From limb loss to robotic augmentation: neurophysiological and cognitive adaptations

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Declaration

I, Elena Amoruso, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

How can the human nervous system deal with the entirely distinct inputs and outputs associated with the loss or the addition of (artificial) limbs? And how can this inform the increasing engineering efforts to interface with it through pioneering neurotechnologies? In my thesis, I have addressed these questions by investigating what happens to the sensorimotor pathways when a hand is congenitally undeveloped, amputated, or robotically augmented. Traditionally, hand representation is considered to be highly plastic even in the adult brain. By taking advantage of a multimodal approach involving the use of brain stimulation, neuroimaging, behavioural and pharmacological techniques, I contribute a more nuanced view on this presumed boundless malleability. First, I offer a new interpretation of classical findings implying that the human sensorimotor cortex can functionally reorganise following the loss of a hand in adulthood, showing that perceptual distortions in amputees are likely to be consequential to uncontrolled psychological biases, rather than cortical plasticity, as traditionally assumed. Next, I show that early-life development may offer a more favourable environment for functional reorganisation, by providing crucial causal evidence for rerouting of motor outputs in cases of congenital deprivation. The fact that, under certain circumstances, sensorimotor resources can be repurposed to support new behavioural needs opens the door to innovative technologies designed to restore or extend sensorimotor function, such as brain-machine interfaces for artificial limb control and robotic augmentation. In the final chapter, I illustrate how existent sensorimotor pathways, such as the functional interconnectedness between the hands and the feet, can be successfully harnessed to support the control of extra robotic digits, and discuss the implications of these findings for augmentative, assistive and restorative technologies. Together, my thesis expands our understanding of sensorimotor plasticity, shedding light on the processes supporting novel forms of human-machine integration, and providing original insights for clinical applications.

Impact Statement

The extent to which our brains can be reshaped by experience throughout life is a key question in neuroscience. The ordered functional architecture of primary sensorimotor cortices has long served as a prime model for studying experience-dependent changes in cortical organisation, with a wealth of evidence from both human and non-human primates linking extreme alterations of typical sensorimotor behaviour, for example following hand loss, to dramatic alterations in cortical maps. It has long been assumed that such cortical remapping is functionally relevant, having direct consequences on perception and action. The findings presented in this thesis shed new light on these processes, highlighting how also in the sensorimotor domain functionally relevant remapping is greatly constrained by the time in which such changes in experience occur, similarly to what previously found in the visual and auditory domain. Congenital one-handers, but not acquired amputees, appear to undergo functional reassignments of cortical resources. These findings were facilitated by adopting a range of different techniques, including the use of causal methods and the thorough consideration of key psychological factors potentially affecting perceptual measures. The importance of embracing a multimodal approach when studying brain plasticity and its behavioural phenotypes is thus greatly emphasized in this thesis and encouraged in future research.

From a translational perspective, the opportunities and limitations afforded by the possibility of dynamically reshaping sensorimotor systems through experience are commonly highlighted when designing clinical treatment, including of phantom limb pain, stroke, spinal cord injury, complex regional pain syndrome, and dystonia. Moreover, plasticity considerations are central to current efforts to restore and extend human sensorimotor function, through brain-machine interfaces to control neuroprosthetics and motor augmentation. Such technologies aim to give independence back to those who have lost it or enhance the functionality of able-bodied individuals in daily life. Increasing our effort on bridging these technologies with a deeper understanding of deprivation and augmentation related plasticity and human sensorimotor control will provide a crucial contribution towards the realisation of these innovations.

To date, our understanding of the human brain's ability to successfully and safely control augmentation devices is particularly limited. A successful implementation of these technologies promises long-term commercial and societal impact, by offering unprecedented opportunities for multi-tasking. Besides, extra robotic arms and fingers could also serve as novel solutions to meet the needs of disabled individuals in daily life, beyond traditional substitution and restoration, considerably innovating the field of upper-limb motor rehabilitation. By identifying and describing fundamental principles for augmentation control and sensory feedback, this thesis aims to bring augmentative technologies a step closer to their effective deployment in real-world environments.

Thus, studying plasticity across both limb loss and augmentations scenarios has considerable clinical impact, in particular for rehabilitation, and strong potential to impact emerging bioengineering applications. It is hoped that the results and ideas presented here will help bridging the gap between physiological and technological knowledge, for the ultimate benefit of the many patients suffering from the debilitating consequences of loss of hand function.

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List of Acronyms

3T	3 tesla
7T	7 tesla
BCI	brain computer interface
BMI	brain machine interface
ANOVA	analysis of variance
BBR	Boundary Based Registration
BET	Brain Extraction Tool
CMAP	compound muscle action potential
DC	direct current
BF	Bayes factor
BOLD	blood oxygenation level dependent
CI	confidence interval
DOF	degrees of freedom
EMG	electromyography
EPI	echo planar imaging
FAST	FMRIB’s Automated Segmentation Tool
FDI	first dorsal interosseus
FEAT	fMRI Expert Analysis Tool
FILM	FMRIB’s Improved Linear Model
FLIRT	FMRIB’s Linear Image Registration Tool
FNIRT	FMRIB’s Nonlinear Image Registration Tool
GABA	gamma-aminobutyric acid
GLM	general linear model

HRA	Health Research Authority
Hz	Hertz
ICMS	intracortical microstimulation
M1	primary motor cortex
MEP	motor evoked potential
MNI	Montreal Neurological Institute
MPRAGE	magnetisation prepared rapid gradient echo
MRI	magnetic resonance imaging
NHS	United Kingdom National Health Service
PCM	pattern component modelling
PLP	phantom limb pain
rmANOVA	repeated measures ANOVA
rB	rank biserial correlation
rMT	resting motor threshold
ROI	region of interest
RSA	representational similarity analysis
S1	primary somatosensory cortex
SD	standard deviation
tDCS	transcranial direct current stimulation
TE	echo delay time
TMS	transcranial magnetic stimulation
TR	repetition time

1 │ INTRODUCTION

1.1 Introduction

One of the key concepts in contemporary neuroscience is that the central nervous system can allocate resources based on experience. The ability of the brain to dynamically adapt its processing properties based on changes in demand is broadly defined as plasticity and underlies our ability to develop, learn and recover from injuries. One of the most insightful ways to explore our brain's ability to undergo plastic changes is by observing what happens in the extreme circumstances where typical inputs to a region are not provided, lost, or dramatically altered.

The functional architecture of the brain grossly follows a prototypical schema across most individuals. This involves a parcellation of sensory inputs from different modalities to distinct cortical regions. A key feature of the cortical areas primarily devoted to vision, hearing, touch, and movement is that they contain orderly maps of their respective epithelial surfaces and body segments. The dominant view is that, in the sensorimotor domain, these representations can change dramatically in response to both peripheral manipulations and behaviourally relevant experiences.

In my research, I've aimed to understand how malleable brain organisation is following extraordinary modulations of sensorimotor needs, along the continuum of limb congenital absence, acquired amputation and augmentation. The emphasis in this thesis will be on *functional* reallocation: if the fact that following such massive alterations of typical sensorimotor experience the brain undergoes some plastic changes is generally well accepted, the extent to which such changes result in behaviourally significant outcomes is still unclear (Muret & Makin, 2021; see Sinh et al., 2018 and Pavani & Bottari, 2012 for analogous debates in in the visual and auditory domain, respectively).

I focus on the hand, which affords many of our physical interactions with the world and benefits from a particularly rich and well-established cortical representation. Hand loss is thus a prime scientific model for studying brain plasticity as it allows to examine the effects of both sensory input loss and altered behaviour on this clear structure. The possibility of comparing brain organisation patterns in cases of acquired and congenital hand loss further offers unique chances to investigate how early-development can favour or constrain neural plastic adaptations. Body augmentation through wearable robotic technology (e.g., extra arms or fingers) is a developing field that allows to look at sensorimotor plasticity from an almost inverse perspective. Here, the system is expected to adapt to an addition, rather than a reduction, of sensory input and to the related motor adjustments. Such extreme case of human-machine integration poses unique considerations, related to the ability of the sensorimotor system to (safely) repurpose existing resources, available for operating our own body, to effectively incorporate the novel ‘body-part’.

In what follows, I will first comprehensively outline the background literature relating to these questions. I will start by sketching out the canonical organisation of the hand representation in the sensorimotor cortex. I will then survey seminal work of cortical plasticity in primates, showing that following hand amputation the neighbouring representations seemingly take over the deprived area. Here, I will also review prior studies suggesting that similar plastic mechanisms may be taking place also in human amputees, and describe their presumed perceptual consequences, particularly in relation with phantom sensations and pain. Next, I will outline how more recent work has expanded on these initial observations in humans, showing that less plasticity may be taking place following amputation in adult humans than previously suggested. Here, I will also show how loss of a hand in early development can give rise to adaptive patterns of plasticity. Finally, I will examine the first body of evidence describing plastic changes accompanying hand augmentation and discuss the theoretical and practical challenges associated with interfacing the sensorimotor system with extra body-parts.

1.2 Canonical representation of the hand

The body is supported by multiple sensory and motor modalities, as such there exists a plethora of relevant representations in the brain. Most notable are the somatosensory and motor body maps in the cortex.

The primary somatosensory cortex (S1) encompasses the postcentral gyrus and the posterior bank of the central sulcus. This region receives direct inputs from the ventroposterior lateral and ventroposterior medial nuclei of the thalamus, as well as a multitude of efferent information from the motor system (Kandel, Schwartz, & Jessell, 2000). Traditionally, S1 includes four Brodmann areas: 1, 2, 3a and 3b, each of which presumably dedicated to different aspects of proprioceptive and tactile processing. Yet, because these areas are highly interconnected, jointly recruited during typical sensorimotor behaviour, and difficult to delineate reliably using non-invasive methods, in what follows I will treat the four regions as a singular functional area. The functional organisation of S1 was first characterised by the pioneering work of Penfield and colleagues (Penfield & Boldrey, 1937), who, by directly stimulating the cortical tissue of awake human patients undergoing surgery and asking them to report the evoked sensations, were able to reveal a detailed body map in S1 (the iconic "homunculus", Figure 1.1, left pane). S1 body map is organised in a somatotopic fashion, where inputs of different body parts are represented separately, with neighbouring body parts often represented adjacently, with some exceptions – notably the hand representation neighbours that of the face.

Primary motor cortex (M1), on the anterior bank of the central sulcus, also shows a topographical organisation, although broader (e.g., crude representation of the legs, hand and mouth) (Figure 1.1, right pane). Typical sensorimotor behaviour requires bidirectional interplay between S1 and M1 (Lee et al., 2008; Perich, Conti, Badi et al., 2020) and both regions are involved in motor planning (Wolpert & Ghahramani, 2000), fine motor control **(**Matyas et al.,2010**)** and motor learning (Ostry & Gribble, 2016; Mathis, Mathis & Uchida, 2017; Kumar, Manning & Ostry, 2019)**.** Despite the apparently clear division of labour between S1 and M1 as input/output regions, drawing orthogonal distinctions between M1 and S1 is thus problematic, as both regions are heavily interconnected and involved in sensorimotor control (Penfield and Boldrey, 1937). For instance, the somatosensory cortex has been reported to send commands to the spinal cord (Rathelot et al., 2017) and the motor cortex contains many neurons that respond to somatosensory stimulation (Meyer, 1987; Adams et al., 2013). Moreover, many contemporary techniques cannot accurately delineate S1 from M1 spatially. Therefore, where the individuated contributions of S1 and M1 cannot be reliably disentangled, I will refer to the evidence as relating to the *sensorimotor* cortex.

Somatotopic topographies are generally preserved, although more loosely, also across other terminals of the sensorimotor system, such as the cerebellum (Hahamy & Makin, 2019; Yeo et al., 2011), basal ganglia (Zeharia et al., 2015), operculum and insula (Brooks et al., 2005), supplementary motor area (Zeharia et al., 2012), and parietal cortex (Huang et al., 2012; Zeharia et al., 2019).

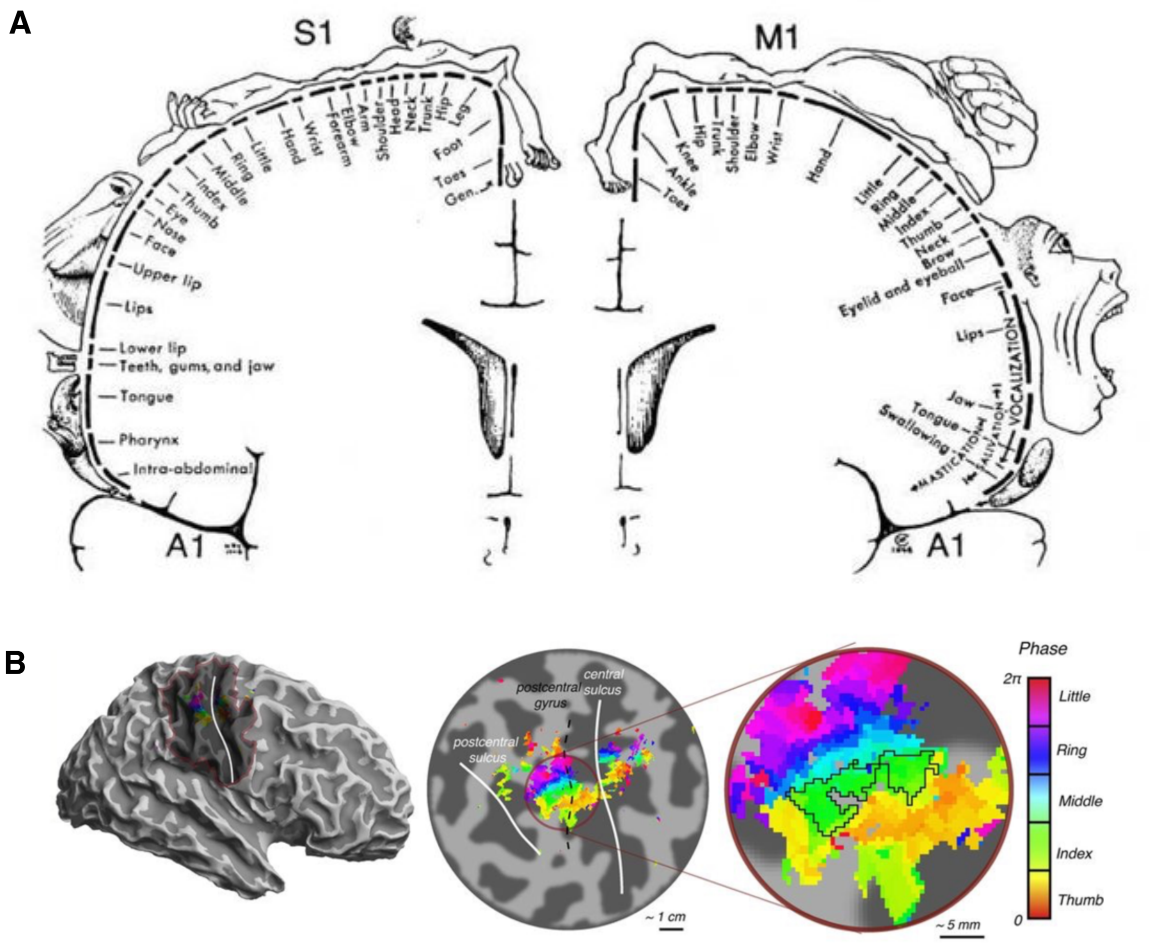

Figure 1.1 Somatotopy in primary somatosensory and motor cortex

A) The sensory homunculus in S1, on the left, and M1, on the right, coronal view. Adapted from Penfield & Boldrey (1937) and Penfield & Rasmussen (1950). **B)** Digit somatotopy within S1 hand area, measured using 7T fMRI, presented on inflated surface, sagittal view. Colours indicate preferred response while stimulating each digit passively in a cycle. Adapted from Sanchez-Panchuelo et al. (2012).

Importantly, the different body parts are not represented across the sensorimotor cortical network in a way that is proportional to body mass. Instead, cortical body maps are highly distorted in favour of the more heavily innervated and actively used body-parts (Sur, Merzenich, & Kaas, 1980). Notably, the representation of the hand is consistently considerably larger than the representation of other body parts, and contains itself (at least in S1) a detailed somatotopic map, where neighbouring fingers are represented separately and adjacently (Kaas et al., 1979). Such feature makes the sensorimotor cortical representation of the hand an especially interesting and informative target for studying brain (re)organisation.

1.3 Deprivation triggered plasticity: the model of hand amputation

What happens to the highly organised hand representation once it becomes functionally redundant following amputation?

1.3.1 Remapping in non-human primates

Our original understanding of what happens to the cortical territories of the hand after sensory input loss comes from foundational research in non-human primates using direct brain recordings. For example, Merzenich and colleagues (1984) found that following a single digit amputation, over the course of weeks and months, the deprived part of the hand map becomes activated by the neighbouring digits. After long term sensory loss (i.e., ‘deafferentation’) of the entire hand, even more extensive changes were observed, with the missing hand area in S1 becoming responsive to touches on the lower part of the face (Pons et al., 1991).

Collectively, these pioneering studies show that when typical sensory input from a body part ceases, the representations neighbouring the ‘freed-up’ cortical territory will expand and ‘invade’ the region that is now deprived of inputs, following the topographic principles of cortical organisation (although see also Wesselink et al., 2022, for an alternative interpretative framework). This phenomenon has been ascribed to multiple plasticity mechanisms, such as unmasking of normally inhibited connections between representational areas (Li et al., 2014), changes in subcortical projections to cortex (Jain et al., 2008) and widespread sprouting of intracortical connections (Florence et al., 1998; Jones & Pons, 1998).

Since the boundaries of the body-part map are now changed, this process is commonly referred to as *reorganisation,* although it would be more accurately described as *remapping* (Muret & Makin, 2021*).* Indeed, the fact that the spatial layout of the map has changed, does not necessarily entail that the native function of the area is lost and reassigned. Establishing that aberrant activity in deprived cortex constitutes a new sensory representation of the displaced input would require causal evidence (e.g., through brain stimulation and optogenetics) or the use of imaging techniques that allow the consideration of functional attributes beyond selectivity of response (e.g., multivariate pattern analyses),

1.3.2 Remapping in human amputees and its functional significance

Interestingly, almost every person who has lost a hand in adulthood report experiencing vivid and continuous sensations of their missing limb (Weeks et al., 2010). These sensations usually emerge immediately after amputation and the vividness of perception can be similar to the perception of one’s intact hand.

Sensations coming from the missing limb are typically described as itching, tingling, or numbness, and are not necessarily bothersome for the individual (Henderson & Smyth, 1948). Most amputees with phantom limb sensations report some level of phantom mobility, although immobility and involuntary movements have also been reported (Weeks et al., 2010). For the majority of amputees, the phantom limb can also be the source of unpleasant, painful and for some even highly debilitating sensations, commonly referred to as phantom limb pain (PLP). To date there is a lack of consensus regarding the basis of this chronic neuropathic syndrome (Makin & Flor, 2020).

An influential model ascribes PLP to *maladaptive* cortical reorganisation, whereby pain arises as a perceptual consequence of cortical reorganisation following limb loss (Flor et al., 1995). The first evidence supporting this model demonstrated a correlation between S1 presumed reorganisation and PLP using MEG (Flor et al., 1995). The authors showed that in amputees' deprived S1 the cortical distance between the lip and an estimated location of the missing hand representation was reduced, i.e. the lip representation was shifted towards the missing hand region. Importantly, a positive correlation was found between the degree of shift in lips representation and the intensity of PLP reported by amputees.

Based on this initial report linking PLP and cortical reorganisation and on the plastic changes documented in primates (Pons et al., 1991), it has been proposed that S1 deprivation after amputation leads to a degradation of the missing hand representation (Ramachandran & Hirstein, 1998). As a result, neighbouring representations 'invade' the missing hand area, causing this deprived region to respond to inputs intended for adjacent cortical territories (i.e., the face). The mismatch between body part representations (i.e., the missing hand and the face) is believed to generate an ‘error’ signal, which is subsequently interpreted as pain arising from the missing hand.

A key implication of the maladaptive model is that reversing cortical remapping by reinstating the missing hand representation in its initial cortical territory, would relieve PLP. Over the years, numerous behavioural therapies have been developed in an effort to normalise the representation of the missing hand by increasing sensory representation and motor control over the phantom. However, these treatments, which include the famous mirror-box treatment (Ramachandran & Rogers-Ramachandran, 1996), virtual reality paradigms (Ortiz-Catalan et al., 2016) and graded motor imagery (Moseley & Flor, 2012), are in actuality of wanting efficacy and have been criticised for lacking a sufficient level of evidence (e.g., they mostly lack a necessary sham control) (Thieme et al., 2016, Colmenero et al., 2018; Aternali & Katz, 2019). Moreover, mounting criticism has been raised to challenge the assumptions underlying these treatments (as discussed below). As such, despite the popularity of these interventions, PLP still remains difficult to treat, suggesting that the mechanisms of the syndrome are still undetermined.

If phantom limb pain is not conclusively a consequence of S1 remapping, are there any other functional consequences to the postulated S1 remapping? Although S1 remapping does not seem to lead to any direct functional benefits in processing the invading input (Vega-Bermudez & Johnson, 2002), a captivating phenomenon relating to distorted phantom sensations has been documented.

For some individuals, phantom sensations can be triggered through tactile stimulation of the residual limb (Grusser et al., 2001), which may simply be a consequence of peripheral reinnervation. A severed sensory axon typically regrows and spontaneously reinnervates intact skin, for example, on the residual arm (Devor, 1997). As a result, it is thought that touch applied to the reinnervated skin can produce signals that are mislabelled by the central nervous system as arising from the missing hand and result in a sensation projected to the missing hand.

A more striking phenomenon that implicates SI reorganisation is when phantom referred sensations are evoked through stimulation of the face. In a famous series of case studies (Ramachandran, 1993; Ramachandran, Rogers-Ramachandran, & Stewart, 1992), three upper-limb amputees reported feeling referred phantom sensations on the missing hand following touches on the face. Importantly, neighbouring sites on the face elicited sensations on neighbouring fingers, suggesting a topographical organisation of referred sensations. These findings were interpreted as the perceptual correlate for the remapping of the face onto the cortical area of the missing hand: if hand neurons in SI become responsive to the face, brain regions receiving input from this area will interpret this sensory signal as coming from the missing hand, resulting in dual sensations on the face and the phantom hand.

Although the phenomenon of referred sensations gained great popularity both in the scientific community and in the popular media (Ramachandran, 1998), this seminal research suffers from severe methodological flaws, and as such should be interpreted with caution (see Chapter 2 for a more extended discussion). Indeed, further studies using more standardised approaches (Grusser et al., 2001; Knecht et al., 1996) indicated that referred sensations do not follow S1 topography, weakening the hypothesis that referred sensations result from S1 remapping and highlighting the need for more systematic research on the aetiology of the phenomenon.

Moreover, in more recent years, a growing body of research has contested some of the fundamental assumptions of the maladaptive plasticity theory, suggesting that there might be less large-scale cortical reorganisation taking place following amputation than previously postulated (Jutzeler et al., 2015). Most notably, evidence for facial remapping in S1, assumed to be the main driver of PLP, has been challenged using more sophisticated approaches, such as fMRI surface-based analyses (which allow to measure cortical distances while taking into account individual brain morphologies). Different groups have shown that amputees' deprived hand area is not remapped by the lower face relative to controls (Kikkert et al., 2018; Makin et al., 2013; Valyear et al., 2020). Shifts in the lip representations are smaller than previously reported, and do not invade the missing hand cortex but seem to be limited to the face region itself (Makin et al., 2015; Raffin et al., 2016), which was recently shown to be organised in an upright manner, with the upper face and not the lips bordering the hand area (Root, et al., 2021). Perhaps most fundamentally, the remapping hypothesis which argues for a freed-up and invaded missing hand area is somewhat in contrast to the phenomenological experience of amputees, who are still vividly feeling the hand, despite it being missing. Amputees experiencing phantom sensations provide a unique opportunity for direct insight into the consequences of sensory input loss and the possibilities of representational preservation.

In a series of more recent studies, in order to characterise the deprived hand region, instead of adopting the classical approach of probing the neighbouring encroaching representations, some groups took advantage of amputee's ability to voluntarily move the phantom limb (Raffin, Giraux, Reilly, 2012; Makin et al., 2013; Kikkert et al. 2016; Bruurmijn, et al., 2017; Kikkert et al. 2018; Wesselink et al., 2019). Phantom movements have been shown to elicit both central and peripheral signals that are different from those found during movement imagery (Raffin et al., 2012a, 2012b; Reilly et al., 2006). Activity elicited by phantom hand movements in the sensorimotor missing hand area was found to be similar in amplitude to controls' hand movements (Kikkert et al., 2018; Makin et al., 2013). Crucially, the organising principles underlying hand representation in the sensorimotor cortex were found to be consistent with the preservation of a normal hand representation. For example, canonical somatotopic representations of the fingers in S1 was uncovered in amputees with vivid phantom kinaesthesia sensations, even three decades after amputation (Kikkert et al., 2016).

Further support for preserved phantom hand representations is provided by studies using brain stimulation, where sensations can be directly produced, thus allowing for causal inference. Stimulating the missing hand M1 area using transcranial magnetic stimulation (TMS) in arm amputees has been shown to elicit phantom movement sensations (Bestmann et al., 2006; Cohen et al., 1991; Hess et al.,1986; Pascual-Leone et al., 1996; Reilly & Sirigu, 2008). Interestingly, even phantom movement not under voluntary control of an amputee can be elicited by TMS (Mercier et al., 2006) and the intensity of these evoked phantom sensations is positively associated with the strength of the stimulator output (Bestmann et al., 2006; Mercier et al., 2006).

Finally, research using brain decoding techniques has further demonstrated the stability of hand representation. For example, a recent study showed that even the typical representational structure of the hand (*representational typicality*, captured by RSA - Representational Similarity Analysis) is preserved in a large sample of amputees after an average of 18 years since amputation (Wesselink et al., 2019). Moreover, amputees with greater phantom kinaesthetic sensations showed a higher representational typicality, suggesting that experience (in the form of phantom hand mobility) could shape the fine-grained aspects of hand representation, although the large-scale functional organisation of the hand area is fundamentally stable. Importantly, missing hand representation was not observed in congenital one-handers, indicating that the formation of the cortical hand representation is predominantly an experience-driven process.

1.4 Adaptive plasticity: the model of congenital hand absence

Hand loss involves two powerful drivers for brain plasticity: sensory deprivation and altered behaviour. Despite this, research on amputees has largely focused on deprivation-triggered mechanisms of cortical plasticity and their maladaptive consequences, namely phantom limb pain (Flor et al., 1995). However, more recent evidence in both congenital and acquired hand loss models suggests the involvement of alternative mechanisms, such as adaptive behaviour, and their potentially beneficial rather than harmful consequences for the individual (Makin et al., 2013; Hahamy et al., 2015,2017).

1.4.1 Early-life experience as a major driver of cortical plasticity

The strongest evidence for experience-dependent plasticity comes from early electrophysiology experiments performed in animals. Pioneering work by Hubel and Wiesel (Hubel & Wiesel, 1965; Wiesel & Hubel, 1965a, 1965b), who investigated reorganisation following visual deprivation in cats, introduced the significance of *critical periods* – temporal windows during childhood where the brain is particularly sensitive to sensory inputs – in influencing brain (re)organisation. In a series of elegant experiments, they showed in fact that input loss to one eye early in development led neurons previously devoted to the occluded eye begin to respond to input from the non-occluded eye. Importantly, the observed reorganisation was much more restricted when visual occlusion was performed in adult cats, as compared to young kittens (Wiesel & Hubel, 1965b), suggesting that the adult brain has a much more limited capacity for reorganisation (for related findings in monkeys see Smirnakis et al., 2005, and in humans: Baseler et al., 2011).

Compensatory patterns of sensorimotor plasticity would therefore be expected to manifest more prominently in people born without hand(s), who never received sensory information of the hand(s) during this crucial time window and have thus adopted compensatory behaviours since early development, when the brain is most plastic. People born with one hand (one-handers) and people born without both hands (no-handers) tend to compensate for their disability by over-using a range of different body parts, most often the feet, mouth, and residual arm. As some of these body part representations are adjacent to the missing hand area (e.g., the arm) while others (e.g., the feet) are not, the repertoire of compensatory strategies allows to address the role of behaviour in driving reorganisation – does intensive usage of these body parts lead to reorganisation that extends beyond the somatotopic layout?

1.4.2 Compensatory patterns of remapping and their functional significance

No-handers tend to display exquisite dexterous abilities with their feet, heavily relying on them to perform typically manual daily tasks since an early age. Using fMRI, Stoeckel and colleagues (2004) found that toes stimulation elicited stronger activity in the sensorimotor putative missing hand areas in congenital no-handers compared to two-handed controls, suggesting usage-dependent reorganisation. The authors further confirmed this foot-to-hand remapping in the deprived primary sensorimotor cortex using TMS (Stoeckel et al., 2009). Single-pulse TMS applied over the deafferented hand area elicited motor-evoked potentials (MEPs) on the feet. Moreover, TMS applied during a reaction time task disrupted participant's foot responses, highlighting the behavioural relevance of the remapped foot representation (see also Nakagawa et al., 2020, for similar findings). As the foot and the hand area are not cortical neighbours, these results demonstrate that cortical reorganisation can expand beyond the limits of typical somatotopic organisation.

As with no-handers, remapping was found to not be limited by somatotopic proximity also in one-handers, with multiple body parts shown to remap onto the missing hand region. In particular, fMRI research (Makin et al., 2013; Hahamy et al., 2017) demonstrated that when congenital one-handers moved each body part used for compensatory purposes (e.g., the feet, residual arm, lips), the induced activity levels in the putative missing hand's sensorimotor cortex were higher than in the non-dominant hand region of two-handed controls. Moreover, increased functional connectivity between the missing hand area and the lips and feet representations were found. Importantly, when congenital one-handers moved their intact hand – which does not typically substitute their missing hand function – this was not the case. Based on this evidence, it has been proposed that opportunities for reorganisation may be predetermined by an area's functional role, rather than by its somatotopic layout. For example, the hand cortex may only support body parts that substitute hand function (Hahamy et al., 2015, 2017).

However, this theory has been contested by other fMRI research which found increased representation for various body parts in the sensorimotor missing hand area of congenital/early life no-handers, regardless of everyday usage strategies (Yu *et al.*, 2014; Striem-Amit et al., 2018). Moreover, despite the increased activation for body parts used for motor compensation, Hahamy and colleagues (2017) found no direct correlation between the amount of activity in the missing hand area and the compensatory frequency of use of each body part to accomplish ecological behavioural tasks. These results suggest that even within the critical period reorganisation may not after all be driven by compensatory experience and support everyday behaviour, but could arise instead from unmasking of functionally-irrelevant inputs. Indeed, Hahamy and colleagues (2017) also showed that the deprived hand area displays reduced levels of GABA, an inhibitory neurotransmitter. Decreased inhibition may allow an 'unmasking' of otherwise suppressed, less specific inputs, such as those coming from other body parts. Such inputs may strengthen the functioning of body parts used to substitute hand function, or, alternatively, this increased excitability and disinhibition may reflect noise. Therefore, further evidence is required to corroborate the functional and/or compensatory nature of this plasticity in congenital handlesseness and its role in supporting behaviour. In particular, there's a lack of causal evidence to date that the deprived cortex functionally supports motor control of other body parts in one-handers.

1.5 Extending physical abilities: the model of wearable robotic augmentation

The fact that brain resources typically devoted to hand representation can under certain circumstances be reassigned to support new behavioural needs has strong potential to impact the development and the adoption of upper-limb assistive technologies. Although artificial arms were originally designed to substitute a missing limb, technological advancements can now allow for built-in enhancement of motor abilities that extend that of a biological body. For example, extra robotic fingers and arms are devices designed to extend and complement, rather than substitute, upper limb functionality (Prattichizzo et al., 2021; Tong & Liu, 2021; Yang et al., 2021; Eden et al., 2022). Unlike traditional tools (e.g. a hammer), which are also designed to extend functionality, such devices are not operated through direct manual manipulation and effective control thus lies in the ability to coordinate seamlessly with the biological body. These technologies carry the potential for substantial innovation across diverse real-world scenarios and at the same time allow us to address, from a radically new viewpoint, fundamental questions on brain plasticity. From a more translational prospective, body augmentation could reshape work environments and healthcare solutions, improving the independence of workers and patients. Successful integration of body augmentative technologies could indeed allow for increased multitasking, with major implications for productivity enhancement in the workspaces. It could also open up entirely novel approaches towards rehabilitation, for example, by augmenting the non-impaired hand (e.g., in stroke, fractures). However, a pressing question remains: how can we effectively control a new robotic body-part from a sensorimotor and neurocognitive perspective?

Figure 1.2 Extra robotic limbs

From top to bottom: *Third Hand* (Stelarc, 1982); *SR Fingers* (Wu & Asada, 2014); *SR Limbs* (Parietti & Asada, 2014); The Soft-SixthFinger (Hussain et al., 2016); *The Thid Thumb* (Dani Clode Design, 2018); *Multiple Wearable Extra Fingers* (Malevezzi et al., 2019); *MetaLimbs* (Sasaki et al., 2017); *Sorbonne arm* (Nima project, 2022).

1.5.1 Allocating motor and sensory resources to the extra limb

As an extension of the user’s sensorimotor system, augmentative technologies are meant to be controlled simultaneously and in close collaboration with our existent limbs. The key feature of augmentative technologies that distinguishes them from restorative prosthetics is that they operate in addition to, rather than instead of, one's own limbs. This implies that we cannot count on any existent dedicated physiological infrastructure (for example, those previously devoted to control an amputated limb discussed earlier) to channel motor commands and sensory feedback to and from the extra body-part. In other words, to operate such devices sensorimotor resources devoted to another body-part need to be employed. The urgent challenge is how to effectively control extra-limbs without overly compromising cognitive resources and the functionality of the rest of the body (Dominijanni et al., 2021).

Presently, all body augmentative technologies use non-invasive control interfaces. The simplest interfaces employ the same limbs directly involved in the task, for example pushing a button to control an extra robotic finger attached to the wrist (Aoyama et al., 2019). These kinds of solutions come at the obvious price of restricting the functionality of the very same body-part they are supposed to augment. A presumably less disruptive control interface seeks to 'hijack' the motor outputs of another body part, such as the foot (Di Pino et al., 2014; Makin, De Vignemont & Micera, 2020). For example, with two degrees of freedom (one per big toe) a user can simultaneously and proportionally flex/extend and adduct/abduct an opposable extra thumb (Third Thumb, Dani Clode Design), extending the overall grasp of the hand. Recent research from our lab (Kieliba et al., 2021) has shown that users can indeed achieve effective control of the Third Thumb within a five-day semi-structured training regime. In particular, repeated practice led to increased motor control, even without visual feedback or for more dexterous movements, as well as increased sense of embodiment.

Interestingly, the study also demonstrated that motor integration of the Third Thumb resulted in changed natural hand kinematics at the behavioural level while using the device and a significant (yet reversible) shrinkage of the canonical hand representational structure in primary sensorimotor cortex, as assessed with pre and post training fMRI scans while participants were not using (or even wearing) the device. Inter-finger dissimilarities, as measured with RSA, are believed to reflect daily hand usage (Ejaz et al., 2015), and, presumably, as people tend to use their hands similarly, the resulting hand representational structure is usually highly invariant across individuals. The fact that short-term training with the extra thumb temporarily altered such typical representations thus highlights the unique motor adaptations and novel coordination patterns required by the usage of extra limbs (see also Ogawa et al., 2019 for related evidence in experienced piano-players). More generally, these findings emphasise the need to examine how longer-term integration of motor augmentation can alter key aspects of biological body representations, and whether such plastic adaptations may carry any maladaptive consequences for the individual.

Keeping the potential risks of maladaptive plasticity mechanisms in mind, how to best channel motor outputs is not the only challenge that the pioneering efforts in augmentation research must deal with. A complementary issue is how to optimally integrate somatosensory information from the robotic device. This would be a crucial technological advancement, not only as it would provide users with a richer sensory augmentation experience but, perhaps more fundamentally, because it has long been known that effective motor behaviour largely depends on close interactions with the somatosensory system. Normal sensorimotor circuits are in fact considered closed control loops, where predicted and actual sensory feedback (mostly somatosensory, but also visual and auditory) are constantly monitored in order to inform the correction of motor commands (sensorimotor loop, Wolpert et al., 1995). A closed sensorimotor loop is considered crucial to guide both online motor planning and error-based motor learning, and a loss of touch or proprioception, in spite of a fully functional motor system, has devastating consequences on the quality of life of individuals (Gordon et al., 1995; Hermsdörfer et al., 2008).

Many studies have highlighted the importance of somatosensory feedback, including both touch and proprioception, for a more efficient control of traditional prosthetics (Bensmaia, Tyler, Micera, 2020; see also Chapter 4 for a more extended discussion). Yet, many augmentation devices lack dedicated somatosensory feedback and movements are often guided solely through visual monitoring. Akin to motor control, it is indeed particularly challenging to deliver somatosensory information from the extra limb without depleting sensory resources allocated to other body parts. Currently, the solutions that have been proposed mainly involve delivering artificial tactile cues on a displaced skin surface (e.g., on a finger: Hussain et al., 2015; or on the contralateral hand: Aoyama et al., 2019), which can be considered suboptimal since these extra cues presumably interfere with the sensory flow coming from the biological body.

1.6 Aims of this thesis

The overarching goal of this thesis is to provide a novel account of how changed demands shape the organisation of the neural systems involved in action and bodily perception, with a special emphasis on the functional significance of such potential neural adaptations. I have tackled this complex issue by employing a multidisciplinary approach, involving the complementary models of sensory input loss (acquired and congenital hand loss) and augmentation (use of extra robotic fingers) and the use of multiple methods (e.g., TMS, psychophysics, pharmacological interventions).

With the ongoing debate surrounding the functional properties of the deprived sensorimotor cortex following input loss, the first two experimental chapters will aim to probe functional reorganisation and its functional correlates in both acquired and congenital one-handers, using brain stimulation, neuroimaging, and behavioural techniques. In light of the evidence described in the preceding sections, I hypothesise that functional reorganisation can take place during early development, implying that the deprived cortex can control body parts that may in turn be used for compensatory purposes. However, once the topographical organisation is established, I expect the hand representation to persist and functional reorganisation (and thus even related behavioural consequences) to be less likely to occur. The third experimental chapter will be dedicated to delving into the limits and opportunities afforded by plasticity in the sensorimotor systems through the model of hand augmentation. As outlined above, successful adoption of augmentative devices requires profound neurocognitive adaptations, particularly in relation to the way we use our own limbs, with clear implications for motor control and sensory processing. Here, I have explored the potential for native sensorimotor resources to be repurposed to support the integration of robotic augmentation.

In Chapter 2, I will show that, in the case of late-onset deprivation, brain remapping may have much more limited functional consequences than classically assumed. Using psychophysics and complementary fMRI evidence, I will demonstrate that a famously reported perceptual consequence of acquired hand amputation (i.e, referral of facial touches on the phantom limb) is likely to be unrelated to brain remapping and represent instead a by-product of uncontrolled experimental conditions.

In Chapter 3, I will examine the scope for adaptive patterns of plasticity in cases of early-life deprivation. Using single-pulse TMS, I will show that the missing-hand motor area can control facial muscles in congenital one-handers, providing crucial causal evidence for functionally relevant brain remapping in cases of early-life input loss.

In Chapter 4, I will look at how fully able-bodied people can integrate a new extra body-part (i.e., a robotic third thumb) into their own existing sensorimotor representations. In particular, using a longitudinal paradigm involving local anaesthesia interventions and behavioural measures of motor learning, I will examine the potentially facilitating role of intrinsic sensory feedback signals for the construction of closed sensorimotor loops.

By studying the scope of sensorimotor plasticity across multiple scenarios, it is hoped that this work will have implications for the development of wearable technologies, rehabilitation strategies, and neuroprosthetics.

2 │ ACQUIRED HAND LOSS

Reassessing the link between perceptual and cortical remapping: the case of referral of touch on the phantom limb

Adapted from:

Amoruso, E.,Terhune D.B, Kromm, M., Kirker S., Muret[1], D., & Makin, T. R. (2021). Reassessing referred sensations following peripheral deafferentation and the role of cortical reorganisation. *medRxiv*

Chapter Abstract

Upper-limb amputees have been famously reported to perceive facial touches as arising from their phantom hand. These so-called "referred sensations" have been since replicated across multiple neurological disorders, and were classically interpreted as a direct perceptual correlate of cortical remapping. Common to all these studies is that participants might have been influenced in their self-reports by the experimental design or contextual biases. Here, we investigated whether referred sensations might be driven by demand characteristics (e.g., compliance, expectation, suggestion) rather than cortical remapping. Unilateral upper-limb amputees (N=18), congenital one-handers (N=19), and two-handers (N=22) were repeatedly stimulated with PC-controlled vibrations on 10 body-parts and asked to report the occurrence of any concurrent sensations on their hand(s). To further manipulate expectations, we gave participants the suggestion that some of these vibrations had a higher probability to evoke referred sensations. We also assessed similarity between (phantom) hand and face representation in primary somatosensory cortex (S1), using fMRI multivariate analyses. We replicated robust reports of referred sensations in amputees towards their phantom hand; however, the frequency and distribution of reported referred sensations were similar across groups. Moreover, referred sensations were evoked by stimulation of multiple body-parts and similarly reported on both the intact and phantom hand in amputees. Face-to-phantom-hand representational similarity was also not different in amputees' missing hand region, compared with two-handed controls. These findings weaken the interpretation of referred sensations as a

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[1] Performed analysis of fMRI data presented in Figure 2.2D and in Appendix

perceptual consequence of S1 remapping and reveal the need to account for demand characteristics when evaluating anomalous perceptual phenomena.

2.1 Introduction

Most amputees experience phantom sensations from their missing limb, typically described as itching, tingling, or numbness (Henderson & Smyth, 1948). These sensations typically manifest spontaneously but can sometimes also be triggered through stimulation of another body-part. Most commonly, phantom sensations can be evoked by touch applied to the residual limb (stump). This is believed to reflect peripheral reinnervation, where the severed sensory nerves, initially targeting e.g. the hand, reinnervate the surrounding tissue (Dhillon et al. 2004). A more curious example of evoked phantom sensations in upper-limb amputees comes from anecdotal reports that touching the face (e.g., while shaving) can elicit tingling sensations on the phantom hand. In a famous series of case studies (Halligan, Marshall, & Wade, 1994; Ramachandran, 1992, 1993; Borsook et al. 1998), a small group of patients reported experiencing referred sensations from the ipsilateral face to the phantom hand. In some cases, the referred sensations were modality specific, with, for example, hot water applied to the face eliciting a warm sensation on the phantom hand. Strikingly, in most of these patients, neighbouring sites on the face elicited sensations on neighbouring fingers, suggesting a shared topographical organisation of the face and phantom hand.

Phantom referred sensations evoked by facial stimulation have been commonly interpreted as the perceptual correlate of primary somatosensory cortex (S1) remapping, observed in classic electrophysiological studies in non-human primates (Pons et al. 1991). Specifically, it was shown that, following long-term arm deafferentation, the deafferented hand territory became responsive to touch applied on the monkey's chin. It was therefore suggested that following a similar remapping process in humans, face-induced activity in the missing hand area will be perceived as arising from the missing hand (hereafter, ‘the perceptual remapping hypothesis’; Collins et al., 2017; Ramachandran, 1992, 1993; Ramachandran & Hirstein, 1998). This theory has been further extended to consider the neural origins of phantom limb pain (Flor et al., 1995), as well as analogous referred sensations described in other neurological disorders (Soler et al. 2010; Moore et al. 2000; Katz and Melzack 1987; McCabe et al. 2003).

However, subsequent studies using more systematic stimulation paradigms found that phantom referred sensations could be evoked by touches on various body-parts. This includes body-parts that have not been considered to invade the missing hand cortical area, such as the feet, trunk, and neck, in some cases even contralateral to the missing hand (Grüsser et al., 2004, 2001; Knecht et al., 1996; (Halligan, Marshall, and Wade 1994; Andoh et al. 2017; Knecht et al. 1998). These reports, as well as recent fMRI studies that dispute the existence of a large-scale S1 facial remapping post-amputation in humans (Makin, Scholz, Henderson Slater, Johansen-Berg, & Tracey, 2015; Root et al., 2021; Raffin et al. 2015; Valyear et al. 2020 - See Introduction), challenge the perceptual remapping hypothesis.

An alternative mechanistic framework of referred sensations is that previous studies, lacking adequate controls, might have been confounded by the demand characteristic which are typical of experimental settings where the desired outcome (or response) is known or can be implicitly inferred by the context (Orne, 1962). Beyond compliance effects, demand characteristics conveyed by a procedure or intervention can also produce genuine experiences. For example, verbal suggestions can elicit robust changes in perceptual states (Oakley et al. 2021; Terhune et al. 2017). It is increasingly recognised that demand characteristics in various forms may function as confounds in a variety of experimental paradigms (Szigeti et al. 2021; Van Dam et al. 2018; Foroughi et al. 2016; Holman et al. 2015; Lush 2020). Therefore, rather than cortical remapping, reports of referred sensations in previous experiments might be driven by both explicit suggestions from experimenters (e.g., “this procedure will produce this experience”) or implicit cues that promote expectations for specific experiences.

Here we used vibrotactile stimulation (used to evoke reliable referred sensations in previous reports, e.g. (Knecht et al. 1996; Grüsser et al. 2001; Knecht et al. 1998)) of ten body-parts in a group of upper-limb amputees experiencing spontaneous phantom sensations. We also tested two control groups who do not report feelings of phantom sensations: two-handed individuals and individuals born with one hand. We manipulated participants’ expectations for referred sensations with explicit verbal suggestions that specific vibro-tactile stimuli were more likely to evoke referred sensations on their hands (and even if that hand is missing). Finally, to more directly test the idea of shared representation between the face and phantom hand representations, we took advantage of a fMRI dataset that was collected in the current study sample and analysed using a multivariate approach. The interested reader can find results from a second fMRI dataset, analysed with a univariate approach, in the Appendix. I decided to not include these results in the main body of the thesis as these analyses were performed by a co-author and not by myself.

We find that referred sensations can be generated and influenced by demand characteristics but no evidence to support the perceptual remapping hypothesis.

2.2 Methods

2.2.1 Participants

18 individuals with acquired unilateral upper-limb amputation (hereafter Amputees; mean age = 52 ± 12.2 (SD) y/o, 6 women, 10 missing the right upper-limb; see Table 1 for details about amputation, phantom limb pain and sensations), 19 individuals with congenital unilateral transverse arrest (hereafter One-handers; mean age = 44 ± 14.3 (SD) y/o, 11 women, 7 missing right upper-limb), and 22 two-handed individuals (hereafter Two-handers; mean age = 45.5 ± 9.5 (SD) y/o, 10 females, 6 left-handed) were tested. One Amputee was not able to participate in the scanning session due to MRI safety concerns and another Amputee only completed the body task due to time constraints. The proportion of participants with intact/dominant right hand was matched between Amputees and both One-handers ($X^2_{(1)}$=.942, p=.503) and Two-handers ($X^2_{(1)}$=2.670, p=.184). Amputees' gender was also matched to both One-handers ($X^2_{(1)}$=2.948, p=.106) and Two-handers ($X^2_{(1)}$=2.948, p=.106). Statistically significant differences for age were found between Amputees and One-handers ($t_{(34)}$=2.280, p=.029) and Two-handers ($t_{(37)}$=2.424, p=.020). Age covariates were therefore included when comparing neuroimaging observations between these groups. The study was designed in accordance with the Declaration of Helsinki and was approved by the UK NHS Health Research Authority (HRA) (18/LO/0474). All participants provided written informed consent prior to participating in the study.

Patient	Age Range	Gender	Handedness (before amputation)	Affected limb	Level of limb deficiency	Years since amputation	PLS intensity	PLS frequency	Chronic PLS	Spontaneous reports of PLS triggers	PLP intensity	PLP frequency	Chronic PLP	Amputation cause
Amp01	50-60	M	R	R	2	43	100	5	100	n-a	60	5	60	Trauma
Amp02	30-40	M	R	R	1	3	50	2.5	14.6	"When sleeping and surprised (e.g. reach out to catch him/herself)"	70*	2	17.5*	Trauma
Amp03	50-60	M	R	R	1	33	90	5	90	n-a	100	1	20	Trauma
Amp04	50-60	M	R	L	2	16	40	1	8	"When relaxed, randomly"	0	1	0	Trauma
Amp05	50-60	M	A	L	1	36	100	5	100	n-a	80	4	40	Trauma
Amp06	40-50	F	R	L	2	18	80	4	40	"When puts on prosthesis, exercises finger movements, or if actively thinking about it"	0	0	0	Electrocution
Amp07 (not MRI safe)	30-40	F	R	R	2	11	95	5	95	"When uses or touches residual arm - wherever on the arm, not just the stump"	50*	4	25	Trauma
Amp08	30-40	F	R	R	1	10	40	3	13.3	"When actively thinking about it"	0	0	0	Trauma
Amp09	40-50	M	R	R	2	5	70	4	35	"When consciously thinking about it or when putting on the prosthesis"	10	4	5	Trauma
Amp10 (fMRI 2 years later)	50-60	F	L	L	1	10	90	5	90	"When drinking hot liquids quickly (but not when touching the skin outside of the throat). The hotter the drink, the more intense the phantom sensation/pain"	60	5	60	Tumour
Amp11	60-70	M	R	R	1	38	60	5	60	"When driving"	0	0	0	Trauma
Amp12	60-70	F	L	L	1	10	90	5	90	n-a	80	4	40	Trauma
Amp13	60-70	M	R	L	1	35	80	2	20	n-a	100	2	25	Trauma
Amp14 (incomplete fMRI session)	50-60	F	R	L	2	4	0	0	0	n-a	0	0	0	Trauma
Amp15	60-70	M	R	R	1	18	75	5	75	n-a	65	5	65	Trauma
Amp16	60-70	M	R	R	1	8	70	5	70	"When actively thinking about it"	0	0	0	Trauma
Amp17	40-50	M	R	R	1	23	85	5	85	"When touching the upper side of the neck on the amputated side, usually when shaving"	65	5	65	Trauma
Amp18	30-40	M	R	L	2	14	30	5	30	"When it is extremely hot or cold"	25	1	5	Trauma

Table 2.1 Demographic details for Amputees

Level of limb deficiency: 1= above elbow (transhumeral), 2= below elbow (transradial); L= left, R= right. PLS= phantom limb sensations; PLP= phantom limb pain. PLS & PLP frequency: 0= no sensation or pain, 1= once or less per month, 2= several times per month, 3= once a week, 4= daily, 5= all the time. PLP intensity: worst PLP experience during the last week or in a typical week involving PLP, with 0= no pain, 100= worst pain imaginable. A chronic measure of PLP for correlation analyses was calculated by dividing the worst PLP intensity in the last week by PLP frequency. Spontaneous reports of PLS triggers: responses to the item: “Does your phantom sensation “come to life” following specific actions or situations (for example, putting on your prosthesis, when you touch your face)?”. *PLP intensity rating was on average

2.2.2 Referred sensations task

2.2.2.1 Procedure

Participants sat in front of a computer screen (2560x1440, 60Hz) and a pedal was positioned underneath each foot for response collection. Vibrating motors (diameter: 10mm, thickness: 2.7mm, operating voltage: 5.5V DC) were secured with surgical tape on ten body-parts (Figure 3.1A). Participants were informed that some of the vibrations were stimulating a newly discovered type of afferent fibres that could evoke sensations not only on the stimulated body-part but also on other areas, most commonly on the hands (see section 'Extended Methods' for verbatim instructions). Participants were told that a red or grey circle display would indicate whether an incoming stimulus had high or low probability, respectively, of evoking these dual sensations (hereafter, "High" and "Low" expectation conditions). All stimuli were, in effect, simple 500ms vibrating trains. To induce the feeling that vibrations may vary, and thus increase the belief in the suggestion, trains were delivered at an intensity well above detection threshold at three frequencies (15Hz, 12Hz, or 9Hz), with equal distribution across the different locations and cues. Participants were instructed to stay still and to focus on potential sensations arising from their hands.

Except for the instruction phase, the experiment ran in a strictly automatised way, with the experimenter leaving the room and visual cues, stimuli and responses delivered and collected by the software (MATLAB r2017a). Each trial began with the red/grey visual cue displayed for 700ms, followed by the 500ms vibration on one body-part. Next, a question appeared on the screen: "Have you just felt one stimulus or more than one?". Participants were instructed to press with the left foot to respond "one" and with the right to respond "more than one". When reporting multiple sensations, participants were further asked to respond, with the corresponding foot pedal, to the question: “Was it on the right or the left hand?”. Amputees and One-handers were briefed that this question also related to their phantom/missing hand (respectively). The experiment included 120 trials, equally distributed across the 10 stimulated locations, 60 trials per expectation cue (High/Low), and 40 trials per vibration frequency (9Hz, 12Hz, 15Hz). Due to technical issues during data collection, two Amputees, one One-hander and one Two-hander performed a reduced number of trials (90).

2.2.2.2 Verbatim task instructions

“In this task you will receive several kinds of stimuli from the vibrotacile stimulators placed on your body. They will feel as gentle vibrations on your skin. Some of these stimulations have been proven to stimulate a newly discovered type of nervous fibre. By doing so, they are able to evoke tactile sensations not only on the actually stimulated area of skin but also on regions of the body further away, and in particular, at the most distal extremities such as the hands or phantom hands. Indeed, due to the configuration of the peripheral nervous system, the hands are the sites at which the dual sensation most likely occurs. In this study we are interested in investigating whether there are sites on your body more responsive to this stimulation, meaning sites that when stimulated are more likely to give rise to these secondary more subtle sensations on the hands. The other types of stimulation stimulate alfa and beta fibres and convey classical information about touch to your brain, without being able to evoke other secondary sensations. On each trial, you will first be cued to which kind of stimulation is coming. Thus, a red circle on the monitor indicates that in the following trial you will receive special stimulation, i.e. the “special” one capable of causing secondary subtle sensations in your hands. A grey circle will appear on the monitor if in the following trial you will receive the classical stimulation. After the coloured circle, you will receive the vibrotactile stimulus. The computer will then ask you whether you felt only one sensation (on the stimulated body site) or more than one. Please press the left pedal to respond that you only felt one and the right pedal to respond that you

felt more than one. Please take your time to respond, as it usually takes a while for this interference process to happen. If you responded that you only felt one, you will start the following trial. If you responded that you felt more than one, another screen will appear asking whether you felt the secondary sensation on the left or right hand. Please respond with the left pedal if you felt it in the left or phantom hand and with the right pedal if you felt it in the right hand. The following trial will then start, and the process will be repeated."

2.2.2.3 Analyses

Trial-level data were aggregated into participant-level data by calculating the proportion of reported referred sensations in each condition. To focus on referred sensations reported on the phantom hand, while accounting for responses on the intact hand, lateralisation scores were calculated by subtracting the proportion of intact/dominant hand to phantom/missing/non-dominant hand referred sensations reports, in Amputees, One-handers and Two-handers respectively. Only participants reporting at least one referred sensation in a given condition of interest were included in this calculation. This resulted in different sample sizes across analyses/groups (see Results).

2.2.3 Functional MRI tasks

2.2.3.1 Procedure

The scanning session was completed on the same day as the behavioural task, apart from one Amputee who was scanned only 2 years later (due to Covid-19 restrictions; Amp10 in Table 1). In brief, the conditions included in the present analysis involved visually-instructed face movements.

Prior to entering the scanner room, participants were thoroughly instructed, and all movements were practiced in front of the experimenter to ensure they were performed correctly.

Participants were instructed to perform one of five movements: raise the eyebrows (i.e., forehead), flare nostrils (i.e., nose), puckering lips (i.e., lips), and flex the left or right thumb (or phantom thumb, if available). When phantom sensations were not present, participants with a missing hand were asked to imagine performing the movement. Note that this dataset was used to determine relationship between the phantom hand and the face representation, and for this reason we did not include the congenital one-handers in this analysis. An additional condition involved tapping the tongue to the roof of the mouth. However, since the inner mouth was not investigated in our behavioural task, we excluded this condition from our analysis in the present study. Instructions and pace were provided visually via a screen, resulting in 5 cycles of movement per 8 seconds block. Each movement block was repeated 4 times per run, which also comprised 5 blocks of rest used as baseline.

Conditions were pseudo-randomly distributed, such that each condition was equally preceded by all other conditions. To confirm that appropriate movements were made at the instructed times, whenever possible – task performance was visually monitored online for both tasks.

2.2.3.2 MRI data acquisition

MRI images were acquired using a 3T Prisma MRI scanner (Siemens, Erlangen, Germany) with a 32-channel head coil. Functional data were obtained using a multiband T2*-weighted pulse sequence with a between-slice acceleration factor of 4 and no in-slice acceleration. The following acquisition parameters were used: TR = 1450 ms; TE = 35 ms; flip angle =70°; voxel size = 2 mm isotropic; imaging matrix = 106 x 106; FOV = 212 mm. 72 slices were oriented in the transversal plane covering the entire brain. Each dataset comprised one and three functional task-related block-design runs (for the body and face tasks respectively). Field-maps were acquired for field unwarping. A T1-weighted sequence (MPRAGE, TR = 2530 ms; TE = 3.34 ms; flip angle = 7°; voxel size = 1 mm isotropic) was used to obtain anatomical images.

2.2.3.3 Functional MRI data pre-processing and analysis

Functional data was pre-processed in FSL-FEAT (version 6.00) and included the following steps: motion correction using MCFLIRT (Jenkinson, Bannister, Brady, & Smith, 2002); brain extraction using BET (Smith, 2002); high-pass temporal filtering with a cut-off of 280s and 119s for the body and face task respectively; and finally spatial smoothing using a Gaussian kernel with a full width at half maximum of 5mm and 3mm for the body and face task respectively (body-task results presented in Appendix). Field maps were used for distortion correction. A midspace between the different functional runs was calculated for each participant, i.e., the average space in which the images are minimally reorientated. Each functional run aligned to the midspace was then registered to each individual structural T1 scan using FMRIB's Linear Image Registration Tool (FLIRT), optimised using Boundary-Based Registration (Greve & Fischl, 2009).

We focused on the S1 hand region, though marginal contribution from M1 may have affected activity profiles due to its spatial proximity. The S1 hand region of interest (ROI) was defined bilaterally for each individual on a template surface using probabilistic cytoarchitectonic maps, by selecting nodes for Brodmann areas (BAs) 3a, 3b, 1 and 2 (Wiestler & Diedrichsen, 2013) approximately 1cm below and above the hand knob. This criterion defined a more conservative hand region than in previous research (Kieliba, Clode, Maimon-Mor, & Makin, 2021; D. B. Wesselink et al., 2019; Wiestler & Diedrichsen, 2013), in order to minimise overlap with the neighbouring face/arm areas. Structural T1-weighted images were used to reconstruct the pial and white-grey matter surfaces using Freesurfer. Surface co-registration across hemispheres was done using spherical alignment. The anatomical hand ROIs were projected into the individual brains via the reconstructed individual anatomical surfaces. For visualisation (Figure 2), S1 ROIs of each participant were projected to MNI152 space using the nonlinear registration carried out by FNIRT. Participant information regarding the side of missing/non-dominant hand were used to sagittal-flip data, such that the ROIs contralateral to the missing hand were always represented in the right hemisphere. ROIs of all participants were then concatenated into a single volume to produce a consistency map (i.e., how many participants have their ROIs overlapping in the MNI space). Resulting consistency maps were then projected to a group cortical surface (Glasser et al., 2016) using Connectome Workbench (v1.4.2).

2.2.3.4 Multivariate representational similarity analysis

The dissimilarity between activity patterns within each S1 hand ROI was computed at the individual level for each pair of movements using cross-validated squared Mahalanobis distance (Walther et al., 2016). Multidimensional noise normalisation was used to increase reliability of distance estimates (noisier voxels are down-weighted), based on the voxel's covariance matrix calculated from the GLM residuals. Due to cross-validation, the expected value of the distance is zero (or negative) if two patterns are not statistically different from each other, and significantly greater than zero if the two representational patterns are different (Diedrichsen, Provost, & Zareamoghaddam, 2016). Larger distances for movement pairs therefore suggest greater information content. The resulting representational pairwise distances between each of the facial conditions and the thumb (phantom/nondominant and intact/dominant, in Amputees and Two-handers respectively) were extracted. The analysis was conducted on an adapted version of the RSA Toolbox in MATLAB (Nili et al., 2014), customised for FSL (D. Wesselink & Maimon-Mor, 2018).

2.2.4 Statistical analyses

All statistical analyses were carried out using JASP (v0.14.1). To identify violations of the normality assumption, Shapiro-Wilk tests were run. No outliers were removed from the analyses to not exclude individuals showing potentially high referred sensations rates and/or large remapping. For non-significant comparisons of interest, Bayesian t-tests (or non-parametric equivalents) were conducted, with a Cauchy prior width set to 0.707 (default). We report Bayes Factors (BF_{10}), showing the relative support for the alternative hypothesis. As measure of effect size, rank-biserial correlations (r_B) or Cohen's d are reported. Kendall's Tau correlations were used to investigate whether referred sensations reports or cortical remapping were related to PLP in Amputees.

For the behavioural task, as normality was consistently violated across conditions, nonparametric tests (i.e., Wilcoxon signed-rank, Mann-Whitney, and Kruskal-Wallis tests) were used to test for within- and between-subject differences in the overall proportions and lateralisation of reported referred sensations.

fMRI data was analysed using mixed ANOVAs with the between-subject factor of Group (three groups) and a repeated-measure factor of Hemisphere (intact/dominant x deprived/non-dominant), and age as a covariate to account for age differences in cortical activation. If assumptions of normality were violated, non-parametric equivalents are also reported. Post-hoc comparisons between groups were conducted with a Bonferroni correction for multiple comparisons (α=.025; uncorrected p-values reported in the text).

2.3 Results

When sharing similar expectations, amputees do not report more referred sensations than One- and Two-handers

First, we examined if Amputees are more prone to report referred sensations, relative to One- and Two-handers. We found that all groups reported experiences of referred sensations significantly above zero ($Z \geq 78$, $p<.005$, $r_B=1.0$, for all groups; Figure 2.1C), with no differences in the proportion of trials where referred sensations were reported across stimulation frequencies ($X^2 \leq 2.311$, $p \geq .315$ for all groups). This result demonstrates that, when given the same instructions, any sample, irrespective of amputation, can report referred sensations. Trials in the High expectation condition evoked more reports than in the Low expectation condition (N=59, Z=206.5, p=.047, $r_B=-.38$), indicating that referred sensations can be enhanced through explicit suggestions. We observed no group differences for this suggestion effect ($X^2=.560$, p=.756), with Amputees' difference (high-low) scores not different from One- and Two-handers (both U>173, p>.499, $r_B<-.124$, $BF_{10}<.403$). Interestingly, referred sensations were reported more frequently relative to zero across all groups even during the Low expectation condition (N=59, Z=351, $p \leq .001$, $r_B=1.0$; 42.4% of participants reported >0 referred sensations), indicating that our experimental setup educed robust demand characteristics effects even when expectations were low (Figure 2.1B). For this reason, further analyses were based on the proportion of referred sensations collapsed across expectancy conditions. Crucially, the overall proportion of reported referred sensations did not differ across groups (X^2=.338, p=.845), and Amputees (N=18) did not report more referred sensations than One- (N=19) or Two-handers (N=22) (both U<220, p>.550, r_B<.111, BF_{10}<.355) (Figure 2.1C). In summary, participants of all groups responded positively, but similarly, to the suggestion/expectation cues, regardless of amputation.

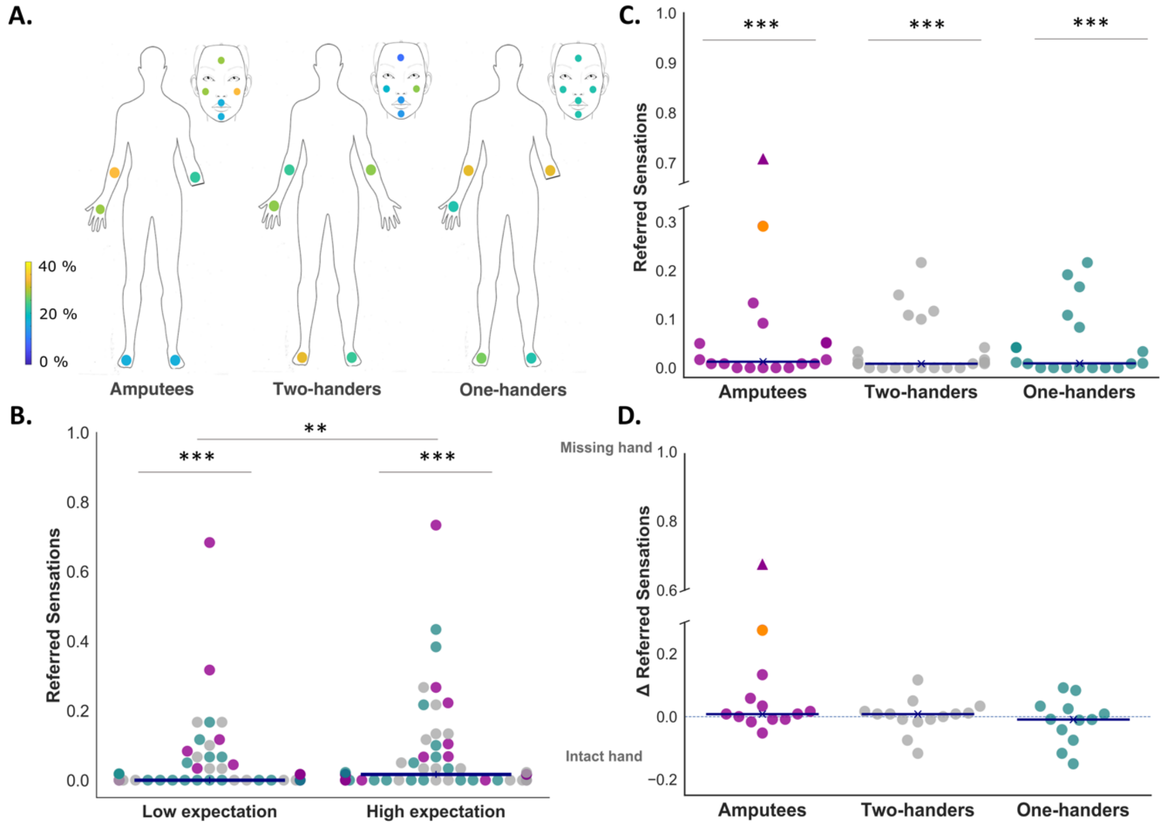

Figure 2.1 Global pattern of referred sensations across groups

A) Circles on the body silhouettes indicate the stimulated locations. The colour code indicates the percentage of participants in each group reporting at least one referred sensation at a given location. **B)** Proportion of referred sensations reported across groups in the Low and High expectation conditions. **C)** Proportion of referred sensations reported in each group across the stimulated locations. All groups reported referred sensations significantly above zero. **D)** Lateralised referred sensations across all stimulated locations, calculated by subtracting the proportion of referred sensations reported on the intact/dominant hand from the proportion of responses on the phantom/missing/non-dominant hand, in Amputees, One-handers and Two-handers, respectively. Positive values reflect referred sensations reported more towards the phantom/missing/non-dominant hand. Participants reporting zero referred sensations across the experiment were excluded (33.9% of the total sample). Each dot represents one participant; horizontal blue lines represent the group medians. In B) and D) participant Amp05, who reported high rates of phantom referred sensations and who could be scanned (contrary to Amp07, triangle symbol), is highlighted in orange to ease qualitative comparison with fMRI results shown in Figure 3.2. ***p<.001, **p<.005, *p<.05.

Sensations are not differentially referred to Amputees' phantom hand

Next, we assessed the specificity of Amputees' phantom hand as a target for referred sensations (Figure 2.1D). Both One- (N=12) and Two-handers (N=14) reported similar proportions of referred sensations across the two hands (both Z≤53.5, p≥.583, $r_B \leq -.192$, $BF_{10} \leq .353$). If referred sensations result from deprivation-triggered cortical remapping, then they should occur more frequently on Amputees' phantom hand. However, we found no significant differences in the proportion of reported sensations across the two hands in Amputees (N=13; Z=59, p=.125, $r_B = .513$, $BF_{10} = 1.11$), or in the lateralisation towards the missing/non-dominant hand across groups ($X^2 = 2.498$, p=.287). Amputees did not show greater lateralisation towards the phantom hand than One- or Two-handers (both U≥106, p≥.134, $r_B \leq .359$, $BF_{10} \leq .940$), indicating that they were not more inclined to report referred sensations on the phantom hand than One- and Two-handers on their missing/non-dominant hand, respectively. Finally, no differences were observed between One-handers' and Two-handers' lateralisation scores (U=70, p=.486, $r_B = -.167$, $BF_{10} = .417$).

Given the presumed mechanistic relationship (i.e. through cortical remapping) between referred sensations and phantom limb pain (Flor et al., 1995; Flor, Diers, and Andoh 2013; although see also Makin et al. 2013), we also explored the correlation between the propensity to report referred sensations on the phantom hand (while accounting for intact hand reports) and chronic phantom limb pain (Table 2.1), and found no significant correlation (N=18, r_{Tau}=.074, p=.692, BF_{10}=.327).

Face-evoked referred phantom sensations are not reflected in shared S1 representation

Phantom referred sensations evoked by facial stimulation have been commonly interpreted as the clearest perceptual correlate of S1 remapping. According to such "perceptual remapping hypothesis" (Ramachandran et al., 1992; Ramachandran 1993; Halligan et al., 1994, Collins et al. 2017), phantom hand referred sensations in amputees should more likely occur following face stimulation, due to the proximity in S1 representations. In accordance with this hypothesis, we found that face-evoked referred sensations were reported marginally more frequently on the phantom/missing/non-dominant rather than on the intact/dominant hand in Amputees (N=10) (Z=46.5, p=.058, r_B=.691, BF_{10}=2.215), but not in One-handers (N=9) and Two-handers (N=10) (both Z≤30, p≥.836, r_B≤.091, BF_{10}≤.330) (Figure 2.2B). However, no significant group difference emerged (X^2=3.417, p=.181), with Amputees' phantom lateralised face-evoked referred sensations not significantly different from One-Handers' (both U≤69.5, p≥.109, r_B≤444, BF_{10}≤.952), and no significant differences between One-handers and Two-handers (U=41.5, p=.805, r_B=-.078, BF_{10}=.414), which could be attributed to reduced statistical power, as indicated by the Bayes factors.

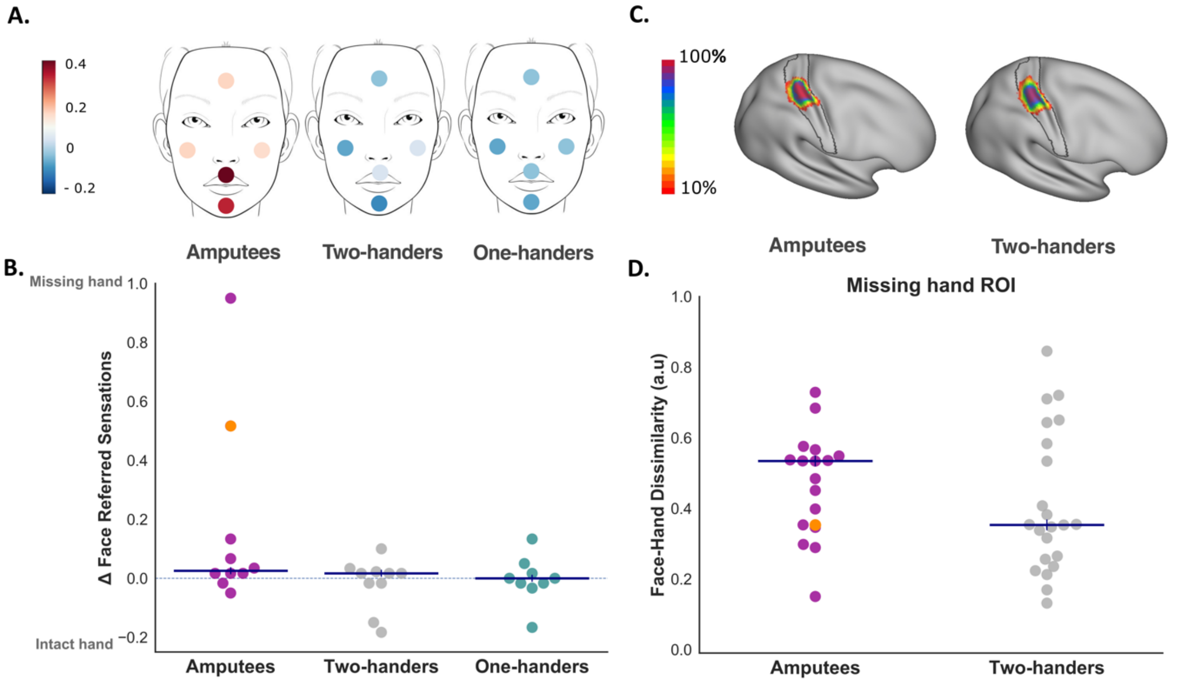

Figure 2.2 Face-evoked referred sensations and face-hand representational content in the missing-hand area

A) Face locations stimulated in the referred sensations task. The colour code indicates the group median lateralisation scores of referred sensations from each location. **B)** Lateralised referred sensations across the five face locations (60 trials). Participants reporting zero referred sensations on any hand across the face trials are excluded (50.8% of total sample). **C)** Inter-participant consistency maps for the missing/non- dominant hand S1 regions of interest (ROIs) across the three groups. The colour code represents the number of participants with overlapping ROIs in standard MNI space. The black contour shows the anatomical delineation of S1 used for ROI definition. **D)** Multivariate representational dissimilarity between activity patterns evoked in the missing/non-dominant hand ROI by facial (lips, nose, forehead) and contralateral thumb movements in Amputees and Two-handers (One-handers excluded as they cannot perform phantom movements). Whole samples are included, each dot represents one participant, horizontal blue lines represent group medians. Participant Amp05 is highlighted in orange: despite reporting high rates of referred sensations, no decreased dissimilarity between face and phantom hand representations is observed.

To provide more direct insight into the relationship between face and phantom hand representation, we used functional neuroimaging. We first replicated the well-known patterns of S1 body-part remapping across the three groups, which are qualitatively, though clearly, distinguishable from the even inter-group profile observed for the referred sensations reports (results presented in Appendix, Figure A.1 and A.2). Nevertheless, this approach relies on net activity levels within the missing hand cortex, while disregarding the well-established finding that the representation of the phantom hand persists in amputees (Raffin et al., 2012a, 2012b; Kikkert et al., 2016; Bruurmijn et al., 2017; Wesselink et al., 2019; see also section 1.3.2)**.** Thus, it may still possible that deprivation-triggered plasticity elsewhere along the somatosensory pathway (e.g. brainstem, Kambi et al., 2014, or thalamus, Jain et al. 2008) generates a neural scaffolding for shared representation between this phantom hand and other body parts, which may not be easily observed using an univariate remapping approach (Wesselink et al., 2020; Muret et al., 2022). We predicted that if referred sensations are associated with deprivation-triggered plasticity along the sensorimotor pathway, this should result in greater representational similarity between amputees' face and phantom hand relative to controls (or to amputees' own intact hand representation in their intact hand region). To test this hypothesis, the multivariate representational dissimilarity between activity patterns evoked by face and contralateral thumb were compared in the hand area across hemispheres and groups (Amputees and Two-handers only, as congenital do not have phantom hand sensations or mobility) (Figure 2.2D). To generate a representation of the (phantom) hand, participants were required to actively move their (phantom) hands, as well as facial sub-parts (Root et al, 2021). No significant differences were found between Amputees and Two-handers ($F_{(1,36)}$=.372, p=.546, η^2=.009), and no interaction with the Hemisphere ($F_{(1,36)}$=1.402, p=.244, η^2=.004). Follow-up comparisons revealed no significant difference between Hemispheres in Amputees ($t_{(16)}$=.619, p=.545, d=.150, BF_{10}=.295), as well as no group difference in the dissimilarities observed in the missing/non-dominant hand area

($t_{(37)}$=-1.047, p=.302, d=-.338, BF_{10}=.483), with Amputees showing, if anything, more face-hand dissimilarity than Two-handers. In other words, we did not find any evidence for shared information content between the phantom hand and the face in the missing hand area (see also Root et al., 2021, for a further characterisation of the lack of facial remapping in this cohort of participants).

2.4 Discussion

Here, we studied referred sensation reports in a group of upper-limb amputees experiencing spontaneous phantom limb sensations, in comparison to two control groups who had not undergone amputation (congenital one-handers and two-handers). Amputees did not report more induced referred sensations than the control groups. In all groups, referred sensations could be evoked from stimulation of multiple body-parts on both sides of the body, irrespective of inter-group differences in S1 remapping (see also Andoh et al. 2017; Flor et al. 2000). When sensations were evoked by face stimulation, we found marginal evidence for amputees differentially referring these sensations to the phantom hand, though this did not drive a significant interaction across groups. Importantly, this marginal effect in amputees is likely not consequential to increased sharing of S1 resources between the face and the phantom hand in the missing-hand territory (Figure 2.2; see also Makin, Scholz, Henderson Slater, Johansen-Berg, & Tracey, 2015; Root et al., 2021; Raffin et al. 2015; Valyear et al. 2020 for evidence of minimal face remapping).

In this context, it is important to note that the only methodology we could harness to reliably trigger phantom hand representation which could be compared across participants and groups includes active movement in the MRI scanner. While recent studies emphasise that representational structure in S1 is comparable across passive and active stimulation of the hand (see Berlot et al., 2019; Sanders et al, 2019), referred sensations are usually predominantly associated with touch, and not movement. As we did not attempt to evoke referred sensation in our fMRI paradigm, our neuroimaging findings are thus not ideally suited to uncover the neural bases of referred sensations. However, considering we found no suggestive evidence for the mainstream hypothesis that referred sensations are the perceptual correlate of post-amputation S1 remapping (Ramachandran 1992, 1993; Ramachandran and Hirstein 1998; Collins et al. 2017), and given that we were able to successfully provide an alternative mechanistic framework to explain previous reports, together our findings call for a general reassessment of this phenomenon and its neural bases. Specifically, while referred sensations might be genuinely and spontaneously experienced by some amputees, the experimental methods used to date to assess this phenomenon clearly contain demand characteristics that at best will contaminate any true effects.

It is well recognized that self-reported phenomena are particularly susceptible to the confounding impact of demand characteristics (Szigeti et al. 2021; Klein et al. 2012; Boot et al. 2013; Turner et al. 1994), yet there have been no attempts to control for these effects in referred sensations testing paradigms. We demonstrate that self-reported referred sensations can be triggered by experimental settings. This is well evidenced not only by the fact that the control groups reported referred sensations in the first place, but also, more directly, by a greater tendency across groups to report referred sensations when they were given the suggestion that these sensations were more likely to occur (‘High’ expectation condition). Expectancy-mediated changes in self-reported experiences can be driven by simple compliance or genuine changes in perception. Although our data does not allow us to dissociate genuine perceptual changes from compliance effects, the observation that congenital one-handers reported referred sensations also on their missing hand, on which touch had never been experienced, and thus no sensations can truly be referred to, suggests that behavioural compliance may have played a prominent role in our results.

The lack of control for demand characteristics poses serious limitations to the interpretation of previous accounts of referred sensations, as well as of other related phenomena that solely rely on self-assessed outcomes without accounting for both experimenter and participants' expectation (e.g., PLP treatment, (Ortiz-Catalan et al. 2016)). It is important to note that our findings do not rule out the spontaneous occurrence of referred sensations in selected amputees. Indeed, one participant reported experiencing referred sensations from the neck in his daily life (Amp18 in Table 2.1) and another (Amp05) reported a "classical" pattern of face-elicited referred sensations in our study (though this did not translate in increased face-hand shared representation or face remapping, as highlighted in Figures 2.2D, A.3). We also did not stimulate the entire skin surface, and thus cannot rule out that some potential 'hot spots' of referred sensations may have been missed. However, our findings clearly show that this phenomenon can be induced by suggestion and expectation. Insofar as referred sensations reports were plausibly driven, or augmented, by such confounds in previous research, they cannot provide a solid perceptual foundation for theories about functional brain reorganisation (Ramachandran & Hirstein, 1998) or for novel efforts to create ecological tactile feedback interfaces for prosthetic limbs (e.g.,Wijk et al. 2019; D'Alonzo et al., 2015). We hope that our findings will promote greater consideration of experimental demand characteristics also in future research on other anomalous perceptual phenomena.

3 │CONGENITAL HAND ABSENCE

Reorganised motor outputs in congenital one-handers: a TMS study

Adapted from:

> **Amoruso, E**., Kromm, M.[2], Spampinato, D., Kop, B., Muret, D., Rothwell, J., Rocchi, L. & Makin, T. R. (2021). Stimulating the deprived motor 'hand' area causes facial muscle responses in one-handers. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation*.

Chapter Abstract

The sensorimotor missing-hand cortical territory of individuals born with one hand can be activated by movements of multiple body parts, such as the lips, as demonstrated with fMRI studies. This cortical remapping has been widely considered to reflect functional reorganisation of the missing-hand cortex to support motor control of another body part (lips), similar to cross-modal reorganisation observed in individuals with congenital blindness and deafness. But activity changes due to input/output loss could result from regulatory processes, such as homeostasis, without invoking representational changes. It therefore remains to be established whether the observed remapping of the lips into the missing-hand area reflects a true functional reassignment. Here, we interrogate the functional significance of the remapping of the lips in the missing-hand motor area in one-handers. To this end, we applied single-pulse transcranial magnetic stimulation (TMS) over the missing-hand area in one-handers and two-handed controls, and used electromyography (EMG) to measure motor evoked potentials (MEPs) in the lower face muscles. Missing-hand cortex stimulation elicited MEPs in the lower face in a subgroup of one-handers, indicating that the missing-hand area can causally engage in motor control of the mouth. Our findings demonstrate that congenital limb differences can trigger patterns of cortical motor plasticity which result in new functional outcomes. It still remains to be determined whether this new motor pathway gets functionally utilised to support adaptive behaviour.

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[2] Co-collected the data with me and performed some analyses under my supervision

3.1 Introduction

How the brain adapts to the absence of sensory inputs and motor outputs from early development is a key question in neuroscience. While congenital sensory deprivation has long been known to trigger changes to cortical maps in sensory and motor cortex, e.g. due to blindness (Singh, Phillips, Merabet, & Sinha, 2018), deafness (Land et al., 2016) or arm malformation (Muret & Makin, 2021), there is an ongoing debate on their functional relevance. For example, using fMRI, our group has previously reported that the deprived sensorimotor hand territory of individuals born without a hand shows increased activity evoked by movements of the lips, feet and residual arm, when compared to two-handed controls (Hahamy et al., 2017; Hahamy & Makin, 2019: see Introduction). Such accounts are consistent with the idea that the missing-hand area may be recruited to support increased representation of alternative intact body parts - stressing the importance of early life experience in shaping sensorimotor cortical maps (Wesselink et al., 2019). However, changes to cortical map boundaries (i.e., remapping), as measured through fMRI, do not necessarily entail changed representational content and readout (Muret & Makin, 2021). As such, the observed remapping may not subserve a functional role. By allowing to directly stimulate the motor cortex and measure the evoked muscular responses in various body parts, TMS provides a unique opportunity to causally assess the functional consequences of sensorimotor remapping.

Few previous studies have used this technique to investigate the functional content of the missing-hand area of individuals with congenital limb deficiencies. In people born without both hands and with exceptional foot dexterity, TMS over the missing-hand area elicited motor evoked potentials (MEPs) in the feet and interfered with foot movements (Nakagawa, Takemi, Nakanishi, Sasaki, & Nakazawa, 2020; Stoeckel, Seitz, & Buetefisch, 2009). In congenital one-handers, studies have focused on the residual arm (Cohen, Bandinelli, Findley, & Hallett, 1991; Reilly & Sirigu, 2011), with mixed evidence: two studies reported an expansion of the residual arm representation (Cohen et al., 1991; Hall, Flament, Fraser, & Lemon, 1990), but this has not been replicated (Reilly & Sirigu, 2011). Here, we used single-pulse TMS to investigate the functional relevance of the previously observed remapping of the lips in the missing-hand motor cortex of one-handers (Hahamy et al., 2017; Hahamy & Makin, 2019). We hypothesised that functional reorganisation should result in facial MEPs when the missing-hand area is activated.

3.2 Methods

3.2.1 Participants

We tested 12 congenital one-handers and 14 two-handed control participants. All participants had no prior history of neurological or psychiatric disorders and passed screening for contraindications to TMS (Rossi, Hallett, & Rossini, 2011). Three control participants (CT2, CT8, CT9) and one congenital one-hander (OH8) had to be excluded from analysis due to technical issues in calibration of the participant's head shape to the neuro-navigation template. This affected the flipping procedure (see 'Definition of the main stimulation area' below), resulting in a defined stimulation location that was far off from the visually estimated hand motor region (Yousry et al., 1997). Two participants (OH7, CT11) were not included in the current analysis as they had a high resting motor threshold (rMT) and they could not tolerate a stimulation at 150%rMT. We therefore included 10 one-handed participants (mean age: 41.9 years (SD=12.9); 7 females; 4 left-handed) and 10 control participants (mean age: 35 years

(SD=11.5); 6 females; 3 left-handed) in our main analysis (for demographic details, see Table 3.1). Participants gave their written informed consent prior to the experimental session and received financial compensation for travel and participation. The study was approved to be in accordance with the Declaration of Helsinki by the NHS Research Ethics Committee (Project ID Number: 18/LO/0474).

Participant	*Sex*	*Age*	*Missing/ Nondominant*	*rMT*	*Amputation Level*
One-Handers					
OH1	F	32	L	34	1
OH2	F	28	R	39	1
OH3	F	52	R	35	1
OH4	M	52	L	45*	0
OH5	M	35	L	40*	1
OH6	F	32	L	36	1
OH9	F	29	L	42	1
OH10	M	63	L	45	1
OH11	F	57	R	46	1
OH12	F	39	R	41	1
Two-handers					
CT1	M	22	R	40	
CT3	M	26	L	39	
CT4	F	22	L	32	
CT5	F	21	R	35	
CT6	F	45	L	58	
CT7	F	48	L	47	
CT10	M	45	L	39	
CT12	M	47	R	38	
CT13	F	42	L	33	
CT14	F	32	L	43	

Table 3.1 Participants' demographic details

Note: Sex: female (F) and male (M); Missing/Nondominant hand on left (L) or right (R) side; Amputation level either above elbow (0) or below elbow (1); rMT is the resting motor threshold of the intact (or dominant) hand hotspot in % of maximum stimulator output (MSO). *The resting motor threshold of the face hotspot was 50% MSO for OH4 and 46% MSO for OH5.

3.2.2 Data acquisition and apparatus

3.2.2.1 Physiological measurements

For all participants, electromyography (EMG) was recorded from the lower face (orbicularis oris), ipsilateral to the missing/non-dominant hand and from the first dorsal interosseous (FDI) of the intact/dominant hand, in one-handers and controls respectively. For one-handers, we selected a prominent stump muscle that was as distal as possible but still well controllable by the participant. In control participants, we recorded from the wrist extensor muscles on the non- dominant side. Additionally, we recorded from the non-dominant FDI in controls to validate the estimated hand stimulation area. We also recorded from a leg muscle (tibialis anterior), as previous studies on people born without both hands showed MEPs in the feet during missing-hand stimulation but we did not observe any leg MEPs during missing-hand stimulation (Nakagawa et al., 2020; Stoeckel et al., 2009).

Self-adhesive electrodes were arranged in a belly-tendon montage. For the orbicularis oris recording, we placed the active electrode on the upper lip, close to the vermillion border, and the reference on the zygomatic bone. A ground electrode was set on the dorsum of the intact/dominant hand. The EMG signals were amplified and band-pass filtered between 20 Hz and 2000 Hz (Digitimer D360, 2015 Digitimer Ltd, United Kingdom) and acquired at a 5000 Hz sampling rate with a data acquisition board (CED-1402, Cambridge Electronic Design Ltd 2016) connected to a PC and controlled with the Signal software (also by CED). The data were stored for offline analysis using the Signal software.

3.2.2.2 Transcranial magnetic stimulation

A Magstim 200 stimulator (Magstim, Whitland, United Kingdom) and a figure-of-eight, 70-mm- diameter TMS coil were used for the stimulation. We adjusted the position and orientation of the coil and the intensity of the single pulses for each participant individually. To determine and monitor the correct coil placement, a neuro-navigation system (BrainSight, Rogue Research) was used. Since not all of our participants were MRI-safe and we could not obtain their structural brain scans, we calibrated the participants' head shape in the neuro-navigation system and fitted an MNI template. In case of a congenital transhumeral arrest (OH4), we used the individual's anatomical scan instead of an MNI template.

3.2.3 Procedure

3.2.3.1 Definition of the main stimulation area

Participants sat in a comfortable chair with both forearms resting on a pillow placed on their lap. Once the electrodes were set, the TMS coil was moved in small steps over the estimated site of the cortical motor area of the intact or dominant hand (in one handers and controls, respectively) in order to find the location on the scalp that consistently yielded the largest MEP amplitudes in the FDI contralateral to the stimulation side (i.e., intact/dominant hand hotspot). This hotspot was saved as a target in the neuro-navigation system. Next, we determined the resting motor threshold (rMT) of the saved hand hotspot. The rMT was defined as the minimal stimulation intensity that produced MEP peak-to-peak amplitudes larger than 0.05 mV in 50% of stimulation trials (Rossini et al., 2015). This threshold was used as a reference to determine the stimulation intensities. In order to define the cortical missing-hand area, we mirrored the hotspot of the intact hemisphere onto the deprived hemisphere by means of the neuro-navigation system. The same procedure was also applied to the control group, where we mirrored the hotspot of the dominant hand onto the nondominant hemisphere to define the cortical non-dominant hand area (corresponding to the missing-hand area in one-handers). The tilt of the coil had to be adjusted in some cases to ensure good contact of the coil with the participants' head, due to the asymmetry of their head shape. We based this procedure on the known symmetry of the M1 somatotopy across both hemispheres (Cicinelli et al., 2003; Civardi, Cavalli, Naldi, Varrasi, & Cantello, 2003). Furthermore, we validated this procedure by observing MEPs in the non-dominant hand of controls during stimulation of the estimated non-dominant hand region. A visualisation of the stimulation location for one one-handed participant is shown on Figure S3.1. The defined missing-hand territory was stimulated at intensities of 120% and 150% of the individual's rMT of the intact/dominant hand. The two intensities were chosen to ensure a sufficiently sensitive stimulation, as the threshold of the missing-hand hemisphere in congenital one-handers is shown to be higher than for the intact side (Reilly & Sirigu, 2011).

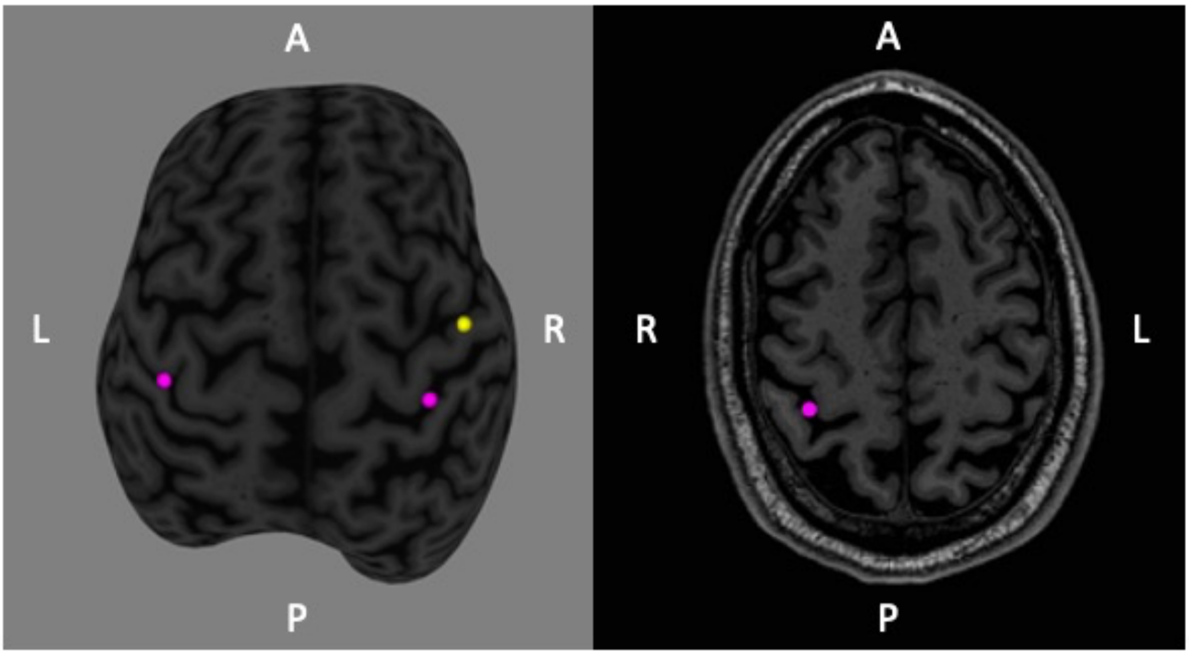

Figure S3.1 Visualisation of stimulation location for OH4

Participant OH4 anatomical scan (left image: 3D-reconstructed, right image: axial slice) with markers representing the approximated stimulation location provided by the neuro-navigation software. The magenta-coloured marker on the right hemisphere shows the stimulated area during the missing- hand stimulation, the yellow marker shows the stimulated area during face hotspot stimulation. The marker on the left hemisphere shows the intact hand hotspot. Images were extracted from BrainSight, Rogue Research.

3.2.3.2 Stimulation at rest

To map the cortical muscle representation in the deprived hand area, single-pulse TMS was applied to the defined missing-hand area while the participant was instructed to stay as relaxed as possible. We administered 30 consecutive pulses with a stimulation intensity of 120%rMT, followed by 30 pulses with an intensity of 150%rMT, with an inter-trial interval of 4±10% s.

3.2.3.3 Stimulation during muscular contraction

Following the measurements at rest, the participant was asked to slightly contract the muscles during the stimulation. The contraction of a muscle recruits the respective cortical neurons that are functionally linked to that muscle, which makes these neurons more sensitive to the magnetic stimulation (Rossini et al., 2015). Thus, the contraction helps to attain a more functional picture of the missing-hand M1 representations.

This part consisted of two blocks - lip contraction and arm contraction. Each block consisted of 30 trials in total (15 trials at 120%rMT; 15 trials at 150%rMT), with an intertrial interval of 4±10% s. However, since we could not identify consistent responses at 120%rMT, we only report results from the stimulation intensity of 150%rMT. The order of the body part contraction blocks was predefined and counterbalanced across participants. To ensure consistent force production, the participants were provided with the online EMG signal for visual feedback. Two horizontal cursors were set around 10% of the individual maximal contraction force in the EMG signal to facilitate the monitoring.

3.2.3.4 *Mouth hotspot*

For some participants, the mouth hotspot of the deprived (non-dominant) hemisphere was determined and stimulated as well. This was done by moving the coil more anteriorly and laterally. The spot that elicited the highest MEPs in the mouth muscles was sampled and the rMT was determined. We administered 20 pulses to this area with a stimulation intensity of 120%rMT. Note that this procedure requires high stimulation intensities as the threshold for the mouth is usually much higher than for the hand. Many participants could not tolerate this stimulation; hence, we could only acquire partial data.

3.2.3.5 *MEP detection*

The peak-to-peak MEP amplitude was determined by finding the global minimum and the global maximum of the EMG signal within a predefined temporal window. We selected the first occurring peak and then chose the temporally nearest peak. To avoid false positive registrations, the temporal window to search for peaks was adjusted for each body part, based on the time period in which an MEP is most likely to occur. The following time windows were used: hand = [15 - 40ms]; arm = [12 - 40ms]; mouth = [7 - 20ms] (Rossini et al., 2015; Roedel, Laskawi, & Markus, 2005). A time window of 150ms before the TMS pulse was used to compute the average and the standard deviation of the baseline noise level. Signals below a threshold of mean ± 4 standard deviations of the noise level were discarded as being indistinguishable from the baseline. Furthermore, MEP amplitudes lower than 0.05 mV were discarded as per general practice (Rossini et al., 2015). As an additional precaution, the single trial wave-plots of the signal for each subject were visually inspected. The latencies of the MEP onset were determined visually on a trial-by-trial basis. The experimenter performing the visual detection was blinded with respect to the participant group.

Some of our participants showed artifacts in the face signal data. During offline analysis, we determined whether signal displayed evidence of peripherally evoked compound muscle action potential (CMAP) or decay electrical artefacts. The CMAPs arise due to direct stimulation of the peripheral facial nerve fibres situated close to the targeted cortical area (Dubach, Guggisberg, Rösler, Hess, & Mathis, 2004). For most of our participants who showed CMAPs in the face EMG recording, the CMAPs ended around 8ms after the pulse onset. Thus, they were not likely to confound potential MEPs. In contrast, the decay artefact results from the voltage induced in the electrodes directly by the TMS pulse, which was of much larger magnitude than the physiological responses and could contaminate potential face MEPs, which complicates their detection. In order to detect eventual MEPs masked by the decay artefact, we fit a 2nd order polynomial function to the signal (which closely resembles the shape of the artefact) and computed the residuals between the fit and the signal. For some participants, the artefact was so strong that the fitting did not result in substantial improvement of the signal legibility. This was the case for three one-handers and one control during missing-hand area stimulation at rest (Figure 3.3), and for the same control during missing- hand area stimulation over the lip-contraction trials (Figure 3.2). Recollection of this data was originally planned, but then halted due to Covid-19 bans on human testing.

3.2.4 Statistical analyses

Peak-to-peak amplitudes and MEP response latencies were examined for all trials showing an MEP. The proportion of individuals showing face MEPs across groups was compared using Fisher's Exact Test. Whenever possible, we also stimulated the face hotspot ipsilateral to the missing hand with an intensity of 120% rMT and compared the face MEP latencies of the two stimulation locations (missing-hand vs. face hotspot) with Welch's t-tests. When non-significant differences were found, we calculated the Bayes factor (Cauchy prior width = 0.707) to obtain the likelihood of observing our data under the null hypothesis. Analyses were performed with custom-made scripts in MATLAB (R2017a, Mathworks), SPSS (Version 25, IBM), and RStudio (R Core Team, 2020).

3.3 Results

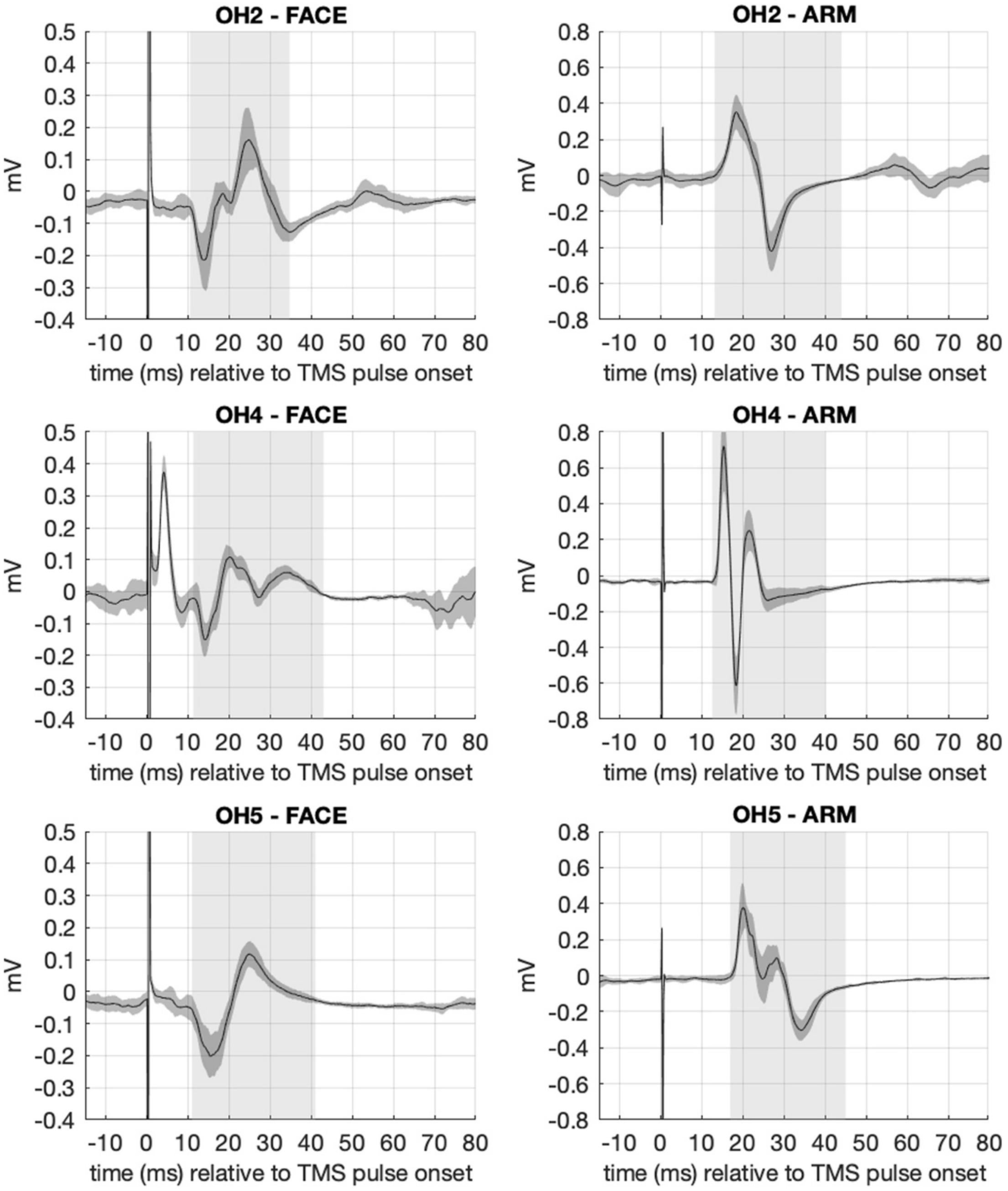

Figure 3.1 Face MEPs during missing-hand area stimulation

Average EMG from the orbicularis oris (left) and residual arm muscle of face-responders for all lip contraction trials during missing-hand stimulation (solid line: mean value; shaded area: ± one standard deviation). The mean MEP-window is highlighted in light grey. For OH4, the spike at around 5 ms reflects a peripherally evoked compound muscle action potential (CMAP).

We observed clear arm MEPs in 6 out of 10 one-handers and in all controls. Of these 6 one-handers, 3 showed face MEPs during lip contraction trials (Figure 3.1) while no face MEPs were found in controls (Figure 3.2).

Average MEP amplitude and latencies were as follows: OH2 (87% MEP-trials): 0.47 mV (SD=0.13 mV) and 11.5 ms (SD=0.4 ms); OH4 (93% MEP-trials): 0.29 mV (SD=0.07 mV) and 12.5 ms (SD=1.0 ms); OH5 (93% MEP-trials): 0.36 mV (SD=0.10 mV) and 12.2 ms (SD=0.9 ms). The remaining one-handers showed either a strong decay artefact in the facial EMG or did not show face MEPs (Figure 3.2). A Fisher's Exact Test confirmed that face MEPs are more common in one-handers than controls (p=0.036). During rest trials, no clear face MEPs were observed (Figure 3.3).

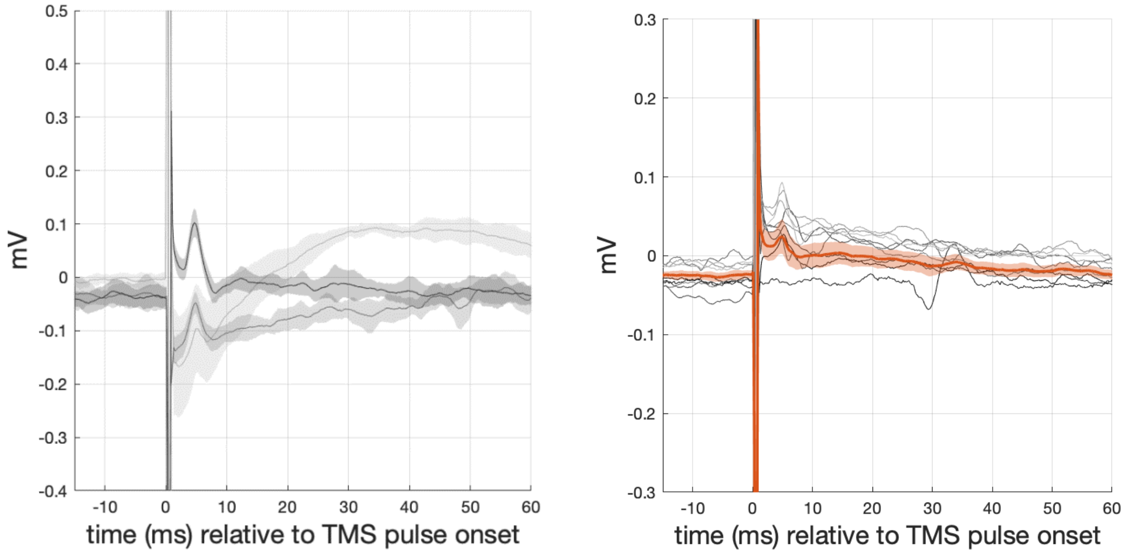

Figure 3.2 Average face EMG signal during lip contraction

Average EMG waveform of contracted lower face muscle during (missing) hand stimulation. The left plot represents one-handers that showed arm MEPs but no face MEPs (n=3), the light grey line shows the detrended signal of one participant. The right plot shows controls (n=9). Each grey line represents a single participant. For controls, the average signal across all participants and the standard error are shown in orange, for each one-hander the standard deviation is shown. The small peaks right after the TMS pulse are CMAPs. One control's face recording is not plotted here because of a strong electrical decay that could not be detrended successfully (for further explanation, see Methods).

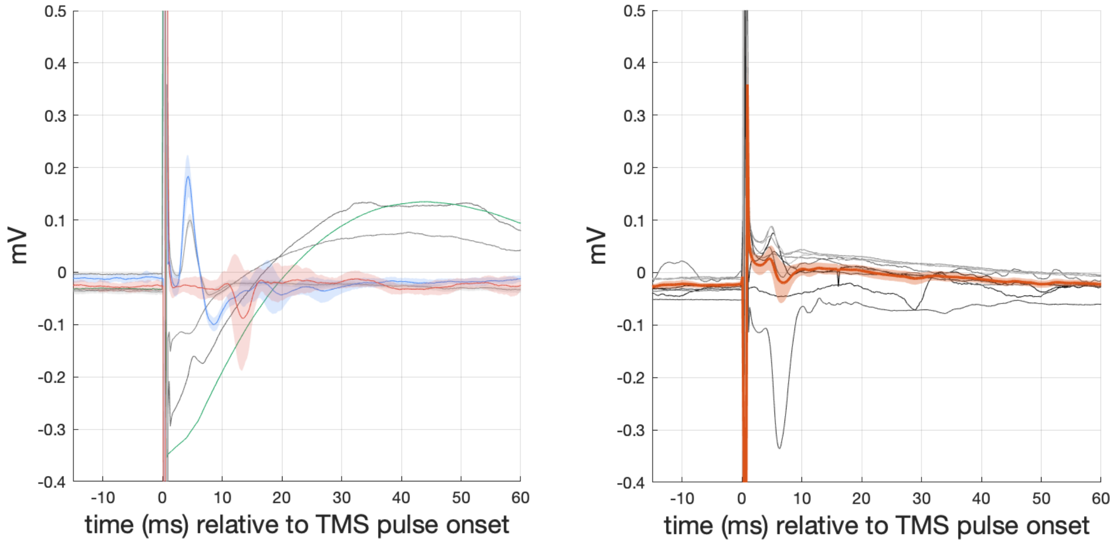

Figure 3.3 Average face EMG signal at rest

Average EMG waveform of the relaxed face muscle during (missing) hand stimulation in 6 one-handers (left) and 9 controls (right). The face responders from the contraction condition are shown in colour (OH2 – red; OH4 – blue; OH5 – green), the other participants in grey. Three one-handers showed an electrical artefact (incl. OH5), here we show the detrended signal without standard deviation (for better legibility). OH2 (red) shows small potential face MEPs at around 11ms. For controls, the average signal across all participants and the standard error are shown in orange. The small peaks right after the TMS pulse (at around 5ms) are CMAPs. One control's face recording is not plotted here because of a strong electrical decay that could not be detrended successfully (for further explanation, see Methods).

Additionally, we stimulated the face hotspot in two of the three face-responders. Average MEP amplitudes and latencies were as follows: OH4 (100% MEP-trials): 0.57 mV (SD=0.13 mV) and 11.2 ms (SD=1.5 ms); OH5 (74% MEP-trials): 0.20 mV (SD=0.07 mV) and 12.5 ms (SD=0.3 ms). In OH4, face MEPs from the face hotspot had shorter latencies compared to the face MEPs from the missing-hand hotspot (W(24.61) =2.9, p < 0.01). OH5 showed no significant differences in latencies across the two stimulation locations (W(15.68) = -1.17, p=0.26, BF(01)

=1.7). Importantly, in both cases the shape of the MEPs looks similar for the two stimulation locations (Figure 3.4) suggesting that the observed facial EMG- signal during missing-hand stimulation are indeed MEPs. No arm MEPs were observed during face-hotspot stimulation.

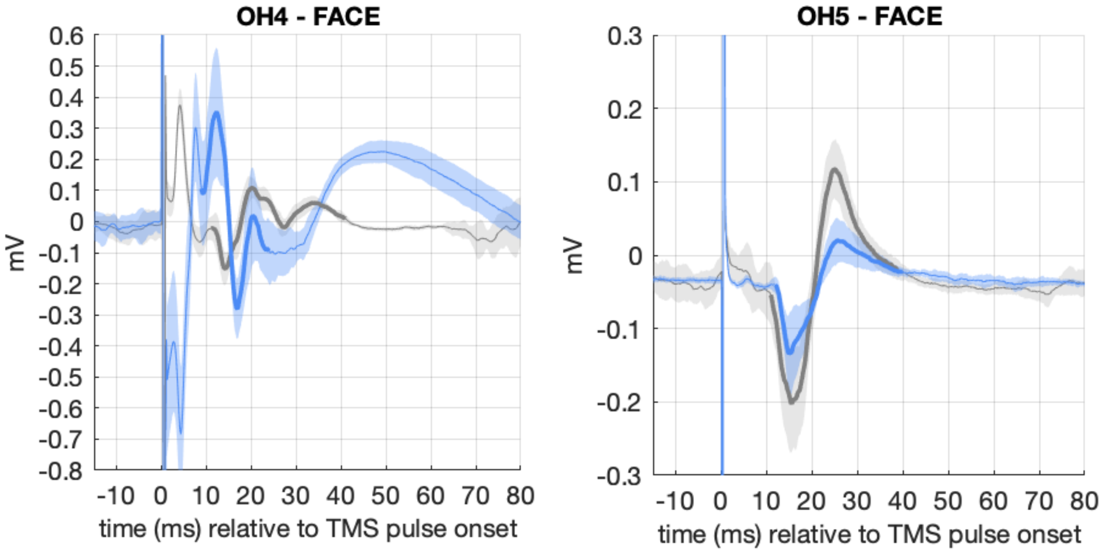

Figure 3.4 Average EMG signal during face area stimulation

Average EMG waveform from the orbicularis oris during face area stimulation for two face-responders. The blue line shows the EMG during face hotspot stimulation, the grey line represents the signal during missing-hand area stimulation. The bold highlight shows the average MEP shape. Note that OH4's recording showed an electrical artefact during face hotspot stimulation, here we show the detrended signal.

All face-responders (OH2, OH4, OH5) exhibited arm MEPs during missing-hand stimulation (Figure 3.1). The Bayesian test for single case assessment revealed no differences in arm MEP latencies between OH5 (M=17.6 ms, SD=0.5 ms) and controls (Z=0.41, p=0.7), with an estimated 65% of the control population falling below OH5's value, and between OH2 (M=14.8 ms, SD=1.4 ms) and controls (Z=1.97, p=0.09), with an estimated 2.5% of the control population falling below OH2's value. For comparing the arm MEP latencies across groups while accounting for the different group sample sizes a Welch's t-test was run (n(OH)=5, n(CT)=10) (Figure 3.5). Note that OH4 had a transhumeral arrest, making the comparison to the controls' forearm muscle inadequate, and was thus not included in the analysis (see Figure S3.1 for visualisation of the stimulation location for this participant).

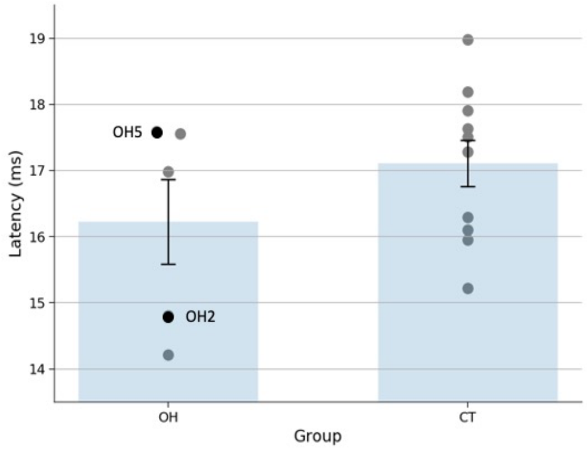

Figure 3.5 Group comparison of arm MEP latencies

Mean and standard error of group arm MEP onset latency in milliseconds. The markers show the mean latency of each participant. The face responders are highlighted in black. OH = one handers; CT = two-handed controls.

We did not observe significant differences between one-handers (*M*=16.23ms, *SE*=0.64ms) and controls (*M*=17.11ms, *SE*=0.35ms, *W*(6.23)=-1.09, *p*=0.32). However, the Bayes factor (*BF*(01)=1.39) does not suggest sufficient evidence for the null hypothesis. Hence, albeit non conclusively, these findings provide indirect evidence that it is unlikely that we stimulated closer to the face region in the face-responders compared to controls.

3.4 Discussion

Here, we show that muscle responses in the lower face can be evoked in a subgroup of congenital one-handers by applying single-pulse TMS over the missing-hand motor area. The normative values and shape of these observed MEPs are in line with previous research directly stimulating the face area (Adank, Kennedy-Higgins, Maegherman, Hannah, & Nuttall, 2018).The fact that MEP latencies evoked from the missing-hand and face sites are not conclusively similar in our sample, with OH4 reporting increased latencies from the missing-hand site, suggests that a mono-synaptic corticospinal projection from the missing-hand area is likely unsuited to explain our findings (Fuhr, Cohen, Roth, & Hallett, 1991). Rather, although our data contain no direct evidence, it appears more plausible that the observed MEPs are generated through cortico-cortical projections through horizontal connections from the missing-hand to the face site (Fang, Jain, & Kaas, 2002).

By showing that the missing-hand motor area can engage in motor control of the lower face muscles, we provide direct evidence for functionally relevant sensorimotor reorganisation in congenital one-handers. We suggest that, in absence of the typical peripheral input/output sensorimotor dynamics during development, the sensorimotor missing-hand area becomes responsive to face inputs, otherwise normally inhibited. This hypothesis is supported by the findings of increased global connectivity and decreased GABA in the missing-hand area, indicating decreased inhibition in one-handers (Hahamy et al., 2017). Our present results suggest that such release from inhibition has functional consequences.

Interestingly, we only detected facial motor responses in a sub-set of one-handed participants. This could be due to methodological reasons (see Methods), but it could also indicate that the patterns of functional reorganisation differ across one-handed individuals. It was suggested that patterns of remapping in the deprived hand area may be dictated by the relative compensatory use of alternative body parts during development, due to mechanisms of Hebbian-like plasticity (Hahamy et al., 2017). Under this framework, only individuals who have used their mouth more frequently to compensate for the missing-hand functions would show functional reorganisation of the face. However, since one-handers predominantly rely on their residual arm for compensatory behaviours, this framework would have predicted shorter arm MEP latencies, which was not consistently observed here (Figure 3.5). Nevertheless, our unique dataset provides a rare opportunity to demonstrate that deprivation-triggered plasticity, which results in new functional outcomes, is possible in the human brain even in absence of exceptional training (Nakagawa et al., 2020; Stoeckel et al., 2009). Further research using longitudinal approaches is needed to better understand the role of motor experience in shaping brain reorganisation as it unfolds across development.

4 │ROBOTIC HAND AUGMENTATION

Exploring the sensorimotor interactions underlying effective control of extra fingers

Adapted from:

> **Amoruso E.** , Dowdall L.[3], Kollamkulam M.T.[3] , Ukaegbu O. , Kieliba P. , Ng T., Dempsey-Jones H., Clode D. , Makin T.R. (2022). Intrinsic somatosensory feedback supports motor control and learning to operate artificial body parts. *Journal of Neural Engineering*.

Chapter Abstract

Motor augmentation is an emerging research domain that aims to extend the physical abilities of any individual through wearable robotics. A key challenge in the field is how to provide motor commands and receive sensory feedback from the new 'body-parts' with minimal neurocognitive costs. In this chapter, I investigate the utility of implicit somatosensory inputs, intrinsically generated by the body part(s) remotely controlling a wearable augmentative device, in supporting motor control and learning. In a placebo-controlled design, we used local anaesthetic to attenuate somatosensory inputs to the big toes while participants learned to operate through pressure sensors a toe-controlled and hand-worn extra robotic thumb (Third Thumb). Motor learning outcomes were compared against a control group who received sham anaesthetic and quantified in three different task scenarios: while operating in isolation from, in synchronous coordination, and collaboration with, the biological fingers. Both groups were able to learn to operate the robotic extra finger within the ~ 2.5 hours training session. Importantly, the availability of displaced somatosensory cues from the distal bodily controllers facilitated the acquisition of isolated robotic finger movements, the retention and transfer of synchronous hand-robot coordination skills, and performance under cognitive load. Motor performance was not impaired by toes anaesthesia when tasks involved close collaboration with the biological fingers, indicating that the motor system can close the sensory feedback gap by dynamically integrating task-intrinsic somatosensory signals from multiple, and even distal, body- parts. Together, our

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[3] LD and MTK collected the data and performed some preliminary analyses under my supervision

findings demonstrate that there are natural avenues to provide intrinsic surrogate somatosensory information to support motor control of wearable augmentation, beyond artificial external stimulation.

4.1 Introduction

In natural conditions, motor control heavily relies on (often subconscious) somatosensory signals for tracking limb state and interactions with objects. The motor control metasystem is in fact often conceptualized as a closed loop, with somatosensory feedback improving motor control by providing online feedback on the environment (Miall & Wolpert, 1996). Tactile signals from the skin mechanoreceptors are especially important for object manipulation, as they convey information about contact timing, size, and location, as well as the optimal amounts of pressure to exert (Johansson & Flanagan, 2009). Proprioceptive signals allow us to plan and guide the dynamics of limbs movements by informing us on current joint position and motion without the cognitively taxing effort of constant visual monitoring (Proske & Gandevia, 2012). Increasing evidence has shown that the somatosensory system plays a fundamental role not only in the online movement planning and correction processes, but also in motor learning, i.e. the acquisition and consolidation of novel motor skills (see Ostry & Gribble, 2016, for a review). Here, an internal (predictor) model is constructed to generate an expectancy about the sensory consequences of a motor action. Following the action, sensory information coming from the environment is compared against the predicted feedback to help update the internal model of action outcomes and improve motor learning (Wolpert, Ghahramani, Jordan, 1995; Parr et al., 2021).

As such, the implementation of motor augmentation in real-life dynamic environments crucially depends on the brain’s ability to integrate somatosensory feedback from the robotic device when constructing the motor plans to operate it, i.e. on the formation of closed sensorimotor loops. In light of this, in parallel with working on solutions for efferent control (Abdi et al, 2015; Salvietti et al., 2016; Parietti & Asada, 2017; Meraz et al, 2018; Baudisch et al., 2018; Guggenheim et al., 2020; Kieliba et al., 2021), augmentation research is also focussing on the development of somatosensory feedback interfaces. These efforts, which build on solutions originally designed for restorative purposes (Bensmaia, Tyler, Micera, 2020), have almost exclusively focussed on artificially induced feedback, where tactile cues are delivered to a displaced skin surface through vibrotactile arrays (Dominijanni et al., 2021). For example, research with a switch-controlled X-digit has shown that event-driven haptic feedback cues, provided via a vibrating ring worn on the contralateral hand, can improve performance in a hand-device coordination task (Hussain et al., 2015). This suggests that the addition of artificial somatosensory feedback can help users efficiently control extra body-parts. However, to represent true augmentation, controlling and receiving feedback from such devices should not deplete sensorimotor resources allocated to other body parts. This unique challenge makes solutions borrowed from the restorative domain not optimally suitable for augmentative purposes. Delivering bespoke feedback through external stimulation can in fact reasonably be expected to interfere with perceptual processing on the patch of skin where the vibrotactile array is positioned. Moreover, monitoring and interpreting such cues requires training and is likely to increase the cognitive load, limiting the opportunities for what augmentation was originally designed for, i.e. effective multitasking.

Given the wearable nature of extra limbs and fingers, it is important to acknowledge that somatosensory inputs are inherently present at the interface between the device and the user's body, providing implicit sensory feedback. For example, in a series of studies, motor control was found to be improved when physically wearing an extra robotic arm compared with controlling a detached virtual version (Guggenheim & Asada, 2020). This suggests that there might be as of yet unexploited opportunities for integrating intrinsic forms of somatosensory feedback to support motor control of augmentative devices, which could overcome the limitations of external signalling.

Here, we investigated whether the intrinsic somatosensory inputs received by the body-part proportionally controlling an extra robotic digit (Third Thumb) are leveraged by users to optimize motor learning, i.e. for correcting movements during the initial training and for further facilitating skills' retention and transfer (Wolpert & Ghahramani, 1995). The Third Thumb (Dani Clode Design, Figure 4.1A-B) is a 2 degrees of freedom (DOF) robotic sixth digit which is worn on the hand and wirelessly operated by the big toes (controllers). To operate the device, the user needs to vary the pressure applied on a pair of force sensors strapped underneath the big toes (right for flexion/extension and left for abduction/adduction).

Over the course of two days, healthy participants were first trained to use the Third Thumb and then tested on a series of motor learning outcomes (Figure 4.1C). To modulate intrinsic somatosensory contributions to the sense of the Thumb's state and position, we used anaesthetic injections to attenuate pressure feedback to the big toes in a test group prior to training. This procedure prevents most tactile signals from the big toes' skin and Golgi tendon receptors, while leaving motor function (enabled by foot/ankle muscles) and proprioception (arising from the ankle's muscle spindles) mostly unaffected. Training performance and learning outcomes were compared against a control group, who received sham injections. We compared participants' ability to acquire, retain and transfer (Kantak & Winstein, 2012) their motor skills to infer the potential benefits of the distal controllers' pressure feedback on motor learning. We hypothesised that, by receiving somatosensory feedback about the amount of pressure exerted with the toes, the Sham group would be able to develop a more accurate internal model of the Third Thumb state and position (i.e., its proprioceptive status), allowing more effective motor learning (Wolpert & Ghahramani, 1995). We also hypothesised that reduced somatosensory information from the device controllers will incur more attention to monitor the Thumb's performance through complementary sensory cues (e.g. visual). As a consequence, the Anaesthetised group would show more deficits in device control when a further cognitive load is added to motor tasks (Poldrack et al., 2005).

4.2 Methods

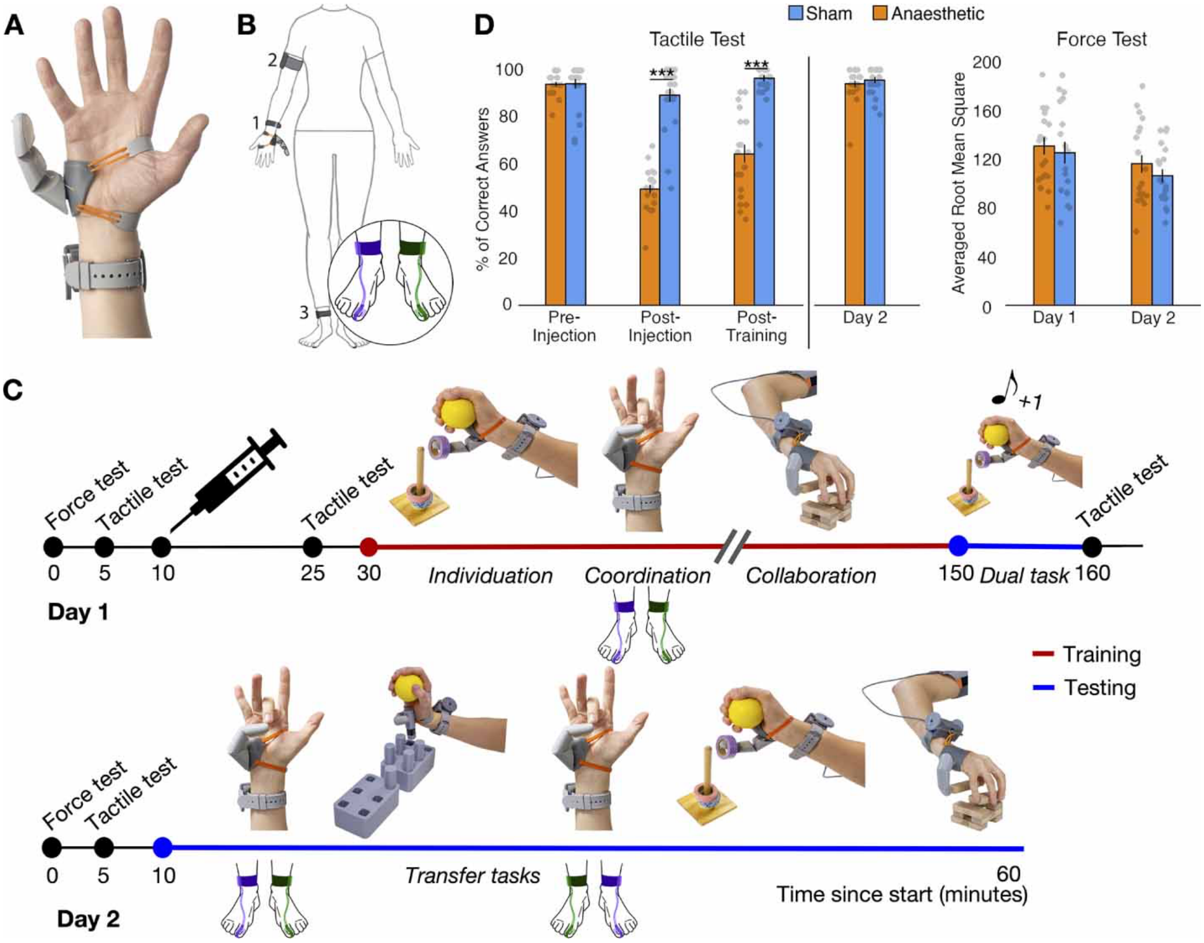

Figure 4.1 Longitudinal experimental design

A-B) The Third Thumb is a 3D-printed robotic extra digit. Mounted on the side of the palm (1), the Thumb is actuated by two servomotors (fixed to a wrist band), allowing for independent control over flexion/abduction. The Thumb is powered by (2) an external battery, strapped around the arm and wirelessly controlled by (3) two pressure sensors strapped underneath the participant's big toes. The colours purple and green represent the sensors controlling the flexion/extension and abduction/adduction movements, respectively. **C)** Timeline depicting the course of the study over the two days. The syringe on Day 1 represents the anaesthetic/sham intervention. Training with the Third Thumb is represented in red and testing in blue. Tasks used for training and testing are displayed on the timeline: Individuation – of the Third Thumb (picking up, transporting and repositioning tape rolls) while keeping the biological hand occupied; Coordination – between the biological fingers and Third Thumb: opposing the tips of the fingers to the tip of the Thumb; Collaboration – between the biological hand and Thumb: lifting and transporting wooden bars to build a tower; Dual task – increasing the cognitive load on the individuation task, by simultaneously conducting a primary working-memory numerical task; Transfer tasks – varying task demands relative to training (1) picking up, transporting and repositioning pegs for Third Thumb individuation and (2) the coordination task with the flexion/extension and abduction/adduction pressure sensors swapped. The training tasks were completed in a randomised order while the testing tasks were completed in the fixed order shown in the timeline. **D)** Participants across groups showed similar performance in the baseline tactile acuity test and confirmed expected changes in tactile sensitivity following the injection intervention according to group assignment. Participants also showed similar performance across groups at baseline and on Day 2 on the Force test, used to assess baseline motor ability and determine the pseudo-randomised group allocations. The bars depict group means; error bars represent standard error of the mean. Individual dots correspond to individual subjects' scores. *Asterisks denote significant effect of Timepoint at *** p<0.001*.

4.2.1 Participants

50 right-handed participants were recruited for the study. Exclusion criteria included allergies to local anaesthetics, needle phobia and history of neurological or psychiatric illness. 4 participants dropped out of the study due to a vasovagal response to the injections and 2 participants did not proceed beyond the injections due to an unsuccessful numbing effect (see: Anaesthetic Intervention). The final participant group included 44 participants with 22 participants in the 'Anaesthetised' group (11 female, mean age = 22.91, SD = 3.85, range = 18 to 35 years) and 22 participants in the 'Sham' group (10 female, mean age = 23.45, SD = 3.60, range = 18 to 33 years). Participants were pseudo-randomly allocated to either the 'Anaesthetised' or 'Sham' group (see: Sampling Validation Tests). Due to updates to the study design after initial data collection, only 40 participants (20 in each group) completed the Dual Task and only 17 participants in the Sham group and 15 participants in the Anaesthetised group completed the retention test for the Collaboration and Individuation tasks. Due to technical issues during data acquisition, pressure sensors recordings from 2 participants in the Anaesthetised group and 5 in the Sham group could not be analysed. All participants provided their informed consent prior to participation. Ethical

approval for the study was granted by the UCL Ethics committee (Project ID: 12921/001).

4.2.2 Third Thumb

The Third Thumb (Dani Clode Design, London, UK) is a robotic extra finger that attaches to the ulnar side of the right hand and is wirelessly operated through pressure sensors strapped underneath the big toes (Figure 4.1A-B). The Thumb has two degrees of freedom that allow a corresponding proportional control: applying pressure to left big toe sensor causes an adduction/abduction movement, whilst pressure on the right one causes a flexion/extension movement (Figure 4.1B). The pressure applied by each big toe when the Thumb was switched on during the experiment was recorded and used for off-line analyses (see section: Toes Pressure Analysis). Both pressure sensors contained an SD-card that logged the position of the Thumb's servomotors (range 10-175 degrees for flexion/extension, range 10-80 degrees for abduction/adduction). The position of the motor was linearly proportional to the pressure applied by the participant. This relationship was fixed for all participants in such a way that the same value of pressure was always associated with the same servo position. When using the Third Thumb, participants placed their big toes on a small platform of a footrest, to minimise somatosensory feedback from the other toes or neighbouring foot surface while controlling the Thumb.

4.2.3 Anaesthetic intervention

All participants received the same deafferentation protocol (Dempsey-Jones et al., 2019). The intervention varied only in the substance injected, which depended on group assignment. Each anaesthetic intervention required an injection of 2.5ml of 2%

Lidocaine Hydrochloride and 2.5ml of 0.5% Bupivacaine Hydrochloride. Lidocaine Hydrochloride is a fast-acting anaesthetic ensuring an almost immediate numbing effect, while Bupivacaine Hydrochloride is a long-lasting anaesthetic ensuring the numbness lasted for the length of the Day 1 sessions. The Sham injection consisted of 3ml of 0.9% Sodium Chloride. The injections were administered on both big toes by medically trained professionals. A 25–gauge sterile needle was inserted into the base of the dorsolateral aspect of the big toe bilaterally and the solution was injected as the needle was withdrawn, achieving a sensory deafferentation of the entire toe. This procedure blocks sensory inputs from the big toe, whereas the main muscles for operating the Thumb (abductor/adductor hallucis and extensor/flexor hallucis brevis) are in the foot/ankle and remain largely unaffected. In the absence of an acceptable numbing effect, indicated by a score of ~50% in the tactile acuity check (see section: Sampling Validation Tests), the medical professional made a clinical decision on whether to administer further injections. To create a sham effect, participants in both groups were informed that they were receiving local anaesthetic, but that the effects were variable, and they therefore may not subjectively perceive a complete anaesthetic effect.

4.2.4 Experimental Design

The study was conducted over the course of 2 days, using the same design across both groups. An overview of the experimental time course is shown in Figure 4.1C. Motor and tactile validation tests on the big toes (black line in Figure 4.1C) were performed at several different timepoints (see Figure 4.1D for outcomes). Prior to the pharmacological intervention, force and tactile tests were performed to establish baseline performance, followed by the anaesthetic/sham injections. A post-injection tactile acuity test was performed by one experimenter. A second experimenter, in charge of administering the subsequent tests, was blinded to the group assignment. A further tactile acuity test was conducted at the end of Day 1. The same force and tactile acuity tests were administered at the beginning of Day 2.

Third Thumb training (red line in Figure 4.1C) was conducted on Day 1 over 3 consecutive sessions, each comprising of 3 different training tasks (Individuation, Coordination, Collaboration; see section: Training Tasks), with task order randomised across participants. Tasks were performed over 10-minute blocks.

Testing tasks (blue line in Figure 4.1C) were performed at the end of Day 1 (Dual Task) and on Day 2 (retention and transfer tests). Task order was fixed on Day 2. Participants completed one 10-minute block of the Coordination task, followed by one block of an adapted individuation task (Individuation Transfer), and one block of an adapted coordination task (Coordination Transfer). Finally, single blocks of the Collaboration and Individuation tasks were completed.

4.2.5 Sampling Validation Tests

4.2.5.1 Tactile acuity

Orientation discrimination tests (adapted from Tong et al., 2013) were conducted at four different timepoints (before the injections, immediately after the injections, at the end of Day 1, at the beginning of Day 2; Figure 4.1D) to assess baseline acuity and the effectiveness of the anaesthetic intervention. A Touch Test® two-point discriminator with a 13mm spacing was presented to the glabrous surface of the distal pad of each big toe for ~2-3 seconds in one of two randomly assigned orientations, with a total of 16 trials per test and inter-trial intervals of ~5 seconds. Participants verbally reported the perceived orientation using a two-alternative forced choice ('down' or 'across'). The outcome measure was the percentage of correct answers.

This data was used to confirm similar group performance during baseline and that the anaesthetic intervention was successful at attenuating somatosensory inputs throughout Day 1. Due to violations of the normality assumption, as well as the presence of several tied values and the lack of true continuity in the data, group differences in tactile acuity were analysed using percentile bootstrap independent-samples tests (see: Statistical Analyses). Before receiving the injections, both groups showed equal tactile acuity on the big toes (mean accuracy Anaesthetised = 93% ± 4.6%, Sham = 94.0% ± 9.6%; bootstrapped 95% CI [-4.12%, 4.47%], p=.935). On Day 2, the Sham and Anaesthetised groups performed similarly and close to ceiling (mean accuracy Anaesthetised = 94% ± 6.6%, Sham = 95.6% ± 5.7%; bootstrapped 95% CI [-5.40%,1.82%]; p=.370), suggesting that the numbing effects had effectively washed out by the following day.

Next, we confirmed that the injections of local anaesthetic affected tactile acuity relative to the sham injections. Following the injections, tactile perception in the Anaesthetised group dropped to chance level (mean accuracy = 49.3% ± 8.8%), whereas performance of the Sham group was relatively high (mean accuracy = 89.2% ± 13.3%), resulting in a significant group difference immediately after the injections (bootstrapped 95% CI [-46.05%, -33.21%], p<.001). A similar effect was also recorded at the end of Day 1 (mean accuracy Anaesthetised = 64.3% ± 17%, Sham = 96.3% ± 5.9.6%; bootstrapped 95% CI [-39.43%, -24.44%], p<.001).

4.2.5.2 *Force*

A force test was administered at the very beginning of each day and repeated on each big toe in order to assess baseline motor abilities and determine the pseudo-random group allocation of the participants. Participants pressed down on a force sensor, taped to their big toe, to control the vertical direction of a horizontally-moving dot displayed on the laptop screen. Their aim was to maintain the appropriate force to hit a series of bars, whose height was determined using 20%, 50% and 80% of the maximum force the participant could apply. The force test consisted of four trials. Each trial contained 6 bars displayed simultaneously on the screen (each bar 20%, 50% or 80% of the participant's maximum applied force). The bars of each trial were arranged in a way that either formed an up/down staircase (20% - 50% - 80% - 80% - 50% - 20%) or in a random order. Participants completed two identical up/down trials and two non-identical randomised trials. The outcome measure was the root mean square error from the ideal force required to maintain the moving dot at the top of each bar, averaged across all four trials. This data was used to confirm that baseline motor abilities were matched across groups and determine group allocation. The first 20 participants were randomly allocated to the Sham or Anaesthetised groups. The group means of the outcome measure were then calculated and participants thereafter assigned to groups based on their performance on the force test, to ensure that baseline motor abilities remained matched. Independent samples t-tests indicated that both Anaesthetised and Sham groups performed similarly in the force test at baseline on Day 1 (t(38)=0.36, p=.720) and on Day 2 (t(38)=0.22, p=.824).

4.2.6 Training Tasks

Training tasks were selected to probe the range of motor skills characterizing unimpaired Third Thumb motor control: using the Thumb in isolation from the hand for dexterous activities, coordinating hand and Thumb movements in a synchronous fashion, continuously collaborating with the hand for objects manipulation (Kieliba et al., 2021; Figure 4.1C). Retention of the trained tasks was additionally measured on Day 2.

4.2.6.1 Individuation

In the individuation task participants had to work on the fine motor control of the Third Thumb, while not relying on any support from the hand. Using only the Third Thumb, participants were required to pick up 6 tape rolls one-by-one and stack them on to a pole, while holding a foam ball to occupy their biological fingers (Figure 4.1C). Each block lasted for either 10 minutes or 10 trials, whichever came first. A trial lasted for however long it took the participant to stack all 6 rolls. If a given trial was still ongoing when 10 minutes had passed, the participant was allowed to complete it for up to an additional 5 minutes. The dependent variable was the time taken to stack all 6 tape rolls averaged across each block.

4.2.6.2 Coordination

To monitor the ability to coordinate the Thumb in synchrony with the hand, we used a finger opposition task (Meraz et al., 2018; Kieliba et al., 2021; Figure 4.1C). Participants were seated in front of a computer screen that displayed task stimuli. They were instructed to move the Thumb to touch the tip of a randomly specified finger of the augmented hand. A MATLAB script was used to randomly select a target finger (thumb, index, middle, ring or little) and to display the finger name on the computer screen. The experimenter manually advanced the program to the next stimulus when the participant successfully touched the tip of the target finger with the Third Thumb (hit), or when a wrong finger had been touched (miss). Participants were instructed to attempt to make as many successful hits as possible within a 1-minute trial, and completed a total of 10 trials per block. The outcome measure was the average number of hits completed in each block.

4.2.6.3 *Collaboration*

To measure continuous collaboration of the Third Thumb with the other fingers, participants were asked to build a 2x2 wooden bar tower with the augmented hand (Figure 4.1C). To do this they had to pick up 2 bars at a time from the table, using the Third Thumb in collaboration with a finger to hold or support one of the bars and two fingers to hold the other. Each block consisted of 10, 1-minute trials, with the dependent variable being the number of 2-bar floors built in 1 minute averaged across each block.

4.2.7 Testing tasks

The testing tasks were variations of the training tasks, designed to examine the automaticity and flexibility of motor learning under different task requirements.

4.2.7.1 *Dual task*

In order to evaluate if pressure feedback from the Third Thumb controllers is associated with a reduced need for cognitive control over performance (i.e. with increased automaticity), participants were asked to complete a dual (motor and numerical) task at the end of Day 1. The task was adapted from previous studies, showing that numerical cognition impacts motor performance while controlling a virtual prosthetic arm (Witteveen et al., 2012) or a brain-computer interface (Guthrie et al., 2019), but does not impact Third Thumb control (Kieliba et al., 2021). The task involved completing an extra 10-minute block of the Individuation task, with a simultaneous counting task. Participants were instructed to complete the Individuation task as described above, but they now had to also complete a simultaneous counting out loud task, as their primary task. At the beginning of each trial a random number would be presented, then a series of high (550 Hz) and low (250 Hz) pitch tones would be sounded at random intervals (between 2 and 6 seconds) in a randomised order. Participants were instructed to add 1 to the current number after hearing a high tone, and subtract 1 from the current number after hearing a low tone. After each mathematical operation, participants were instructed to verbally respond with the resulting number. The outcome measure was the time taken to complete the secondary Individuation task, under the interference effects of the primary numerical task.

4.2.7.2 Coordination Transfer task

This task was aimed at probing transfer of learning by modulating task demands from training. The transfer version of the Coordination task was conducted using the same procedures as described above, but with the force sensors controlling the Third Thumb swapped onto the opposite feet. This resulted in having to adapt to a new mapping between the controlling movements of the toes and the Third Thumb responses, i.e. pressing with the left toe for a flexion/extension movement and with the right toe for adduction/abduction.

4.2.7.3 Individuation Transfer task

This task aimed to get participants to transfer the fine motor techniques developed in the Individuation task to a distinct setting. The task was inspired by grooved pegboard tasks widely used to assess motor functioning and dexterity in clinical neuropsychology (Rabin et al., 2005). A brick of 6 3-D printed pegs was placed to the participants' left and a brick of 6 holes for the pegs to fit in was placed to their right. Participants were instructed to pick up each peg using only the Third Thumb and plant it in a designated hole, while holding a foam ball to occupy their biological fingers. They were instructed to move the pegs in a set order, starting from the right back peg, ending on the left front peg. The block was completed when all 6 pegs had been placed, or when 15 minutes had passed, whichever came first. Participants completed one block of this task. The outcome measure was the time taken to complete the block.

4.2.8 Toes motor control analysis

As a follow-up analysis, we investigated potential differences in how the two groups used the big toe pressure sensors to control the Third Thumb at the very first stages of motor learning. We focused on Block 1 of the Collaboration task, where no group differences on the main outcome measure were observed. The data corresponding to the first block of the Collaboration task was identified using the timestamps and manual logs and was then imported into MATLAB. The average servomotor position (linearly dependent on the force applied) for both the flexion/extension and the abduction/adduction sensors for the duration of the Collaboration task was extracted for each participant. This was then normalised by dividing the average value by the maximum servomotor angle (respectively, 175 and 80 degrees). The total time spent making a flexion/extension or abduction/adduction movement was then calculated by first identifying the timestamps at which participants applied force, moving the servomotor out of its baseline position of 10 degrees. This was then used to find the proportion of task time corresponding to each of the movements. Using the same approach, we also calculated the time spent making bilateral movements (moving both servomotors at the same time), as a proportion of the total movement time.

4.2.9 Statistical analyses

To identify violations of the normality assumption Shapiro-Wilk's values were inspected. Within- and between-participants comparisons in task performance across acquisition, retention and transfer of coordination and collaboration skills were assessed using mixed analysis of variance (ANOVA). Significant interactions were followed up with confirmatory comparisons using independent and paired samples t-tests, where appropriate. For repeated-measurement factors with more than two factor levels, if the assumption of sphericity was violated, Huynh–Feldt corrected p-values are reported. Due to violations of normality, data from the individuation tasks, the tactile acuity tests and the toes pressure sensors were analysed using independent-samples percentile bootstrap tests, which do not require any assumption on the underlying distribution of the data. For each variable of interest, we calculated the difference in group means on each iteration (10000) and estimated a 95% confidence interval and p-value of the difference in group means from the resulting distribution. Multivariate Mixed ANOVA was used to compare group differences in performance across the different tasks and blocks. All task scores from the first three blocks of training on Day 1 and the retention test on Day 2 were first normalised by subtracting the lowest score on the task during all four sessions by the range of task scores across the sessions, resulting in scores ranging from 0 to 1 across all tasks. The normalised scores for the Individuation tasks were subtracted from one so that a higher score reflected a better performance, as in the Coordination and Collaboration tasks. On all datasets, data points were removed separately for each task and/or block if they exceeded the outlier threshold of 2 standard deviations above or below the group mean. Less than 2% of data points across all tasks were removed for this reason. All parametric tests were performed on SPSS 25 (IBM, Chicago, Illinois), percentile bootstrap tests on RStudio 1.3 (RStudio PBC, Boston, Massachusetts).

4.3 Results

Faster acquisition of Third Thumb individuation skills

We first considered the role of pressure feedback while controlling the Third Thumb individually, i.e. independently from the rest of the hand. Here, without any complementary information from the biological fingers about interaction with objects, task success is dependent on the development of dexterous motor control with the robotic device alone. We found that under these conditions, the anaesthetic intervention impacted the early stages of motor learning. Due to violations of the assumption of normality, we used bootstrapping approaches. To obtain a measure of the improvement on the task for both groups through Day 1 training, we subtracted the score (time taken to stack all the rolls) of Block 3 from Block 1, akin to the interaction term of a mixed ANOVA (Figure 4.2A). A bootstrapped independent-samples test performed on the obtained difference score was significant (bootstrapped 95% CI [28.297, 144.501], p=.003). Additional bootstrapped tests revealed a significant group difference in performance at Block 1 [Mean Anaesthetised (N=20) = 262s, Mean Sham (N=21) = 175s; bootstrapped 95% CI [13.216, 158.814], p=.018], but only marginally at Block 3 [Mean Anaesthetised (N=22) = 78s, Mean Sham (N=21)

= 72s; bootstrapped 95% CI [-21.455, 29.160], p=.064]. Therefore, the Anaesthetised group showed initial deficits on performance when first approaching this dexterous task, but eventually appeared to reach a similar skill level to the Sham group by the end of the training day (see further results in the next section).

We next examined whether a similar level of retention of learning was obtained across both groups. The Bootstrap test on the subtracted scores of Day 2 from Block 3 of Day 1 revealed no significant group differences (bootstrapped 95% CI [-21.370, 144.501], p=.919). An additional Bootstrap test performed on the retention test scores confirmed that both groups retained their acquired skills similarly [Mean Anaesthetised (N=15) = 53s, Mean Sham (N=16) = 46s; bootstrapped 95% CI [-11.283, 24.983], p=.419]. We also tested for the transfer of the individuation skills, and found no significant group differences [Mean Anaesthetised (N=20) = 241s, Mean Sham (N=21) = 196s; bootstrapped 95% CI [-41.971, 135.916], p=.324], suggesting that the Anaesthetised group was able to transfer the individuation skills learned on Day 1 to similar tasks' settings as effectively as the Sham group. Together, these findings suggest that, once learned, the individuation skills were retained and transferred similarly by the two groups.

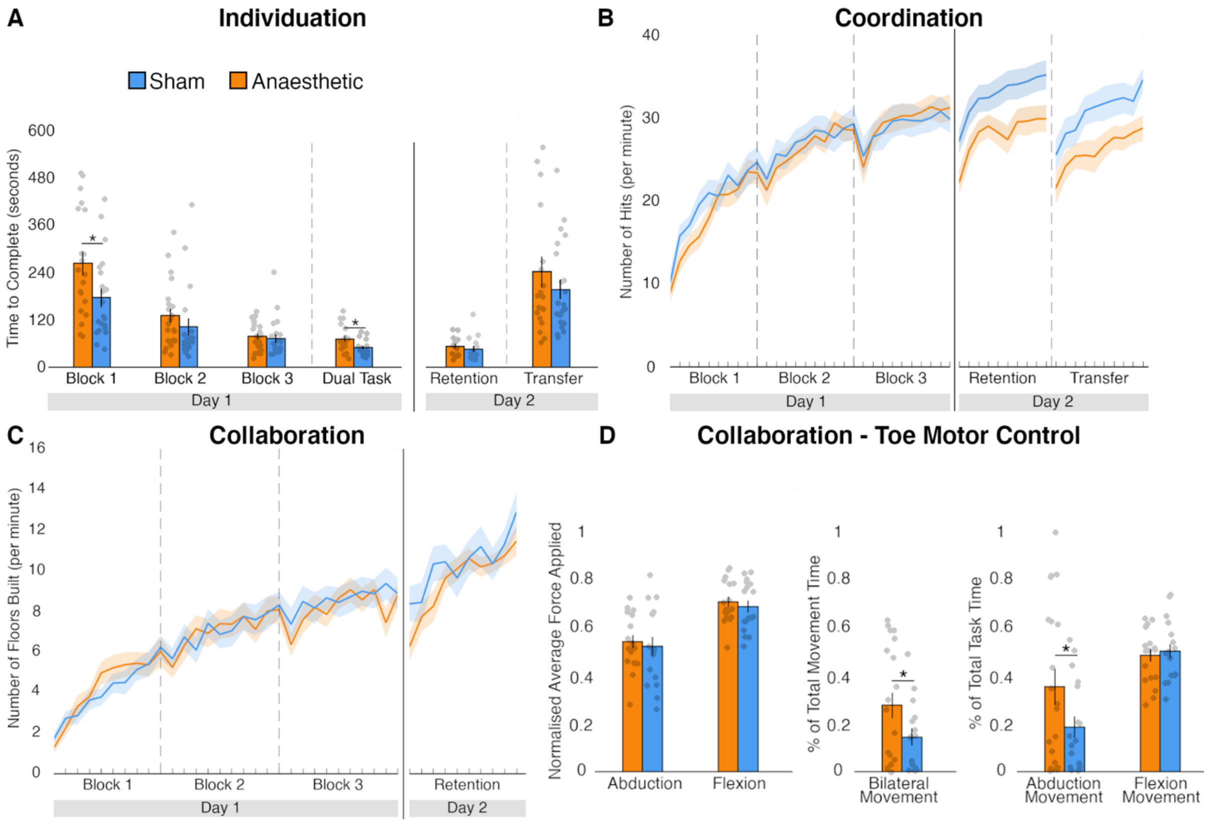

Figure 4.2 Task outcomes

A) In the Individuation task, the Anaesthetised group had significant deficits at the beginning of training and when a further cognitive load was added (Dual Task), but groups performed similarly in the retention and transfer tests of Day 2. **B)** In the Coordination task, despite similar Day 1 training performance, the Anaesthetised group showed significant deficits in the Day 2 retention and transfer tests. **C)** Both groups showed similar performance throughout the Collaboration task. **D)** Toe motor control analysis for Block 1 of the Collaboration task: no group differences in the amount of force applied; the Anaesthetised group used significantly more bilateral movements than the Sham group; the Anaesthetised group used significantly more adduction/abduction movements throughout the task, whereas there was no difference for flexion/extension movements. The bars depict group means; error bars represent standard error of the mean. Individual dots correspond to individual participants' scores. *Asterisks denote significant effects at * p<0.05.*

Lower cognitive demands during Third Thumb control

We also examined differences in skill learning by increasing cognitive task demands, using a simultaneous counting task (Figure 4.2A). Whilst there was no difference in the proportion of incorrect answers given by each group on the counting task (bootstrapped 95% CI [-0.16688, 0.23674], p=.783), we found that the increased cognitive load uncovered a deficit in motor performance induced by the anaesthetic intervention. A direct comparison of performance on the Dual Task was significant [Mean Anaesthetised (N=19) = 71s, Mean Sham (N=19) = 41s; bootstrapped 95% CI [5.579, 39.263], p=.008], indicating an increased interference effect of the primary task. In other words, with attenuated sensory feedback, motor performance may reach less automaticity and require higher-order resources. It is important to note that no group differences were observed when comparing the difference scores between the Dual Task and Block 3 (bootstrapped 95% CI [-7.473, 32.211], p=.277), indicating that this observed deterioration of performance in the Anaesthetised group might have already been present, though latent, regardless of the cognitive load task.

More effective retention and transfer of hand-Third Thumb coordination skills

A unique challenge for extra limbs control is the need to coordinate the movements of the robotic body part synchronously with our biological body. Overall, the anaesthetic intervention did not significantly affect the acquisition of hand-Thumb coordination skills throughout the training day (Figure 4.2B). To assess the acquisition of coordination skills across groups, we first ran a mixed ANOVA with Training Time (all training blocks of Day 1: Blocks 1, 2 and 3) and Group (Anaesthetised and Sham) as predictors of performance. Here, we found a statistically significant main effect of the Training Time, F(2,82)=186.242, p<.001, confirming improvements in performance over the training day, as expected. However, we did not observe a significant main effect of Group (F(1,41)=.231, p=.634), or interaction (F(2,82)=.934, p=.380), suggesting that, despite a qualitative (non-significant) disadvantage observable in the Anaesthetise group at Block 1 (Figure 4.2B), the two groups showed overall a similar performance during the first stages of motor learning, independently of the anaesthetic intervention.

We did find, however, that the anaesthetic intervention impacted the retention of the acquired coordination skills. For this purpose, we ran a mixed ANOVA with Day (the final training block of Day 1 and the testing block on Day 2) and Group (Anaesthetised and Sham) as the independent variables. The ANOVA revealed a significant interaction (F(1,42)=15.429, p<.001), and no significant main effects of Day (F(1,42)=2.269, p=.139), or Group (F(1,42)=1.210, p=.278). Confirmatory paired samples t-tests indicated that the Sham group improved from Day 1 to Day 2 (t(21)=4.090, p=.001), whereas the Anaesthetised group did not (t(21)=1.620, p=.120). This resulted in a significant group difference on Day 2 (t(42)=2.285, p=.027), which indicates that the Sham group retained the hand-robot coordination skills more effectively than the Anaesthetised group over the ~24 hours interval. Furthermore, the anaesthetic intervention impaired the transfer of coordination skills, resulting in a worse performance in the Anaesthetised group on the Transfer Coordination task relative to the Sham group (t(41)=-2.10, p=.042). Collectively, these findings indicate that, despite similar acquisition, pressure feedback from the toes may afford a stronger and more flexible learning to coordinate movements between the Third Thumb and the fingers of the hand. However, given that retention and transfer on Day 2 were assessed without anaesthetics, it is possible that these findings may be influenced by the changed context relative to training. Note, though, that, as seen below, similar retention and transfer were observed in the Individuation and Collaboration task even while the context in Day 2 (no anaesthetics) was varied relative to training, making this interpretation less likely.

No benefits to acquisition and learning of hand-Third Thumb collaboration skills

Finally, to complete the picture of hand-robot motor interactions, we examined the impact of the anaesthetic intervention on the ability to use the Third Thumb in close collaboration with the biological fingers to grip, lift and transport objects, while maintaining constant pressure. This task contains elements of coordination, but in addition also allows for increased reliance on sensorimotor control of the biological fingers, that maintain constant pressure on the object to afford the joint grip with the Thumb. Under such cooperating conditions, we did not find any significant impact of the anaesthetic intervention on task performance and learning (Figure 4.2C).

To assess the acquisition of collaboration skills, we first ran a mixed ANOVA with Training Time (all training blocks of Day 1: Blocks 1, 2 and 3) and Group (Anaesthetised and Sham groups) as predictors of performance. Here, we found a significant main effect of Training Time (F(2,78)=114.749, p<.001), but no significant main effect for Group (F(1,39)=.044, p=.834), or an interaction (F(2,78)=.116, p=.884), suggesting the groups did not improve differently across the training day. To test for group differences in retention, we ran a mixed ANOVA with Day (the final training block of Day 1 and the testing block on Day 2) and Group (Anaesthetised and Sham) as the independent variables. We found a significant main effect of Day (F(1,29) = 6.717, p=.015), but no significant effects for Group (F(1,29)=.003, p=.960) or for the interaction (F(1,29)=.320, p=.576). Overall, these results suggest that the skills needed to perform the Collaboration task were acquired and retained similarly by the two groups.

Different somatosensory demands across tasks and learning stages

The results presented so far suggest that pressure feedback was not equally important across the three tasks and stages of motor learning. This was statistically assessed by running a Multivariate Mixed ANOVA on normalised performance scores, which revealed a significant three-way interaction between the factors Task (Individuation, Coordination, Collaboration), Time (the three training blocks of Day 1 and the testing block of Day 2) and Group (Anaesthetised and Sham): $F(6,16) = 2.806$, $p = .046$, Pillai's Trace = .513. This result further confirms that various sources of somatosensory feedback might differentially support the device's proprioceptive status, resulting in different learning profiles.

Different toe motor control despite similar task performance during hand-Third Thumb Collaboration

It is possible that while overall performance appears similar between the two groups, the underlying strategies for achieving the same skill level are divergent. We therefore investigated differences in how the two groups used the pressure sensors to control the Third Thumb at the very first stages of motor learning. We focused on performance during Block 1 of the Collaboration task, where, as seen in Figure 4.2C, no performance differences were apparent. We found that the motor action patterns shown by the Anaesthetised group to achieve the same scores as the Sham group were less lateralised (Figure 4.2D).

Due to violations of the assumptions of normality, group differences were analysed with bootstrap tests. Both groups did not differ in the amount of average force applied during flexion/extension and abduction/adduction movements (Flexion: Mean Sham (N=17) = 0.691 ± 0.10, Mean Anaesthetised = 0.710 ± 0.09, bootstrapped

95% CI: [-0.045, 0.081], p = .555; Abduction: Mean Sham (N=17) = 0.525 ± 0.16, Mean Anaesthetised (N=17) = 0.544 ± 0.12, bootstrapped 95% CI: [-0.074, 0.111], p = .685). However, the Anaesthetised group spent a significantly longer proportion of time making bilateral movements (movements including the simultaneous flexion and abduction of the Third Thumb) – Mean Sham (N=16) = 14.5% ± 14.3%, Mean Anaesthetised (N=20) = 27.8% ± 24%, bootstrapped 95% CI: [0.011, 0.258], p=.033. Further analyses show that this can be attributed to the Anaesthetised group spending a larger proportion of the task time making adduction movements (Mean (N=20) = 35.5% ± 33.9%), when compared to the Sham group (Mean (N=17) = 18.7% ± 18.6%, bootstrapped 95% CI: [0.006, 0.342], p=.043). The proportion of the task time spent making flexion movements did not differ between the Anaesthetised (Mean (N=19) = 48.6% ± 11.1%; and Sham (Mean (N=17) = 50.4% ± 12.6%) groups - bootstrapped 95% CI: [-0.096, 0.055], p=.636). These findings indicate that even when a task can be equally performed with attenuated sensory feedback from the Thumb controllers, this is achieved through arguably more complex and energy-consuming control patterns.

4.4 Discussion

Learning to act efficiently in the world depends on complex interactions between the motor and somatosensory systems, forming a closed control loop for the sensory consequences of motor commands. When controlling augmentative wearable technologies this closed loop is impaired, which has prompted the need to develop external interfaces for the delivery artificial somatosensory feedback.

Here, we tested if pressure feedback readily available from the body part proportionally controlling an extra robotic finger (Third Thumb) can support device control and skill learning, presumably by acting as a proxy for inferring the device state and position (device proprioceptive status). Using local anaesthesia in a placebo-controlled design, we show that attenuating intrinsic somatosensory inputs from the robotic digit's controllers results in impoverished motor control and learning. Deficits in motor control were found both during skill acquisition (for the Individuation task), and at the delayed retention and transfer tests (for the Coordination task). Importantly, our findings also demonstrate that despite these deficits, participants were able to learn to control the Thumb even when these intrinsic pressure signals were not available. Most strikingly, performance was not impaired by anaesthesia when tasks involved close collaboration with the biological fingers, indicating that the brain could 'close the gap' of the missing state and position cues by complementary means, including through continuous task-relevant somatosensory feedback from other body parts involved in the task. Nevertheless, the impairments in multitasking and the somewhat less economical control patterns underlying the performance of the Anaesthetised group highlight the unique advantages that the displaced and intrinsic pressure feedback from the device controllers can provide for more automatic and efficient control. Together, our findings indicate that there are multiple available avenues – involving somatosensory signals from both distal controllers and collaborating body-parts – that could be harnessed to increase the bidirectionality of extra limbs' control.

Initial deficits in skill acquisition were seen in the Individuation task (Figure 4.2A), which required dexterous control of the Third Thumb in isolation from the biological fingers, highlighting the benefits of supplementary sensory feedback when first approaching a task where extra precision is needed (Blank et al., 2008). Such early deficits, however, seemed to resolve by the end of the training sessions. Moreover, this effect was not observed in the Collaboration task and did not reach statistical significance for the Coordination task, which both required using the Thumb in conjunction with the other fingers, likely allowing for an increased reliance on sensorimotor control of the biological hand for task success (Zhu et al., 2019). These findings suggest that, during the initial stages of motor learning, other sensory cues can probably sufficiently compensate for the lack of pressure feedback from the controllers of the device. Such complementary cues include visual and auditory inputs from the Thumb, but also, perhaps most notably, somatosensory inputs from the collaborating hand.

Nevertheless, such increased reliance on complementary feedback modalities appears to result in more pronounced cognitive demands for motor control, at least during the early stages of learning. This is well evidenced by the impairments shown by the Anaesthetised group on a Dual Task (Figure 4.2A), requiring participants to multi-task by simultaneously carrying out the Individuation task and numerical operations (Witteveen et al., 2012; Guthrie et al., 2019). When fewer executive resources were available due to the need to prioritise the numerical task, the poorer motor performance in the Anaesthetised group appeared to resurface. This finding suggests that automaticity in device control can more readily be achieved when users can rely on task-intrinsic pressure feedback. A related finding on the distinct control patterns characterising the performance of the two groups was revealed in the Collaboration task (Figure 4.2D). Here, we uncovered a potentially redundant, and thus less efficient, use of the pressure sensors to operate the Third Thumb by the Anaesthetised users in the early stages of learning. Specifically, to achieve equal task performance as the Sham group, Anaesthetised participants used more complex and energy-consuming bilateral movements. These findings show that even when the advantages afforded by the controllers' pressure feedback are not reflected in task performance, they provide opportunities for optimisation that are not as readily available to users relying only on other complementary sensory cues.

Further clues for the potential advantages of surrogate state and position cues for motor learning were revealed during retention and transfer. Performance on the retention tests, which involved the repetition of the practiced tasks on the following day, reflects the relative strength of the motor memory representation over time, whereas performance on the transfer tests reflects its flexibility. Here, the impact of practice with reduced pressure feedback was evident for the Coordination task (Figure 4.2B), where the Sham group continued to show improvements whereas the Anaesthetised group did not, even if toes sensitivity was by then restored for both groups. It is possible that the pressure feedback from the Thumb controllers during task training may have enabled a more accurate internal model of the motor plan, thereby resulting in a more effective error-based learning (Wolpert & Ghahramani, 1995), and, in turn, a more robust and flexible motor memory representation. However, no group differences in retention or transfer were observed in the Collaboration task (Figure 4.2C), and, perhaps most surprisingly, in the Individuation task (Figure 4.2A), where users could not rely on complementary information from the biological fingers. Given the importance of somatosensory feedback for motor learning, as highlighted in previous literature (Ostry & Gribble, 2016), and the noticeable initial deficit in skill acquisition we documented here, this finding calls for a more detailed understanding of the circumstances (e.g., task demands or learning stages) where users can in fact benefit from intrinsic somatosensory information about the proprioceptive state of the device.

It is important to consider that both groups were receiving normal pressure feedback during the retention and transfer tests of Day 2. Therefore, it is possible that although participants learnt effectively to perform the Coordination task without pressure feedback, the incoming somatosensory inputs on Day 2 may have required them to re-learn how to integrate this information into their internal model. It was impossible for us to empirically test the theory that under anaesthesia retention would have been more complete, due to unsafe toxicity build-up that could result from consecutive-day anaesthetic blocks. Nevertheless, considering that performance during Day 2 did not vary across groups for the other tasks, we believe this interpretation is less likely.

By showing that users can, to a certain extent, intrinsically benefit from somatosensory feedback through the current wearable designs, these results are expected to inform on the utility of adding complex artificial feedback interfaces to motor augmentative technologies. But our findings also bear important implications for the development of assistive technologies. Considerable resources are in fact being invested to provide bidirectional sensorimotor control of artificial limbs (Bensmaia et al., 2020). Most of the progress in this work has involved providing information about contact between the device and objects (i.e., touch), either by providing tactile stimulation on a displaced skin surface (Antfolk et al., 2013) or by directly activating the neural pathways originally supporting the sensory function, e.g. the peripheral nerves (Dhillon and Horch, 2005; Raspopovic et al., 2014; Tan et al., 2014), the spinal cord (Chandrasekaran et al., 2020), or the somatosensory cortex (Romo et al., 1998; Tabot et al., 2013; Flesher et al., 2016; Salas et al., 2018; Flesher et al., 2021). Although the development of such tactile afferent interfaces is at a much earlier stage than their efferent counterparts and critical challenges remain (e.g., cognitive training efforts and the quality of the evoked percepts), the benefits of artificial touch while using bionic limbs are being documented in both lab and home settings (Ortiz-Catalan et al., 2020; Graczyk et al., 2018; Flesher et al., 2021). Conversely, very little progress has been made in reproducing proprioception to provide kinaesthetic feedback, owing to the complexity of the distributed and multilabelled nature of proprioceptive signals (Tomlinson & Miller, 2016). Indeed, attempts to restore (London et al., 2008; Suminsky et al., 2010; Dadarlat et al., 2015) or substitute (Blank et al., 2008; Schiefer et al., 2018; D’anna et al., 2019) proprioceptive feedback in artificial limbs haven’t so far been able to show consistent benefits for motor control.

The challenges currently posed by reproducing artificial forms of somatosensory, particularly proprioceptive, feedback prompt us to (re)consider those simplified approaches that can potentially take advantage of intrinsic bodily signals. For example, in the case of traditional myoelectric or body-powered prostheses, when motor commands are sent out by the bodily controller (residual arm and shoulder, respectively), amputees receive a range of somatosensory inputs (Markovic et al., 2018). These are not only sensations on the patch of skin interfacing the prosthetic actuator that can inform about contact with objects but could also include implicit cues about the device state and position, similar to those investigated here. It has indeed been speculated that such intrinsic, although limited, forms of sensory feedback may contribute to amputees' general preference for body-powered prostheses over more sophisticated ones (e.g. myoelectric), due to the increased sensory information that is received on their body when operating the device (e.g. from extending the cable/harness with the shoulder) (Antfolk et al., 2013). Another example relates to patients with spinal cord injuries. While the body itself might only mediate minimal sensory input, intrinsic skeletal vibrations arising from a restorative device (e.g. exoskeleton) might still afford some level of sensory input that could be useful for guiding motor control, even if the patient might not be aware of these implicit inputs. The benefits of such physiologically built-in feedback systems, which may circumvent the need for complex substitutionary or restorative interfaces, have long been highlighted in clinical and rehabilitative settings (Mann & Reimers, 1970; Hirsch & Klasson, 1974; Simpson, 1974; Prior & Lyman, 1975), yet seldom considered when designing novel sensory feedback technologies.

Our findings with the body-controlled Third Thumb suggest that body-powered and myoelectric interface may already provide closed-loop control with good performance. Therefore, artificial feedback needs to be more thoughtfully designed to outperform this baseline to become truly effective. Moreover, these results emphasize the need to carefully examine and weigh the disadvantages that more ground-breaking technologies for movement restoration may pose for opportunities of harnessing intrinsic feedback, i.e. most neuroprosthetics (brain-machine interfaces - BMIs), that completely bypass the body and any related tactile and proprioceptive processing (see section 5.2.3 for a more extended discussion on current BMIs). We hope that future research on bidirectional (neuro)prosthetics will benefit from considering the advantages of keeping “close to the body” when trying to interface with the sensorimotor system, for either restorative, substitutive, or augmentative purposes.

5 │ DISCUSSION

5.1 Overview of thesis studies

This thesis comprises of a set of experiments designed to provide a better understanding of the neurocognitive and physiological adaptations accompanying extreme alterations of typical sensorimotor experience. In particular, I have examined the scope for sensorimotor systems to functionally reassign neural resources in response to hand loss and augmentation. To this end, I have employed a multimodal approach involving the use of brain stimulation, neuroimaging, behavioural and pharmacological techniques.

In Chapter 2, I offer a new interpretation of classical findings implying that the sensorimotor cortex can functionally reorganise following the loss of a hand in adulthood. I demonstrate that the referral of facial touches on the phantom limb, repeatedly acclaimed as the direct perceptual consequence of putative face-to-hand remapping in S1, is likely to be consequential to poorly controlled experimental conditions, rather than cortical reorganisation. While large-scale sensorimotor reorganisation might thus be more limited in adults than originally considered, in Chapter 3 I show that early life development may offer a more favourable environment for functional reorganisation. Here, I show in fact that the motor cortex missing hand area can be recruited in motor control of the mouth in individuals born without a hand, providing crucial causal evidence for functionally meaningful reorganisation in cases of congenital deprivation. The fact that, under certain circumstances, sensorimotor resources can be repurposed to support new behavioural needs opens the door to several fascinating technological developments, such as brain-machine interfaces for artificial limb control and robotic hand augmentation. In chapter 4, I illustrate how existent sensorimotor pathways, such as the functional interconnectedness between the hand and the toes, can be harnessed to support the control extra robotic digits.

5.2 Implications & future directions

The experimental results and theoretical frameworks described in this thesis can inform basic neuroscience and deepen our knowledge of the principles underlying the plasticity of sensorimotor representations. Moreover, although the studies presented here were not designed to directly inform clinical practice, their results can offer insights that might be useful in developing future therapeutic approaches and rehabilitation protocols. Finally, with technological progress, we must think of all the tremendous opportunities that the novel efforts to restore movement through neuroprosthetics might offer to patients affected by amputations or paralysis. Bridging these neuroengineering advances with a deeper understanding of sensorimotor plasticity could help overcome pressing stalls in the next generations of bionic limbs. In what follows I will discuss the relevance of my work to the three perspectives of 1) Brain plasticity, 2) Clinical rehabilitation, and 3) Neurotechnologies.

5.2.1 Theories of brain plasticity

Seminal work on sensorimotor plasticity established the notion that the adult cortex is extremely plastic. For example, following deafferentation of a finger (Merzenich et al., 1983, 1984) or an arm (Pons et al., 1991), the deprived primary somatosensory cortex (SI) of monkeys becomes activated by inputs of the cortically neighbouring fingers or face, respectively, resulting in shifted body maps boundaries (see section 1.3.1). It has long been assumed that such cortical remapping is functionally relevant, having direct consequences on perception and action, for example in the context of tactile remapping, through referred sensations from the face to the phantom limb (Ramachandran et al., 1992). The evidence I presented in Chapter 2 contributes to dispute this classical view, arguing that deprivation-triggered remapping, as identified in amputees, may lack behavioural relevance. How can we relate the classical notion of functionally relevant remapping with the findings in Chapter 2 and the newly accumulating evidence suggesting that primary sensorimotor areas maintain a persistent representation of the missing hand, rather than reorganising their read-out properties?

Increasing evidence has revealed that body-part information may be more distributed along the homunculus than classically thought, in both M1 (Graziano, 2006; Zaharia, Hertz, Flash et al, 2012; Mayer, Baldwin, Cooke et al., 2019; Huber, Finn, Handwerker et al., 2020; Willett, Deo, Avansino et al., 2020) and S1 (Tal, Geva, Amedi 2017; Muret, Root, Kieliba et al., 2022). This implies that, even in physiological conditions, some local activity may be latent relative to a more dominant activity elicited by the primary body-part (e.g., mouth and feet information in the hand motor area; Willett et al., 2020). Considering such distributed latent activity, the structure of the maps is going to be affected in the first place by methodological choices. For example, both electrophysiological (Merzenich et al., 1984; Pons et al., 1991) and neuroimaging studies (e.g., Flor, Elbert, Knecht,1995) usually engage a limited set of body-parts to define remapping and assign a neuron/cortical unit to the most responsive body-part among the ones tested (*winner-takes-all* manner). In this way, other (and potentially even more dominant) inputs may be ignored (e.g., phantom hand representation), in favour of a bias for selecting for further testing those body-parts that were previously shown to be implicated in remapping (e.g., lower vs upper face; Root et al., 2021). Indeed, it was recently elegantly demonstrated that an apparent remapping obtained by leaving out a finger from a finger map analysis is very similar to the remapping observed following a nerve block of that finger (Wesselink et al., 2022), which may call for a reinterpretation of the classical electrophysiological findings obtained in monkeys (Merzenich et al., 1983, 1984). In this framework of distributed body-part information and somewhat biased methodological choices, deprivation may therefore simply uncover pre-existing latent activity, which can manifest as remapping, without entailing reorganised functional representations for other body-parts, in lack of causal evidence or solid associations with functional effects. It has been recently suggested that the remapping observed in amputees may be potentiated by the intervention of homeostatic mechanisms, that would come into play to increase the normal levels of latent activity, in order to maintain network stability after the dramatic loss of inputs to the cortex (Muret & Makin, 2020). As such, and in line with the evidence presented in Chapter 2, uncovering this increased latent activity in amputees would not imply that the brain has modified the way it reads out this activity from the deprived area. In other words, this process would not necessarily change the representational content of the remapped activity, which would be consistent with a lack of evidence for behaviourally relevant deprivation-triggered remapping (see also Haak et al. 2015 for analogous considerations in the visual domain).

Importantly, the findings presented in Chapter 3 suggest that such principles may be different for a developing brain, allowing for functional reorganisation in cases of early life deprivation. Using TMS, we provided in fact a rare demonstration of a causal relation between local processing in the missing hand motor area and reorganised motor outputs in congenital one-handers. This functional reorganisation of sensorimotor networks could result from the fact that brain organisation is particularly sensitive to experience during critical developmental windows, when white matter tracts are still underdetermined, as suggested in the visual domain (Hubel & Wiesel, 1965b; Levelt & Hubener, 2012). Still, a question remains of what drives the reorganisation of certain body-parts in this special population. While past research in people born without both hands has shown that reorganisation in such instances may not be restricted to cortical neighbours (Stoeckel et al., 2009; Nakagawa et al., 2020), we weren't able to replicate this observation in our sample of one-handers, where we only observed MEPs from missing-hand stimulation on the mouth area and residual arm, but not on more cortically distant body-parts such as the legs. This could be due to a variety of reasons, including our small sample size or more idiosyncratic reasons related to the specific patterns of compensatory behaviour adopted by our one-handed participants.

One attractive hypothesis is in fact that, if not exclusively driven by somatotopy, one potential driver for reorganisation in one- or no-handers could be adaptive behaviour, i.e., the overuse of alternative body-parts to substitute for the original function of the missing hand (Hahamy et al., 2015, 2017; see also section 1.4.2). Specifically, it has been proposed that remapping may be facilitated or even constrained by the functional role of the deprived brain area, such that only inputs by body parts that engage in the same functional role of the missing limb would be consolidated, through Hebbian-like plasticity mechanisms. In the emerging framework of a sensorimotor homuncular organisation shaped by functionality rather than pure somatotopy (e.g., Graziano, 2016), the cortical territory of a certain body-part (e.g., the hand area) would be in fact responsible for facilitating the functional role of that body-part (e.g., grasping, feeding) rather than just representing the body-part per se. Since some of these typical hand functions could also be performed through other means (e.g., feet or mouth), this framework suggests that the hand area would already have the capacity to support these other effectors. Under the extreme circumstances of congenital hand absence, this normally more latent activity from effectors sharing some of the functionality of the missing hand would boosted to ensure functional stability at the network-level and possibly reinforced through daily adaptive behaviour, ultimately revealing itself as functional reorganisation, as evidenced in Chapter 3. Importantly, early-life experience seems crucial for such functional reorganisation to take place. Indeed, while a similar process of adaptive reorganisation was initially suggested also for the increased activity observed in the missing hand sensorimotor area for the intact hand in amputees (for whom this effector, rather than the mouth or feet, is the primary means of compensation) (Makin et al. 2013), later research revised this hypothesis, by showing with RSA very little information content for this remapped body-part in the missing hand area of amputees (Wesselink et al., 2019; see also section 1.3.2).

It is important to note that the theory of function-dependent remapping driven by compensatory mechanisms has been contested (Yu et al., 2014; Striem-Amit, Vannuscorps, Caramazza, 2018) and awaits more direct empirical evidence. Crucial demonstration of the role of behaviour in driving brain plasticity (and/or vice versa) would imply not only the use of methods that allow to test for causality (such as brain stimulation), but also better behavioural measures of adaptive behaviour. Many standardized tests in the lab, such as video-recordings of participants carrying out a limited set of instructed tasks (e.g., Hahamy et al., 2017), cannot capture everyday behaviour in ecologically meaningful ways. Better tracking equipment, for example based on automatic optical motion capture (Aristidou & Lasenby, 2013) and markerless pose estimation (Mathis, Mamidanna, Cury et al., 2018) or wearable biometric sensing (Blasco, Chen, Tapiador et al., 2016), may allow for more naturalistic, freely moving experiments. The sheer amount of data provided by these novel tools, along with advances in machine learning, could facilitate insights into how idiosyncratic behavioural repertoires map onto cortical remapping patterns. Possibly even greater insights could be gathered by looking at how such measures unfold across development, through longitudinal studies in children. Further, experiments involving participants who underwent amputations at very young ages could help specify the developmental time window in which organisation may be guided by behaviour, if not from birth. Obvious difficulties in recruitment make these experiments particularly challenging to run in reality. Nevertheless, causal methods and multivariate neuroimaging measures of body representation (e.g., RSA), possibly coupled with detailed behavioural tracking, can still yield informative results with a small number of participants (e.g., Dempsey-Jones et al., 2019). As such, in time, the small number of young amputees participating in a study may span the entire range, from infantile to adolescent, giving unprecedented insights on the temporal constraints of sensorimotor plasticity.

Similar considerations also apply to research trying to shed light on the relation between behaviour and neural representations in late life amputees. Here, with sophisticated high-density EMG equipment becoming more widely available (Farina, Merletti & Enoka, 2014), it will be possible to track the movement of the phantom limb in more detail. This will allow, for example, to investigate how motor control over the phantom can relate to a more or less degraded representation of the missing limb and/or remapping of other body parts in the sensorimotor cortex, as well as phantom limb pain (Kikkert et al., 2017, 2019).

The importance of embracing a multimodal approach when studying brain plasticity is reinforced by the findings presented in this thesis. Neuroimaging alone, especially when relying only on univariate analyses, does not appear suited by itself to answer all the important questions about (sensorimotor) plasticity, particularly when it comes to considering its potential functional implications. To establish that abnormal activity in the deprived cortex constitutes a new sensorimotor representation of the displaced input requires causal evidence. When working at inferior levels of inference (e.g, correlations), it is then imperative to first rule out alternative explanations before functionally attributing phenomena to brain (reorganisation) processes. Based on our findings in Chapter 2, we recommend that researchers account at the very least for the insidious effects of psychological biases when designing experiments aimed at probing the behavioural phenotypes of brain plasticity.

The model of augmentation can also offer other valuable insights on the ability of our sensorimotor system to plastically adapt to novel demands, in this case the integration, rather than the loss, of an extra (robotic) body-part. As illustrated in Chapter 4 and by previous research (see sections 1.5.1 and 4.1), repurposing another body-part for controlling the extra limb provides a simple and intuitive means for developing successful coordination with the device. Moreover, as detailed by our study, the proportional pressure afforded by the bodily controller can feed rich information to the sensorimotor loop by providing intrinsic somatosensory cues. Given that in our model (Third Thumb) the bodily controller (i.e., the big toes) is a body part physically distant from the effector (i.e., the extra thumb worn on the hand), our findings suggest that a common infrastructure between even distal body parts can be somehow shaped for sensorimotor control of an extra body-part. However, there is currently no reliable account of how such adaptation in the sensorimotor network takes place, and this is most likely due to technical issues concerning robotic technology in the MRI scanner environment. The evidence reviewed above in cases of hand loss suggests that neural body representation may not be malleable enough to integrate the extra body-part along with biological limbs. Future research with MRI-safe devices should seek to clarify if the brain is able to integrate the contribution of the “hijacked” body-part’s (e.g., toes) motor command into the biological hand representation or if instead it coordinates the toe and hand movements independently. Modern neuroimaging brain encoding methods (e.g, Pattern Component Modelling, PCM; Diedrichsen & Kriegeskorte, 2017) would be particularly suited for clarifying if the representation of coordinated hand-device movements is indeed distinct from that of the hand and toes separately.

Exploiting the unique similarities and functional interconnectedness between the fingers and the toes (Manser-Smith et al., 2019) for extra-limb control has undoubtedly proven successful in lab testing scenarios. However, a considerable disadvantage of toe-controlled extra digits is that they cannot be used easily during locomotion, limiting their general utility in real-life settings. To circumvent all such issues related with repurposing another body-part, the field is trying to find ways to achieve dedicated and independent motor control of the extra body-part by exploiting the redundancies of our motor system (Dominijanni et al, 2021). Such solutions are likely to prove very challenging, due to the extensive training needed to generate isolated muscle activity patterns to control the extra body-part, without triggering a motor response in the hosting body part (Formento, Botros, Carmena, 2021). Ultimately, from a brain plasticity perspective, a successful integration of extra robotic body-parts will require concerted efforts and close cooperation between neuroscientists and engineers, to create and evaluate safe, intuitive, and effective motor augmentation devices.

5.2.2 Clinical rehabilitation

The findings presented in Chapter 2 and 3 can bear potential implications for clinical research on the neuropathic pain affecting acquired amputees and derived therapeutics. Classical research on sensorimotor remapping after amputation has promoted interventions for phantom limb pain that target the presumed maladaptive brain remapping of the face onto the missing hand territory (see section 1.3.2), with mixed results (Thieme et al., 2016, Colmenero et al., 2018; Aternali & Katz, 2019). As highlighted above, however, the traditional focus on sensorimotor remapping due to changed selectivity to certain body-parts is likely incomplete, considering that net activity changes might not adequately reveal the underlying functional processing. The results presented in Chapter 2 strengthen this cautionary view, by warning against the overinterpretation of self-reported facial referred sensations as evidence for functionally meaningful remapping. Additionally, we did not find evidence of any correlation between such putative perceptual remapping and phantom limb pain. At the same time, using the model of congenital limb absence, in Chapter 3 I demonstrated that even when face-to-hand reorganisation does seem to effectively occur, this is not sufficient to cause phantom limb pain. Collectively, therefore, these findings and the research presented in the preceding sections highlight the need to consider potential contributions of additional brain mechanisms, beyond sensorimotor remapping, for understanding and alleviating phantom limb pain.

Beyond the debilitating effects of neuropathic pain affecting amputees, loss of hand functionality has other devastating consequences on the quality of life of an individual. For example, 69% of people who lose their hand due to amputation, become unemployed or have to change their profession (Jang et al., 2011). A key challenge in restoring hand function is that it is exceedingly complex, involving many degrees of freedom. Currently, efforts for restoring long-term loss of hand function (e.g., due to amputation or stroke) are through rehabilitation or via substitution technologies (prosthetic limbs and more recently pioneering efforts with exoskeletons). The research presented in Chapter 4 and elsewhere (see section 1.5.1) on the relatively easy sensorimotor integration of extra robotic fingers invites to reflect on some of the potential advantages that employing augmentative technologies for assistive purposes may offer. In certain cases, in fact, it may be more efficient to focus on enhancing the motor abilities of an existing intact hand, rather than attempting to substitute or restore the functionality of a missing or impaired hand. By focusing on augmenting the motor abilities of the unimpaired hand, wearable extra robotic fingers such as the Third Thumb may provide a solution for those who cannot use a traditional prosthesis (e.g., due to high level of amputation) and to other clinical populations that are unable to wear a prosthesis at all (e.g., stroke patients). This approach of attempting to augment the intact hand may benefit not only people affected by long-term or permanent disability, but also a large range of patients suffering from transient loss of hand function, such as those sustaining temporary hand and/or arm immobilisation due to orthopaedic injuries. Indeed, unlike lower-limb assistive options, such as wheelchairs and crutches, there are currently no assistive technologies for temporary upper-limb immobilisation. Given that during the acute phase of an injury mobilising the injured hand may be painful and impractical, augmenting the unimpaired hand may offer a readily available means to enhance functionality and alleviate the temporary disability.

The idea of increasing the daily functionality of disabled individuals through robotic augmentation could thus considerably innovate the field of motor rehabilitation, for the benefit of diverse patients' groups. The effective deployment of augmentative technologies across real-world (clinical) scenarios, however, will first inevitably require a thorough understanding of how the sensorimotor system can best interface with such devices.

5.2.3 Emerging neurotechnologies

The last three decades have seen dramatic advances in the development of interfaces that can substitute or restore human motor function. Such interfaces allow people with absent or severely impaired hand function to operate assistive technologies by decoding intended movements at different levels along the motor system. Current designs allow the extraction of motor commands not only from the user's muscle activations and movements (e.g., myoelectric prostheses), but also directly from neuronal signals (Brain Machine Interfaces - BMI) (Bensmaia & Miller, 2014). Typically, invasive BMIs allow movement restoration by translating the activity of pools of neurons recorded with implanted electrodes into motor commands, which are then directly fed into a table-mounted robotic artificial arm (or virtual cursors). Most BMIs decode movement intentions from M1 neurons, but occasionally also from the premotor (Shanechi et al., 2012) or parietal cortex (Aflalo et al., 2015; Guan et al., 2022; see Gallego, Makin & McDougle, 2022 for an extensive discussion of efferent interfaces options). These ground-breaking technologies offer to the many patients suffering the consequences of limb amputation and paralysis promising opportunities to regain independence. However, it is widely acknowledged that the performance of unidirectional (i.e., efferent only) BMI systems is limited by the inadequate sensory information available to users, whose movements are guided mainly using vision (Bensmaia, Tyler, Micera, 2020). As such, BMI-controlled movements still require considerable attention and do not typically achieve near-natural levels of dexterity and fluidity (Tomlinson & Miller, 2016).

The evidence reviewed above for preserved functional layout of sensorimotor cortical processing following amputation (or spinal cord injury, Kikkert et al., 2021), further supported by the findings presented in Chapter 2, upholds current efforts for creating bidirectional BMIs and restoring tactile feedback. The somatosensory cortex can in fact be electrically interfaced through chronically implanted electrode arrays (intracortical micro stimulation, ICMS) to produce percepts localized to a patch of skin (Tabot et al., 2013; Flesher et al., 2016; Flesher et al., 2021). The most straightforward strategy to restore (quasi)naturalistic sensations through ICMS is to mimic natural patterns of cortical activity and an intuitive way to attempt this is by respecting and exploiting the native topographies (Bensmaia, 2015). For example, the location of contact between the prosthesis and an object can be signalled by electrically stimulating the neurons that responded to that part of the hand before the injury. When the prosthetic fingertip touches the object, electrical current is delivered to an electrode that stimulates the corresponding fingertip representation in the somatosensory cortex, thereby evoking a sensation referred to that fingertip. However, if those topographies get completely remapped after injury (e.g by face invasion), as the classical theory of cortical reorganisation suggests, such “biomimetic” approaches would no longer be viable. It is therefore clear how, from the standpoint of BMIs and ICMS delivered feedback, a preservation of the native topographies after amputation or paralysis is advantageous.

Beyond artificial stimulation, though, there may be other even more “natural” approaches to provide surrogate somatosensory information to support motor control of a BMI-controlled prosthetic limb. Almost by definition and unlike traditional prostheses, BMIs are designed to completely bypass the body and any related tactile and proprioceptive processing. In Chapter 4, I have shown that leveraging task-intrinsic somatosensory inputs can substantially enhance motor performance and learning with wearable robotic limbs, even when such inputs arise from displaced locations. By reading motor commands from the cortex and bypassing the body, current BMIs may be missing important opportunities for harnessing such task-intrinsic somatosensory signals for optimising control. Further, engaging the body in some form of device control may not only optimise motor control through increased task-relevant sensory feedback, but also serve rehabilitation by providing physical activity, with the potential to prevent muscle atrophy and maintain any residual mobility (Pierella et al., 2015). Future developments could explore letting paralyzed patients with residual sensorimotor abilities try to use their overt movements concurrently with BMI controlled movements (Bashford et al., 2018). BMI systems may potentially benefit from such hybrid approaches even when neuronal recordings are coupled with very minimal forms of bodily engagement (Gurgone et al., 2022). BMIs that restore movement by reanimating the patient’s own paralyzed limbs through neuromuscular stimulation (Bouton et al., 2016, Ajiboye et al., 2017; Bockbrader et al., 2019; Ganzer et al., 2020) may already be benefiting from such opportunities.

5.3 Concluding remarks

Exploiting some of the key mechanisms of sensorimotor plasticity promises tremendous opportunities for clinical and technological breakthroughs. This vast research landscape appears however as narrowly framed by the influence of old interpretative models, inherited from the animal electrophysiology literature and early neuroimaging work in humans. This thesis has illustrated how the study of such a multifaceted phenomenon as the capacity for our brains to adapt can greatly benefit from the adoption of a wider conceptual and methodological framework. This includes not only the use of neuroscience methods that can go beyond the limitations posed by traditional imaging techniques, but also a commitment to asking (old) questions through innovative experimental models and in close dialogue with other disciplines. For example, here I have illustrated how results obtained in healthy samples can feed rich insights to research on disability, or how, similarly, studying augmentation through the lenses of deprivation (and vice versa) can prove mutually informative. As scientists and engineers progressively join forces to give body function back to those who have lost it, such multidisciplinary frameworks may soon be considered the prime approach to the study of sensorimotor plasticity. Eventually, neuroscience and technology are set to become increasingly intertwined, warranting new and potentially pivotal cross-fertilisation opportunities.

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APPENDIX

5.4 Extended Results Chapter 2

Extended fMRI results

Remapping in S1 missing-hand area

To gain further insights into the hypothesized link between referred sensations and S1 remapping, we assessed BOLD average activity levels evoked by movement of multiple body-parts in the missing/non-dominant hand area.

The task involved movement of one of five body-parts: intact/dominant hand (open/closing the fingers), residual/non-dominant arm (flexing the most distal residual joint, the elbow for Two-handers), right or left toes (wiggling the toes) or lips (puckering the lips) (Figure A.1). Movements were repeated at a constant instructed pace for a period of 12s, interleaved with 12s of rest. Each condition was repeated 4 times in a pseudo-random order (see sections 2.5.2.2 and 2.5.2.3 for full details on fMRI data acquisition and pre-processing and analysis).

Compared to Two-handers (N=22), increased activity levels (i.e., remapping) were found in the missing-hand area of One-handers (N=19) (U=86, p<.001, r_B=-.589), with only a marginal difference between Two-handers and Amputees (N=17) ($t_{(37)}$=-1.928, p=.062, d=-.623, BF_{10}=1.315), resulting in a significant interaction between Groups and Hemispheres ($F_{(2,54)}$=8.753, p<.001, η^2=.039; non-parametric equivalent: X^2=10.497, p=.005; Figure 2C). No difference in activity levels in the deprived hemisphere was found between Amputees and One-handers (U=198, p=.257, r_B=.226, BF_{10}=.506). These group differences are qualitatively, though clearly, distinguishable from the even inter-group profile observed for the referred sensations reports. In other words, the non-significant group difference for reported sensations on the phantom hand is unlikely to be due to lack of remapping (see Supplementary Material in Appendix for a replication of these and subsequent results using the reduced samples matched to the referred sensation task analyses). In addition, no significant correlation was found between fMRI activity levels in the missing-hand area and chronic PLP in Amputees (N=17, r_{Tau}=.103, p=.582, BF_{10}=.360).

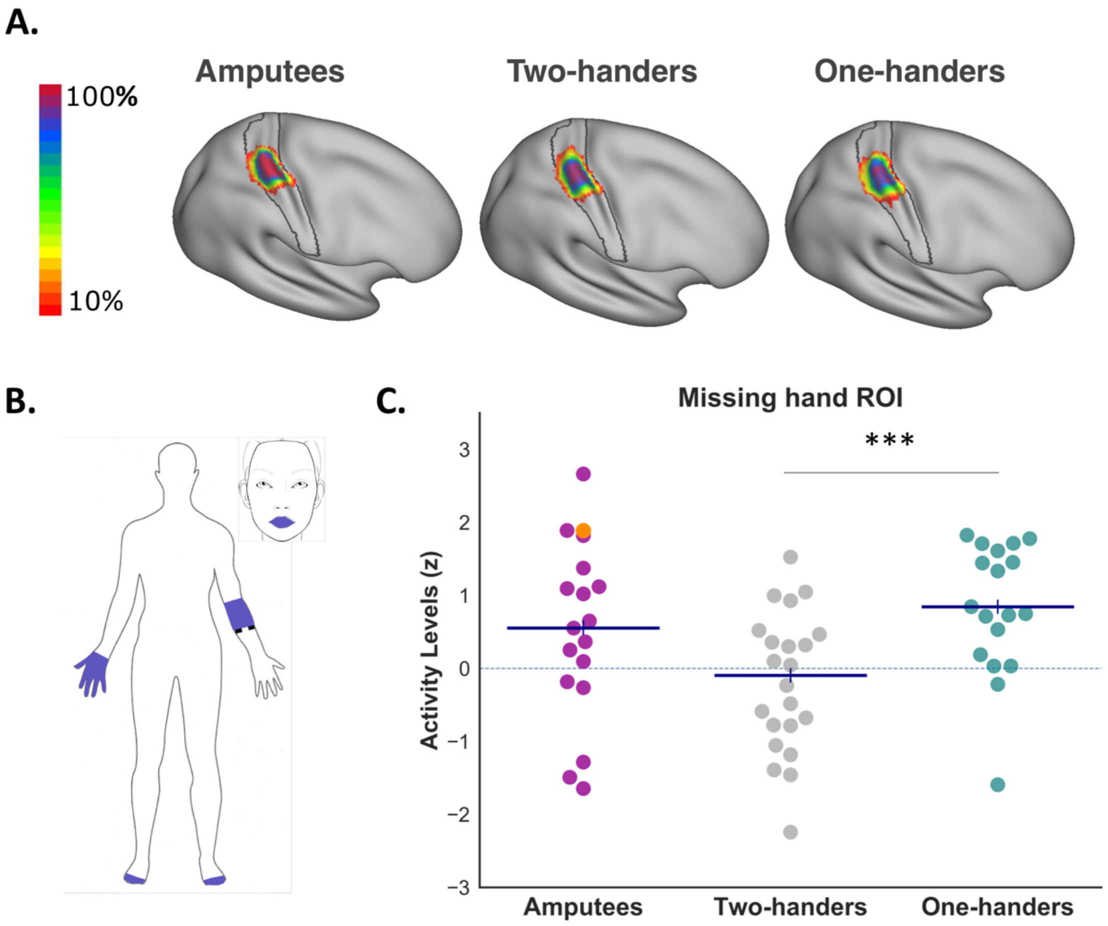

Figure A.1 Remapping in S1 missing-hand area
A) Inter-participant consistency maps for the missing/non-dominant hand S1 regions of interest (ROIs) across the three groups. The colour code represents the number of participants with overlapping ROIs in standard MNI space. The black contour shows the anatomical delineation of S1 used for ROI definition. **B)** Body-parts included in the fMRI body task. **C)** Average BOLD activity levels in the missing/non-dominant hand ROI, evoked by averaged movement of the body-parts shown in B. participant Amp05, who reported high rate of referred sensations in the behavioural

task (see Figure 2.1), is highlighted in orange. Asterisks indicate significant group differences: ***p ≤ 0.005.

Body-parts undergoing S1 remapping

To identify which body-parts were driving the observed remapping in the missing-hand area (non-dominant in Two-handers), independent samples t-tests were used to assess group differences between one-handed groups and Two-handers. Since we were considering the contributions of five body-parts, we adjusted our significance (alpha) levels to 0.01. Compared to Two-handers (N=22), Amputees (N=17) exhibited increased activity for the intact Hand ($t_{(37)}$=-4.422, p<.001, d=-1.428) and decreased activity for the residual Arm ($t_{(37)}$=2.999, p=.005, d=.969). No increased activity was found in Amputees for the Lips ($t_{(37)}$=-1.664, p=.105, d=-.537, BF_{10}=.920). We also found a significant increase in activity for the Foot of the intact side ($t_{(37)}$=-2.978, p=.005, d=-.962) but not for the Foot of the missing side ($t_{(37)}$=-2.242, p=.031, d=-.724, BF_{10}=2.137). Taken together, we only find clear and consistent contribution from the intact hand to the identified group differences in activity profiles involving Amputees.

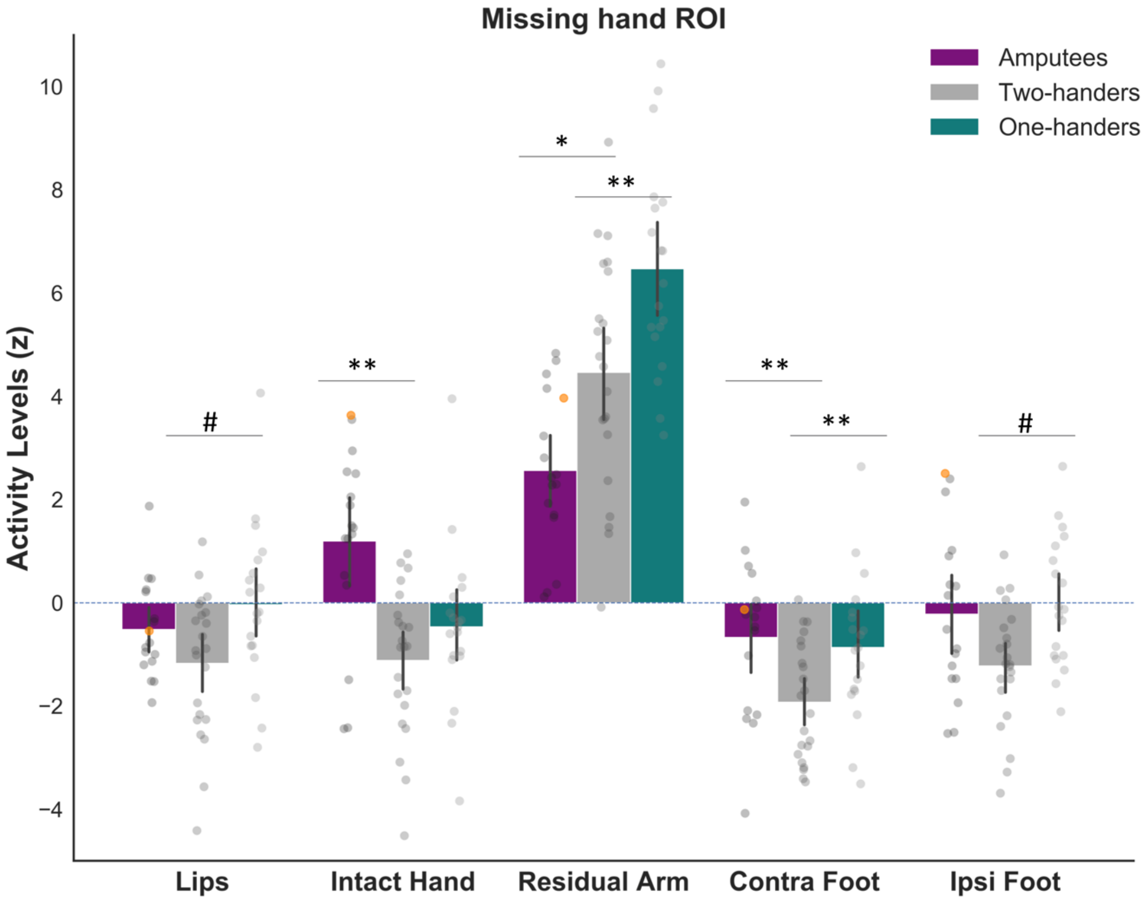

Figure A.2 Breakdown of the fMRI activity evoked by different body-parts in the missing-hand area
Average BOLD activity levels in S1 missing/non-dominant hand area, evoked in each group by movement of the Lips, Intact/Dominant hand, Residual/Non-dominant Arm, Foot Contralateral and Ipsilateral to the missing/non-dominant hand. Each grey dot represents one participant. Note that, overall, increased remapping can be qualitatively observed in Figure A.1 for participant Amp05 (highlighted in orange), who reported high rates of phantom referred sensations (Figure 2.1). Such increased activity, however, is unlikely to be driven by remapping of the face, as shown here. Asterisks indicate significant group differences: # trend, *$p < 0.05$, **$p \leq 0.005$

Sub-sample analysis based on referred sensations reports

Results in Figure 2.2D and above are reported independent of individual participant's responses in the behavioural task, i.e. all participants, regardless of whether they reported referred sensations or not, were included in those analyses (for statistical power considerations). Here, we considered whether we can find evidence for group differences in activity levels while specifically focussing on the sub-set of individuals in each group who reported experiencing referred sensations during the behavioural task. Compared to Two-handers (N=14), both Amputees (N=12) ($t_{(24)}$=-2.707, p=.012, *d*=-1.065) and One-handers (N=12) (U=18, p<.001, r_B=-.786) showed increased activity levels in the missing-hand area (i.e., remapping), resulting in a significant interaction between Groups and Hemispheres ($F_{(2,34)}$=5.604, p=.008, η^2=.045; age as covariate; non-parametric equivalent: X^2=11.676, p=.003). No difference in activity levels in the missing-hand area was found between Amputees and One-handers (U=82, p=.590, r_B=.139, BF_{10}=.419). No significant correlation was found between fMRI activity levels in the missing-hand area and chronic PLP in Amputees (N=12, r_{Tau}=.162, p=.482, BF_{10}=.461).

We then assessed which body-parts were driving this increased activity in the missing-hand area. As above, alpha levels were adjusted to 0.01 to correct for the five comparisons across body-parts. Compared to Two-handers (N=14), Amputees (N=12) showed increased activity levels for the intact Hand ($t_{(24)}$=-4.032, p<.001, d=-1.586) and for the Foot on the intact side (U=31, p=.005, r_B=-.631). A trend for increased activity was also found for the Foot on the missing side ($t_{(24)}$=-2.690, p=.013, d=-1.058, BF_{10}=4.274). No differences were found for the Lips ($t_{(24)}$=-1.246, p=.225, d=-.490, BF_{10}=.640) or the residual Arm ($t_{(24)}$=1.022, p=.317, d=.402, BF_{10}=.533). Compared to Two-handers (N=14), One-handers (N=12) exhibited increased activity for the residual Arm ($t_{(24)}$=-3.802, p<.001, d=-1.496) and for the Foot of the missing side ($t_{(24)}$=-2.875, p=.008, d=-1.131). No differences were found for the Lips ($t_{(24)}$=-2.285, p=.031, d=-.899, BF_{10}=2.245), for the Foot of the intact side (U=50, p=.085, r_B =-.405, BF_{10}=1.067) or for the intact Hand (U=59, p=.212, r_B=-.298, BF_{10}=0.643).

Finally, we also compared the representational dissimilarity between activity patterns evoked by face and the contralateral thumb movement and we found no significant differences between Amputees and Two-handers ($F_{(1,17)}$=.176, p=.680, η^2=.009), and no interaction with the Hemisphere ($F_{(1,17)}$=.008, p=.929, η^2<.001). Follow-up comparisons revealed no significant difference between Hemispheres in Amputees ($t_{(8)}$=.316, p=.760, d=.105, BF_{10}=.336), as well as no group difference in the dissimilarities observed in the missing/non-dominant hand area ($t_{(17)}$=-.405, p=.690, d=-.186, BF_{10}=.429).