dati hanno rivelato le principali caratteristiche delle percezioni della mano evocate da TMS (in termini di soglia e percezioni soggettive evocate) e mostrano che queste sono distinguibili dai MEPs e dai movimenti evocati da TMS a impulso singolo su M1.

Infine, abbiamo combinato la configurazione TMS-VR (**Studio 1**) e il nostro protocollo di stimolazione (**Studio 2**) per studiare se la consapevolezza degli stimoli corporei periferici è necessaria per indurre cambiamenti nella BSC (**Studio 3**). Le nostre scoperte rivelano un ruolo preminente di percezioni corporee coscienti nell'innescare l'embodiment, anche in assenza di stimoli periferici.

Inoltre, sono stato coinvolto in altri progetti che studiano la plasticità del corpo e la rappresentazione dello spazio in partecipanti giovani e anziani sani (elencati nelle appendici 1 e 2) e l'induzione robotizzata delle allucinazioni di presenza (appendice 3).

Il presente lavoro fornisce importanti risultati e strumenti promettenti per quanto riguarda il ruolo della stimolazione cerebrale non invasiva e della tecnologia virtuale nello studio di BSC. In particolare, ho dimostrato che è possibile manipolare BSC stimolando direttamente la rappresentazione corticale M1 della mano inducendo embodiment per una mano virtuale. Inoltre, ho studiato il ruolo delle sensazioni della mano evocate da TMS e il loro ruolo nell'embodiment mostrando che il senso di proprietà della mano è principalmente guidato dal rilevamento degli stimoli TMS sulla mano.

**Parole chiave**

Coscienza del corpo, rappresentazione del corpo, proprietà del corpo, illusione della mano di gomma, TMS, integrazione multisensoriale.

# Contents

# 1.     Introduction

Right now, I am completing my thesis in neuroscience and I realize that the time is short. My hands are moving extremely fast, pressing hard on the keys generating much noise. At the same time, my heartbeat is increasing due to the stress. In our daily life we are exposed to a constant flow of bodily information (e.g. auditory, visual, tactile, motor, and visceral) that are integrated to generate a functional representation of our body that allows us to interact with the environment.

A large amount of scientific work has focused on higher-order aspects of self-consciousness (e.g. language, memory, and social cognition) based on explicit cognition and language. However, this line of studies does not consider an important property of consciousness, which is its relation with the self of a given experience. Recently, we observed a growing interest in the study of the pre-reflective form of self-consciousness (Blanke et al., 2009) closely linked to the neural body representation. This experience of being a unified entity residing in a physical body, perceiving as its own and being able to control it, is known as bodily self-consciousness (BSC) (Blanke, 2012; Blanke et al., 2015) and relies on the integration of the constant flow of multisensory bodily signals.

The BSC is investigated in the field of cognitive neuroscience by studying how we perceive our body through the manipulation of the brain mechanisms underlying the integration of bodily signals (Ehrsson, 2012; Blanke, 2012; Blanke et al., 2015; Park et al., 2019). Multisensory stimulation has been combined with virtual reality (Sanchez-Vives et al., 2010; Yuan et al., 2010) and robotic technologies (Arata et al., 2014) to investigate the behavioural, neural, and physiological correlates of BSC. This approach has led to advancements in the understanding of the neural mechanisms of self-consciousness (Blanke et al., 2015b) and have suggested relevant clinical applications (Bolognini et al., 2014; Pozeg et al., 2017; Solcà et al., 2018; Rognini et al., 2019).

In this introduction, I will first present the theoretical background, multisensory mechanisms, and the experimental manipulation of the BSC. Then, I will provide an overview of the current

approaches used to investigate the embodiment with non-invasive brain stimulation (NIBS) techniques.

## 1.1. Theoretical background: multisensory mechanisms underlying BSC

One of the first studies to successfully induce illusory states of BSC (Botvinick & Cohen, 1998) demonstrated that healthy individuals can experience the illusion that a fake hand is part of their body after few minutes of visuo-tactile stimulation. This study was carried out by Botvinik and Cohen in 1998 and is considered a milestone in the study of self-consciousness. In this paradigm, also known as the rubber hand illusion (RHI), the participants are sitting on a chair with their left hand resting palm-down upon a table (Figure 1.A). The experimenter places a rubber hand beside the participants' left hand and a standing screen between the real and the rubber hand, to preclude vision of the real one. During the experiment, the participants are asked to keep the eyes fixed on the rubber hand while the experimenter proceeds with simultaneous stimulation of the fake and the real hand using two paintbrushes. At the end of the procedure, the participants report the sensation that the rubber hand is their own hand.

The RHI was the first experimentally controlled demonstration that body ownership, i.e. the sense that a body part belongs to me (De Vignemont, 2011), can be manipulated with visuo-tactile stimulation. Since then, this experimental procedure has been extended to various body parts like faces (Sforza et al., 2010), legs (Kokkinara et al., 2014), feet (Crea et al., 2015), the belly (Normand et al., 2011) and also to the full-body (Lenggenhager et al., 2007), providing new insights on the mechanisms of plasticity in the multisensory integration of bodily signals.
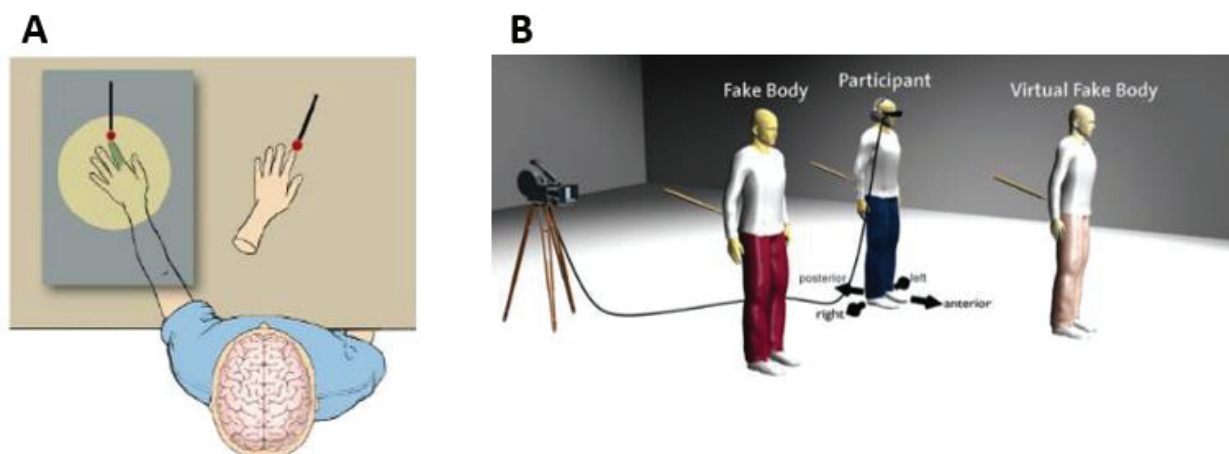
Figure 1. (A) Rubber hand and (B) full-body illusion (Lenggenhager et al., 2007) setups

The RHI has opened the opportunity to investigate intriguing scientific questions regarding the neural processes that contribute to body representation, as well as the functional changes in the brain that allow perceiving a sense of ownership for a fake hand. Functional magnetic resonance imaging (fMRI) studies on the RHI have revealed a distributed frontoparietal network involved in the integration of tactile, proprioceptive and visual feedback (Makin et al., 2007; Tsakiris et al., 2007, 2010; Brozzoli et al., 2011; Gentile et al., 2011, 2013), by suggesting that areas like the ventral premotor cortex (vPMC) and the insula might play a crucial role in generating the experience of body ownership (Ehrsson, 2004; Tsakiris et al., 2007; Limanowski et al., 2015; Grivaz et al., 2017). Electroencephalography (EEG) studies have shown that the RHI is associated with the attenuation in the somatosensory-evoked responses (Zeller et al., 2015) and decreases in fronto-parietal alpha and beta power (Faivre et al., 2017; Rao et al., 2017). Also, gamma oscillations in the temporoparietal junction (TPJ) and in the premotor cortex might play a role in the visuo-tactile integration process underlying the RHI (Kanayama et al., 2007, 2009; Lenggenhager et al., 2011).

## 1.1.1. Neurological alterations of BSC

In the neurological and neuropsychological literature, it has been reported that the damages or dysfunctions in areas involved in the process of multisensory signals, and in particular in the representation of the body, can generate explicit pathological alterations of body ownership

(Haggard & Wolpert, 2005; Ronchi et al., 2018). Post-stroke patients may report emotional indifference (i.e. Anosodiaphoria, Langer et al., 2014), unawareness (i.e. Asomatognosia, Critchley 1953, Arzy et al., 2006; Feinberg et al., 2010), or feeling of hatred and aggressive behaviour (i.e. Misoplegia, Critchley, 1974; Pearce, 2007) towards their affected contralesional limb, in absence of premorbid psychiatric disorders. In extreme cases, patients can experience a disturbance of BSC called somatoparaphrenia, a condition in which patients verbally deny the ownership of their affected limb, ascribing it to another person by means of verbal confabulations (Romano et al., 2019). Somatoparaphrenia has been associated with fronto-temporo-parietal lesions, with a prominent role of the TPJ in the genesis of the delusion (Vallar & Ronchi., 2009), a brain area that is known to play a crucial role in the integration of bodily signals (Ionta et al., 2011). Another disorder related to the sense of ownership is the pathological embodiment (Pia et al., 2016), where patients erroneously report that someone else's limbs belong to their body (Gerstmann, 1942). This condition occurs mainly when the patient observes an alien limb that is placed in a body-congruent position with his/her visual first-person perspective ( Pia et al., 2013; Garbarini et al., 2013, 2014). Also, in this case, the underlying brain lesions involve portions of the TPJ, the frontal operculum, and the inferior frontal gyrus (Martinaud et al., 2017). Finally, rare clinical cases report supernumerary limb perception (Halligan et al., 1993; Miyazawa et al., 2004; Cipriani et al., 2011), where the patients may experience the presence of an additional limb originating from their body (usually from the contralesional shoulder), usually associated with subcortical lesions (Khateb et al., 2009).

## 1.1.2. BSC constraints to hand ownership

The multisensory mechanisms of BSC that allow the induction of illusory bodily states pose, at the same time, some constraints to those illusions. Four main constraints have been identified that determine ownership for a fake hand (Blanke et al., 2015) (Figure 2): (i) *The proprioceptive constraint*: The observed body part must be placed in a coherent position with respect to the own body. Studies on the RHI have shown that when the fake hand assumes an implausible orientation, the illusion is reduced or abolished (Ehrsson, 2004; Tsakiris & Haggard, 2005, Ide, 2013); (ii) *The body-related visual information constraint*: To successfully achieve an illusory sense

of ownership, the observed object has to resemble a body part. Different studies demonstrated that, if the rubber hand is substituted with an object other than a body part (like a wooden stick), the illusion is not evoked (Tsakiris and Haggard, 2005; Haans et al., 2008; Tsakiris et al., 2010). These results may suggest that the brain stores a supramodal body representation that contains information of visual, anatomical, and structural properties of the body (Tsakiris, 2010), providing top-down limitations to multisensory integration processes. Accordingly, neuroimaging studies found that the extrastriate body area (EBA) in the occipital cortex preferentially responds to the vision of body parts with respect to objects (Orlov et al., 2010), and specific subpopulations of neurons in the EBA may distinguish between own and other's body parts; (iii) *The PPS constraint:* multisensory integration presents a spatial limitation and are strongly integrated when spatial proximity is respected. This space has been defined as peripersonal space (PPS) (G. Rizzolatti et al., 1981a; 1981b; 1997) and studies investigating the spatial body representation have shown that the reaction times to tactile stimuli applied over the hand are faster when touch is associated with static (Làdavas & Serino, 2008; Serino et al., 2007, 2011) and dynamic sounds (Canzoneri et al., 2012), or visual stimuli (Serino et al., 2015) close to the body. This shows that multisensory stimuli influence tactile processing as a function of the proximity with the body. However, the speedup of the tactile processing is body-part specific (Serino et al., 2015), limited in space, and when stimuli are far (beyond 30-45 cm for the hand) from the body the advantage in the tactile processing is abolished (Serino, 2019). Accordingly, the strength of the RHI is reduced as a function of the distance between the participant body and the rubber hand, both on the horizontal (Lloyd, 2007) and vertical plane (Kalckert et al., 2014); (iv) *The embodiment constraint:* to achieve a successful manipulation of the BSC is necessary to apply synchronous multisensory bodily stimulation over a prolonged period of time. The embodiment constraint allows linking the stimulation coming from the own body to objects other than the physical body. More in detail, it allows to process information through an external object at the sensory, motor, and affective levels in the same way as the properties of one's body (De Vignemont, 2011; Makin et al., 2017). The embodiment of artificial body parts has been interpreted in light of the properties of neurons in multisensory brain regions. In fact, these areas contain bimodal and multimodal neurons that integrate visual, auditory, and tactile information. It has been shown that in non-human primates

the firing rate of the premotor cortex (PMC) neurons is modulated when visual stimuli approach the upper limb, but also when are directed toward a fake hand placed in a body congruent position (Graziano, 1999). A similar pattern of activity has been observed in humans through non-invasive methods. A recent review (Grivaz et al., 2017), pointed out the presence of consistent clusters localized in the posterior parietal cortex, PMC, and in the left anterior insula related to body ownership.
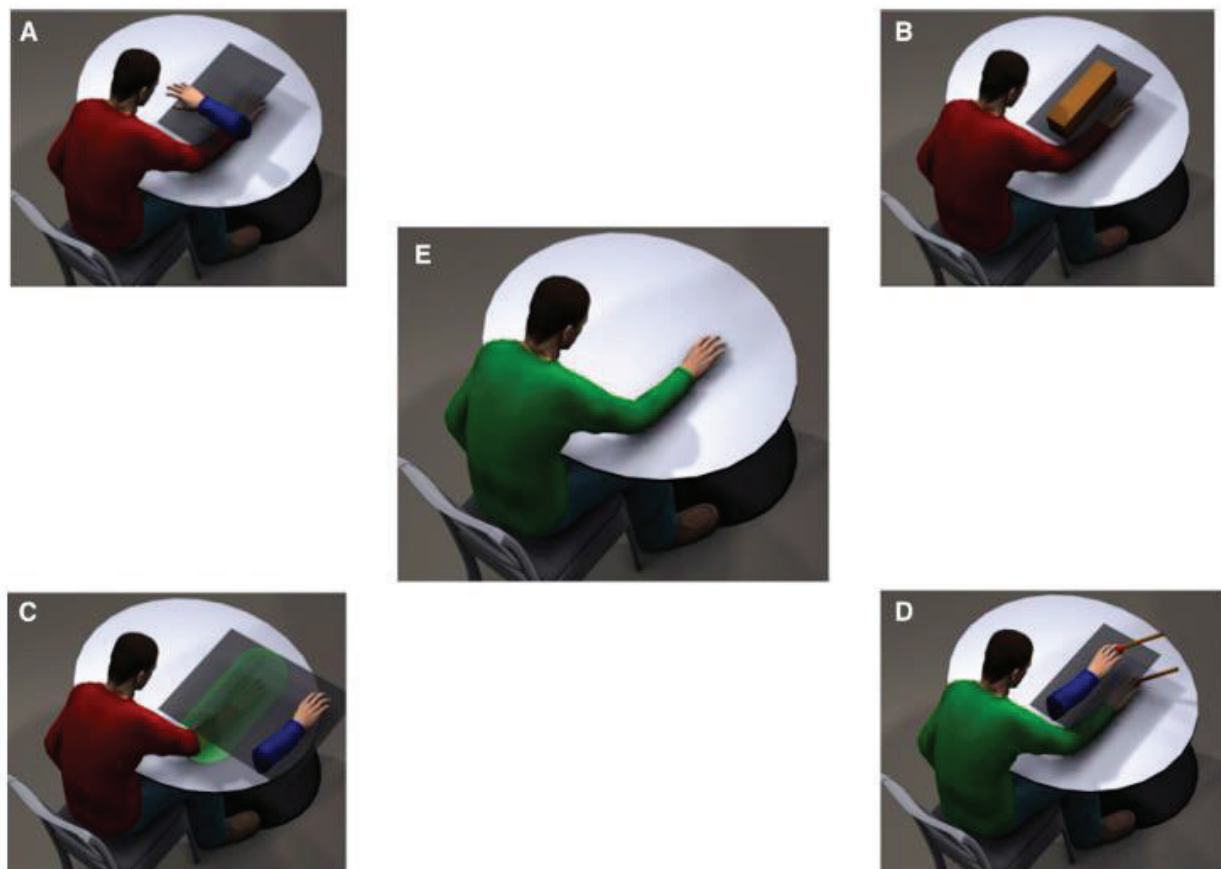


Figure 2. Four constraints determining the hand ownership (Blanke et al., 2015). (A) proprioceptive, (B) body-related visual information, (C) PPS, (D) embodiment constraint.

To summarize, each of the four constraints defines the specific conditions that must be met to manipulate BSC according to the principles of multisensory integration investigated in the literature. Principles of multisensory integration indicate that stimuli from different modalities are effectively integrated when they are congruent in time (temporal law) and space (spatial law), and more efficiently if the stimulation in each modality is weak (inverse effectiveness) (Stein et

al., 2008). BSC can be linked to a virtual or fake hand when (i) the latter is congruent with the subject body posture (*proprioceptive constrain*), (ii) the visual appearance resembles a body (*body-related visual information*), (iii) when the stimuli are in proximity of the body (*PPS constraint*) (iv) during a synchronous and prolonged stimulation (*embodiment constrains*). Vice-versa, the conditions that do not respect those constraints conflict with the multisensory principles described above, and are usually applied as a control condition in the experimental practice. Most of the studies contrast the synchronous stimulation by introducing a delay of around 500 ms (known as asynchronous condition). Other studies place the prosthetic body part rotated of 90°, 180°, or 360°; others replace the visual body with a non-corporal object or place the fake hand far from the body.

The principles described above are not only related to the specific case of hand ownership illusion. Other lines of research have applied the same multisensory principles focusing on more global aspects of BSC (Blanke et al., 2009) that entail changes in the self-identification, self-location (the experience of where 'I' am in space) (Lenggenhager et al., 2007; Blanke, 2012), reshaping and extending the PPS (Noel et al., 2015). These manipulations can be achieved by showing a virtual avatar being stroked simultaneously with the own body, paradigm named full-body illusion (FBI) (fig1.B). Full-body BSC is built on a trunk reference frame, and the underlying neural circuits extend beyond the hand ownership circuits.

## 1.2. Experimental manipulations of the BSC

Embodiment has been measured with questionnaires, behavioural and physiological measures. The first proposed behavioural measure (Botvinick & Cohen, 1998) of embodiment, later defined proprioceptive drift (Tsakiris et al., 2006), consists in a pointing task after the illusion onset, in which the participants have to point to the perceived position of the stimulated hand using the non-stimulated one. Higher displacement error towards the rubber hand has been interpreted as an implicit measure of embodiment. Also, physiological measures have been proposed as implicit measures of embodiment (Kilteni et al., 2012), like the skin conductance (Armel et al., 2003), the body temperature (Moseley et al., 2008), reaction times to tactile stimuli (Folegatti et al., 2009)

and motor evoked potential amplitude (della Gatta et al., 2016). Most of those studies have shown that, after the illusion onset, the normal flow of physiological activity is decreased in the real arm. This data has been interpreted as if the bodily representation of the real hand is altered by the fake one, leading to a general downregulation of the real arm's physiological activity. However, we have to acknowledge that these implicit measures have not been always replicated (De Haan et al., 2017; Isayama et al., 2019) and further work is required to understand what is the relation between explicit (questionnaire) and implicit (behavioural and physiological) measures (Rohde et al., 2011).

The RHI is a paradigmatic case where a bodily stimulation is adopted to induce illusory states of BSC through the manipulation of exteroceptive signals like vision and touch. Here, I will summarize some of the methods that have been adopted to induce embodiment over a fake body part.

From the technical point of view, a direct way to modulate the BSC is the synchronous application of visuo-tactile stimuli as in the RHI. In most of the early studies, it was directly the experimenter to administer the stimulation with paintbrushes or other tools. Despite its effectiveness, this method does not allow a precise synchronization/desynchronization of the delivered stimuli. However, it has been shown that the perceptual threshold to detect asynchrony between stimuli is around 230 ms (Shimada et al., 2010), ensuring the reliability of this method. Another procedure to induce illusory embodiment is based on active (Kalckert et al., 2012; Riemer et al., 2013, 2014) and passive movements (Tsakiris et al., 2006). In those cases, the illusion is induced by synchronizing the voluntary (or involuntary) hand movements generated by participants with the observed rubber hand movements. The sensorimotor stimulation opens the possibility to investigate the BSC emphasizing the role of the sense of agency (Patrick Haggard, 2017). Agency, in this context, is defined as "the sense that I am the one who is causing or generating an action" (Gallagher, 2000). The sense of agency, together with body ownership, is considered another component of embodiment that could be modulated thanks to the RHI(Longo et al., 2008).

What I listed so far are methods that involve only exteroceptive stimulation, i.e. mainly based on the simultaneous presence of tactile/motor and visual stimuli. However, there are several evidences that visceral interoceptive signals (sensory signals originating from the internal body,

such as visceral input from the stomach, heart, or lungs) contribute to the BSC (Park et al., 2019). For instance, Suzuki et al (2013) demonstrated that projecting the participant's heart-beat as visual flash on the hand through an augmented reality head-mounted display can induce the feeling of ownership for the fake hand. Similarly, other studies attempted to combine self-identification with an avatar when the cardiac or respiratory bodily signals are coupled with a congruent visual cue on a virtual avatar (Aspell et al., 2013; Betka et al., 2020).

One of the challenges of the present thesis work is the attempt to induce embodiment by using NIBS, specifically TMS to investigate BSC and the role peripheral signals at the explicit and implicit level in the modulation of BSC. in the next chapter, I will provide an overview of the current application of NIBS to investigate embodiment.

## 1.3. NIBS for embodiment investigations

The core element of TMS (Figure 3) is a stimulator that produces a magnetic field that can generate a current when applied over conductive materials such as the cortical brain tissue. In order to generate a magnetic pulse, a capacitor is discharged across a coil placed over a subject' scalp. The TMS has a temporal resolution of a few milliseconds, and a spatial resolution sufficient to provide a mapping of the motor cortex (Walsh and Cowey, 2000; Shea and Walsh, 2007). For instance, when a TMS supra-threshold pulse is applied over the M1-hand motor area, it can reach the brain layer V and be propagated through the corticospinal tract until the peripheral hand muscles (Gentner et al., 2006). By means of electromyographic (EMG) and accelerometer systems placed over the hand, motor-evoked potentials (MEPs) from a target hand muscle and hand twitches (TMS-evoked movements) can be recorded around 20 ms after the pulse onset.

The TMS was introduced in 1985 (Barker et al., 1985) as an alternative clinical tool to transcranial electrical stimulation (TES) (Merton et al., 1980; Rossini et al., 1994) given that it is pain-free and causing minimal discomfort. Nowadays, TMS is widely used in clinical practice as a diagnostic tool and (Groppa et al., 2012) for the treatment of specific neurological and psychiatric disorders (Lefaucheur et al., 2014). In parallel, it became a very popular technique in cognitive neuroscience to investigate how the transient stimulation of brain areas can modify their activity and therefore

its function (Sandrini et al., 2011, Rossini et al., 2015). TMS has been used as an inference technique for the contribution of brain regions in specific cognitive functions, for example by using TMS to interfere with cognitive tasks (Pascual-Leone et al., 2000) or to investigate the reactivity of the motor system to certain conditions by measuring the MEPs amplitude (Sato et al., 2007).



Figure 3. TMS (left) and tDCS (right) devices.

Another NIBS technique introduced in clinical and cognitive neuroscience is the transcranial direct current stimulation (tDCS) (Figure 3) (Nitsche et al., 2000, 2008) that is used with the purpose of upregulating or downregulating the brain activity to improve and alter the cognitive functioning (Parkin et al., 2015). Differently from TMS, tDCS consists of a battery-powered current-controlled stimulator that generates the stimulation waveform (Truong et al., 2018) of a few mA delivered through a cathodal (inducing membrane hyperpolarization) or anodal (inducing membrane depolarization) electrode over the participants' scalp.

Recently, TMS and tDCS have been also applied to investigate BSC, and especially hand ownership, with different approaches. One line of research attempts to down-regulate or up-regulate brain areas involved in body ownership. The first studies showed that stimulating specific brain regions can affect the proprioceptive drift towards the rubber hand. Concretely, the stimulation of the IPL led to a decrease in proprioceptive drift (Kammers et al., 2009), whereas it was increased if the target was the EBA (Wold et al., 2014). and increasing it if the target area is the EBA. A recent study (Fossataro et al., 2018) suggests that when M1 is inhibited, the overall ownership for the contralateral hand decreases, boosting the susceptibility to the RHI. Similar

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findings have been reported after cathodal tDCS attenuation of the primary sensory-motor cortex activity (Hornburger et al., 2019), while the anodal tDCS stimulation of the posterior parietal cortex can speed-up the illusion onset. Altogether, these studies corroborate the previous observation that different areas of the fronto-parietal network play a crucial role in generating the conscious experience of hand ownership.

Other studies have used non-invasive stimulation to assess the effect of the illusions on the corticospinal excitability by measuring the MEP amplitude after the illusion induction period (Dilena et al., 2019). Those studies reported a systematic decrease of the MEP amplitude after the synchronous stimulation (della Gatta et al., 2016; Kilteni et al., 2016), which has been interpreted as a possible inhibitory effect due to the disownership of the real hand in favour of the fake hand, i.e. the disownership may affect the motor reactivity (Folegatti et al., 2009) and downregulate M1 activity.

## 1.4. Thesis outline

My thesis work aims to investigate the role of cortical motor signals in the representation of the own body. In particular, I investigated the influence of motor cortical stimulation in healthy participants by using non-invasive brain stimulation with a specific focus on the possibility to modulate the BSC. For this purpose, I carried out a series of studies combining VR and TMS stimulation to induce a RH-like state in healthy participants.

In the first study (**Study 1**), I contributed to develop a setup to induce a RH-like state (TMS-VR induced RHI) in healthy participants. In this experiment, we tried to validate for the first time our protocol, combining the visual presentation of a virtual hand in VR with the simultaneous supra-threshold TMS of the motor cortex (M1-hand area). To this aim, we measured the illusion with questionnaires and we managed to successfully demonstrate that the TMS-VR induced RHI is effective when the visual stimuli are time-locked with the TMS of the M1-hand area, i.e. the observed hand movement mimicked the TMS-evoked movement of the participants' real hand. This illusion is ineffective when the TMS is subthreshold and the visual feedback is asynchronous.

In the second study (**Study 2**), I applied a psychometric approach to refine the TMS-VR induced RHI protocol. The purpose was to investigate if the TMS can activate the cortico-spinal tract eliciting TMS motor-evoked potentials (MEPs) in the absence of a conscious perception of the hand stimulation. We found that a specific intensity of stimulation, slightly higher than the MEPs threshold, is more likely to evoke hand perceptions (TMS-evoked hand perceptions).

In the third study (**Study 3**), we combined the TMS-VR protocol with the newly found intensity of stimulation evoking hand perceptions to investigate whether the awareness of the peripheral stimuli, evoked by the TMS, is necessary to modulate the participant's BSC.


# 1.5. Personal contribution

Papers included in my thesis

**Study 1:** M. Bassolino, M. Franza, J. Bello Ruiz, M. Pinardi, T. Schmidlin, M.A. Stephan, M. Solcà, A. Serino, O. Blanke.
*Non-invasive brain stimulation of motor cortex induces embodiment when integrated with virtual reality feedback*. Eur J Neurosci. 2018 Apr; 47(7): 790–799. doi: 10.1111/ejn.13871

**Personal contribution:** data collection, data analysis.


**Study 2:** M. Franza, G. Sorrentino, M. Vissani, A. Serino, O. Blanke, M. Bassolino.
*Hand perceptions induced by single pulse transcranial magnetic stimulation over the primary motor cortex*. Brain Stimul. 2019 May - Jun; 12(3):693-701. doi: 10.1016/j.brs.2018.12.972.

**Personal contribution:** study design, paper writing, data collection, data analysis.


**Study 3:** M. Franza, G. Sorrentino, N. Faivre, O. Blanke, M. Bassolino.
*Move or believe to move? The role of bodily signals perception in triggering illusory feelings of embodiment towards a virtual hand* (In preparation).

**Personal contribution:** study design, paper writing, data collection, data analysis.


## Papers in the appendix

**Appendix 1:** G. Sorrentino, M. Franza, C. Zuber, O. Blanke, A. Serino, M. Bassolino

*How ageing shapes body and space representations: a comparison study between healthy young and elderly participants.* Submitted to Cortex (In revision).

**Personal contribution:** data collection, manuscript revision.


**Appendix 2:** M. Galigani, N. Castellani, B. Donno, M. Franza, C. Zuber, L. Allet, F. Garbarini, M. Bassolino
*Effect of tool-use observation on metric body representation and peripersonal space. (*Submitted to Neuropsychologia).

**Personal contribution:** data collection.


**Appendix 3:** F. Bernasconi, E. Blondiaux, J. Potheegadoo, G. Stripeikyte, J. Pagonabarraga, H. Bejr-Kasem, M. Bassolino, M. Akselrod, S. Martinez-Horta, F. Sampedro, M. Hara, J. Horvath, M. Franza, S. Konik, M. Bereau, J.A. Ghika, P. Burkhard, D. Van De Ville, N. Faivre, G. Rognini, P. Krack, J. Kulisevsky, O. Blanke.
*Sensorimotor hallucinations in Parkinson's disease*. (Submitted to Science translational medicine).

**Personal contribution:** data collection.

# 2. Papers

## 2.1. Study 1: Non-invasive brain stimulation of motor cortex induces embodiment when integrated with virtual reality feedback

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**ABSTRACT**

Previous evidence highlighted the multisensory-motor origin of embodiment – that is, the experience of having a body and of being in control of it – and the possibility of experimentally manipulating it. For instance, an illusory feeling of embodiment towards a fake hand can be triggered by providing synchronous visuo-tactile stimulation to the hand of participants and to a fake hand or by asking participants to move their hand and observe a fake hand moving accordingly (rubber hand illusion). Here, we tested whether it is possible to manipulate embodiment not through stimulation of the participant's hand, but by directly tapping into the brain's hand representation via non-invasive brain stimulation. To this aim, we combined transcranial magnetic stimulation (TMS), to activate the hand corticospinal representation, with virtual reality (VR), to provide matching (as contrasted to non-matching) visual feedback, mimicking involuntary hand movements evoked by TMS. We show that the illusory embodiment occurred when TMS pulses were temporally matched with VR feedback, but not when TMS was administered outside primary motor cortex, (over the vertex) or when stimulating motor cortex at a lower intensity (that did not activate peripheral muscles). Behavioural (questionnaires) and neurophysiological (motor-evoked-potentials, TMS-evoked-movements) measures further indicated that embodiment was not explained by stimulation per se, but depended on the temporal coherence between TMS-induced activation of hand corticospinal representation and

the virtual bodily feedback. This reveals that non-invasive brain stimulation may replace the application of external tactile hand cues and motor components related to volition, planning and anticipation.

**INTRODUCTION**

The brain constantly receives, sends and updates information from and to the body, thus building association rules between different multisensory bodily signals (i.e. tactile, proprioceptive, kinesthetic, visual, auditory, vestibular), motor commands and related external events (e.g. Medina & Coslett, 2010; Serino & Haggard, 2010; De Vignemont, 2011; Held et al., 2011). (Medina et al., 2010)Integrated signals between motor intention, execution and multisensory feedback have been pro- posed to lead to a sense of control for one's own movements (sense of agency, 'I am the one who generated that hand movement') and to a feeling of ownership for one's own body ('the hand which is moving is my hand'). The sense of ownership and sense of agency are fundamental components of embodiment (i.e. the experience of having a body and being in control of it), and several experimental procedures to manipulate embodiment have been described (e.g. Jeannerod, 2003; Kannape & Blanke, 2013; Blanke et al., 2015). For instance, embodiment for a fake hand can be induced through multi- sensory stimulation using the rubber hand illusion (RHI) (Botvinick & Cohen, 1998; Tsakiris, 2010). During the RHI, synchronous visuo-tactile stimulation is applied and participants observe touches on a fake hand, while receiving concurrent tactile stimuli on their hidden hand. Many other RHI-like protocols have been proposed, such as by providing visuo-motor stimulation based on participants' movements and congruent visual feedback of a fake or a virtual hand moving accordingly (e.g. Slater et al., 2008; Sanchez-Vives et al., 2010; Kil- teni et al., 2015). Thus, most RHI work has been based either on the direct application of somatosensory stimuli to participant's skin or limb (i.e. tactile, Botvinick & Cohen, 1998 or proprioceptive cue, Walsh et al., 2011) or on subjects' movements (Tsakiris et al., 2006; Riemer et al., 2013, 2014; Kalckert & Ehrsson, 2014). One intriguing possibility is to induce embodiment by providing artificial stimulation able to activate the corticospinal hand representation. Despite recent clinical evidence in this direction (in two epileptic patients

undergoing invasive stimulation of somatosensory cortex evoking artificial somatic sensations in patients' hand; Collins et al., 2017), the possibility to induce hand embodiment in healthy participants by artificially activating the corticospinal hand representation non-invasively, that is through transcranial magnetic stimulation (TMS), has been never investigated, so far. Here, we linked the stimulation of the hand corticospinal representation by non-invasive transcranial magnetic stimulation (TMS) over the motor cortex (M1) with visual hand feedback provided by an immersive VR system (TMS-VR induced RHI) to investigate embodiment induction for a virtual hand. If applied over hand M1, a TMS pulse, at a sufficient intensity, induces measurable twitches and short involuntary hand movements and in the corresponding hand muscle (i.e. motor-evoked potentials, MEPs). In our new experimental set-up, TMS automatically triggered the VR system, so that participants observed an animated virtual hand mimicking the TMS-induced movements. We hypothesized that the combination between the activation of the hand corticospinal representation (induced through TMS), leading to involuntary hand twitches, time-locked with visual feedback provided via VR (synchronous condition), induces illusory embodiment for the virtual limb. Thus, we compared the strength of subjective feelings of ownership for the virtual hand against a control condition where TMS and visual feedback were decoupled (asynchronous condition). To study the specificity of these effects for the location and intensity of TMS, we run two further conditions, where we applied TMS over a site outside M1 (vertex, supra-threshold vertex) or over M1, but with reduced intensity (subthreshold condition, 80% of resting motor threshold), not evoking muscle contractions or peripheral movements.

**MATERIALS AND METHODS**

*Subjects*

Thirty-two subjects were recruited (mean age 29.7 years, SD ± 4.7, 16 females). They all performed the main experimental condition (supra-threshold M1 stimulation, 130% of the resting motor thresh- old) and in addition one of two other conditions. A first group of 16 'vertex' control condition (supra-threshold vertex), to test the alter- subjects (mean age 29.8 years, SD ± 4.8, 8

females) underwent the native hypothesis that unspecific TMS effects unrelated to the activation of the M1 could induce embodiment for the virtual hand (experiment 1). The second group of 16 participants (mean age 29.6 years, SD ± 4.6, eight females) underwent the 'subthreshold' control condition (subthreshold M1), in which TMS over M1 was set not to evoke any muscle twitches or movements (experiment 2). All participants were right-handed, as determined by the Flinders Handedness survey (Nicholls et al., 2013). They had normal or corrected-to-normal vision, touch and hearing and no contraindication to TMS (Rossi et al., 2009). Participants were naive to the purpose of the study and participated after giving an informed consent. The study was conducted with the approval of the local ethics committee (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM 017/14).

### *General procedure*

The experimental procedure is illustrated in Fig. 1. Subjects were seated in a comfortable TMS chair (Brainsig, Rogue Research) with their arms resting in a prone position on a white table. First, all TMS parameters were set: the area to stimulate, the intensity of the stimulation and the movements evoked (see below). Next, participants were familiarized with the VR scenario by wearing a head-mounted display (see below for details about VR): they were instructed to keep their right hand still and as relaxed as possible and to observe the virtual hand lying palm down on a white table. In an initial calibration phase performed for every subject and experimental session, the position of the virtual hand was carefully set in order to match the perceived position of the participants' right limb. After VR calibration, we first recorded motor-evoked potentials (MEPs, 12), as a control measure for cortical excitability at baseline, while participants observed a neutral virtual scenario (a white table). After this, participants performed a hand location task (drift PRE, six trials; see below). At that time, they underwent the TMS-VR stimulation for about 2 min (12 TMS pluses). Next, participants repeated the drift (POST, three trials). Then, short blocks of TMS- VR stimulation (three TMS pulses, approximately 30 s) were followed by one trial of the drift. This loop was repeated six times. After this, another TMS-VR stimulation followed (six TMS pulses, approximately 1 min) and at the end of this block of stimulation, participants were asked

to answer to two questionnaires (see below), one related to embodiment (Embodiment questionnaire) and one regarding the sensations induced by TMS (TMS questionnaire). Half of the subjects (16 subjects in total, balanced in supra-threshold vertex and subthreshold M1 conditions) performed the Embodiment questionnaire after the drift-stimulation loop as just described, while the other half (16) performed first the Embodiment questionnaire and then the loop. The TMS questionnaire was always per- formed at the end of the entire procedure. After the TMS questionnaire, we recorded MEPs (12 trials), as control measure for cortical excitability after the TMS-VR stimulation, while participants observed the virtual white table. This overall procedure remained identical for all conditions. The subjects performed the main experimental condition (supra-threshold M1) on 1 day and one additional condition (either supra-threshold vertex or subthreshold M1, depending on the group) on a different day, in a counterbalanced order, and with an interval of approximately 1 week between the two testing days. In each session, participants were exposed both to a condition in which TMS and VR feedback were time-locked (synchronous condition) and to a condition in which a delay was inserted between the TMS pulses and visual feedback provided by the VR system (asynchronous condition, see below for details). There was a 10-min break between the two stimulation blocks. The order between conditions and blocks was counterbalanced across participants.
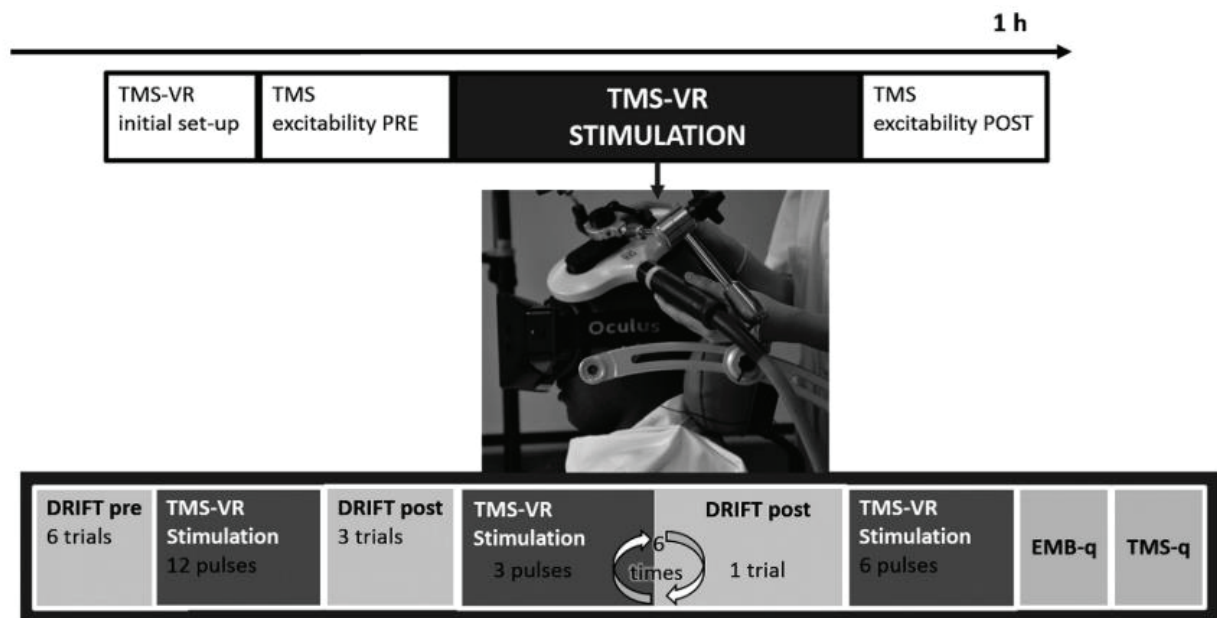


Fig. 1. Transcranial Magnetic Stimulation (TMS) and virtual reality (VR) were fully integrated to induce rubber hand illusion (RHI): TMS-VR induced RHI. It shows the experimental procedure: after the initial set-up for TMS and VR (see main text), excitability of motor cortex was recorded at rest before and after the TMS-VR stimulation (white squares). During the TMS-VR stimulation (grey

squares), participants received single TMS pulses, over motor cortex or the vertex, and at a specific intensity of stimulation, supra- or subthreshold, accordingly to the experimental conditions. Through a head-mounted display (small photo), they observed a virtual hand mimicking the TMS-evoked movements. The temporal congruency between TMS pulse and the movement of the virtual hand was manipulated in a synchronous or asynchronous condition. Before and after the first block of TMS-VR stimulation (12 TMS pulses), participants performed the hand location task in VR (drift). Then, brief blocks of TMS-VR stimulation (three pulses) followed by a drift measure were repeated six times. At the end, participants responded to a questionnaire related to embodiment (Embodiment questionnaire, in the figure EMB-q) and to one related to the sensations induced by the TMS (TMS questionnaire, in the figure TMS-q). This procedure was repeated twice in the same session, once for synchronous and once for asynchronous condition (in a counterbalanced between-participants order). On a separate day, a second identical session was performed, which differed for the site (experiment 1) or the intensity (experiment 2) of TMS according to the experimental condition.

*Transcranial magnetic stimulation*

TMS was delivered through a figure-eight coil (wing diameter of 70 mm) connected to a single Magstim monophasic stimulator (Magstim 2002; Magstim Co., Whitland, UK). To determine the optimal position for activation of the right first dorsal interosseous (FDI) muscle (i.e. the scalp position from which the largest MEPs were elicited), the coil was positioned at an initial estimate 5 cm lateral and 1 cm anterior to the vertex (Groppa et al., 2012). TMS pulses at slightly supra-threshold intensity were then applied by moving the coil in 0.5 cm steps around this initial estimate. Resting motor threshold of FDI muscle was determined according to standard procedure using the software based 'adaptive method' (Awiszus, 2011) (TMS Motor Threshold Assessment Tool, http://www.c linicalresearcher.org/software.htm) (Groppa et al., 2012). During the procedure to define resting motor threshold, participants were asked to relax their muscles and wore the head-mounted display to observe a neutral virtual scenario (a white empty table, i.e. without the virtual hand). During supra-threshold M1 and supra-threshold vertex conditions, the intensity of the TMS pulse was set at 130% of the resting motor threshold to ensure stable MEPs and TMS-evoked movements. During subthreshold M1 condition, the intensity was set at 80% of the resting motor threshold, according to previous studies showing that this intensity is not sufficient to induce MEPs (peak-to-peak amplitude higher than 0.05 mV) in the hand (e.g. Nakamura et al., 1997). The absence of any MEPs at 80% of resting motor threshold has been verified at the beginning of the experiment for every subject, and the amplitude of MEPs during the entire experiment has been recorded. During supra-threshold M1 and subthreshold M1 conditions, the coil was placed on the optimal position for activation of the right FDI, while for the supra-threshold vertex condition, the coil was centred over the vertex (e.g. Sandrini et al., 2011), at the electrode position Cz as defined by the International 10–20 system (Jasper, 1958).

The coil was placed tangentially to the scalp with the handle pointing 45° postero-laterally away from the midline during supra-threshold M1 and subthreshold M1, while the handle was pointing 0° posterior during supra-threshold vertex (Duecker et al., 2013; Case et al., 2016). In all conditions, the optimal position of the coil was marked on the scalp with a pen to ensure the correct coil placement throughout the experiment. During the entire experiment, the coil was fastened to an articulated mechanical arm. The intertrial interval between two consecutive TMS pulses was randomly varying from 9.8 to 12.2 s to ensure no change in cortical excitability (Chen et al., 1997) and to avoid expectancy.

### *Motor-evoked potentials (MEPs)*

To assess the excitability of the motor system, we measured the peak-to-peak MEP amplitudes elicited by TMS in the FDI muscle before, during and after the illusion. MEPs were recorded by means of a surface electromyographic system (EMG) through wireless electrodes positioned on the FDI in a tendon-belly configuration. EMG signals were amplified and band-pass filtered (1 Hz to 1 kHz) by a Noraxon DTS amplifier (Velamed; GmbH, Köln, Germany). The signals were sampled at 3000 Hz, digitized using a laboratory inter- face (Power1401; Cambridge Electronics Design CED) and stored on a personal computer for display and later offline data analysis using the Signal software. During the initial TMS calibration and MEP registration (see above), each recording epoch lasted 1500 ms, from 300 ms before to 1200 ms after the TMS pulse. During the TMS-VR illusion, we recorded the EMG traces continuously and a trigger was given to the Noraxon system for every TMS pulse. The absence of voluntary contractions was continuously verified by visual monitoring of the EMG signal. Trials with EMG background activity (peak-to-peak amplitude > 0.05 mV in the 100 ms preceding the TMS pulse) were excluded from analysis. To assess the similarity between the movements evoked by the TMS and the observed virtual movements, we recorded the TMS-evoked hand movements by means of a three-dimensional accelerometer fixed over the middle finger knuckle (Noraxon (Velamed; GmbH). Accelerometric data for the separate axes were acquired in parallel with the EMG data, at the same sampling frequency. Accelerometric data were filtered (0.4–100 Hz) and analysed by a custom-made soft- ware written in MATLAB, following methods already proposed in previous works (Classen et al., 1998;

Finisguerra et al., 2015). The acceleration modulus (i.e. the square root of the sum of the squares of the axes) was first computed for a 200-ms window starting from TMS delivery. According to previous studies, we calculated the acceleration onset as the time when 5% of the peak acceleration was detected. Trials were included in the analysis if peak acceleration appeared between 20 and 55 ms after the TMS pulse (Finisguerra et al., 2015) and its amplitude was equal to or higher than 0.09 m/s2 in one axis (Classen et al., 1998). Given that the scope of this control measure was to verify whether the movements induced by TMS were mimick-ing the virtual ones, we focused on Z-component being sensitive to capture hand-closing movements similar to those displayed in VR. We compared the percentage of the movements indicating hand-closing in all experimental conditions.

### *Virtual reality (VR)*

Participants wore a head-mounted display (Oculus Rift Development Kit 1, 640 x 800 resolution per eye, 110° Field of View (nominal), refresh rate 60 Hz; Oculus VR, Menlo Park, CA, USA). An in-house software (ExpyVR, EPFL, http://lnco.epfl.ch/expyvr, frame- work for designing and running experiments in virtual reality) was used for stimulus presentation, to collect subjects' answers and to generate triggers for TMS pulses. For this later purpose, the laptop running the VR software was connected to the laboratory interface (Power1401; Cambridge Electronics Design CED), by means of a laptop-parallel-adapter-card to send triggers to the TMS stimulators. On the head-mounted display, participants observed virtual right hand movements mimicking their own hand movements evoked by supra-threshold M1 TMS pulses. For this purpose, we controlled a realistic three-dimensional virtual hand lying on a virtual white table in real time. The virtual hand's movements were animated to move with the same kind of movements that would be evoked by the supra-threshold M1 TMS pulses, every time that a trigger was sent to the TMS stimulators. During the TMS-VR stimulation, the virtual hand was displaced leftward with respect to the perceived position of the real hand as defined in an initial calibration phase. The inclination of the real table was adjusted to match the subjects' perceived inclination of the table in VR. In the synchronous condition, the animated movement of the virtual hand started right after the trigger

was sent to the TMS stimulators to temporally match the real hand evoked movement. An intrinsic delay of about 65 ms, due to the connection between the TMS and VR systems, was however present but did not have any effect on the perceived synchronicity. Indeed, visual animation occurred before the value of 150 ms that is generally necessary for detecting visuo-motor conflicts (Blakemore et al., 1999; Tsakiris et al., 2006) and it was longer than the physio-logical latency between TMS pulse and peripheral motor effects in the hand [in healthy young subjects, the latency of muscle twitches in the FDI muscle is usually around the 20 ms (Groppa et al., 2012), while the onset of evoked hand movements is usually between 20 and 55 ms after the TMS pulse (Finisguerra et al., 2015). In the asynchronous condition, three different delays of either 600, 1200 or 1800 ms were inserted between TMS pulse and visual VR feedback (the three delays were applied in random order and balanced between blocks and conditions). Importantly, when debriefing participants at the end of the experiment, they confirmed that (i) in the synchronous condition, the virtual hand movements appeared at the same time as the TMS pulses and as the perceived real hand movements, while (ii) in the asynchronous condition, they appeared as delayed with respect to the TMS pulses.

### *RHI induced by coupling of TMS with VR (TMS-VR-induced RHI)*

The general procedure remained identical in all conditions (see above), which differed for the site (M1 or vertex) or the intensity (supra-threshold or subthreshold) of stimulation and the temporal congruency between virtual and real hand movements (synchronous and asynchronous). We assessed the TMS-VR-induced RHI by means of consolidated measures previously used in RHI studies (Botvinick & Cohen, 1998; Tsakiris et al., 2006): (i) standard questionnaires assessing the subjective experience of embodiment (Embodiment questionnaire); and (ii) a hand location task evaluating the proprioceptive drift. Moreover, MEPs and TMS-evoked movements were recorded during the whole duration of the stimulation to measure how cortical excitability and peripheral TMS effects varied across conditions.

## Embodiment questionnaire

The items of the Embodiment questionnaire were selected from those used previously to test different embodiment components (Longo et al., 2008). Our main component of interest was related to ownership for the virtual hand ('it seemed like the virtual hand was part of my body'; 'it seemed like the virtual hand was my hand'). Considering previously reported dissociations between ownership and other embodiment-related components (e.g. Longo et al., 2008; Serino et al., 2013), we also included in the questionnaire the following additional components: disownership for the physical hand ('it seemed like my hand had disappeared'; 'it seemed like the experience on my hand was less vivid than normal');location ('it seemed like the virtual hand was in the location where my hand was'; 'it seemed like my hand was in the location where the virtual hand was') and agency ('it seemed like I was in control of the virtual hand'; 'it seemed like I could have moved the virtual hand if I had wanted'). The questionnaire included two statements for each component, plus two control questions ('it seemed like I had more than two hands'; 'it seemed like I had three hands') (10 items in total). The two statements referring to the same component (Longo et al., 2008) were collapsed together for the analysis. Subjects were asked to indicate agreement or disagreement with the statements of the questionnaire using the keyboard to move a cursor on a continuous vertical scale displayed through the head- mounted display. The top extreme of the scale, indicated by a green dot, represented a complete agreement (score 6), while the bottom extreme of scale, indicated by a red dot, corresponded to a complete disagreement (score 0).

## Proprioceptive drift

The hand location task (proprioceptive drift) was similar to that described elsewhere (Tsakiris, 2010 for a review). Before and after the TMS-VR stimulation, participants were asked to indicate the perceived position of their real right hand in VR using a keyboard with their left hand to move a cursor on a white empty table. Given that during the TMS-VR stimulation the virtual hand was displaced with respect to the perceived position of participants' real hand, we verified whether

after the stimulation, participants reported the perceived position of their hand as drifted towards the location of the virtual hand.

*Control measures*

In order to assess our subjects' perception to the different TMS conditions (supra-threshold M1, supra-threshold vertex, subthreshold M1), participants responded also to a TMS questionnaire about general sensations induced by the pulse ('Did you perceive any sensation on your head induced by TMS?'; 'Did you hear the click sounds induced by TMS?') and about specific perceptions regarding the hand and the evoked movements ('Did you perceive any sensation on your hand induced by TMS?'; 'Did you perceive your hand moving?'). The general sensations induced by TMS are expected to be similar in all the conditions, because of the contact between the coil and the scalp and because of the TMS sound clicks. In contrast, the sensations regarding the hand are specific to the supra-threshold M1 condition. Participants used the same continuous scale used for the Embodiment questionnaire to rate the intensity of the induced sensations (from very high, green dot, to very low, red dot). One subject's answers at TMS questionnaire were not recorded due to technical problems in experiment 1. Moreover, MEPs and TMS-evoked movements were recorded for the entire duration of the stimulation, as control measures of excitability and of the peripheral effects, respectively. One subject's accelerometric data were not recorded in experiment 1 due to a technical problem. To reduce the perception of the click sound produced by TMS, during stimulation and also MEP recording, and to exclude sur- rounding auditory cues, participants were listening to white noise presented through noise-cancelling ear plugs (Bose Quiet Comfort 20) during the experiment. For every subject, the sound volume was adjusted at the beginning of the session. Due to the different click sounds volume produced by TMS pulses at the different intensities of stimulation (i.e. lower sound volume in the subthreshold M1 versus higher sound volume in supra-threshold M1), during the sub- threshold M1 condition a second sham coil was connected to the stimulator in order to discharge at the same time of the real coil and mask any possible difference between the conditions. In addition, subjects' perception of the TMS sound was assessed with the already described TMS questionnaire. Finally,

to assure subjects' attention during the TMS-VR stimulation, they were requested to count red dots randomly appearing on the virtual index finger in the interval between two consecutive TMS pulses. The performance at this task was always very high (> 90% of correct responses).

*Similarity between virtual stimuli and TMS-evoked movements*

To tests the protocol duration and to prepare the stimuli in VR, we performed preliminary tests (six subjects, about 140 TMS stimuli per subject in total considering the whole procedure). We recorded the movements evoked by TMS pulses applied at the same intensity and coil location as in the main experiment (supra-threshold M1, 130% resting motor threshold, stimulation over the optimal position for the right FDI muscle activation, see above). Preliminary tests revealed that the movements typically evoked with that type of stimulation are a closing hand twitch, involving the whole hand, while in few cases (few trials in two subjects) an index abduction or extension occurred. Before the actual experiment, we inspected for every subject the TMS-evoked movements, to select the most appropriate stimulus in VR. TMS induced a closing hand movement in almost all subjects of our sample (i.e. in 31 of 32 subjects).

*Statistical analysis*

A repeated-measures ANOVAs were run on subjective ratings of the four components related to the embodiment (ownership, disowner- ship, location, agency) with 'embodiment components' (four levels), 'temporal congruency' (synchronous vs. asynchronous) and 'site' (experiment 1, M1 vs. vertex) or 'intensity' (experiment 2, supra-threshold vs. subthreshold) of stimulation as within-subjects factors. Similar repeated-measures ANOVAs with the within-subjects factors 'temporal congruency' and 'site' (experiment 1) or 'intensity' (experiment 2) of stimulation were performed on (i) the ratings related to the control component of the Embodiment questionnaire; (ii) on the perceived position of participants' hand reported in the proprioceptive drift task (difference between post and pre); (iii) on ratings to the four items of the TMS questionnaire with the additional within-subjects factor 'sensations' (four levels: sensations related to the hand, to the

head, to the TMS- evoked movements and to the sound clicks); (iv) on the MEPs amplitude before and after the stimulation with the supplementary within-subject factor of 'time' (two levels: pre vs. post). If the ANOVA revealed a significant interaction, we corrected for multiple comparisons using Newman-Keuls post hoc tests. The percentage of TMS-evoked movements and the MEP amplitude during the stimulation were compared in synchronous versus asynchronous condition by means of paired t-tests, Bonferroni corrected. An additional analysis about the supra-threshold M1 stimulation put- ting together data from experiment 1 and from experiment 2 has been included in Appendix S1 (see also Fig. S1 and S2).

**RESULTS**

***Experiment 1: combining VR with supra-threshold TMS over motor cortex, but not over the vertex, induces embodiment for a virtual hand***

***Embodiment questionnaire***

We found a significant interaction 'site of stimulation x temporal congruency' ($F_{1,15}$ = 5.82, P = 0.029), independently of the four components related to the Embodiment ('site of stimulation x temporal congruency x embodiment components': $F_{3,45}$ = 0.99, P = 0.40). Post hoc test revealed higher ratings in the synchronous as compared to the asynchronous condition after M1 stimulation (P = 0.005) and not after vertex stimulation (P = 0.49). As expected, no synchronous–asynchronous difference emerged in the control questions, neither in M1 nor in vertex condition (main effect 'temporal congruency': $F_{1,15}$ = 0.11, P = 0.75; 'site of stimulation x temporal congruency': $F_{1,15}$ = 0.08, P = 0.78). These data show that supra-threshold stimulation over M1 induces the illusory embodiment for the virtual hand selectively when combined with a temporally congruent visual feedback in virtual reality (see Fig. 2; means and standard errors are reported in Table S1, Supporting Information).

*TMS questionnaire*

A significant interaction 'site of stimulation x sensations' was found ($F_{3,42}$ = 19.76, $P < 0.0001$). Importantly, no differences between synchronous and asynchronous condition emerged ('site of stimulation x temporal congruency x sensations': $F_{3,42}$ =0.69, $P = 0.57$; 'site of stimulation x temporal congruency': $F_{1,14}$ = 0.08, $P = 0.77$). Post hoc test showed no difference in general TMS effects, such as the TMS-induced somatosensory sensation on the head ($P = 0.45$) and the TMS sound clicks ($P = 0.80$), reported by participants during M1 and vertex stimulation. In contrast, as expected, higher ratings to hand sensations induced by TMS ($P < 0.001$) and the perception of the TMS-evoked hand movements ($P < 0.001$) were found in M1 than in vertex stimulation (see Fig. 2; means and standard errors are reported in Table S1, Supporting Information). Altogether these data suggest that participants gave different ratings after M1 and vertex stimulation related to the hand effects evoked by the TMS (sensation and movement), but similar scores to the general TMS effects, such as the TMS-induced somatosensory sensation on the head and the TMS sound clicks. Importantly, no difference was noticed between synchronous and asynchronous stimulation, thus indicating that the subjective changes found on embodiment in synchronous supra-threshold M1 stimulation are not due to any intrinsic difference in sensation induced by synchronous and asynchronous conditions.

**Experiment 1**
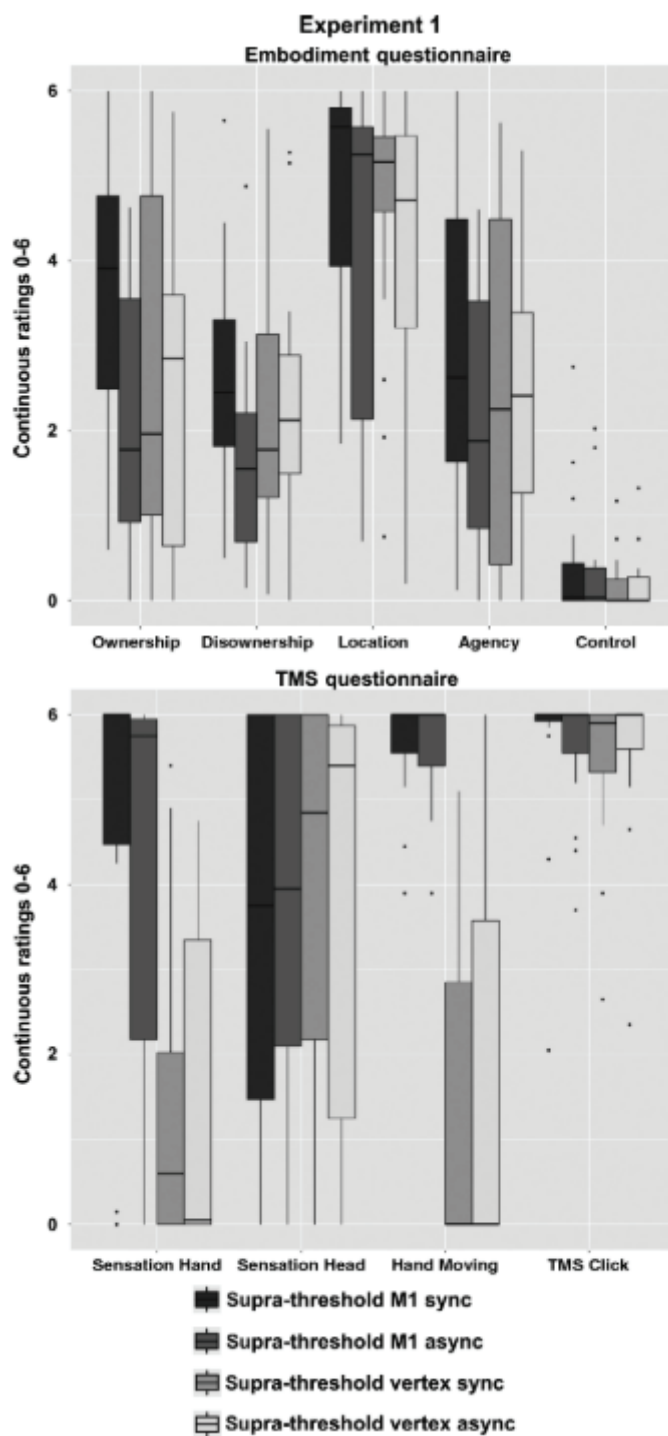**Embodiment questionnaire**
**TMS questionnaire**

Fig. 2. Experiment 1. Supra-threshold transcranial magnetic stimulation (TMS) over the M1 vs. supra-threshold TMS over the vertex. Figure shows results on subjective ratings at the Embodiment questionnaire (upper panel) in the supra-threshold M1 vs. supra-threshold vertex condition (16 subjects). 'Boxes' are based on the first and third quartiles (interquartile range, lower and upper 'hinges'), the median (line), the largest and the smallest value no further than 1.5 x the interquartile range (upper and lower whiskers), data beyond the end of the whiskers (points). While higher ratings to the 'embodiment questions' after synchronous (in the figure, sync) rather than asynchronous (async) condition were reported in supra-threshold M1, no synchronous–asynchronous difference was found in supra-threshold vertex. This suggests that no illusion was induced after vertex stimulation. No synchronous–asynchronous or M1-vertex difference emerged in the control questions. As expected, subjects' ratings on sensations induced by TMS (TMS questionnaire, lower panel) on the hand (somatosensory sensation on the hand and perception of TMS-induced movements) were different between supra-threshold M1 and supra-threshold vertex conditions, while general TMS sensations related to the somatosensory sensation on the head or the TMS sound clicks were not different between conditions.

*Drift*

A positive drift towards the virtual hand was present across all conditions (always different from zero, $P < 0.0125$, alpha set at 0.05/ 4 = 0.0125 following Bonferroni correction), both in synchronous and asynchronous conditions and this occurred in the supra-thresh- old M1 and supra-threshold vertex conditions (main effect 'temporal congruency': $F1,15 = 0.72$, $P = 0.41$; interaction 'site of stimulation x temporal congruency': $F1,15 = 0.52$, $P = 0.48$).

*MEPs and TMS-evoked movements*

As expected, no movements were evoked during TMS over the vertex, while the percentage of evoked movements mimicking the hand movements observed in virtual reality during the supra-threshold M1 was always very high in both the synchronous (mean = 92%, SE = 3.51) and asynchronous (mean = 90%, SE = 5.7) conditions [paired t-test, $t(14) = 0.75$, $P = 0.47$]. Similarly, MEP amplitudes during synchronous (mean = 2.59 mV, SE = 0.45) and asynchronous (mean = 2.85 mV, SE = 0.52) condition were comparable in supra-threshold M1 [paired t-test, $t(15) = 0.97$, $P = 0.35$], while as expected no MEPs were recorded during the supra-threshold vertex condition. Moreover, MEP amplitudes did not differ before and after supra-threshold M1 and supra-threshold vertex in both synchronous and a synchronous condition ('site of stimulation x temporal congruency x time': $F1,15 =0.061$, $P = 0.81$, all main effects $P > 0.05$) (Fig. S3).

*Experiment 2: subthreshold TMS over M1 does not induce RHI*

**Embodiment questionnaire**

We found a significant interaction 'intensity of stimulation x temporal congruency x embodiment components' ($F3,45 =3.23$, $P = 0.031$) on the ratings related to the embodiment. Thus, to understand the source of this interaction, we run four separate ANOVAs on each embodiment component with 'intensity of stimulation' and 'temporal congruency' as within-subjects factors.

Significant 2 x 2 interactions were found for the main component of ownership ($F_{1,15} = 12.19$, $P = 0.003$), as well as for agency ($F_{1,15} = 7.19$, $P = 0.017$) and location statements ($F_{1,15} = 9.73$, $P = 0.007$). For both ownership and agency components, we found significantly higher ratings in the synchronous rather than in the asynchronous condition in the supra-threshold, but not in the subthreshold stimulation (paired t-test, ownership: supra-threshold, $P = 0.0004$, subthreshold, $P = 0.37$; agency: supra-threshold, $P = 0.023$, subthreshold, $P = 0.28$). Higher scores for location were found in the asynchronous rather than in the synchronous condition after the subthreshold stimulation, but not after the supra-threshold one (supra-threshold, $P = 0.07$; subthreshold, $P = 0.01$). Only a main effect of temporal congruency with no inter- action statistically emerged on disownership, with higher score in the synchronous condition, suggesting that this effect was independent from the pattern of stimulation (main effect 'temporal congruency': $F_{1,15} = 8.27$, $P = 0.012$; 'intensity of stimulation x temporal congruency': $F_{1,15} = 0.15$, $P = 0.70$). As expected, no difference was found for the control questions in supra-threshold or subthreshold conditions (main effect 'temporal congruency': $F_{1,15} = 2.13$, $P = 0.17$; 'intensity of stimulation x temporal congruency': $F_{1,15} = 0.08$, $P = 0.79$; see Fig. 3; means and standard errors are reported in Table S2, Supporting Information). These data suggest that supra-threshold, but not subthreshold M1 stimulation, induced illusory embodiment for the virtual hand when combined with synchronous visual feedback in virtual reality.

### TMS questionnaire

A significant interaction 'intensity of stimulation x sensations' was found ($F_{3,45} = 9.59$, $P < 0.0001$). Importantly, no differences between synchronous and asynchronous condition emerged ('intensity of stimulation x temporal congruency x sensations': $F_{3,45} = 0.37$, $P = 0.78$; 'intensity of stimulation x temporal congruency': $F_{1,15} = 0.13$, $P = 0.72$). Post hoc test revealed that participants reported higher ratings in supra-threshold M1 rather than subthreshold M1 at the TMS questionnaire about TMS-induced hand sensations ($P = 0.0001$) and on the perception of the TMS-induced movements ($P = 0.0001$). In contrast, similar ratings concerning the general effect of TMS were given during supra-threshold M1 and subthreshold M1 (head sensation: $P = 0.06$;
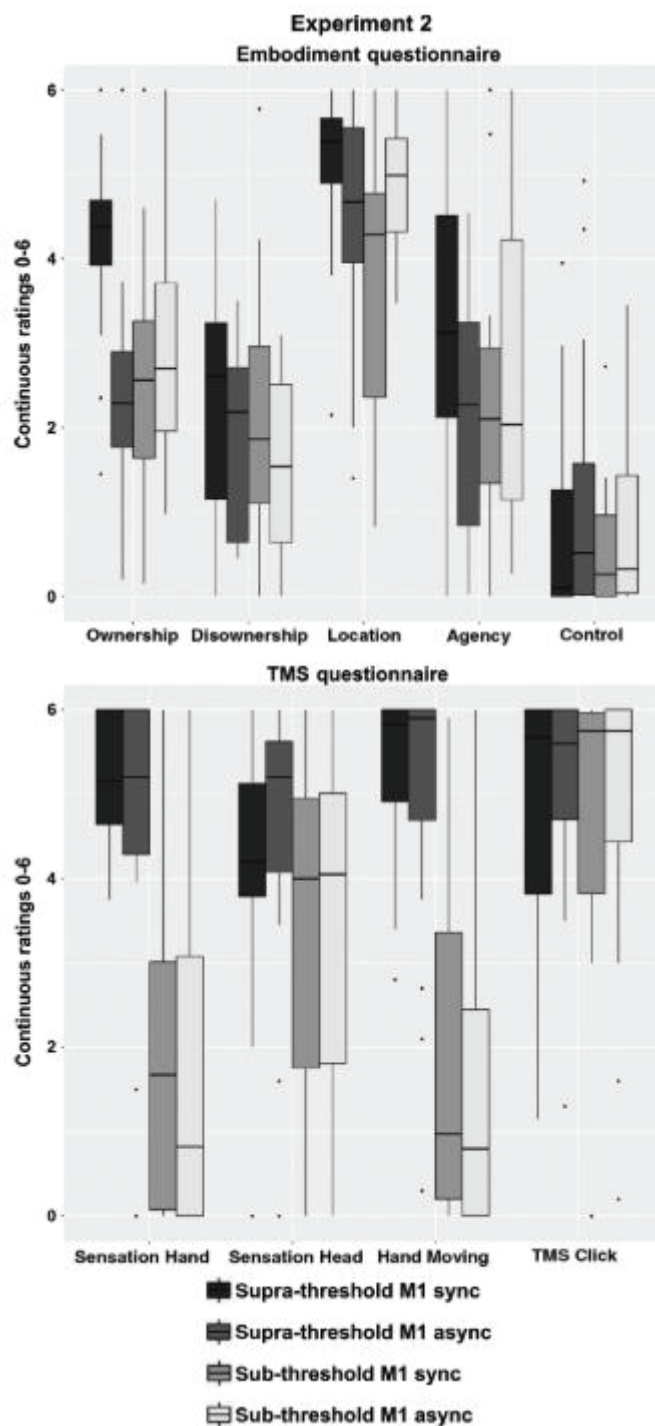
**Fig. 3.** Experiment 2. Supra-threshold vs. subthreshold transcranial magnetic stimulation (TMS) over M1. Figure shows findings related to subjective ratings (Embodiment questionnaire, upper panel) in the supra-threshold M1 vs. sub- threshold M1 condition (16 subjects). 'Boxes' are based on the first and third quartiles (interquartile range, lower and upper 'hinges'), the median (line), the largest and the smallest value no further than 1.5 x the interquartile range (upper and lower whiskers), data beyond the end of the whiskers (points). While higher ratings after synchronous (in the figure, sync) rather than asynchronous (async) condition were reported in supra-threshold M1 for the 'ownership for the virtual hand' and 'agency' component, this was not the case in subthreshold M1 condition. No synchronous–asynchronous or supra-threshold/sub-threshold stimulation difference emerged in the control questions. As expected, and similar to experiment 1, subjects' ratings on sensation induced by TMS (TMS questionnaire, lower panel) on the hand (somatosensory sensation on the hand and perception of TMS-induced movements) were different between supra-thresh- old M1 and subthreshold M1 conditions, while general TMS sensations related to somatosensory sensation on the head or the TMS sound clicks were not different between conditions.

sound clicks: $P = 0.81$). This confirms that subjects rated differently the sensations related to the hand during supra-threshold M1 and subthreshold M1 stimulation, despite similar scores reported to general effect of TMS (sensation on the head and sound clicks). Again, crucially, no synchronous–asynchronous difference emerged, thus excluding that the embodiment effects in the synchronous supra-threshold M1 condition were due to intrinsic difference related to the

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stimulation in synchronous and asynchronous conditions (see Fig. 3; mean and standard error are reported in Table S2).

*Drift*

Regardless of the conditions (synchronous, asynchronous) and the intensity of stimulation (supra-threshold M1; subthreshold M1), a positive drift towards the virtual hand was present (different from zero, Bonferroni corrected, $P < 0.0125$, alpha set at $0.05/4 = 0.0125$) (main effect 'temporal congruency': $F_{1,15} = 2.21$, $P = 0.16$; interaction 'intensity of stimulation x temporal congruency': $F_{1,15} = 0.008$, $P = 0.93$).

*MEPs and TMS-evoked movements*

As expected, movements or MEPs were evoked very rarely during subthreshold M1 stimulation (one movement of 36 delivered TMS pulses in two subjects, in synchronous and in asynchronous condition, respectively). In contrast during supra-threshold M1, the percentage of evoked movements mimicking hand movements observed in virtual reality were very high in the synchronous (mean = 99%, SE = 0.36) and asynchronous (mean = 99%, SE = 0.31) conditions [percentage of movements: paired t-test, $t(15) = 0.15$, $P = 0.89$]. The amplitude of MEPs was comparable in the synchronous (mean = 3.18 mV, SE = 0.76) and in the asynchronous (mean = 2.69 mV, SE = 0.50) conditions [paired t-test, $t(15) = 1.07$, $P = 0.30$]. Moreover, MEP amplitude was equal before and after the supra-threshold M1 or subthreshold M1 stimulation in both synchronous and asynchronous conditions ('intensity of stimulation x temporal congruency x pre-post': $F_{1,15} = 4.12$, $P = 0.06$, all main effects $P > 0.05$) (Fig. S3).

**DISCUSSION**

The present findings demonstrate that a TMS supra-threshold artificial signal over M1 triggering hand twitches, when combined with time-locked visual feedback, is able to induce embodiment

for a virtual hand in healthy participants. This novel form of RHI was driven by the temporal congruency between the TMS pulse activating the hand corticospinal tract and VR visual feedback and did not occur if TMS was administered outside M1 (over the vertex) or using an intensity of M1 stimulation below the motor threshold (subthreshold). This is the first report of the experimental induction of illusory embodiment using non-invasive brain stimulation.

*Coupling TMS with VR to induce embodiment*

While both VR and TMS have been previously applied in RHI protocols, this is the first study in which TMS is fully integrated with VR to induce embodiment. Several VR techniques have been employed to animate virtual hands based on tracked participants' active movements (Sanchez-Vives et al., 2010; Yuan & Steed, 2010; Kilteni et al., 2012). Previous studies used TMS to record the MEP amplitude as marker of ownership (Della Gatta et al., 2016) and of agency (Weiss et al., 2014) or to interfere with circumscribed cortical areas to investigate their role in RHI-related processes (e.g. right temporo-parietal junction, (Tsakiris et al., 2008); inferior posterior parietal lobule, (Kammers et al., 2009); left extrastriate body area, (Wold et al., 2014). Here, we directly coupled visual hand feedback via a fully controlled VR system with the TMS-induced activation of the hand corticospinal tract to induce the RHI. The pre- sent effects confirm the possibility of inducing embodiment for a fake hand via brain stimulation and are in line with a recent proof- of-concept clinical study whereby the RHI was induced using synchronized cues between invasive S1 stimulation (via implanted subdural electrodes) and visual feedback provided manually to a physical fake hand in two epilepsy patients undergoing pre-surgical epilepsy monitoring (Collins et al., 2017). Our approach further extends those findings by proposing a novel, non-invasive, protocol that is applicable in healthy subjects and different patient populations (i.e. amputation, chronic pain, stroke), limits experimenter and subject biases (allowing double-blind protocols), and enables fine- grained control of a large number of experimental factors (various stimulation intensities and sites, different spatiotemporal couplings with VR feedback, and more complex VR scenarios).

*Inducing illusory embodiment by triggering artificial hand twitches through TMS*

Previous work induced illusory feelings of embodiment for a virtual or fake hand by linking vision of the artificial limb with congruent bodily inputs of a real arm, using tactile and proprioceptive or motor information (Tsakiris, 2010; Blanke et al., 2015; Kilteni et al., 2015) or even sensory expectation (Ferri et al., 2013); here, we induced embodiment by artificially activating the hand corticospinal representation with TMS, without providing any direct cues on the physical hand. Moreover, the present protocol has several differences compared to earlier visuo-motor version of RHI involving active and passive movements (Kalckert & Ehrsson, 2012, 2014; Riemer et al., 2013, 2014; Kilteni et al., 2015). First, the brief and involuntary TMS-induced movements are weaker than prolonged, repetitive and large-amplitude active movements and are smaller in amplitude and typically shorter than a passive movement (due to short-lasting activity in corticospinal pathways of 10/15 ms (Gentner & Classen, 2006). Second, active movements are generally accompanied by voluntary motor commands, by motor planning and by anticipation (e.g. Wolpert & Ghahramani, 2000). As these latter aspects were absent (or at least strongly diminished), the present TMS-RHI data show that these features characterizing active physical movements are not necessary to induce hand embodiment. Nevertheless, it is important to acknowledge that the afferent information evoked by TMS-induced movements could be very similar to those evoked during passive movements. These inputs could have a role in triggering the illusion when combined with congruent visual feedback of a moving virtual hand (see below the comparison between supra- and sub threshold condition).

*Main features of the TMS-VR induced RHI*

The present supra-threshold M1 stimulation when combined with temporally congruent virtual feedback induced an illusory feeling of ownership towards the virtual hand (experiment 1 and 2). Illusory embodiment involved all main components of hand embodiment as described for the RHI induced by manual stimulation protocols (Longo et al., 2008) (ownership, agency, location, disownership for the physical hand) when a larger sample size is considered (see the analysis in

32 subjects, Appendix S1 and Fig. S1). The lack of any effects on control questions excludes compliance or suggestibility confounds. Crucially, the RHI occurred only when supra-threshold TMS pulses over M1 were temporally linked with a virtual hand movement (synchronous), but not when a temporal delay was inserted between the TMS pulse and the movement of the hand shown on the head-mounted display (asynchronous). This confirms previous RHI findings about the critical role of temporal congruency between the visual fake hand stimulus and the movement of the participant's hand (Tsakiris et al., 2006; Riemer et al., 2013, 2014) or tactile (Tsakiris, 2010) or cardiac (Suzuki et al., 2013) cues. Our data exclude that the RHI is caused by any difference in the perception of the TMS across these conditions, as indicated by the TMS questionnaire. Importantly, we also found no differences in TMS-evoked movements or MEPs amplitude between synchronous and asynchronous supra-threshold M1 conditions (see Fig. S3). Thus, subjective perceptions (TMS questionnaire), behavioural (TMS-evoked movements) and neurophysiological (MEPs) data comprehensively argue against a role of the movement per se in explaining the present embodiment effects, but confirm the role of temporal congruency. Moreover, our data show that the experimental induction of illusory embodiment is anatomically specific, in that it occurred only when TMS pulses were administered over M1, only in the synchronous condition, and not when TMS was applied over the vertex, ruling out the possibility that any generic effect of TMS (such as the sound clicks or any sensation on the scalp induced by the stimulation) and the temporal congruency between TMS and visual feedback could induce the illusion. In addition, no illusory embodiment was elicited by applying a subthreshold TMS pulse over M1 that did not evoke any sensory or movement effects at the hand (no MEPs, no muscle twitches), further under- lining the selectivity of the obtained embodiment effects for the activation of the hand corticospinal representation. This shows that the illusory ownership in the present study was only triggered if hand twitches are evoked by the TMS and are combined with congruent visual feedback of a virtual hand moving accordingly to the evoked movements (synchronous condition). Alternatively, one could hypothesize that the subthreshold TMS intensity was not sufficient to activate M1 in order to trigger the illusion (in line with studies showing differences in M1 activation depending on TMS intensity, e.g. Shitara et al., 2011). At present, we do not exclude the possibility that slightly higher subthreshold stimulation (e.g.

around the resting motor threshold) could induce illusory ownership even without inducing muscle twitches, an issue that should be investigated in future studies. Finally, the same proprioceptive drift was present in all conditions as compared to baseline (compatible with condition-independent high ratings of the 'location' item; Embodiment questionnaire), although the virtual hand was presented as displaced with respect to the perceived position of participants' real hand as in earlier RHI studies (e.g. Tsakiris, 2010). Previous findings on the drift after RHI based on active and passive movements, however, reported condition-specific drift (Tsakiris et al., 2006; Kammers et al., 2010; San- chez-Vives et al., 2010; Riemer et al., 2013; Kalckert & Ehrsson, 2014). We argue that this lack of synchronous–asynchronous difference of the drift might be related to strong visual capture of proprioception due to the high level of immersion of the VR system, and in line with other studies, it was independent of the pattern of stimulation (Kilteni et al., 2015) and the ownership modulation (Longo et al., 2008; Serino et al., 2013).

**CONCLUSION**

The state of the motor system and a limb's current state of embodiment are mutually tied (Miller & Farné, 2016). On the one side, sensorimotor information together with available multisensory inputs is crucial to enable the sense of body ownership and agency (De Vignemont, 2011; Blanke et al., 2015; Bolognini et al., 2015; Kilteni et al., 2015). On the other side, embodiment modifications have been shown to affect motor excitability of the hand at rest (della Gatta et al., 2016) or during action observation (Schütz-Bosbach et al., 2006, 2009). Here, we further extend this concept by showing that is possible to induce embodiment for a virtual hand by activating the corticospinal sensorimotor system via TMS, without motor- related components of volition, planning or anticipation. The present form of non-invasive brain stimulation over hand M1 coupled to VR can replace the application of tactile cues on the hand's skin (as typically used in the RHI), minimizes associated movements (only involuntary, small-amplitude artificial movements), and fully automatizes the coupling between brain stimulation and visual feedback (mediated by the TMS-coupled VR system). We argue that illusory embodiment in the present experiments is due to neuro-visual integration between TMS-induced M1 activation (and

connections with sensory cortex, e.g. Jacobs et al., 2012) and hand twitches with visual VR feedback. Finally, our protocol may be clinically relevant for the evaluation and treatment of motor and embodiment disorders (Berti et al., 2005; Schwoebel & Coslett, 2005; Vallar & Ronchi, 2009; Bartolo et al., 2014; Bassolino et al., 2015; Bolognini et al., 2015), avoiding direct application of bodily cues and movement that may be perceived as painful and limit therapeutic options (i.e. allodynia in complex regional pain syndrome).

**Supplementary Information. Additional analysis: supra-threshold M1 in experiment 1 and 2**

We analyzed the data of the 32 subjects who were exposed to the TMS-VR induced RHI by applying supra-threshold TMS stimulation (130% of resting motor threshold) over M1 in experiment 1 (16 subjects) and 2 (16 subjects). We compared the effects induced by supra-threshold stimulation when the movement of the virtual hand was temporally congruent (synchronous) or delayed (asynchronous) with respect to the TMS pulse. We applied the same statistical analysis used in experiment 1 and 2, with the exception of the factor "intensity" or "site" of stimulation that was absent here. Embodiment-questionnaire. We found a significant interaction "temporal congruency X embodiment components" ($F_{(3, 93)}=3.64$, $p=0.016$) on the ratings related to the embodiment. Post hoc tests revealed significantly higher ratings in the synchronous versus asynchronous conditions for all embodiment components (ownership: $p=0.0001$, disownership: $p=0.0496$, location: $p=0.008$, agency: $p=0.001$). The interaction was driven by the fact that the ratings among the 4 components were significantly different in the synchronous conditions (ownership > disownership: $p=0.0001$; ownership > agency: $p=0.014$; but ownership < location: $p=0.003$) while in the asynchronous condition the ratings were similarly low in the ownership, disownership and agency components (ownership versus disownership: $p=0.11$; ownership versus agency: $p=0.45$), with the exception of higher score in the location rather than in ownership (ownership < location: $p=0.001$). As expected, responses to the control questions did not differ across conditions (paired t test, $t(31)=-0.68$, $p=0.50$). In line with results of experiment 1 and 2, this analysis suggests that supra-threshold M1 stimulation activating the hand corticospinal representation induces embodiment for the virtual hand if combined with

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synchronous visual feedback in virtual reality (Supplementary Figure 1). TMS-q. No differences were found between synchronous and asynchronous conditions in subjects' ratings to sensations induced by TMS assessed through the TMS-questionnaire ("temporal congruency": $F_{(1, 30)}=0.20$, $p=0.66$; "temporal congruency X questions": $F_{(3, 90)}=2.34$, $p=0.08$). This suggests that the different effects found on embodiment in the synchronous and asynchronous conditions are not due to any intrinsic difference in the stimulation conditions (Supplementary Figure 1). Drift. A positive drift towards the virtual hand position versus baseline was present in all conditions (synchronous: mean=0.68, SE=0.11, different from zero, $p>0.0001$; asynchronous: mean=0.62, SE=0.12, different from zero, $p>0.0001$), without any significant difference between synchronous and asynchronous (paired t-tests, $t_{(31)}=0.41$, $p=0.69$). MEPs and TMS-evoked movements. During stimulation, the percentage of TMS-evoked movements was high and, importantly, not different between synchronous (mean=96%, SE= 1.76) and asynchronous (mean=95%, SE=2.54) conditions (paired t-test, $t_{(30)}=0.72$, $p=0.47$). Similarly, during stimulation, the MEP amplitudes did not differ either (synchronous: mean=2.88 mV, SE=0.44; asynchronous: mean=2.77 mV, SE=0.36; paired t-test, $t_{(31)}=0.41$, $p=0.68$). Moreover, MEP amplitudes was equal before and after the RHI stimulation in both synchronous and asynchronous conditions, excluding a modulation of the overall cortical excitability due to the illusion or the course of the experiment (repeated measures ANOVA: interaction "temporal congruency X time": $F_{(1, 31)}=0.074$, $p=0.79$) (Supplementary Figure 2).

**SUPPLEMENTARY FIGURES AND TABLES**



**Supplementary Figure 1: supra-threshold M1**
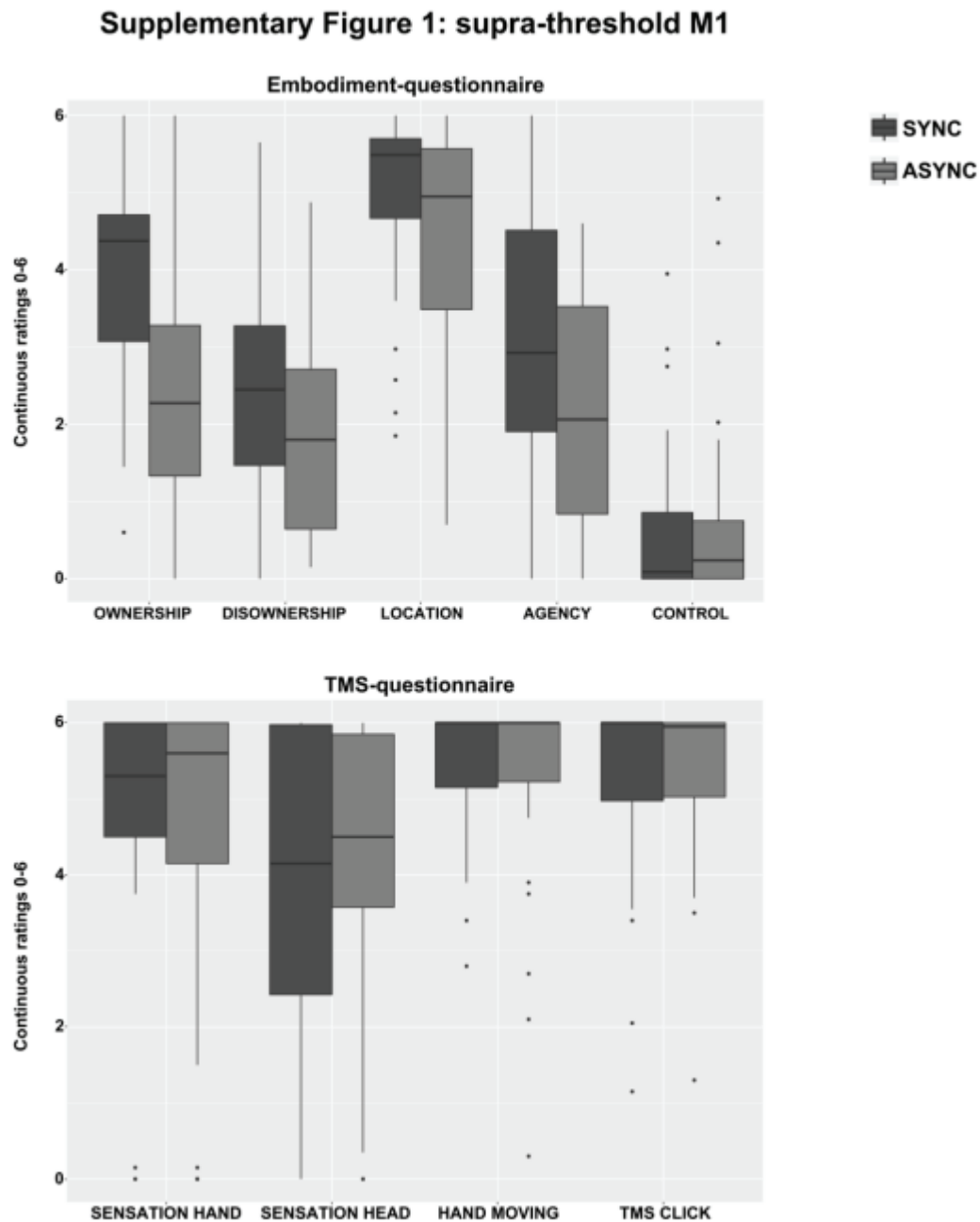
**Supplementary Figure 1.** Main experiment. Supra-threshold TMS over motor cortex (supra-threshold M1) time-locked with virtual reality feedback induces embodiment for a virtual hand Figure shows results on subjective ratings on Embodiment-questionnaire (upper panel) and TMS-questionnaire (lower panel). "Boxes" are based on the first and third quartiles (inter-quartile range, lower and upper "hinges"), the median (line), the largest and the smallest value no further than 1.5 * the interquartile range (upper and lower whiskers), data beyond the end of the whiskers (points). Higher ratings (32 subjects) after synchronous (sync, dark grey) than (async, light grey) condition were reported for all embodiment

statements, but not for the control questions (upper panel). Subjects' ratings on any sensation induced by TMS were comparable in synchronous and asynchronous conditions, excluding any generic effect of TMS between conditions (lower panel).



**Supplementary Figure 2: supra-threshold M1 MEPs**
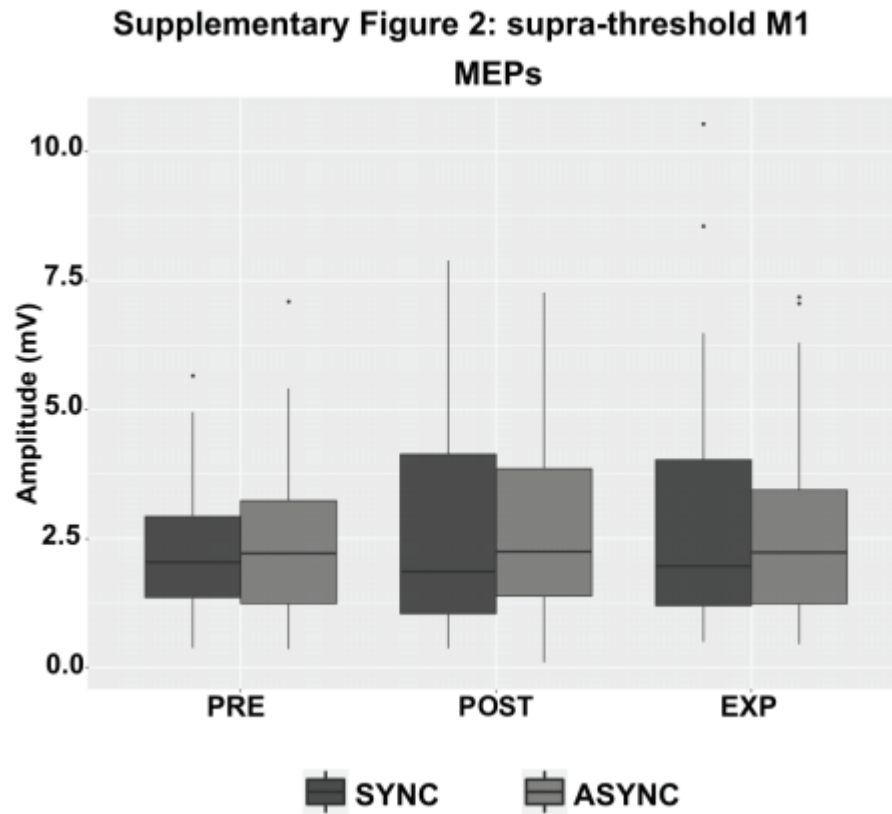
**Supplementary Figure 2.** Motor evoked potentials (MEPs) did not differ in synchronous or asynchronous condition "Boxes" are based on the first and third quartiles (inter-quartile range, lower and upper "hinges"), the median (line), the largest and the smallest value no further than 1.5 * the inter-quartile range (upper and lower whiskers), data beyond the end of the whiskers (points). The amplitude of MEPs (32 subjects) was comparable in the synchronous (sync, dark grey) and asynchronous (async, light grey) condition when recorded before (PRE) and after (POST) TMS-VR stimulation (supra-threshold M1), as well as during the stimulation (EXP).

**Supplementary Figure 3: MEPs**

**Experiment 1**
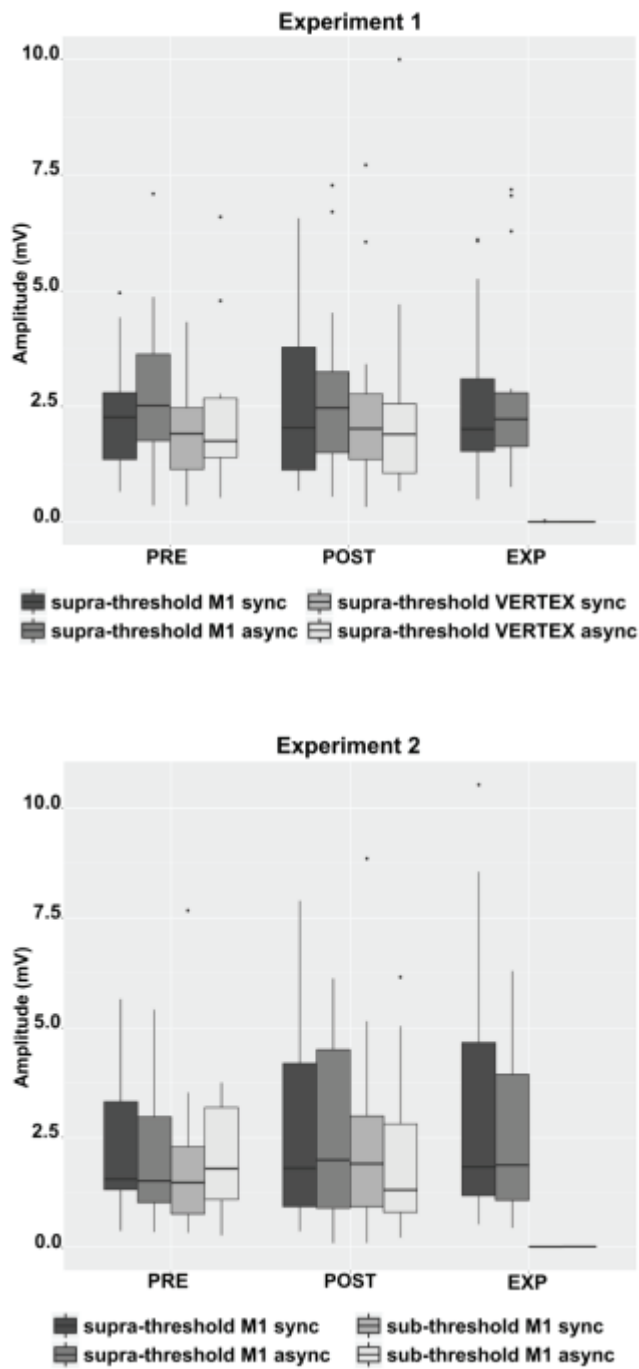
**Experiment 2**

**Supplementary Figure 3**. Motor evoked potentials (MEPs) in experiment 1 and 2 Figure represents the amplitude of motor evoked potentials (MEPs) before (PRE), during (EXP) and after (POST) the TMS-VR stimulation in experiment 1 (supra-threshold M1 versus supra-threshold vertex, upper panel) and in experiment 2 (supra-threshold M1 versus sub-threshold M1, lower panel) (16 subjects). "Boxes" are based on the first and third quartiles (inter-quartile range, lower and upper "hinges"), the median (line), the largest and the smallest value no further than 1.5 * the inter-quartile range (upper and lower whiskers), data beyond the end of the whiskers (points). Differently from supra-threshold M1 stimulation, as expected, MEPs were absent during TMS-VR stimulation in supra-threshold vertex and in sub-threshold M1.

## SUPPLEMENTARY TABLE 1: Experiment 1

| Embodiment-questionnaire | OWNERSHIP | DISOWNERSHIP | LOCATION | AGENCY | CONTROL |
|---|---|---|---|---|---|
| supra-threshold M1 SYNC | 3.72 ± 0.93 | 2.60 ± 0.65 | 4.83 ± 1.21 | 2.90 ± 0.73 | 0.45 ± 0.11 |
| supra-threshold M1 ASYNC | 2.13 ± 0.53 | 1.70 ± 0.43 | 4.11 ± 1.03 | 2.06 ± 0.52 | 0.37 ± 0.09 |
| supra-threshold VERTEX SYNC | 2.81 ± 0.70 | 2.30 ± 0.57 | 4.60 ± 1.15 | 2.49 ± 0.62 | 0.20 ± 0.05 |
| supra-threshold VERTEX ASYNC | 2.51 ± 0.63 | 2.34 ± 0.59 | 4.15 ± 1.04 | 2.51 ± 0.63 | 0.19 ± 0.05 |

| TMS-questionnaire | HAND | HEAD | MOVEMENT | CLICK |
|---|---|---|---|---|
| supra-threshold M1 SYNC | 4.47 ± 0.61 | 3.40 ± 0.61 | 5.63 ± 0.17 | 5.60 ± 0.28 |
| supra-threshold M1 ASYNC | 4.13 ± 0.67 | 3.67 ± 0.57 | 5.63 ± 0.16 | 5.58 ± 0.20 |
| supra-threshold VERTEX SYNC | 1.47 ± 0.52 | 3.97 ± 0.56 | 1.40 ± 0.48 | 5.38 ± 0.25 |
| supra-threshold VERTEX ASYNC | 1.48 ± 0.53 | 3.85 ± 0.65 | 1.58 ± 0.58 | 5.55 ± 0.25 |

**Supplementary Table 1. Experiment 1 (supra-threshold M1 versus supra threshold vertex)**

Means ± standard error of the ratings reported at the Embodiment-questionnaire (upper part) and TMS-questionnaire (lower part) in the experiment 1.

## SUPPLEMENTARY TABLE 2: Experiment 2

| Embodiment-questionnaire | OWNERSHIP | DISOWNERSHIP | LOCATION | AGENCY | CONTROL |
|---|---|---|---|---|---|
| supra-threshold M1 SYNC | 4.16 ± 0.28 | 2.29 ± 0.38 | 5.08 ± 0.24 | 3.21 ± 0.44 | 0.80 ± 0.31 |
| supra-threshold M1 ASYNC | 2.49 ± 0.32 | 1.91 ± 0.26 | 4.49 ± 0.34 | 2.18 ± 0.39 | 1.17 ± 0.40 |
| sub-threshold M1 SYNC | 2.57 ± 0.39 | 2.16 ± 0.37 | 3.84 ± 0.41 | 2.31 ± 0.41 | 0.59 ± 0.19 |
| sub-threshold M1 ASYNC | 2.90 ± 0.33 | 1.58 ± 0.25 | 4.90 ± 0.18 | 2.62 ± 0.48 | 0.87 ± 0.27 |

| TMS-questionnaire | HAND | HEAD | MOVEMENT | CLICK |
|---|---|---|---|---|
| supra-threshold M1 SYNC | 5.16 ± 0.21 | 4.22 ± 0.39 | 5.27 ± 0.25 | 4.92 ± 0.35 |
| supra-threshold M1 ASYNC | 4.74 ± 0.44 | 4.55 ± 0.42 | 4.92 ± 0.44 | 5.06 ± 0.32 |
| sub-threshold M1 SYNC | 1.88 ± 0.50 | 3.51 ± 0.58 | 1.76 ± 0.48 | 4.52 ± 0.51 |
| sub-threshold M1 ASYNC | 1.79 ± 0.53 | 3.40 ± 0.52 | 1.58 ± 0.47 | 4.83 ± 0.45 |

figure not included in the published manuscript:
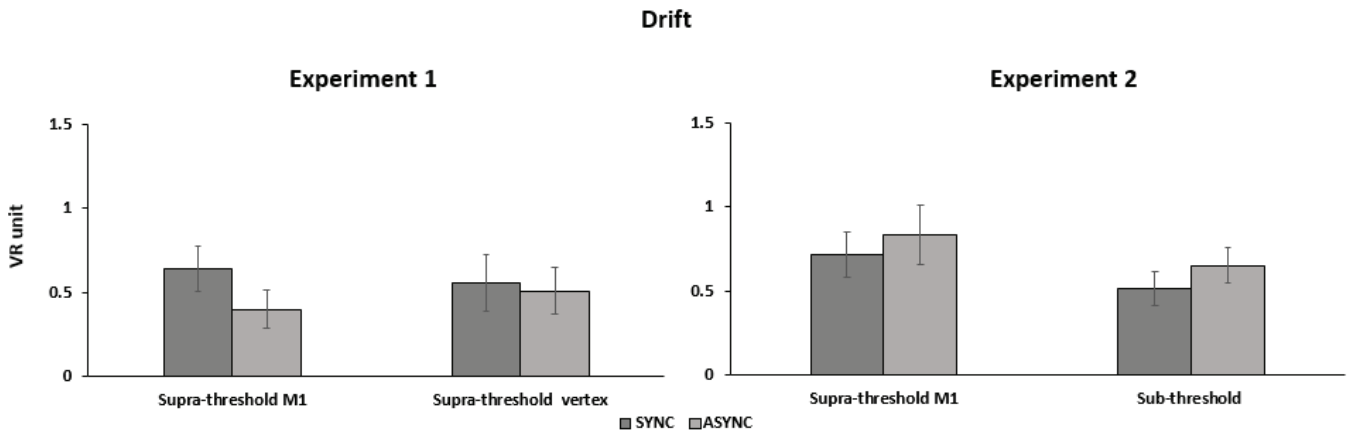
**Drift**



The present figure represents the proprioceptive drift measured in virtual reality.

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## 2.2. Study 2: Hand perceptions induced by single pulse transcranial magnetic stimulation over the primary motor cortex

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Personal contribution: study design, paper writing, data collection, data analysis.

**INTRODUCTION**

Single-pulse transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) has been extensively used to study the functional organization and the plasticity of the corticospinal motor system [1–5]. When single pulse TMS is applied over primary motor cortex, different corticospinal volleys are elicited and can be measured [6]. At the cortical level, a first descending volley is called direct wave and it is generated by fast-conducting pyramidal tract neurons and is followed by later volleys (indirect waves) mainly reflecting the transynaptic activation of pyramidal tract neurons. Spinal cord mechanisms are also recruited, involving spinal motor neurons. This cascade of events is classically evaluated at its bottom end by using motor-evoked potentials (MEPs) recorded through electromyography from contralateral muscles of upper or lower limbs.

Based on many studies, broadly accepted guidelines have been established [7,8]. Thus, MEPs are defined as muscular twitches characterized by liminal electromyographic (EMG) activity (peak-to-peak amplitude of 0.05 mV) and specific latencies depending on the addressed muscles, leading, for instance, to shorter latencies for the hand than for leg muscles [7]. MEP amplitude can be modulated by motor [9–11], sensory and cognitive processes. For instance, action observation/motor imagery [12,13] or stimuli presented within the peripersonal space [14–16] has been shown to increase MEP amplitude, whereas immobilization [17,18] or peripheral nerve block [19] decreases MEP amplitude. Thus, MEP amplitude is a crucial parameter to measure corticospinal excitability in a variety of experimental protocols (e.g. motor mapping, recruitment curves, [4,20,21,22]), with a large range of applications in clinical and experimental studies [6,23,24].

In addition, single pulse TMS over M1 has also consistently been shown to induce rapid involuntary movements in the contralateral limb (i.e. TMS-evoked movements). TMS-evoked movements have been typically measured by attaching accelerometers to the fingers and hand [10,16] or by using glove-embedded sensors [25,26]. These are smaller in amplitude and typically shorter than a passive movement [25]. Next to MEPs, TMS-evoked movements have also been used to study the functional organization of the motor system, for example after intensive motor training [11] or during action observation[27].

In the TMS field, specific parameters have been identified to quantify MEPs and TMS-evoked movements and facilitate the comparison among studies. For instance, the "resting motor threshold" (rMT) indicates the minimum intensity to elicit an MEP in a target muscle in half of the administered trials for a given participant at rest [7,28]. Similarly, a threshold value related to the amplitude of the recorded acceleration has also been proposed to discern actual TMS-evoked movements of the hand from signal noise (e.g. 0.09 $m/s^2$ in Classen et al., 1998 [10]).

Moreover, TMS-induced activation of the corticospinal motor tract may also elicit hand sensations (here called TMS-evoked hand perceptions), previously reported in seminal studies as sensations like "paraesthesias", "tingling", "kinesthesia" or "touch" [29–31].

However, while MEPs and TMS-evoked movements have been widely studied, leading to accepted guidelines, TMS-evoked hand perceptions were mostly neglected apart from few studies approaching this topic in amputee patients [32–34] or in healthy participants with protocols based on one specific intensity of stimulation [35,36], on anesthetic block [30,37–39], or on repetitive TMS (e.g. [37]). Systematically measuring TMS-evoked hand perceptions in healthy participants under standard conditions would be important to quantify the physiology and variety of TMS effects and investigate whether such subjective sensations following TMS over M1 lead to reliable responses.

Here we specifically investigate the occurrence of TMS-evoked hand perceptions in healthy participants after single pulse TMS administered over the dominant hand area in M1 and at different stimulation intensities. With respect to previous studies [29–31,37], we adopted a standardized protocol and a bigger sample size allowing to apply robust statistical approaches to investigate the relationship between the subjective evoked hand perceptions and objective

outcomes of the TMS such as MEPs and TMS-evoked movements. We expected that M1 stimulation, at intensities that are higher than those evoking MEPs and movements, would also induce TMS-evoked hand perceptions. Moreover, we aim at investigating the exact relation between MEPs, movements, and hand perceptions. For this, we used two different approaches. First, we compared the number of MEPs, movements, and hand perceptions evoked at different intensities of M1 stimulation and, secondly, we determined the intensity necessary to elicit each response in at least half of the administered trials (absolute thresholds and logistic curve fitting). Finally, we determined the minimal amplitude of MEPs and the minimal acceleration of TMS-evoked movements sufficient to elicit TMS-evoked hand perceptions.

## Methods and materials

### *Subjects*

23 subjects took part in the study (mean age 27.1 ± 3.2 years, 13 females). All of them were right-handed, as determined by the Flinders Handedness survey [32]. No one showed any contraindication to TMS [33,34]. Participants were naive to the purpose of the study and participated after giving an informed consent. The study was conducted with the approval of the local ethics committee (PB_2016-02541, CCVEM 017/14). In a subgroup of subjects (final 7 participants out of the total included 23 subjects), we asked participants to specify if the reported TMS-evoked hand perception corresponded mainly to kinesthetic, somatosensory or mixed sensations (see Supplementary Materials, Figure 1) and we further recorded TMS-evoked movements by a second accelerometer placed on the index finger (see below and Supplementary Materials, Figure 2).

### TMS and recording procedure

Subjects were seated in a chair (Brainsight, Rogue Research) with their arms resting in a prone position on a table (elbow flexion of about 90 degrees). TMS was delivered through a figure-eight coil (wing diameter: 70 mm) connected to a single Magstim monophasic stimulator (Magstim 200$^2$, Magstim Co., Whitland, UK) as described previously [35]. The coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle to the sagittal plane

inducing a posteroanterior current in the brain [36,37]. In order to determine the optimal position for activation of the right First Dorsal Interosseus (FDI) muscle (hotspot), the coil was initially positioned 5 cm lateral and 1 cm anterior to the vertex over the left hemisphere [7]. Then, TMS pulses slightly above threshold intensity levels (45% of the maximal stimulator output, MSO) were applied by moving the coil in 0.5 cm steps around this initial estimate (around 5 pulses for each stimulated point). The hotspot was defined as the point over the scalp from which the largest and more stable MEPs were observed. The position of the hotspot was marked on the scalp with a pen and was carefully checked by the experiment to ensure the correct coil placement throughout the experiment. Resting motor threshold (rMT) of FDI muscle was determined according to standard procedure by using the software based 'adaptive method' developed by Awiszus et al. 2003 (TMS Motor Threshold Assessment Tool, http://www.clinicalresearcher.org/software.htm)[7].

MEPs were recorded by means of a surface EMG system through wireless electrodes positioned on the FDI in a tendon-belly configuration on both hands. EMG signals were amplified and bandpass filtered (1Hz-1kHz) by a Noraxon DTS amplifier (Velamed, GmbH, Köln, Germany). The signals were sampled at 3000 Hz, digitized using a laboratory interface (Power1401; Cambridge Electronics Design CED), and stored on a personal computer for display and later off-line data analysis (Signal and Matlab software). Each recording epoch lasted 1500 ms, from -300 ms before to 1200 ms after the TMS pulse. Trials with EMG background activity (> 0.05 mV) preceding the TMS pulse of 100 ms in the stimulated or the non-stimulated hand were excluded from the present analysis in order to avoid possible biases ascribable to uncontrolled MEPs facilitation [23,38,39].

In addition to the EMG data, we also recorded movements evoked by the TMS pulse (TMS-evoked movements) through a 3-dimensional accelerometer (Noraxon Sensors DTS 3D Accelerometer) fixed over the middle finger knuckle (but see the comparison with a second accelerometer placed on the index finger, Figure 2, Supplementary results 2). Data were acquired for the 3 separate axes (x, y, z) at the same sampling frequency of the EMG, were filtered (0.4 Hz to 100 Hz) and analyzed by a custom-made software written in MATLAB (MATLAB R2016b), following methods already proposed in previous works [10,16,35]. The acceleration modulus was first computed for

a 200 ms window starting from TMS delivery. We then calculated the acceleration onset as the time when 5% of the peak acceleration was detected. Trials were included in the analysis if peak acceleration appeared between 20 and 55 ms after the TMS pulse [40] and its amplitude was equal to or higher than 0.09 m/s$^2$ in one axis [10].

**Experimental design**

Participants were instructed to keep their right hand still and as relaxed as possible, palm down on the table. A bandage around the wrist assured the contact between subjects' arm and the table. The position of the hand was visually checked by the co-experimenter for the entire duration of the stimulation. To standardize what participants observed during the stimulation, subjects wore a head-mounted display (Oculus rift DK1) and observed a virtual scene that consisted of a table in an otherwise empty room.

The four blocks of stimulation were separated by a 1-minute break. In each block, single pulses were delivered over the left M1 hand area at 5 different intensities of stimulation in a pseudorandom sequence (20 pulses each intensity, a total of 100 pulses split into 4 blocks, each including 5 pulses per intensity) to avoid as much as possible hysteresis effects [41]. Five intensities of stimulation were used: 90%, 100%, 105%, 110% and 130% of the individual rMT (in % of maximum stimulator output). By definition, the absolute threshold for MEPs (MEPs present in half of the trials) thus corresponds to the intensity of 100% of rMT. The interval between two consecutive pulses was of a minimum of 10 seconds (range 10-12 seconds), to ensure no changes in motor cortex excitability [42,43].To quantify the TMS-evoked hand perceptions elicited at the right hand, after each pulse, subjects were instructed to report if they had perceived "any kinesthetic or somatosensory perception including (but not limited to) contractions, movements, changes in hand position, tingling sensation, or any other perceptions at the right hand" with a yes/no answer. We chose to include any type of sensation because we wanted to avoid an underestimation of the phenomenon by just focusing on one specific sensation or to introduce any bias due to possible different perceptions evoked at different intensities of stimulation (see Supplementary results 1).

**Data analysis**

We separately calculated the percentage of trials in which a TMS pulse evoked (i) MEPs (peak-to-peak amplitude higher than 0.05 mV, e.g. Groppa et al., 2012 [7]; P. Rossini et al., 2015 [8]); (ii) a TMS-evoked movement (peak acceleration between 20 and 55 ms after the TMS pulse, e.g. Finisguerra et al., 2015; Bassolino et al., 2018) with an amplitude equal to or higher than 0.09 $m/s^2$ [10]; (iii) a TMS-evoked hand perception (i.e. "yes" response) reported by the subject regarding a kinesthetic or somatosensory perception evoked by the TMS on the hand when a MEP was present.

The occurrence of the TMS-evoked responses (MEPs, movements or hand perceptions) was calculated as a percentage of valid trials, i.e. all administered pulses (i.e. 100) after the rejection of trials with EMG background activity higher than 0.05mV (3.1% considering the all administered stimuli among all participants). We did not include in the calculation of the TMS-evoked perceptions those trials in which participants answered "yes" but no MEPs were present (i.e. false positives, 3.4% of the total stimuli, less than 1.5% in each intensity of stimulation).

First, we compared the number of responses evoked at the different intensities of stimulation for MEPs, movements or hand perceptions expressed as a percentage of the total valid trials through a generalized linear mixed model with a logit link function on the occurrence of the evoked responses with "intensity of stimulation" (5 levels) and "type of responses" (3 levels) as within-in subject factors. This approach has been already demonstrated to be effective to treat proportional data, by overcoming the limitations of the ANOVA (e.g. [44]). To correct for multiple comparisons, we used Tukey post hoc test. Then, we determined an "absolute threshold", that is the intensity of stimulation (in % of maximum stimulator output with respect to the individual resting motor threshold) necessary to evoke each response in half of the trials (50%, chance level) by comparing the percentage of MEPs, TMS-evoked movements, and TMS-evoked hand perceptions averaged among all subjects at each stimulation intensity with respect to the chance level (one sample Wilcoxon test, Bonferroni corrected, the α value was set at 0.05 divided by the 5, i.e. five intensities of stimulation, the response was considered not different from the chance level if $p > 0.05$ ). See figure 3 for further explanation.

Second, to further analyze these aspects and compare the absolute threshold among the three TMS-evoked responses, we investigated the relation between MEPs, TMS-evoked movements, and TMS-evoked hand perceptions, by fitting logistic curves (maximum likelihood method) to each TMS-evoked response as a function of the used stimulation intensities (expressed as percentage of individual rMT). For each curve, we computed the central point defined as the point where the function crossed 50% (half of the trials, chance level); in other words, it represents the point (that is the intensity of stimulation with respect to individual rMT) where the presence or absence of the specific TMS-evoked responses was equally likely. Furthermore, we computed the "semi-interquartile range" (as one half the difference between the 75th percentile and the 25th percentile) that can be interpreted as the minimum increase of the stimulation intensity (expressed as percentage of individual rMT) that makes a response detectable in half of the trials, i.e. a stimulation intensity sufficient to leap from responses rarely evoked (25% of the total administered pulses) to responses evoked at chance level (50%). Specifically, it can also be interpreted as an index of "detectability" of the three TMS-evoked responses. The present "central point" and "semi-interquartile range" correspond respectively to the point of subjective equality (PSE) and just noticeable difference (JND) of a putative psychometric function (a term not applicable here for MEPs and TMS-evoked movement because these are neurophysiological and not subjective responses). According to the results of the Shapiro-Wilk test used to check for normality, individual central point and "semi-interquartile range" were compared by means of a one-way ANOVA (main factor: TMS-evoked responses, 3 levels) or by applying the equivalent non-parametric test (Friedman test). Post hoc test was corrected for multiple comparisons (Tukey correction or Wilcoxon test Bonferroni corrected).

Finally, to explore the relationship between TMS-evoked hand perceptions and the MEP amplitude, we conducted a further analysis by fitting a psychometric curve (maximum likelihood method) for the percentage of TMS-evoked hand perceptions with respect to the MEP amplitude. This procedure enables us to estimate the MEPs amplitude necessary to reach a 50% detection rate for the hand perceptions (point of subjective equality, PSE). The same procedure was adopted to assess the relationship between TMS-evoked hand perceptions and the amplitude of the TMS-evoked movement (accelerometric data). Considering that the peak of movement

acceleration was acquired in three spatial axes (x, y, z), the Euclidean norm was computed to obtain a single value representing the global hand acceleration (movement norm).

Statistical analysis, pictures and curve fitting was performed by using R Studio (R Core Team, 2017. R: a language and environment or statistical computing. R Foundation for statistical computing, Vienna, Austria. URL http://www.R-project.org/) and custom-made scripts written in MATLAB (MATLAB R2016b).

# Results

**Preliminary results.**

All participants easily completed the experiment, without any adverse effects to TMS.

All participants reported kinesthetic (e.g. muscles contractions, movements, changes in hand position or posture), somatosensory (e.g. tingling, touch sensations, pins and needles sensations) or mixed (kinesthetic + somatosensory) hand perceptions due to stimulation. No participant reported unpleasant perceptions.

In the subgroup of subjects specifically asked to specify if the reported sensation corresponds to one of the above mentioned three categories, it emerged that the judgement was influenced by the intensity of stimulation, with the percentage of kinesthetic and mixed sensations augmenting at higher intensities (see Figure 1, Supplementary results 1). Rarely (3.4% of the total amount of trials), participants reported some TMS-evoked hand perceptions, even when no MEPs and movements were evoked ("paresthesia-like perceptions").
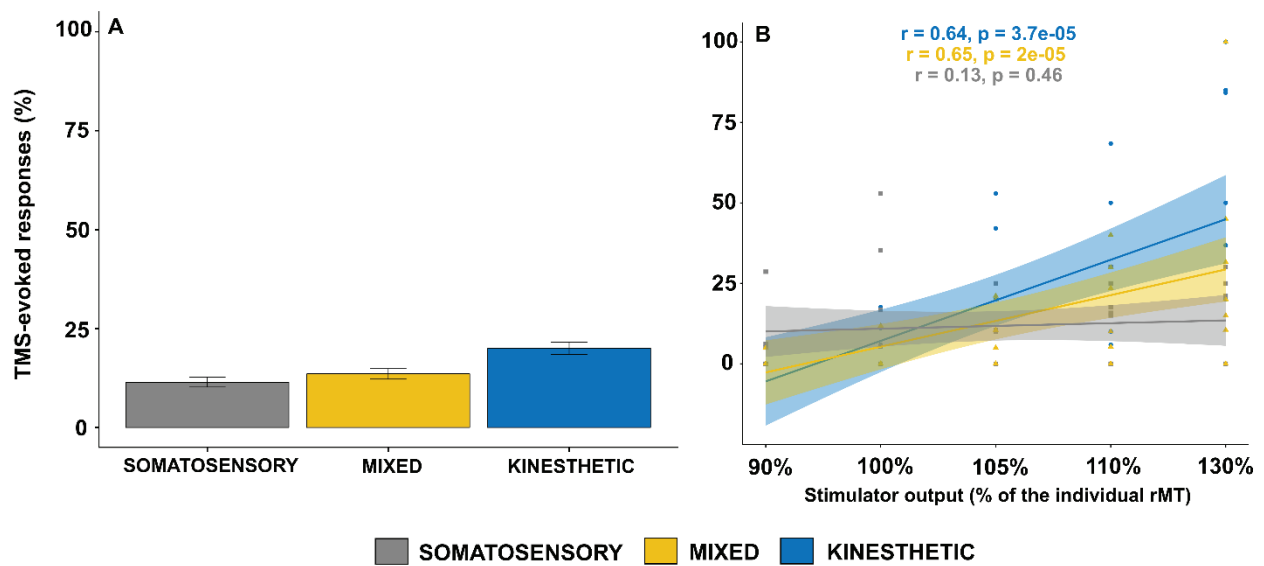
Fig. 1. Qualitative report of TMS-evoked hand perceptions. TMS-evoked hand perceptions respectively referred as kinesthetic (blue), somatosensory (grey) or mixed (yellow) sensations are here expressed as percentage of total administered pulses (A) or as a function of the intensities of stimulation (B). See Supplementary Materials for a detailed description of the procedure and the results. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Moreover, in the same subgroup of subjects, the percentage of movements recorded by the accelerometer placed on the middle finger was comparable to that of movements recorded by a second additional accelerometer placed on the index finger (see Figure 2, Supplementary results 2).
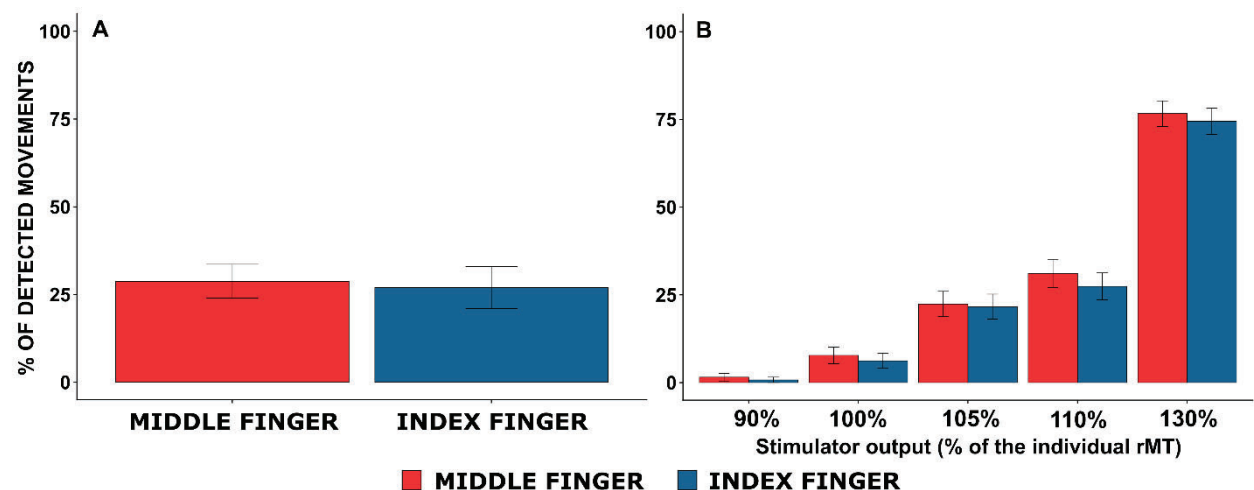


Fig. 2. TMS-evoked movements as a function of the placement of the accelerometer. TMS-evoked movements recorded from the accelerometer placed over the middle finger knuckle (red) and from the accelerometer placed on the metacarpal bone of the index finger (blue) expressed as percentage of total administered pulses (A) or divided for each stimulation intensity (B). See

### *TMS-evoked responses at different intensities of stimulation*

The model (generalized linear mixed model) on the percentage of evoked responses revealed an interaction between the type of response (MEPs, movements, hand perceptions) and the intensity of stimulation ($\chi 2$ (15) = 3547.9, $p < 0.001$). Post hoc (Tukey correction) showed that MEPs, TMS-evoked hand perceptions and movements increased with increasing intensities (all p values < 0.001). At the maximum intensity of stimulation (130%), the percentage of responses was equally high for MEPs and hand perceptions (all p values = 0.14), while these were higher than the percentage of TMS-evoked movements (both p-values $p < 0.001$). For all other intensities, the percentage of MEP was higher than that of hand perceptions and movements (all $p < 0.001$). The percentage of MEP at 100% was comparable to that of perceptions at the intensity of 105% (p = 0.54) and of movements at 110% (p = 1) (please see below the comparisons against the chance level). The percentage of hand perceptions was similar to that of evoked movements at the lowest intensity (i.e. 90% p = 0.54), while it was higher than the percentage of the movements at the intensity of 100%, 105% and 110% (all p values < 0.01) (Figure 3).

The analysis of the percentage of the evoked responses with respect to chance level (absolute threshold, Wilcoxon test against the chance level, Bonferroni corrected alpha set at 0.05/5 stimulation intensities) revealed that hand perceptions were evoked in half of the trial at 105% (Z = 81.5, p = 0.148). This differed for evoked movements, which were evoked in 50% of trials at 110% of the subjective rMT (Z = 102, p = 0.435). As defined, MEPs were evoked in half of the trials (50%, chance level) when the intensity of the TMS pulse corresponded to the rMT (100%, Z = 125, p = 0.974) (Figure 3, Table A for a summary).

Finally, we note that these results did not change if we included in the analysis the "false positives" (3.4% of the total amount of trials). The main interaction between the type of response (MEPs, movements, hand perceptions) and the intensity of stimulation remained significant ($\chi 2$ (15) = 3407, p < 0.001), with similar post hoc comparisons except for the percentage of hand perceptions that turned out as significantly higher than that of evoked movements at the lowest

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intensity (i.e. 90% p = 0.004). Importantly, even when the false positives were included, the analysis of the percentage of the evoked responses with respect to chance level (Wilcoxon test, Bonferroni corrected) showed that hand perceptions were evoked in half of the trial at 105% (Z = 121, p = 0.615, all the other p values <0.002).
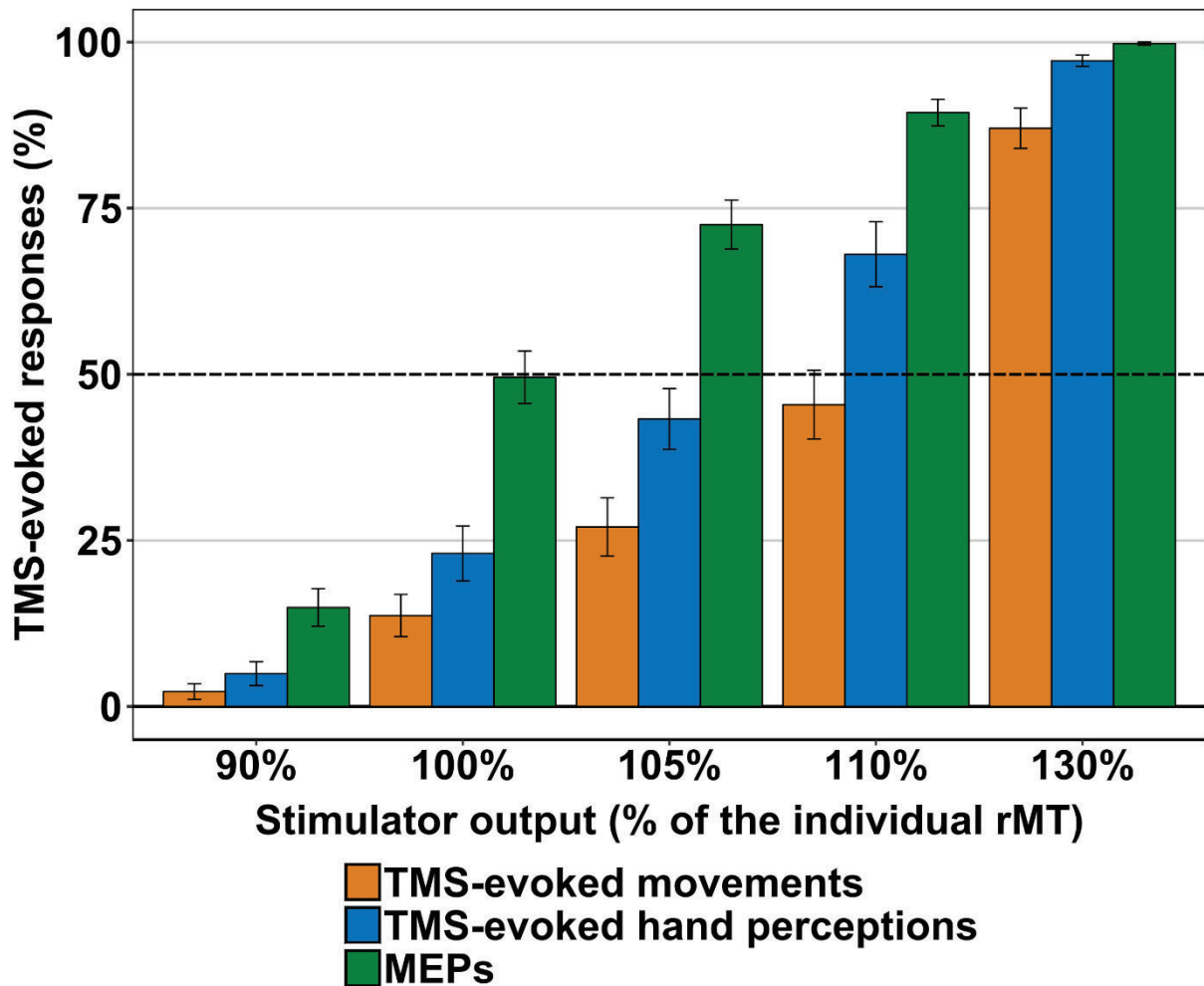


Fig. 3. MEPs, TMS-evoked movements and hand perceptions at different intensities of stimulation. The figure illustrates on the ordinate the percentage of evoked MEPs (green), TMS-evoked hand perceptions (blue) and TMS-evoked movements (orange) with respect to the total of valid trials (all administered trials excluded the trials with EMG background activity > 0.05). On the abscissa, the stimulus intensities are shown in term of percentage of MSO with respect to the individual rMT. The error bars indicate the standard error. The comparison against the chance level (black broken line) revealed that different intensities of stimulation are necessary to evoke MEPs, TMS-evoked movements and TMS-evoked hand perceptions at threshold. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

*Logistic curve fitting between TMS-evoked responses (MEPs, movements and hand perception) and TMS intensity.*

To investigate if the three TMS-evoked responses have different absolute thresholds, i.e. they require different intensities of stimulation to be elicited, we compared the central point for the MEPs, hand perceptions, and movements obtained by the fitting of the percentage of the evoked responses as a function of the stimulation intensities. Shapiro-Wilk test showed that all the data were normally distributed (MEPs: p = 0.274, TMS-evoked perceptions: p = 0.948, TMS-evoked movements: p = 0.900A). One-way ANOVA revealed a significant difference among the three obtained central point values ($F_{(2,66)}$ = 31.38, $p < 0.001$). Post hoc comparisons (Tukey correction) showed that the mean intensity to evoke the MEPs at chance level (M= 99.62, SD= 3.83) was significantly lower than the intensity to elicit hand perceptions (M= 106.52, SD= 5.66, $p < 0.001$) and hand movements (M= 113.80, SD= 8, $p < 0.001$). Furthermore, the intensity to elicit TMS-evoked hand perceptions at chance level was lower than that used to induce movements (p= 0.001, Figure 4).

Concerning the semi-interquartile range, obtained by the same fitting of the percentage of the evoked responses as a function of the stimulation intensities, data were not normally distributed for the TMS-evoked hand perceptions (TMS-evoked perceptions $p < 0.001$). Significant differences emerged among the three responses at the Friedman test ($\chi^2$ (2) = 11.217, p = 0.004). Post hoc comparisons (Wilcoxon test Bonferroni corrected, alpha set at 0.05/3 comparisons) revealed lower values for the TMS-evoked hand perceptions (p = 0.0156) and MEPs (p = 0.006) with respect to TMS-evoked hand movements, while we found similar values of the semi-interquartile range between TMS-evoked hand perceptions and MEPs (p= 1, see Figure 4).
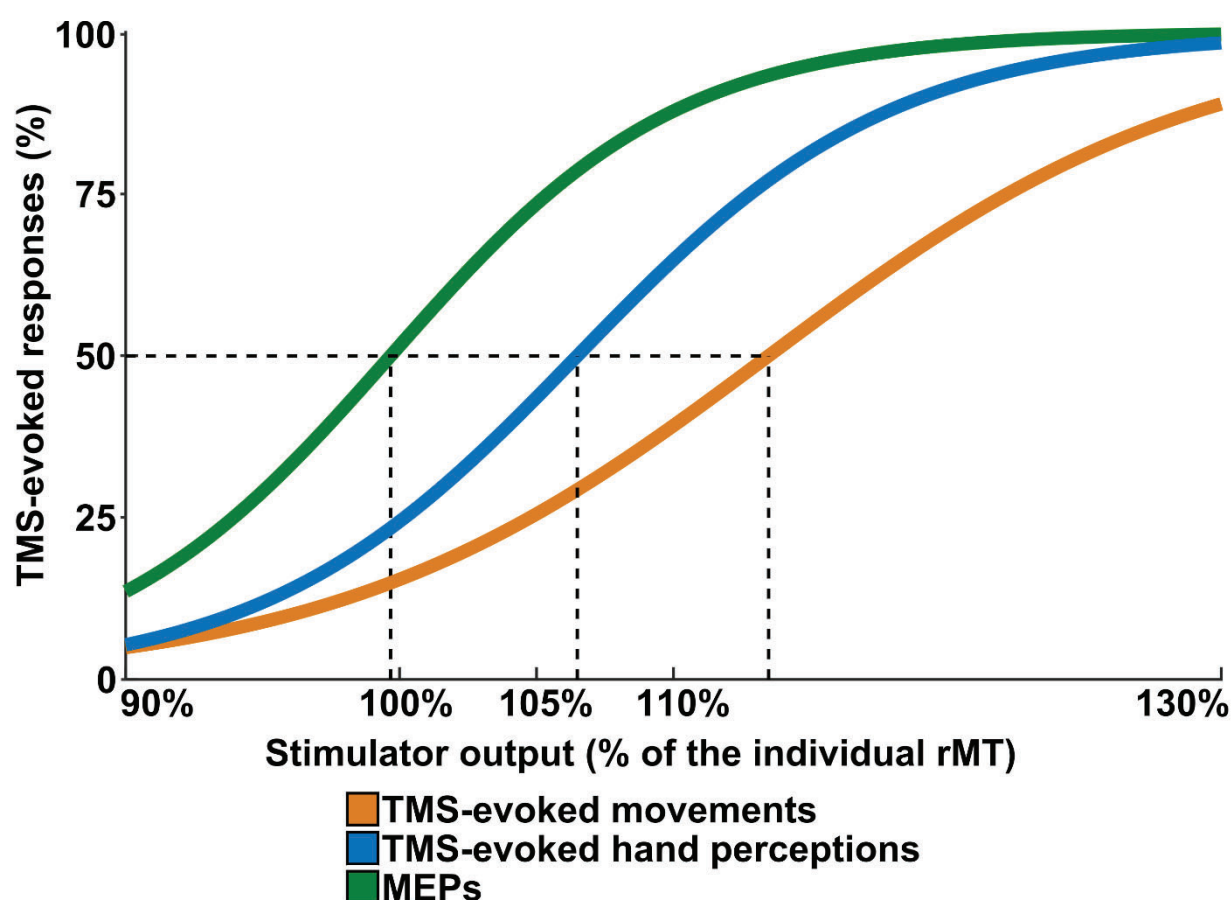
Fig. 4. Comparison among TMS-evoked responses (MEPs, movements and hand perceptions). The figure shows the results of the logistic curve fitting between (on the ordinate) the percentage of the evoked responses in terms of MEPs (green), TMS-evoked hand perceptions (blue) and TMS-evoked movements (orange) and (on the abscissa) the intensity of stimulation (percentage of MSO with respect to the individual rMT). TMS-evoked hand perceptions required an intensity of stimulation higher than MEPs, but lower than TMS-evoked movements to be elicited at the 50% (ANOVA on central point values). TMS-evoked movements require a bigger increase of stimulation intensity to be detected at threshold compared to the other two TMS-evoked effects (Friedman test on the semi-interquartile range). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

***Psychometric curve on the relationship between TMS-evoked subjective response (hand perception) and neurophysiological responses (MEPS and movements amplitude).***

Fitting between TMS-evoked hand perceptions and MEPs amplitude revealed a PSE of 0.62 mV ($R^2$= 0.68, JND= 0.37). This indicates that only MEPs with amplitude higher than 0.62 mV, approximately 10 times higher than the amplitude used to define a discernible MEPs (0.05 mV), induces reliable hand perceptions (Table B.1 for a summary). The same approach on the movement norm revealed a PSE of 0.42 m/s$^2$ ($R^2$= 0.64, JND= 0.25). This suggests that the

acceleration of a TMS-evoked movement has to be higher than 0.42 m/s$^2$ to elicit hand perceptions at threshold (Table B.2 for a summary).
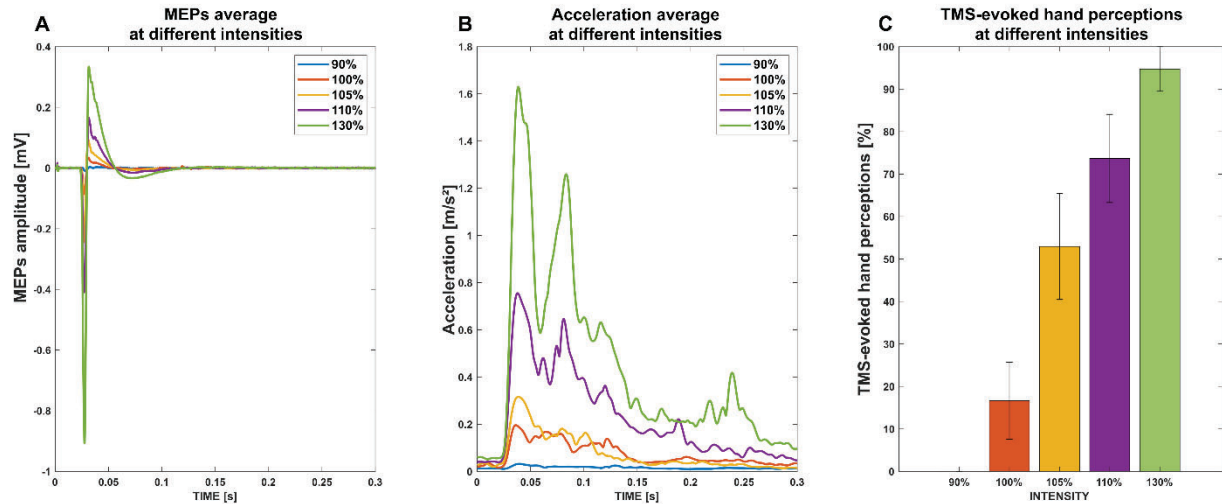


Fig. 5. TMS-evoked responses in a representative subject. The average MEPs amplitude (mV), acceleration profile (movement norm of the acceleration on the 3 axes, m/s2) and percentage of reported TMS-evoked hand perceptions (%) for each intensity of stimulation (represented in different colors) in one representative subject are respectively shown in panel A, B and C. The x axis in panel A and B represents the time (s) and the zero refers to the TMS pulse. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

# Discussion

## *Main features of TMS-induced hand perception*

This is the first study that systematically investigates hand perceptions elicited by TMS over M1 at different intensities of stimulation. Interestingly, a recent work quantified the number of hand movement perceptions in healthy participants after single pulse TMS over M1 at threshold [45] and used this measurement as an index of participants' ability in monitoring involuntary actions. They showed that participants' detection ability of TMS stimuli was altered by concomitant tDCS over posterior parietal cortex, suggesting that this area is involved in movement awareness during involuntary actions. Differently, here we compared subjective hand perceptions with the well-known and widely used neurophysiological TMS parameters of MEPs and TMS-evoked movements and found that different stimulation intensities are necessary to evoke TMS-evoked hand perceptions as compared to MEPs and movements. Specifically, the stimulation necessary to induce hand perceptions was (≈105% of the individual rMT) between the threshold for MEPs

(corresponding to the individual rMT 100% according to MEPs' definition) and TMS-evoked movements (≈110%). Then, a second analysis based on logistic curve fitting on each TMS-evoked response further confirms that MEPs, hand movements and hand perceptions are associated with different stimulation intensities.

These results show that healthy participants are able to report hand perceptions after a single pulse of TMS over M1 if the stimulation is applied above the MEP threshold. It also shows that TMS-evoked hand perceptions can be dissociated from the presence of MEPs: considering only MEPs to estimate hand perceptions is not valid and would lead to an overestimation of the amount of TMS-evoked hand perceptions, given that not all the MEPs correspond to a TMS-evoked perception. Our results also differentiate TMS-evoked hand perceptions from the presence of significant TMS-evoked movements. Dependence on the latter would result in an underestimation of such subjective responses, given that hand perceptions occurred even without any recorded movements. Accordingly, we claim that TMS-evoked hand perceptions should be considered as a separate TMS-evoked response, modulated by stimulation intensity that does not correspond to the presence of MEPs or TMS-evoked movements. This suggests that hand perceptions reported by the participants were not directly linked to the amplitude of the muscular contractions or the acceleration of movements, respectively recorded by the EMG and accelerometer. We instead hypothesize that TMS-evoked hand perceptions could be mainly driven by somatosensory and kinesthetic sensations related to skin and muscle stretch, these latter likely captured by muscle spindles. In support to this, it has been recently demonstrated that differences in the firing of muscle spindle could be independent from differences in kinematics or EMG activity and could have a role in sensory forward models [46,47].

Moreover, the result that hand perceptions may arise in the absence of peripheral movements or muscle contractions is in line with previous studies using TMS [48,49] as well as direct brain stimulation in epileptic patients [50]. For instance, movement perceptions evoked by TMS have been reported in subjects undergoing ischemic block and even in the absence of MEPs [48]. In the case of invasive brain stimulation, stimulation of parietal areas elicited an illusory sense of motion, even in the absence of EMG activity, while it was reported that stimulation of premotor areas induces involuntary movements [51,52].

Finally, our data revealed another important feature of TMS-evoked hand perceptions, indicated by the semi-interquartile range, a measure of detectability obtained from the fitting between the three TMS-evoked responses and the intensities of stimulation (expressed as a percentage of individual rMT, see Figure 4). Namely, similar semi-interquartile ranges were found between MEPs (semi-interquartile range= 5.7%) and TMS-evoked hand perceptions (semi-interquartile= 6.3%), thus suggesting that for both types of responses a small increase of intensity is sufficient to leap from responses rarely evoked (25%) to responses evoked at chance level (50%). This proposes a similar detectability between the two responses, and more precisely indicates that TMS-evoked hand perceptions are sensitive to even small changes of stimulation intensity ($\approx$6% of rMT), similarly to what we observed for MEPs. This result points out that the precision of the present verbal reports about TMS-induced hand perceptions could be comparable to well-established objective TMS measures, like MEP detection through EMG activity. This further supports the use of hand perceptions as reliable TMS-evoked responses.

***The neurophysiological responses evoked by TMS: MEPS and TMS-evoked movements***

We observed that even the two well-known neurophysiological responses evoked by TMS, MEPs and movements, did not fully overlap. Indeed, the intensity of stimulation to elicit TMS-evoked movements was higher than the intensity needed to induce MEPs. This is in line with previous studies reporting higher threshold for TMS-evoked movements than MEPs, both when the movements were evaluated with the accelerometer [10], as in this present study, and by means of the visual observation. In particular, previous authors [53] found that an 11.3% increase of the stimulation (expressed in % of maximum stimulator output) was necessary to determine the movement threshold, if the judgment was based on visual observation of movements instead than MEPs, a result very close to the present findings (113.47%). Moreover, a new result concerns the detectability of the TMS-evoked movements. We found that the change in stimulation intensity was higher for evoked movements (at chance level) than those found for MEPs and TMS-evoked hand perceptions, suggesting that evoked movements are less sensitive to changes in

intensity. This could be considered as a methodological constraint in the design of protocols aiming at recording MEPs or TMS-evoked movements.

Finally, in a further analysis, we have evaluated the amplitude of MEPs and TMS-evoked movements necessary to induce a liminal hand perception (chance level). Surprisingly, our findings reveal that the MEP amplitude necessary to perceive a discernible TMS-evoked hand perception at threshold is ≈10 times higher than the EMG activity used to define liminal MEP detection (0.05 mV). In addition, we found that the acceleration profile of a TMS-evoked movement corresponding to hand perception is ≈5 times higher than the movement threshold differentiating movements from signal noise. These data provide new reference values linking TMS-evoked hand perceptions to the neurophysiological parameters of MEPs and TMS-evoked movements (at least in healthy young participants under similar experimental conditions). These values could have implications in single pulse TMS protocols measuring MEPs or TMS-evoked movements, in which TMS-evoked hand perception could play an important role (e.g. studies in which an unwanted difference among conditions could emerge because of different TMS-evoked hand perceptions could be elicited).

***Limitations***

One possible limitation of our study is that we recorded MEPs only from the FDI muscle. Thus, we cannot exclude that the presence of MEPs in more or other hand muscles would alter the estimated relationship between TMS-evoked hand perceptions and MEPs. Indeed, in our study, the presence of MEPs in any other hand muscle could lead to an underestimation of participants' abilities to report TMS-evoked hand perception with respect to MEPs, by excluding such "false positives" (reported hand perception when no MEPs at the FDI, but MEPs at other muscles were present). However, this hypothesis seems unlikely given that false positives occurred in a very low percentage of trials (3.4% of the total amount of trials) and that the results did not change if those trials were integrated in the analysis. Moreover, this aspect does not affect the results related to the TMS-evoked movements, which absolute threshold is in any case higher than the one related to hand perception. In addition, the presence of TMS-induced sensations in the absence of MEPs, is not new, being already reported by previous studies [30]. Furthermore, since the present

results refer to the dominant hand, in a precise posture (e.g. palm down on the table, thus preventing closing movements against the table surface) our claims should be mainly limited to these conditions. Indeed, one could hypothesize that TMS-evoked hand perception could be affected by handedness, use, motor skills or different postures leading to other evoked movements (e.g. closing movements) or different sensory feedback.

## Conclusions

Our results showed that neurophysiological (MEPs), kinematics (TMS-evoked movements) and subjective (TMS-evoked hand perceptions) responses to TMS stimulation are three discernible components of single pulse TMS over M1. We argue that the evoked hand perceptions reported by the subjects could be based on somatosensory and kinesthetic perceptions elicited by TMS. In addition, we provide reference values in terms of stimulation intensities to elicit the three TMS-evoked responses and in terms of minimal MEPs amplitude and acceleration of TMS-evoked movements required to elicit TMS-evoked hand perceptions in young healthy participants under similar experimental conditions. The protocol described in the present work could be adopted as a simple task to study hand movement perception, but also more cognitive aspects such as body awareness or sense of agency in different experimental conditions that could specifically alter MEPs, TMS-evoked movements or perceptions. Thanks to its simplicity, the present protocol could also be theoretically applied in neurological patients with the aim of assessing sensorimotor and bodily functions.

**SUPPLEMENTARY MATERIAL**

**ADDITIONAL DATA**

In a subgroup of subjects participating in the main experiment (7 subjects out of the total included 23 participants, mean age 26.1 ± 2.3 years, 2 females) we recorded additional data to further address two aspects.

First, we investigated the qualitative characteristics of the hand perceptions evoked by TMS. While our main focus was to quantify the presence of TMS-evoked hand perceptions, in this subgroup of participants we also asked to specify the quality of the reported perception, by attributing it to one of the following categories: kinesthetic, somatosensory or mixed sensations. The experimental procedure remains identical, except for this additional instruction (Supplementary results 1, Figure 1 in the main text).

Moreover, we tested if a reduced percentage of TMS-evoked movements with respect to MEPs can be due to a discrepancy in the placement of the electrode used to record movements, i.e. the accelerometer, and the EMG electrode used to record muscular activity. Indeed, the accelerometer was placed over the middle finger knuckle, while MEPs were recorded from the FDI muscle. One thus could expect a higher movement detection by placing the accelerometer on the index finger because of the anatomical connection and proximity with the FDI. To test this hypothesis, in the subgroup of participants, a second identical accelerometer (Noraxon Sensors DTS 3D Accelerometer) was added on the index finger during the TMS recording performed with the same procedure described in the main text (Supplementary results 2, Figure 2 in the main text).

***Supplementary results 1: Qualitative report of TMS-evoked hand perceptions***

We investigated the quality of the TMS-evoked hand perceptions. In the case in which the subjects reported a TMS-evoked hand perception (yes answer, see the main text) in one trial,

immediately after this positive answer, they also had to categorize such perception as kinesthetic (e.g. muscles contractions, movements, changes in hand position or posture), somatosensory (e.g. tingling, touch sensations, pins and needles sensations) or mixed (kinesthetic + somatosensory) sensation, by verbally indicating one of these categories. Such categories were largely explained to participants before the beginning of the experiment by showing them a written description.

Results revealed that the reported sensations were spread among the three categories, with the majority of subjects (6 out of 7) reporting all the three types of sensations during the experiment. The total amount of perception reported for each category did not statistically differ (Wilcoxon test: all p values > 0.6, see Figure 1 in the main text, panel A).

Further, to investigate the relation between TMS-evoked hand perceptions and the intensity of stimulation, we correlated the 3 possible responses (i.e. kinesthetic, mixed and somatosensory) to the intensities of stimulation. The Spearman's rank correlation coefficient revealed a significant positive correlation between the intensity of stimulation and the amount of perceived kinesthetic (r = 0.64, p < 0.001) and mixed sensations (r = 0.65, p < 0.001). Differently, somatosensory responses were not linearly related with the intensity of stimulation (r = 0.13, p = 0.46). This suggests that the number of TMS-evoked kinesthetic and mixed perceptions tend to increase at the increasing of the intensity of stimulation, while pure somatosensory sensations are not influenced by the intensities (see Figure 1 in the main text, panel B).


*Supplementary results 2: TMS-evoked movements as a function of the placement of the accelerometer*


Here we compared the amount of movements recorded by the two accelerometers respectively placed on the metacarpal bone of the index finger and over the middle finger knuckle by means of the Wilcoxon signed-rank test. The total amount of movements recorded by the two accelerometers at all intensities of stimulation was comparable (Wilcoxon test: Z = 0.50456, p-value = 0.617, Figure 2 in the main text, panel A). Similarly, considering each intensity separately, the percentage of TMS-evoked movements recorded by the two accelerometers was equal and

none of the statistical comparisons resulted significant (Wilcoxon test: all the p-values > 0.5, see Figure 2 in the main text, panel B). Lastly, we checked if the acceleration profile of the movement could be affected by the different placements of the accelerometers, by comparing the total movement norm collected among all the intensities. Again, no differences between the accelerometers emerged (Wilcoxon test: Z = 0.78902, p-value = 0.4358).

Together, these data indicate that the accuracy in the movement detection was comparable between the two electrodes, independently of the placement. In other words, TMS-evoked movements were reliably acquired even from the middle finger knuckle without significant differences in the amount of movements recorded and in the acceleration profile. These data make very unlikely the hypothesis that the discrepancy of the percentage of TMS-evoked movements and MEPs in our data could be related to the accelerometer's position. Instead, the reduced numbers of TMS-evoked movements with respect to MEPs is in line with previous works ([53], see Discussion in the main text).

**Table A**

| MEPs | | | | | |
|------|------|--------|--------|---------|--------|
|      | Mean | Std.dv | Std.err | t-value | p |
| **90%** | 15.15 | 14.13 | 3.58 | -9.742 | <0.001 |
| **100%** | 47.78 | 20.01 | 5 | -0.444 | 0.664 |
| **105%** | 70.90 | 17.78 | 4.45 | 4.701 | <0.001 |
| **110%** | 90.64 | 9.52 | 2.38 | 17.070 | <0.001 |
| **130%** | 99.67 | 1.32 | 0.33 | 151.091 | <0.001 |
| **TMS-evoked hand perception** | | | | | |
|      | Mean | Std.dv | Std.err | t-value | p |
| **90%** | 4.30 | 7.98 | 1.99 | -22.915 | <0.001 |
| **100%** | 22.29 | 16.44 | 20.03 | -6.744 | <0.001 |
| **105%** | 46.04 | 20.03 | 5.01 | -0.791 | 0.441 |
| **110%** | 71.81 | 20.50 | 5.13 | 4.255 | <0.01 |
| **130%** | 97.57 | 4.19 | 1.05 | 45.385 | <0.001 |
| **TMS-evoked movements** | | | | | |
|      | Mean | Std.dv | Std.err | t-value | P |
| **90%** | 2.59 | 6.37 | 1.59 | -29.759 | <0.001 |
| **100%** | 16.27 | 16.56 | 4.14 | -8.150 | <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| **105%** | 28.86 | 22.28 | 5.57 | -3.795 | <0.01 |
| **110%** | 51.83 | 25.03 | 6.26 | 0.292 | 0.774 |
| **130%** | 89.71 | 10.06 | 2.52 | 15.786 | <0.001 |

*Table A. Two tailed one sample T-test against chance level, Bonferroni correction: α level set at 0.01 (0.05/5).*

**Table B.1**

| Logistic curve fitting between TMS-evoked responses and TMS intensities | | | | | |
|---|---|---|---|---|---|
| | **P25** | **CP** | **P75** | **R$^2$** | **sIQR** |
| **MEPs** | 94.11 | 99.83 | 105.56 | 0.83 | 5.72 |
| **TMS-evoked hand perceptions** | 100 | 105.94 | 111.75 | 0.83 | 5.81 |
| **TMS-evoked movements** | 103.43 | 111.45 | 119.46 | 0.75 | 8.01 |

**Table B.2**

| Psychometric curve | | | | | |
|---|---|---|---|---|---|
| | **P25** | **PSE** | **P75** | **R$^2$** | **JND** |
| **TMS-evoked hand perceptions as function of MEPs amplitude (mV)** | 0.29 | 0.67 | 1.06 | 0.74 | 0.38 |
| **TMS-evoked hand perceptions as function of movement norm (m/s$^2$)** | 0.21 | 0.46 | 0.70 | 0.69 | 0.25 |

*Table B. CP (central point), P25 (25% of logistic curve fitting), P75 (75% of logistic curve fitting), sIQR (semi-interquartile range), PSE (point of subjective equality), JND (just noticeable difference).*

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## 2.3. Study 3: Move or believe to move? The role of bodily signals perception in triggering illusory feelings of embodiment towards a virtual hand

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**ABSTRACT**

The bodily self-consciousness (BSC) is defined as the implicit and pre-reflexive experience of being the subject of a given experience. BSC is highly malleable and can be altered with experimental manipulations, like the rubber hand illusion (RHI), in which the simultaneous application of tactile stimuli on the participant's hidden hand and on a visible rubber hand leads to an illusory feeling of embodiment towards the fake hand. However, most of the paradigms rely on supra-threshold tactile or motor stimuli on the participants' hand, thus leaving unsolved the question if peripheral bodily signals and their conscious perception are necessary to induce embodiment. To solve this issue, here we adopted a modified version of the RHI based on transcranial magnetic stimulation (TMS) over the motor cortex to evoke peripheral hand twitches perceived in half of the trials. In all trials, participants observed a virtual hand mimicking the movements typically evoked by the TMS (Figure 1). We compared the illusory feeling of embodiment towards the virtual hand depending on the presence of peripheral hand twitches and their conscious perception. Results showed higher illusory feelings of embodiment towards the virtual hand when participants reported the conscious perception of bodily signals, even regardless the presence of actual hand twitches. Our findings reveal a prominent role of conscious bodily perception in triggering illusory embodiment, even in the absence of peripheral stimuli.

**INTRODUCTION**

The human brain constantly integrates multisensory exteroceptive and interoceptive stimuli from the body. These signals give rise to the phenomenological experience of the self, termed bodily self-consciousness (BSC) [1,2,3]. Studies on multisensory integration have shown that the manipulation of peripheral stimuli can induce illusory states of bodily self-consciousness (BSC), in which participants experience owning fake body parts, like a rubber hand [4], the face [5] or even the full body [6]. A well-known approach to manipulate BSC is the Rubber Hand Illusion (RHI) [7], in which the simultaneous application of tactile stimuli on a participant's hidden hand and on a visible rubber hand leads to the illusory embodiment for the fake hand [8]. Although myriad variations of the RHI protocol have been investigated [9,10], the large majority of these paradigms rely on tactile or motor stimulation where participants are fully aware of the ongoing stimulation. However, as for other cognitive phenomena [11], the integrative processes underlying the BSC occur mainly in the absence of perceptual consciousness [12]. Salomon and coworkers [13] investigated this issue inducing the full-body illusion in a group of subjects exposed to a visual masking paradigm. During the experiment, the participants were stroked on the back while observing a virtual avatar with a Mondrian on the back to mask the visual stimuli synchronized with the stroking. Despite the subjects were unaware of the presence of visual stimuli, they reported higher self-identification with the avatar when the "invisible" visual stimuli were synchronized with the back stroking, in agreement with the multisensory principles of BSC.

These results remind what has been reported in pathological conditions such as the numbsebse [14] and blindsight [15] where patients show a dissociation between explicit and implicit awareness of the stimuli. Patients affected by numbsense are unable to verbally report tactile stimuli on the affected hand, while they are quite accurate in localizing the same stimuli pointing with the unaffected hand. Blindsight patients show a similar dissociation but in the visual domain, namely, they can elaborate visual properties of objects that they claim to not see. Altogether, these experimental and clinical data suggest that the mechanism of BSC acts in the absence of awareness of the stimulation.

To investigate this issue, we took advantage of a recently proposed RHI protocol relying on the combination of transcranial magnetic stimulation (TMS) and virtual reality [19]. Here we induced

the RHI by using TMS-induced hand movements and immersive VR and personalized the level of M1 stimulation, so that we evoked conscious perceptions in approximately 50% of the trials [20]. Thanks to this approach we can combine psychophysics with embodiment, investigating how the subjective ratings are modulated by the perception of bodily stimuli. During the stimulation, participants observed a virtual hand mimicking the movements typically evoked by TMS. In addition, participants were exposed to sham (control) stimulation in which they received a single TMS pulse over a non-motor area, i.e. the vertex (V), never evoking hand twitches. In both conditions, during the stimulation, participants were presented with a virtual hand on a head-mounted display (HMD).

The role of peripheral stimuli (MEPs) and awareness of stimuli perception in driving the illusory feeling of embodiment was assessed by comparing ownership and agency ratings across four categories of trials: 1) HITS, when participants reported conscious TMS-evoked hand perception (yes answer) in case an MEP was evoked; 2) CORRECT REJECTIONS (CRJ), when participants did not report any TMS-evoked hand perception and no MEP was evoked; 3)FALSE ALARMS (FA), when participants reported TMS-evoked hand perception in the absence of an MEP; 4) MISSES, when participants did not report TMS-evoked hand perception while a MEP was evoked.

We hypothesize that perceived MEPs (HITS) would be more effective to induce embodiment compared to when no stimuli are evoked (FALSE ALARMS, CORRECT REJECTIONS) as expected from the literature where most of the RHI studies adopt supra-threshold tactile or motor stimuli. Mainly, we expect that when MEPs are evoked but not perceived (MISSES), i.e. when participants are unaware of the ongoing stimulation, this may result in a higher embodiment for the conditions with no peripheral stimulation indicating a modulation of embodiment in absence of perceptual awareness.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Participants recruitment

Seventeen healthy participants took part in the present study (mean age 23.9 years, SD $\pm$ 3.0, 5 females). All participants were right-handed according to the Flinders Handedness Survey [21]

and had a normal or corrected-to-normal vision. None of them showed contraindication to TMS [22] and took part in the study after giving informed written consent. The present study was conducted with the approval of the local ethics committee (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM 017/14). All participants were remunerated with 30 Swiss Francs for their participation.

**METHODS**

**Transcranial magnetic stimulation (TMS) and electromyographic recording (EMG)**

Single-pulses TMS was delivered through a figure-eight coil (wing diameter of 70 mm) connected to a single Magstim monophasic stimulator (Magstim 200$^2$, Magstim Co., Whitland, UK). To evoke the optimal activation of the corticospinal tract from the right First Dorsal Interosseous muscle (FDI), we positioned the coil over the left primary motor cortex tangentially to the scalp with the handle oriented 45° postero-laterally away from the midline. During the vertex condition (V), the coil was centered over the vertex (e.g. [39,37]), at the electrode position Cz as defined by the International 10-20 system [25]. To record the electromyographic activity evoked by the stimulation (motor evoked potentials, MEPs), we used a surface electromyographic system (EMG) through wireless electrodes positioned on the FDI muscles of participants' right hand. According to published guidelines (e.g. Groppa et al. [26], Rossi et al. [27]), we initially set the TMS parameters: the hotspot for the stimulation of the hand area of the left primary motor cortex, the resting motor threshold (rMT) and the intensity of the stimulation to be used during the experiment (i.e. 105 % of the rMT; see below for details).

To find the hotspot of the stimulation, we initially positioned the coil 5 cm laterally and 1 cm anteriorly to the vertex. We delivered TMS pulses at a slightly supra-threshold intensity (45% of the maximal stimulator output, MSO) moving the coil of 0.5 cm steps around the initial estimate point. We choose as the hotspot of the stimulation the position in which we recorded from FDI the most stable and with the maximal amplitude MEPs (peak-to-peak > 0.05 mV [26,28]). Once defined the hotspot, we marked with a pen the exact position on the scalp to make sure to place the coil always in the same position during the whole session. For the definition of the Resting

Motor Threshold (rMT) of the FDI muscle, we followed a standard procedure developed by Awiszus et al. [29] using a software-based "adaptive method" (Motor Threshold Assessment Tool, http://www.clinicalresearcher.org/software.htm). We used as the intensity of stimulation the 105% of the individual rMT to elicit TMS-evoked hand perception in half of the trials [20]. We verified this in each participant by delivering 10 TMS pulses before starting the experiment.

We assessed the rMT and the intensity of stimulation at the beginning of each condition (M1 and V). Mean values of rMT were 42.2 (SD $\pm$ 6.2) and 41.6 (SD $\pm$ 6.2) for M1 and V condition, respectively (paired sample two-tailed T-test, t (16) = 1.492, p = 0.155).

MEPs were recorded from the FDI muscle of both hands using a surface electromyographic system (EMG) through wireless electrodes positioned in a tendon-belly configuration. EMG signals were amplified and band-pass filtered (1 Hz to 1 kHz) by a Noraxon DTS amplifier (Velamed, GmbH, Köln, Germany). The signals were sampled at 3000 Hz, digitized using a laboratory interface (Power1401; Cambridge Electronics Design CED), and stored on a personal computer for display and later off-line data analysis using the Signal software. Each recording epoch lasted 1500 ms, from 300 ms before to 1200 ms after the TMS pulse. Trials with EMG background activity (peak-to-peak > 0.05 mV) preceding the TMS pulse of 100 ms in the stimulated hand were excluded from the present analysis to avoid possible biases ascribable to uncontrolled MEPs facilitation [30–32].


**Virtual reality (VR) setup**

The same virtual reality set-up previously adopted in Bassolino et al.,2018 was used [33]. Participants were asked to wear a Head Mounted Display (HMD, Oculus Rift Development Kit 1, 640 x 800 resolution per eye, 110° Field of View (nominal), refresh rate 60 Hz, Oculus VR, Menlo Park, CA, USA).

The virtual scenario was controlled by an in-house software (ExpyVR, EPFL, http://lnco.epfl.ch/expyvr, a framework for designing and running experiments in virtual reality), also used for collecting participants' ratings and generating triggers for TMS pulses. For this latter purpose, the laptop running the VR software was connected to the laboratory interface

(Power1401; Cambridge Electronics Design CED) using a laptop-parallel-adapter-card to send triggers to the TMS stimulator.

First, the position and dimension of the virtual hand were calibrated in VR by each participant to match the felt position and dimension of their real right hand, while they were wearing the HMD. As previously reported [34,35], during the experiment the virtual hand was displaced toward the body midline with respect to initial calibration.

**General procedure**

We adapted the procedure previously described in Bassolino et al. [33]. In brief, participants were instructed to observe on the HMD a virtual right hand, displaced toward the body midline with respect to initial calibration, lying palm down on a white table. The virtual hand was set to move synchronously with the TMS pulse, mimicking the typical movement evoked by the supra-threshold TMS stimulation on participants' contralateral hand. The inter-trial interval between two consecutive TMS pulses was always above 10 s. This was designed to minimize changes in cortical excitability during the experiment [36].

In a trial-by-trial design, after each TMS pulse, participants were instructed to rate the level of agreement/disagreement with two embodiment statements regarding the illusory feeling of ownership ("It seemed like the virtual hand was my hand") and agency ("It seemed like I was in control of the virtual hand") for the virtual hand (Figure 1). The statements selected among those proposed in the literature [8] and used in our previous study [33] were read by the experimenter in a randomized order. Participants indicated their level of agreement/disagreement on a continuous vertical scale (a vertical line) that appeared on the HMD screen by using a keyboard that they controlled with their left hand (Figure 1). The upper extreme of the line, represented with a green dot, indicated a total agreement (maximum score 6) with the statement, while the lower extreme of the line, represented with a red dot, indicated a complete disagreement (minimum score 0) with the statements. After the two embodiment ratings, participants had to answer a third question related to TMS-evoked hand perception. More precisely, they had to report verbally (yes/no answer) if they had perceived any kinesthetic or somatosensory

perception including (but not limited to) contractions, movements, changes in hand position, tingling sensation, or any other perceptions at the right hand related to the TMS pulse [20].

All participants underwent the two conditions in two separate sessions performed in a randomized order within the same day and separated by a 10 minutes' interval. In a session, TMS was applied over the hand area of the left primary motor cortex (M1, experimental condition), while in the other session it was applied over the vertex (V, control condition). In both conditions, the intensity of stimulation was set at 105% of the rMT (see above). We delivered a total of 50 pulses for each condition (M1 and V), with a break of one minute every twenty pulses.

At the end of the experiment, we debriefed the participants by asking if they had perceived differences between the two conditions (e.g. if they perceived a change in the position of the coil). It turned out that the 76 % of the group did not notice any differences between experimental and control conditions in terms of the site of stimulation.
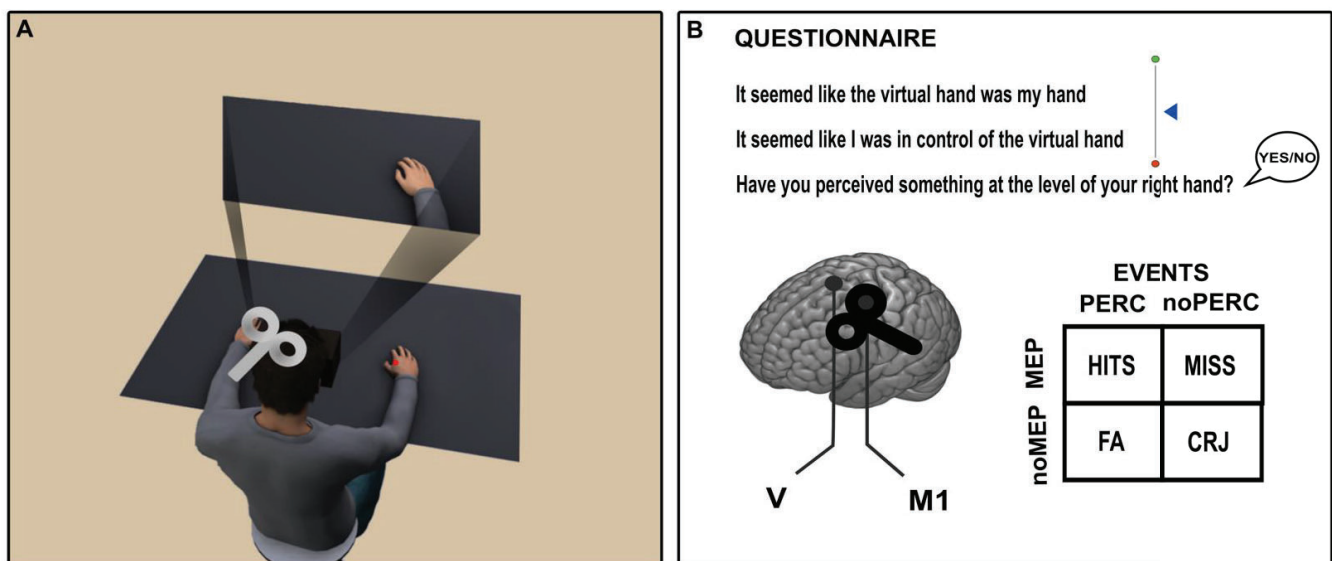


**Figure 1. Combining transcranial magnetic stimulation and virtual reality to study the contribution of body signals perception in illusory embodiment.** The figure shows the set up (panel A) and a summary of the experimental procedure (panel B). While receiving TMS pulses over the left motor cortex (M1) or the vertex (V), participants wore a virtual reality headset (head mounted display, HMD) and observed a virtual right hand moving as mimicking hand movements typically evoked by supra-threshold TMS over M1 (panel A).After each pulse, participants had to rate the level of agreement (green dot) or disagreement (red dot) with two embodiment statements (ownership and agency) by operating on a keyboard to move a cursor (blue triangle) on a continuous vertical scale that appeared on the HMD screen (panel B, above). They also had to verbally report the presence of TMS-evoked hand perception (yes/no answers, panel B, above). The intensity of stimulation was set at 105% of the resting motor threshold so that TMS over M1 elicited TMS-evoked hand perception in half of the trials, while in all trials the virtual feedback (i.e. the moving

hand) remained identical. With the same intensity and in the presence of the same virtual stimuli, TMS was also applied on the V never evoking MEPs (panel B, lower part, on the left). Based on the peripheral activity (MEP) and the reported perception (TMS-evoked hand perception), four different events could be evoked: HITS, MISSES, FP and CRJ (panel B, lower part, on the right). The picture in panel A was modified with permission from Solcà et al., 2018 (Neurology, 91(5)).

## Quantification and statistical analysis

Behavioral ratings were collected trial by trial and analyzed using linear mixed-effects models [37], with participants used as the random factor. Additional random effects were added based on a model selection with Akaike's Information Criterion (AIC). For fixed effects, p-values were obtained by likelihood ratio tests, and the degrees of freedom were approximated using the Satterthwaite method. Additional random effects were added based on a model selection with AIC. The significance threshold was set at 0.05. The statistical analysis and the graphs were realized with the software R packages.

## RESULTS

The model on ownership ratings ($R^2$ = 0.78) collected during M1 stimulation revealed a main effect of Perception (F (1, 16.72) = 15.91, p < 0.001) with higher ratings for ownership obtained when participants reported a TMS-evoked hand perception. However, this effect was not influenced by the presence of MEPs (Perception X Condition: F (1, 732.25) = 2.34, p = 0.125; MEP: F (1, 141.87) = 3.24, p = 0.073). We found a similar result for agency ratings ($R^2$ = 0.75), with higher agency for trials when participants perceived TMS-evoked hand sensations (Perception: F (1, 17.17) = 8.28, p = 0.010), again regardless of MEPs (Perception X Condition: F (1, 740.60) = 0.80, p = 0.369; MEP: F (1, 151.36) = 1.25, p = 0.264). Altogether, these results show that illusory embodiment (ownership and agency) for the virtual hand depends on the presence of hand perceptions, but does not depend whether TMS was associated with a MEP or not. Surprisingly the same finding was observed for the V control condition where we expected no difference between FP and MISSES. We found for both ownership and agency a significant difference in the ratings with higher ratings when stimuli when perceived (ownership t.test t (27) = -3.220, p < 0.001; agency t.test t (27) = -3.663, p < 0.001) (Figure 2). Stimuli frequencies are reported in table 1.
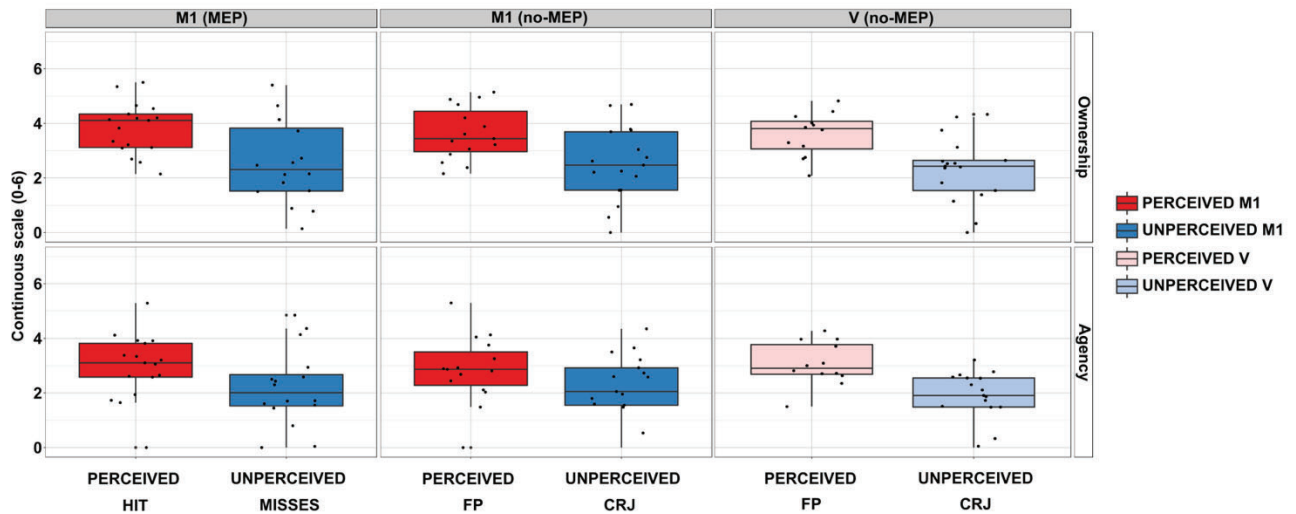
**Figure 2. Conscious perception of bodily signals triggers illusory feelings of embodiment.** Ratings about the illusory feeling of ownership (upper panels) and agency (lower panels) were significantly higher when participants reported TMS-evoked hand perception (red versus blue boxes) in both M1 (MEP) (left panel) and M1 (no-MEP) (central panel). The same result was found by comparing trials in which no MEPs were evoked after V stimulation (V (no-MEP), right panel, light red versus light blue box). Boxes represent the first (lower hinges) and third quartiles (upper hinges), the line within the box is the median, the whiskers represent the largest (upper) and the smallest (lower) value no further than and at most 1.5* the inter-quartile range. Single subject averaged responses are also shown (black points).

We also compared the EMG activity in the different trials as a function of reported TMS-evoked hand perception, by using paired sample two-tailed T-tests, Bonferroni corrected (alpha set at $0.05/6 = 0.008$). During M1 stimulation, the evoked muscular activity in the HITS trials (MEPs and perception) was significantly higher than MISSES (MEPs no perception; $t(15) = 5.0304$, $p < 0.008$), FALSE POSITIVE (no MEPs and perception; $t(14) = 9.858$, $p < 0.008$) and CORRECT REJECTION (no MEPs no perception; $t(16) = 8.8812$, $p < 0.008$). No significant difference was found in the comparison between FALSE POSITIVE and CORRECT REJECTION neither during M1 ($t(14) = 2.8491$, $p > 0.008$), neither V stimulation (paired sample two-tailed T-test, $t(11) = -1.161$, $p > 0.008$) (Figure 3). Please note that the average EMG activity in FALSE POSITIVE and CORRECT REJECTION during M1 or V was always clearly below the 0.05 mV (red line, Figure 3).
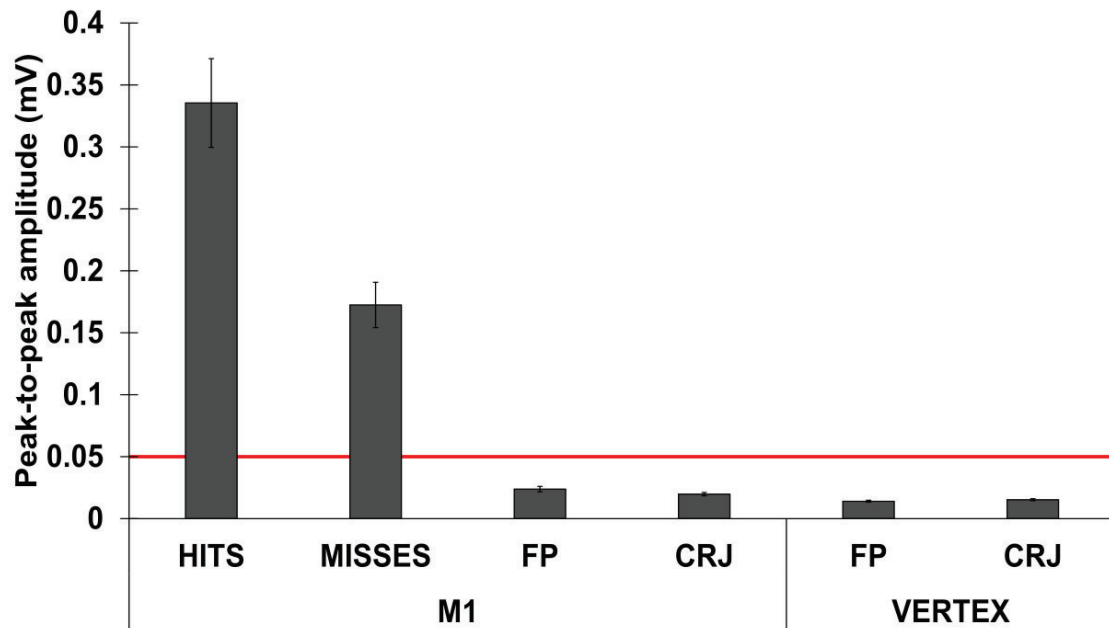
**Figure 3. Comparison of the recorded EMG activity as a function of reported TMS-evoked hand perception.** The figure shows the evoked muscular activity (on the abscissa) expressed as the average peak-to-peak EMG amplitude for M1 and vertex stimulation across the four categories of trials (on the ordinate), depending on participants' perception. As expected, the EMG activity evoked in the HITS (TMS-evoked hand perception was reported in the presence of a MEP) is significantly higher than in the MISSES (MEPs but no TMS-evoked hand perception), the FALSE POSITIVE (FP; no MEPs but TMS-evoked hand perception) and the CORRECT REJECTION (CRJ; no MEPs and no TMS-evoked hand perception) trials. No differences were found in the EMG activity in the comparison between the FP and the CRJ trials within M1 stimulation and within vertex stimulation. The error bars indicate the standard error. The red line represents the threshold (0.05 mV) to define the evoked EMG activation as a motor-evoked potential (MEP) [28,38]. All comparisons were Bonferroni corrected (alpha set at 0.05/6= 0.008).

| M1 | |
|---|---|
| HITS | 27% |
| MISS | 19% |
| FP | 23% |
| CRJ | 31% |
| VERTEX | |
| FP | 24% |
| CRJ | 76% |

**Table 1. Frequency table.**

**DISCUSSION**

In the present work, we found that the embodiment for a virtual hand is mainly driven by the perception of the stimulation, i.e. when participants are aware of the MEPs. When MEPs were perceived, both the sense of ownership and agency ratings were higher compared to when no perception was reported. Surprisingly, we found that even in the trials where no MEPs were evoked (i.e. FA) the ratings tended to be higher compared to trials where no perception (CRJ, MISSES) was reported regardless of the presence of MEPs. This last result is confirmed by those observed during the control condition, where participants produced higher ratings when they reported a perception even if MEPs were never elicited.

Previous studies showed that the stimulation over the rubber hand is not always necessary to induce embodiment while the real hand is stimulated. In those studies [39,40], they showed that the expectancy of tactile stimuli approaching the rubber hand is enough to elicit embodiment in healthy participants when they are synchronous with the tactile stimulation on the real hand. Similarly, it has been demonstrated that the RHI may occur even if the stroking is performed without contact with the rubber hand but performed at mid-air [41], inside the hand peripersonal space [42]. Here, we extended those results showing that the stimulation of the real hand is not necessary to induce embodiment but is sufficient that the participant believes that the stimulation occurred. Importantly, in our study, the observed virtual hand always moved as if it was stimulated, while the stimulation on the real hand was uncertain.

In this study, we also observed more FP than what we observed in a previous study were the subjects observed a static virtual scenario without a moving virtual hand. The observation of the moving hand may enhance the presence of false TMS-evoked hand perceptions, as suggested by previous works [43,44]. For instance, Kaneko and coworkers showed that in healthy participants the observation of a moving hand overlapping the real hand position can induce kinetics illusion, mainly involving the premotor dorsal and the inferior parietal cortex, even in the absence of M1 and somatosensory activations. This is in agreement with another work [45] reporting kinetics illusion in the absence of real movement after stimulation of the parietal lobe. This might suggest that the high subjective ratings during the FP might be interpreted as the effect of similar kinetics illusions occurring in our participants. Anyway, we have to acknowledge that this interpretation

is highly speculative since we do not have sufficient data to confirm that our setup can induce such kinetics illusions.

Differently from Salomon [13], we did not found BSC modulations due to unconscious multisensory integration. When participants were not aware of the peripheral TMS-induced MEPs, their ratings tended to be lower than when the stimuli where perceived. Surprisingly, high ratings were reported even when no MEPs were evoked but participants claimed to have perceived something. This discrepancy can be accounted claiming that the neural mechanisms and the properties of full-body BSC and hand ownership are different [2]. Indeed, full-body BSC manipulations imply changes in self-location [46] and self-identification [47], associated with activation of brain areas that extend beyond those involved in body ownership [48]. According to this observation, our study might be not directly comparable with Salomon's study due to the different processes underlying full-body and body-part specific manipulations. Second, in our study, we used an intensity of stimulation at the perceptual threshold, while in Salomon's work the visual stimuli were unconscious. Accordingly, we may speculate that artificially evoked sub-threshold TMS is harder to be integrated with other stimuli with respect to some more ecological visual subthreshold stimuli. Previous works have rarely studied this contribution of the awareness of bodily signals in BSC, mainly focused on supra-threshold peripheral signals [9] where participants are constantly aware of the ongoing stimulation. An exception to this lack in literature is a recent study by Salomon and coworkers [13] where the bodily illusion was induced by coupling unconscious visual stimuli with conscious bodily stimuli. Differently from our research, in that study the external visual stimulus was unconscious, while the tactile stimulation on the participants' body was consciously perceived.

In conclusion, we suggest that the perception of peripheral stimuli plays a crucial role in driving the embodiment illusion for a virtual hand even when no stimuli are delivered but the participant believed that the stimulation occurred. This effect was mostly independent of peripheral and cortical contributions, being equally present regardless of TMS-evoked muscular contractions on participants' physical hand and despite the locus (M1 or V) of the cortical stimulation.

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# 3.    Discussion

## 3.1.  Summary of scientific contributions

In the first study (**Study 1**), I contributed to developing an innovative setup to combine TMS and VR stimulation to induce illusory states of BSC, and specifically illusory embodiment of a virtual body part by providing neural and peripheral stimulation. We showed that supra-threshold TMS evoking hand twitches time-locked with synchronous visual feedback of a moving hand can effectively induce embodiment for a virtual hand. This was the first study to show that the BSC can be modulated by combing artificially generated motor brain signals and virtual reality, without involving a direct stimulation of the participants' body. However, differently from our original hypothesis, the sub-threshold stimulation was not sufficient to modulate embodiment. We believe that this might be due to the absence of an evoked corticospinal activity because of the low-intensity sub-threshold stimulation, indicating a predominant role of peripheral signals in inducing illusory states of the embodiment.

In the second study (**Study 2**), we focused our research on the TMS-evoked hand sensations related to the TMS. Using our protocol, we were able to find a specific threshold needed to evoke hand sensations and to show that MEPs, TMS-evoked hand sensations, and TMS-evoke movements are three discernible components of single-pulse TMS over M1. This study provides new insight into the underlying sensory process involved in our TMS-VR protocol for embodiment, and more generally in TMS studies and proposes that TMS can be used as a psychophysical tool to investigate the monitoring of evoked hand sensations.

In the third study (**Study 3**), we tackled the impact of multisensory integration of unconscious stimuli on BSC. We combined our TMS-VR induced RHI protocol with a stimulation around the perceptual threshold to investigate the contribution of unconscious body signals to the embodiment illusion. We found that, in the absence of conscious perception, TMS is not sufficient to induce embodiment for a virtual hand. We rather found that the main factor driving the questionnaire ratings is the perception of stimuli, regardless of the presence of actual hand twitches.

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## 3.2. The role of peripheral and unconscious multisensory stimuli on BSC

### 3.2.1. Peripheral vs central stimulation

So far, most of the studies investigating BSC studied multisensory brain mechanisms by manipulating sensory or motor stimuli (Kilteni et al., 2015; Riemer et al., 2019). Those studies share the same methodological approach, where a bodily stimulation is coupled with synchronous stimulation to efficiently induce illusory embodiment, thus implying that peripheral bodily signals are necessarily involved. This is the typical case of the RHI, where a visuo-tactile stimulation is performed on the participants' hands. An intriguing hypothesis is, whether it might be possible to "bypass" the afferent bodily signals to manipulate BSC (Collins et al., 2017), directly stimulating the cortical representation of the body. This issue has been already investigated in patients during invasive brain stimulation. It has been observed that stimulation of the right angular gyrus systematically evoked out-of-body experiences (Blanke et al., 2002), i.e. the experience in which a person seems to be awake and to see his body and the world from a location outside the physical body (Blanke et al., 2004). In a patient affected by central post-stroke pain, the perception of a supernumerary limb on the left contralesional side of the body (Canavero et al., 1999) was elicited during extradural cortical stimulation of M1. Also, the invasive stimulation of the left temporoparietal junction leads to the experience of presence hallucinations (Arzy et al., 2006), during the presurgical evaluation of an epileptic patient. Furthermore, recent data collected in an amputee patient showed that sub-threshold neurotactile stimulation coupled with automatized visual illumination of a virtual hand can induce embodiment for the virtual hand (Rognini et al., in preparation, see figure 4).
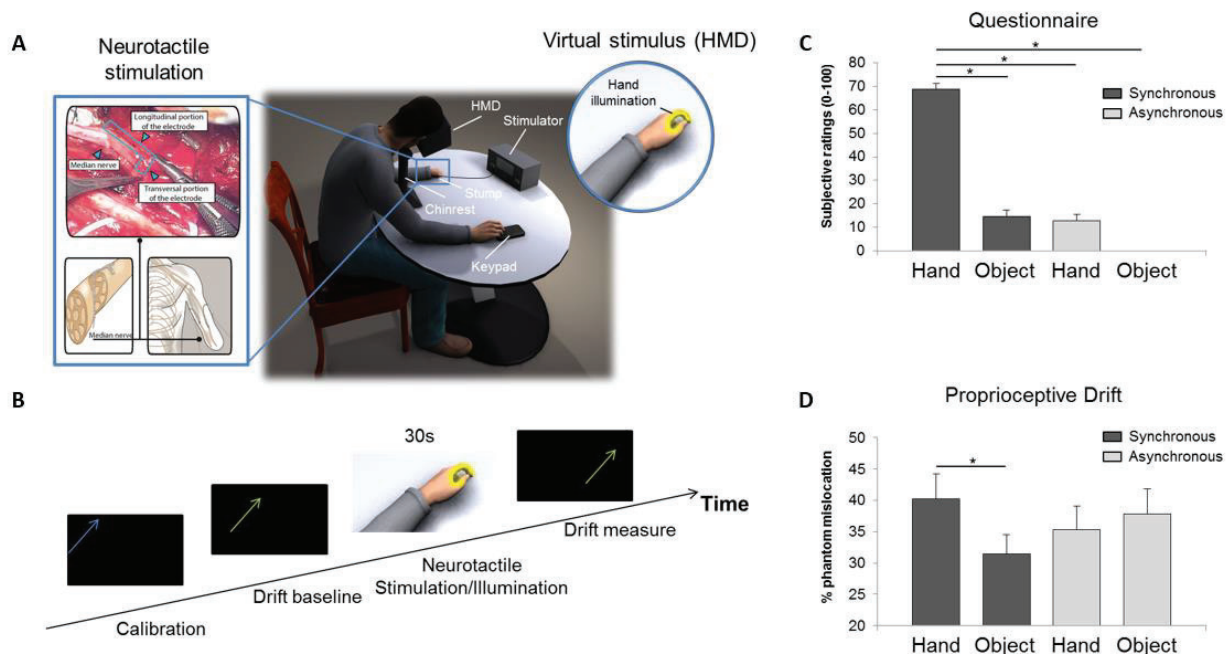
Figure 4. Experimental Setup and Procedure. (A) An illustration of the experimental setup is shown. During the experiment, neurotactile stimulation was coupled with a visual stimulus on a PC display where a virtual hand is represented with the illumination of the same skin region where neurotactile stimulation was experienced. Illumination was presented in virtual reality on a HMD and consisted in a static picture of a left virtual arm, in a posture mimicking the positions of the patient's phantom hand and fingers (right inset). The virtual hand was generated by using a mirror-reversed picture of the patient's right arm. In order to set the position of the virtual hand in space, the patient was asked to orient his eyes and head (i.e. his visual perspective) in such a way that a blue line appearing on the HMD matched the felt position of the index finger of his phantom limb. A chin-rest was used to maintain the correct positioning throughout the experiment and the patient was asked to keep gazing towards the virtual stimulus. (B) During drift block trials, the patient was asked to localize the felt position of his phantom limb - before (baseline) and after neurotactile stimulation/illumination - by moving a green arrow through a keypad. Embodiment questionnaire and drift results from sub-threshold stimulation are shown. (C) The average of 3 embodiment questions ('I felt the touch where I saw the flash', 'It seemed as if the flash caused the tough sensations that I was feeling', 'I felt as if the hand/object that I saw was my left hand') is shown for all the experimental conditions. (D) Drift results across all the experimental conditions are shown. Scores are reported as percentage of phantom hand mislocation: 0% indicates no difference between the perceived positions of the phantom limb before and after neurotactile stimulation/illumination; positive scores indicate a proprioceptive drift toward the virtual hand (index of embodiment); negative scores indicate a drift away from the virtual stimulus.

Those cases provided a priceless contribution to the understanding of the central mechanism of BSC. However, this kind of evidence collected in patients is limited by the time and ethical constraints associated with the invasiveness of the stimulation in a clinical setting. To overcome this limitation, in the first study (**Study 1**) of my thesis, I targeted the M1-hand brain area with transcranial magnetic stimulation while using a novel TMS-VR induced RHI protocol. Specifically, I tested if it is possible to induce embodiment by bypassing the body adopting a sub-threshold stimulation where the TMS pulse does not elicit peripheral effects (i.e. neither MEPs nor TMS-evoked movements) but is sufficient to activate the motor cortex (Kujirai et al., 1993).

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Our results showed that, in line with the existing literature (Riemer et al., 2019), we successfully reproduced the embodiment illusion with our TMS-VR setup using a supra-threshold TMS over the M1-hand area. We showed a modulation for the body ownership (De Vignemont, 2011) and the sense of agency (Haggard, 2017) due to neuro-visual synchronous stimulation between TMS-induced M1 activation and the hand twitches shown by visual VR feedback.

However, differently from our original hypothesis, activation of the motor cortical representation in M1 without any peripheral activity (neither MEPs nor TMS-evoked movements) with sub-threshold stimulation over M1 did not elicit embodiment for a virtual hand.

A study by Collins et al., (2017) showed that hand ownership can be manipulated with invasive brain stimulation in patients undergoing invasive electrocorticographic (ECoG) monitoring. Differently from our study (**Study 1**), they electrically stimulated the somatosensory cortex (SI) at threshold of perception while the patients were observing a rubber hand being stroked. Their results showed higher ownership ratings when the stroking was synchronized and congruent with the electrical somatosensory evoked hand sensation, in line with our supra-threshold TMS stimulation. They concluded that electrical brain stimulation can be used to bypass the peripheral nervous system to induce BSC illusions. This claim is in contrast with our results obtained during sub-threshold TMS, where we showed that merely evoking brain activity in the M1-hand motor area is not sufficient to elicit illusory embodiment. Indeed, as reported by Collins, the ECoG stimulation generated paresthesia on the patients' hand, which makes their stimulation similar to our supra-threshold condition. Furthermore, in another study, I also showed that illusory ownership is mainly driven by the TMS-evoked hand sensations (**Study 3**). Indeed, using a TMS stimulation at perceptual threshold we found that subjective ownership and agency ratings are mainly modulated when participants perceive the stimulation (in form of somatosensory and/or kinesthetic perceptions) even in the absence of peripheral muscular contractions due to TMS. However, other studies (Ferri et al., 2013, 2017) have suggested that tactile stimulation might not be necessary to induce embodiment when the participants are prompted to generate tactile expectancy while observing tactile stimuli approaching a rubber hand superimposed to their own hand. However, this effect due to tactile expectancy seems to be less consistent when compared with the classical visuo-tactile RHI procedure (Guterstam et al., 2019). Altogether, the claim that

it is possible to bypass the body to induce hand ownership modulations does not seem to be fully justified at the current stage. Nevertheless, we must acknowledge that our results are mainly based on questionnaires and the proprioceptive drift. We might speculate that other physiological measures (Armel et al., 2003; Moseley et al., 2008) might provide different results. Furthermore, we cannot claim with certainty what was the extent of the cortical activation driven by our sub-threshold stimulation on the motor cortex, raising the hypothesis that our intensity of stimulation was insufficient to activate the motor representation of the hand. Differently, another study (Rognini et al., in preparation, see figure 4) found embodiment for a virtual hand in an amputee patient affected by phantom limb during intrafascicular multichannel sub-threshold stimulation of the stump. In their study, the stump stimulation was combined with synchronous visual feedback in the corresponding hand part to the somatotopic location of circumscribed touch sensations on the phantom hand, as induced by electrical stimulation. Noteworthy, their electrical stimulation consisted of a neurotacile stimulation of the brain, while our TMS included a predominant neuro-motor involvement mediated by the corticospinal tract. Thus, we can hypothesize that the sensory and motor representations in the brain may have a different sensitivity to sub-threshold stimulation.

A general limitation of the results discussed above is that they are mainly based on questionnaires. In study 1 we proposed an adapted VR version of the proprioceptive drift task (Botvinick & Cohen, 1998; Tsakiris et al., 2006) where, after the illusion onset, participants had to move a virtual cursor to indicate the position of their right index finger. However, a general drift was observed in all the conditions and the task was not repeated in study 3. We proposed that this result might be interpreted as a visual capture effect of proprioception (Kilteni et al., 2015). Noteworthy, there are no standardized procedures for the proprioceptive drift task and the different response modality (verbal or motor) might differently affect the results (Riemer et al., 2019) and it has also been suggested that ownership and proprioceptive drift could be two dissociated phenomena (Rohde et al., 2011). Furthermore, the proprioceptive drift does not necessarily represent an objective measure of proprioception and it has been demonstrated to be susceptible to demand characteristics (Tamè et al., 2018) similarly to questionnaires (Lush, 2020).

# 3.2.2. Novel applications of TMS in the study of BSC

In the first study (**Study 1**) we also extended the results from previous literature investigating the application of NIBS (such as TMS and tDCS) to the topic of embodiment, demonstrating that TMS can be used to induce embodiment. Differently, previous studies rather adopted non-invasive stimulation techniques to measure physiological correlates (della Gatta et al., 2016), or modulate embodiment (Fossataro et al., 2018; Kammers et al., 2009).

The results of our second study (**Study 2**) provide further indications on the relationship between the intensity of TMS and evoked effects. We found that MEPs, TMS-evoked hand sensations and TMS-evoked movements have a different threshold (respectively 100%, 105% and 110% of the individual resting motor threshold), by highlighting the importance of specific somatosensory perceptions evoked by TMS on the stimulated hand. This aspect has been neglected in the literature with only one recent study investigating the detection of TMS stimuli (Bruno et al., 2017), which should be carefully considered in further experiments, here somatosensory perceptions evoked by TMS could play a role. We also investigated the qualitative perceptions evoked by TMS over M1 at the different intensities and we found that lowest intensities mainly evoke mixed (somatosensory and kinesthetic) sensations, while high intensity of stimulation mainly corresponds to kinesthetic perceptions. Overall, in the context of BSC, the stimulation intensity plays a crucial role in defining the central (MEPs, hand perception, or movements) and subjective perceptions (somatosensory or kinesthetic) evoked during M1 stimulation.

In the third study (**Study 3**), we attempted to bypass the conscious perception of the peripheral stimulation using TMS at perceptual threshold, monitoring the subjective detection of the stimuli throughout the experiment. In fact, BSC in normal conditions rely on the constant flow and integration of interoceptive and exteroceptive signals (Park et al., 2019) that are under the threshold for perceptual consciousness (Faivre et al., 2015). Moreover, psychophysics studies on multisensory integration have pointed out that integrative processes are still possible in the absence of consciousness (Mudrik et al., 2014). Related evidence has been reported in pathological conditions such as the numbsense (Rossetti et al., 1995), where stroke patients affected by hemianesthesia for the upper limb are unable to verbally report tactile stimuli on the affected limb, but they could still localize the stimuli pointing with the left unaffected hand.

Similarly, some patients with lesions of the primary visual cortex are not able to consciously process visual stimuli, but their clinical and experimental observation demonstrates that some features of the stimuli are still accessible to their consciousness, affecting their behavior (blindsight, Weiskrantz 1996). This suggests that even BSC might be modulated when the participant does not have conscious access to the stimuli manipulated by the experimenter. This has been first tested by Salomon and coworkers (Salomon et al., 2017) who successfully elicited the full-body illusion while subjects were exposed to a visual masking paradigm. During the experiment, the participants were stroked on the back while observing a virtual avatar, with a Mondrian on the back to mask the visual stimuli, being stroked synchronously. Despite the subjects were unaware of the presence of visual stimuli, they reported higher self-identification with the avatar when the "invisible" visual stimuli were synchronized with the back stroking, in agreement with the multisensory principles of BSC. Similarly, in the case of the amputee patient reported in the previous chapter, he was not aware of the neurotactile stimulation, but the embodiment for the virtual hand was successfully achieved.

Thus, evidence from the literature suggests that the manipulation of bodily signals when participants are unaware of the current manipulation can induce illusory states of BSC. In study 3 (**Study 3**) we attempted to tackle the issue of multisensory integration of unconscious stimuli to induce illusory embodiment for a virtual hand. We adopted the intensity of stimulation levels found in study 2 (**Study 2**) to elicit TMS-evoked hand sensations in half of the trials while participants observed a moving virtual hand. Differently from Salomon (Salomon et al., 2017), we did not find BSC modulations for unconscious multisensory stimulations. When participants were not aware of the peripheral TMS-induced MEPs, their ratings tended to be lower than when the stimuli where perceived. Surprisingly, high ratings were reported even when no MEPs were evoked but participants claimed to have perceived something. This finding could be accounted for by neural mechanisms and the properties of full-body BSC and hand ownership are different (Blanke et al., 2015). Indeed, full-body BSC manipulations imply changes in self-location (Lenggenhager et al., 2011) and self-identification (Ionta et al., 2011) associated with activation of brain areas that extend beyond those involved in body ownership (Grivaz et al., 2017). According to this observation, our study might be not directly comparable with Salomon's study

due to the different processes underlying full-body and body-part specific manipulations. Second, in our study, we used an intensity of stimulation below the perceptual threshold, while in Salomon's work the visual stimuli were unconscious. Accordingly, we may speculate that artificially evoked sub-threshold TMS is harder to be integrated with other stimuli with respect to some more ecological visual subthreshold stimuli.

## 3.3. Methodological relevance

The proposed TMS-VR protocol can provide important technical and methodological advancements concerning the previous rubber hand experiments, allowing fine-grained control of a variety of crucial experimental factors.

First, our setup can accurately control the timing between the stimulation and the visual feedback which can be kept constant across different experimental sessions. Second, the intensity of the stimulation is controlled and individualized based on objective parameters such as the individual resting motor threshold. These two parameters (timing and intensity of the stimulation), are usually neglected in the field, leading to potential issues in the reproducibility of the experiments (Riemer et al., 2019). Indeed, the visuo-tactile stimulation is often directly administered manually by the experimenter, and different stoking tools are used across studies which might have a different impact on hand mechanoreceptors.

Our TMS-VR induced RHI setup not only provides better control over the experimental parameters but also prevents possible interferences due to the experimenter. In psychology, it is known that the experimenter itself is part of the experimental setup and can be a crucial factor in determining the results when his/her behavior affects the participant's performance. The experimenter's behavior, often even not voluntarily, may lead the participant to create demand characteristics (Orne, 1962) influencing the responses based on expectations and interpretation of the experiment purposes. In this regard, our TMS-VR setup is fully automated and may avoid any form of interaction between the experimenter and the subject during the whole experiment. Last, our paradigm allows performing double-blind experiments that otherwise would be hardly possible. Indeed, when the experimenter has to manually perform the stimulation, he also has to know the current condition of stimulation.

## 3.4. Clinical considerations

In the recent years, we have observed an increasing interest for clinical application of BSC illusions (Martini, 2016; Tsay et al., 2015) associated with robotic (Rognini et al., 2016) and VR supports (Pozeg et al., 2017; Rognini et al., 2019; Solcà et al., 2018). For instance, Solcà and coworkers (Solcà et al., 2018) recently showed that immersive virtual reality and multisensory integration based on cardiac signals have an analgesic effect in patients affected by Complex regional pain syndrome (CRPS) for the upper limbs. Similar analgesic effects have reported also in neuropathic pain related to spinal cord injuries (Pozeg et al., 2017).

The proposed TMS-VR setup (**Study 1**) might be a possible solution to face some of the current challenges in the field of neuroprosthetics. The technological advancements have provided sophisticated bionic prosthetics to replace amputated limbs or nonfunctional body parts. However, only 45% of those patients (Jang et al., 2011) decide to use it in everyday life and prefer low-tech devices. Patients that reject the embodiment of the prosthesis (Makin et al., 2017; Pazzaglia et al., 2016) may benefit from our TMS-VR stimulation to facilitate the incorporation of the prosthesis providing the sufficient integration of neural-visual signal that can facilitate the embodiment (**Study 1**).

Another issue in amputees is the painful representation of the missing limb, i.e. the phantom pain (Flor et al., 2001; Ramachandran et al., 1995), where treatments based on multisensory integration or TMS have already been proved to be effective in the pain reduction (Töpper et al., 2003). Our TMS-VR protocol might be a solution to combine those two forms of treatment integrating the benefits of both. Moreover, given that the TMS-VR can be fully automatized, it also minimizes the therapist's intervention allowing the treatment of multiple patients with prolonged stimulation.

Furthermore, we also propose that the detection of TMS-evoked hand sensations (**Study 2**) might be worthy of further investigation in the clinical context of voluntary actions. This could be applied to assess the compromised self-monitoring (Fleminger et al., 2005) in people after traumatic brain injuries and also constitute a measure of post-stroke sensory recovery.

To conclude, we propose future opportunities to integrate and extend our TMS-VR protocol with other therapeutically approaches by providing an automatized and controlled multisensory stimulation protocol to restore the regular flow of sensory information in neurological patients.

## 3.5. Conclusions and outlook

In this present thesis work, I presented a novel application of TMS combined with immersive VR to approach the challenging topic of BSC. I mainly focused on the possibility to induce illusory embodiment for a virtual hand, replicating and extending the previous literature. Thanks to this protocol I was able to investigate the role of bodily signals and their awareness in the context of the embodiment. I also worked on the subjective perception of the peripheral effects of the TMS. Future research should try to extend these results to other body parts and more global aspects of BSC, namely self-identification and self-location. Furthermore, more work is required to better understand the integration of artificially generated stimuli with ecological multisensory stimuli and their neural correlates.

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# Appendix

## Appendix 1: How ageing shapes body and space representations: a comparison study between healthy young and elderly participants

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**Abstract**

To efficiently interact with the external world, the brain needs to represent the size of the involved body parts - body representations (BR) - and the space around the body in which the interactions with the environment take place - peripersonal space representation (PPS). BR and PPS are both highly flexible, being updated by the continuous flow of sensorimotor signals between the brain and the body, as observed for example after tool-use or immobilization. The progressive decline of sensorimotor abilities typically described in ageing could thus influence BR and PPS representations in the elderly. To explore this hypothesis, we compared BR and PPS in healthy young and elderly participants. By focusing on the upper limb, we adapted tasks previously used to evaluate BR and PPS plasticity, i.e., the body-landmark localization task and audio-tactile interaction task, together with a new task targeting explicit BR (avatar adjustment task, AAT). Results show significantly higher distortions in the elderly rather than young participants in the perceived metric characteristic of the upper limbs. We found significant modifications in the implicit BR of the global shape (length and width) of both upper-limbs, together with an underestimation in the arm length. Similar effects were also observed in the AAT task. Finally, both young and elderly showed equivalent multisensory facilitation in the space close to the hand, suggesting an intact PPS representation. Together, these findings demonstrated significant alterations of implicit and explicit BR in the elderly participants, probably associated with a less efficient contribution of bodily information typically subjected to age-related decline, whereas the comparable PPS representation in both groups could be supported by preserved multisensory abilities in elderly participants. These results provide novel empirical insight on how multiple representations of the body in space, subserving actions and perception, are shaped by the normal course of life.

**Keywords: ageing; body representation; multisensory integration; peripersonal space**

## 1. Introduction

We directly interact with the external world via our physical body; to do so, our brain needs an internal representation of the dimension of the body parts involved in those interactions (i.e. body representations, BR) (e.g. de Vignemont, 2010; Longo, Azañón, & Haggard, 2010a; Schwoebel & Coslett, 2005), as well of the space where those interactions occur, i.e., a representation of the space immediately surrounding our body, called peripersonal space (PPS) (Rizzolatti, Fadiga, Fogassi, & Gallese, 1997; Serino, 2019). Several behavioral, neurophysiological and imaging

studies have described these representations in young, healthy individuals (for recent reviews Bufacchi & Iannetti, 2018; Cléry & Ben Hamed, 2018; Noel, Blanke, & Serino, 2018; Riva, 2018; Serino, 2019; Serino et al., 2015). Regarding the size and the shape of the different body parts, authors have suggested that an implicit model of the metric proprieties of the body is stored in the brain (Longo & Haggard, 2010, 2012b) and it is updated through on-line peripheral signals, such as sensorimotor, proprioceptive and kinesthetic inputs coming from the skin, the muscles and the joints as well as visual bodily information (de Vignemont, 2010; Longo, Azañón, & Haggard, 2010; Medina & Coslett, 2010; Serino & Haggard, 2010). Concerning the PPS, single neuron data in non-human primates (Cléry, Guipponi, Wardak, & Ben Hamed, 2015; Graziano & Cooke, 2006) as well as neuropsychological (Pavani, Làdavas, & Driver, 2003), neuroimaging (e.g., Grivaz, Blanke, & Serino, 2017; Makin, Holmes, & Ehrsson, 2008) and behavioral (Serino, 2019) studies in humans indicate that the representation of the PPS is coded by the special interaction between somatosensory signals coming from a specific body part (e.g. face, hand and trunk (Serino et al., 2015)) and external visual or acoustic stimuli presented close, but not far from that specific body part.

Furthermore, studies also indicate that both BR and PPS are built and continuously updated by signals from different sensory modalities (Dijkerman & Lenggenhager, 2018; Kandula, Van der Stoep, Hofman, & Dijkerman, 2017; Maravita, Spence, & Driver, 2003; Salomon et al., 2017), implying that BR and PPS are not fixed, but plastically modified through the continuous flow of sensorimotor information arising from the interactions with the environment.


A paradigmatic example of the plasticity of BR and PPS is the use of the tools allowing to reach objects located in the far space (e.g., Maravita & Iriki, 2004; Martel, Cardinali, Roy, & Farnè, 2016; Miller et al., 2018). Studies in non-human primates, patients and healthy participants have demonstrated that short or long experiences with tools (Bassolino, Serino, Ubaldi, & Làdavas, 2010; Biggio, Bisio, Avanzino, Ruggeri, & Bove, 2017; Maravita & Iriki, 2004; Serino, Bassolino, Farnè, & Làdavas, 2007) affect PPS representation, for instance by increasing multisensory interactions between stimuli on the body and in the far space (see for a review Maravita & Iriki, 2004). Similarly, plasticity of BR after tool-use has been reported both in terms of kinematic changes and modifications of the perceived limb dimensions (Canzoneri et al., 2013; Cardinali et al., 2011, 2009; Garbarini et al., 2015; Romano, Uberti, Caggiano, Cocchini, & Maravita, 2018; Sposito, Bolognini, Vallar, & Maravita, 2012). Moreover, BR and PPS are also modifiable by

reduced use of the upper limb as during immobilization in healthy participants (Bassolino, Finisguerra, Canzoneri, Serino, & Pozzo, 2014; Toussaint, Wamain, Bidet-Ildei, & Coello, 2018).

The plastic properties of BR and PPS, driven by sensorimotor experiences, also suggest that these representations could be modified and updated during the entire life span (Bremner, 2017; Lewkowicz & Ghazanfar, 2009). For instance, considering the important changes in body size in children (Bremner, Holmes, & Spence, 2008), BR and PPS representation have to evolve across the development in childhood. Similarly, it is possible to suppose that BR and PPS could be affected in elderly people by the functional decline of primary sensory inputs and/or motor function during normal ageing. For instance, functional, physiological and anatomical changes in the hand have been reported in the elderly, such as a reduction of the muscles mass and strength ("sarcopenia of old age"), together with a decrease of sensitivity and motor performance (Carmeli, Patish, & Coleman, 2003). Moreover, it has been shown that tactile and auditory thresholds are significantly increased in elderly compared to young people, probably due to a decrease in density or distributions and degeneration of the dedicated receptors (Wickremaratchi & Llewelyn, 2006; Liu & Yan, 2007). Similarly, several findings suggest that proprioception (Adamo & Brown, 2007; Goble, Coxon, Wenderoth, Van Impe, & Swinnen, 2009; Shaffer & Harrison, 2007) and vision (Owsley, 2011) are vulnerable to ageing.

Together these data show that sensory and motor functions that impact BR and PPS are prone to ageing-related changes (Costello & Bloesch, 2017). However, whether BR and PPS are also affected by natural ageing is still unknown, since research about their plastic properties has mainly focused on children or young adults. To investigate this topic, in the present study, we compared BR and PPS of the upper limbs in young and elderly healthy participants. To evaluate BR, we used: (i) an adaptation of an implicit task, the body-landmark localization task (BL), already validated in the literature to implicitly capture the perceived dimensions of one's own upper-limbs (Bassolino et al., 2014; Canzoneri et al., 2013); and (ii) a new explicit task, assessing the explicit estimation of one's own upper-limbs dimensions by asking participants to adjust the width and the length of the upper-limbs of a realistic human avatars presented on a screen (avatar adjustment task, AAT, see paragraph 2.2.2 for details). To study the PPS representation, we used an adaptation of the audio-tactile interaction task previously used in young adults to capture PPS

representation's plasticity after tool-use (Canzoneri et al., 2013) and immobilization (Bassolino et al., 2014).

## 2. Methods

### 2.1. Participants

A group of 30 young participants and a group of 52 elderly participants were recruited in total for the study. To avoid fatigue that could have affected the performances, elderly participants were divided in two groups: a first group of 29 participants undergoing the BL task and the AAT in two separate randomized sessions performed on the same day, and a second group of 29 participants performing the PPS task (only 23 subjects were finally included in the analysis, see paragraph 2.3 for explanations). Young participants performed the three tasks in different sessions on the same day. The order among the tasks and the testing side (right or left upper-limb) was randomized among participants. Information related to the age, gender and education in the groups of participants performing each task is shown in Table 1.

All the participants were right-handed as confirmed by the Flinders Handedness Survey (Nicholls, Thomas, Loetscher, & Grimshaw, 2013), had a normal or corrected-to-normal vision, hearing and touch, no psychiatric or neurological deficits, no pain or sensorimotor pathologies in the upper-limbs or fractures in the previous 12 months. Participants in the elderly group did not show any cognitive impairment as assessed by the Montreal Cognitive Assessment (MOCA) (all equivalent scores $\geq 2$) (Conti, Bonazzi, Laiacona, Masina, & Coralli, 2015).

Participants were all naïve to the purpose of the study and participated after giving informed consent. The study was conducted with the approval of the local ethics committee (Commission Cantonale Valaisanne d'Ethique Médicale, CCVEM 017/14).

| TASK | GROUP | AGE (years) | EDUCATION (years) | GENDER (female) |
|---|---|---|---|---|
| BL | Young (n=29) | 25.24 ± 3.31 (20-33) | 14.93 ± 2.98 (9-20) | 21 |
| | Elderly (n=29) | 71.76 ± 7.81 (53-86) | 12.62 ± 3.62 (6-18) | 24 |
| AAT | Young (n=30) | 25.48 ± 3.34 (20-33) | 14.74 ± 2.98 (9-20) | 20 |
| | Elderly (n=29) | 71.52 ± 8.07 (53-86) | 12.45 ± 3.47 (6-18) | 24 |
| PPS | Young (n=29) | 25.31 ± 3.34 (20-33) | 14.72 ± 3.03 (9-20) | 19 |
| | Elderly (n=23) | 73.48 ± 8.20 (61-91) | 12.30 ± 4.22 (3-23) | 18 |

**Table 1.** Demographic information. For each task, it is indicated the number of participants included in the two groups (young and elderly) with the related average age and education (mean values ± standard deviation and the range in brackets) as well as the gender distribution (number of female participants).


## 2.2. The experimental tasks

### 2.2.1. Implicit body representation task: the body-landmark localization task (BL)

To assess the implicit perceived dimension of the upper-limbs, we adapted the body-landmark localization task (BL) (Bassolino et al., 2014; Canzoneri et al., 2013; Longo, 2018). The BL task can be considered implicit because participants had to indicate only the locations of some anatomical landmarks, while not providing explicit judgments about width or length of the body parts, that are then reconstructed a posteriori during the data analysis (e.g., Fuentes, Longo, & Haggard, 2013; Longo, 2015). Precisely, participants were instructed to verbally indicate when a moving marker reached the felt position of one of five possible non-visible anatomical landmarks that were: the tip of the index finger, the tip of the annular finger, the internal part of the wrist (the radius styloid), the external part of the wrist (the ulnar styloid) and the elbow joint (the olecranon).

Participants were seated on a chair with the forearm (left or right, depending on the condition) resting palm down on a table, aligned with the shoulder and positioned 20 cm far from the body midline and 10 cm far from the border of the table. To avoid movements and to standardize the position, the participants' forearm was fixated to the table for the entire duration of the experiment

while the hand was resting on a computer mouse. Before starting the experiment, while participants were blindfolded, we recorded the actual position of the five anatomical landmarks. Afterward, subjects removed the eyeshades to perform the task. During the task, to prevent participants from viewing their arm, we positioned a wooden table (80 cm x 80 cm) above the arm, and we put an additional cloth to impede the view of the shoulders. During each trial, the experimenter showed on her body the target landmark to judge. Then, the experimenter manually moved a marker attached to a wooden stick over the surface of the table, along the longitudinal axis of the forearm. Participants were instructed to say "Stop" when the marker was perceived just above the felt position of the target anatomical landmark. At that signal, the experimenter stopped the movement, leaving the marker where indicated. Importantly, participants were allowed to further adjust the position of the marker by asking the experimenter to move it backward, forward or laterally and, following the final confirmation, the marker's location was recorded.

The task comprised five blocks in which we recorded the five landmarks, randomized between blocks, for a total of five repetitions for each landmark (see Figure 1). Data were collected for both left and right upper-limb in randomized order among participants.

We used retro-reflective markers (1 cm of diameter) recorded using an optical motion capture system (OptiTrack V120: TRIO; Motive 1.7.5 Final 64-bit, 2015) and a custom-made script in Matlab (R2018a). Propaedeutic data analysis was performed using a custom-made script written in Matlab.
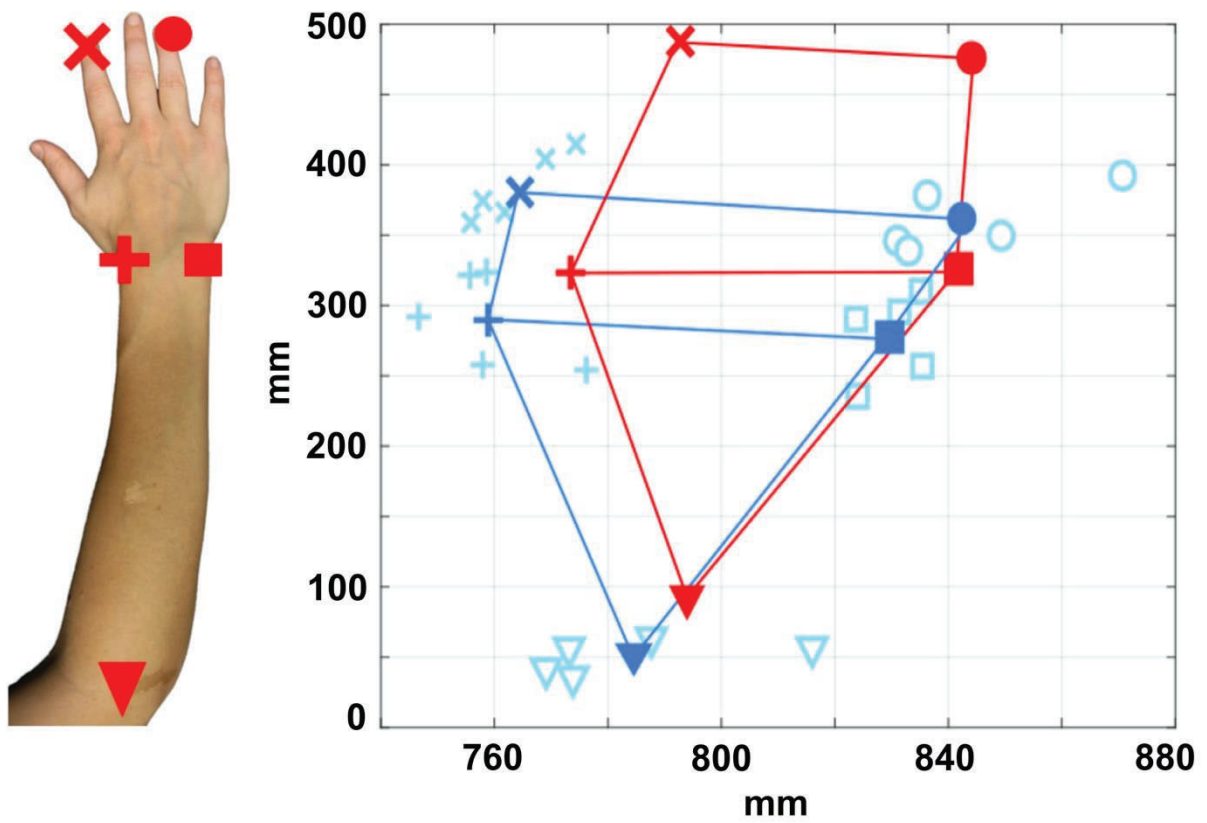
**Figure 1. The body-landmark localization task.**

Panel A shows the five anatomical landmarks that were recorded during the task: the tip of the index finger (oblique cross), the tip of the annular finger (dot), the internal part of the wrist (the radius styloid, plus), the external part of the wrist (the ulnar styloid, square) and the elbow joint (the olecranon, triangle). Panel B shows the reconstruction of the actual positions of the anatomical landmarks, recorded at the beginning of the experiment (in red) as well as the reconstruction of the perceived position recorded for each landmark on every single trial (five repetitions for each landmark, light blue) and averaged among repetitions (dark blue) in one simulated subject (the horizontal displacement is depicted on the x, mm, while the vertical ones on the y, mm).

## 2.2.2. Explicit body representation task: the avatar adjustment task (AAT)

Here, we propose a novel procedure to explicitly assess the perceived dimension of the participants' upper-limbs with respect to a visual model. During the experiment, participants were seated in front of a monitor (52X32 cm) with a white cloth covering their shoulders, arms and

hands. A distorted body model on an avatar matching the participant's characteristics of age and gender was showed in diagonal view (see Figure 2). In each trial, participants were instructed to modify the dimensions (i.e., the width or the length) of the observed avatar's hands or arms to resemble their occluded body parts. To do so, they had to verbally guide the experimenter who operated on a keyboard to adjust the avatar's body parts dimensions, making them longer or shorter, fatter or narrower. The starting dimension of the body model was extremely enlarged in one-half of the trials and extremely shrunk in the other half. Divided into two blocks, we recorded four trials for each dimension (width and length, two trials with each starting position) and each body parts (arm and hand), for both left and right side, for a total of 32 trials (16 for each block).

The avatars were designed by using an open-source tool capable of producing realistic virtual humans (Make Human, http://www.makehumancommunity.org/ , Accessed 11 October 2018). The avatar respected anatomical characteristics of the general population considering the age and gender (Bastioni, Re, & Misra, 2008).

The software developed for the task was written in Phyton programming language (http://www.makehumancommunity.org/frontpage/makehuman_110_has_been_released.html/ , Accessed 11 October 2018).
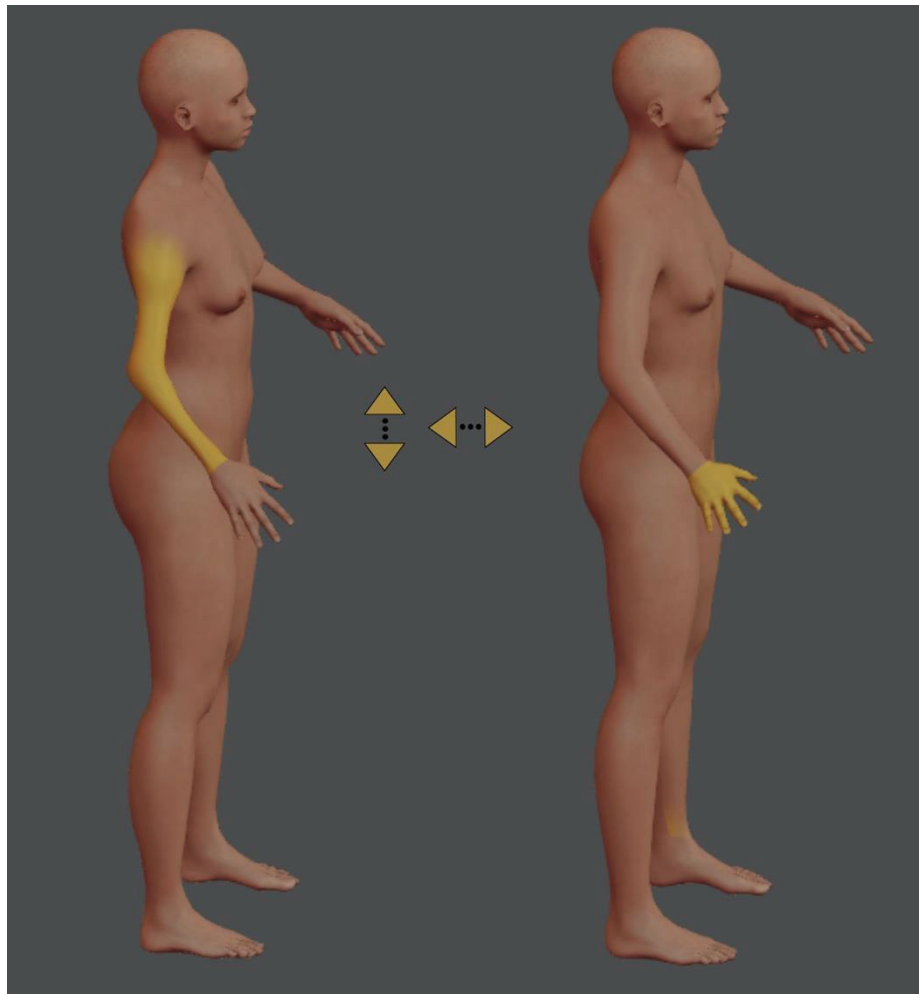
**Figure 2. The avatar adjustment task.**

The figure shows an example of the avatar used in the avatar adjustment task (AAT). At the begging of the experiment, the avatar's dimensions were set to match the anatomical characteristics of the general population groups of the same age and gender of each participant. During the task, the participant guided the experimenter who operated on a keyboard to adjust the avatar's body parts (hand or arm) dimensions (in yellow) by making them longer or shorter (length), fatter or narrower (width), to resemble his/her body parts.

### 2.2.3. Peripersonal space task (PPS)

We adopted the audio-tactile interaction task previously used in Canzoneri et al. (Bassolino et al., 2014; Canzoneri, Magosso, & Serino, 2012; Canzoneri et al., 2013; Serino et al., 2015) to evaluate PPS representation around the left and right hand. Participants were blindfolded and seated in a comfortable position with their arm (left or right, depending on the condition) resting on a table.

The acoustic stimuli consisted of a dynamic broadband noise looming toward the participants' hand. Two loudspeakers generated the sound: one was positioned near the participant's hand (0 cm), and the other one placed 100 cm distant from the near speaker (i.e., far from the participants' hand). To give the impression of approaching to the subject's body, the sound was manipulated in intensity (Canzoneri et al., 2012). For the tactile stimulation, we used a single vibrotactile device that was placed on the dorsum of participant's hand (Precision MicroDrives shaftless vibration motors, model 312–101, 3 V, 60 mA, 9000 rpm, 150 Hz, 5 g). Tactile stimulation lasted 100 ms. Acoustic and tactile stimuli were delivered in a controlled manner using an in-house software (ExpyVR; http://lnco.epfl.ch/expyvr/ , Accessed 11 October 2018), also used to store the data.

Participants were informed that during the task, they would feel a single tactile vibration and hear a sound coming from the speakers. They were asked to answer as fast as possible to the tactile stimulation by pressing a foot pedal with their right foot and to ignore the non-informative looming sound. The crucial manipulation of this task consists on the fact that the tactile stimulus was randomly presented at one out of three temporal delays (D3, D2 and D1) from sound onset, i.e. when the sound was perceived at one out of three possible distances from the body (D3/far= 0.3 s; D2/medium= 1.5 s; D1/near= 2.7 s). The correspondence between the temporal interval from the sound onset and the spatial distance between the sound and the touch location matched linearly and negatively (Serino et al., 2015). Similarly, also unimodal trials (only tactile) were administered at three different delays, corresponding to the equivalent timing of the farthest, medium and the nearest distance of sound. According to previous works (Serino et al., 2015, 2018), unimodal trials were considered as a baseline and were used to normalize audio-tactile trials. The rationale of this task is based on the fact that a multisensory facilitation effect in bimodal versus unimodal trials is expected on tactile RTs due to sounds presented close, i.e., within the PPS, but not far from the body. Such multisensory effects have been used as a proxy of PPS representations (Noel, Pfeiffer, Blanke, & Serino, 2015; Serino et al., 2015). In total, the trials consisted of 20 bimodal trials for each temporal delays (20x3), in which participants heard a sound and at a given moment in time they received the tactile stimulation; 10 unimodal trials for each temporal delays (10x3), in which the tactile stimulation was delivered in the absence of auditory stimulation. In addition, 20 catch trials with only auditory stimuli were included to control for automatic motor response. During each trial, the sound lasted 3 s and participants had 2 s, starting from the moment in which the tactile stimulus was delivered, to answer to the stimulation. Inter-stimulus intervals were randomized between 0.5, 0.75 and 1 s.

*Unimodal sound localization task*. In order to demonstrate that participants actually perceived the looming sound in the three different locations (far, medium and near) at the three possible delays (D3, D2 and D1) in both groups, all participants from elderly group (N= 23) and a subgroup from the young group (N= 23) underwent a sound localization task, following a procedure already used in previous works with young participants (e.g. Canzoneri et al., 2012; Finisguerra, Canzoneri, Serino, Pozzo, & Bassolino, 2014). During the task, participants were seated in a chair with the eyes closed and with their arm resting on a table in front of them. While listening to the looming sound, participants received a tactile stimulation on the right hand at one of the three temporal delays in a randomized order. After each trial, participants opened the eyes and were instructed to verbally report at which distance was the sound when they perceived the tactile stimulation. To answer, they referred to a meter positioned on the table with visible digits ranging from 0 cm to 100 cm. This task was always done after having performed the audio-tactile interaction task on both sides.

## 2.3. Data analysis

*Body-landmark localization task*. To calculate the width and length of the two body parts (hand and arm), we considered the position (real and perceived) of the five landmarks (see Figure 1). The hand length was calculated as the mean of the distance (considering both horizontal and vertical coordinates) between the markers on the fingers and the wrist. More precisely, it was calculated as the mean value between two distances, i.e., the distances between the tip of the index and annular finger and the internal and external part of the wrist, respectively. The arm length was obtained calculating the mean between the markers on the wrist and the elbow. Specifically, it was computed as the mean between two distances, i.e., the distances between the internal and external parts of the wrist and the elbow. The hand width was calculated as the distance between the tip of the index and annular fingers, while the arm width was obtained calculating the distance between the internal and external parts of the wrist. Then, for each participant, we calculated an index of the bias in the perceived dimension with respect to the actual one (estimated dimension, e.g. Peviani & Bottini, 2018), as the ratio between the perceived and the real size for each body parts (arm length, arm width, hand length, hand width). In this way, values below 1 represent an underestimation of the perceived dimension with respect to the real one, and values above 1 indicate an overestimation. Moreover, similarly to previous studies (Longo et al., 2010), we calculated a global index of the perceived shape of the arm and the hand. In the present work, we calculated for each subject the ratio between the estimated dimension

(i.e., the ratio between the perceived and the real size) on the width and the length, for both the arm and hand (from here on, Normalized Shape Index). In this case, values higher than 1 indicate a higher estimated dimension for the width with respect to the length of the body segment. For this analysis, we excluded data from one subject in the elderly group that turned out to be an outlier (+ 2 SD) with respect to the mean of his group.

*Avatar adjustment task.* Data analysis was performed on the estimated length and width of the arm and hand expressed as a percentage of overestimation or underestimation with respect to the average (i.e., unbiased) size of the body model (i.e., the size corresponding to the reference population of equal age and gender provided by the software (Bastioni et al., 2008, see Figure 2). Similarly to the BL task, values above 1 indicated an overestimation and values below 1 indicated an underestimation with respect to the average size of the reference population (=1). We calculated the mean of the data collected in all trials for each dimension and body parts (arm length, arm width, hand length, hand width). Finally, as in the BL task, we calculated the Normalized Shape Index as a general index of the perceived shape of the arm and the hand, by dividing the estimated dimension between the width and the length. As defined above, values higher than 1 indicate higher values on the width with respect to the length. Namely, participants showed a higher estimated dimension for the width than for the length.

*Peripersonal space task.* Before proceeding with the analysis of interest based on RTs, we had to ensure that the accuracy was comparable in the two groups. Thus, we calculated a general index of accuracy considering the percentage of correct trials participants answered to, with respect to the total of the administered tactile trials. Note that the PPS task has been designed to assess speeded RTs to easy-to-detect tactile stimuli, to avoid the confounding effect of speed-accuracy trade-off or post-perceptual metacognitive decision processes. Thus, it is important that accuracy from participants involved in the RTs analysis demonstrated a sufficiently high accuracy. Based on this index, we decide to exclude 4 participants from the elderly group because they had an accuracy level below 60%. Moreover, we decided to exclude the data from other 2 participants from the elderly group because they turned out to be outliers (+ 2 SD) with respect to the mean of the group. Once these subjects were excluded, the accuracy was 99.2% for the young group and 97.8% for the elderly group, with no difference between the two groups (Wilcoxon rank sum test:

W= 1156, p= 0.09).

Data analysis was performed on RTs. We expect that, when the acoustic stimulus reach and exceed the boundaries of the hand-PPS, sounds interact in a multisensory way with tactile processing, resulting in faster RTs to tactile stimuli delivered to the hand, compared to unimodal tactile stimulation (Canzoneri et al., 2012; Serino et al., 2015, 2018). For each subject, we first calculate the mean RT to tactile stimuli for every temporal delay both for unimodal and bimodal trials. We removed from the analysis all the trials exceeding 2 standard deviations from the mean RT (outlier trials). We then identified, on the individual basis, the baseline unimodal condition, which is the fastest mean RT among the unimodal tactile conditions, and we subtracted this value from the mean RT to tactile stimulus in the audio-tactile conditions for each distance. In this way, we show a facilitation effect on tactile RTs due to auditory stimulation with respect to the fastest unimodal tactile RT. Accordingly, negative values (below the baseline, that by definition is zero) indicate a multisensory facilitation effect (Serino et al., 2015).

## 2.4. Statistics

To compare performances between young and elderly groups, we ran the main statistical analysis using Linear Mixed Effect Models (LMM) with the software R Studio (R Core Team, 2017, http://www.R-project.org/). The use of LMM is justified by a model selection based on Akaike's Information Criterion (AIC) and Bayesian information criterion (BIC), always showing better parameters for LMM rather than ANOVA. In all the analyses, we considered participants as random effect. Additional random effects were added based on a model selection with AIC and BIC values. For fixed effects, p-values were obtained by likelihood ratio tests and degrees of freedom were approximated by using the Satterthwaite method. In the BL and AAT tasks, for the main analysis run on the estimated dimension, we considered as fixed effects the body parts dimensions (4 levels: arm length, arm width, hand length, and hand width), the side (2 levels: left and right) and the group (2 levels: young and elderly). For the Normalized Shape Index, we considered as fixed effects the group, the side and the body parts (2 levels: arm, hand).

In the PPS task, for the task on sound localization, we considered as fixed effects the temporal delays (3 levels: D3, D2, and D1) and the group (2 levels: young and elderly), while for the main analyses on RTs to the unimodal tactile stimuli and the bimodal audio-tactile stimuli, we considered as fixed effects the hand (2 levels: left and right), the temporal delays (3 levels) and the group (2 levels). After significant triple interaction, further analyses were applied to explore

triple interactions. Otherwise, the Tukey post hoc test was used to check for multiple comparisons. In addition, linear fittings were performed on the responses obtained at the unimodal auditory localization task and unimodal tactile perception task using a custom-made script written in Matlab. The estimated individual slopes were compared between the two groups with the appropriate statistical tests (i.e., two sample t-test or Wilcoxon rank sum test depending on results of the Shapiro-Wilk normality test).

Finally, as additional analysis, responses to bimodal audio-tactile stimuli were fitted with linear and step-like functions. The root mean square error (RMSE) was considered as an index of the best fit between the models within groups. RMSE was compared by using the Wilcoxon signed rank test. In all analyses, the significance threshold was set at 0.05.

## 3. Results

*Body-landmark localization task.* The model ($R^2$= 0.75) on the estimated dimension (i.e. ratio between the perceived and the real size) revealed a significant interaction between the body parts and the group (F $(3, 71.67)$ = 6.551; p= 0.001), regardless of the side (body parts X side X group: F $(3, 290)$ = 0.378; p= 0.769). Tukey post hoc comparisons revealed an underestimation of the arm length in the elderly participants that was significantly different from the young participants (p= 0.0001). No differences emerged in the comparison between the two groups in the arm width (p= 0.056), hand length (p= 0.879) and hand width (p= 0.228) (see Figure 3 A/C).

We then ran a model on the Normalized Shape Index (the ratio between the normalized width and the length) for the arm and the hand. The model ($R^2$= 0.81) revealed a main effect of body parts (F $(1, 57.45)$ = 40.57; p < 0.001) with a higher bias for the hand with respect to the arm, independently from the side and group (side X group X body parts: (F $(1,136.6)$ = 0.075; p = 0.784). Crucially, also the main effect of the group emerged (F $(1, 51.24)$ = 13.05; p = 0.001), with a higher bias in the elderly group compared to the young participants, without any significant difference in the two body parts (group X body parts: F $(1,57.45)$ = 0.08; p= 0.779) or side (group X side: F $(1,136.6)$ = 0.117; p= 0.733) (see Figure 3 B/D).

Taken together, these data indicate that elderly participants perceived their arms as shorter than the actual size and than the younger participants (Figure 4), a bias that seemed common between the two sides (left or right arm). Moreover, we found a distortion in the global shape of the arms,

indicating a higher bias on the overall aspect ratio between the estimated width (slightly underestimated) and length of the arm (clearly underestimated) in the elderly with respect to the young group. This higher distortion of the global shape of the arm in elderly rather than in young seems true also for the hand given that no significant effects between body parts (hand, arm) emerged, although the analysis on the estimated dimension of the length or width did not differ between the two groups for the hand.
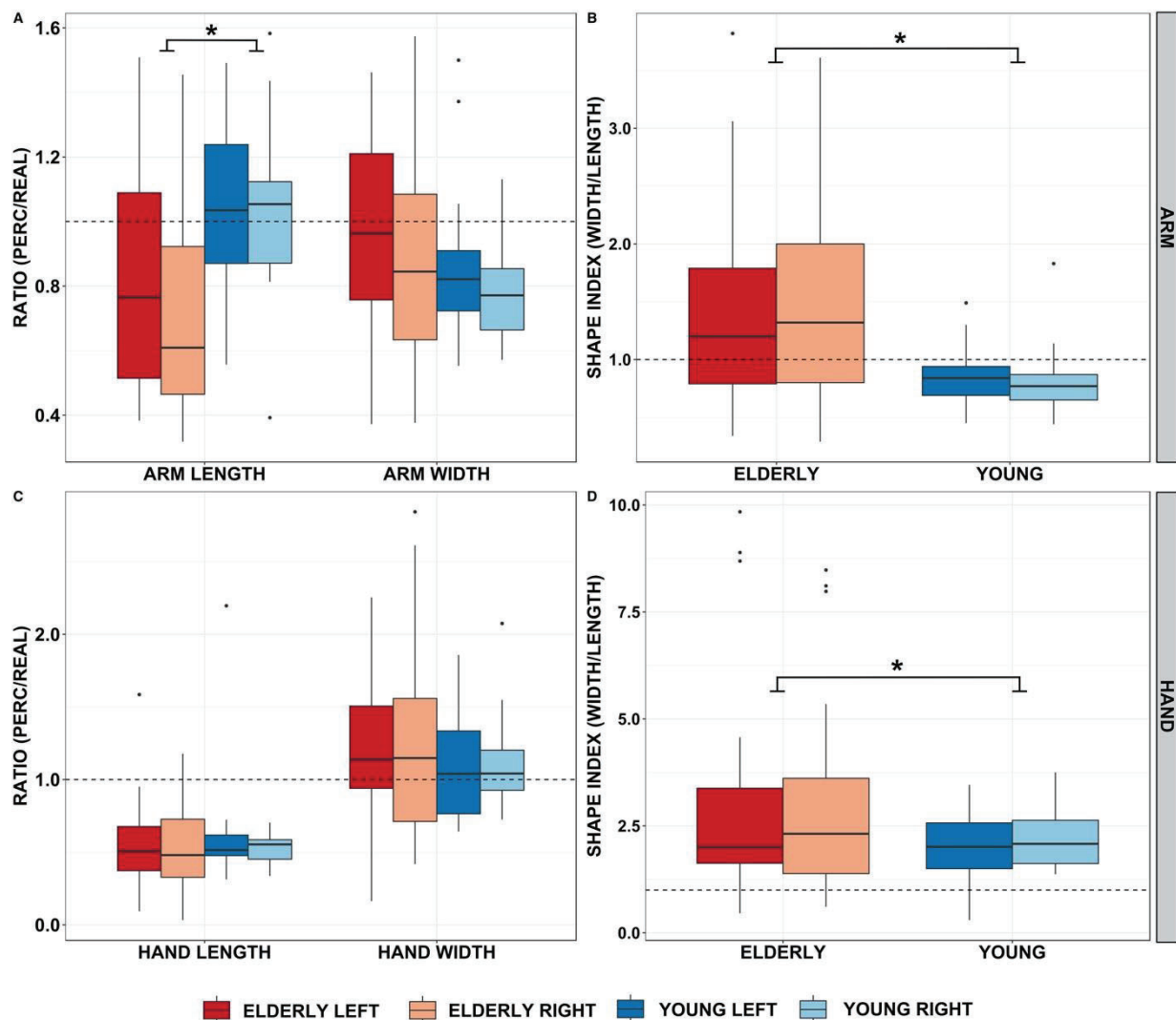


**Figure 3. Distortions in implicit body representation in healthy ageing.**

The panels on the left represent the estimated width and length (i.e. ratio between the perceived and the real size) of the arm (A) and hand (C). Values below 1 (thin dashed line) indicate an

underestimation of the perceived dimension with respect to the real one, while values above 1 indicate an overestimation.

The panels on the right (B and D) show the Normalized Shape Index of the arms (above) and the hands (below), respectively, expressed as the ratio between the estimated width and length. Values higher than 1 indicate a higher estimated dimension on the width with respect to the length. In all panels, black brackets with asterisks above the boxes indicate a significant main effect between the groups. Data are represented through boxes indicating the first (lower hinges) and third quartiles (upper hinges), with internal lines for the median, whiskers for the largest (upper) and the smallest (lower) value ≥ 1.5 * the inter-quartile range, and black points for data beyond the end of the whiskers.
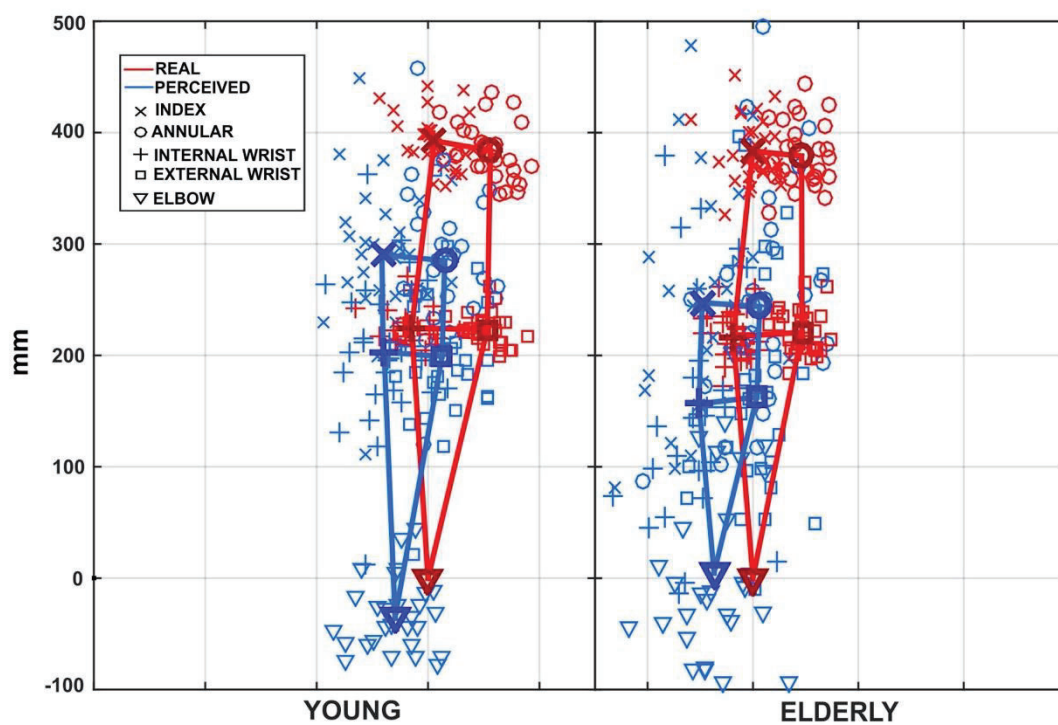


**Figure 4. Elderly participants show greater distortions in implicit body representation**

The figure presents the localization of the real (red) and of the averaged perceived (blue) position of every anatomical landmark on the right upper-limb for each participant. The five landmarks are represented with 5 different symbols (see the legend and Figure 1). The symbols in bold indicate the group average position.

*Avatar adjustment task.* The model ($R^2 = 0.79$) on the estimated length and width of the arm and the hand revealed a significant triple interaction between body parts, side, and group ($F_{(3,295)} =$

6.491; p < 0.001). To further explore this interaction, we ran separated analyses for each body part (arm and hand) and dimension (width and length) (see Figure 5).

ARM LENGTH. Elderly participants underestimate the length of their left arm with respect to their right arm (p < 0.001) and to the young participants' left arm (p = 0.002) (model: $R^2$ = 0.80; interaction: F (1,59) = 13.62; p < 0.001). No difference between sides was present in the young group (p= 0.1554) (Figure 5 A).

ARM WIDTH. No difference between the two groups emerged (model: $R^2$ = 0.87; group: F (1,59) = 2.021; p= 0.16; group X side: F (1,59) = 0.398; P= 0.531), while a main effect of the side (F (1,59) = 39.21; p < 0.001) was found indicating that the arm width of the left side is overestimated with respect to the right side (p < 0.001) in elderly and young participants . (Figure 5 A).

HAND LENGTH. The model ($R^2$ = 0.79) on the hand length revealed a main effect of the side (F (1,59) = 18.16; p < 0.001) and a main effect of group (F (1,59) = 15.66; p < 0.001). Besides a similar difference in the two groups between the left and right hand (i.e. a higher underestimation for the right than the left hand), a greater underestimation appeared in elderly than in young, on both sides (side x group: F (1,59) = 1.765; p= 0.189) (Figure 5 C).

HAND WIDTH. A side-related distortion emerged in the young group, with a higher underestimation of the width for the right hand with respect to the left hand (p < 0.001), not present in the elderly group in which the perceived width of the two hands was comparable (p= 0.379) (model: $R^2$ = 0.66; interaction: F (1,59) = 26.13; p < 0.001) (Figure 5 C).

NORMALIZED SHAPE INDEX. We first considered the Normalized Shape Index of the arm and the hand together. The model ($R^2$ = 0.92) revealed a significant triple interaction between the body parts, side and group (F (1,59) = 34.1; p < 0.001). To further explore this interaction, we ran separated analyses for each body parts (see Figure 5 B). The model ($R^2$ = 0.81) on the shape index for the arm showed a significant interaction between side and group (F (1,59) = 4.717; p= 0.034). Post hoc comparison revealed that both elderly and young participants have a higher distortion in the overall aspect ratio between width and length for their left arm with respect to the right arm (all p < 0.01). Crucially, elderly participants' left arm was more distorted then the homologous of the young group (p < 0.001), while this was not the case for the right arm between the two groups (p= 0.202) (Figure 5 B). The model ($R^2$ = 0.63) on the Normalized Shape Index for the hand revealed a significant interaction between side and group (F (1,59) = 10.99; p = 0.002). Post hoc comparison showed that young participants show a bigger bias for the left than the right hand (p= 0.004), similarly to the arm, while the two sides are similarly perceived in the elderly (p=

0.082). Comparing the two groups, the right hand seems more biased in the elderly than in the young group (p < 0.001), while this was not the case for the left (p= 0.933) (Figure 5 D).

Taken together, these data show a distortion in the length (underestimation) and the global shape of the arm in the elderly participants with respect to the young participants, for the left arm. Moreover, elderly participants perceived both hands as shorter than young participants with a higher distortion of the global shape of the hand that was higher on the right side in the elderly than in young participants.
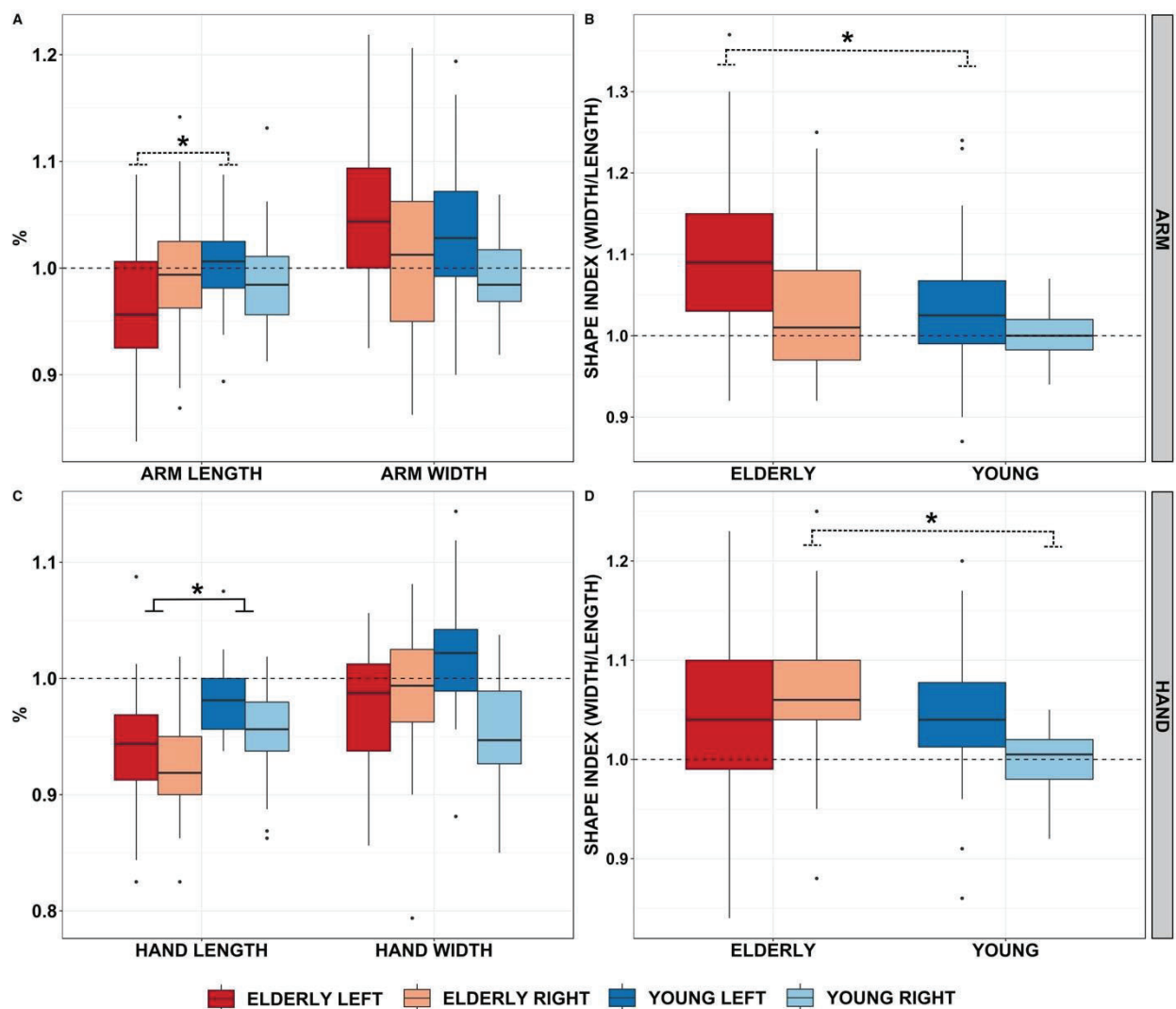
**Figure 5. Distortions in the explicit body representation in healthy ageing.**

The panels on the left, represent the estimated dimensions of the arms (A) and the hands (C), expressed as a percentage of overestimation (above 1) or underestimation (below 1) with respect to the average size of the body model (1, thin dashed line) at the avatar adjustment task.
The panels on the right show the Normalized Shape Index of the arms (B) and the hands (D), calculated by dividing the ratio between the width and the length. Values higher than 1 indicates higher values on the width with respect to the length.
Asterisks highlight only significant main effect between groups (black brackets) and interactions between the groups and sides (black dashed brackets). Data are represented through boxes indicating the first (lower hinges) and third quartiles (upper hinges), with internal lines for the median, whiskers for the largest (upper) and the smallest (lower) value ≥ 1.5 * the inter-quartile range, and black points for data beyond the end of the whiskers.

*Peripersonal space task.*

The PPS task involves audio-tactile stimulation and thus, before assessing any multisensory interaction, we firstly analyzed participants' ability to process auditory (auditory localization task) and tactile (unimodal tactile perception) stimuli constituting the multisensory PPS task.

*Auditory localization tasks.* For the task of sound localization, we calculated, for each subject, the mean of the distances at which the sound was perceived when the tactile stimulus was given at each of the three temporal delays (see Figure 6 A). The model ($R^2$= 0.77) on the perceived distances revealed a significant interaction between temporal delays and group (F (2, 91.999) = 14.448; p < 0.001). Post hoc comparisons revealed that both groups were able to distinguish the source of the sound at the three different distances according to the different temporal delays (all p < 0.001, see Figure 6 A). Moreover, it showed that young and elderly participants perceived at the same distance sound at the medium temporal delays (p= 0.3718), but they perceived the first and the last ones differently. In particular, elderly perceived sounds at the first temporal delay (D3) at a closer distance (p < 0.001, difference between elderly and young: 18.22 cm), and at the last (D1) at a farther distance (p < 0.003, difference between elderly and young: 12.85 cm) with respect to the young. To better characterize these effects, the perceived distances were fitted with a linear function (young: $R^2$= 0.99; elderly: $R^2$= 0.99) and individual slopes were compared between the two groups. Data revealed flatter slopes for elderly participants than for young participants (Shapiro-Wilk normality test, elderly: W= 0.90138, p= 0.027; young: W= 0.94243, p= 0.3284; Wilcoxon rank sum test: W= 119.5, p= 0.001). To summarize, data from the auditory localization task confirmed that elderly participants were able to perceive the three different distances of the

sounds in space corresponding to the three temporal delays, although with a decreased precision in sound distance estimation, as indicated by flatter slopes.

*Unimodal tactile perception.* We compared between young and elderly participants RTs to unisensory tactile stimuli for each temporal delay with respect to sound onset. The model ($R^2$= 0.94) on unimodal RTs revealed a significant interaction between side and group ($F_{(1, 52)}$ = 4.193, p = 0.046) and crucially, between group and temporal delays ($F_{(2, 208)}$ = 4.336, p = 0.014) (Figure 6 B). Post hoc comparison revealed slower RTs for the right side with respect to the left side in the elderly group (p= 0.0110), but no difference in the young group (p= 0.9064) and more importantly, faster RTs at near distance (D1) than at the medium (D2, p = 0.002) and the far distance (D3, p < 0.001, D2 vs D3: p = 0.7891) in elderly but not in young participants (all p > 0.70). Also, elderly participants were always slower in answering than young participants at all temporal delays (all p values < 0.001), as also shown by a significant main effect of group ($F_{(1, 52)}$ = 23.51, p < 0.001). Moreover, after checking that RTs were not different in the three temporal delays between the left and right side in the young (Wilcoxon Signed Ranks Test: D3: Z= -0.422, p= 0.673; D2: Z= -0.205, p= 0.837; D1: Z= -0.205, p= 0.837) and elderly group (Wilcoxon Signed Ranks Test: D3: Z= -1,934, p= 0.053; D2: Z= -1.846, p= 0.065; D1: Z= -1.025, p= 0.306), unimodal RTs were merged between the two side for every temporal delay in each group. Then, unimodal RTs were fitted with a linear function (young: $R^2$= 0.154; elderly: $R^2$= 0.866), and we compared individual slopes between the two groups. Data showed steeper slopes in elderly than in young participants (Shapiro-Wilk normality test: young: W= 0.97591, p= 0.7266; elderly: W= 0.96007, p= 0.4648; Two sample t-test: t= -2.8065, p= 0.007), with slopes having lower values in the young participants. This result confirms a speeding up effect in RTs to later temporal delay (D1) in elderly participants, in line with the previous analysis (linear mixed model, see above). Taken together, these results indicate that elderly participants present a strong expectancy effect for unimodal tactile stimuli (i.e., faster reactions for later tactile targets – see Kandula et al., 2017), that was not present in young participants. Furthermore, not surprisingly, elderly participants were slower in responding to tactile cues than young participants, independently from temporal delays.
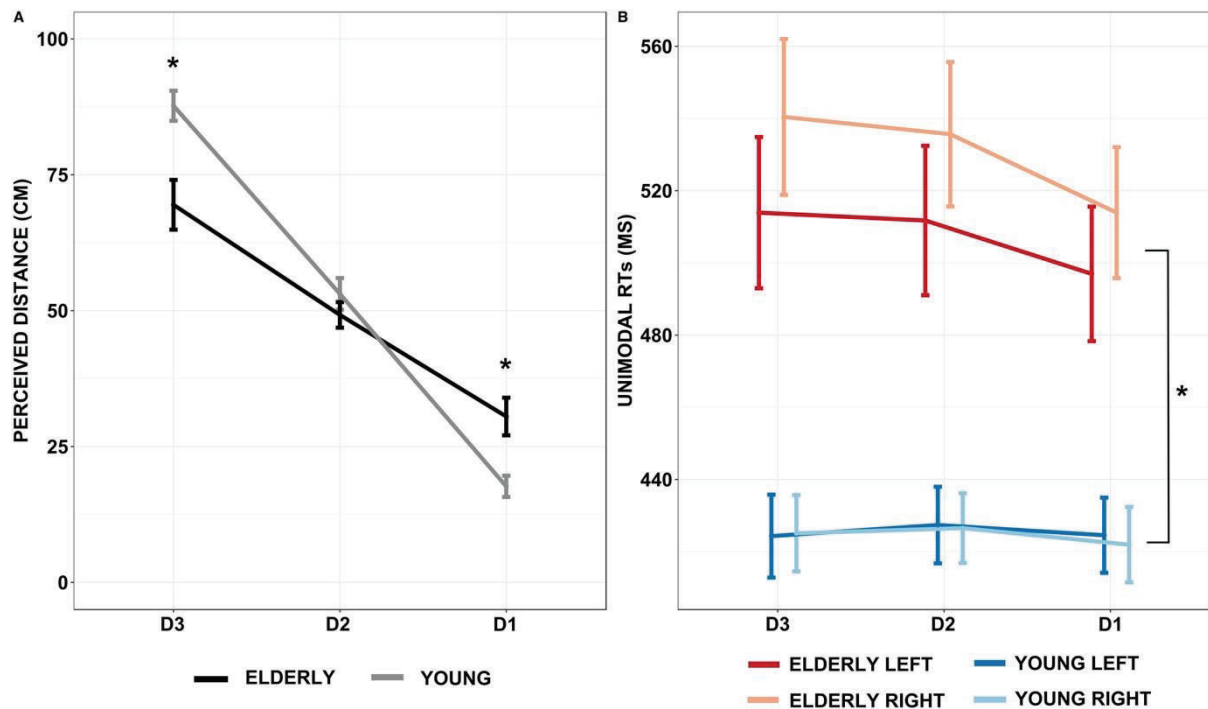
**Figure 6. Age-related differences in auditory (left) and tactile (right) processing**

The figure on the panel A shows the results of the localization task. Data represent the mean (± standard errors) of the estimated distances (cm) at which the sound was perceived when the tactile stimulus was given at each of the three temporal delays (D3, D2, and D1) corresponding to three sound distance (D3= far; D2= medium; D1= near). The asterisks indicate significant differences between the two groups.
The figure on the panel B shows means (± standard errors) RTs (ms) at unimodal tactile stimulus as a function of the three temporal delays (D3, D2, and D1). The black bracket with asterisk indicates a significant main effect between the two groups.

*Multisensory PPS task: audio-tactile interaction*. Audio-tactile RTs were corrected by subtracting the baseline of the unimodal condition in each group (i.e., the fastest mean RT among the unimodal tactile conditions, see Method). Given the differences in unimodal RTs between elderly and young participants, the baseline correction allows us to calculate a facilitation effect on tactile RTs due to auditory stimulation with respect to the unimodal tactile RTs as an index of multisensory PPS (see e.g., Serino et al., 2015) to be compared between groups. In particular, the model ($R^2$= 0.87) on baseline corrected multimodal RTs revealed a significant interaction

between temporal delays and group (F (2, 71.418) = 3.5414, p = 0.034) regardless of the stimulated hand (hand X temporal delays X group: F (2,156.003) = 0.5592; p = 0.572). Post hoc comparisons revealed slower RTs for the elderly group in the far distance (D3), with respect to the young group (p= 0.0154). In contrast, the distance at which sounds were able to affect the tactile RTs was similar in the two groups: indeed, in both groups, RTs at medium distance (D2, p= 0.8381) and the near distance (D1, p= 0.9799) were not different from each other, whereas, in both groups, RTs at medium distance were significantly faster with respect to the far distance (both p < 0.03), but similar to the near one (both p > 0.98) (see Figure 7). Considering that the model showed no effect between the two sides, we merged the RTs between the two sides for each temporal delay and group. We then compared multimodal RTs against zero (i.e., the fastest reaction time for unimodal tactile stimuli taken as a baseline) at each of the three temporal delays, by using one-sample T-tests (Bonferroni corrected, alpha set at 0.05/3= 0.017). Comparisons showed that RTs in the young group are always different from the baseline (all p < 0.001), i.e., young participants show multisensory facilitation at each of the three considered delays, while in elderly participants RTs differ from the baseline only at medium (D2) and at near (D1) distances (all p < 0.001), i.e. elderly do not show multisensory facilitation (t= 0.17727, p= 0.8609) when they have to react to tactile stimuli on the hand while a sound is presented at far distance (D3).

*Additional analysis*. Considering that in elderly, differently from young participants (see above), a speeding up effect in D1 was already present in RTs to unimodal stimuli as revealed by linear mixed model analysis and linear fitting results, a further control analysis was performed by fitting the bimodal data not only with linear but also with step-like functions. Two step-like functions were considered by imposing a step between D1 and D2 (near-medium, step1) and between D2 and D3 (medium-far, step 2) distance. RMSE between the three models were compared in elderly participants to exclude that bimodal responses just resemble the linear model previously obtained for unimodal responses. Because the normality assumption was not respected (Shapiro Wilk normality test: W= 0.91263, p < 0.001), Wilcoxon signed rank test was used. The analysis revealed that data in elderly participants were best fitted by a step-like functions with a step between the D2 and D3 (medium-far, step 2) distance compared to both the linear function (Wilcoxon signed rank test: V= 222, p= 0.009) and the step-like function with a step between the D2 and D1 (medium-near, step 1) distance (Wilcoxon signed rank test: V= 213, p= 0.021). This analysis confirms that bimodal data do not just represent the expectancy effect already observed in unimodal response in this group.

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Taken together, and in line with previous works (Noel et al., 2015; Serino et al., 2015), these findings indicate that a higher facilitation in the RTs to tactile stimuli occurred when the sound was perceived at near or at a medium distance from the hand, with respect to far location. Importantly, this effect was similar in both groups. Nevertheless, while young participants seem to show multisensory facilitation already at the farthest distance, even if this was less pronounced than at the medium or at the near distance, elderly participants did not show this facilitation with respect to the baseline in the far space. These results emerged beside a general slowing down effects in the processing of unisensory tactile stimuli in the elderly with respect to young participants.
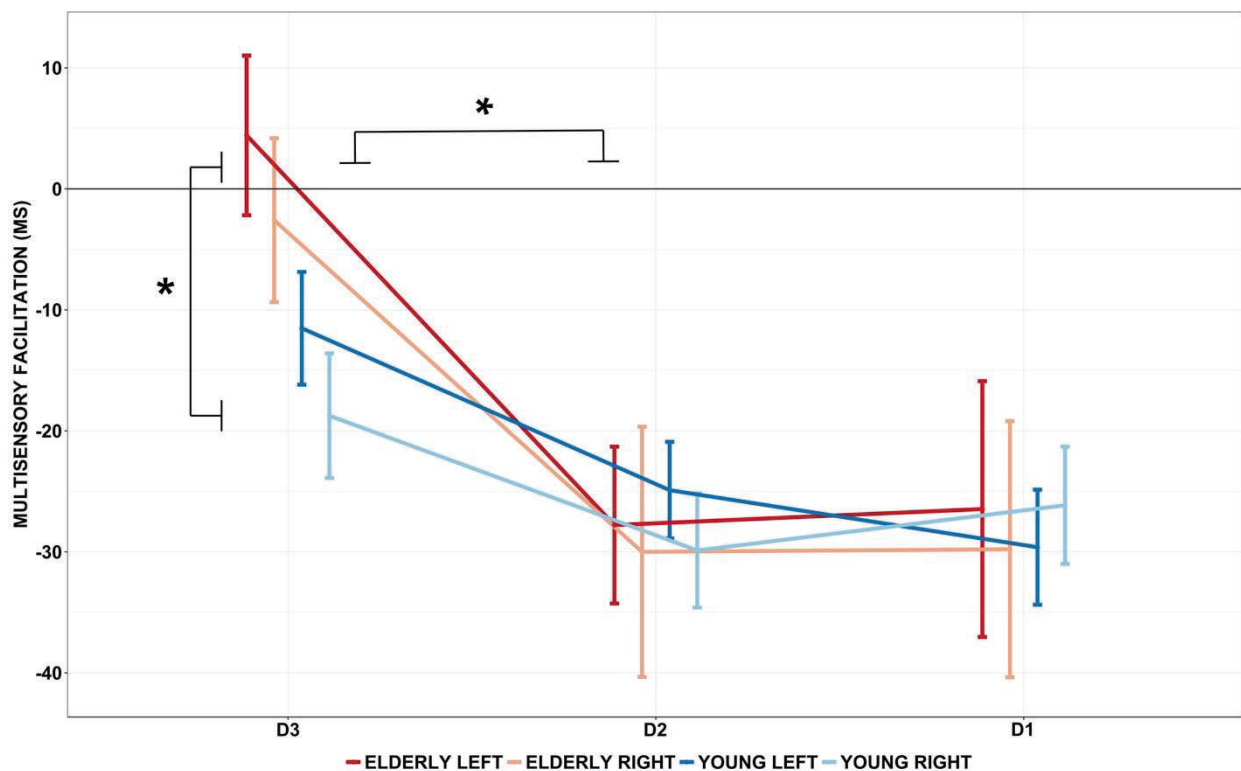


**Figure 7. Peripersonal space representation is resistant to ageing.**

The figure in panel B shows the results of the peripersonal space task. Data represent mean (± standard errors) RTs (ms) as a function of the temporal delays from the sound onset (D3, D2, and D1). Data are corrected for the baseline (i.e., the fastest RTs to unimodal tactile stimulation, baseline, 0 solid line), so that negative values indicate a multisensory facilitation effect. Slower RTs, not different from the baseline, emerged in the far distance (D3) in the elderly rather than young participants (vertical bracket). Crucially, in both groups, higher facilitation in the RTs to tactile stimuli occurred when the sound was perceived at near (D1) or at a medium (D2) distance from the hand, with respect to far location (D3) (horizontal bracket).

# 4. Discussion

A decline in the processing of sensorimotor information that seems fundamental to maintaining updated body and space representations (Bassolino et al., 2014; Berlucchi & Aglioti, 2010; Canzoneri et al., 2013; de Vignemont, 2010) has been described previously in elderly (Costello & Bloesch, 2017; Kuehn et al., 2018). This would suggest the presence of less accurate body and space representations in elderly participants. In the present study, by focusing on the upper limbs, we explored this hypothesis by comparing the performance of healthy young and elderly participants in three tasks aiming to assess implicit and explicit perceived body dimensions and the PPS representations. Overall, our findings show more substantial distortions in BR observed both in the implicit and explicit tasks in the elderly rather than in young participants. Comparable multisensory facilitation for stimuli presented near the body was found in young and elderly individuals, suggesting a similar PPS representation around the hand, while a reduced multisensory interaction for far stimuli, less accurate auditory localization and slower tactile processing was found in elderly participants.

## 4.1. Distortions in the implicit and explicit metric body representation in normal ageing

### 4.1.1. Implicit body metric

The findings from the BL task, aiming to capture implicit metric representations of the upper limbs, suggest that with respect to the young group, elderly participants underestimated the arm length (Figure 4) and have a greater distortion in the global shape of the upper limbs (hands and arms), with a greater bias on the overall ratio between the estimated width and the estimated length (see Figure 3).

The present results show a distorted global shape of the hand (with overestimation of the width and underestimation of the length) and are in agreement with several earlier studies evaluating hand representations in young participants (Longo & Haggard, 2010, 2011, 2012a; Longo, Morcom, Pia, Preston, & Romano, 2016; Peviani & Bottini, 2018). Our findings expand this work by showing that the hand bias seems even higher in the elderly rather than in young participants. Also, while global shape distortions have been reported mainly for the hand with similar effects for the forearm in tasks based on tactile stimuli perception (Canzoneri et al., 2013; Longo, 2017; Miller, Longo, & Saygin, 2014), the present results extend the bias to the arm only in elderly participants, thus suggesting that those distortions involve the whole upper limb (i.e., hand and arm) in normal ageing.

Although the overall distorted characteristics of the hand shape have been replicated under different versions of the body-landmark localization task (e.g. motor versus purely perceptual responses; Longo, Long, & Haggard, 2012; Longo, 2018; Peviani & Bottini, 2018) by different laboratories (e.g. Cocchini, Galligan, Mora, & Kuhn, 2018; Longo & Haggard, 2010; Longo, Mattioni, & Ganea, 2015; Medina & Duckett, 2017; Peviani & Bottini, 2018; Saulton, Dodds, Bülthoff, & de la Rosa, 2015) and, even using other tasks (e.g. Canzoneri et al., 2013; Ferrè, Vagnoni, & Haggard, 2013; Longo & Haggard, 2011; Longo, Mattioni, & Ganea, 2015b; Lopez, Schreyer, Preuss, & Mast, 2012), the mechanisms underlying such a bias remain uncertain (Ambroziak, Tamè, & Longo, 2018; Longo, 2018). Multiple, non-exclusive, factors have been proposed to be responsible for these distortions (Longo, 2017; Longo et al., 2015; Saulton, Longo, Wong, Bülthoff, & de la Rosa, 2016). One hypothesis suggests that this distorted global shape of the hand found in the general population relies on a stored implicit body model which is mostly influenced by somatosensory maps in the primary somatosensory cortex (Longo et al., 2015), such as the greater tactile acuity on the medio-lateral rather than proximo-distal axis of the hand dorsum and the anisotropy of the receptive fields of somatosensory neurons (Cody, Garside, Lloyd, & Poliakoff, 2008; Longo & Haggard, 2011). This effect might be even magnified in elderly people. Evidence, indeed, suggests that primary somatosensory maps decline with age, resulting in a decrease of tactile acuity (e.g., Kalisch, Ragert, Schwenkreis, Dinse, & Tegenthoff, 2009) and associated with a degradation of peripheral mechanoreceptors on the skin (e.g., Kuehn et al., 2018). This is also supported by studies about ageing in animal models which reported an increase in the dimension of the receptive fields of somatosensory neurons (David-Jürgens, Churs, Berkefeld, Zepka, & Dinse, 2008; Spengler, Godde, & Dinse, 1995). These probable changes in primary somatosensory maps during ageing may explain the observed amplified distortions in the BL task in the elderly.

Moreover, in the general population, it has been proposed that such a stored body model is maintained and updated through multiple sensory and motor inputs, i.e., proprioceptive, tactile, visual and efference copies (Longo & Haggard, 2010; Schwoebel & Coslett, 2005). An age-related decline is also observed in peripheral mechanoreceptors at the levels of muscles and joints associated with alterations in proprioception (Adamo & Brown, 2007; Carmeli et al., 2003; Costello & Bloesch, 2017; Kuehn et al., 2018; Shaffer & Harrison, 2007), which in turn could affect the accurate updating of the implicit body model. In this sense, the body model in elderly participants could result more distorted because not efficiently supported by the contribution of on-line afferent proprioceptive and somatosensory information. The hypothesis that the distortions observed in the implicit body metric representation in elderly participants could be linked to a not efficient

updating through the information coming from the body is in line with studies on motor imagery in normal ageing. Authors have demonstrated that the accuracy in mental imagery (i.e. the temporal correspondence between executed and imagined movements, e.g. Collet, Guillot, Lebon, MacIntyre & Moran, 2011; Marchesotti, Bassolino, Serino, Bleuler, & Blanke, 2016) declines with ages (Personnier, Kubicki, Laroche, & Papaxanthis, 2010; Skoura, Papaxanthis, Vinter, & Pozzo, 2005; Skoura, Personnier, Vinter, Pozzo, & Papaxanthis, 2008), in particular in cases in which a newly changed state of the body has to be taken into account during action simulation, as when a load is worn on the arm (Personnier, Paizis, Ballay, & Papaxanthis, 2008). This behavior has been interpreted as difficulty in accurately updating the changed configuration of the musculoskeletal system through peripheral signals. Such a decline in updating the body model could also be linked to a decrease in the flow of sensorimotor information from/to the body due to relative underuse of limbs in the elderly because of different motor capabilities and reduced daily life necessities. This hypothesis was also proposed in a previous study using an arm bisection task and showing an underestimation of the perceived arm length in elderly participants, in line with our results (Garbarini et al., 2015). A comparable underestimation of the perceived arm length has also been described in a few studies in pathological conditions of reduced or absent sensorimotor information such as amputees or stroke patients with motor deficits (e.g., Rognini et al., 2018; Tosi, Romano, & Maravita, 2018). However, while in patients the bias was restricted to the affected side, in our study elderly participants showed a similar underestimation of the perceived arm length for both the left and the right sides, in line with the non-lateralized hand distortions typically reported in young healthy participants (see Longo & Haggard, 2010, Experiment 3). This common result on both limbs suggests that the distortions reported in elderly participants at the BL task are not related to manual dominance or any cognitive lateralized mechanism.

Another factor recently proposed to explain perceived distortions in body metrics is related to general conceptual distortions linked to mistaken beliefs or visual memory of the location of different landmarks (Longo et al., 2015; Saulton et al., 2016). Although it is not possible to discard this hypothesis at the moment, this proposal is unlikely in explaining the age-related effects for at least two reasons. First, typically conceptual distortions have been reported at the level of the hand knuckles (Longo et al., 2015), while the present distortions in the elderly also refer to the arm. Secondly, similar bias was found in a different task, the AAT, not based on landmark localization. Finally, previous studies have demonstrated that visual-spatial memory of simple task mainly requiring maintenance of spatial information is not altered with age (Iachini, Iavarone, Senese, Ruotolo, & Ruggiero, 2009), not supporting the hypothesis that a decline in this function could be at the basis of the observed higher distortions in elderly participants.

### 4.1.2. Explicit body representation

Results similar to that obtained in the BL task have also been found in a more explicit task aiming to evaluate the perceived dimension of the upper-limb by manipulating the corresponding body parts on an avatar (AAT). In particular, in line with BL findings, elderly participants showed a greater bias (underestimation) for the arm length and the global shape of the upper limbs. Also, the underestimation of the hand length was higher in the elderly than in young participants. However, while in the BL task differences between the two groups of participants emerged independently of the side, in the AAT, the difference between the two groups was limited to one of the two limbs, i.e. the left arm concerning the arm length and the Normalized Shape Index, and the right hand for the Normalized Shape Index. Thus, overall, although the age-related effects observed in the two tasks go in the same direction, the degree of distortions seems different, with modifications in elderly participants limited to one side in the explicit task. We can speculate that such differences in the results obtained at the AAT and the BL are related to the contribution of different sensory modalities sub-serving these tasks and the underlying body representations. It has been demonstrated that while evident distortions in the representation of the hand emerge using implicit tasks (Longo & Haggard, 2010, 2012b), similar effects but with reduced magnitude have been reported in an *explicit metric* task, the "line length task" (Longo & Haggard, 2012b; Longo et al., 2015) where participants have to provide explicit judgment of the hand dimension, by referring to their own body. In this sense, the present AAT may resemble the "line length task" by tapping into a combination of somatosensory representations of one's own body and *explicit metric* judgment in terms of width and length. Moreover, in both tasks, participants have to provide their metric judgments by modifying the visual characteristic of a model (the avatar in our task, lines in the "line length task"). It is thus possible that in AAT with respect to BL, the influence of somatosensory representation is reduced in favor of additional visual information that typically appears to be less biased (see "the template matching task", Longo & Haggard, 2010; Longo et al., 2015b; Longo & Haggard, 2012), thus leading to a reduced pattern of distortions. This interpretation could also account for higher bias in the elderly than young participants because the additional contribution of the visual component in this task could be not sufficient to completely compensate for the already higher distorted somatosensory representations captured at the BL task in the older group. It is also possible that elderly participants could not properly decrease the weight of the declined somatosensory inputs in favor of the visual information, in line with evidence showing difficulties in adjusting the weights of sensory bodily signals with ageing (Kuehn et al., 2018). We also note that probably visual characteristics of the avatar matching the participants' age and gender could have had a role, accordingly to previous studies showing distortions in the

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estimation of the length of others persons' body parts with greater distortions if the others were of the same gender (Linkenauger, Kirby, McCulloch, & Longo, 2017; Linkenauger et al., 2015).

Concerning the lateralization of the distortions, and differently from the results at the BL task, in the AAT we found an overall reduced distortion of the dominant right side, evident in the estimated global shape of the limbs (Normalized Shape Index) in both groups, except for the hand in the elderly participants. This seems to mimic the dominant-hand advantage typically reported in the motor domain in young participants (Kalisch, Wilimzig, Kleibel, Tegenthoff, & Dinse, 2006). Such hand dominance has been described to decline with ageing, so that performances of the two hands become balanced and comparable in the elderly (Kalisch et al., 2006), in line with the similar Normalized Shape Index of the left and right hand, found here only in elderly participants. Although, considering the visuo-somatosensory nature of the AAT, the fact that participants did not execute any upper-limb movement to perform this task, and the absence of such lateralization at the BL task (according to Longo & Haggard, 2010, Experiment 3), this side–related effect is unlikely to be directly linked to motor information contributing to the task, but could reflect some visual dominance–related differences, as visual feedback during motor experience. Alternatively, one could hypothesize that the side differences found in the elderly can be due to attentional bias. However, a rightward attentional bias (i.e. lower attention on the left) in the visual domain recently proposed in ageing (Zeller & Hullin, 2018) would predict opposite results with always higher distortion for the left side in elderly participants (effect present here in both groups, but with the exception of the hand for the elderly).

## 4.2 Age-related differences in tactile and auditory processing, but comparable peripersonal space representation in young and elderly participants

Our results show significant differences between elderly and young participants in auditory and tactile processing. More precisely, in the auditory distance perception task assessing sound localization, elderly participants were able to perceive the source of the sounds at the three different distances, but they were less sensitive to discriminate the three locations, as they perceived the farthest distance as significantly closer and the closest distance as more distant (with no difference at the middle position), with respect to the young participants (see Figure 6 A). This resulted in a flatter slope in the linear function between temporal delays and sound localization in the elderly rather than in young participants. Changes in the sound localization ability from young adulthood to old age (Abel, Giguère, Consoli, & Papsin, 2000; Dobreva, O'Neill,

& Paige, 2011) and a reduction of perceived spatial volume have been reported in elderly (Ghafouri & Lestienne, 2000). However, it is difficult to compare this result with related work on age-related effects in distance perception because, so far, different protocols have mainly employed static visual objects (e.g., Bian & Andersen, 2013), whereas the present study is one of the few studies using acoustic dynamic stimuli. Age-related unimodal perceptual differences were confirmed in the perception of unimodal tactile stimuli. First, tactile RTs were, in general, slower in the elderly, in line with previous studies showing age-related changes in tactile perception (Carmeli et al., 2003; Costello & Bloesch, 2017), as well as in reaction times and speed processing in different modalities (e.g. Cespón, Galdo-Álvarez, & Díaz, 2013; Kuehn et al., 2018; Murray et al., 2018). Also, in elderly participants, and not in young participants, RTs to unimodal tactile stimuli varied as a function of the delay of tactile stimuli administration with respect to sound onset. In particular, in elderly people RTs became faster with longer delays, an effect indicating a form of cognitive expectancy (Kandula et al., 2017): as the trial's time increases, the probability of receiving touch also increases, and thus subjects are more ready to respond for late delays. The two effects on auditory and tactile processing might not be fully independent of each other. Given that participants had to judge sound distance by indicating at which location the sound was when they perceived a tactile cue, a slower general tactile processing, and an increased expectancy could have a role in explaining the results in the auditory localization task. Slower tactile processing might explain why the auditory space is contracted for the far stimuli, as touch is processed later. This effect, however, could be compensated when the tactile cues were administered at further delays during the trial (i.e., medium position), and it could even produce the opposite effect (the closer position is perceived further away) because of increasing expectancy in the latest delay (near, i.e., the tactile processing is anticipated, and thus the sound is perceived farther away).

Crucially for this study, despite these differences in the elaboration of auditory and tactile stimuli in the two groups, we found comparable multisensory facilitation in elderly and young participants for bimodal stimuli at near and at medium distance from the body. Importantly, in both groups, such facilitation was numerically higher in these two closer positions than at the far location. Further analysis of the responses to bimodal stimuli in the audio-tactile task shows a better fitting of the step-like rather than the linear function in explaining the relationship between temporal delays and reaction time. This finding further supports the fact that the multisensory facilitation observed in the elderly does not just mimic the linear effect, probably due to expectancy, already observed in unimodal responses in this group. Previous multisensory studies (Noel et al., 2015; Serino, 2019; Serino et al., 2015) have interpreted such multisensory facilitation as a proxy of PPS

boundaries. This result could thus suggest similar hand-PPS boundaries in the elderly and young participants. Although few previous studies have tried to describe elderly abilities in processing stimuli within the PPS (Costello & Bloesch, 2017; Kuehn et al., 2018), so far, this is the first work directly comparing PPS representations in young and elderly participants by exploring its multisensory proprieties.

The similar facilitation observed in the two groups could be based on comparable multisensory abilities. This is supported by results demonstrating that multisensory integration processes seem to be spared in elderly people (Laurienti, Burdette, Maldjian, & Wallace, 2006; Mahoney, Li, Oh-Park, Verghese, & Holtzer, 2011) and similar to younger subjects in the audio-tactile task (Mahoney et al., 2011). Besides reduced unisensory abilities in processing external and bodily stimuli in elderly, an efficient PPS representation could play an important role to support actions in the near space and to protect the individual from incoming potential dangerous stimuli, in line with the proposed function of PPS (for recent reviews Bufacchi & Iannetti, 2018; Cléry & Ben Hamed, 2018; Serino, 2019).

An intriguing hypothesis is that spared multisensory processing in the elderly is due to decrease in unisensory processes. This could be linked to one principle of multisensory integration: two stimuli in the two modalities more strongly interact when unimodal processing is weak – inverse effectiveness (Stein & Stanford, 2008). Even if the current results are compatible with it, our paradigm was not designed to test this hypothesis directly. Alternatively, it could be possible that efficient, or even greater, multisensory processing in elderly compensates for the age-related decline in unisensory abilities (de Dieuleveult, Siemonsma, van Erp, & Brouwer, 2017; Diaconescu, Hasher, & McIntosh, 2013; Diederich, Colonius, & Schomburg, 2008), in line with studies showing a more extended recruitment of brain areas during multisensory tasks in elderly versus young adults (Heuninckx, Wenderoth, & Swinnen, 2008; Townsend, Adamo, & Haist, 2006; Venkatraman et al., 2010).

Despite similar effects in elderly and young participants in PPS representation, differences between the two groups were found in the far space. Elderly participants showed less multisensory facilitation as compared to young participants when the tactile stimulus was coupled with far sound (see Figure 7). This could be interpreted as a weaker effect of sound in the far space, not able to compensate for the slower RTs to the tactile stimuli in far rather than in near location in the elderly. However, a similar multisensory integration in the two groups has been observed at the medium distance, besides equivalent slow RTs to unimodal tactile stimuli in medium and far position in the elderly. Such difference in the far space between the two groups could also be seen as if young

participants showed higher multisensory facilitation with respect to elderly people in the far space, to more efficiently anticipate potential contact with external stimuli during their likely more frequent and more dynamic interactions with the environment, in contrast with decrease upper extremities range of motion and disuse in elderly (Daley & Spinks, 2000; Schultz, 1992).

## 5. Dissociation between distortions in metric body representation and mainly unaltered PPS representation in elderly

The present results show stronger BR distortions in the elderly in perceiving the metrics of the upper limb using implicit and explicit BR tasks. This differs from the PPS representation, which was similar in young and elderly participants, even though associated with age-dependent differences in tactile and auditory perception. The present dissociation between alterations in BL, with mainly unaltered PPS representation in elderly participants, is opposite to that we found after immobilization. After immobilization, the perceived arm length remained unchanged (BL task), while multisensory facilitation within PPS decreased. In the case of immobilization, we interpreted those changes as due to unaltered afferent static information from the immobilized limb maintaining BR, associated with reduced possibility to act in space, driving modifications in the PPS (Bassolino et al., 2014). In normal ageing, the opposite effects could be related to a decline in afferent information from the body (Costello & Bloesch, 2017; Kuehn et al., 2018) not properly updating BR, and preserved actions in the close space, even if likely reduced in terms of frequency, supporting a comparable multisensory facilitation within PPS boundaries. The unaltered PPS representations could also be linked to compensation for the decline of unisensory and bodily information through preserved multisensory mechanisms, and to the protective function of predicting dangerous stimuli near the body, particularly necessary when perceptual processing is altered or slowing down as in the case of elderly.

## 6. Conclusion

To our knowledge, this is the first study describing BR and PPS representations in normal ageing by adopting tasks previously used to demonstrate the plasticity of these representations after tool-use and immobilization (Bassolino et al., 2014; Canzoneri et al., 2013). The present findings suggest the importance of introducing those tasks aiming to evaluate BR and PPS representations in the assessment of elderly people. Although the present sample of healthy, cognitively unimpaired and active elderly participants could not allow us to make inferences about

pathological conditions during ageing and PPS or BR distortions, a topic that requires future research, the present study has the advantage to show that even in the absence of diseases, age-related effects are evident in BR and PPS representations. More generally, this work extends the concept of plasticity underlying BR and PPS by showing that these representations are not only modifiable after short or long experience of tool-use or disuse (Bassolino et al., 2014, 2010; Biggio et al., 2017; Maravita & Iriki, 2004; Martel et al., 2016; Serino et al., 2007), but even during the normal course of life.

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## Declaration of interests

The authors report no commercial or competing interest.

## Author contributions

MB and AS designed the study; GS, MF and CZ performed the experiments; GS, MF and MB analyzed the data; GS, MB, OB and AS wrote the manuscript.

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# Appendix 2: Effect of tool-use observation on metric body representation and peripersonal space.

**Personal contribution:** data collection

**Manuscript submitted in Neuropsychologia**

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- **Highlight**

1. Does observational tool-use affect body and peripersonal space representations?
2. A longer perceived arm length is reported after active but not observational tool-use
3. Only after active tool-use near and far sounds induce similar effect on tactile RTs
4. Observational tool-use is not sufficient to affect body and space representations
5. Motor intention and sensory feedback seem necessary to alter these representations

- **Keywords (5):** body representation, peripersonal space, tool-use, action observation, motor intention

**Abstract**

In everyday life we constantly act and interact with objects and with others' people through our body. To properly perform actions, the representations of the dimension of body-parts (metric body representation, BR) and of the space surrounding the body (peripersonal space, PPS) need to be constantly updated. Previous evidence has shown that BR and PPS representation are highly flexible, being modulated by sensorimotor experiences, such as the active use of tools to reach objects in the far space. In this study, we investigate whether the observation of another person using a tool to interact with objects located in the far space is sufficient to influence the plasticity of PPS and BR representation in a similar way to active tool-use. With this aim, two groups of young healthy participants were asked to perform twenty minutes trainings based on the active use of a tool to retrieve far cubes (active tool-use) and on the first-person observation of an experimenter doing the same tool-use training (observational tool-use). Behavioural tasks adapted from literature were used to evaluate the effects of the active and observational tool-use on BR (body-landmark localization task- group 1), and PPS (audio-tactile interaction task – group 2). Results show that after active tool-use, participants reported an extension in the perceived length of their arm, while no significant differences appear after observation. Similarly, comparable multisensory facilitation on tactile responses due to near and far sounds was seen only after active tool-use, while this does not occur after observation. Together these results suggest that a mere observational training could not be sufficient to significantly modulate BR or PPS. The dissociation found in the active and observational tool-use points out differences between action execution and action observation, by suggesting a fundamental role of the motor planning, the motor intention and the related sensorimotor feedback in driving BR and PPS plasticity.

**Introduction**

To efficiently interact with the environment, as to plan and execute properly the action of reaching for an object positioned in front of the body,  the brain needs updated representations related to the shape and to the dimension of the involved body parts (i.e. metric body representations, BR) (de Vignemont, 2010; Longo et al., 2010; Schwoebel and Coslett, 2005), and of the space closely surrounding the body in which the interactions with the environment take place (i.e peripersonal space, PPS) (Rizzolatti et al., 1997; Serino, 2019). During the last years, many studies have been

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dedicated to investigating these representations, that contribute, in different ways, to the conscious experience of the self as an acting body (Garbarini et al., 2015).

As far as concerns BR, since no unique sensory signal directly convey to the brain information about the size and the shape of the different body parts, authors have hypothesized that an implicit representation of the body metric is stored in the brain (Longo and Haggard, 2012, 2010; Tamè et al., 2019). This representation is constantly updated through on-line peripheral signals related to body parts, such as somatosensory, proprioceptive and kinaesthetic inputs coming from the skin, the muscles and the joints, as well as through visual bodily information, during the interactions with the environment (de Vignemont, 2010; Longo et al., 2010; Medina and Coslett, 2011; Riva, 2018; Serino and Haggard, 2010).

On the other hand, PPS representation has been originally studied in primates, where specific populations of multisensory neurons integrating visual and/or auditory stimuli near the body with tactile information on the body surface (Duhamel et al., 1997; Fogassi, 1996; Graziano et al., 1997; Graziano and Cooke, 2006) have been identified within a fronto-parietal network. Evidence for this has been corroborated by results also obtained in humans through neuropsychological (Di Pellegrino et al., 1997; Ladavas, 1998; Làdavas et al., 1998), neuroimaging (Grivaz et al., 2017; Makin et al., 2008) and behavioural (Bassolino et al., 2010; Canzoneri et al., 2012; Teneggi et al., 2013) studies. These works demonstrated a speed-up effect in responding to tactile stimuli when these were associated to visual or auditory stimuli presented close (i.e. within PPS), but not far from the body (Cléry and Ben Hamed, 2018; de Vignemont and Iannetti, 2015; di Pellegrino and Làdavas, 2015). This form of multisensory facilitation  within PPS allows the brain to detect and anticipate potential interactions between the body and external objects and to trigger appropriate motor responses both in terms of defensive behavior (e.g. prevents a potential threat) or approaching (reaching/grasping) actions (Bufacchi and Iannetti, 2018; Serino, 2019).

Taking together, previous studies indicate that both BR and PPS have a multisensory nature, being built and constantly updated thanks to the integration of signals from different sensory modalities (Dijkerman and Lenggenhager, 2018; Kandula et al., 2017; Maravita et al., 2003; Salomon et al., 2017). This implies that BR and PPS are not fixed, but could be plastically modified through actions, and specifically through changes in the in- and out- flows of sensorimotor information arising from the interactions with the environment (e.g. reaching for an object). From this

perspective, the nature of those representations is not only multisensory but also sensorimotor in the sense that the action execution can modulate both PPS and BR (Gallese and Sinigaglia, 2010).

A classic example of the plasticity of BR and PPS after action execution is the use of the tools allowing to reach objects located in the far space (Canzoneri et al., 2013; Cardinali et al., 2009; Maravita and Iriki, 2004; Martel et al., 2016). Using a tool to reach far objects allows to act outside PPS making outside-reach objects ready-to-hand (Iriki et al., 1996), and modifies the functional dimension of the effector holding the tool (e.g. the arm) (Martel et al., 2016). More specifically, it has been shown that tool-use re-shapes BR, by extending the estimated length of the body part (arm/hand) using the tool or by altering the subsequent hand free movement kinematic profile (Bassolino et al., 2014; Canzoneri et al., 2013a; Cardinali et al., 2009; Garbarini et al., 2015; Romano et al., 2018; Sposito et al., 2012). Analogously, previous research has shown that, after tool-use, PPS representation is modified. In primates, PPS neurons normally coding tactile stimuli on the hand and associated external visual or auditory stimuli presented close to the hand started also to respond to associated visual/auditory stimuli located in the more distant space of the tool's reach (Iriki et al., 1996; Maravita and Iriki, 2004; Radman, 2013). Similarly, studies with both healthy participants and patients have found that after tool-use, it is possible to extend the representation of the PPS, by increasing the multisensory interaction between tactile stimuli on the body and visual or auditory cues presented in the far space, in particular at the functional location where the tool was used (Farnè and Làdavas, 2000; Galli et al., 2015; Holmes and Spence, 2004; Maravita et al., 2001). This effect was reported after short experience with a tool (around 15 minutes) as well as after persistent use of specific tools in different populations, such as blind people using the cane (Serino et al., 2007),  computer mouse users (Bassolino et al., 2010)  or professional tennis players (Biggio et al., 2017). In line with this, it has been argued that the space is accurately represented in relation to action capabilities by allowing the brain to determine whether a certain spatial sector is accessible and to select the most appropriate motor actions in the accessible space (Bufacchi and Iannetti, 2018; Serino, 2019).

The evidence of BR and PPS modifications after tool-use would drive the question if mere observation of someone else acting with a tool in far space may impact on bodily and spatial representations as execution. Action observation would indeed activate a shared representation of the movement between the observer and the agent (e.g. Rizzolatti et al., 2001) that would be

sufficient to drive plastic effects on PPS and BR similar to action execution. Coherently, the only study on space representation after observational tool-use so far (Costantini et al., 2011), reported an extension of the explicit perceived reaching space of the observer in a visual distance judgment task, in which participants had to judge the distance of a graspable object with respect to their body. Importantly, these authors found that observing tool actions can extend the representation of reaching space only when observers shared the same action potentialities with the agent, namely holding a tool compatible with the goal and the spatial range of the observed action. However, Garbarini and colleagues (2015) did not find any modification in the perceived length of the arm (BR) evaluated with a "body bisection task" (Sposito et al., 2012) after observational tool-use. These contrasting results would lead to the hypothesis of a possible dissociation in the effects of tool-use observation on BR and on PPS. Nevertheless, the different results previously reported on reaching space and BR modifications after observational tool-use could be related to participants' age. Indeed the study by Garbarini and colleagues was performed in young adults, while the one by Costantini and collaborators was done in healthy elderly controls, who could potentially show reduced plasticity after tool-use because of age (Costello et al., 2015).

To solve this issue, the present study aims to investigate the effects of active and observational tool-use on BR and PPS representations in young healthy adults. Besides the previously demonstrated similar effects of the extension of both BR and PPS representations after active tool-use (Canzoneri et al., 2013), it is possible to hypothesise dissociable effects after observational tool-use. Indeed, if BR modifications could be mainly mediated by multisensory and sensorimotor information related to *one's own* body (Bassolino et al., 2014), the mere visual observation of *another person* using the tool could be not enough to induce alterations of BR in the observer. In contrast, if plastic changes in PPS are mostly dependent on the motor representation of the space in which the body potentially acts, the activation of a shared motor representation between the person using the tool and an observer holding the same tool (Costantini et al., 2011) through action observation (Rizzolatti and Craighero, 2004) could be sufficient to affect PPS. However, alternative hypotheses could be considered; first, given that PPS is strictly anchored to one's own body and related somatosensory information (Serino, 2019), the mere observation of someone else acting in the same space could be not sufficient to modify the representation of the observer's PPS, as in the case of BR. Second, we can anticipate that the mere visual observation of *another person* using the tool could be enough to drive a plastic change of both PPS and BR, suggesting that the

lack of modification of the BR after observational tool-use found by Garbarini and colleagues (2015) was mainly due to the age of their sample.

## 2. Materials and methods

### 2.1 Participants

Two groups of twenty-one healthy, right-handed participants were included in the study. Participants in group 1 (age: 24.50 ± 3.02, range: 19-31, gender: 57% of female) underwent a task previously reported to assess the implicit perceived length of their arm, the body-landmark localization task (BL) (Bassolino et al., 2014; Longo, 2017), while subjects in group 2 (Age: 23.71 ± 1.49, range: 20-26 gender: 67% of female) performed a task previously described to capture multisensory characteristics of PPS representation around their right hand. i.e. audio-tactile interaction task (e.g. Bassolino et al., 2010; Ronga et al., *under review*.; Serino et al., 2007). The subjects' handedness was evaluated with the Flinders Handedness survey (FLANDERS) (Nicholls et al., 2013). The following exclusion criteria were considered: the presence of neurological or psychiatric diseases or any other deficits impairing their capacities to perform the tasks (e.g. visual deficits, acoustic deficits, the presence of chronic pain in the upper limbs, sensorimotor deficits or recent fractures < 1 year). All the participants were naïve to the experimental procedures and the purpose of the study and participated after having signed the informed consent. The study was conducted with the approval of the local ethics committee (group 1: Commission Cantonale Valaisanne d'Ethique Medicale, CCVEM 107/14, group 2: Ethics Committee of the University of Torino, prot. n. 125055, 12/07/16).

### 2.2 Procedure

#### 2.2.1 Active tool-use training

During the training session, participants were comfortably seated on a chair in the experiment room and they were asked to place their left hand on their left leg and the right one in a prone position on a table by holding a standardized tool (Aluminium rake, length: 100 cm, width: 8 mm diameter, with at the end a 15 x 10 cm plastic plate with two rectangular 6 x 10 cm sides at 90°, total weight of the tool: around 1kg) in the starting position (i.e. on the right side) (see Fig.1.A). They had to then perform a tool-use training session, inspired by similar works (Canzoneri et al., 2013; Costantini et al., 2011; Garbarini et al., 2015; Sposito et al., 2012). The training consisted in using

the tool to retrieve 30 wooden coloured (red or blue) cubes (5.5 cm$^3$) that had to be placed into the coherent coloured squares (blue or red depending on the colour of the cube) (see.1.A). Participants could freely decide which objects to reach. They were asked to retrieve an object every time they heard a "bip" sound coming from an audio track, made to emit a "bip" every 5 seconds. This procedure was chosen to standardize the duration of the training among participants. Before the training, participants were familiarized with the tool to ensure that they could perform the task easily (a few minutes). Overall, participants retrieved all the objects in 150 seconds and had a 60 second break while the experimenter recomposed the initial objects' composition on the table. During the break, participants were asked to hold the rake in their hand in the starting position. The task was performed in 6 blocks lasting 20 minutes in total.

### 2.2.2 Observational tool-use training

The observational procedure was the same as for the active condition, but in this case, the experimenter actively retrieved the objects at each "bip" by using the tool, while the participant observed the experimenter's actions while holding an identical tool with his/her right hand in the starting position (i.e. on the right side). The experimenter stood behind the participant during this condition (see Fig.1.B).

To maintain participants' attention during the training, the subjects were specifically asked to carefully observe the action performed by the examiner and orient their gaze to the left or to the right, according to the location of the target, as already described elsewhere (Garbarini et al., 2015).
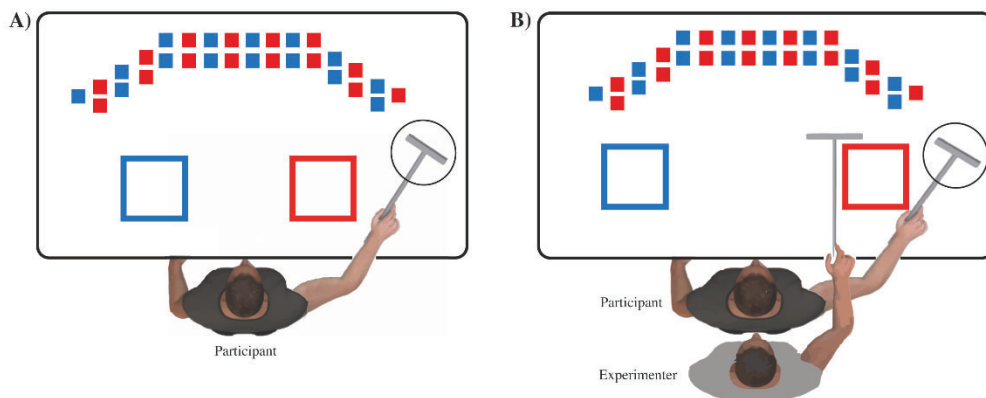
Fig.1. Experimental task: (A) Active tool-use training: schematic aerial view of the experimental setting depicting the participant holding the tool in the starting position (black circle); (B) Observational tool-use training: schematic aerial view of the experimental setting depicting the participant holding the tool in the starting position and the experimenter actively using the tool.

### 2.2.3 Group 1: Body-landmark Localisation task (BL)

In group 1, the implicit perceived dimension of the upper limb (arm length) was measured before (pre) and after (post) the training (active and observational) with the body-landmark localisation task (BL), already described in previous works (Bassolino et al., 2014; Canzoneri et al., 2013). The order of the sessions was balanced between participants, with half of the participants doing the observational training as first, and the other half beginning with active tool-use training.

The BL task can be considered implicit because participants had to indicate only the locations of some anatomical landmarks, without explicit judgements about the perceived length of the body parts (Fuentes et al., 2013). To evaluate the perceived arm length, we considered two anatomical landmarks: the external part of the wrist (ulnar styloid) and the elbow joint (olecranon). The perceived arm length was then reconstructed a posteriori during the data analysis and compared with the individual real arm length captured at the beginning of the experiment, while participants were blindfolded.

During the task, participants were seated on a chair with the right forearm resting palm-down on a table in front of them, aligned with the shoulder and positioned 20 cm away from the body midline and without contact between the elbow and the edge of the table. To avoid movements and standardize the position, the participants' right forearm was fixed to the table during the duration of the whole recording session while the hand was resting on a computer mouse, a position used to standardize participants' posture during the task. The left forearm was kept relaxed on the left leg.

After having acquired the actual position of the 2 landmarks, the experimenter positioned a wooden table (80 cm x 80 cm) above their arm and put an additional cloth to occlude the shoulders, in order to prevent participants from viewing their own arm during the task. Afterwards, subjects removed the eyeshades, and, in every trial, the experimenter showed to the participant the location of the

target landmark on her body. Participants were instructed to verbally indicate, by saying "stop", when a retro-reflective marker (see below) attached to a wooden stick and moved by the experimenter along the table's longitudinal axis, reached the felt position of the target non-visible anatomical landmarks (wrist or elbow depending on the trial). Before recording the marker position, subjects were allowed to adjust their judgement, by verbally asking the experimenter to move it backward or forward, to the left or to the right. 10 randomized trials were repeated for each landmark. This exact procedure was reproduced after the training (post), taking care of placing the participants' upper limb in the same position of the pre-training session.

Retro-reflective markers (1 cm of diameter) captured by means of an optical motion capture system (Optitrack V120: TRIO; Motive 1.7.5 Final 64-bit, 2015) and a custom-made script written in Matlab (R2018a) were used for the recording. The positions of the markers on the limb and of the limb on the table were also marked to be used for the post training session.
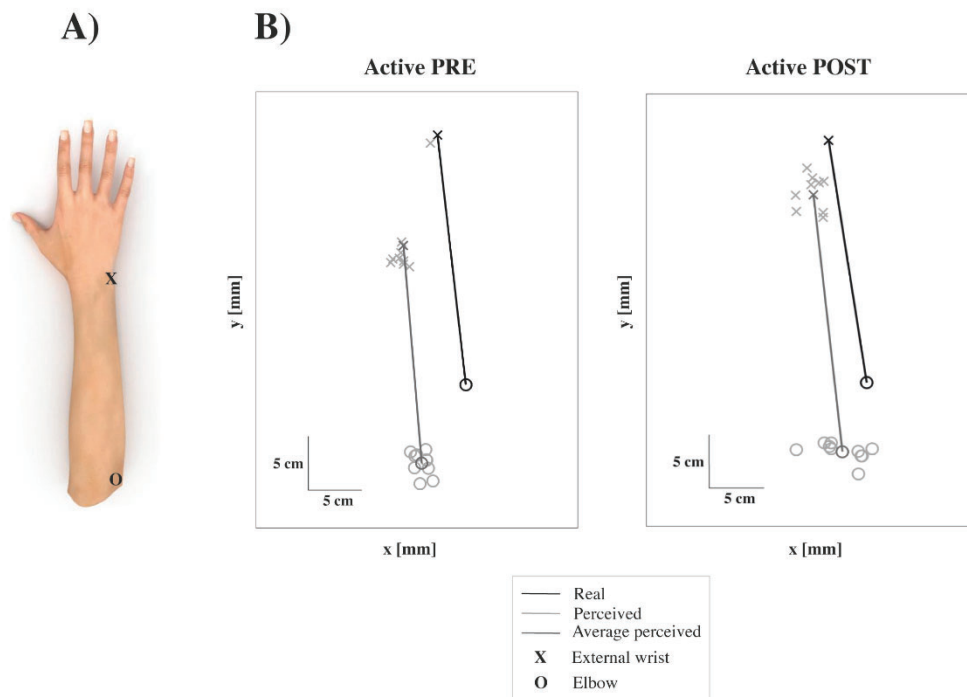


Figure 2. Panel A) The anatomical landmarks recorded during the body landmark (BL) task: the external part of the wrist (ulnar styloid, cross) and the elbow joint (olecranon, circle). Panel B) The reconstruction of the anatomical landmarks, recorded at the beginning of the experiment

(black) as well as the reconstruction of the perceived position recorded for each landmark on every single trial (ten repetitions for each landmark, light grey) and averaged among repetitions (dark grey) in one representative subject (the horizontal displacement is depicted on the x, mm, while the vertical ones on the y, mm).

## 2.2.4 Group 2: Audio-tactile interaction task

In group 2, to investigate plastic modulations of PPS induced by tool-use, we adopted a procedure similar to those used in previous studies (e.g. Bassolino et al., 2010; Sambo and Forster, 2009; Serino et al., 2007) to exploit multisensory integration phenomenon occurring when bimodal stimuli appear simultaneously within PPS, and in particular when static auditory stimuli near the hand speed up reaction Times (RTs) to tactile stimuli on the hand. Participants underwent the audio-tactile interaction task (Fig.3) after three different trainings (active, observational and cognitive), performed in three experimental sessions separate by an interval of one week. We opted to include a third session (cognitive training) as control condition rather than a *pre* vs *post training* paradigm, to avoid possible unspecific learning effects due to perform the same task multiple times in different sessions and twice in a day (Ronga et al., *under review*). Indeed, the cognitive training can be considered as a control condition, because subjects underwent a task in the far space (see below) without performing any motor action. Moreover, such training allows to control for possible unspecific attentional shifts, merely driven by the performance of training in a specific portion of space (Holmes, 2012).

During the cognitive session, participants performed a visual task, in which they were asked to judge whether two sequentially presented (50 ms of duration; 1 s of interstimulus interval) configurations were identical or different. Visual stimuli consisted of four configurations of three dots, forming triangles pointing upwards, downwards, rightwards or leftwards, and were presented on a computer screen placed at a 100 cm of distance from the hand (a distance corresponding to the length of tool-use).

In the audio-tactile interaction task, participants were seated on a chair with their right hand placed on the table while holding the tool, and tactile and auditory stimuli were administered by an Arduino system (https://www.arduino.cc) – E-Prime system.

*STactile stimuli* consisted of non-painful transcutaneous electrical, constant current square-wave

pulses (duration: 200 μs, delivered by DS7A, Digitimer) applied to the right-hand dorsum, using surface bipolar electrodes (1 cm between electrodes). The stimulus intensity, adjusted according to participants' sensitivity, corresponded to the individual threshold * 2. The individual sensory threshold was estimated before each experimental session, using the methods of limits (Gescheider, 1997). The mean stimulus intensity was 3.14 ± 0.97 mA (Active session: 3.55 ± 1.24 mA; Observational Session: 3.1 ± 0.88; Cognitive session: 3.18 ± 0.71 mA). To prevent habituation, three electrodes were placed at a constant distance between each other (i.e. about 1 cm) and connected to the electrical stimulator, so that the one with the negative polarity was kept always active, whereas the other two electrodes with positive polarity were activated on at a time. In this way, participants might perceive the stimulation coming from two distinct sites of the hand dorsum as if the stimulation was randomly shifted by displacing the electrodes' position of about 1 cm.

*Auditory stimuli* consisted of 784 Hz tones (intensity ≅ 65 dB; 50 ms duration) delivered by two different loudspeakers: the first loudspeaker was placed near (< 5 cm) to participants' right (stimulated) hand (henceforth *near position*), the second loudspeaker was positioned 100 cm (i.e. a distance corresponding to length of tool-use) from subjects' right hand (henceforth *far position*).

To explore multisensory integration effects within PPS, tactile and auditory stimulations could occur either in isolation (i.e. *unimodal conditions*: Touch, henceforth *T*; Auditory stimulus, catch trials, coming from near position, henceforth *ANear*; Auditory stimulus coming from far position, henceforth *AFar*) or combined (i.e, *bimodal conditions*: Touch+Auditory stimulus coming from near position, henceforth *TANear*; Touch+Auditory stimulus coming from far position, henceforth *TAFar*). Between each stimulation, the inter-trial interval was randomly jittered between 7 and 9 s, in a way that participants could not anticipate stimulus occurrence.

Participants were asked to respond as fast as possible to tactile stimuli, ignoring auditory ones, by pressing a button on the response box with their right index finger. The audio-tactile interaction task consisted of a 16 minutes' experimental block and 24 trials per condition were delivered. Stimulus delivering and RTs were controlled and recorded by Eprime V2.0 software (Psychology Software Tools Inc., Pittsburgh, PA, USA).

During the piloting phase we ensured that subjects perceived synchronously the tactile and the auditory stimuli and we calculated that our Arduino-E-Prime system administered the two stimuli with a maximum delay of 40 ms, with the auditory stimulus occurring later.
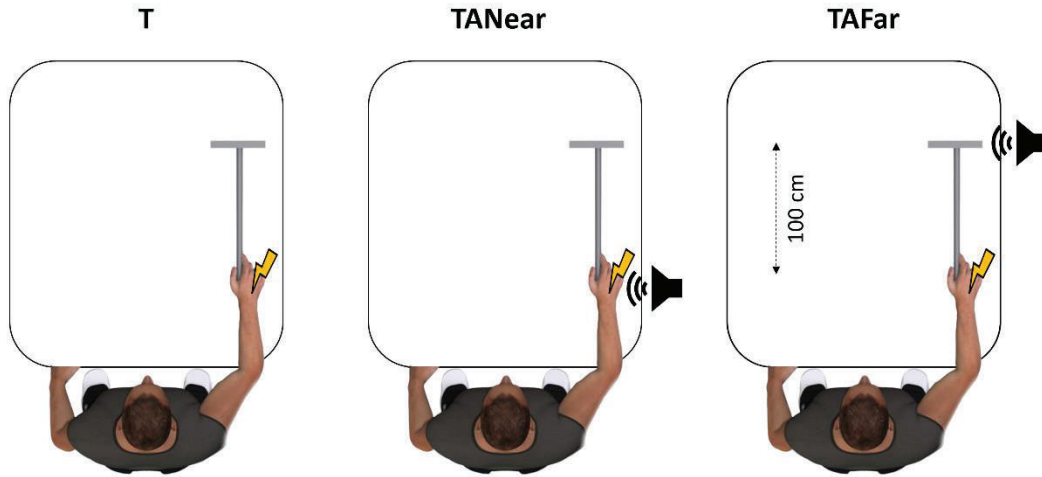
Fig.3. Audio-tactile interaction task, setup: tactile stimulation was administered alone (T condition) or simultaneously with an auditory stimulation coming from near position (TANear condition) or coming from far position (TAFar condition). During the stimulation, participants always hold the tool.

## 2.2.5. Data analysis

*Body-landmark localization task.* For each participant, the mean estimated location of the elbow and wrist among trials was computed and the distance between the two landmarks was considered as an indirect measure of the perceived arm length. We then calculated an index of the bias in the perceived dimension with respect to the actual one (estimated dimension, e.g. Peviani and Bottini, 2018), as the ratio between the perceived and the real length of the arm. In this way, we obtained an index of *estimated arm length* with respect to the real length of the arm, with values > 1 indicating an overestimation of the perceived arm length with regard to the real one and values < 1 referring to an underestimation (see Fig. 4). One subject was excluded from the final analysis

because his index of *estimated arm length* at baseline (A_pre and A_post) was greater than 2 standard deviations from the group mean. In addition, another subject was excluded because of a technical error during the acquisition of the real length of the. To compare the *estimated arm length* of the remaining 19 participants before and after the active and observational tool-use, we ran a 2x2 RM- ANOVA (Statistica Software 7.0 – StatSoft Inc.) with the within-in subject factors "Session" (pre or post) and "Training" (active or observational). Post hoc tests (planned comparisons) were used to explore significant interactions. Moreover, one sample t-tests against the value of 1, where 1 indicates the equivalence between the perceived and the real dimension, have been performed on each condition: active_pre, active_post, observational_pre, observational_post (significance level set at 0.05/4 comparisons, Bonferroni corrected).

*Audio-tactile interaction task.* First, the accuracy of each participant was calculated to ensure that they detected correctly at least the 97% of the trials (bimodal and unimodal) (e.g. Bassolino et al., 2010; Serino et al., 2015, 2007). Second, outliers were discarded if participants' RTs exceeded two standard deviations from the average of RTs collected within all the repetitions of any specific distance. This procedure was applied for both bimodal and unimodal trials. The average number of discarded responses among all the types of stimulation in all conditions (active, cognitive and observational) was around 5%. Then, subjects' RTs in response to T, TANear and TAFar conditions were averaged.

Multisensory integration effect. To investigate the multisensory integration effect (i.e. significant differences between unimodal and bimodal stimulation) and to explore the presence/absence of a space-dependent effect (i.e. significant differences between near and far positions), we run three separate (one for each training) one-way repeated measures ANOVAs (Statistica Software 7.0 – StatSoft Inc., Tulsa, OK) with RTs as dependent variable and "Conditions" as within-subject factor (three levels: T, TANear and TAFar). Then, to explore the presence of possible differential effects among conditions (T, TANear and TAFar), in each ANOVA planned comparisons were performed as post-hoc tests. Statistical threshold was set at p<0.05.

Space-dependent effect. To directly compare results obtained after the three different trainings (i.e. active, observational and cognitive) with the aim to highlight possible PPS modulations related to active and observational tool-use, we calculated an index of space-dependent effect. The index was obtained by subtracting, after each training, RTs in response to TAFar condition to those in

response to TANear condition (i.e. TANear *minus* TAFar). According to the spatial congruency law of multisensory integration mechanism (i.e. greater RT facilitation when bimodal stimulation occurs close in space – TANear – as compared to when bimodal stimulation occurs far in the space – TAFar), the smaller the index, more similar are RTs in response to TAFar condition and those in response to TANear condition, i.e. the more effective is the training in widening the boundaries of PPS. To compare possible PPS modulations after the three training, we performed a one-way repead measures ANOVA with the index of space-dependent effect as dependent variable and "Training" as within-subject factor (active, observational, cognitive training). Then, to explore the presence of possible differential effects among conditions, planned comparisons were performed as post-hoc tests. Statistical threshold was set at p<0.05.

### 3. Results

3.1. **Differentials effects on active and observational tool-use on BR and PPS representation**

*Body-landmark localization task.* Results to the body-landmark localization task are represented in Figure 4.

A repeated measure ANOVA performed on the estimated arm length, with "Training" (active or observational) and "Session" (pre and post training session) as within subjects factors, revealed a significant interaction between "Training and Session" ($F(1,18) =7.11$, $p =.016$) (Main significant effects: training [$F(1,18) =8.27$, $p =.010$], session [$F(1,18) =15.4$, $p <.001$]). Post-hoc planned comparisons revealed that the arm length before (pre) the active tool-use training and after (post) were significantly different (*active_pre* vs *active_post*: p= 0.001; mean±SD: *active_pre*: 0.89±0.12 mm; *active_post*: 1.03±0.18 mm), with the arm length perceived significant longer after active tool-use than at baseline. In contrast, the perceived arm length before and after (post) the observational tool-use training were not significantly different (*observational_pre* vs *observational_post*: p= 0.91; mean±SD: *observational_pre*: 0.86±0.16 mm; *observational_post*: 0.86±0.21 mm). This finding indicates that the observational tool-use training does not induce a significant change in the perception of the arm length. Accordingly, further planned comparisons show that even if the perceived arm length at the baselines was not significantly different (*active_pre* vs *observational_pre*: p= 0.35), the perceived arm length after the active training was significantly

larger than after the observational tool-use (*active_post* vs *observational_post*: p= 0.003, see Fig.4).

We noted also that the perceived arm length was statistically different from 1-value (where 1 indicates the equivalence between the perceived and the real length of the arm, see Fig.4) at baseline (active_pre, p value<0.0125, significance level set at 0.05/4 comparisons, Bonferroni corrected), while this was not the case after the active tool-use (p=0.47). This indicates that the significant underestimation observed at the baseline was no significant after active tool-use. This effect was not found after observational tool-use, where the perceived arm length remained statistically different from 1-value both before and after the training (all p values<0.0125, significance level set at 0.05/4 comparisons, Bonferroni corrected).
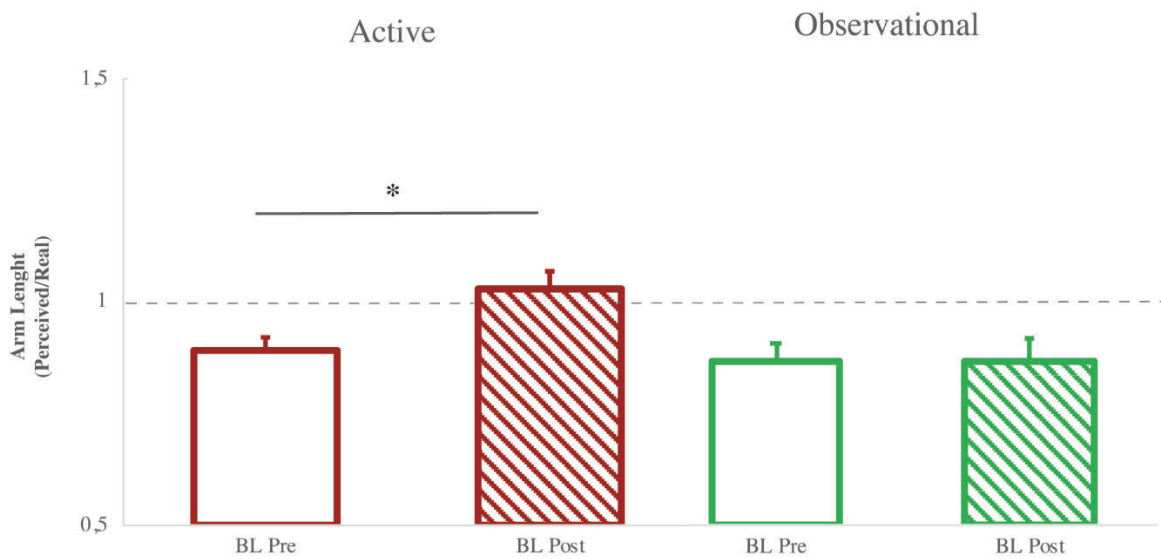


Fig. 4. Results of the body landmark (BL) localization task. The figure shows the results of the body-landmark localization task, expressed as the ratio between the perceived and the real arm length. Values below 1 (dashed line) indicate an underestimation of the perceived dimension with respect to the real one, while values above 1 indicate an overestimation. After (post) active tool-use (dark red) the arm length was perceived significantly longer than before (pre), while no significant changes emerged after observational tool-use (green). The perceived arm length was

statistically smaller than 1 (i.e. underestimation) at baselines and after observational tool-use, but not after the active training.

*Audio-tactile interaction task with corrected RTs*

Behavioural Results to the audio-tactile interaction task are represented in Figure 5.

*Post Cognitive training.* The one-way ANOVA on RTs revealed a main effect of Stimuli [$F_{(40;2)}=14.561$; $p<0.001$]. Post-hoc planned comparisons showed that TANear (bimodal near condition) and TAFar (bimodal far condition) were significantly different from T (TANear vs T: $p<0.001$; TAFar vs T: $p=0.031$); crucially, TANear and TAFar significantly differed ($p<0.001$), with faster RTs in bimodal near than in bimodal far condition (mean±SD: T: 404.49±129.88 ms; TANear: 358.95±132.99 ms; TAFar: 380.35±138.69ms – Fig. 5A). Overall, these results indicate that a greater RT facilitation occurred when the sound originated from near position rather than in the far position, in line with the spatial congruency law and as expected according to multisensory facilitation within PPS (e.g. Serino 2019).

*Post Active tool-use training.* The one-way ANOVA on RTs showed a main effect of Stimuli [$F_{(40;2)}=14.561$; $p<0.001$]. Post-hoc planned comparisons revealed that TANear and TAFar were significantly different from T (TANear vs T: $p<0.001$; TAFar vs T$<0.001$); crucially, TANear and TAFar did not significantly differ ($p=0.26$) (mean±SD: T: 403.67±126.28 ms; TANear: 380.04±136.62 ms; TAFar: 383.96±133.00ms – Fig. 5A). These findings indicate that RT facilitation in the near position and in the far position was not significant different after active training, suggesting that active tool-use induced a PPS remapping, eliminating the space-dependent effect of multisensory integration found after cognitive training.

*Post Observational tool-use training.* The one-way ANOVA on RTs revealed a main effect of Stimuli [$F_{(40;2)}=14.561$; $p<0.001$]. Post-hoc planned comparisons showed that TANear (bimodal near condition) and TAFar (bimodal far condition) were significantly different from T (TANear vs T: $p<0.001$; TAFar vs T: $p=0.028$); crucially, TANear and TAFar significantly differed ($p<0.001$), with faster RTs in bimodal near than in bimodal far condition (mean±SD: T: 383±99.99 ms; TANear: 350.95±111.11 ms; TAFar: 365.80±111.35ms – Fig. 5A). Overall, these results indicate

that a greater RT facilitation occurred in the near position than in the far position, pointing out that observational tool-use did not broaden the PPS, maintaining the space-dependent effect observed after cognitive training.

*Space-dependent effect.* The one-way ANOVA directly comparing the three indexes of space-dependent effect (computed from the subtraction between RTs of the far position from those of the near position after each training) displayed a main effect of Training [F=(40;2)=3.666; p=0.034]. Crucially, post-hoc planned comparisons revealed that the active tool-use space-dependent effect was significantly smaller both from the observational tool-use (p=0.034) and the cognitive index (p=0.016) (Fig. 5B). On the contrary, the observational tool-use and the cognitive tool-use space-dependent effect did not differ (p=0.457). This finding, in accordance with one-way ANOVAs presented above, suggested that the differential response between near and far positions was significantly reduced following active tool-use as compared to following cognitive training and observational too-use training. Hence, the smaller space-depending effect after active-tool use indicates a remapping of PPS induced by the training, highlighted by a comparable multisensory facilitation on tactile responses associated with near and far sounds.
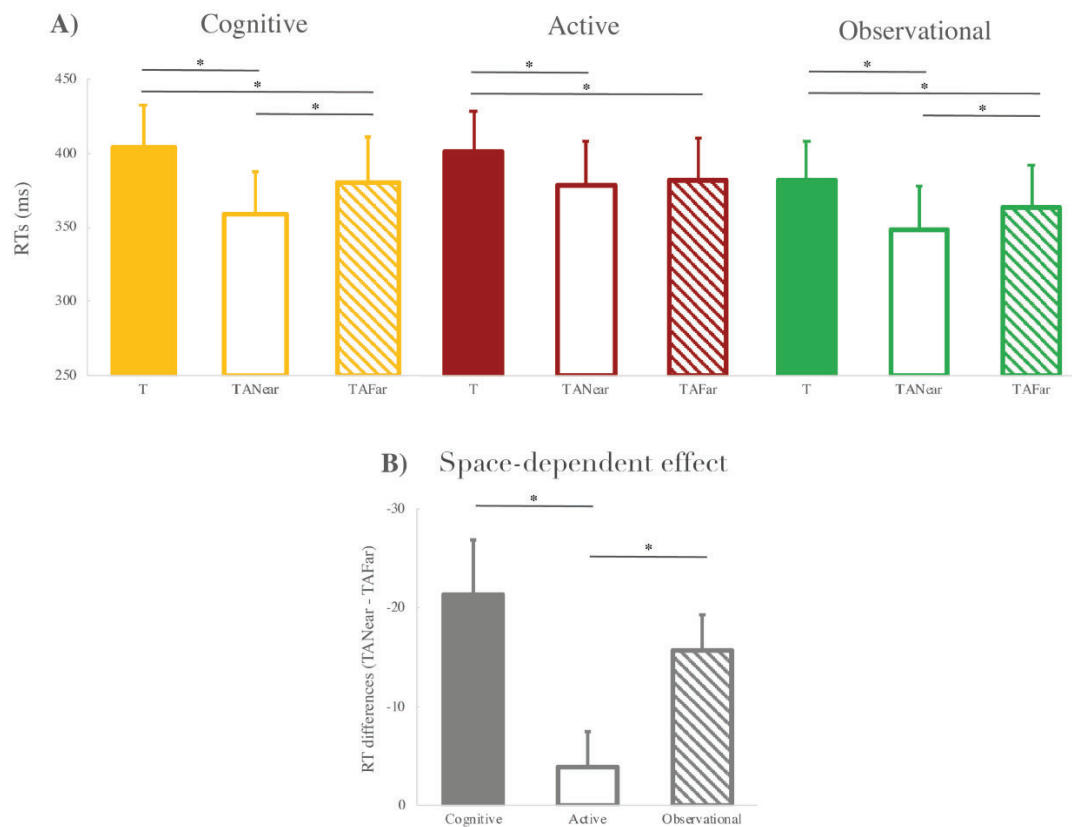
Fig. 5.A. Panel (A) Mean of reaction times (RTs) in the three conditions: after cognitive training (on the left), after active-tool use training (on the middle); after observational tool-use training (on the right). Only after active tool-use training, the two bimodal conditions (TA, tactile+auditory stimuli) did not significantly differ, suggesting that the PPS remapping occurs only when the subject actively perform the tool-use. B. Space-dependent effect. The figure represents the space-dependent effect index (i.e. RTs of TANear condition *minus* RTs of TAFar condition) after the three trainings. The index after active tool-use training is significantly smaller than both indexes obtained after cognitive and observational tool-use training, suggesting that only active tool-use induced a remapping of the PPS.

## 4. Discussion

The present study aimed at investigating whether the mere observation of someone else acting with a tool in far space impacts on bodily and spatial representations as execution. To solve this issue, BR and PPS were assessed with a body-landmark localization task and an audio-tactile interaction

task. Our results show that, as expected, active tool-use induced a modulation of BR and PPS, respectively highlighted by an increased perceived length of the arm, and a comparable multisensory facilitation on tactile responses due to near and far sounds after active training. On the contrary, such modulations were not found after observational tool-use, pointing out that a mere observational training is not sufficient to affect BR and PPS.

*Body-landmark localization task.* The findings from the BL task, aiming to capture the implicit metric representations of the upper limb, suggest that participants underestimated the arm length (i.e. perceived length smaller than real length) at baseline (before the training) similarly in both conditions in agreement with earlier studies (e.g. Longo, 2017).

As expected, after the active condition, a significantly longer perception of the arm length after the training compared to the baseline was found. This is in line with an extension of the arm length after tool-use demonstrated in previous studies using the same task as in the present work (Canzoneri et al., 2013), an arm bisection task (Garbarini et al., 2015; Sposito et al., 2012), or by analysing free hand movements kinematics (Cardinali et al., 2009). In the present work, the increased perceived length of the used arm in the active condition could be also interpreted as a bias reduction (see Fig. 4), considering the fact that the post session was not statistically different from the 1 ratio representing the correct estimation of the perceived arm length. Importantly, the bias reduction in the arm length perception after active tool-use (Bassolino et al., 2014; Canzoneri et al., 2013; Cardinali et al., 2009; Sposito et al., 2012), could be interpreted as driven by the flow of sensorimotor information, as well the motor planning and intention, related to the active movement performed during the training, which contribute to update the representation and to correct the underestimation found at the baseline.

In contrast, after the observational condition, the arm length was not statistically different from the baseline: both pre and post assessment demonstrated an underestimation of the arm length (values significantly different from 1). This result is also line with a previous study demonstrating no changes in BR after observational tool-use in older adults (Garbarini et al., 2015). Considering together the two studies, it is possible to suggest that observing an actor using a tool while holding the same tool could not be sufficient to modify BR neither in young nor in elderly participants. It has been demonstrated that action observation could activate motor areas (Jeannerod, 2001), but here these results suggest that a central brain activation of motor region through observation is not

enough to shape BR. If BR modifications could be mainly mediated by multisensory and sensorimotor information related to *one's own* body, it is possible that the mere visual observation of *someone else* using the tool could not be sufficient to induce alterations of one's own BR (Bassolino et al., 2014), because of a lack of updated afferent information from ones' own body. In line with this assumption, a previous study on a patient with proprioception impairment demonstrated that only visual information of the movement in absence of the perception of one's own arm in motion is not sufficient to induce an incorporation of the tool, pointing out the role of afferent information in shaping BR (Cardinali et al., 2016). However, recently Bruno and colleagues (Bruno et al., 2019)) showed that the mere sensorimotor feedback of the arm movement action is no sufficient either to induce plastic changes of BR. Indeed, authors found no plastic changes in BR when participants performed a passive tool-use. In that study, the active session consisted of the execution of "enfold-and-push" movements with a tool in order to place cubes in a target area; instead, in the passive session, participants were asked to be completely relaxed, and the movements towards the target area were performed with robotic assistance. Results displayed a significant increase of the perceived arm length only after active training, suggesting that the passive execution of tool action is not enough to shape the BR. Together, these two studies in line with the present results seem to suggest that sensorimotor feedback are necessary to induce plasticity of BR (Cardinali et al. 2006), although not sufficient (Bruno et al., 2019). This may indicate that the congruency between sensorimotor feedback, and motor planning and intention are crucial in order to induce a plastic modulation of BR.

*Audio-tactile interaction task.* The audio-tactile interaction task aimed at investigating the effect of active and observational tool-use on the PPS plasticity exploiting the multisensory integration phenomenon, i.e. a speeding up in RTs to tactile stimuli due to simultaneous auditory stimuli appearing near the hand, within PPS (e.g. Bassolino et al., 2010; Sambo and Forster, 2009; Serino et al., 2007). As expected, after the active tool-use condition, we found comparable RTs in near and in far position (see Fig 5A), pointing out that following tool-use the auditory stimulus delivered in the far space induced a similar multisensory facilitation as in the near space. The present results are fully in agreement with previous studies (Bassolino et al., 2010; Biggio et al., 2017; Iriki et al., 1996; Neppi-Mòdona et al., 2007; Ronga et al., n.d.; Serino et al., 2007), showing that tool-use results in an extension of PPS by extending the typical multisensory integration of the space surrounding the body to the farther spatial sector where the tool is used. In contrast, after cognitive

training (i.e. a visual discrimination task performed at a distance from participants' chest corresponding to the length of tool radius action), we found a greater multisensory facilitation effect in the near space as compared to the far space (see Fig. 5A), revealed by significantly faster RTs when the auditory stimulus occurred close to the stimulated hand as compared to when it occurred in far positions. This finding excludes that an attentional shift towards the far space is the only determinant of PPS remapping after tool-use (Holmes, 2012). Similarly to cognitive training, also following observational tool-use we found a differential behavioural performance between bimodal near and bimodal far conditions (see Fig. 5A). These results suggest that the observation of another individual performing a tool-use does not modify the PPS representation. As supporting evidence, the *index of space-dependent effect (*calculated by subtracting, in each session, RTs in response to TAFar condition to those in response to TANear condition - TANear *minus* TAFar) of the post-active tool-use session was significantly smaller than both indexes obtained post observational tool-use and post cognitive training (see Fig.5B), confirming that only active tool-use widened the boundaries of PPS. However, some effects of tool-use observation on space representation were found in previous works. In particular, Costantini and colleagues (2011) showed that observing an alien arm performing actions extends the reaching space of the observers, if they hold a similar tool in the hand. It could be then possible that during the observation of goal-oriented actions in the extrapersonal space, a mirror mechanism is activated (Rizzolatti et al., 2001) that is robust enough to remap a spatial representation of the observer in an explicit reachability task such as that employed in the Costantini's et al. (2011) study, but not sufficient to significantly modify the implicit multisensory representation of the observers' PPS as evaluated with the present paradigm. Accordingly, in the Costantini and colleagues' work, the mirrored movement experienced during the training (i.e. grasping with a rake) reflects the same movement involved during the reachability judgment task (i.e. grasping); thus, it is reasonable to hypothesize that the remapping effect may be due to the fact that the "grasping network" is recruited both in the training and in the task phase. In contrast, our task specifically focused on PPS as the preferential space for multisensory integration, thus directly contributing to the emergence and maintenance of a coherent multimodal bodily self-representation [i.e., self-consciousness purpose – for a recent review see e.g., (Noel et al., 2018)]. Hence, we can suppose a dissociation between a reaching-related spatial representation, assessed by Costantini and co-authors' task, and a multisensory PPS representation, assessed by the task in the present study, assuming a different effect of observation of another agent

performing the tool-use in modifying such representations.

The lack of remapping of multisensory PPS after observational tool-use may indicate that PPS plasticity could rely on the sensory feedback originating from performing the effects of the action with the tool in the far space, coupled with the sensory feedback arising from the hand during this movement. In line with this, (Serino et al., 2015) proposed that the plasticity of multisensory PPS is triggered by the association between synchronous tactile stimulation at the hand, due to holding the tool, and multisensory -auditory or visual stimulation - from the far space, where the tool is operated.

*Similar dissociable effects of active and observational tool-use in BR and PPS representation*

To sum up, the present findings suggest different effects both on the BR and PPS representation during the active and observational tool-use. In line with previous studies (Bassolino et al., 2014; Berti and Frassinetti, 2000; Biggio et al., 2017; Canzoneri et al., 2013; Cardinali et al., 2009; Sposito et al., 2012), after active tool-use, BR and PPS were modified. In particular, after active tool-use participants reported a longer perceived length of the arm than at baseline (group 1) and equally facilitated RTs to tactile stimuli when combined with near and far sounds (group 2). Crucially, no significant plastic effects in BR or PPS occur after a training of the same duration based on observational tool-use. More precisely, after observational tool-use, no significant modification of the perceived length of the arm occurred (group 1), and a higher facilitation in RTs to tactile stimuli associated with near sounds as compared to far sounds occurred as in the control condition (cognitive) (group 2).The absence of effects on BR and PPS in the observational condition suggests that, at least in our sample, active tool-use is necessary in order to induce plastic changes of these representations, whereas tool-use observation is not sufficient. In line with this assumption, previous studies demonstrated that sensorimotor feedback is necessary, but not sufficient, to drive BR plasticity (Bassolino et al., 2010; Bruno et al., 2019; Cardinali et al., 2016). This evidence seems to highlight a fundamental role of motor intention and planning in reshaping own BR and PPS. This is also supported by evidence provided by Garbarini and coauthors (2015). They showed that brain damaged hemiplegic patients, manifesting a pathological embodiment of someone else's arm, exhibited an increase of the perceived length of their forearm after a training phase in which an experimenter was aligned to them and performed movements with a tool in the

far space. The crucial aspect of this study is that these patients, while observing the experimenter's arm performing the tool-action, were firmly convinced to perform it with their own (paralyzed) arm. It has been proposed that the pathological embodiment of the experimenter's arm movement automatically triggers intentional motor processes of the own arm that, in turn, induces a forearm length remapping comparable to that found in healthy subjects actually performing the tool-use training. Thus, these findings point out that having real motor intentions to move the tool, even in absence of actual movement execution, induces a modulation of BR. Coherently, BR and PPS have been shown to be affected by the sense of agency (D'Angelo et al., 2018); in this study, BR and PPS were assessed after a training phase, in which participants virtually grasped objects by controlling the virtual hand in a 3D environment. In the training phase, the sense of agency was modulated introducing a synchronous condition, wherein participants were shown virtual hands movements responding in real time to their own movements, and an asynchronous condition, wherein a 3-second delay was interposed between the participant's actual hand and the virtual hand movements. Crucially, only when subjects sensed agency for the virtual hand, induced by the synchronicity between motor and visual feedbacks, BR and spatial representation (evaluated with a reaching distance estimation task) enlarged. Therefore, the modulation of body and space metrics is strictly dependent to the sense of congruency between the intention to perform an action and the resulting sensorimotor feedback. Overall, this would suggest that motor planning and intention related to performing tool-actions and consequent sensorimotor feedback play a crucial role in driving BR and PPS plasticity.

In view of the foregoing, further studies would be addressed to investigate whether the mere motor intention and planning are sufficient to induce plastic changes of BR and PPS, or whether the congruency between the intention to perform an action and the resulting sensorimotor feedback are necessary to cause these modulations. Motor imagery could help to disentangle between the role of motor intention and sensorimotor consequences, allowing to isolate the contribution of motor planning. Motor imagery can be considered as a promising tool, also in light of previous results showing that kinematics of free-hand movements was affected after tool-use imagery, in a similar way to that previously documented after active tool-use (Baccarini et al., 2014). Then, if motor intention and motor planning are sufficient to induce a tool-related BR and PPS broadening, we should expect a modulation of these representations following tool-use imagery. Alternatively, if PPS, and also BR, plasticity is triggered by the congruency between the intention to perform an

action and the resulting actual sensorimotor feedback, we should expect any change on these representations after motor imagery-based tool-use, as found here after observational tool-use.

## 5. Conclusion

In conclusion, the present findings provide evidence that the observation of another person using a tool to interact with objects located in the far space is not sufficient to influence the plasticity of PPS and BR. Thus, the dissociation found in the active and observational tool-use highlights differences between action execution and action observation, pointing out a crucial role of motor intention and planning and the related sensorimotor feedback in driving BR and PPS plasticity.

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# Appendix 3: Sensorimotor hallucinations in Parkinson's disease

**Personal contribution:** data collection

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# Sensorimotor hallucinations
# in Parkinson's disease

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FB designed Study 1 and 3, collected & analyzed data, conducted clinical interviews, wrote paper; EB designed Study 2, collected & analyzed data, wrote paper; J. Potheegadoo collected data, designed questionnaire for semi-structured interview, conducted clinical interviews and clinical evaluations for study 1; GS analyzed data for study 3; J. Pagonabarraga, HB and JK recruited patients, conducted clinical interviews, collected data for study 3; MA and NF analyzed data for study 2; MB collected data, conducted clinical interviews and clinical evaluations for study 1; MF collected data for study 1; SK coordinated the recruitment for study 1; SMH designed and conducted clinical interviews for study 3; FS collected data for study 3; MH designed and developed the robotic systems; JH, JG, PB

recruited patients and conducted clinical evaluations for study 1; DV designed study 2; PK designed study 1; GR and OB designed study 1, 2 and 3, wrote paper. All authors provided critical revisions and approved the final version of the paper for submission.

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## Summary

Hallucinations in Parkinson's disease (PD) are one of the most disturbing non-motor symptoms, affect half of the patients, and constitute a major risk factor for adverse clinical outcomes such as psychosis and dementia. Here we report a robotics-based approach, enabling the induction of a specific clinically-relevant hallucination (presence hallucination, PH) under controlled experimental conditions and the characterization of a PD subgroup with enhanced sensorimotor sensitivity for such robot-induced PH. Using MR-compatible robotics in healthy participants and lesion network mapping analysis in neurological non-PD patients, we identify a fronto-temporal network that was associated with PH. This common PH-network was selectively disrupted in a new and independent sample of PD patients and predicted the presence of symptomatic PH. These robotics-neuroimaging findings determine the behavioral and neural mechanisms of PH and reveal pathological cortical sensorimotor processes of PH in PD, identifying a more severe form of PD associated with psychosis and cognitive decline.

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**Introduction**

The vivid sensation that somebody is nearby when no one is actually present and can neither be seen nor heard (i.e. sense of presence or presence hallucination, PH), has been reported from time immemorial and found its way into the language and folklore of virtually all cultures[1–3]. Following anecdotal reports of PH by extreme mountaineers[4], solo-sailors and shipwreck survivors[5], PH have also been described in a variety of medical conditions including schizophrenia[1,6], epilepsy, stroke, brain tumors[7–9] and Parkinson's disease (PD)[10–12].

Whereas PH are rare manifestations in most medical conditions, they are frequent and may occur regularly, even on a daily basis, in patients with PD. Hallucinations, including PH, are not only frequent, occurring in up to 60% of PD patients, but increase in frequency and severity with disease progression and are one of the most disturbing non-motor symptoms[11–13]. Importantly, PH and other hallucinations in PD are associated with major negative clinical outcomes such as chronic psychosis, cognitive decline and dementia, as well as higher mortality[10,11,14–16]. PH are generally grouped with so-called minor hallucinations and are the most prevalent and earliest type of hallucination in PD[11,12], often preceding the onset of structured visual hallucinations[17], and may even be experienced, by one-third of patients, before the onset of first motor symptoms[18]. Despite their high prevalence and strong association with major negative clinical outcome, PH (and other hallucinations) remain underdiagnosed[12,14,19,20], caused by patients' reluctance to report hallucinations and difficulties to diagnose and classify them[21,22].

Past research described changes in visual function, cognitive deficits and related brain mechanisms in PD patients with hallucinations, yet these studies focused on patients with structured visual hallucinations[23]. Comparable studies are rare or lacking for PH (or other minor hallucinations) and very little is known about the early brain dysfunction of PH in PD and how they lead to more severe and disabling structured visual hallucinations and cognitive deficits[11,24]. Early neurological work investigated PH following focal brain damage and classified PH among disorders of the body schema, suggesting that they are caused by abnormal self-related bodily processes[9,25].

More recent data corroborated these early findings and induced PH repeatedly by electrical

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stimulation of a cortical region involved in sensorimotor processing[8]. By integrating these clinical observations with human neuroscience methods inducing bodily illusions[27–30], we have designed a method able to robotically induce PH (robot-induced PH or riPH) in healthy participants[26]. This research demonstrated that specific sensorimotor conflicts, including bodily signals from the arm and trunk, are sufficient to induce mild to moderate PH in healthy participants, linking PH to the misperception of the source and identity of sensorimotor signals of one's own body.

Here, we adapted our robotic procedure to PD patients and elicited riPH, allowing us to characterize a subgroup of patients that is highly sensitive to the sensorimotor procedure, and to identify their aberrant sensorimotor processes (study 1). We next determined the common PH-network in frontal and temporal cortex, by combining MR-compatible robotics in healthy participants with brain network analysis in neurological non-PD patients with PH (study 2). Finally, we recorded resting-state fMRI data in a new and independent sample of PD patients and identified pathological functional connectivity patterns within the common PH-network, which were predictive for the occurrence of PD-related PH (study 3).

## Results

### riPH in patients with PD (study1.1)

Based on semi-structured interviews, patients with PD were grouped into those who reported symptomatic PH, sPH (PD-PH; n=13), and those without sPH (PD-nPH; n=13) (Supplementary S1-2, Tab.S1-2). Patients were asked to actuate a robotic device and were exposed to repetitive sensorimotor stimulation that has been shown to induce PH in healthy participants in a controlled way[26]. In study1.1, we assessed whether robotic sensorimotor stimulation induces PH in patients with PD and whether such riPH differ between PD-PH and PD-nPH, hypothesizing that PD-PH patients are more sensitive to the robotic procedure.

In the robotic sensorimotor paradigm, participants were asked to perform repetitive movements to operate a robot placed in front of them, which was combined with a back robot providing tactile feedback to participants' backs (Fig.1A). Based on previous data[26,28,31], tactile feedback was delivered either synchronously with patients' movements (synchronous control condition, a spatial conflict is present between movement in front and touch on the back) or with a 500ms delay (asynchronous condition) associated with an additional spatio-

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temporal sensorimotor conflict shown previously to induce PH[26,36] (Supplementary S3).

The robotic procedure was able to induced PHs in patients with PD. Importantly, PD-PH patients rated the intensity of riPH higher than PD-nPH patients (main effect of Group: permutation p-value=0.01) (Fig.1B). Confirming the general importance of conflicting asynchronous sensorimotor stimulation[26] for riPH, both sub-groups gave higher PH ratings in the asynchronous versus synchronous condition (main effect of Synchrony: permutation p-value=0.045) (Fig.1C) (Supplementary S4 for additional results). Other robot-induced bodily experiences (e.g. illusory self-touch) also confirmed previous findings[26] (Supplementary S5) and no differences were observed for the control items (all permutation p-values>0.05). These results show that PH can be safely induced by the present robotic procedure under controlled conditions in patients with PD. Such riPH were modulated by sensorimotor stimulation with asynchronous robotic stimulation resulting in higher ratings in all tested groups, and, importantly, PD-PH (vs. PD-nPH) reported stronger riPH, linking the patients' usual sPH to experimental riPH and showing that PD-PH patients were more sensitive to our robotic procedure.

Post-experiment debriefing revealed 38% of PD-PH patients who reported riPH that were comparable (or even stronger) in intensity to the patients' usual sPH in daily life. One PD-PH patient, for example, described his riPH as "an adrenaline rush. Like something or someone was behind me, although there is no possibility to have someone behind" (for additional reports Supplementary S6). Interestingly, all such instances were reported after asynchronous stimulation. Moreover, PD-PH patients often experienced riPH on their side (and not on their back, where tactile feedback was applied), revealing a further phenomenological similarity between riPH and PD patients' usual sPH[10] and suggesting that we induced a mental state that mimics sPH (Supplementary S7-8).

Data from study1.1 reveal that riPH can be safely induced by the present procedure, are stronger in patients who report sPH (PD-PH), and that such riPH share phenomenological similarities with PD-related sPH. These findings cannot be related to a general response bias related to PD, because riPH were absent or weaker in PD-nPH and because the control items showed no effects in any of the participant groups.

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**riPH in PD-PH patients depend on sensorimotor delay (study1.2)**

Previous work investigated the effects of systematically varied sensorimotor conflicts (i.e. delays) on somatosensory perception, enabling the induction and modulation of different somatic experiences and illusions[31–33]. Sensorimotor processing and the forward model of motor control[34,35] are prominent models of hallucinations[36,37] and it has been proposed that deficits in predicting sensory consequences of actions causes abnormal perceptions and hallucinations[36–38]. In study1.2, we assessed whether riPH depend on the degree of conflict applied during sensorimotor stimulation, by inserting variable delays between the movements of the front robot (capturing movements of the forward-extended arm) and the back robot (time of tactile feedback on the back). In each trial, participants (Supplementary S9) were exposed to a randomly chosen delay (0-500ms, steps of 100ms). After each trial, participants were prompted whether they experienced a riPH or not (yes-no response, Supplementary S10). We investigated whether the intensity of riPH increases with increasing delays in PD patients (showing that PH are modulated by increasing spatio-temporal conflicts) and whether PD-PH have a higher spatio-temporal delay sensitivity than PD-nPH.

As predicted, study1.2 shows that the intensity of riPH increased with increasing spatio-temporal conflict (main effect of delay: permutation p-value=0.014) and that this delay dependency differed between the two patient groups, showing a higher delay sensitivity in PD-PH patients (interaction Group*delay: permutation p-value=0.039) (Fig.1D) (Supplementary S11, Fig.S1). Control analysis (Supplementary S12) (Fig.1E-F, Fig.S2) allowed us to exclude that the observed differences (in riPH ratings between patient groups) are due to differences in movements of the arm and related tactile feedback during the robot actuation (Supplementary S13). In addition, these differences in riPH between PD-PH and PD-nPH cannot be explained by differences in demographic or clinical variables (including anti-parkinsonian medication, motor impairment; all permutation p-values>0.05) (Supplementary S14, Tab.S1).

Based on previous results using robotics and conflicting sensorimotor stimulation to alter somatosensory perception[31–33], these data extend those of study1.1 and reveal abnormal perceptual processes in PD-PH patients when exposed to different sensorimotor conflicts, characterized by experiencing stronger riPH and a higher sensorimotor sensitivity. These findings are compatible with an alteration of sensorimotor brain processes associated with the forward model and its role in hallucinations in PD-PH patients[36,37,39].

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**Brain mechanisms of PH**

Neuroimaging work on sPH and other minor hallucinations in PD patients has described structural alterations and aberrant functional connectivity in different cortical regions[24,40]. Despite these clinical neuroimaging findings, it is not known whether the regions associated with sPH of neurological non-parkinsonian origin[26] are also altered in PD patients with PH. Moreover, because the brain networks of riPH have never been investigated, it is also not known whether the abnormal sensorimotor mechanisms described in PD-PH patients (study1) are associated with a disruption of brain networks of riPH. To determine the brain mechanisms of PH, we first adapted an MR-compatible robot[41] (Supplementary S15) and applied sensorimotor stimulations while recording fMRI during riPH in healthy participants and identified the associated brain networks (study2.1). We then combined this network with evidence from sPH of neurological non-parkinsonian origin (study 2.2) and, finally, applied this common network to PD patients (study 3).

**Brain mechanisms of riPH in healthy participants using MR-compatible robotics (study2.1)**

Based on behavioral pilot data (Supplementary S16-S17, Tab.S5), we exposed 25 healthy participants to asynchronous and synchronous robotic stimulation while recording fMRI (Fig.2A, Supplemental S15, Fig.S3). Our behavioral data replicated previous results ([26], study1 and pilot study) and we found that asynchronous vs. synchronous robotic stimulation induces stronger PH (main effect of Synchrony: permutation p-value=0.0082, Fig.2B) and another bodily experience (Tab.S6), but did not modulate control items (all permutation p-values>0.08, Supplementary S18, Tab.S6). As for study1.2, riPH were not related to movement differences across conditions (permutation p-value=0.99) (Fig.2C), confirming that sensorimotor stimulation (and not movement differences) applied with the MR-compatible robot modulated PH intensity across conditions.

To identify the neural mechanisms of riPH, we determined brain regions that were (1) more activated during the asynchronous vs. synchronous condition (spatio-temporal sensorimotor conflict) and (2) activated by either of the sensorimotor conditions (synchronous, asynchronous) vs. two control conditions (motor and touch) (Supplementary S19, conjunction

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analysis). Regions more activated during asynchronous vs. synchronous sensorimotor stimulation were restricted to cortical regions (Fig.2D, Tab.S7) and included the inferior frontal gyrus (IFG), anterior insula, medial prefrontal cortex (mPFC) and the posterior part of the middle temporal gyrus (pMTG, bordering on angular gyrus and adjacent occipital cortex). Conjunction analysis (between contrast synchronous>motor+touch and contrast asynchronous>motor+touch) (Supplementary S20, Fig.S4) revealed a subcortical-cortical network in left sensorimotor cortex (contralateral to the hand moving the robot, including M1, S1 and adjacent parts of premotor cortex and superior parietal lobule), in bilateral supplementary motor area (SMA), right inferior parietal cortex, left putamen, and right cerebellum (Fig.2E, Tab.S8).

Collectively, these fMRI results constitute the first delineation of the neural underpinnings of riPH in healthy participants that is unrelated to movement differences across conditions and distinct from activations in two control conditions, revealing a network of brain regions that have been shown to be involved in sensorimotor processing and in agency (such as M1-S1, pMTG[42,43], PMC[44,45], SMA[43,46], IPS[47,48], as well as the cerebellum[42,49] and putamen).

**Common PH-network for sPH and riPH (study2.2)**

To determine neural similarities between riPH and sPH and confirm the sensorimotor contribution to sPH, we first applied lesion network mapping (Supplementary S21) and identified network connectivity mapping in neurological non-parkinsonian patients, in whom sPH were caused by focal brain damage (study2.2), and then determined the common network (cPH-network) between the riPH and sPH. Lesion network mapping[50] extends classical lesion symptom mapping by considering each lesion as a seed (region of interest, ROI) and computing its connectivity map (in normative resting state fMRI data, publicly available database, 126 healthy participants[51]) (Fig.S5).

This analysis revealed that all lesions had functional connectivity with bilateral posterior superior temporal gyrus/temporo-parietal junction (pSTG/TPJ), bilateral middle cingulate cortex (MCC), bilateral insula, and right IFG, constituting the sPH-network (Fig.3A, for all regions see Tab.S9) and did not overlap with connectivity patterns of a control hallucination network (Supplementary S22-S23, Tab.S10). We then determined the common regions between the sPH-network (non-parkinsonian neurological patients) and the riPH network (healthy participants). This cPH-network consisted of three regions, including right IFG, right

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pMTG, and left vPMC (Fig.3B, Supplementary S24) and is the first neuroimaging evidence that riPH and sPH recruit similar brain regions, even if both types of PH differ in several aspects such as frequency, intensity, trigger mechanism, supporting a link between sensorimotor robotics inducing hallucinatory states with neuroimaging in healthy participants and in patients.

### Disrupted functional connectivity in cPH-network accounts for sPH Parkinson's disease (study3.1)

To assess the relevance of the cPH-network for PD patients' usual sPH in daily life, we analyzed resting state fMRI data in a new group of PD patients and investigated whether functional connectivity of the cPH-network (as defined in study2, projected bilaterally, Fig.3C) differed between PD-PH and PD-nPH (new cohort of 30 PD patients) (Supplementary S25-26, Tab.S11). Based on the disconnection hypothesis of hallucinations[52], evidence of decreased connectivity for hallucinations of psychiatric origin[37], and aberrant functional connectivity in PD patients with minor hallucinations including PH[24], we predicted that the functional connectivity within the cPH-network differs between both PD patient groups and that the connectivity within the cPH-network is reduced in PD-PH vs. PD-nPH patients. We found that the functional connectivity within the cPH-network, predicted with 93.7% accuracy whether a patient was clinically classified as PD-PH (kappa:0.86, permutation p-value=0.0042). Moreover, within the cPH-network, the functional connectivity between the left IFG and left pMTG contributed mostly to the classification of the two sub-groups (Tab.S12). PD-PH had reduced IFG-pMTG connectivity (permutation p-value<0.0001; Fig.4A-B). These changes were selective because (1) the same analysis in a control network (Fig.S7) (same size, same number of connections) did not predict the occurrence of hallucinations based on the functional connectivity (accuracy:27.7%, kappa:-0.43, permutation p-value=0.24) and (2) no changes in functional connectivity were observed when analyzing whole brain connectivity. These data show that reduced fronto-temporal connectivity within the cPH-network distinguishes PD patients with sPH from those without hallucinations, in accordance with the disconnection hypothesis of hallucinations[52–54].

### Functional disconnection within the cPH-network correlates with cognitive decline for PD-PH (study3.2).

It has been suggested that PH (and minor hallucinations) are indicative of a more severe and

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rapidly advancing form of PD, evolving towards structured visual hallucinations and psychosis[11,17], as well as faster cognitive deterioration including dementia[16,55–57]. We therefore tested whether functional connectivity between the left IFG and the left pMTG within the cPH-network relates to cognitive dysfunction in the present PD-PH patients. Results show that stronger decreases in left IFG-pMTG connectivity are associated with stronger cognitive decline (PD-CRS[58]), reflecting differences in frontal-subcortical function (p-value=0.01,rho=0.69,Fig.4C), but not on posterior-cortical function (p-value=0.66, rho=-0.15, the two correlations differed significantly: t=3.87, p-value<0.01). These results reveal an association between fronto-subcortical cognitive alterations and specific decreases in fronto-temporal connectivity within the cPH-network in PD-PH patients, compatible with a more severe form of PD associating PH and cognitive decline.

**General Discussion**

Having developed a robotic procedure that can induce PH in PD patients under safe and controlled sensorimotor conditions, we report that PD patients with sPH are highly sensitive to the procedure and reveal abnormal sensorimotor mechanisms leading to PH. Using MR-compatible robotics in healthy participants combined with lesion network mapping analysis in patients with sPH of neurological non-parkinsonian origin, we identify the common network associated with PH and show that fronto-temporal connectivity within this cPH-network is selectively disrupted in a new and independent sample of PD patients. Disruption of the cPH-network was only found in PD patients suffering from sPH (PD-PH) and the degree of this disruption further predicted the severity of cognitive decline.

The present behavioural findings show that stronger sensorimotor conflicts result in stronger riPH, supporting and extending previous evidence in favor of an alteration of self-related sensorimotor processing as a fundamental mechanism underlying PH[33]. Importantly, we show that this mechanism is especially vulnerable in PD-PH patients, revealed by their stronger bias and sensitivity when exposed to conflicting sensorimotor stimulation. These results extend the sensorimotor forward model to hallucinations in PD-PH patients[36,37,39] and support earlier evidence in neurological non-PD patients that PH are self-related body schema disorders associated with altered sensorimotor self-monitoring[7–9].

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By including fMRI data from healthy participants experiencing riPH and from non-parkinsonian neurological patients with sPH, we mapped common brain structures between both types of PH, which we showed to be selectively disrupted in PD patients with sPH. The imaging results within this cPH-network further revealed aberrant functional connectivity decreases between fronto-temporal regions that have been associated with outcome processing of sensorimotor signals and the forward model[54,59], further linking PH in PD to the fronto-temporal hallucination disconnection model[52,54,60]. The present account - involving sensorimotor mechanisms and brain structures in fronto-temporal cortex rather than posterior brain functions and regions - is functionally and conceptually distinct from earlier proposals that hallucination in PD are caused by visuo-spatial deficits[23] or that sPH are caused by abnormal social-cognitive brain mechanisms[10] in parietal or occipital cortex[23,61,62]. Our finding that the decreased fronto-temporal connectivity within the cPH-network is associated with stronger cognitive decline of PD-PH patients in fronto-subcortical (but not posterior-cortical, functions) lends support to clinical suggestions about the importance of PH (and other minor hallucinations) as a major risk factor not only for the occurrence of structured visual hallucinations and psychosis[17], but also for a more severe and rapidly advancing form of PD[11,16,55,57].

Because the phenomenology of riPH resembles those of sPH and PD-PH patients were found to be more sensitive to riPH, the present procedure provides researchers and clinicians with new objective possibilities to assess the occurrence and intensity of subjective hallucinatory phenomena by quantifying delay-sensitivity and the repeated online induction of hallucinatory states across controlled conditions in PD patients, as well as the association of these measures with cPH-network activity. This is not possible in current clinical practice that is based on clinically important, but post-hoc interviews between physician and patient, often about hallucinations that have occurred many days or weeks ago, and that many patients hesitate to speak about[21]. The detection of specific behavioural and imaging changes associated with specific hallucinatory states that are observed online during the robotic procedure will improve the quantification and prediction of a patient's proneness for hallucinations and psychosis and may facilitate targeted pharmacological interventions that limit side effects[63].

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## Methods

### Study 1
### Participants (study1.1-1.2)

All participants provided written informed consent prior to the experiments. The study was approved by the Cantonal Ethics Committee of Geneva (Commission Cantonale d'Ethique de la Recherche sur l'Être Humain), the Cantonal Ethics Committee of Vaud. Participants of study1 consisted of patients with PD (n=26) and age-matched healthy controls (HC, n=21) (Supplementary S1-S4). Based on an extensive semi-structured interview (conducted after the experimental sessions) about hallucinations (including sPH), PD patients were separated into two sub-groups: patients who reported sPH as part of their PD (PD-PH) (n=13) and PD patients without sPH (PD-nPH) (n=13). Patients were considered as having sPH if they answered affirmatively to the question that previous investigators have used: "do you sometimes feel the presence of somebody close by when no-one is there?" The hallucinated presence could be located behind, on the side (left or right) of the patient, or in another room and was generally not seen (see [2,7,8,10,26]). All PD patients, who were included in study1 presented idiopathic PD diagnosed by trained neurologists. No patient was suffering from a neurological disorder other than PD (more details in Supplementary S2).

### General experimental procedure (study1)

Each PD patient underwent study1 at a similar time (10am), after having received their usual anti-parkinsonian medication and were in their "best ON" state. To investigate riPH, we adapted the experimental method and device as our previous research[26]. Briefly, sensorimotor stimulation was administered with a robotic system consisting of two robotic components (front-robot, back-robot) that has previously been used to induce PH. For each experimental session, we applied the following conditions: synchronous sensorimotor stimulation (the participants were asked to move the front-robot via either their right or left hand that was actuating the movements of the back-robot to apply tactile feedback to their back); asynchronous sensorimotor stimulation (same as synchronous stimulation, but with an additional temporal delay between the front-robot and the back-robot; see below for details of each experiment; Fig.1A). During sensorimotor stimulation, participants were always asked to keep their eyes closed and were exposed to continuous white noise through headphones (Supplementary S3).

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**Procedure, design, and analysis (study1.1)**

Participants were asked to insert their index finger in the haptic front-robot and carry out repeated poking movements while they received tactile cues on their backs, delivered by the back-robot. Thus, sensorimotor stimulation included motor, tactile, and proprioceptive signals from the upper limb moving the front-robot and additional tactile signals from the back-robot. Stroking was applied either synchronously (0ms delay) or asynchronously (500ms delay) (*Synchrony*: asynchronous vs. synchronous). Additionally, we measured the effect of the side of the body (i.e. hand moving the front-robot) that was most strongly affected by PD versus the other hand (*Side*) to investigate if the hemisphere predominantly affected by PD influenced riPH[64,65]. The factors (*Synchrony*; *Side*) and the order of testing were randomized across participants. Each participant randomly started with one Side first, for which the two *Synchrony* conditions (random order) were tested, and then the second Side was tested with the two *Synchrony* conditions (random order). In total, each participant performed four sessions (one per condition) lasting two minutes each. At the end of each of the four sensorimotor stimulation conditions, all participants filled a questionnaire (see below). Each PD-PH, PD-nPH, and HC included in the study was able to perform the entire study1.1.

*PH and other subjective ratings*

To measure PH and other illusions, we administered a questionnaire (6 questions) that was adapted from[26]. Participants were asked to indicate on a 7-point Likert scale, how strongly they felt the sensation described by each item (from 0 = not at all, to 6 = very strong). For questions see Supplementary S5.

*Data analysis*

Each question was analyzed with linear mixed effects models (lme4 and lmerTest both R packages[66,67]). Models were performed on the subjective ratings in each of the four conditions with *Synchrony* (synchronous vs. asynchronous), *Groups* (i.e., PD-PH vs. PD-nPH, and PD-PH vs. HC) and *Side* as fixed effects, and random intercepts for each subject. The significance of fixed effects was estimated with a permutation test (5000 iterations; predictmeans[68] R package).

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**Procedure, design, and analysis (study1.2)**

To complement and extend study1.1, we applied a Yes/No task, following sensorimotor stimulation, in which participants were asked to report whether they experienced a PH or not, on a trial-by-trial basis. On each sensorimotor stimulation trial, the delay between the movement and the stroking on the back was randomly chosen from a delay between 0 and 500ms (steps of 100m). One trial started with an acoustic signal (400 Hz tone, 100ms duration) indicating the beginning of the trial: at this point the participant started with the poking movements. Once the number of pokes reached a total of six (automatically counted), two consecutive tones (400 Hz, 100ms duration) indicated to the participant to stop the movements and to verbally answer with either a "Yes" or a "No" to the PH question, (Question: "Did you feel as if someone was standing close by (behind you or on one side)?"). The investigators where always placed > 4 meters away and in front from the participants during the experiment. Each participant was asked to perform three sessions; each session consisted of 18 trials (3 repetitions per delay (9 repetitions in total)). Between each session, the participant could take a break according to his/her needs (Supplementary S10).

*riPH rating analysis*

First, to investigate how the degree of sensorimotor conflict modulates PH, we analyzed the behavioral responses as a function of different delays (i.e., 0-500ms, steps of 100ms) across groups (i.e., PD-PH vs. PD-nPH). Here, the data was analyzed with a linear model, fitted for each participant independently. We assessed (1) the main effect of the delay (on the intensity of riPH) with a permutation test (5000 iterations) between slopes of the individual fit vs. zero; (2) the difference between the slopes of PD-PH vs. PD-nPH with a permutation test between the slopes of the two subgroups; (3) the main effect of group with a permutation test on the intercepts between the two subgroups.

**Study 2**
**Participants, ethics, and informed consent (study2.1)**

All healthy participants had no history of neurological or psychiatric disorders. All participants provided written informed consent prior to the experiment. The study was approved by the Cantonal Ethics Committee of Geneva (Commission Cantonale d'Ethique de la Recherche sur l'Être Humain - CCER). Twenty-five healthy participants (10 women, mean age±SD: 24.6±3.7 years old; age range: 18-32 years old, Edinburg Handedness Inventory mean index: 64.8±23.7 and range: 30-100) took part in study2.1.

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**Experimental procedure (study2.1)**

The experimental procedure was based on a pilot study performed in a mock scanner (Supplementary S16). Participants were blindfolded during the task and received auditory cues through earphones to start (1 beep) and to stop (2 beeps) the movement. The paradigm was implemented using an in-house software (ExpyVR, http://lnco.epfl.ch/expyvr) and Visual studio 2013 interface (Microsoft) was used to control the robotic system.

Participants underwent two runs of 12 min each, during which they repeatedly had to move the front robot for 30s with their right hand followed by 20s of rest for a total of 16 repetitions per condition (8 repetitions for the motor and touch control tasks) (Supplementary S15-S19 and Fig.S3). Synchronous and the asynchronous conditions were randomized across runs. The questionnaire was presented at the end of the scanning session and after a randomized repetition of 30s of each condition. The questionnaire was based on the pilot study (Supplementary S16-S18) and on a previous study[26]. Participants were asked to indicate on a 7-point Likert scale, how strongly they felt the sensation described by each item (from 0 = not at all, to 6 = very strong).

**Questionnaire analysis**

Questionnaire data were analyzed in the same way as in study1.1. Synchrony (synchronous and asynchronous) was used as a fixed effect and the subjects as random intercepts.

**fMRI experiment**

*fMRI data acquisition*

The imaging data was acquired with a 3T Siemens Magnetom Prisma MR scanner at Campus Biotech MR Platform (Geneva). The functional data were acquired using an Echo Planar Imaging (EPI) sequence with a full brain coverage (43 continuous slices, FOV=230mm, TR=2.5s, TE=30ms, flip angle=90°, in-plane resolution=2.5x2.5mm2, slice thickness=2.5mm using a 64-channel head-coil) containing 320 volumes for the experimental runs and 160 volumes for the localizer runs. For each participant, an anatomical image was recorded using a T1-weighted MPRAGE sequence (TR=2.3s, TE=2.32 ms, Inversion time=900ms, flip angle=8°, 0.9mm isotropic voxels, 192 slices per slab and FOV=240mm).

*fMRI data analysis*

All the fMRI data analysis reported were pre-processed using SPM12 toolbox (Wellcome

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Departement of Cognitive Neurology, Institute of Neurology, UCL, London, UK) in Matlab (R2016b, Mathworks). Slice timing correction and spatial realignment was applied to individual functional images. The anatomical image was then co-registered with the mean functional image and segmented into grey matter, white matter and cerebro-spinal fluid (CSF) tissue. Finally, the anatomical and the functional images were normalized to the Montreal Neurological Institute (MNI) brain template. Functional images were then smoothed with a Gaussian kernel with full-width half-maximum of 6mm. Head motion was assessed based on framewise displacement (FD) calculation[69]. All participants had a mean FD value inferior to 0.50mm (mean FD=0.12±0.05 mm). The two experimental runs were filtered with a high-pass filter at 1/300 Hz to remove low frequency confounds, while the two localizers were filtered with a high-pass filter at 1/100 Hz.

*Activation contrasts*

The experimental runs and functional localizers were submitted to a general linear model (GLM) analysis. In all runs, the periods corresponding to a given robotic stimulation (i.e., synchronous, asynchronous, motor task, touch task (Supplementary S19 and Fig.S3)) and the periods corresponding to the auditory cues were modelled as separated regressors. The six realignment parameters were modelled for each run as regressors of no interest. In order to avoid confounding effects due to the amount of movement performed in each trial, the quantity of movement of the front robot (synchronous and asynchronous for the experimental runs and movement condition for the motor localizer, see above) was included as parametric modulators of each condition (see above).

Second-level analyses were performed using the first-level contrasts defined for each subject. In order to determine which brain regions were involved in sensorimotor conflicts (spatio-temporal conflict and fixed spatial conflict), the following contrasts were computed: asynchronous>motor+touch and synchronous>motor+touch. A conjunction between those two contrasts was performed to identify the regions involved in the fixed spatial sensorimotor conflicts. For the experimental runs, two sample t-tests (asynchronous>synchronous and synchronous>asynchronous) were performed to assess brain activations activated during a specific sensorimotor conflict. Results were thresholded at p<0.001 at voxel level and only the clusters surviving p<0.05 FWE-corrected for multiple comparison were reported as significant. The obtained clusters were labelled using the AAL atlas[70] and the Anatomy

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toolbox[71].

**Lesion network mapping analysis (study2.2)**

In order to identify the brain regions functionally connected to each lesion location causing PH in neurological patients, we used lesion network mapping analysis[50,72]. Briefly, this method uses normative resting state data from 151 healthy subjects obtained from the publicly available Enhanced Nathan Kline Institute Rockland Sample[51] and uses the lesion locations as seed ROI. The fMRI acquisition parameters are described in the Supplementary S21.

*Resting state fMRI analysis*

For the pre-processing steps see above and Supplementary S21. The anatomical T1-weighted image was segmented into grey and white matter and CSF. Spatial realignment was applied to individual functional images. The six realignment parameters and their first-degree derivatives were added in addition to the averaged signals of the white matter and cerebro-spinal fluid. Subjects with the excessive motion were excluded from the analysis, this comprised 25 subjects which had a mean FD higher than 0.5mm and where more than 15% of scans were affected by movement. In total, 126 subjects were included for the analysis. Then, fMRI data was bandpass-filtered in the range of 0.008-0.09Hz.

The resting state data was analyzed using the CONN-fMRI Functional Connectivity toolbox[73] (v.18.a, http://www.nitrc.org/projects/conn). The lesion masks were used as seed ROIs and their mean time course was extracted and correlated to all other brain voxels. Each lesion-seed yielded a brain network thresholded at $p<0.001$($t±3.37$) with $p<0.05$ whole brain FWE peak level corrected. The 11 networks were then binarized and overlapped to determine the regions of shared positive and negative correlations (Fig.S5). The network overlap was thresholded at 90% (at least 10 cases out of 11) with a minimal cluster extent of 50 voxels. This procedure was repeated with the visual hallucinations (VH) lesions (Supplementary S22-S23 for further analyses).

**Study 3**
**Participants (study3.1)**

Data from thirty PD patients were analyzed in this study. All patients were prospectively recruited from a sample of outpatients regularly attending to the Movement Disorders Clinic

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at Hospital de la Santa Creu i Sant Pau (Barcelona) based on the fulfilling of MDS new criteria for PD. Informed consent to participate in the study was obtained from all participants. The study was approved by the local Ethics Committee. Patients were diagnosed by a neurologist with expertise in movement disorders. Each patient was interviewed regarding years of formal education, disease onset, medication history, current medications, and dosage (levodopa daily dose). Motor status and stage of illness were assessed by the MDS-UPDRS-III. All participants were on stable doses of dopaminergic drugs during the 4 weeks before inclusion. Patients were included if the hallucinations remained stable during the 3 months before inclusion in the study. No participant had used or was using antipsychotic medication (Supplementary S24). Details of image acquisition and data processing are in Supplementary S25.

*Regions of interest*

The cPH-network as defined in Study 2 *(*right posterior middle temporal gyrus (pMTG; x =54, y=-54, z=0), the right inferior frontal gyrus (IFG; x=51, y=18, z=29) and the left ventral premotor cortex (vPMC; x=-53, y=1, z=37) was transposed bilaterally to ensure that the cPH-network is not affected by any effects of movement-related laterality of activation observed in the riPH-networks (Fig.3B). Clusters were built using FSL (https://fsl.fmrib.ox.ac.uk/fsl/). A control network was derived by shifting each region (x±0/20; y+30; z-15) of the cPH-network (Fig.S7). This approach allowed controlling for the exact same shape and number of voxels as original cPH-network areas.

*Statistical analyses*

To assess whether the functional connectivity of the cPH-network predicted if a patient was clinically classified PD-PH (or PD-nPH), we conducted a leave one out cross-validation procedure with a linear discriminant analysis (LDA) (using Caret R packages[81]). To ensure that the kappa value was above chance-level we conducted a permutation test (5000 iterations). At each iteration, functional connectivity values were permuted between sub-groups and the cross-validation procedure was repeated. Post-hoc analyses for the between group differences were performed using a permutation tests (5000 iterations) on the connection which mostly contributed to the decoding. Connectivity outliers (8.75% of all data points) were identified based on 1.5 IQR from the connectivity median value for each connection. Spearman 2-tailed correlation analyses were performed between functional

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connectivity within cPH-network areas and neuropsychological measures of the PD-CRS (Parkinson's disease – Cognitive Rating Scale). Significance between the two correlations was assessed using the Steiger Tests (psych R package[76]).

**Code & Data availability**

Matlab and R code, behavioral and MRI data of this study are available from the corresponding author (Olaf Blanke) upon reasonable request.

**Figure Legends**

**Figure 1. Robot-induced PH (PD patients). A.** Setup for study 1. Responses in synchronous and asynchronous conditions are shown. During the asynchronous condition, the sensorimotor feedback on the participants' back was delayed by 500 ms (study1.1) or with a random delay (0-500ms, steps of 100ms) (study1.2). **B.** Study1.1. riPH in PD-PH are stronger than in PD-nPH. Each dot indicates the individual rating of the intensity of the riPH (PD-PH (purple) and PD-nPH (yellow)). The dot with the bar on the left and right side indicate the mixed effects linear regression between PD-PH and PD-nPH. Error bar represent 95% confidence interval. **C.** Study1.1. Asynchronous condition induced stronger riPH. Each dot indicates the individual rating of the intensity of the riPH. The dot with the bar on the left and right side indicate the mixed effects linear regression between Asynchronous (black) and Synchronous (gray) sensorimotor stimulation. Error bars represent 95% confidence interval. **D.** Study1.2. riPH were modulated by delay (permutation p-value=0.014) and PD-PH vs. PD-nPH were more sensitive to the sensorimotor stimulation (slope permutation p-value=0.039, intercept p-value=0.016). The thicker line indicates the mean of the fitted models, the shaded are indicates the 95% confidence interval, thinner lines indicate single subject fit. **E.** Study1.2. Exemplary movements executed by one patient during sensorimotor stimulation. **F.** Study1.2. Mixed effects linear regression between the Euclidean distance between pokes for PD-PH (purple) and PD-nPH (yellow). Error bar represent 95% confidence interval.

**Figure 2. Neuroimaging results of robot-induced PH (healthy participants). A.** MR-compatible robotic system is shown. Participants were instructed to move the front robot with their right hand and the back robot delivered the touch to the participant's back either synchronously or asynchronous (500ms delay between their movement and the sensory

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feedback received on the back). **B.** Asynchronous vs. synchronous condition induced stronger riPH. Each dot indicates the individual rating of the intensity of the riPH in healthy participants. The dot with the bar on the left and right side indicate the mixed effects linear regression between asynchronous (black) and synchronous (gray) sensorimotor stimulation. Error bar represents 95% confidence interval. **C.** Movement data from the fMRI experiment: no movement differences were found between the two conditions. **D.** Brain regions sensitive to the delay. **E.** Brain areas present in the conjunction analysis between the contrast synchronous>motor+touch and the contrast asynchronous>motor+touch. The coronal slices are at Y = -1 and Y = -53. There was no anatomical overlap between both networks (D and E).

**Figure 3. Symptomatic PH-network and common PH-network. A.** sPH network connectivity in neurological non-parkinsonian patients. **B.** Common regions between the riPH-network and sPH-network (cPH-network) were found in three regions: left vPMC, right IFG and right pMTG. **C.** Schematic display of the cPH-network projected bilaterally.

**Figure 4. Functional connectivity in the sensorimotor network. A.** Connections showing differences in functional connectivity between PD-PH vs. PD-nPH within the cPH-network are shown (yellow). **B.** Mixed effects linear regression between the functional connectivity for PD-PH (purple) and PD-nPH (yellow) between left IFG and left pMTG is shown. PD-PH vs. PD-nPH patients have a significantly reduced functional connectivity. Error bar represents 95% confidence interval, and the dot represents the mean functional connectivity. Dots represent the functional connectivity for each patient. **C.** Degree of functional disconnection is correlated with the cognitive decline (fronto-cortical sub-score of PD-CRS) in PD-PH patients. Lower connectivity was correlated with lower frontal cognitive fronto-subcortical abilities.

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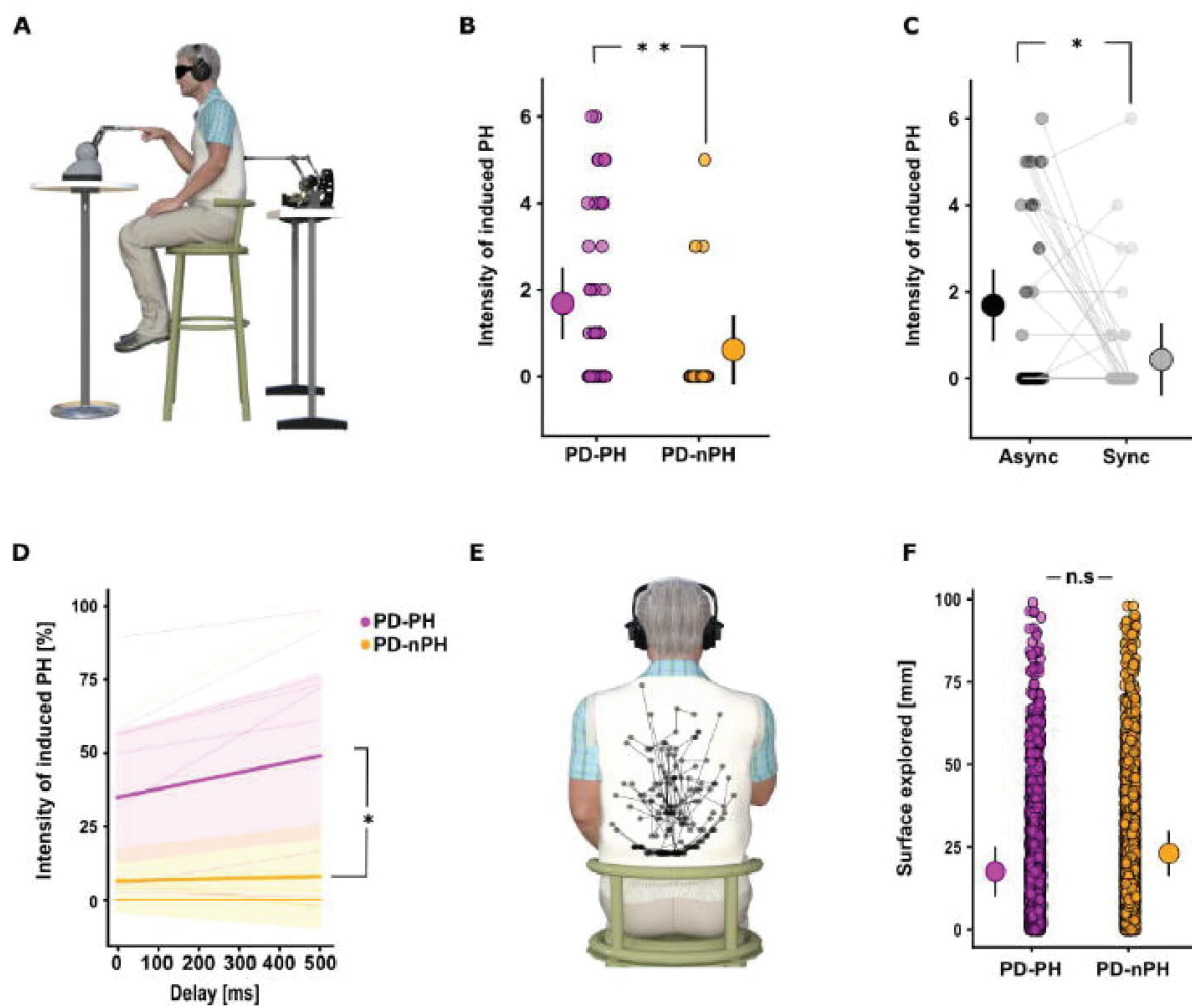76. Revelle, W. R. psych: Procedures for Personality and Psychological Research. (2017).

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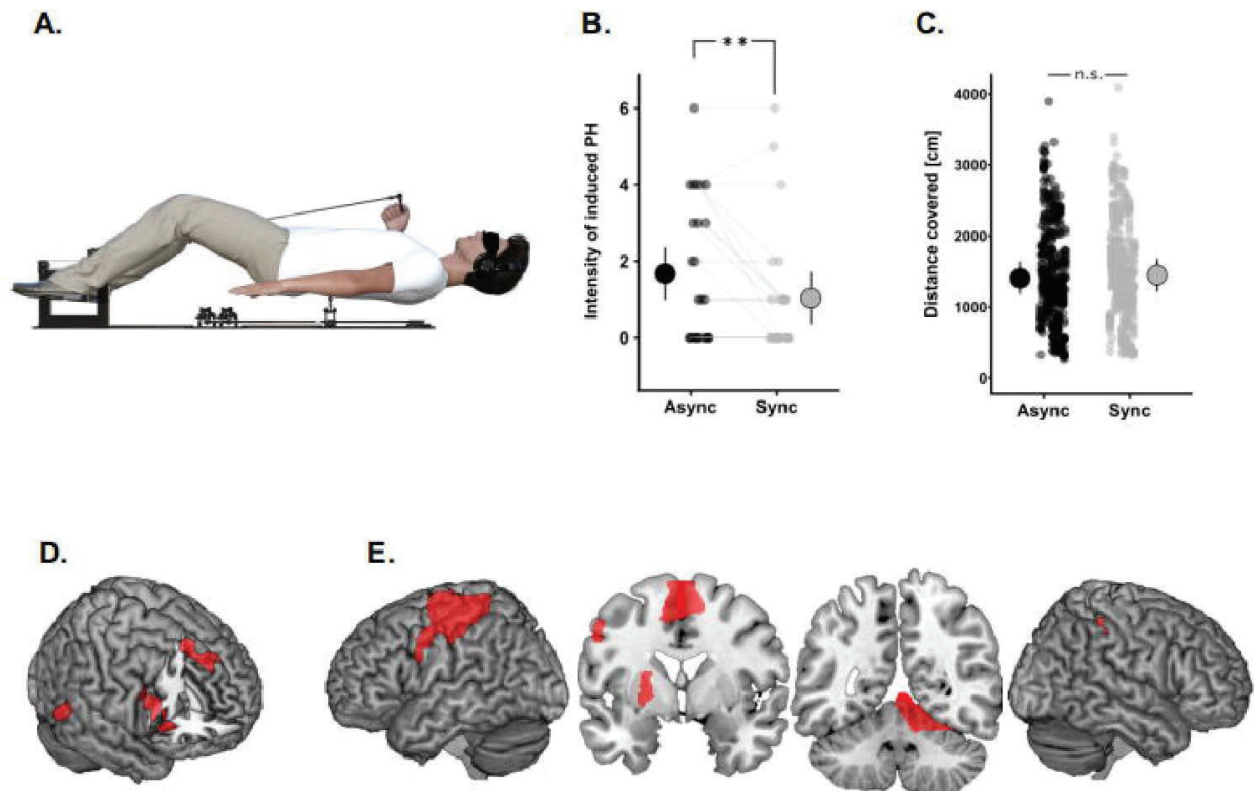Figure 1. Robot-induced PH (PD patients).

Figure 2. Neuroimaging results of robot-induced PH (healthy participants)

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**A.**

N = 10/11        N = 11/11
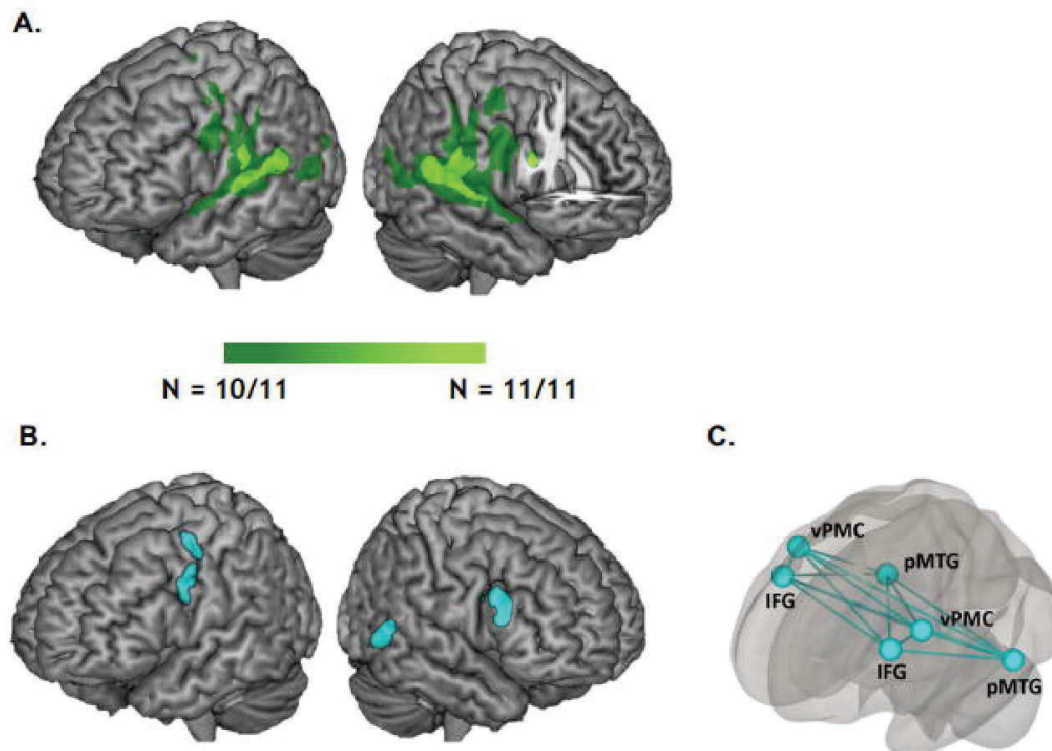
**B.**

**C.**

vPMC
pMTG
IFG
vPMC
IFG
pMTG

Figure 3. Symptomatic PH-network and common PH-network.
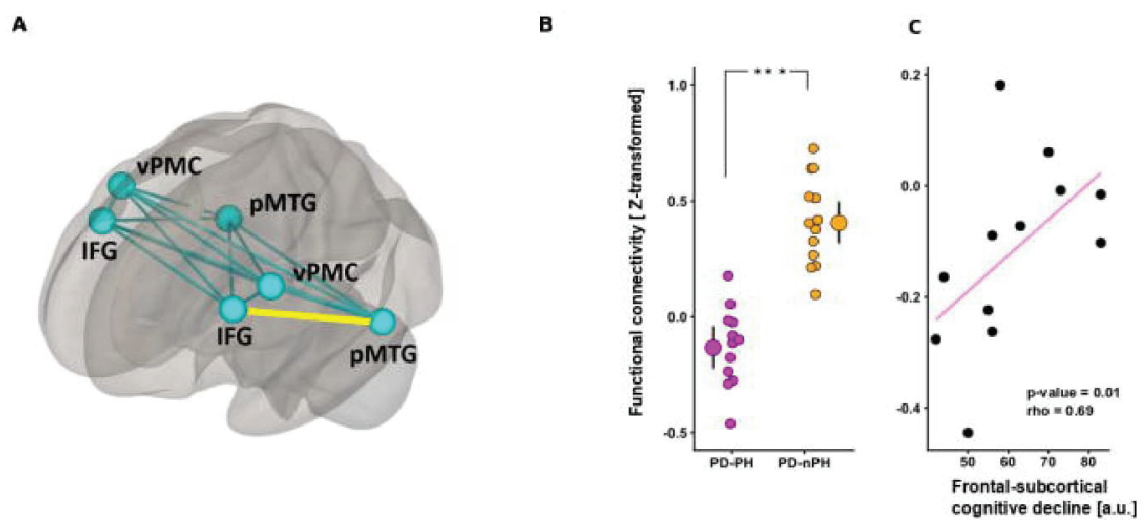
Figure 4. Functional connectivity in the common PH-network.

# Abbreviations

BSC: bodily self-consciousness

TMS: transcranial magnetic stimulation

MEPs: motor evoked potentials

VR: virtual reality

M1: primary motor cortex

RHI: rubber hand illusion

tDCS: transcranial direct current stimulation

TES: transcranial electrical stimulation

FBI: full-body illusion

PMC: premotor cortex

PPS: peripersonal space

EBA: extrastriate body area

EEG: Electroencephalography

vPMC: ventral premotor cortex

fMRI: Functional magnetic resonance imaging

# Curriculum Vitae

## PERSONAL INFORMATION

**Name:** Matteo Franza

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**Date of birth:** 17/05/1991

**Nationality:** Italian

## EDUCATION

**2016 - present**  PhD student at EPFL

**2013 - 2015**  Master's Degree in Psychology -110/110 cum laude

University of Turin, Turin (Italy)

Thesis title: "Feeling pain on an alien hand: EEG study in pathological embodiment".

Supervisors: Annamaria Berti, Francesca Garbarini

**2010 - 2013**  Bachelor's Degree in Psychology –110/110 cum laude

University of Turin, Turin (Italy)

Thesis title: "Grasping movements in hemiplegic patients: a control study".

Supervisor: Annamaria Berti

**2015-2016**  Internship in Clinical Neuropsychology at San Camillo Hospital of Turin
Tutor: Patrizia Gindri

| 2005 - 2010 | Diploma magistrale |
| | Istituto Magistrale E. De Amicis, Cuneo (Italy) |

## TRAINING

| 05/2017 | Lesion Analysis Workshop (Tubingen, Germany) |
| 06/2016 | 4th Science Factory: TMS–EEG Summer School and Workshop (Aalto university, Finland) |
| 2015-2016 | Internship in Clinical Neuropsychology at San Camillo Hospital of Turin<br>Tutor: Patrizia Gindri |
| 01/2013 - 06/2013 | Internship in Clinical Neuropsychology at San Camillo Hospital of Turin<br>Tutor: Laura Gullone, Lorenzo Pia |

## PUBLICATIONS

1. Ronga, I., Franza, M., Sarasso, P., and Neppi-Modona, M. (2017). Oculomotor prismatic training is effective in ameliorating spatial neglect: a pilot study. Exp. Brain Res. *235*. doi:10.1007/s00221-017-4923-6
2. Bassolino, M., Franza, M., Bello Ruiz, J., Pinardi, M., Schmidlin, T., Stephan, M.A., Solca, M., Serino, A., and Blanke, O. (2018). Non-invasive brain stimulation of motor cortex induces embodiment when integrated with virtual reality feedback. European Journal of Neuroscience, Vol. 47, pp. 790–799. doi:10.1111/ejn.13871
3. Franza, M., Sorrentino, G., Vissani, M., Serino, A., Blanke, O., and Bassolino, M. (2019). Hand perceptions induced by single pulse transcranial magnetic stimulation over the primary motor cortex. Brain Stimul. doi.org/10.1016/j.brs.2018.12.972

## PRESENTATIONS AND POSTERS

- "The contribution of conscious perception in the rubber hand illusion: a transcranial magnetic stimulation and virtual reality study" ASSC 22, 26/06-29/06, 2018 Kraków
- "Non-invasive brain stimulation to study the role of peripheral and central contribution in illusory embodiment" SIPF, 15/11-17/11 2018, Turin.
- "Combining TMS, virtual reality and psychophysics to investigate bodily self-consciousness" OHBM, 09/06-13/06 2019, Rome.
- "New applications of transcranial magnetic stimulation (TMS): embodiment & psychophysics" Rockefeller neuroscience institute 14/05/2019, Morgantown (WV).