THESIS FOR THE DEGREE OF DOCTOR OF PHILOSPHY

Investigating Phantom Motor Execution as Treatment of Phantom Limb Pain

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Cover:
A ghostly little man © Eva Lendaro
The image on the cover represents the Penfield motor homunculus. The hands of this homunculus shine of inner light as they turn into phantoms.

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Abstract

Phantom Limb Pain (PLP) is commonly suffered by people with amputations and even though it has been studied for centuries, it remains a mysterious object of debate among researchers. For one thing, despite the vast number of proposed PLP treatments, no therapy has so far proved to be reliably effective. For another, studies attempting to provide a mechanistic explanation of the condition have produced mixed and inconsistent results, thus providing unreliable guidance for devising new treatment approaches.

Phantom Motor Execution (PME) – exertion of voluntary phantom limb movements – aims at restoring control over the phantom limb and the exercise of such control has been hypothesized to reverse neural changes implicated in PLP. Preliminary evidence supporting this hypothesis has been provided by clinical investigations on upper limb amputees. The main purpose of this doctoral thesis was to provide high quality and unbiased evidence for the use of PME as a treatment of PLP, by probing its efficacy with a Randomized Controlled Trial (RCT) on both upper and lower limb amputees. However, the implementation of this clinical investigation required of additional technology development related the extraction of motor volition via Myoelectric Pattern Recognition (MPR). In practice, this doctoral work consisted in the extension of PME technology to lower limb amputations by proposing and validating a new and more user-friendly recording method to acquire myoelectric signals. The use of PME was then shown to be efficacious in relieving PLP even in the lower limb population with a case study.

Another necessity for providing unbiased evidence was to ensure that the highest standards were met when designing, conducting, analysing and reporting the results of the RCT. For this reason, the protocol for the RCT and the prospective Statistical Analysis Plan (SAP) were designed and published. The RCT was established as an international, multi-center effort in 2017 and it is expected to reach its conclusion in September 2021. Preliminary results of the RCT regarding the primary outcome showed reduction of PLP above what is considered clinically relevant, and whereas a higher reduction was obtained with PME, this was not statistically significant over the control treatment. The available evidence at this stage indicates that the RCT will not be able to rule out the role of contextual factors other than PME in providing pain relief.

Having at hand a way to alleviate PLP provided a unique opportunity to investigate and identify its neural correlates, therefore this became a secondary aim of this thesis. In particular, patients suffering from PLP were followed regarding their pain trajectory through the therapy and brain imaging studies with functional Magnetic Resonance Imaging (fMRI) and electroencephalography (EEG) were performed. The present doctoral thesis reports part of this work by showing the early results of a cross-sectional study on the EEG correlates of PLP. The results show that it is possible to use machine-learning techniques to discriminate EEG recorded from patients with and without PLP. The findings further point to this technique as a promising target for future longitudinal research aiming at elucidating the neural mechanisms underlying PLP.

Keywords: Phantom Limb Pain, Phantom Limb Sensations, Phantom Motor Execution, Amputation, Randomized Controlled Trial, EEG.

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List of Publications

This thesis is based on the following publications:

[A] **Lendaro E**, Mastinu E, Håkansson B, Ortiz-Catalan M, "Real-time classification of non-weight bearing lower-limb movements using EMG to facilitate phantom motor execution: Engineering and case study application on phantom limb pain", *Frontiers in Neurology, 2017;8(SEP):1-12*

[B] **Lendaro E**, L Hermanson., H Burger, C Van der Sluis C., B McGuire, M Pilch, L Bunketorp-Käll, K Kulbacka-Ortiz, I Rignér, A Stockselius, L Gudmundson, C Widehammar, W Hill, S Geers, and M Ortiz- Catalan, "Phantom motor execution as a treatment for phantom limb pain: protocol of an international, double-blind, randomised controlled clinical trial", *British Medical Journal Open, 2018;8(7):e021039.*

[C] **Lendaro** E, Nilsson S, and Ortiz-Catalan M, "Differential Activation of Biceps Brachii Muscle Compartments for Human-Machine Interfacing", *Proceedings of the 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2018, 4705-4709.*

[D] **Lendaro E**, Guo L, Novoa MJM, Sandsjö L, and Ortiz-Catalan M, "Seamless Integrated Textrode-Band for Real-time Lower Limb Movements Classification to Facilitate Self-Administrated Phantom Limb Pain Treatment", *Proceedings of the 41st Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2019, 1753-1756.*

[E] **Lendaro E**, Earley EJ, Ortiz-Catalan M, "Statistical analysis plan for an international, double-blind, randomised controlled clinical trial on the use of phantom motor execution as a treatment for phantom limb pain."*, PREPRINT (Version 1), Research Square*

[F] **Lendaro E**, Balouji E, Baca K, Muhammad AS, and Ortiz-Catalan M, "Common Spatial Pattern EEG decomposition for Phantom Limb Pain detection. *, Submitted to 43rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2021*

Other publications by the author, not included in this thesis, are:

Lendaro E, Middleton A, Brown S, Ortiz-Catalan M, "Out of the Clinic, into the Home: The in-Home Use of Phantom Motor Execution Aided by Machine Learning and Augmented Reality for the Treatment of Phantom Limb Pain", *Journal of Pain Research, 2020;13:195-209.*

Lendaro E, and Ortiz-Catalan M, "Classification of Non-Weight Bearing Lower Limb Movements: Towards a Potential Treatment for Phantom Limb Pain Based on Myoelectric Pattern Recognition", *Proceedings of the 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2016;5457-5460.*

Mastinu E, Ahlberg J, **Lendaro E**, Hermansson L, Hakansson B, Ortiz-Catalan M, "An Alternative Myoelectric Pattern Recognition Approach for the Control of Hand Prostheses: A Case Study of Use in Daily Life by a Dysmelia Subject"., *IEEE Journal of Translational Engineering in Health and Medicine*, 2018;6: 1-12.

Zbinden J, **Lendaro E**, Ortiz-Catalan M, "Prosthetic embodiment: Review and perspective on definitions, measures, and experimental paradigms.", TechRxiv. Preprint. 2021, https://doi.org/10.36227/techrxiv.14450058.v1

Acronyms

CNS:	Central Nervous System
EMG:	Electromyogram
fMRI:	Functional Magnetic Resonance Imaging
ITT:	Intention To Treat
M1:	Primary Motor Cortex
MPQ:	McGill Pain Questionnaire
MPR:	Myoelectric Pattern Recognition
PAP:	Post Amputation Pain
PME:	Phantom Motor Execution
PMI	Phantom Motor Imagery
PNS:	Peripheral Nervous System
PLP:	Phantom Limb Pain
PLS:	Phantom Limb Sensations
PP:	Per Protocol
RLP:	Residual Limb Pain
RCT:	Randomized Controlled Trial
RLP:	Residual Limb Pain
S1:	Primary Somatosensory Cortex
S1M1	Primary Sensorimotor Cortex
S2:	Secondary Somatosensory Cortex

Contents

Part I

Overview

CHAPTER 1

Introduction

Motivation

Phantom Limb Pain (PLP) — "painful sensations referred to the lost body part"[1] — is a common complaint among people with acquired amputation, with different sources attesting its prevalence between 50% and 88% [2]. As other chronic neuropathic pain conditions, PLP has negative effects on a person's well-being. For example, amputees with PLP are less likely to wear a prosthesis thus resulting in additional disability [3]. Moreover, most amputees report PLP to affect their sleep and episodes can be so intense to wake the sufferers through the night [4]. This causes the sufferer to be sleep deprived, condition which has been shown to reduce pain tolerance [5]. PLP can also have social implications. For examples, it has been shown that PLP decreases employment and satisfaction with working life [6]. Despite being a known condition since centuries, first appearing in the literature in 1551 [7], the mechanisms that originate and maintain PLP have yet to be identified.

As a consequence, when faced with the task of helping a patient suffering of PLP, clinicians do not have access to strong evidence-based guidelines specifically for treating PLP [8], despite the large number of treatments described in the literature [9]. This can largely be attributed to the scarcity of Randomized Controlled Trials (RCTs) assessing these treatments which have been found to be of poor quality [10]. To obviate this problem, research groups working on new approaches for the treatment of PLP should strive for meeting the highest standards when gathering evidence and testing their hypotheses.

Phantom movements have been consider as a potential treatment of PLP [11]–[14]. In particular, according to a recent hypothesis attempting to explain the mechanistic aspect of pain relief [12], engaging the affected sensorimotor circuitry in phantom movements is expected to alleviate PLP. Preliminary evidence in support of this hypothesis has been provided by a clinical investigation on upper limb amputees by using a novel way to enable the voluntary control of phantom movements [15]. The approach, first proposed by Ortiz-Catalan *et al.* [16] and dubbed Phantom Motor Execution (PME), makes use of Myoelectric Pattern Recognition (MPR) as a way to decode motor volition while at the same time providing real-time feedback via virtual and augmented reality (VR-AR). Exercising PME is hypothesized to reengage the motor neural circuitry in the central and peripheral nervous systems, ultimately resulting in PLP reduction [12], [13]. Clinical evidence in support of PME has been obtained through non-controlled studies on upper limb amputees, which are however ill-equipped to determine the extent to which pain relief is due to the active treatment component, rather than to any other contextual factor. The next consequential step was therefore to study the use of PME for the treatment of PLP with a RCT. This objective on one hand presents technical challenges connected to the implementation of MPR in clinical settings and on the other hand requires careful planning and design to reduce biases and confounding factors.

For over twenty-five years, starting from the seminal work of Flor *et al.* [17], PLP has been studied with neuroimaging tools with the objective of unraveling its underlying mechanisms. Yet, after three decades and numerous studies, mostly conducted with fMRI, the underlying mechanism of PLP remain unveiled. Findings by different groups have often shown to be inconsistent with one another [18], even pointing to diametrically opposed directions possibly owing to methodological discrepancies [19]. An approach to overcome the current shortcomings and move the field forwards would be to widen the scope of the research. One way to do this is by exploring the use of different techniques, such as electroencephalography (EEG), which could help in providing evidence needed to break the current deadlock of opposing hypotheses. Another way to achieve this would be to run longitudinal brain imaging studies, which are regarded as scientifically more rigorous than cross-sectional ones but are still scarce. Having access to a method to relieve PLP is therefore instrumental in creating the experimental manipulation required for a longitudinal study of the neural correlates.

Phantom sensations (painful and non-painful) are a peculiar phenomenon that raises a host of challenging questions relevant to philosophy, psychology, and neuroscience, and the long standing fascination of researchers with the experience of phantom limbs comes to no surprise:

"I think that whoever solves the puzzle or problem of the phantom limb will also solve the problem of perception . . .That is what I like so much about the phantom: I think of it as a window into the central nervous system"

Katz 2005 [20].

Scope

This doctoral thesis was focused on the following aims:

1. To provide an overview of the current state of the art in phantom limb pain research (Part I)
2. To solve some of the technological challenges connected to the extraction of motor volition via MPR in people with amputations, by:
    a. Developing a more suitable electrode configuration for myoelectric recordings in lower limbs (Paper A).
    b. Studying the feasibility of textile electrodes in substitution to disposable electrodes for the clinical application of PME using MPR (Paper D).
    c. Studying the viability of subjects learning to independently contract muscles with different heads innervated by separated branches of the same nerve while provided with real-time biofeedback, to increase motor dexterity. (Paper C).
3. To provide evidence for the use of PME as treatment of PLP, by:
    a. Demonstrating that PME is a viable option for PLP relief in lower limb amputees (Paper A).
    b. Developing the protocol of a large-scale, international RCT to gather high-quality evidence of PME as treatment of PLP (Paper B).
    c. Establishing a public Statistical Analysis Plan (SAP) prior to analyzing the resulting data of the RCT, in order to reduce bias and further increase rigor (Paper E).
    d. Presenting the result of the RCT (Paper F).
4. To explore the use of EEG in a cross-sectional study of the neural correlates of PLP (Paper G).

Thesis outline

This thesis is divided in two parts. *Part I* is constituted by eight introductory chapters, including the present one which is intended to give an overview of the research field and the reasons that motivated this work. Chapter 2 describes the historical context, the major contributors to the field and the prevailing neuroscientific framework that throughout time have provided the boundaries for the epistemology of phantom limbs. Chapter 3 describes the experience of phantom phenomena from a phenomenological perspective. Chapter 4 provides a brief introduction on the neurological basis of phantom limb sensations (PLS) and PLP. Chapter 5 gives an overview of the neurology of motor control and phantom motor control. Chapter 6 discusses the contribution of brain imaging to the study PLP. Chapter 7 lays out an overview on the prevailing theories of PLP and PLS. Chapter 8 gives an overview of the currently available treatments, giving special attention to PME. Chapter 9 looks deeper into the implementation of PME for both clinical practice and for the RCT. Here some preliminary results of the RCT are also provided. Chapter 10 presents a summary of the contributions of the included papers and finally Chapter 11 presents the conclusive remarks and outlines the work ahead. *Part II* contains the appended publications of this thesis.

CHAPTER 2

Historical Context

Amputation is one of the most serious surgical interventions and the thought that early societies, which we often call "primitive", were able to survive it successfully is fascinating. We find evidence of this in 27,000-years old cave paintings showing the imprint of hands with missing phalanges (**Figure 1**) [21], or in the oldest successful trans-humeral amputation found in a Neolithic site (4900-4700 BC) in France [22]. Moreover, survival after amputation is also well documented throughout history as abundant archeologic findings of prosthetics devices can confirm [23], [24]. Yet, perhaps even more fascinating is the total absence of phantom limbs from medical records until the 16th century: quoting Price and Twombly "there is every reason to suppose, and no reason to doubt, that individuals with an amputation have, in all times, experienced phantom sensations of some kind . . ."[25]. These observations rise the question of why certain phenomena are reported in medical literature while others remain unnoticed. For instance, one could legitimately wonder how come migraine was already known by the Ancient Egyptians (2500 BC) [26], whereas phantom pain was never mentioned before 1551, the year when Paré (1510–1590) made the first documented reference [27]. Following up on this thought, one could also be intrigued by the fact that, starting from that first report onwards, phantom limbs became a topic of high scientific interest to the point that the literature available today has past reached forbidding proportions.

Figure 1: Negative hands*. Impressions of hands made by stencil technique from the Upper Paleolithic period (about 27,000 years old). The paintings are found in the Caves of Gargas in the Pyrenees region of France.*

One interpretation is to regard this as an example of how the scientific and medical community may not be open to investigation of a sensorial phenomenon, unless it can be integrated in the body of theories of the time. This implies that the first accounts might have provided a paranormal interpretation of the perceptual experience instead of a scientific one. For example, while examining medieval folklore accounts describing the loss and miraculous restoration of body parts, Price and Twombly [25] came across what they judged to be clear

metaphorical allusions to phantom limb phenomena, thus pushing back the recorded history of phantom limbs to the tenth century. In his historical account of the process that brought phantom limbs from being a miraculous phenomenon to an instrument for investigating neural plasticity and consciousness, Wade [28] ascribes these early folkloristic descriptions to the first phase of the process. It is only in later phases that descriptions are furnished with theoretical speculations. Noteworthy is Finger and Hustwit's work [29] where they addressed the history of phantom limbs by reviewing the contributions made to the medical literature before the 20th century, thus giving an insight of how many people from a variety of different backgrounds were writing on the topic, each with different motives in mind.

Paré (1510) provided potentially the first report of phantom limbs to the medical field. He was a French military surgeon that made considerable progress in the surgical amputation technique in a time when the most common cause for this intervention was gangrene [28]. As a result of his improved technique, patients were now more likely to survive, which led him to work more closely with amputees. For instance, he designed several ingenious prostheses with movable parts (**Figure 2**). Through his work with amputees, he also discovered that they tended to have sensations in their lost limbs. In line with his primary purpose of improving the surgical procedure for amputation, he eventually wrote a commentary warning to other surgeons of the existence of deceptive sensations (phantom sensations) in dead tissue which may dissuade from performing a lifesaving amputation required to stop the gangrene from spreading [29].

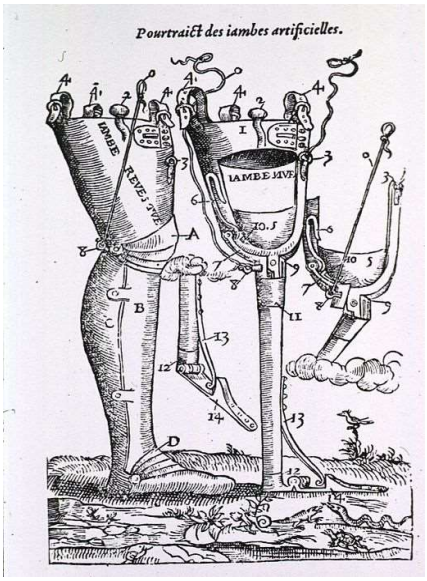

***Figure 2: Artificial leg**. Leg designed by Ambroise Paré (1575) available in the collection of Images from the History of Medicine, which is a library of the U.S. government's National Institutes of Health, Public Domain.*

By ascribing the phantom feelings to the stimulation of the severed nerves, Paré's initial accounts were integrated with the prevailing theory of perception of his time [30]. In contrast, just few decades later, Descartes decided to take his theoretical speculations beyond the commonplace knowledge of his time and exploited the phenomenon to corroborate his dualistic philosophy of body and mind, specifically as a proof of the fragmented and unreliable nature of the senses and as a further evidence of the unity of the mind. These two first reports remained rather isolated until the nineteenth century when other scientists also incorporated phantom limbs into their work. Reasonably this incorporation process was catalyzed by the establishment of basic neuroscientific concepts such as the existence of nerve cells and "animal electricity" [31], the "law of specific nerve energies" (Muller [32]), the idea of pain as an independent tactile quality (Frey [32]), or the existence of a sixth sense associated with the muscles (Bell [33]). Further, it was during this period that Silas Weir Mitchell (1829–1914) [34], one of the founders of neurology in the United States, coined the name "phantom limb" unequivocally introducing the concept in the scientific discourse.

Throughout the nineteenth century most views on the mechanisms underlying phantom sensations converged on the general idea that activity in the severed nerves alone could account for the manifestation of phantoms [35]. However, with the turn of the twentieth century and the rise of neurology, the peripheral theory started to be challenged by a dichotomous central interpretation, in which the central nervous system came to assume the primary role. Precisely, this view can be traced back to the early 1900s when Head and Holmes (1911) coined the concept of "body schema" to describe the spatial model of the body that the brain constructs based on sensory inputs. Building on this, Pick (1915) proposed that phantom phenomena are perceptual manifestations of the persistence of the lost limb in the body schema [36]. In accordance with this view, he also remarked that children with congenital absence of limbs, or after amputation in the first years of life, do not have phantom limbs due to the lack of afference required to build the body schema.

Two decades after Head and Holmes, Schilder (1935) began what Crawford renamed as the psychologization of the body schema. Namely, he deemed that the emotional processes are necessary in order to guide the sensations and perceptions that form the body schema [20]. Within this framework, the phantom represented "a reactivation of a given perceptive pattern by emotional forces" [37]. This view, combined with the concept of denial in psychology, popularized by Anna Freud, eventually led to psychogenic explanations of phantom pain such as the view of pain as the narcissistic inability to renounce the integrity of the body and adapt to the defect [20][38]. In clinical practice, psychogenic explanations tended to convene to the conclusion that phantom pain is the interpretation of phantom sensations by individuals who show psychopathology [39].

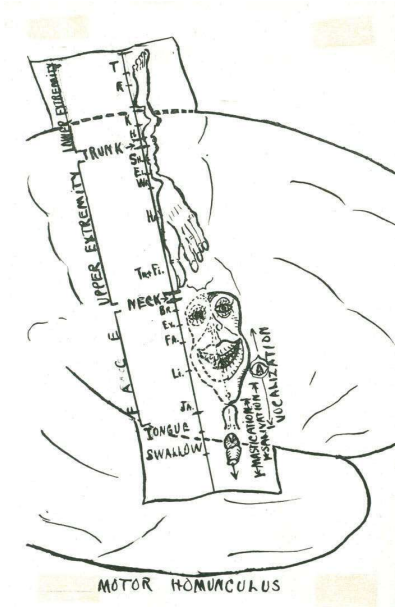

Figure 3: The motor homunculus. *Sketch by Penfield, circa 1951. ©Osler Library of the History of Medicine.*

The notion of phantom limbs as expression of psychological trouble remained unchallenged till post second world war, however further advances in neurology created favorable conditions for a shift. The cortical homunculus (**Figure 3**), discovered by Wilder Penfield and colleagues in 1937 [40], is the key concept that allowed phantom limbs to take official residence in the cerebral cortex of amputees. Early references to Penfield's homunculus regarded the somatic and motor cortical maps as the physical manifestation of it. Subsequently the homunculus was used to account for the phenomenological peculiarities of phantom limbs. For example, it was used to explain why sensations in extremities, such as phantom hands or feet tended to be more vivid than those arising from other parts. Namely, most vivid phantom sensations were perceived in those body areas with the largest cortical representations. In this light, the fact that phantom experiences in

people with congenital limb absence were not reported until 1961 [42] does not surprise. Phantom sensations in congenital limb absence were irreconcilable with the early body schema theories. Indeed, congenital sensations were explicable only by admitting that the body schema was at least partly built in the central nervous system. Melzack later commented that previous reports of phantom experience in congenital amputees were probably rejected due to the lack of a conceptual framework to make sense of these accounts [43].

Although the idea of an innate neurophysiological structure of the phantom was first provided by Penfield's work in 1937, it was not until the 1990s, also thanks to extensive foundation work conducted during the 1980s on monkeys [44]–[50], that the homunculus was used to provide empirical evidence of the properties of these maps in relation to phantom sensations. The microelectrode mapping done on monkeys investigated the effect of deafferentation and amputation on cortical sensorimotor maps and came to challenge the idea that the adult brain is hardwired with stable neuronal connections.

By the mid-90s, thanks to the advance of medical imaging technologies, it became possible to examine whether adult plasticity takes place also in humans and to further study the perceptual correlates of such plasticity. This led to phantom phenomena being attributed to plastic changes in the cortex taking place after amputation. For example, cases of phantom limb referred sensations triggered by far-removed trigger points (for example referring sensations to the hand when touching the face), previously unexplainable by peripheral theories and by fixed neural connections, started to be documented only then. This type of sensations is also known as "dual percept" because it is perceived as if it were applied simultaneously at the actual stimulation point and at a location on the missing limb [51]. Perhaps the most known report of this dual percept phenomenon comes from Ramachandran and others [52]–[55] demonstrating that referred sensations are evoked in phantom limbs by stimulating topographically organized hand maps in the lower face and stump. They explained the referral of sensations as a result of cortical plasticity where face and stump representations invade the deafferented area, as corroborated by the layout of Penfield's homunculus where the hand area is flanked by the face representation on one side and the wrist representation on the other. They further suggested that this remapping could explain the very existence of phantom limbs: spurious discharges from neurons innervating the trigger zones could be interpreted as originating from the missing limb. It was later pointed out that this phenomenon is actually exceptionally rare (present in <7% of the cases) [56] and therefore could not explain the presence of phantom phenomena, which are virtually universal in amputees. Moreover, it has been shown that the topography of referred sensation is rather dynamic over time while the invasion into the deafferented cortical zones was said to be a very robust phenomenon [57].

Twenty-five years have passed from that first paper presenting the idea that PLP magnitude correlates with the amount of cortical reorganization in S1 [17], and yet we're still looking for an answer to the question of how phantom limbs and phantom pain originate and become chronic. Through the years new brain imaging studies have tried to bring more clarity, yet some of them have failed to replicate earlier findings and have even added new inconsistent details (see [18] for a review, and [58] for a more recent example). Perhaps we have reached the point where we need to acknowledge the boundaries imposed by the current research

paradigm. Brain imaging, and especially fMRI, has been the undisputed protagonist of the last two decades, which however has confined us to the cortex. It is important to keep in mind, in Wall and colleagues' words [59], that *"peripheral injuries cause widespread neurochemical/molecular, functional, and structural alterations in subcortical and cortical substrates in the brain, and that cortical changes are but one reflection of global mechanisms that, beginning from the moments after injury, operate at multiple sub-cortical and cortical levels of the somatosensory core.".* Perhaps time has come for starting to look for answers elsewhere and with different methodologies.

The purpose of this chapter was to give an historical context to phantom limbs and pain. This should provide a sense of how tightly linked the etiology and manifestation of phantoms limbs are to what is considered legitimate by the current theoretical framework. As we have seen, the context seems to set the limits of what is possible for phantom limbs to be, feel and do. The following chapter is dedicated to the phenomenology of phantom limbs, however in the light of what has just been pointed out, I encourage you to read the rest keeping in mind that although the literature has exploded in size, and the characteristics of phantom limbs have already been investigated far and wide, the story we are telling probably remains incomplete.

"Normal science does not aim at novelties of fact or theory and, when successful, finds none"

Thomas Kuhn

CHAPTER 3

Phenomenology of phantom limbs

The purpose of this chapter is to provide a description of the experience of phantom phenomena. Yet, it must be noted that this is a challenging task since the study of phantom limbs is tightly linked to the study of the content of conscious experience, which is subjective.

A common and natural consequence of amputation is the perception of phantom phenomena [60]. Phantoms have also been reported following mastectomy [61], [62], amputation of genitals [63], rectum [64], and removal of other body parts such as eyes [65], bladder [66], uterus [67], tongues [68], or teeth [69]. Moreover, phantom are associated with conditions other than amputation. For example supernumerary phantom limbs have been reported following nerve avulsion [70], spinal cord injury [71], stroke [72], head injurie [73], anesthetic nerve block [74]. Finally, it has been found that phantom limbs were experienced also by about 20% of children with congenital limb absence [42], [43].

Phantom limbs can be perceived with the vividness of a real limb. For example, it has long been documented that amputees may forget the loss of the limb and reflexively attempt to step out of bed, answer the phone, or shake hands with the missing limb [75]. The remarkable reality of phantom limbs owes to the wide range of sensations that can be experienced.

Phantom sensations are not the only consequence of amputation and often subjects perceive also pain in the residual limb, which is known as Residual Limb Pain (RLP). Non-painful phantom sensations do not pose a clinical problem, however they often coexist with RLP and PLP [76], making it potentially difficult for the patient to distinguish them. In order to disambiguate the descriptions of phantom phenomena, Danke [77] introduced a taxonomy, later popularized by Nikolajsen *et al.* [2] as the “phantom complex”. In 2014, the taxonomy

was also adopted by the International Association for the Study of Pain (in occasion of the global year against neuropathic pain). Components of the phantom complex are *phantom limb pain* (PLP) - painful sensations referred to the absent limb; *phantom limb sensation* (PLS) - any sensation in the absent limb, except pain; and stump or *residual limb pain* (RLP) - pain localized in the stump. Beyond being by far the most popular definition adopted in the literature, its simplicity makes it also rather handy when approaching patients with an initial diagnosis. Yet, this is not a perfect definition. The first challenge is connected to the fact that phantoms limbs are sometimes perceived as retracted into the residual limb (**Figure 4**) [78]–[80]: the pain is still perceived in the phantom, but the phantom is technically located within the boundaries of the stump. The second challenge is related to the fact that with a classification based solely on the perceived location, the pathophysiology is neglected. Not only is this not optimal for treatment (*i.e.*, a RLP due to infection requires a different approach from a sensitized neuroma) but it can also be confusing when looking for biomarkers, as they reasonably differ depending on the subtype of pain. This issue specifically has been recognized by Clarke *et al.* [81], who expanded the "phantom complex" to include several subtypes of RLP (see **Figure 5**). Even though it is a step forward, this taxonomy still does not consider that also PLP has a multifactorial pathophysiology that can in some cases overlap with RPL, as for example with neuromas which have shown to play a role in PLP [82]–[84].

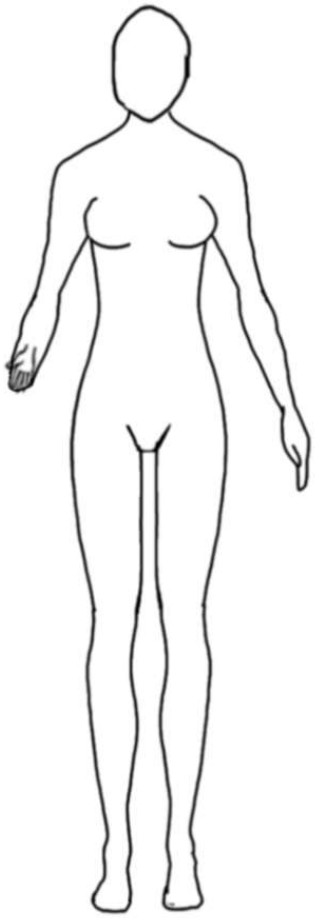

***Figure 4: Telescoping**. Patient with phantom retracted into her stump.*

The focus of Clarke *et al.*, was limited to RLP terminology. Yet, it is important to recognize that PLP is also associated with a multitude of different factors, so that PLP could be further classified in subcategories.

Indeed Griffin *et al.* [85] proposed that PLP is not a single disorder but rather a family of conditions and advocated for a mechanism-based classification of PLP aimed at specifying the distinct mechanisms and treatments for each subtype of PLP. In more recent work, Ortiz-Catalan [12] made a step in this direction by proposing a distinction based on the mechanism of perception, namely nociception and neuropathic pain, similarly to the nociceptive/neuropathic RLP classification proposed by Clarke and colleagues. Specifically, Ortiz-Catalan defines *Neuropathic PLP* as the "pain perceived as arising from the missing limb due to sources other than stimulation of nociceptive fibers that used to innervate the missing limb"; and *Nociceptive PLP* as the "pain perceived as arising from the missing limb deterministically by stimulation of nociceptive fibers".

However, in practice, it is hard to draw a sharp line between neuropathic and nociceptive perception of PLP and one could argue that PLP is by definition a form of neuropathic pain

since amputation always involves nerve damage and several processes triggered by it. The distinction between *nociceptive* and *neuropathic PLP* was initially proposed to overcome the fact that the "phantom complex" does not acknowledge the heterogeneous etiology of PLP and fails to recognize that stimulation of a neuroma could give rise to PLP. Yet, it is difficult to establish the extent to which this type of phantom-referred neuroma pain is due to stimulation of nociceptive fibers. For example, neuromas can also lead to central sensitization that causes pain perception in response to innocuous mechanical stimulation (e.g. signals coming from fibers other than nociceptive) [86].

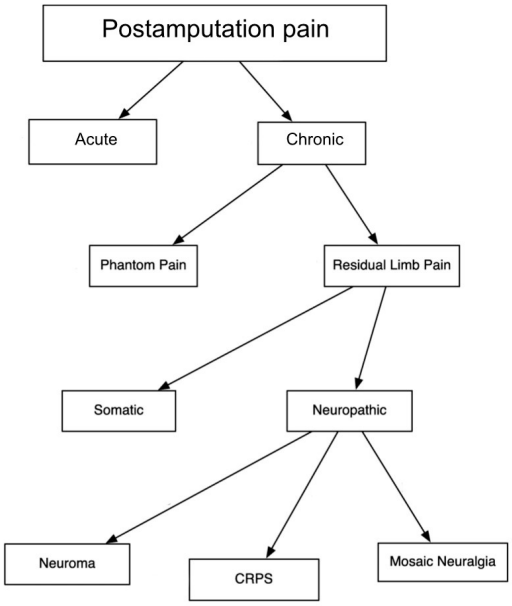

Figure 5: Classification of postamputation pain*. Chart showing the classification of different phenotypes of postamputation pain proposed by Clarke et.al. [81].*

All in all, it is important to keep in mind that etiology and manifestation of the condition are separate aspects of PAP, but both are important when it comes to proper diagnosis, treatment, and investigation. The purpose of the rest of this chapter is to describe the phenomenology of phantom sensations and phantom pain and for this purpose the "phantom complex" taxonomy is the most useful.

Phantom sensations

The prevalence of non-painful sensations has been reported in 70% of the acquired amputees [53], [87]–[91]. Weinstein recognized three types of PLS, namely exteroceptive, kinesthetic, and kinetic sensations [92].

Exteroceptive sensations include a wide range of sensory aspects, such as tingling, itching, pressure, warmth, or cold [51], and super-added phantom features, such as the sensation of wearing a shoe or a glove [93].

Kinaesthetic sensations, also known as proprioception, are the most basic type of sensation that phantom limbs are endowed with. Phantom limbs are perceived to occupy a plausible body space, usually aligning with the stump and moving with it [94]. They also are perceived to be of a particular size, shape and posture [95]. For example, phantoms may feel perfectly normal in all respects, retaining a shape and form of the former limbs [89], [96], however with time they may also fade away leaving the phantom with missing parts or gaps [69]. In some cases phantom limbs can be in an habitual and normal position, conversely they might also occupy an abnormal position which can be constantly fixed or anatomically impossible [90][53]. Phantoms can be weightless or be perceived as heavier than normal limbs [89], and often they are reported to shrink in size or shorten in length in a process first described by Guéniot in 1861 [99] as telescoping. Nevertheless, telescoped phantoms can also grow back and return to their full length, for instance when wearing a prosthesis (**Figure 6**) [98]. Likewise, they can actively telescope back when doffing the prosthesis [90]. Amputees tend to perceive predominantly the distal parts of the limb, although perception of exclusively proximal portions is also possible [90].The perception of distal parts of the phantom, in combination with the dropping out of the proximal parts leads to the perception of the phantom as detached from the residual limb, floating in air (**Figure 7**) [70].

Finally, amputees can perceive kinetic sensations, of voluntary or involuntary movements [100]. Ramachandran and Altschuler [101] reported that roughly half of the amputees reported their phantom as immobile or frozen, leading the researchers assimilate this to the "learned paralysis" that commonly affects stroke patients. Controllable phantoms have been described as "intentionally exploitable", as illustrated by Poeck (1964) with the example of an 11-year old girl with bilateral peromelia who learned to solve simple arithmetic problems by counting on her phantom fingers [102]. Another famous example is provided by pianist Paul Wittgenstein, whose right arm was amputated during WWII. After the war he learned to play the piano with his left hand resuming his concert pianist career. It is believed that the movements of his phantom hand played a crucial role in the acquisition of his unusual left-handed dexterity, as he allegedly used the phantom hand to select the strategy for pressing the piano keys [103]. Phantoms can also move involuntarily and automatic phantom movements have been described as jerking, jolting, spasm, or tremor movements [104]. An example of these is provided by McGrath and Hiller's patient who experienced an unusual sensation referred to as nerves jumping, by saying "weird tingling that starts in your toes and goes up to your stump and the nerves jump. The stump jumps up and down (1 or 2 inches) for a few seconds."[105].

It has also been reported that phantom limbs interact with the surrounding world and have different adaptation strategies. The most exhaustive study in this regard was authored by Jalavisto [106]. In this study, 173 subjects were asked to give an accurate account of what happens to their phantom when they are placed near a wall or table and had to move the stump so that the phantom, if unchanged, would occupy some place within the wall or the table. Two main strategies emerged, namely an adaptive strategy, where the phantom

disappears, shuns the obstacle or moves, and a fixed strategy in where the phantoms do not adapt and penetrate objects instead. Interestingly, it was found that that amputees younger than twenty-five were more likely to described their phantoms as disappearing or moving when approaching the obstacle (adaptive phantoms), while amputees over the age of twenty-five tended to describe their phantoms as passing into the wall (fixed phantoms) [106].

The temporal evolution of phantom limbs are remarkably variable. Among the people experiencing PLS, the vast majority reports of being aware of PLS within the first week post amputation, while the remaining part perceives them within the first few weeks [89][91]. Some patients experience the missing limb for only a few days or weeks, while others, an estimated 30%, continue to experience it for decades [107]. Finally, the PLS can be spontaneous (independent from any kind of stimulus) or they can be stimulus-dependent (i.e. evoked) by a discrete event or condition [108], [109].

Giummarra *et al.* [90] points out that one of the limitations of the literature on PLS is the anecdotal nature of the descriptions, which usually explores only isolated features of the phantom or are based on extraordinary cases. To overcome this shortfall, they ran a systematic phenomenological study on 283 amputees reporting the prevalence of each of the various features of PLS. **Table 1** reports the results of the study. The authors found that telescoping was more common among upper limb amputations and less common among amputees who underwent vascular or diabetic amputation. The size of the phantom was not influenced by the amputation level; however, the size was perceived more clearly when pain was also present. Participants reporting anatomically impossible postures were more likely to be traumatic amputees, while normal posture was more common in those patients with some form of functional impairment prior amputation. No differences in exteroception and proprioception of phantom limb was found considering cause of limb loss, functional impairment prior to amputation, and infection or gangrene prior to amputation. Upper limbs were however more likely to report temperature in their phantom limbs. Finally, prosthesis "embodiment" was more frequent in amputees with an extended phantom compared to a telescoped phantom [90].

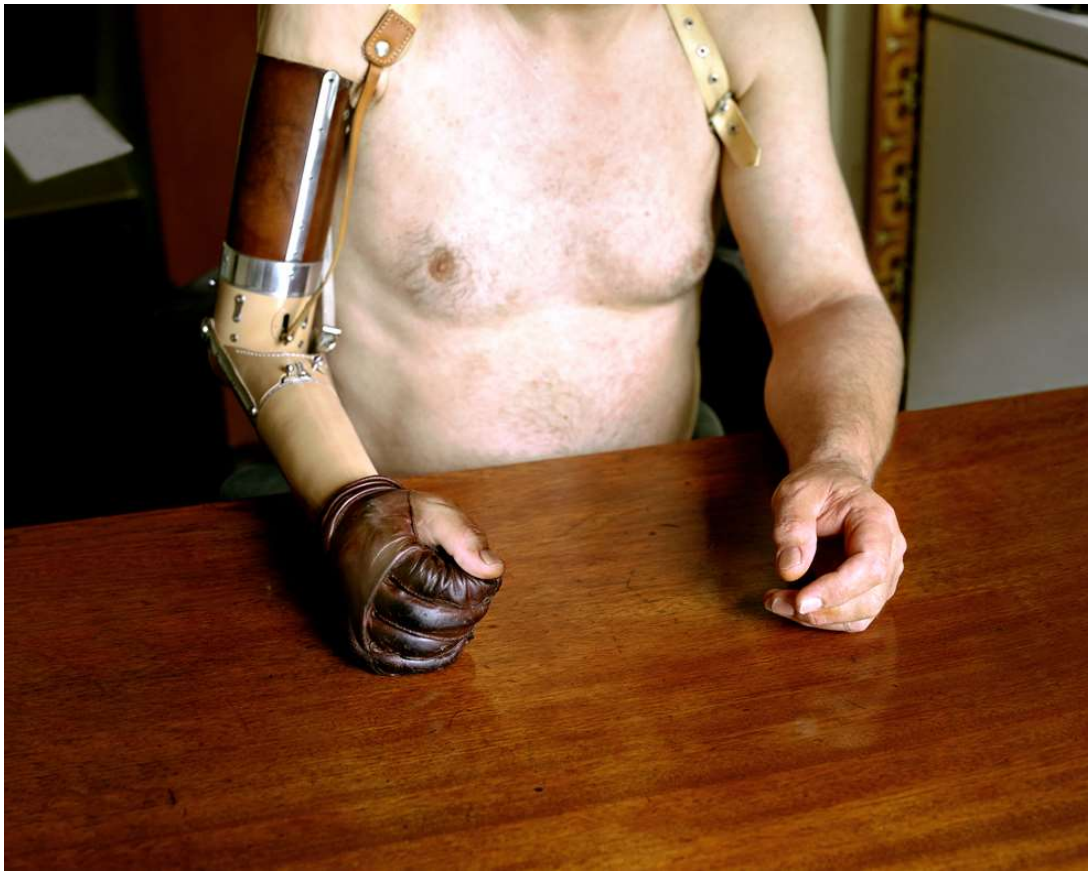

Figure 6: After Image GN2.
(Caption and photo reprinted with permission from Wright, Alexa. 1997. After Image. London, England: www.alexawright.com.)

GN
Date of amputation: 1964
Time since amputation: 33 years
Age: 52 Male
Motor cycle accident: brachial plexus lesion
Arm amputated 4 months after accident
No previous damage to limb

"At first I had a phantom limb whilst the arm was still there, because the arm was paralyzed. The phantom used to float away from where the arm was. I was in a hospital bed and it would float through the bedclothes and get cold, so I developed this habit of sleeping on my right side so the phantom limb drifted into the mattress and stayed warm. At the beginning used to believe I could get the arm back.
Now nearly all of the arm has disappeared, but if I am wearing the artificial arm and I swing my arms as I walk, the right arm swings. If I can see the artificial hand out of the corner of my eye or I can feel it up against my leg the phantom hand is inside the glove. If I can't see the artificial hand, I can be wrong; I could be six inches out as to the location of the hand: the phantom hand can miss the artificial one in terms of spatial placing.
There is an intermittent crushing pain, but the phantom is always there. It's part of me; it will never go away completely. I will always be this; I will always have two arms, it's just that one of them is missing. The real me is without the prosthesis; its uncomfortable; it's not me. It is surprising how one armed I look when I see photographs of myself; my self-image is two armed."

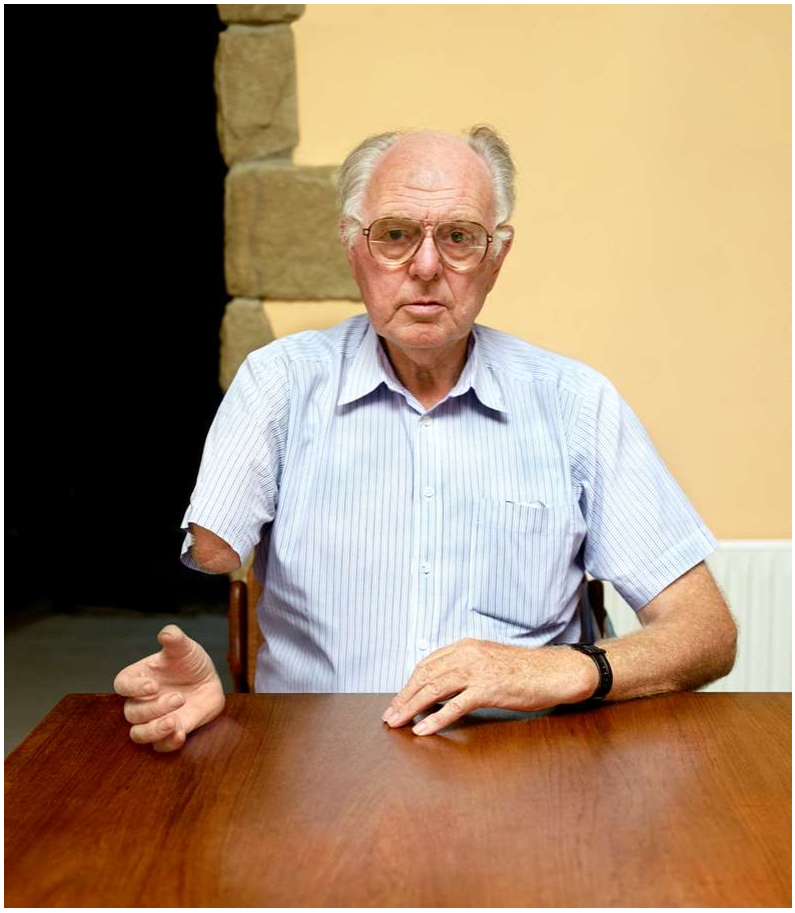

Figure 7: After Image RD2
(Caption and photo reprinted with permission from Wright, Alexa. 1997. After Image. London, England: www.alexawright.com.)

Date of amputation: October 1995
Time post amputation: 21 months
Age 71: Male
Road accident in which arm was crushed
No previous damage to arm

"As our car bowled over in collision with another car, my arm went out of the window and was crushed. X-rays later revealed that the arm was severely damaged, but the hand was left intact.
The phantom is continuous; it takes the form of my hand. It is sometimes painful and sometimes just sensation. I feel I can control the movements of the hand until I suddenly realize that it isn't there. The hand is slightly clenched fist, and that doesn't really change; it can only go about three quarters unclenched. The pain is mostly in the third finger; that sometimes hurts and is painful as though I had broken it. The hand is the same size as my real hand, but much heavier. It itches a lot of the time and I want to scratch it.
I can kid myself that I can make the phantom limb move. It's really just a sort of opening & closing: the hand moves from the wrist downwards, but rotation of the wrist isn't available. I have only got finger and hand joint movements. When I haven't moved it for a while it becomes stiff.
I can't imagine being without the phantom because it is there all the time and it is very much like eating or breathing: I can put up with it quite adequately and would probably miss it if it went away. I might wish it wasn't so irritating, but I think I would rather keep it as it is than risk losing it."

Phantom limb pain

PLP has been long described in the literature however the many accounts are often inconsistent and contradict each other. The contradiction starts when trying to determine the prevalence of the condition. Early reports were more contradictory, with some studies indicating very low prevalence rates of PLP (1-5%) [38], [39], [41], [110] and others rates as

Table 1 :Prevalence of Phantom Limb Sensations. *Summary of the results of the survey conducted by Giummarra et al. [87]. The table reports the number (per cent) of participants of the study who perceived a phantom limb with various sensation.*

	N (%)
Phantom limb perception and pain	
Non-painful phantom limb sensations (PLS)	207 (73.1)
Phantom limb pain (PLP)	191 (67.5)
PLP and PLS (partially overlapping data above)	135 (47.7)
Frequency of PLS at present	
Constantly	102 (35)
A few times an hour	10 (4)
A few times a day	38 (14)
A few times a week	16 (6)
A few times a month	24 (9)
A few times a year	23 (8)
Very infrequently	33 (12)
Never	35 (12)
Parts of the phantom limb	
Whole – as it was before amputation	100 (39.1)
Whole – deformed unlike prior to amputation	17 (6.64)
Whole – deformed as it was prior to amputation	2 (0.8)
Distal parts only	107 (47.8)
Proximal parts only	11 (4.3)
Posture of the phantom	
Normal position	203 (79.3)
Abnormal position	30 (11.7)
No perception	23 (8.1)
Size of the phantom limb	
Smaller	4 (1.4)
Normal size	213 (75.3)
Larger	4 (1.4)
No perception	34 (13.3)
Telescoping	55 (21.6)
Exteroceptive sensations	
Itching	129 (50.0)
Pressure	92 (36.7)
Touch	41 (16)
Temperature–heat	43 (16.6)
Temperature–cold	40 (15.6)
Temperature–heat and cold	12 (4.7)
Temperature–warm	14 (17.6)
Electric sensations	120 (43)
Vibration	32 (11.5)
Pins and needles	49 (17.6)
Prosthesis embodiment	
Phantom embodies prosthesis	23
Phantom disappears when wearing prosthesis	34
Phantom does NOT embody prosthesis	5
No change	111
Other	
Increase in PLS/PLP	25
No phantom limb	30
Missingand/or do not wear prosthesis at all or enough	55
Phantom limb movement	
Voluntary phantom limb movement	132 (47.0)
Spontaneous phantom limb movement	107 (37.8)

high as 50% [94]. Recent reports are more homogeneous and attest rates between 60% and 80% [20], [111],[112]. Table 2 is taken from Crawford's book "Phantom limb: Amputation, embodiment, and prosthetic technology" (2015) [20] and shows how the prevalence of PLP has steadily increased over the years up until the 1990s, then reducing slightly with the turn of the twenty-first century. The large discrepancies and low rates in early reports have been attributed to the confusion of terminology (RLP,PLP and PLS) and poor sample selection, probably due to the social stigma attached to the condition [113]. Factors including age, gender, side, level and cause of amputation do not seem to have an influence on the prevalence of PLP [1], [76] although a prospective study on 85 amputees showed that female upper limb amputees are associated with higher risk of PLP [114]. A clear predisposing factor related to PLP seem to be the presence of RLP [113]. Early literature excluded the presence of phantom limbs and PLP in young children and congenital amputees [41], however it has been later found by others that to a small extent, they occur even in this patient group [43]. Older children and adolescents have been found to suffer from PLP as much as adults [115].

Table 2: Prevalence of Phantom Limb Pain. *Prevalence over time. This table is an adapted version of the one appearing at page 81 in [15].*

Period	Prevalence
1910–1919	Unknown
1920–1929	Unknown
1930–1939	Not Infrequent
1940–1949	1%
1950–1959	1%
1960–1969	5%–15%
1970–1979	35%–50%
1980–1989	50%–85%
1990–1999	70%–85%
2000–2009	50%–80%

There is great uncertainty regarding the onset and duration of PLP. Most often it starts immediately after amputation; however, some authors have reported late onset. For example Rajbhandari *et al.* [116] who described a case of PLP starting forty-four years after amputation. Late onset can happen in presence of a precipitating factor such as injury to the stump, or development of pathology to the nerves [20]. The long-term time course of PLP is also rather unclear. Whereas some studies report decreased intensity over time [117]–[119], others report higher likelihood of PLP when longer time since amputation has passed [87]. Our group has worked with patients reporting constant or increased intensity levels of PLP up to 48 years post amputation [16], [120]. In a prospective study on 526 veterans, PLP disappeared over time in 16% of the subjects, decreased significantly in 37%, remained similar in 44%, and increased in 3% [121]. The frequency of PLP is also extremely variable having constant pain on one end of the spectrum to sporadic short-lasting painful shocks [79]. The pain is usually perceived in the distal part of the phantom. For upper limb amputees this means the palm of the hand and fingers, whereas for lower limb amputees it is the toes, foot, or ankle [97], [118], [119].

The introduction of the McGill Pain Questionnaire (MPQ) by Melzack [122] has played an important role in standardizing the language of qualitative descriptors of PLP. **Figure 8** reports the pain descriptors used in the classic version of the MPQ. The most common descriptors applied to PLP are burning, stabbing, throbbing, cramping, numb, smarting, stinging,

throbbing, piercing, and tearing [60], [123]. However, Crawford pointed out that before the advent of MPQ it was common to find more vivid and colorful descriptions. For example the wrinkled, raw flesh, red-hot needles, wet, slimy, swollen, glowing, dry, and furry qualities of phantoms, were largely documented prior to 1975, the year when the MPQ was institutionalized [20], [110]. Accounts of PLP with a more detailed narrative were also more frequent in the past. An example is the one provided by Russell (1949) reporting a sensations of "reopening of the old wound of his foot, followed by a sensation of blood welling up between his toes" [124].

Oftentimes patients report that the pain resembles pre-amputation pain both in quality and location. Katz and Melzack [125] coined the term "somatosensory pain memories" in 1990 to indicate painful sensation in the phantom which resemble somatosensory events experienced in the limb before amputation. They interviewed 68 amputees and a total of 57% of those who reported having had pain before the amputation claimed that their PLP was indeed similar in quality and location. Nikolajsen *et al.* [119] asked patients to describe their pain before and after amputation and although 44% of patients claimed that their PLP was similar to the preamputation pain, the character of PLP was actually similar to preamputation pain only in a minority of patients [119]. Some studies found that PLP was significantly more frequent in the first months post amputation but not later in those patients who suffered from preamputation pain compared with those who did not [118], [119]. Therefore, although pre-amputation pain seems to play a role in the short-term development of PLP, it is not the only mechanism involved, and in the long term, the correlation between pre-amputation pain and PLP is not evident.

It has been reported that painful experiences in the phantom limb can be modified or triggered by spontaneous events, autonomic reflexes (e.g. micturition), physical (e.g. weather changes), and psychological or emotional factors [126]. Giummarra *et al.* [75] explored triggers of phantom phenomena by surveying 264 upper and lower limb amputees with phantom sensations. The results showed for example that upper limb amputees were more likely to experience weather-induced phantom phenomena than lower limb amputees; traumatic amputees were more likely to report emotional triggers. The correlation between stump pain and phantom pains and phantom painless sensations has been shown by different authors [88], [127], [128]. Finally, it has been reported of cases of referred phantom pain in which pain in a phantom arm was associated with myocardial ischemia [129].

To summarize, the purpose of the current chapter was to describe the main features of the experience of phantom phenomena, which can be divided in painful and non-painful sensations. What emerges from this account is that the literature is characterized by contradiction and uncertainty. A possible reason for the contrasting accounts is that PLP is not a single syndrome but a class of syndromes with distinct etiologies, that often share a diagnosis based only on the perceived location of pain. Another reason is the subjectivity of phantom phenomena.

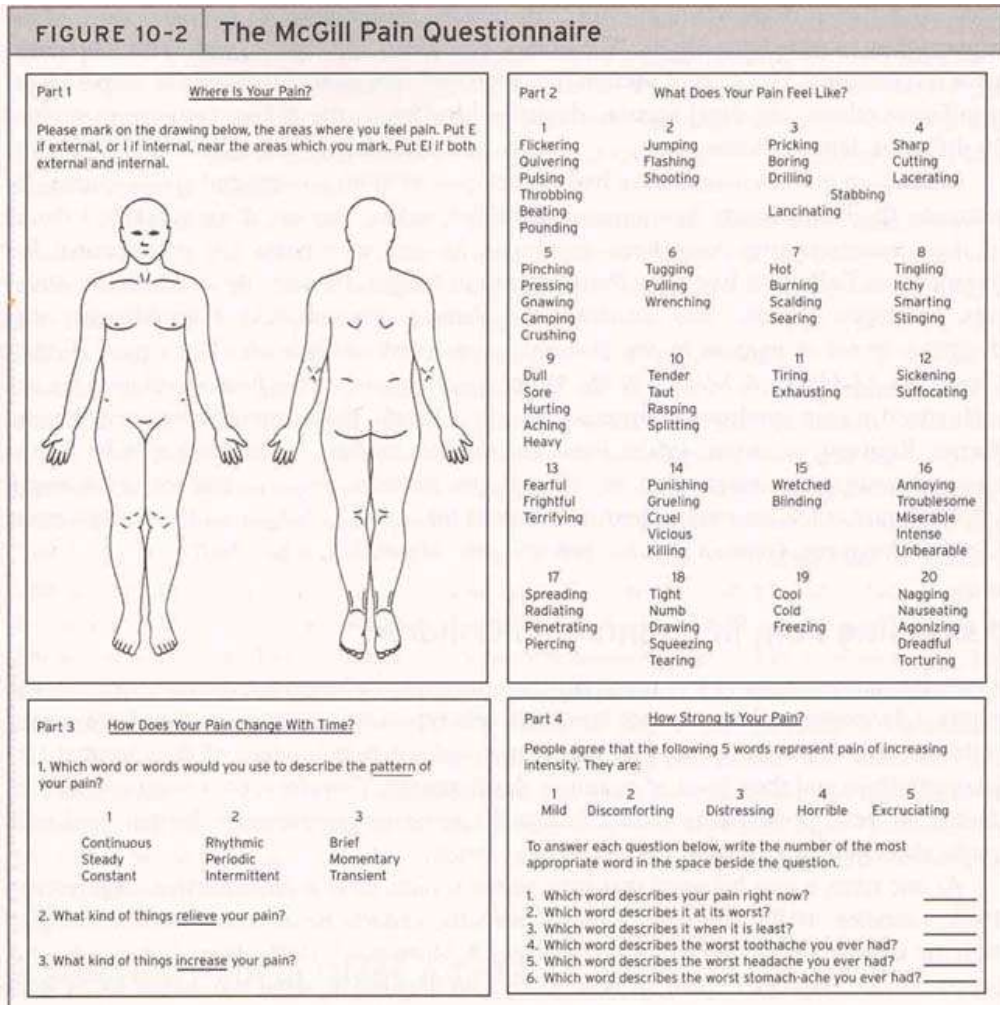

FIGURE 10-2 The McGill Pain Questionnaire

Part 1 Where Is Your Pain?

Please mark on the drawing below, the areas where you feel pain. Put E if external, or I if internal, near the areas which you mark. Put EI if both external and internal.

Part 2 What Does Your Pain Feel Like?

1	2	3	4
Flickering	Jumping	Pricking	Sharp
Quivering	Flashing	Boring	Cutting
Pulsing	Shooting	Drilling	Lacerating
Throbbing		Stabbing	
Beating		Lancinating	
Pounding			
5	6	7	8
Pinching	Tugging	Hot	Tingling
Pressing	Pulling	Burning	Itchy
Gnawing	Wrenching	Scalding	Smarting
Camping		Searing	Stinging
Crushing			
9	10	11	12
Dull	Tender	Tiring	Sickening
Sore	Taut	Exhausting	Suffocating
Hurting	Rasping		
Aching	Splitting		
Heavy			
13	14	15	16
Fearful	Punishing	Wretched	Annoying
Frightful	Grueling	Blinding	Troublesome
Terrifying	Cruel		Miserable
	Vicious		Intense
	Killing		Unbearable
17	18	19	20
Spreading	Tight	Cool	Nagging
Radiating	Numb	Cold	Nauseating
Penetrating	Drawing	Freezing	Agonizing
Piercing	Squeezing		Dreadful
	Tearing		Torturing

Part 3 How Does Your Pain Change With Time?

1. Which word or words would you use to describe the pattern of your pain?

1	2	3
Continuous	Rhythmic	Brief
Steady	Periodic	Momentary
Constant	Intermittent	Transient

2. What kind of things relieve your pain?

3. What kind of things increase your pain?

Part 4 How Strong Is Your Pain?

People agree that the following 5 words represent pain of increasing intensity. They are:

1	2	3	4	5
Mild	Discomforting	Distressing	Horrible	Excruciating

To answer each question below, write the number of the most appropriate word in the space beside the question.

1. Which word describes your pain right now? _____
2. Which word describes it at its worst? _____
3. Which word describes it when it is least? _____
4. Which word describes the worst toothache you ever had? _____
5. Which word describes the worst headache you ever had? _____
6. Which word describes the worst stomach-ache you ever had? _____

Figure 8: The McGill Pain Questionnaire*. Reproduction of the McGill Pain questionnaire introduced by Melzack in 1975, reprinted from [119].*

CHAPTER 4

Perception of phantom limbs

The neurological mechanisms underlying phantom phenomena are not completely understood. In the case of amputation, phantom limbs occur when parts of the peripheral nervous system (PNS) are disconnected from the central nervous system (CNS), causing changes at every level of the nervous system. The purpose of this chapter is to present an overview of the literature on perception of painful and non-painful phantom limbs. However, in order to understand what mechanisms are involved in an abnormal condition such as amputation, this chapter will first provide an overview of the normal functioning of the somatosensory system.

Perception

Cutaneous sensation, proprioception, and nociception

The somatosensory system provides our brain with information coming from the external world as well as from our own body. This is made possible by the presence of receptors located all over the body, from the surface of our skin to the depth of our internal organs. Somatosensation comprises three different systems: the cutaneous sensory system that senses stimuli applied to the skin; the interoceptive system that provides general information about internal body conditions; and the proprioceptive system that senses the position of body parts. Proprioception and cutaneous senses are particularly relevant to the discussion of what happens when a limb is amputated [130].

The skin mediates a wide range of sensation thanks to the presence of specific receptors that transduce a stimulus into electrical impulses. The type of stimuli that can be transduced are pressure, vibration, skin stretch, heat, cold and chemical. The receptors transducing these stimuli are usually classified into three categories: mechanoreceptors, transducing mechanical stimuli; thermoreceptors, transducing the temperature information of the stimulus; and chemoreceptors, responding to chemicals. Nociceptors are a subtype chemoreceptors and mechanoreceptors that responds to stimuli potentially damaging to tissue. The experience of pain usually starts with activation of nociceptors. When a receptor is activated by a sufficiently strong (supraliminal) stimulus, it will send the transduced information along the ascending pathway to which it belongs. Nociceptive, temperature, itch sensations, and crude touch follow the spinothalamic tract (in the anterolateral column of the spinal cord) (**Figure 9**.**B** [131]) which crosses the midline in the spinal cord and ascend the nervous system in the contralateral side. Conversely fine touch, vibration, and proprioception

follow the dorsal column–medial lemniscus pathway (**Figure 9.A**) which crosses the midline more rostrally at the level of the medulla, thus ascending the spinal cord on the ipsilateral side. Because of this special arrangement, spinal hemisection causes a dissociated sensory loss of contralateral pain and temperature sensations, and ipsilateral of fine-touch perception. Both spinothalamic tract and the dorsal column-medial lemniscus pathway consist of a chain of three neurons to convey information from periphery to cerebral cortex. The first order neuron is in the dorsal root ganglia (DRG) and enters the spinal cord via dorsal horns, then following its specific pathway.

First order neurons belonging to the mechanosensory pathway, once in the dorsal horn of the spinal cord continue to ascend the nervous system following the ipsilateral dorsal column up to the brainstem where they then synapse in the caudal medulla with the second order neurons. As already mentioned, here the second order neurons shift to the contralateral side and ascend to the thalamus where they synapse again. From here, the pathway continues bringing the information to the primary somatosensory cortex (S1), in the postcentral gyrus.

The S1 is subdivided into four Brodmann's areas (BA), namely 3a, 3b, 1, and 2, which are somatotopically organized—that is, the sensory signals are represented according to where in the body they come from (**Figure 10** [132]). BA 3b and BA 1 receive information from receptors in the skin, and BA 3a and BA 2 receive proprioceptive information from muscles and joints, but there are extensive interconnections between these areas.

First order neurons synapse with the second order already in the dorsal horn of the spinal cord. From that synapsis, the second order fibers cross the midline and ascend the contralateral anterolateral column of spinal cord, projecting to several different structures in the CNS. This broad array of central targets forms an extensive network, also known as pain matrix, that contributes to different aspects of how pain is processed, making pain a multidimensional experience (**Figure 10** [133]). In particular, the different central structures that are part of the pain matrix can be grouped into two main systems: one system mediating the sensory-discriminative aspects of pain perception and the other conveying information about the affective-motivational aspects [134].

The sensory-discriminative system processes location, intensity, and quality of the noxious stimulus. Secondary neurons ascending the anterolateral column of the spinal cord project to the thalamus where they make synapse with third order neurons which in turn distribute the signals to S1, respecting the somatotopic arrangement, and secondary (S2) somatosensory cortex [135]. The affective-motivational system is instead responsible for mediating unpleasant feelings and autonomic activations that accompany the exposure to nociceptive stimulation. Second order neurons belonging to this system project to targets in the reticular formation, the superior colliculus, the periaqueductal grey, the hypothalamus, and the

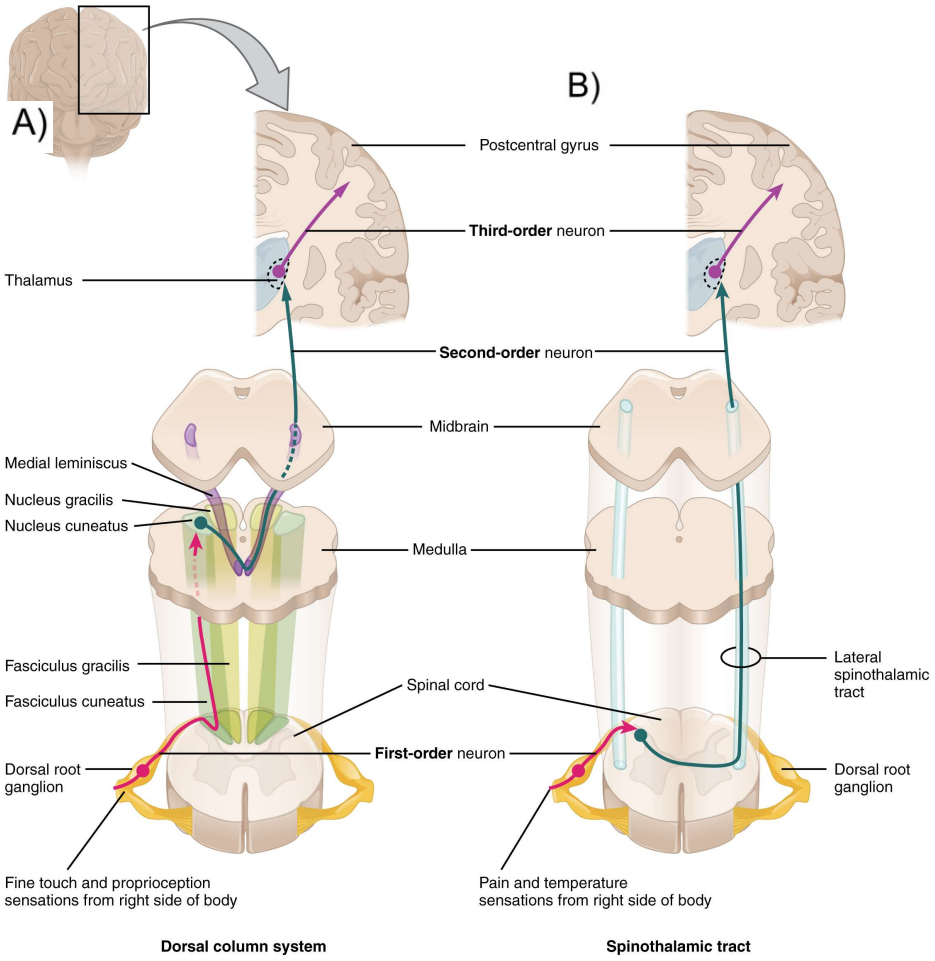

***Figure 9: Ascending sensory pathways**. A) the dorsal column-medial lemniscal pathway, which carries mechanosensory information from the posterior third of the head and the rest of the body. Information from the face is carried by the trigeminal portion of the mechanosensory system. B) The discriminative pain pathway mediating aspects of pain and temperature for the body. Source of the image [128]*

amygdala. Another important target is the thalamus from which third order neurons depart and reach the anterior cingulate cortex (ACC) and the insular cortex (IC) [135].

This view of pain being mediated by two separate systems is consistent with the results of brain imaging studies that have been able to separate the relative contribution to pain perception. By using hypnosis directed to increasing or decreasing the perceived intensity of the burning sensation produced by submerging a subject's hand in painfully hot water, it was found that pain-related activation S1 was modulated [135]. Conversely, hypnosis directed to change the unpleasantness of the perceived sensation had no effect on S1 but produced robust modulation of the activation of ACC, directly correlated to the perception of unpleasantness [136], [137].

Top-down modulation of pain

The perception of pain is also subject top-down modulation, in which higher order brain functions can suppress or amplify sensory information coming from lower order mechanisms.

This is possible thanks to descending pathways where several brain areas including the ACC and IC, the amygdala and the hypothalamus, project to the periaqueductal grey, which in turn regulates the transmission of nociceptive information. For example, it was found that maintaining attention to pain can worsen it [138], whereas distraction can alleviate it [139]. Another factor that is thought to significantly worse pain is pain catastrophizing, defined by Sullivan as “an exaggerated negative mental set, brought to bear during actual or anticipated painful experience”[140]. Distraction was found to be particularly efficacious in relieving pain in these patients. Several studies have also investigated the effect of expectation on pain experience indicating that expecting a pain stimulus exacerbate the actual experience. Similarly, expecting pain relief can ameliorate pain in what is known as placebo effect. The placebo effect is a physiological response following the administration of an intervention, relief is at least partially due to the brain’s own descending modulation circuit [141]. The effects of placebo are real and brain imaging studies have been able to show reduced activity in areas usually involved in pain processing [142] suggesting that this effect is due to the release of endogenous opioids [143].

Finally, another mechanisms for pain modulation initially proposed by Melzack and Wall as gate theory of pain, consists in the modulation of information coming from nociceptive fibers at the level of the spinal cord by the interaction with mechanoreceptive afferences and the circuitry within the dorsal horns [144].

The multitude of areas and targets at all levels of the nervous systems that are involved in pain perception suggests that the full experience of pain is mediated by a cooperative distributed network of brain areas that are often referred to as pain neuromatrix. It should not be surprising then that pain is a multidimensional subjective experience with sensory, emotional, affective, and cognitive components.

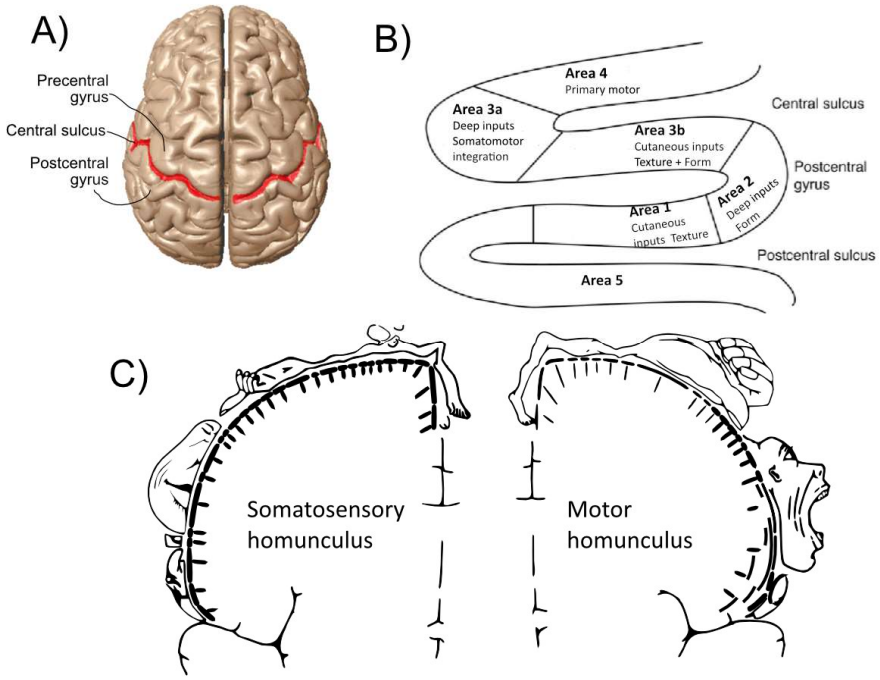

***Figure 10: Sensorimotor maps**. A) Dorsal view of the central sulcus (highlighted in red). B) Sagittal section (along the longitudinal fissure) of precentral and postcentral gyri to highlight the subdivision in Brodman areas. C) Division of sensory (left) and motor (right) functions in the cerebral cortex. Adapted from Penfield and Rasmussen, 1950 [129])*

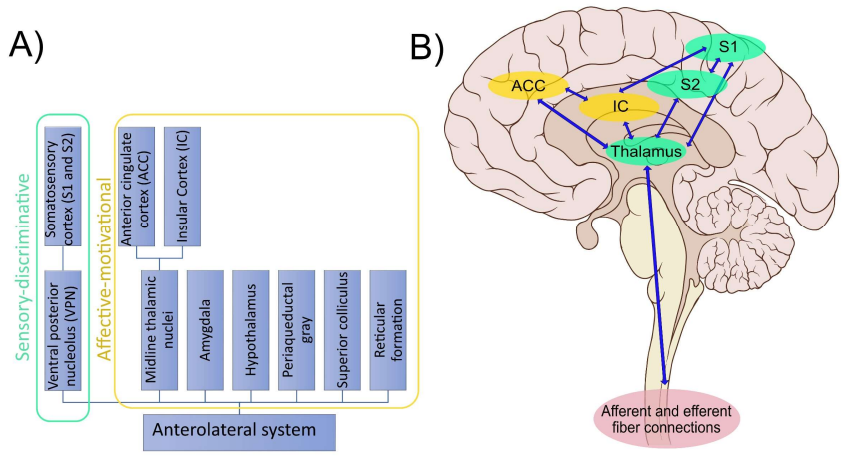

***Figure 11: The experience of pain.** A) A flow diagram showing how the anterolateral system supplies information to different parts of the brainstem and forebrain. B) Detail of the cortical target of the sensory-discriminative (green) and affective motivational (yellow) system. Sagittal view of the brain adapted from [130]*

Neuropathic pain

The International Association for the Study of Pain (IASP) defines nociceptive pain as the pain occurring with a normally functioning somatosensory nervous system in presence of actual or threatened damage to non-neural tissue and is due to the activation of nociceptors. This definition is meant to emphasize by contrast the abnormal function seen in neuropathic pain, which is instead defined as pain arising from lesion or disease of the somatosensory nervous system (Definitions last updated on December 14, 2017).

Nociceptive pain is a physiological sensation aimed at protecting the organism by preventing injury. This is achieved with two strategies: a withdrawal strategy in which a reflex automatically removes the body from the source of the noxious stimulus; and with a protective strategy in which an unpleasant sensation induces the organism to implement complex behavior to avoid further exposure to the source of pain [145]. Another mechanism that further enhances the protective approach is sensitization of the nociceptive system, known as peripheral sensitization, in which repeated, or particularly intense noxious stimuli bring the nociceptors to be more sensitive. A nociceptive stimulus is still necessary to trigger pain; however, the firing threshold of the nociceptors is lower, making them fire following nociceptive stimuli that would normally not be perceived. This condition of heightened sensitivity is also known hyperalgesia. As the injured tissue heals the sensitivity of the nociceptors goes back to normal levels [130].

Neuropathic pain arises from a damage to the neural tissue, for example when a nerve is severed due to amputation. When this happens, the normal pattern of afferent nerve input to the spinal cord gets disrupted causing maladaptive changes. One of these changes is for example known as central sensitization. This type of sensitization is initiated by activity in nociceptors; however, the effects generalize to other inputs that arise from low threshold mechanoreceptors (allodynia). This feature of central sensitization is caused by means of neural plasticity in the CNS, which changes the sensory response elicited by normal inputs, including those that usually evoke innocuous sensations. Since this effect is cause by plastic changes in the neurons, pain might be experienced long after.

Neuropathic pain reflects both peripheral and central sensitization mechanisms [146].

The effects of amputation on the nervous system

Amputation deprives the nervous system of the sensory inputs originating from the detached body part and causes changes at every level of the nervous system. The dynamic ability to change and adapt is called plasticity and can take place both in the periphery [147] and in the central nervous system [130]. This section attempts to outline the consequences of amputation on the PNS and CNS in order to give a brief account of their contribution to postamputation pain (PAP) and PLS.

Postamputation pain (PAP) is a composite phenomenon that can have two stages: an acute phase sometimes followed by a chronic phase. Two types of acute postamputation pain may occur. The first is the pain in the amputated stump, or RLP, and the second is the pain perceived in the missing limb (PLP). Acute postoperative pain is due to the damage of tissue

and nerves, and it should resolve itself once healing is complete. However, both acute RLP and PLP can become chronic. Chronic RLP can have both nociceptive (somatic) and neuropathic origins [148]. Neuropathic mechanisms include the presence of a neuroma, development of Complex Regional Pain Syndrome, heterotrophic ossification, or mosaic neuralgia [149]. Nociceptive/somatic mechanisms are instead connected to the failure of the stump to heal and might involve infection, failure of flap closure, bone spurs, vascular insufficiency, or soft tissue inflammation around the prosthesis [81].

Among the different phenotypes of RLP, neuroma pain deserves special attention since it can be perceived in the missing limb and thus considered as PLP. After disruption of a nerve, axons regenerate and sprout often in an unregulated fashion resulting in a tangle containing axons (both A and C fibers), Schwann cells, endoneurial cells, perineurial cells in a dense collagenous matrix and fibroblasts [123]. Wall and Gutnick [150] showed ongoing spontaneous activity in neuromas, in terms of increased firing rate, generated mechanically (by applying pressure) or chemically (by noradrenaline). Sherman [151] later showed that pain in the phantom can be initiated by spasms in muscles surrounding the neuroma, which would be analogous to applying direct pressure. Further, Nyström and Hagsbarth [152] demonstrated with microelectrode recordings in the peripheral nerves of human amputee, that when using anesthetic block the increased activity evoked by taps on the neuroma was eliminated together with the associated increase of PLP. In contrast, the spontaneous impulse activity in the nerve fascicle was left unaltered by anesthesia together with the spontaneous background PLP. Finally, neuromas can also lead to central sensitization thus causing pain perception in response to stimuli that normally would not provoke pain (e.g. signals coming from fibers other than nociceptive) [86].

As argued by Ortiz-Catalan [12], it is important to distinguish if the pain referred to the missing limb is actually due to a neuroma, since at present there are rather effective ways to treat this type of PLP [83], [84].

Beyond neuroma pain referred to the missing limb, PLP is a neuropathic pain condition with a multitude of factors playing a role into its origin and persistence. In the following a brief overview of these factors is given.

Peripheral mediators of PLP

Early theories attributed the PLS and PLP exclusively to the irritation of peripheral nerves. However, these theories have been abandoned over time in light of solid evidence showing that no anesthetic block can universally abolish PLS and/or PLP [153]. Further contributing to the dismissal of PNS as the only cause of PLS and PLP, anesthesia was also used in healthy subjects to actually induce phantom limbs [74].

Yet, as mentioned earlier, it has been observed that neuroma pain (and ectopic discharge at the severed end of the nerve) can contribute to PLP and nowadays it is acknowledged that peripheral factors do play an important role in initiating a cascade of events that lead to neuropathy.

For example, recent research has shed light on an additional site of ectopic firing in the dorsal root ganglia (DRG). The DRG contain the cell bodies of the first order afferent neurons and are located on the dorsal roots of the spinal nerve near the entry to the dorsal horn of the spinal cord. Following amputation, the DRG lose their receptors and nerve endings and might start firing spontaneously (ectopically), which in turn can amplify the discharges coming from the residual limb or initiate depolarization of neighboring neurons. Vaso *et al.* [154], by performing blockade of the DRG, showed a dramatic relief PLP and a decrease in PLS, thus suggesting that the PNS could be indeed considered as a viable component for a theory of PLP.

Spinal mediators of PLP

As briefly outlined earlier, the spinal cord has a major role in the development of neuropathic pain, with central sensitization as one of the main mechanisms that might be taking place following amputation. With specific reference to PLP, evidence of the contributions of the spinal cord to the perception of phantom limbs has been presented by Aydin *et al.* [155] who reported a case of a woman who had suffer from PLP for 60 years and experienced a progressive decrease in PLP in parallel with the growth of an intraspinal tumor. PLP gradually reappeared after resection of the tumor. Further, spinal anesthesia has been implicated in the development of PLP, causing it in patients who were previously pain-free [156].

Supraspinal mediators of PLP

Cortical reorganization is perhaps the most cited factor mediating PLP at the level of the brain. Kaas and Merzenich (1984) were the first ones to demonstrate that after digit amputation the deafferented cortex would undergo functional remapping, responding to stimulation of neighboring body regions [157]. Functional remapping has been shown to take place also in the thalamus [158], the brainstem [159] and the spinal cord [160], thus suggesting that some of the cortical reorganization might be indeed induced by the plasticity of subcortical structures [161], [162]. However, it has also been shown that changes at subcortical levels originate in the cortex, thanks to connections to the thalamus and lower structures [163].

Several brain imaging studies have later demonstrated a shift of the mouth representation into the hand representation in S1 of upper limb amputees [17], [52], [153], which have been used to explain phantom phenomena in the context of postamputation reorganization. For example, Flor *et al.* [17] also showed that the perceptual correlated to this reorganization is PLP: the larger the invasion of the mouth into the hand representation, the more intense the pain. This cortical shift, together with PLP, completely disappeared in some patients by using brachial plexus anesthesia that eliminated the peripheral input. This suggests that at least in some cases, cortical reorganization and PLP are maintained by the periphery [153]. However other studies using cortical stimulation have found loci on the S1 cortex of patients that if stimulated can elicit PLP and if removed abolish pain [164].

Contributions of subcortical structure to the perception of phantom limbs have been shown with studies performing thalamic micro stimulation and recordings in human amputees have shown that the reorganizational changes occurring at the thalamic level are closely related to the perception of PLS and PLP. Thalamic stump representation was found unusually large.

Moreover, in amputees with phantom limbs, thalamic stimulation could reliably evoke PLP and PLS even by stimulating those areas responsive to the stump, consistent with the hypothesis that the deafferented neurons remain functionally related to the missing limb.

In this chapter, the main aspects involved in the perception of phantom limbs and PLP were summarized. At the current state, it seems that both peripheral and central mechanisms are involved these phantom phenomena. Recent findings showing dramatic relief from both PLP and PLS following blockade of the DRG [154] suggest that role of peripheral factors in PLP might have been so far underestimated, however further evidence is needed in order to affirm the PLP depends solely on ectopic discharges from the PNS. Central aspects of phantom phenomena are more contradictory and involve reorganization of S1 and S2 (maladaptive or experience-dependent) and possible reorganization at every subcortical level.

Motor control

Under normal conditions, both voluntary and involuntary movements are the result of contracting muscles, which in turn are directed by the activity of neural circuits both in the brain and spinal cord. Voluntary movements of the limbs are made possible by said skeletal muscles, innervated by lower motor neurons (LMN), that have cell bodies in the ventral horn of the spinal cord grey matter. LMN activation is controlled by local circuits within the spinal cord, which receive direct input from sensory neurons (to mediate the sensory-motor reflexes) and are tightly interconnected. The local circuits of LMN are modulated by upper motor neurons (UMN), whose cell bodies are situated in brainstem centers (such as vestibular nuclei, superior colliculus, reticular formation) as well as in the cerebral cortex which controls the volitional aspect of the movements. This descending pathway, containing the projections of the UMN to the spinal cord, is called corticospinal tract (**Figure 12**, [165]), which is the largest descending tract present in humans [166]. The corticospinal tract is divided into anterior and lateral components. The anterior corticospinal tract innervates both contralateral and ipsilateral axial and proximal limb muscles, securing control of posture and balance. The lateral component of the corticospinal tract innervates the contralateral distal limb muscles thus mediating skilled movements. The decussation of the corticospinal tract takes place at the level of the caudal medulla.

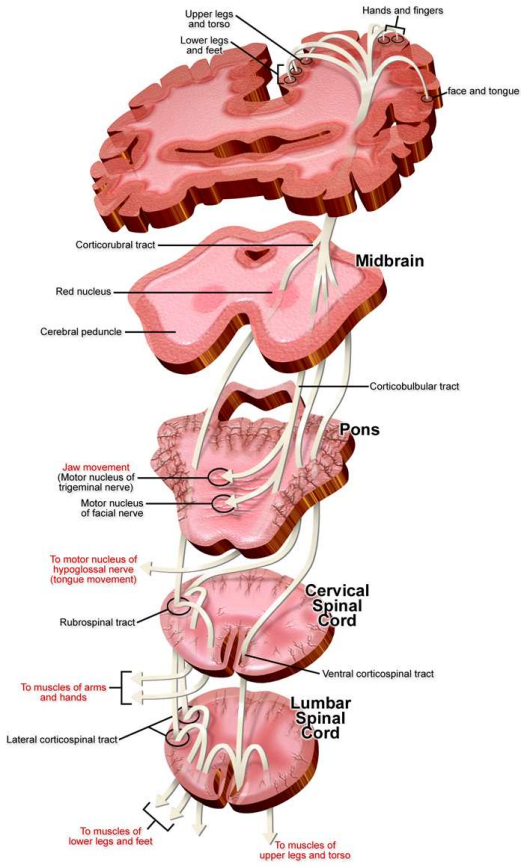

***Figure 12: Descending motor pathways**. Corticospinal and corticobulbar tracts. Source of the picture [165].*

Among the cortical areas involved in motor control, the primary motor cortex (M1) and a collection of

premotor areas in the frontal lobe, are responsible for planning and controlling complex sequences of voluntary movements. Two big subcortical structures, the basal ganglia and cerebellum, are also involved both in motor planning and execution. The basal ganglia regulate the UMN, by projecting to M1 via relays in the ventrolateral thalamus and mediating initiation of movements and selection of actions on the of the balance of risk and reward from possible behaviors [167]. The cerebellum oversees the detailed execution of each movement on a millisecond timescale and forms internal sensorimotor models used to predict the outcome of motor programs in terms of their sensory consequences. This enables procedural learning and acquisition of motor skills [167].

In the literature on phantom phenomena, M1 occupies a central role [11] and, for the purposes of this thesis, it is useful to describe its structure and function with more detail. M1 consists of BA 4 (see **Figure 10.B**), which takes up most of the precentral gyrus [168] and it presents a gross somatotopic organization matching that of S1 in most regions [168]. Evidence from a recent cortical stimulation study, the largest systematic mapping since Penfield [40], suggests that neurons in M1 represent specific movements or groups of agonist muscles involved in a movement [168]. Movements elicited by the stimulation are isolated (e.g., selective flexion of a single finger, raising of the contralateral eyebrow, etc), basic (i.e. no complex movements such as bringing the hand to the mouth as found by Graziano *et al.* [169] or grimacing as found by Penfield *et al.* [40]) and stereotyped (i.e., repeated stimulation induced the same movement). A well replicated finding is the presence of spatially separated sites activating the same individual movement or muscles, indicating that at a fine-grained scale there is no clear somatotopy, differently from what one could think by looking at the well-defined boundaries of the motor homunculus (see **Figure 10.C**) [168], [170].

Mirror neurons are a particular class of neurons that are activated not only by one's own actions but also by the actions of others [171]. They were originally found in the premotor cortex of macaques by showing that a small portion of cells in this area were responding both during the monkey's own grasping action and during observation of the same action carried out by a human [172]. Later studies have found evidence for the presence of mirror-like activity in the corticospinal neurons in M1, in both monkeys [173]–[175]and humans [176]–[178], leading to the conclusion that activity of these neurons must have consequences for spinal networks supporting voluntary movements [179]. Finally it is now thought that mirror neurons are widespread throughout the motor system, including ventral and dorsal premotor cortices as well as in different regions of the parietal cortex [171]. Regarding their functional role, a lot has been written about it to the point that they have been dubbed as "the most hyped concept in neuroscience" [171]. Given that they discharge shortly after the beginning of the observed action they seem to contribute to a rather low-level motor processing, rather than to goal interpretation [180]. In this regard, it has been found that action observation modulates motor corticospinal [178], intracortical [176] and spinal excitability [181] and it has been proposed that mirror neurons play a role in this facilitation.

Phantom movements are real movements

At amputation, motor neuros are severed, and their target muscles are removed. Studies on monkeys have shown that severed motor neurons previously supplying the distal muscles have the ability to reinnervate the stump musculature [182], however it is unclear the extent to which this takes place in humans. What remains uncontested however is that the representation of the amputated body part is preserved in the cortex, although it might undergo some degree of reorganization.

Phenomenologically, amputees can perceive kinetic sensations of voluntary or involuntary phantom movements [100]. The implication is that, when performing phantom movements, motor commands can still be issued and sent to periphery, even if the effectors are missing and no overt movement is performed. Neurological evidence for this comes for example from intraneural recordings of severed nerves formerly innervating the hand, showing motoneuron activity associated with missing limb movements [183]. Moreover, neuroimaging studies have shown that correlates of phantom limb movements are comparable to those of movements performed by intact limbs. For example a study showed that phantom execution activates the set of brain regions normally active during the execution of intact limb movements [184]. Another study has also shown that phantom movement produces the typical EEG signatures of positive volition required to initiate action and negative volition required to inhibit an action [185].

Motor mapping with Transcranial Magnetic Stimulation (TMS) has shown that stimulation of the deafferented M1 results in sensations of movement in the phantom hand accompanied by motor evoked potentials in stump muscles [186]. The same study also showed that phantom movements that were not accessible by the amputee under normal conditions, could be executed under TMS. Amputation causes reorganization even in the motor system and a systematic review on the use of TMS to map M1 showed an enlarged and shifted representation of the neighboring areas into the deafferented representation [187]. Similar results have been found also using cortical stimulation in an upper limb amputee [188] and animals [47].

Phantom mobility and PLP

Epidemiological studies trying to establish what are the protective and risk factors of PLP have repeatedly found a relationship between PLP and ability to perform phantom movements, however the results are contradictory. For example, some studies have indicated phantom movements as a protective factor [189], whereas others have indicated that phantom movements could be a risk factor [88].

A recent study has tried to uncover more information on phantom limb mobility by quantifying and characterizing its occurrence in a rather large sample of amputees [190]. The results suggested an interaction between the two phantom phenomena, although the relationship was not clear, leading the authors to concluded that the existence of pain does not exclude the possibility to perform phantom movements. For example, it was found that some patient reported either pain appearance or increase during voluntary movement,

whereas other used them to relieve pain. The study also reported that for repetitive phantom movements, the speed and amplitude were progressively limited by fatigue, eventually leading to a block of the movement, although with no implication for PLP.

Yet, other studies have found slower movements in conjunction to PLP, indicating that pain might generally influence the quality of phantom movements [191]–[195]. With regard to this, Kawashima *et al.* [196], have shown that movements carried out at a pace different than the preferred one, reach a lower level of attainment, thus become harder to execute. Finally, training phantom movements has been shown to reduce PLP [15], [197], make phantom execution easier and lead to improved speed and endurance [190].

In order to fully understand the relationship between phantom motor execution and pain however, it is also important to characterize when the phantom limb is frozen and incapable to move. This has been done in a recent survey [198] in which almost all amputees included in the study had PLP.

In general, the relationship between phantom motor control and PLP is still unclear, although there seems to be high interindividual variability. Furthermore, additional studies would be needed in order to establish what is the role of perceived effort, difficulty, fatigue and training in phantom movements and PLP modulation.

CHAPTER 6

Contribution of neuroimaging to the understanding of PLP

The past thirty years have seen considerable advances in the field of neuroimaging, where functional magnetic resonance imaging (fMRI) has taken up the role of main character. This has been also the case within the community researching PLP. In this particular context, fMRI has been especially used to re-address the question, originally answered using magnetoencephalography (MEG) [17], of what is the relationship between cortical reorganization after deafferentation and PLP. This chapter aims at giving an overview of the main findings of this specific line of research, discussing also what are the current challenges and how could the future of the field look like.

As already mentioned previously, Flor *et al.* [17] were the first to show, with a sensory stimulation paradigm and MEG, that after an amputation the severity of PLP and the degree of topographic reorganization in the somatosensory cortex were positively correlated. Since then, the study has been replicated with fMRI in patients with spinal cord injury [199] and CRPS [200] and in the motor domain [192], [201], using also EEG [202] and TMS [203][187].

More recently however, in a fMRI study comparing PLP patients with congenital one-handers and healthy controls, Makin *et al.* [204] found that stronger activations in S1M1 following phantom execution were correlated with the amount of PLP experienced by amputees. In follow up studies by the same group, it was also shown that higher PLP correlates with worse motor control of the phantom limb, which in turn produced stronger S1M1 activation [193]. PLP relief was found to be associated with decreased activity in M1 [14]. The authors of these studies concluded that the best way to explain these results was to challenge the main notion that cortical reorganization and PLP are correlated. To this end, they introduce the idea that persistent PLP is associated with preserved structure and functional organization in the former hand area.

As exhaustively argued in a series of three papers, Flor, Andoh and colleagues [19], [205], [206], explained why the methods adopted by Makin *et al.* in their series of studies, are not readily comparable to what was previously done in the literature. Briefly, choice of the regions of interest, patient sample, and type of stimulus adopted for the mapping are all confounding factors that need to be carefully evaluated before it is possible to lend support to the persistent representation idea. For example, Makin and colleagues used active phantom movements to map the motor cortex whereas previous studies have mostly used imagined or observed movements or brain mapping techniques that are not affected by the sensory reafference caused by muscles contractions (such as ERPs and TMS). Execution of phantom movements might involve activation of the M1 region representing the residual limb, which is anatomically different from subject to subject, and lead to reafference, which again activate

S1 in a way that is dependent on the individual anatomy, and different from the activation that would occur following external stimulation. Further phantom movements might activate also neuromas, yielding to a different pattern of S1 activation.

Another challenge is posed by the choice of the region of interest, which was shown to be inconsistent with previous literature and might explain why representational shift were not found (for more details, see the full study by Andoh *et al.* who analysed the impact of different methodological choices on the results [19]).

The main challenge with the choice of the task however is that phantom motor execution is an internally generated stimulus, rather than an external one (as for example movement observation is) and therefore it is contingent upon individual performance. As seen in the previous chapter, it is rather established that pain affects performance and that there are inter-individual differences in the relationship between PLP and motor control, although there seem to be consensus on the fact that movements are slower and of worse quality [191]–[195]. The measure used to indicate persistent representation is increased intensity of the activation within the former hand area. Yet this measure might reflect increased effort/difficulty or increased fatigue during motor execution, especially because movements are externally paced, rather than represent a direct relationship to pain. For example, it has been shown that there is a strong positive correlation between motor input (activity in motor regions in the brain) and motor output (muscle activity and contraction force) [207] and that the motor fatigue leads to increased fMRI activation [208].

All in all, these considerations suggest that the results shown in [193], [204], [209] might be best explained by the differences in the methodology rather than by PLP.

Challenges and road ahead

The cortical reorganization correlate to PLP has been widely investigated with fMRI and mass univariate analysis. This type of approach allows us to arrive only to correlative results, meaning that by itself it doesn't support conclusions related to the causality of the effects observed. Related to this, it must be considered that maladaptive plasticity related to pain is not the only change taking place in the nervous system following amputation, and that experience-dependent plasticity related to the compensatory use of the amputated and the intact limb (which could be affected by pain) might also take place and confound the results. Therefore, after all these years studying cortical reorganization and PLP, there is still no idea of how pain and reorganization are linked.

This type of analysis is concerned with determining whether there exists a significant population-wide effect, however with small samples (<20) this effect might be overestimated [210] while at the individual level it remains unclear what is the exact correspondence between cortical reorganization and pain. Cortical reorganization is not a measure that can be used diagnostically and when looking at the brain scan of a patient in pain, we would not be able to quantify the amount of PLP (*i.e.*, it is not possible to make reverse inferences).

Overcoming these challenges, probably entails a shift of focus to new experimental designs, imaging techniques, and analytical methodologies. For example, the use of causative

techniques such as TMS or tDCS might help to establish how PLP relates to cortical reorganization or any other brain change. The use of machine learning instead of mass univariate analysis could help to move from group results to biomarkers of PLP that can be used to detect and quantify pain in individuals. Finally, it has been recently advocated that the experience of PLP might be best captured by adopting a dynamic network perspective accounting for the dynamic interaction of both central and peripheral factors [211]. In this respect, resting state paradigms and EEG, which has a better time resolution compared to fMRI, might be more appropriate methods to study the temporal and spatial pain-related changes affecting brain networks. As a part of this doctoral thesis, the use of machine learning for detection of PLP in resting state EEG was explored.

CHAPTER 7

Explanatory hypotheses of phantom limb pain

As mentioned in the chapter dedicated to the historical context, numerous theories and models of PLP have been proposed through the years as our understanding of the nervous system progressed. Strictly speaking, most of these have not been properly formulated as hypothesis, theories, or models, yet these words are commonly found in the literature and are used in interchangeably. Here, I use the word model unless it has been properly formulated in other way. Whereas some models have been completely abandoned, the scientific conversation on the main factors and mechanisms of PLP is more alive than ever. The present chapter provides an overview of some of the mechanistic theories for the origin and maintenance of PLP, which are reported by the recent scientific literature [9], [12], [13].

Maladaptive plasticity and cortical reorganization

The cortical reorganization model is possibly the most popular among the proposed ideas on PLP, and it was initiated by the seminal work by Flor *et al.* (1995) on neural correlates of PLP. In their work, Flor and colleagues found a strong positive relationship between the intensity of PLP and the amount of cortical reorganization, and the observed reorganizational changes consisted in the invasion of the deafferented S1 cortex by neighboring cortical representations[17]. Other studies replicated the finding in S1 [212], [213] and further showed similar reorganizational patterns into M1 cortex [203]. The findings of Flor *et al.* themselves could not establish a causal link between reorganization and PLP, however later studies have shown that reduction of PLP was accompanied by a rather fast normalization of the cortical representation [153]. Further, it was also found that functionally relevant sensory discrimination training [214] and intensive use of a myoelectric prosthesis [201] led to reduced reorganization and PLP, strengthening the relationship between cortical reorganization and pain. Yet, a recent systematic review of fMRI studies, found that the positive correlation between PLP and cortical reorganization was not always replicated [18]. The cause for this might be found in methodological differences [19], [205], [206], and it has been highlighted the need for further studies, in particular longitudinal ones, capable of assessing the effect of pain-modulating interventions and to establish a causal link [18].

Much has been written on this topic, to the point that the cortical reorganization as a neural correlate of PLP has started to be considered -erroneously- as a standalone model for PLP (see for example [9], [13], [215]). In contrast, several reviews and opinion articles by Flor (and colleagues) [79], [123], [211], [216], [217] have repeatedly argued that cortical reorganization was one, among others, important variable at play and have further proposed comprehensive models of PLP. For example, early work ([79], [123], [216], [217]) has highlighted the possible

role of nociceptive input prior the amputation in priming the nervous system and leading to reorganization, thus subscribing to the painful memory model of PLP (reviewed later in this chapter).

Painful memories

Katz and Melzack (1990) are often credited with being the first to describe the concept of pain memories in phantom limbs. They observed, both by reviewing the literature and by conducting a retrospective study, that patients often report their PLP to resemble distressing pre-amputation pains experienced *at* or *near* the time of amputation [125]. This led the authors to hypothesize that the experience of pain at or near the amputation time causes lasting changes in central neural structures and that these changes are in turn associated with cognitive-evaluative memories of the pre-amputation pain. The model was later adopted by Flor, who used it to interpret the cortical reorganization found in PLP patients as the neural footprint of such painful memories [216]. Flor and colleagues [79] further elaborated the model clarifying that pain memories can also be implicit, i.e. consisting of changes in the brain that are not open to conscious awareness but can still lead to behavioral and perceptual alterations.

Evidence in support of Katz and Melzack's version of the hypothesis is mixed: some prospective studies have shown that chronic pain before the amputation predicts later PLP [79], whereas others have found weak [88] or no relationship at all [218]. Yet, formulating the hypothesis in terms of implicit memories, as Flor did, makes this kind of evidence irrelevant for the purposes of testing its validity. In general, the essence of the hypothesis is the idea that nociceptive input *prior* or *at* the amputation can prime the subsequent neural changes taking place peripherally, spinally, and supraspinally and that would later sustain PLP chronically [79], [206]. Support for this view comes from a recent work with TMS showing that compared with no pain, application of tonic pain prior to temporary ischemic hand deafferentation (induced by inflation of an arm cuff) increased corticospinal excitability in healthy participants [219], which has been related to phantom limb pain [203].

Cortical reorganization then is not the direct cause of PLP but subtends the establishment of painful memories. Understanding cortical reorganization in these terms is in agreement, and not in opposition as reported elsewhere [215], with the observation that stimulation of S1 in healthy subjects does not normally elicit pain. Indeed, for this to happen, electrical stimulation has to be applied on a pathologically altered S1, such as that one of a PLP patient. To date, the available evidence suggest that this not only is the case, but resection of those sites might abolish pain, at least temporarily [164].

A corollary to this hypothesis is that not only the circumstances of that led to amputation (whether pathology or traumatic event), but also the surgery itself might matter for the development of pain, as it is confirmed by the fact that persistent postsurgical pain is known major complication of a wide variety of procedures such as thoracotomy, mastectomy, and c-section to name a few [220].

Neuromatrix

During the early 90's Melzack proposed the neuromatrix framework as an attempt to explain the brain activity underlying the perception of phantom limbs [98], [221], theory that was later adopted to account for general perception of the intact body [222]. Melzack noted that there was already general agreement on the notion that phantom phenomena cannot be explained by peripheral mechanisms alone and that their origin ought to be sought in the brain. However, he also believed that it was wrong to solely focus on S1. For this reason, he proposed a distributed network of neurons, the neuromatrix, as the substrate for the neurosignature, characteristic pattern of activity encoding the state of the body. Melzack acknowledged that his formulation of the neuromatrix echoed Hebb's theory of cell assembly, but he clarified that the neuromatrix departs from the former in that connections between neurons of the matrix are genetically dictated and not forged by experience [98], [221]. Still, he did not exclude a role for Hebbian plasticity, hypothesizing that the hardwired matrix could still be partially sculpted by experience-dependent plasticity (explaining the painful memories). Melzack's neuromatrix encompasses several structures including thalamus, somatosensory cortex, reticular formation (in the brain stem), the limbic system, and the posterior parietal cortex. The neuromatrix takes several different sources of input while outputting the neurosignature, which accounts for everything we feel (see **Figure 13**). The landmark feature of this framework is that sensory inputs can only modulate the neurosignature, but do not directly cause perception. As Melzack put it:

"Phantoms become comprehensible once we recognize that the brain generates the experience of the body. Sensory inputs merely modulate that experience; they do not directly cause it." [221]

Although this framework offers a complete account of the complexity of pain by considering its genetic, emotional, cognitive, and sensory aspects, it does so by positing that the neuromatrix - as a whole - is involved in pain perception: a claim too broad and unspecific to be either falsifiable or of practical utility. Melzack himself put forward a couple of more specific and testable ideas on the cause of PLP, which fit within the neuromatrix framework. For example, he hypothesized that the neuromatrix, when deprived of its modulating sensory inputs from the limbs or body, produces an abnormal neural pattern that is interpreted as heat or burning pain. He also proposed that cramping pain is instead the result of efforts from the neuromatrix to produce limb movements. In absence of reafference, which is a form of modulating sensory input, the neuromatrix issues stronger and stronger commands that eventually are perceived as cramping pain. The prediction of the first hypothesis clearly is that restoring the normal sensory input to the neuromatrix should eliminate heat and burning PLP. The second hypothesis instead should lead to the conclusion that providing feedback to the execution of phantom movements should reduce the cramping quality of PLP. Finally, since input to the neuromatrix could come also from the emotional and cognitive components, this framework also speaks to the need of a holistic approach for treating PLP, which encompasses all the components.

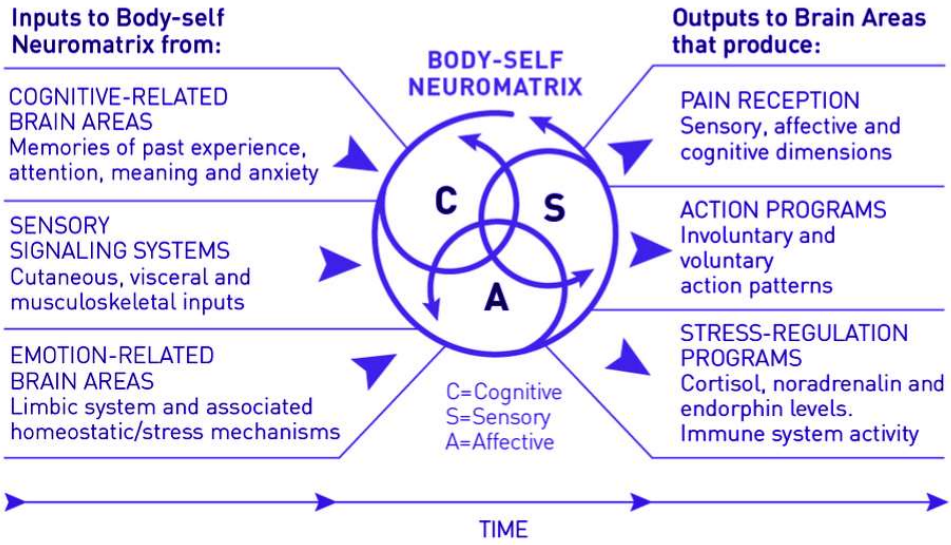

Figure 13: Pain neuromatrix. *Factors that contribute to the patterns of activity generated by the body-self neuromatrix, which comprises sensory, affective, and cognitive neuromodules. The output patterns from the neuromatrix produce the multiple dimensions of pain experience as well as concurrent homeostatic and behavioral responses. Source [187].*

Stochastic entanglement

The stochastic entanglement hypothesis, proposed by Ortiz-Catalan [12], tries to explain aspects of PLP that have been left uncovered by other theories, by accounting for example for how changes in the sensorimotor systems come to be experienced as pain. The hypothesis regards the motor and sensory circuitry as a complex dynamical system that if seriously perturbated (i.e. by amputation) enters a susceptible state in which it becomes "entangled" with the circuitry dedicated to pain perception due synchronized stochastic firing: firing coinciding temporally and spatially. In this framework, PLP arises when the pain neurosignature is "entangled" with the sensorimotor network and, similarly to Melzack's neuromatrix theory, the stochastic entanglement can fully account for the multidimensionality of the experience of pain, including emotional and cognitive components.

A fundamental feature of this hypothesis is that the evolution of such a complex system, depends on its initial conditions upon entering the susceptible state (at amputation), thus accounting for the portion of patients that never develop PLP. Another important feature of the hypothesis is the prediction that PLP is relieved by 1) undoing the entanglement, which is understood in terms of Hebbian cell assembly (i.e. "stochastic entanglement can be conceived as a function of Hebb's law"), and 2) repurposing the affect neural circuitry and thus prevent it from underside entangling with pain processing. It is proposed that one way of undoing the entanglement consists in the inverse of Hebb's cell assembly theory "neurons that fire apart wire apart". For example, it is proposed that reengaging the sensorimotor network in a functionally meaningful way, either by exercising phantom movement or by sensory restoration through sensory feedback. In this view, pain arises from co-activation of the

sensorimotor and pain circuitry and pain relief would require of activation of one network without the other. Yet, a point to be clarified is how purposeful training can lead to less pain rather than more if it is carried out when a person is in pain: PLP is a chronic condition, often constantly present, and at times it is triggered by phantom motor execution [190], training under this condition should entangle the two networks even further rather than disassociate them.

The stochastic entanglement hypothesis has the merit of widening the framework for understanding pain: the mechanisms underlying PLP are probably too complex to be understood solely by using neuroimaging in search of correlates. Regarding the nervous system as a complex dynamical system is an approach that could do justice to its complexity and such an alternative focus seems timely and much needed. Ongoing work is concerned with the project of grounding the hypothesis in more precise neurophysiological terms. This is done in conjunction with the development of a computational model that could help with formulating testable predictions [223].

Sensorimotor incongruence

Based on Ramachandran's work [53], Harris (1999) suggested that PLP and many other chronic pain states are the result of a mismatch between motor intention and the perception of movement which is realized, for example, in presence of conflicting information about motor intention, awareness of movement, and visual feedback [224]. Harris emphasized the importance of visual feedback bought in mediating the incongruence and in treating pain states that arise from it. Yet, incongruent visual feedback is only one possible way of realizing sensorimotor incongruence, even though it is certainly the most adopted one in experimental paradigms owing to the simplicity of its implementation (for experimental paradigm without the use of visual feedback see [225]). Related to this, Reilly and Sirigu, based on the observation that severed motoneurons previously supplying distal muscles can reinnervate stump muscles [182], hypothesized that the reafference from these muscles could restore a modified version of the sensorimotor loop and help reduce the mismatch between motor commands and the expectation of their sensory consequences [11]. This, according to Reilly and Sirigu [11], could also explain why the ability to move the phantom voluntarily is associated to low levels of PLP.

When it comes to the evidence in support of the hypothesis, the notion that the nervous system has areas responsible for multimodal processing that can detect sensorimotor incongruences has been long established, and the importance of this ability has been charted in detail for motor control and proprioception (for review see [226]). Yet, to date its role in the generation of pain remains to be convincingly established. The first attempt to test the sensorimotor incongruence hypothesis was carried out by McCabe and colleagues, who induced sensorimotor incongruence between motor intention and actual movement by placing a mirror or whiteboard between the limbs of healthy subjects while they were moving their limbs [227]. During the task some patient reported low level of pain, leading the authors to support the possibility that sensorimotor incongruence can evoke pain. The far reaching conclusions of the study were challenged by Moseley and Gandevia [228] who referenced problems in the methodology as potential sources of bias. Following this, several studies have

been carried out and beyond some successful replication, there have found minimal reports of pain when visually mediated sensorimotor incongruence is induced in healthy volunteer [229], [230]. More importantly, no study has found a positive relationship between the intensity or frequency of pain and the extent of sensorimotor incongruence.

Finally, in order to explain the mechanism linking sensorimotor incongruence to activation of the pain circuitry, Harris noticed that it is the same mechanism that gives rise to nausea when there is a discrepancy between vestibular and proprioceptive sensations of body balance and displacement and vision [231]. Yet even this point lacks validation, for example by showing that the same medications used for motion sickness can be used for PLP.

Adversarial collaboration

The models presented in this chapter are a non-exhaustive selection of the many ideas that cohabit the literature at this stage. Although each one of them adds new insights to the debate and all of them find some level of support in the experimental data, at present no model is capable to fully account for all the aspects of the disease in a testable way. Perhaps, the ultimate theory of PLP will involve a combination of many models, yet in order to achieve this, it is important to understand which aspects of the different ideas hold up to scientific scrutiny and which do not.

Currently, as data accumulates with new studies, models evolve to better fit the new evidence. This however is done without much crosstalk apart from the scientific debate carried out in the format of critique–reply–rejoinder, usually aimed at highlighting own strengths and/or the other's shortcomings. However, solving a complex problem such as understanding PLP might require a completely different dynamic among advocates of opposing hypotheses. Championed by Kahneman in the field of behavioral economics [232], adversarial collaboration is the approach currently adopted by a group of researchers trying to solve the hard problem of consciousness [233].

Briefly, adversarial collaboration consists in having scientific adversaries working together for identifying the most diagnostic point of divergence among competing theories. Researcher would then reach precise agreement on the predictions of every theory so that experiments aimed at directly testing the diverging predictions can be designed. In order to avoid any source of bias, other researchers than those directly associated with the theories would design the experiments and finally the experiments would be conducted simultaneously and in the same way in several independent labs, which would then make the data publicly available.

In the worst-case scenario in which no theory prevails, this type of work would end with some new facts accepted by all and narrower differences of opinions. Under the best of the circumstances, adversarial collaboration can instead lead to an agreed-upon theory of PLP. In either case, research on PLP would greatly benefit from this approach, if anything in terms of cost efficiency and speed with which we come up new ways to treat or even prevent PLP.

CHAPTER 8

Treatments for phantom limb pain

Over the past 50 years, numerous approaches have been attempted for treating PLP: a survey carried out in the '70s identified 68 different methods of which as many as 50 were in use [234]. More recently, it has been estimated that more than 25 treatments for PLP are currently available [235], yet there is still no consensus on whether there exists a treatment clearly superior to others, probably due to the scarcity of high-quality RCTs [10], [235]. Generally, treatments for PLP are divided into pharmacological and non-pharmacological, which can be further distinguished between surgical approaches and conservative therapies [10]. In the following, an overview of different approaches for all the three categories is given.

The evidence in favor of pharmacological interventions for PLP is currently limited. The best available evidence suggests that morphine, gabapentin, and ketamine provide pain relief compared with placebo only in the short-term, at the cost of moderate side effects [236]. Furthermore, pharmacological interventions carry the non-negligible risk of establishing an addiction.

For a long time, surgical approaches have been considered a costly and ineffective way to treat PLP [10]. Nonetheless, more recent techniques such as targeted muscle reinnervation (TMR) have been advocated as an effective way to treat neuropathic pain following amputation [237], [238], with evidence from an RCT supporting the use for PLP relief. Yet, the improvement in PLP by TMR remains partial.

When it comes to conservative non-pharmacological techniques however, the landscape is more heterogeneous. Some approaches for example try to tackle the psychological mechanisms maintaining PLP, such as eye movement desensitization and reprocessing; hypnotherapy; and cognitive behavioral therapy. Other approaches are based on guided-plasticity and try to reverse the maladaptive changes that sustain PLP. In this category techniques such as repetitive transcranial magnetic stimulation; mirror therapy; sensory discrimination training, phantom exercises, and graded motor imagery (GMI) can be found. Finally approaches based on alternative medicine are also common and examples are limb covers; reflexology; and acupuncture.

Two recent systematic reviews [10], [235] have looked at all RCTs available and concluded that there is only limited evidence in support of any of these intervention. Among all, GMI and hypnosis; however due to a lack of high-quality consistent findings, no firm clinical conclusions could be made. All in all, making progress in the field requires high quality research and new treatments need to pass the bar of a placebo control.

Phantom Motor Execution as a treatment for PLP

Pure phantom exercises, without visual feedback from mirrors or VR, were used to treat PLP by Ülger *et al.* [197]. In this work, repetitive phantom movements were carried out simultaneously with the intact limb and it was shown to be more effective than routine prosthetic training and a general exercise. The concept was later formalized by Ortiz Catalan [15], who dubbed it as phantom motor execution (PME). PME is a technique to treat PLP which consists in producing phantom movements. As previously mentioned, this leads to recruitment of the appropriate central and peripheral circuits, ultimately resulting in muscular activation at the stump.

The working hypothesis of PME is based on the stochastic entanglement model of PLP [12]. Briefly, by executing real physiological movements the subjects engage both the original motor circuitry corresponding to the missing limb, as well as circuitry related to the control of the stump musculature. This would potentially lead to invasion of the stump representation into the phantom cortex, and in the case of phantom hands, this would also lead to preservation of the border with the lip representation since the phantom cortex is now activated. By reengaging the phantom cortex in a purposeful manner, PME has further the capacity of subtracting neural resources that would otherwise be entangled and utilized for pain processing.

The main point of the therapy is to exercise volitional phantom mobility, and anthropomorphic visual feedback is not required for this, although it can facilitate the execution. For the purposes of facilitation even a mirror could be used, however it provides little feedback to the subject concerning the performance of the movement. For this reason, PME facilitated by virtual/augmented reality or serious gaming controlled via motor decoding techniques, has the potential of yielding better clinical outcomes. This implementation of PME was first evaluated in a patient with chronic intractable PLP in 2013 [16] and later in a multi-center clinical trial on a similar patient population of chronic intractable PLP sufferers [15].

Yet further evidence coming from a properly controlled trial is required in order to be able to draw clinical conclusion about the suggested use of PME. The mechanism of action of PME is the complete engagement of the motor system, which ultimately yields muscle contractions. Action observation, as described previously in Chapter 5, engages the same cortical structures (though to a lesser extent) but does not result in muscle contraction: hence, it does not engage the motor infrastructure comprehensively as PME. Therefore, action observation can be a good control condition to establish the analgesic effect of engaging in volitional control of the phantom limb. Further, an RCT pitting the two approaches against each other would help in gaining further insight into the condition itself. Speculatively, superiority of PME to action observation for example could mean that the structures that are relevant for PLP relief are found in the spinal cord or periphery. No difference between the two could instead mean that the mechanisms responsible for pain relief are equally engaged and thus must be found in supraspinal structures. Much of the work of this doctoral thesis has been concerned with the implementation of this RCT. The following chapter gives more background on the technique and presents some preliminary results available at the time of writing

CHAPTER 9

Providing clinical evidence for the use of PME as a treatment of PLP

Preliminary evidence of a PME's efficacy and safety has been found in two earlier studies. To make these findings confirmatory and to establish whether they provide adequate basis for the use of PME in clinical practice, a randomized controlled trial is necessary.

The main objectives are to guard against the effect of possible sources of bias such as improvements in pain that are due to contextual factors of the therapy rather than to the active treatment component. Another objective is to generalize the findings to a broader population (i.e. do the preliminary efficacy and safety results from earlier studies apply also to lower limb patients?). Most of the publications appended to this thesis are concerned, directly or indirectly, with the aim of providing unbiased evidence for the use of PME as treatment of PLP. The rest of the chapter is concerned with giving a background and motivation to this work, together with giving a brief overview of some preliminary results of the trial.

Enabling Phantom Motor Execution

The clinical implementation of this treatment uses myoelectric pattern recognition (MPR) to decode the myoelectric (EMG) signals produced during the execution of phantom movements and enable the control of a VR/AR environment.

Briefly, a typical MPR control system usually consists of the following components (see **Figure 14**):

- Data segmentation: EMG signals, due to their randomness, cannot be used directly (sample by sample) as input of the classifier, but they must be reduced to a more suitable form. This simplification of the signal is implemented by apply a transformation to a larger "chunk" of the signal, also called as "window".
- Feature extraction: The segmented data is then mapped into smaller dimension vectors by computing a set of pre-determined features. The feature vectors are used as input of the classifier.
- Classification: A pattern recognition algorithm classifies the features into pre-defined categories.
- Controller: Uses the output of the classifier to generate commands to control an artificial device. Post-processing techniques, such as majority voting, can be applied

after classification to dampen the effect of misclassifications and smoothen out the output

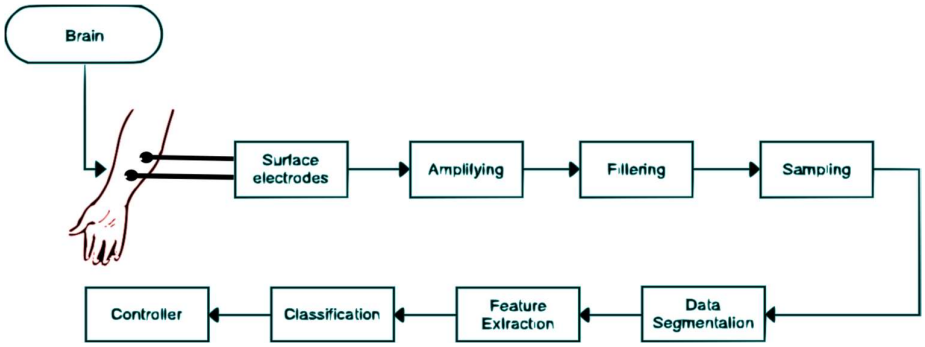

Figure 14: *Flowchart of a typical myoelectric pattern recognition system*

Successful MPR and successful implementation of the therapy are crucially dependent on the diversity of the patterns produced during different motions and the quality of the EMG signals recorded from the stump musculature. Diversity of the patterns depends in part on the level of amputation: the more proximal the amputation, the fewer muscles and control sites are available. Quality of the EMG signals instead is more elusive: it is known that even different electrode locations over the same muscle can yield signals with considerably different features and quality (e.g. Signal to Noise Rratio (SNR)). This is due to the fact that the signals are heavily influenced by several factors such as:

- Location of the electrodes over the muscle: surface EMG signals are affected by the placement of the electrodes with respect to the innervation zones (IZs). When recording with a bipolar channel (see Figure 15) over an IZ, any small displacement of the sensors with respect to the IZ will affect the signals [239]. It is therefore advisable to place the two surface electrodes between the IZ and the tendon. This is easily done in muscles having fibers running parallel to each other where the innervation zones are generally distributed in a narrow band around muscle belly [240]. More challenging could be the electrode placement on muscles with complex structure, such as pennate muscles, where the innervation zones are scattered over the muscle [240].
- Thickness of the subcutaneous tissue layers: EMG signals are particularly influenced by the depth of the subcutaneous tissue over which the surface electrodes are placed. In particular, surface EMG (sEMG) signals are attenuated in the subcutaneous tissue and its thickness, which greatly varies among subjects and among body parts, partly explains the variance among individuals in sEMG amplitude [241].
- Inter-electrode distance: In a bipolar configuration, two electrodes are placed in proximity; the distance between them is called inter-electrode distance (IED) and can

also impact the quality of the signals. As the IED increases, the magnitude of the target signal and the magnitude of the crosstalk signals (signals coming from muscles other than the targeted one) also increase [242]. The ideal IED is reached with a trade-off between maximizing the target signal and minimizing the crosstalk signals.

- Orientation of the bipolar channel relative to the direction of the muscle fiber: Optimal surface EMG signals are recorded with surface electrodes orientated parallel to the muscle fibers and therefore parallel to the direction of the action potential propagation [243]. By using a monopolar configuration (see Figure 16), the problem of the electrode alignment with the fibers is bypassed.

Consideration of all these factors allows to understand why correct placement of electrodes can be challenging. To tackle this problem a widely referenced report from the Surface EMG for Non-invasive Assessment of Muscles (SENIAM) initiative contains a set of guidelines covering exhaustively the subject [244], and its use is highly recommended in the common practice of sEMG.

Yet, within clinical practice, electrode placement can be complicated by additional factors. For example, people with amputation have fewer muscles and altered anatomy. Often, parts of the stump are covered by scar tissue resulting from the amputation: scar tissue increases the skin impedance, while pre-gelled adhesive electrodes do not stick well detaching easily. Recording signals form patients with lower limb amputation has additional challenges connected to the higher amount of soft tissue and to the muscle's fibers orientation (for example in quadriceps which are pennate muscles), which is hard to follow when placing electrodes.

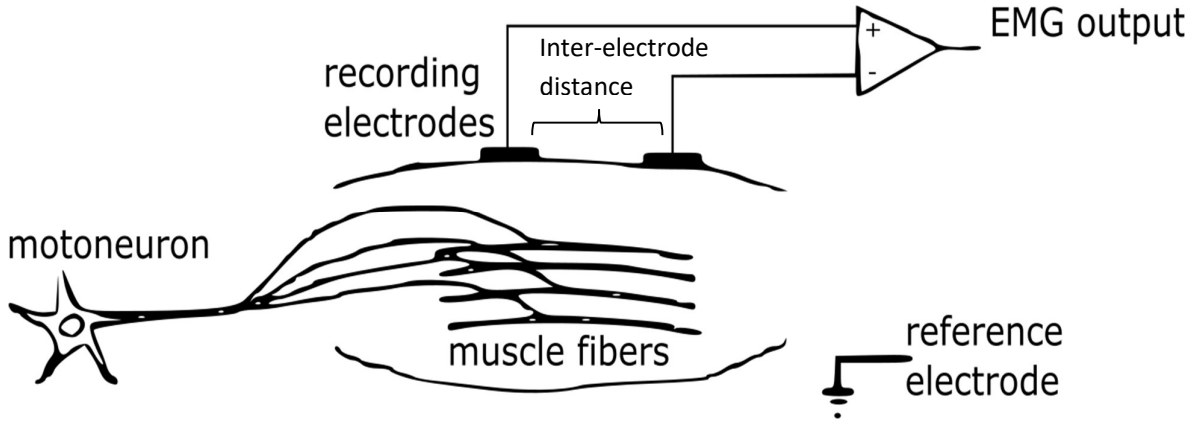

Figure 15: Bipolar configuration*. Image re-elaborated from* [248]

In the perspective of a rehabilitative application used routinely, the electrode placement procedure can become too challenging and time consuming and hinder not only wider clinical adoption, but also the implementation of the RCT. More time spent placing electrodes means less time spent doing the therapy, while lower quality of the signals means increased difficulty for the patient and less reliable results. Solving the challenges connected to the extraction of motor volition via MPR was instrumental for the implementation of the RCT, and the work related to this is presented in papers A, C and D.

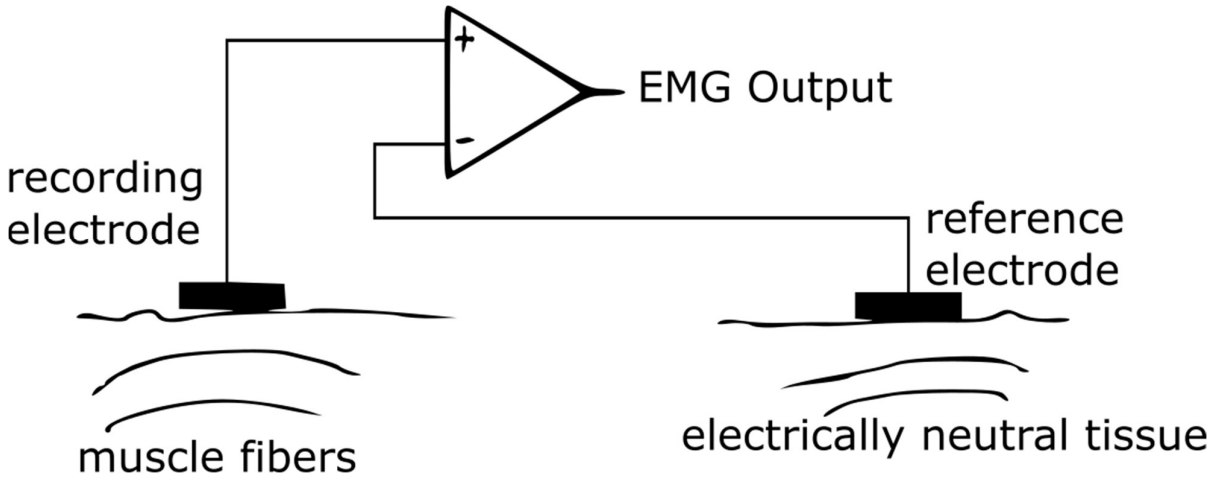

Figure 16: Monopolar configuration*. Image re-elaborated from* [248]

Implementation of the RCT

Preliminary evidence for the use of the PME treatment in a lower limb amputee has been shown in Paper A. This has supported the implementation of an RCT comprising patients with both upper and lower limb amputations. When planning and implementing the study, we have strived to identify and control for any possible source of bias. The trial has been prospectively registered on www.clinicaltrials.gov before the beginning of enrolment and the protocol has been published as a journal publication (Paper B). This has been done in order to provide the research field with comprehensive details, making the methods easily replicable. Finally, to guard off from selective outcome reporting and post-hoc analyses we have prospectively defined and made public the Statistical Analysis Plan (SAP) (Paper E).

Preliminary results

Currently the enrolment phase of the RCT has been concluded and all the patients enrolled have received 15 treatments sessions, thus allowing to lock the database containing data from visit 0 to visit 15. The follow up phase is still ongoing and prospected to conclude in September 2021. As specified in the SAP, the theory of fixed sequential multiple testing is used for accounting of the multiple comparisons, and it is also used to determine whether results are confirmatory. However, at the time of writing not all the secondary outcomes required to carry out the full sequence of tests are available. Therefore, only results pertaining to the

primary outcome are reported in this thesis. To fully makes sense of the results, the reading of Paper B and E is suggested before moving to the next section.

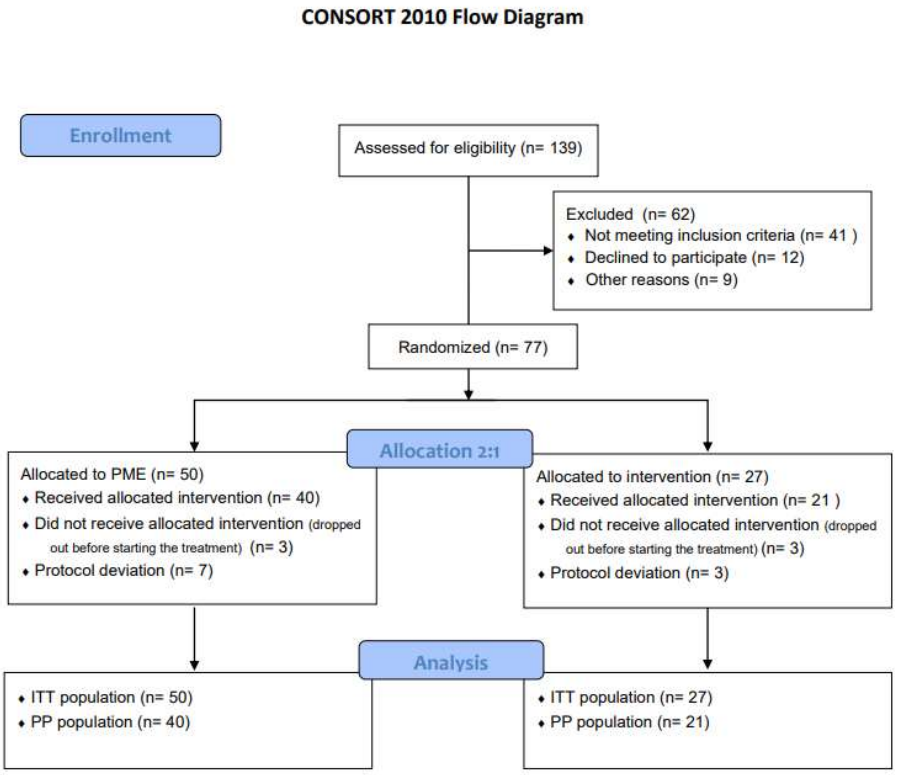

Figure 17: CONSORT flowchart. *Abbreviations: PME: Phantom Motor Execution, PMI: Phantom Motor Imagery.*

Primary outcome and statistical methodology

Information about screening, randomization, treatment adherence and inclusion in the analysis populations is presented in the CONSORT Flowchart in Figure 17.

The primary efficacy variable of the RCT is the Pain Rating Index (PRI). The PRI is a continuous variable computed as the sum of the scores for all descriptors of the Short Form of the McGill Pain Questionnaire (SF-MPQ). The outcome measure is evaluated as change in PRI score between baseline (visit 0) and end of treatment (visit 15). The Table 3 summarizes the PRI distributions by reporting the mean, SD, median, Q1, Q3, minimum and maximum. As specified in advance in the study protocol [245], the main analysis was performed using the intention to treat (ITT) population and is unadjusted. The comparison of the difference between the two randomized groups was carried out using the Fisher's non-parametric permutation test. In order to carry out the analyses on the ITT population, missing data (patients that dropped out of the study before completion of the 15 treatment sessions) was imputed and for this the last observation carry forward (LOCF) approach has been used (as per protocol).

Table 3:Summary of PRI scores. *Comparison of values between pre- and post-treatment in the groups*

		PME			PMI		
		Mean (SD)	*Median (Q1-Q2)*	*(Min - Max)*	*Mean(SD)*	*Median (Q1-Q2)*	*(Min - Max)*
PRI (ITT)	*Baseline (Visit 0)*	14.8 (8.68)	15.5 (8 -20.75)	(0-33)	14.85 (7.24)	16 (9-19.5)	(1-31)
	End of treatment (Visit 15)	5.9 (7.79)	4 (0-7)	(0-33)	7.44 (9.31)	5 (1-8)	(0-37)
PRI (PP)	*Baseline (Visit 0)*	14.48 (7.69)	15.5 (8-20)	(0-30)	14.05 (7.21)	16 (8-19.5)	(1-31)
	End of treatment (Visit 15)	4.95 (5.87)	4 (0-7)	(0-28)	4.62 (5.7)	3(1-7)	(0-26)

Abbreviations: *PRI: Pain Rating Index, PME: Phantom Motor Imagery, ITT: Intention To Treat; PP: Per Protocol*

The following sensitivity analyses were also carried out:

1. Assessment of the robustness of the treatment effect, consisting of an adjusted comparison between two groups, using the analysis of covariance (ANCOVA). Intervention/control were set as independent variable and the baseline characteristics used for randomization as covariates. This analysis was conducted on the ITT population and using the LOCF for handling missing data.
2. The impact of the imputation method, consisting of the same analysis as point 1 but using multiple imputation with 50 datasets.
3. The impact of missing data, consisting of an unadjusted comparison on the Full Analysis Set (FAS) using the same statistical methods as in primary analyses without imputing missing data.

Finally, to complement the analyses changes within groups were also assessed, and this has been done using Fisher's non-parametric permutation test for paired observation. All statistical tests conducted were two sided and at the 5% significance level.

Table 3 contains a summary of all the analyses that have been carried out, while Table 4, Table 5, Table 6 and Table 7 report the results of these analyses. In particular, the results are reported as p-values, effect sizes and 95% CI between the two groups when applicable. Analysis #5 should be performed on the FAS population, however in this clinical trial the FAS population is the same as the PP population. For this reason, analysis #5 yield the same results as analysis #6.

Table 4: Summary of the statistical analyses.

Outcome	Population	Missing data	Comparison	Test	Analysis #
PRI	ITT	LOCF	between groups	two-sided Fisher's non-parametric permutation test	1
PRI	ITT	LOCF	*within group*	Fisher's non-parametric permutation test for paired observation	2
PRI	ITT	LOCF	between groups	ANCOVA (PLP NRS, Level of amputation)	3
PRI	ITT	multiple imputation	between groups	ANCOVA (PLP NRS, Level of amputation)	4
PRI	FAS		between groups	two-sided Fisher's non-parametric permutation test	5
PRI	PP		between groups	two-sided Fisher's non-parametric permutation test	6
PRI	PP		*within group*	Fisher's non-parametric permutation test for paired observation	7

Abbreviations: *PRI: Pain Rating Index, ITT: Intention To Treat; PP: Per Protocol; FAS: Full, Analysis Set, NRS: Numeric Rating Scale.*

***Table 5: Results** of the between group comparisons listed in Table 4.*

Analysis #	Between-groups Results		
	95% CI	**p-value**	**effect size**
1	N.A.	0.51	1.58
3	[-3.2924, 6.3509]	0.53	1.53
4	[-4.9721,4.3674]	0.85	0.44
6	N.A.	0.93	0.21

Table 6 contains the complete results from the ANCOVA analyses. In particular, analysis #4 utilizes the multiple imputation method in order to make up for the missing data. In this case CI, p-value, and effect size are all calculated by taking an average of ANCOVA outcomes from 50 imputed data sets where final trial PRI is drawn from a distribution containing only data from the last trial. In this ANCOVA analysis, interaction terms were insignificant, thus affirming the ANCOVA assumption, so these terms were removed, and only simple main effects were analyzed.

***Table 6: Detailed results of the ANCOVA analyses**. Treatment is considered as the independent variable while Amputation Level and Pain Level are the covaries used for the adjustments.*

	Analysis #3			**Analysis #4**		
	95% CI	***p-value***	***effect size***	***95% CI***	***p-value***	***effect size***
Treatment	[-3.29, 6.35]	0.53	1.5292	[-4.97, 4.37]	0.85	0.43641
Amp. Level	[-5.5, 5.32]	0.97322	-0.09149	[-6.9, 3.59]	0.36853	-2.4131
Pain Level (NRS)	[-0.76, 0.94]	0.83586	0.088474	[-0.72, 0.92]	0.84425	-0.082386

***Abbreviations**: Amp. Amputation; NRS: Numeric Rating Scale.*

***Table 7: Results** of the within- group comparisons listed in Table 4.*

Analysis #	**Within-groups Results (PMI)**		**Within-groups Results (PME)**	
	p-value	***effect size***	***p-value***	***effect size***
2	1.60E-03	7.22	3.03E-06	8.8
7	8.72E-04	9.19	4.84E-06	9.4

***Abbreviations:** PME:Phantom Motor Execution, PMI: Phantom Motor Imagery.*

Finally, Figure 18 shows the trend of the difference of the PRI score in the ITT population: in this figure the PRI score has been normalized by subtracting from each value the PRI at baseline and by dividing by the maximum PRI score registered over the course of treatment. In this way it is possible to visualize how the two groups position themselves with respect to the 50% reduction threshold required to reach a clinically meaningful reduction in pain [246], [247].

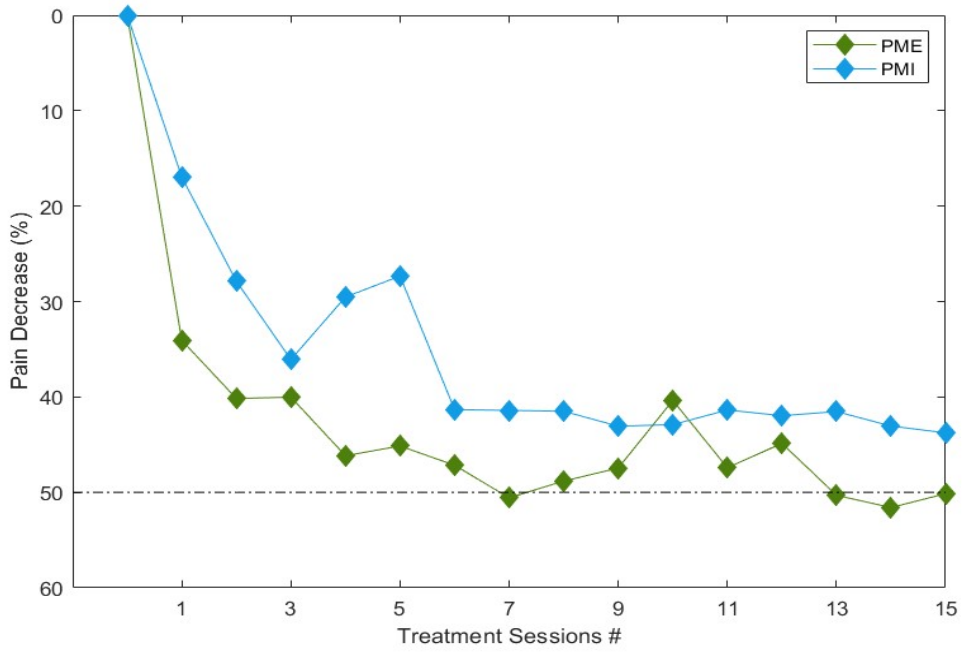

***Figure 18: Trend in PRI** over the treatment sessions. To plot this figure the PRI has been normalized. The dotted line indicates a 50% reduction in pain, which is what is considered to be clinically meaningful.*

Preliminary conclusion

In this last section, preliminary results regarding the primary outcome measure of the RCT were presented. At this stage, we have ascertained that there is no statistically significant difference in the effect of the two therapies when it comes to the primary outcome. Since we follow the theory of fixed sequential multiple testing, we can already state that the results of the analyses of all the remaining outcome measures, if significant, will be regarded as explorative.

On the other hand, the within group comparison shows that both treatments lead to a significant reduction in pain. When looking at the main analysis, pain reduction in the PME group reaches the threshold of 50% reduction required for being considered clinically meaningful, whereas the PMI treatment does not reach the threshold. However, when considering the PP population, this difference is no longer present (see for example mean values in Table 3).

CHAPTER 10

Summary of Included Papers

This chapter provides a brief summary of the papers that constitute the basis for this thesis. Full versions of the papers are included in Part II.

Paper A

E Lendaro, E Mastinu, B Håkansson, M Ortiz-Catalan

Real-time classification of non-weight bearing lower-limb movements using EMG to facilitate phantom motor execution: engineering and case study application on phantom limb pain *Published in Frontiers in Neurology, 2017, 8:470*.

DOI: 10.3389/fneur.2017.00470

The evidence in support of PME as an effective way to treat PLP was initially obtained only on upper limb amputees. However, lower limb amputees represent the vast majority of cases of limb loss. In order to investigate the effectiveness of PME in lower amputees, the system in use for treating upper limb patients needed to be adapted. The first aim of this study was to enable PME aided by MPR and VR/AR in lower limb amputation. This resulted in the proposal and validation of a new recording configuration that is a more user-friendly to record EMG signals from the lower limb. Further, the second aim of the paper was to provide evidence that PME is a viable option for PLP relief in lower limb amputees, and therefore the successful treatment of the first lower limb patient was conducted. Enabling and verifying the treatment for lower limb patient was an instrumental step for the RCT on the use of PME, the protocol of which is presented in paper B.

Paper B

E Lendaro, L Hermanson., H Burger, C Van der Sluis C., B McGuire, M Pilch, L Bunketorp-Käll, K Kulbacka-Ortiz, I Rignér, A Stockselius, L Gudmundson, C Widehammar, W Hill, S Geers, and M Ortiz- Catalan

Phantom Motor Execution as a treatment for Phantom Limb Pain: Protocol of an international, double-blind, randomized, controlled clinical trial *Published in British Medical Journal Open, 2018, 8:e021039*

DOI:10.1136/bmjopen-2017-021039

Despite the large number of treatments described in the literature to treat PLP, none of them has proven to be decisively effective for treating the condition, and at present, guidelines for the treatment of patients in this situation are absent. This can be largely attributed to the scarcity of RCTs on such treatments. In this paper, we designed the protocol for a double blind, international, multi-sited RCT on the use of PLP in order to gather unbiased and stronger evidence of the actual effect of PME. This is to the best of our knowledge, the largest international clinical trial on PLP ever conducted.

Paper C

E Lendaro, S Nilsson, and M Ortiz-Catalan

Differential Activation of Biceps Brachii Muscle Compartments for Human-Machine Interfacing, *Published in Proceedings of the 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2018, 4705-4709.*

DOI: 10.1109/EMBC.2018.8513103

Extracting motor volition of distal joints using proximal muscles is a challenge when intending to predict motor intent in amputated limbs. As the level of amputation becomes more proximal, more movements need to be decoded, the number of muscles available to collect input signals for control decreases. A way to increase the quantity of decodable movements and the quality of MPR, would be to exploit muscle portions that are innervated by separated branches by learning to contract them independently. In this paper the viability of learning independent contraction of the biceps brachii's heads while providing real-time biofeedback was investigated. We showed that this was indeed possible in able-bodied subjects.

Paper D

E Lendaro, L Guo, MJM Novoa, L Sandsjö, and M Ortiz-Catalan

Seamless Integrated Textrode-Band for Real-time Lower Limb Movements Classification to Facilitate Self-Administrated Phantom Limb Pain Treatment, *Published in Proceedings of the 41st Annual*

International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2019, 1753-1756.

DOI: 10.1109/EMBC.2019.8856979

A technical drawback of PME using MPR is that this is commonly done with disposable surface electrodes, which aggregated cost can be prohibiting in many health care systems and for application in home settings. In collaboration with University of Borås, the feasibility of using textile electrodes that can be more easily applied and reusable when conducting PME was verified.

Paper E

E Lendaro, EJ Earley EJ, M Ortiz-Catalan

Statistical analysis plan for an international, double-blind, randomised controlled clinical trial on the use of phantom motor execution as a treatment for phantom limb pain., *PREPRINT (Version 1) available at Research Square*

DOI:10.21203/rs.3.rs-798862/v1

The vast majority of RCTs on PLP do not meet the necessary criteria and present flawed design, conduct, analysis, and/or reporting. Standardized reporting of results and publication of the study protocol have become ever more common also thanks evidence-based instruments such as the SPIRIT and CONSORT guidelines. Yet, the risk for selective reporting of outcome and analysis persists (decision of which analyses to conduct, and which results to report) is still large and not fully acknowledged. To this end, JAMA published a statistical analysis plan (SAP) guidance document in 2017 containing a checklist of minimum items to include when reporting details of the statistical analysis of RCTs. To ensure the much-needed high quality evidence in the field of PLP research, not only protocols, but also SAPs should be published. Here the pre-specified SAP for the international, double-blind, randomized controlled clinical trial on the use of phantom motor execution as a treatment for phantom limb pain was described.

Paper F

E Lendaro, E Balouji, K Baca, AS Muhammad, and M Ortiz-Catalan

Common Spatial Pattern EEG decomposition for Phantom Limb Pain detection, *Accepted to Proceedings of the 43rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), 2021*

Phantom Limb Pain (PLP) is a chronic condition frequent among individuals with acquired amputation. PLP has been often investigated with the use of functional MRI focusing on

determining the link between pain and the plasticity taking place in the sensorimotor cortex after an amputation. In the present study we investigated whether a different type of data, namely electroencephalographic (EEG) recordings, can be used to study the condition. Resting state EEG data were acquired from people with and without PLP and used for a binary classification task. The study showed that is possible, with great accuracy, to detect PLP using EEG data, which is a promising target for future research aiming at elucidating the neural mechanisms underlying PLP.

CHAPTER 10

Conclusions and future work

The work conducted in this doctoral thesis was focused on four main objectives. The first objective was to provide an overview of the field also outlining some of the current challenges. The second objective was to solve some of the technological challenges connected to the clinical implementation of PME. This objective was propaedeutical to the third objective, concerned with the implementation of a RCT aimed at establishing PME as an evidence-based treatment for PLP. Finally, the last objective was to explore the use of EEG in a cross-sectional study of the neural correlates of PLP. The RCT was established as an international, multi-center effort in 2017 and is expected to close in September 2021.

Preliminary results of the RCT regarding the primary outcome showed reduction of PLP above what is consider clinically relevant, and whereas a higher reduction was obtained with PME, this was not statistically significant over PMI. The available evidence at this stage indicates that the RCT will not be able to rule out the role of contextual factors other than PME in providing pain relief. Future work will involve publishing the complete results of the trial.

The lack of statistical difference between PME and PMI can be seen as negative result. In science, negative results are less likely to award glory to who conducted the research, and they are hardly celebrated. Yet, negative results are fundamental in moving the field forward and as they are of great interest for informing future research. For instance, the field can use these results to frame new thinking regarding PLP, arriving for example to new explanatory hypotheses for the condition or new ideas of how to provide treatment.

Double-blind RCTs are considered providing one of the highest levels of scientific evidence in the evaluation of treatment interventions, second only systematic reviews and metanalyses of RCTs. Further, well conducted, and standardized RCTs are fundamental to enable systematic review and metanalyses. This trial has been designed and conducted striving for transparency and with the ambition of enabling easy replication of the methods for future research. In the ideal scenario, future studies on treatment for PLP based on similar principle (i.e. guided plasticity) could, for example, use the same control intervention in one of the arms. Another area that would benefit from standardization would be the outcome assessment. Not only should outcome measures be standardized but also the timing of the assessments.

Similar considerations apply to the neuroimaging field, where studies present replicability challenges, focusing on adopting standardized functional tasks, outcome measures, analytical approaches and, most importantly, making the imaging data publicly available would go a long way in speeding up the research. This would also make it more efficient and cheaper to

conduct. The work carried out within this thesis regarding the neural correlates for PLP is ongoing. We have shown that EEG holds promise for bringing further insight into the condition, yet more detailed analyses are need in order to assess the robustness of the findings. Moreover, the study carried out in Paper E is a cross-sectional study and does not look into whether different levels of pain can be discriminated within the same subject. This is the focus of future studies together with the completion of an ongoing fMRI investigation. In the future, both EEG and fMRI data gathered by us will be made publicly accessible.

In conclusion, some exciting years could be lying ahead in the field of PLP if researchers are to rely more on adversarial collaboration (as outlined in Chapter 7), standardized protocols and open science practices.

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