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Neural activity and motor behaviours in subjects with congenital limb deficiency and traumatic amputees

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ABBREVIATIONS

%BSC = percent BOLD (blood oxygenation level dependent) signal change
ADS = Allgemeine Depressions-Skala
fMRI = functional magnetic resonance imaging
GABA = γ-aminobutyric acid
M1 = primary motor cortex
MPI = Multidimensional Pain Inventory
MRI = magnetic resonance imaging
nonPLP = amputees without phantom limb pain
PLP = phantom limb pain
PLPamp = amputees with phantom limb pain
RLP = residual limb pain
ROI = region of interest
S1 = primary somatosensory cortex
TMS = transcranial magnetic stimulation

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INTRODUCTION

Plastic changes in body representation can be influenced by many factors, like learning a specific motor skill (Elbert et al., 1995) or deprivation of sensory input (e.g. missing limb, blindness, deafness) (Hahamy et al., 2017; Houde et al., 2016; Renier et al., 2014). Neural plasticity is the basis of learning and learning leads to reorganization in the cortex (Elbert et al., 1995; Molina-Luna et al., 2008; Recanzone et al., 1992). But reorganization does not only occur after positive activities, it can also be maladaptive and can cause chronic pain (Maihofner et al., 2003).

Phantom limb pain (PLP) is a frequent phenomenon occurring after an amputation (Jensen et al., 1983; Kooijman et al., 2000; Sherman & Sherman, 1983) and has been associated with cortical alterations, especially in primary somatosensory and motor cortices (Andoh et al., 2017; Elbert et al., 1994; Flor et al., 1995; Makin, Filippini, et al., 2015; Montoya et al., 1998). The drivers of cortical plasticity and how they relate to PLP remain however debated.

Moreover, after an amputation, individuals are forced to adopt different motor behaviours (e.g. physical rehabilitation) to compensate for the missing limb or to perform specific movements to avoid PLP (Hahamy et al., 2015). Such motor behaviours might induce use-dependent changes in cortical plasticity. Furthermore, in the case of a planned amputation (e.g. related to a chronic medical illness, infection), individuals can experience anxiety and depression disorders (Darnall et al., 2005; Noble et al., 1954). Such psychological factors can also influence cortical plasticity and should be accounted for. Cortical reorganization after an amputation results therefore of a complex interplay between peripheral, central mechanisms and psychological factors, and its assessment should take into account all these factors.

An alternative and a relatively easier population in which one could investigate cortical organization related to a missing limb is individuals with a congenital limb deficiency (or “amelics”). Amelics learn from an early age to compensate for the missing limb, often use other body parts such as lips or feet compensatorily and have a different motor behaviour compared with amputees (Makin, Cramer, et al., 2013). Unlike amputees, in whom PLP is a common phenomenon (Jensen et al., 1983), PLP is very rare in amelics (Melzack et al., 1997; Weinstein & Sersen, 1961). With

amelics, one can therefore determine the neural correlates related to the deprivation of sensory input without the influence of PLP.

The present thesis aims to investigate the various drivers of cortical plasticity and their interaction. For this purpose I decided to study amelics because they do not suffer PLP, and to compare this population to amputees with PLP and amputees without PLP as well as to two-handed controls. Furthermore, the relationship between cortical reorganisation and motor behaviours will be examined. The following sections give an overview about congenital and acquired limb deficiency especially regarding PLP and neural correlates in both populations.

1.1 Congenital and acquired limb deficiency

Dysmelia or congenital limb deficiency is an umbrella term for several congenital malformations of one or multiple limbs. It can range from affecting the separation of fingers and toes to the absence of entire limbs. The prevalence of congenital limb deficiency varies strongly in the literature, e.g. 4.4 per 10.000 births (Klungsoyr et al., 2019), and up to 21.1 per 10.000 births (Vasluian et al., 2013).

According to the German medical dictionary Pschyrembel, Dysmelia can be divided into four types depending on the type of congenital malformation (Figure 1)(Pschyrembel Online).

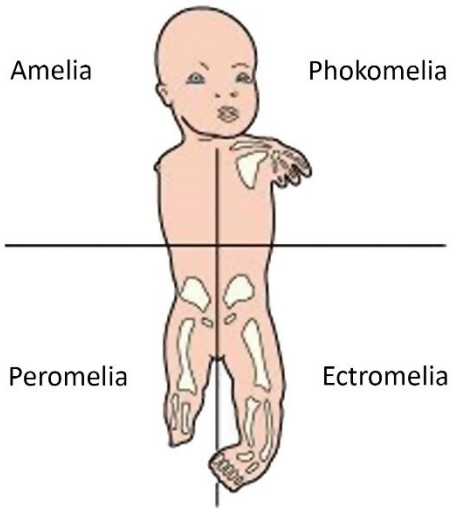

Figure 1 Types of Dysmelia, modified from Pschyrembel Online

The total absence of a limb is named Amelia, whereas the term Peromelia is used if only part of the limb is missing, like in intrauterine truncation of limbs. Phocomelia

describes a hand or foot directly attached to the trunk. If the limb is fully developed but the long bones are hypo- or aplasic (e.g cleft hand or clubfoot), the term Ectromelia is utilized. To facilitate comprehension, I will use the term “amelics” to describe all individuals with dysmelia.

The aetiology of dysmelia is still unclear. Causes of dysmelia can be divided into hereditary and non-hereditary (e.g. mechanical, external noxae) causes. Hereditary causes include chromosome abnormality or syndromes which are related with limb malformations and are often accompanied by other organ malformations (Klungsoyr et al., 2019), for example the Apert-syndrome (Wenger et al., 1993) or the ectrodactyly-ectodermal dysplasia-cleft syndrome (EEC-syndrome) (Rudiger et al., 1970).

One example for non-hereditary causes is the constriction band syndrome (CBS) (Tada et al., 1984), also known as constriction ring syndrome (CRS) or uterine band syndrome (UBS). Fibrous bands in the uterus lead to constrictions of foetal body parts. This can result in malformations and amputations of arms, fingers, legs or toes. The pathogenesis of these fibrous strings is still unclear. Other reasons for dysmelia are teratogenic substances like radiation, infections or drugs (Klungsoyr et al., 2019). Thalidomide, also known as “Contergan”, is certainly one of the most well-known causes of congenital malformations. Prescribed to pregnant women for the treatment of sleep disturbances and morning sickness, it induced severe malformations in children (Franks et al., 2004). Shortly afterwards, thalidomide was withdrawn from the market (Lenz, 1988). Nowadays, it is used in the treatment of cancer, especially of multiple myeloma (Franks et al., 2004).

Amputation is defined as a surgical or traumatic removal of a limb due to trauma, dysvascular disease (e.g. peripheral arterial disease), frostbite or cancer (Pschyrembel Online). Dysvascular disease usually leads to lower limb amputation and is more common while trauma usually causes upper limb amputations (Ziegler-Graham et al., 2008). In the last years, the absolute number of amputations has increased slightly in Germany. This observation could be due to an increasing prevalence of diabetes mellitus which can lead to vascular disease (Kroger et al., 2017). According to a study about amputees in the USA in 2005, most of the amputees are men, and nearly 40% were older than 65 (Ziegler-Graham et al., 2008).

1.2 Phantom limb sensation and pain

Sensations in the phantom limb are a common phenomenon after amputation and can be divided into painful and non-painful sensations. Both types have a high prevalence six months after amputation (non-painful sensations up to 90%, painful sensations up to 80%) (Jensen et al., 1983). Amputees can describe the phantom limb by length, volume or other spatial sensations (Jensen et al., 1983). Non-painful phantom sensations involve exteroceptive sensations (e.g. itching, tingling, cold or heat), voluntary or spontaneous phantom limb movements and kinaesthesia sensations (e.g. normal or abnormal length and volume) (Jensen et al., 1983; Kooijman et al., 2000).

Painful sensations are often reported as an exaggeration of non-painful phantom limb sensations and thereby described as knifelike, sticking, shooting, pricking, burning, cramplike or pressing-like sensations (Jensen et al., 1983; Kooijman et al., 2000; Sherman & Sherman, 1983; Weeks et al., 2010). About 30% report a feeling of telescoping, e.g. a shrinking of the phantom limb towards the residual limb which has been shown to be positively correlated with cortical reorganization and PLP (Flor, 2002; Jensen et al., 1983).

The prevalence and intensity of PLP seem to be influenced by several factors. Pain before amputation is believed to lead to PLP (Jensen et al., 1985; Larbig et al., 2019; Ramachandran & Hirstein, 1998) and stump pain has been shown to be positively correlated with PLP (Kooijman et al., 2000; Montoya et al., 1997). The usage of protheses however seems to reduce PLP (Lotze et al., 1999; Weiss et al., 1999). Another factor which is believed to be related to PLP is time since amputation (Jensen et al., 1983, 1985; Sherman et al., 1984), however this finding has not been consistently reproduced (Flor et al., 1995; Sherman & Sherman, 1983). Additionally, psychological factors such as depression or anxiety before amputation could be predictors of the development of PLP (Larbig et al., 2019). The literature is however scarce and longitudinal studies are desperately needed.

1.2.1 Theories about PLP

The pathophysiology of PLP is still unclear and several different theories about the origins of PLP exist. Acquired amputation leads to changes in the peripheral and central nervous system (Figure 2) (Andoh et al., 2018; Flor et al., 2006; Kuffler, 2018). There are several theories for the development of phantom limb pain, arguing

for cortical or peripheral mechanisms, but the exact underlying mechanisms remain unclear and need further investigation.

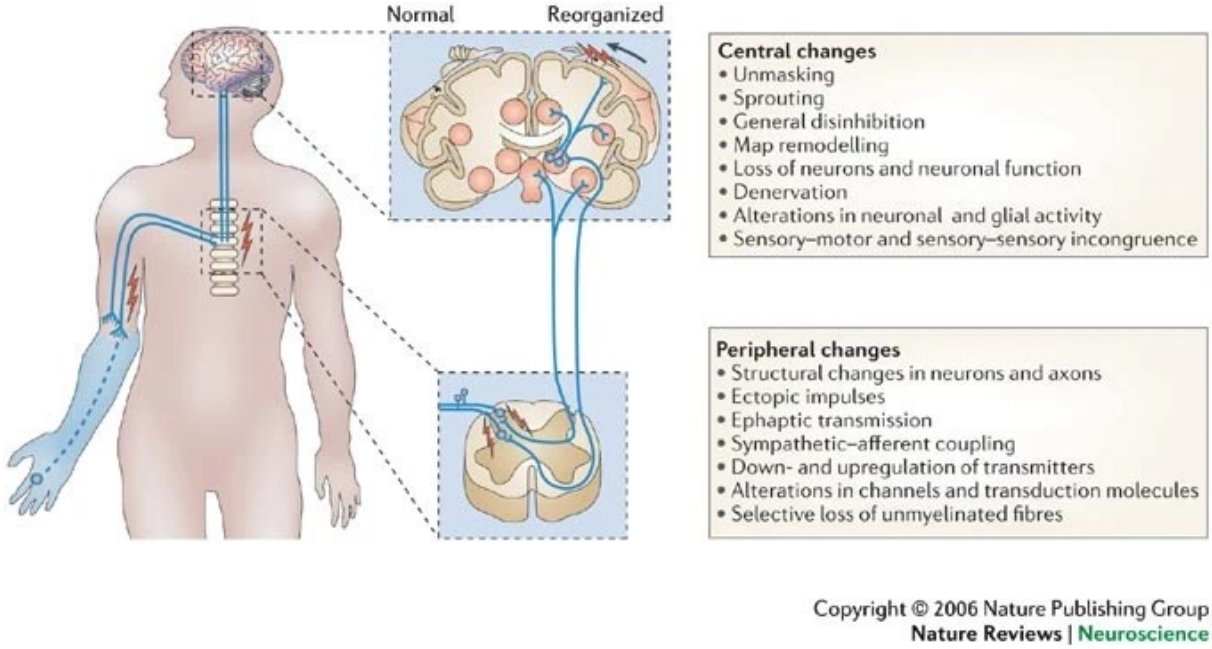

Figure 2 Changes in the peripheral and central nervous system are correlated with the development of PLP.
Figure reprinted from Flor et al. (2006)

It needs to be investigated whether cortical or peripheral changes cause PLP, or vice versa. For this reason, a group like amelics is interesting, because they do not suffer from PLP. If amelics do not undergo cortical changes, then PLP would be the main driver of cortical changes in amputees.

1.2.1.1 Role of the central nervous system

It is known that the human body is represented in the motor and somatosensory cortex according to a certain scheme. The Canadian neurosurgeon Penfield created a map, today known as the “Penfield’s Homunculus” showing the representation of each body part in the human cortex, weighted by the innervation (Figure 3). The map differentiates between the somatosensory homunculus in the postcentral gyrus and the motor homunculus in the precentral gyrus (Penfield & Boldrey, 1937).

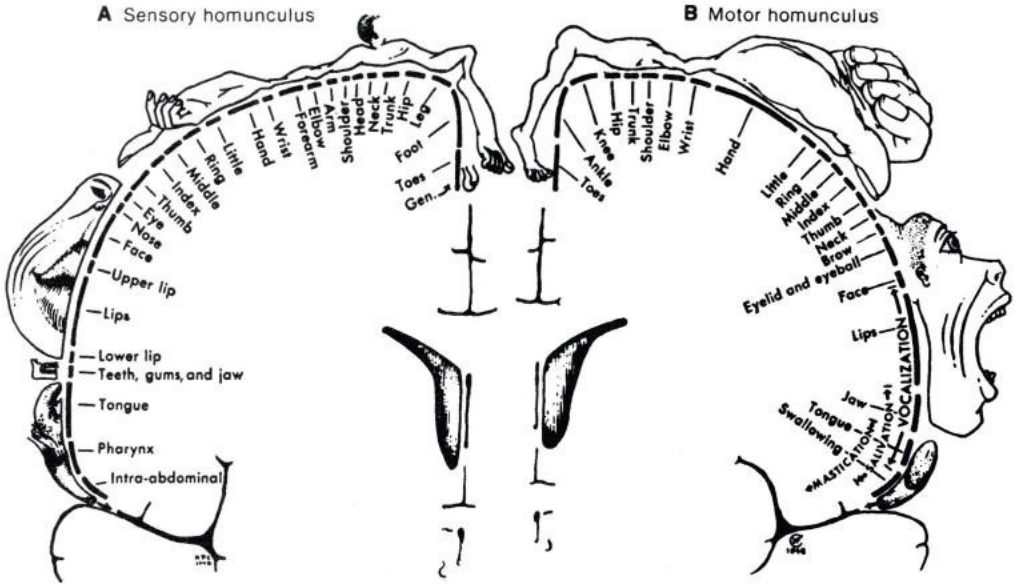

Figure 3 The Penfield homunculus with the somatosensory (left) and motor (right) cortex. The hand territory is next to the lip and shoulder. Reprinted from Penfield, W., & Rasmussen, T. (1950). The Cerebral Cortex of Man. New York, NY: Macmillan Company

Within in the theory arguing for central changes related to PLP, two main models have been proposed.

First, the maladaptive plasticity model, which suggests that adjacent areas invade the missing body part. For example, when an arm gets amputated, the area of the lips and shoulders which are somatotopically close to the hand area, would expand and invade the cortical area of the missing arm. This model is supported by several studies that have shown that upper limb amputees have an asymmetry in the cortical representation of the face, which was related to PLP but not to non-painful phantom sensations (Elbert et al., 1994; Flor et al., 1995; Flor et al., 1998; Makin, Scholz, et al., 2015; Montoya et al., 1998; Raffin et al., 2016). In amputees, a cortical shift of the representation of the lip was found which was positively correlated to phantom limb pain (Flor et al., 1995). Additionally, sensory input of the lips leads to neural activity in the former arm area in the cortex, but only in amputees with PLP and not in amputees without PLP (Birbaumer et al., 1997). Moreover, regional anaesthesia of the axillary brachial plexus can lead to a decrease in the intensity of PLP in some amputees and also to a reduction of the cortical reorganization (Birbaumer et al., 1997). The idea of the maladaptive plasticity model is supported by evidence from prothesis-based therapies showing normalization of cortical plasticity and reduced PLP after prosthesis use (Karl et al., 2004; Lotze et al., 1999; Weiss et al., 1999). However, it is not clear whether amputees who have less pain tend to use protheses more or if prothesis usage leads to a decrease of PLP (Collins et al., 2018).

The second model argue for a maintained representation of the missing limb in primary motor and somatosensory cortices. In this model, there is no reorganization, but rather a persistent limb representation. Pain is correlated with preserved structure and function in the former hand rather than with the invasion of adjacent areas. The neural activity in the former hand area was shown to be positively related to PLP (Makin, Scholz, et al., 2013).

Although both models, the maladaptive plasticity and the persistent representation models, seem contradictory, some differences between these two models could be accounted for by methodological differences between these two models. In Makin, Scholz et al., (2013), neural activity in the former hand area was measured using an active motor task, in which participants had to move their phantom hand. Not all the participants could however perform such task. The amelics for example do not experience phantom limbs and were therefore asked to imagine moving their phantom hand. A control group (two-handed controls) was also included, in which participants were asked to move the non-dominant hand. Despite the heterogeneity in task-related execution, the authors defined a hand motor area based on common neural activation between amputees (with and without PLP), amelics and controls. We believe that such definition of a phantom hand area could be erroneous because of different processes that might be happening when participants execute different hand movement tasks. For example, in amputees, movements of the phantom limb activate muscles in the stump. Therefore, the activation in the motor cortex could be evoked concomitantly by movements of the phantom limb and by muscles in the stump. Moreover, the presence of PLP might be also be a confound when investigating movements of the phantom hand, such that specific movements of the phantom limb might trigger PLP and amputees with PLP might use adaptive strategies to move the phantom in specific positions to avoid PLP (Anderson-Barnes et al., 2009).

Makin, Scholz et. al also assumed that non-dominant hand movements in controls are comparable to phantom hand movements in amputees (Makin, Scholz, et al., 2013). Furthermore, in amelics, performing imagery of phantom hand movements might activate different areas than performing execution of phantom hand movements (Raffin, Giraux, et al., 2012).

Bostrom et al. suggested that both models, the maladaptive plasticity model and the persistent representation model, could coexist in the cortex (Boström et al., 2014).

The authors designed a simulation based on a self-organizing map, also called Kohonen map (Kohonen, 1982), about neural network and reorganization. The simulation predicts that the degree of reorganization is stronger in the pain state and would support the maladaptive plasticity model. On the other hand, the study also estimated that the phantom limbs are still preserved after amputation and are correlated with pain. This would be in accordance with the persistent representation model. As this is only based on computational assumptions, no study showed experimental evidence that both models could coexist simultaneously so far (Boström et al., 2014).

1.2.1.2 Role of the peripheral nervous system

Afferent nerve pathways conduct sensory information like fine touch or vibration from the peripheral body parts to the central nervous system (Trepel, 2017). Nociceptive inputs like residual limb pain are discussed as a possible determinant of PLP as they are positively correlated with PLP (Flor, 2002; Jensen et al., 1985; Kooijman et al., 2000; Montoya et al., 1997).

If an amputation occurs, these peripheral neurons are injured and the axons are severed. This leads to inflammation and therefore to sprouting and formation of neuroma in the residual limb (Collins et al., 2018). After injury, nerves are hyperexcitable as thresholds are lower and stimuli that normally do not lead to activity provoke an action potential (Zheng et al., 2007). Severed nerves generate ectopic activity as the activity does not come from the physiological end point of the axons. It is assumed that PLP is provoked by activity in neuroma (Collins et al., 2018; Flor, 2002). Axotomy leads also to changes in the metabolic system, the distribution of receptors and in postsynaptic nerves (Collins et al., 2018; Kuffler, 2018). If inflamed peripheral nerves were the main cause for phantom limb pain, anesthetizing these nerves would lead to a complete pain relief or even a decrease (Collins et al., 2018). Local anaesthesia which was injected intrathecal and intraforaminal lead to significant reduction of PLP intensity and also nonpainful phantom limb sensations (Vaso et al., 2014). The authors suggested ectopia of dorsal root ganglia as a possible reason for PLP. In a study of Birbaumer et al., only three out of six amputees with PLP experienced a pain relief after anaesthesia by a brachial plexus blockade. However, in these three patients with pain relief, a reduction of cortical reorganization was also observed (Birbaumer et al., 1997). Liu et al. showed that

peripheral input can modulate PLP intensity in amputees with PLP but is not able to create PLP in amputees without PLP. The authors suggested that PLP during nerve stimulation is provoked by a central misinterpretation but not by peripheral input itself (Liu et al., 2020).
While mechanisms of the peripheral nervous system are not fully understood, peripheral input certainly influences the cortical reorganization and thereby might play an important role in the development and maintenance of phantom limb pain.

1.2.2 Phantom limbs in people with congenital limb deficiency

Phantom limb pain occurs rarely in amelics and its etiology has not yet been fully resolved. Reorganization plays a key role in all theories about PLP, but it is unclear if cortical changes occur also in amelics. Amputees generally suffer a traumatic event, (physically and emotionally) and have to adapt rapidly to drastic changes in their lives. Amelics on the other hand are born without a limb, and they possibly adopted adaptive behaviours before they were born.
Initially, amelics are believed to not experience phantom limbs because the missing limb was never part of the normal body representation (Browder & Gallagher, 1948) and most studies reported that amelics did not perceive phantom limb sensations (Flor et al., 1998; Hahamy et al., 2017; Montoya et al., 1998; Wesselink et al., 2019). But there is also evidence, although rarely, that some amelics can experience phantom limb sensations (Price, 2006; Weinstein & Sersen, 1961). Melzack et al. even suggested a prevalence of 20% (Melzack et al., 1997). However, it is important to distinguish between phantom sensations of amelics who never underwent an amputation and amelics who developed phantom limb sensations after an amputation (e.g. for better fit of the prothesis) in childhood or adulthood. For example, Saadah et al. reported about phantom limb sensations in amelics but all of the participants experienced phantom sensations only after injuries or additional amputation at the stump (Saadah & Melzack, 1994).
The discussion why amelics experience phantom limbs leads to the question how the foetal brain and the body schema develop in the mothers' womb. Using ultrasound readings, Hepper and colleagues have reported that embryonic arm movements are usually right lateralised by 10 weeks, before any signs of asymmetry in the brain (Hepper et al., 1998). It was suggested that motor behaviours may precede and determine brain organization (Price, 2006). These findings suggest that at the

embryonic stage, motor contractions might originate in the muscles or spine, rather than the brain and that therefore motor behaviour determine the body representation (Hepper et al., 1998). Melzack et al. suggested that the body representation and possibly also the presence of congenital phantom limbs could be in a large part genetically determined (Melzack et al., 1997). Nevertheless, Melzack et al. postulated that genetic predisposition cannot be the only factor that provokes phantom sensations, otherwise every person or no person born with congenital limb deficiency would report about phantom limb sensations.

Some phantom sensations however can appear after an amputation, or in two-handed individuals using sensory illusions such as the rubber-hand illusion (Ehrsson et al., 2005; Giummarra et al., 2010). The rubber hand illusion has not yet been shown in amelics. Another possible method to induce phantom sensations is transcranial magnetic stimulation (TMS). TMS applied over the motor cortex led to phantom sensations in amputees (Mercier et al., 2006). In amelics who never experienced phantom sensations, it was not possible to induce phantom sensations (Reilly & Sirigu, 2011). Reilly and colleagues concluded that motor function in amelics might follow a different cortical development compared with the one's of two-handed individuals. However, in the case of an amelic woman without forearms and legs who experienced phantom sensations in all four limbs as long as she remembered, it was possible to elicit phantom sensations with TMS (Brugger et al., 2000). These findings indicate that phantom limb sensations are a multifactorial and complex process that occurs early in life, and can then possibly be altered by motor behaviours at a later stage in one's life.

A possible theory about the development of the body representation and therefore phantom limbs is suggested by E.H. Price. He proposed multiple hypotheses for the development of body image and classified congenital phantom limbs in two categories: phantom limbs that match to the opposite limb and are experienced from birth and phantom limbs that are experienced later in life and are based on visual or sensory input. The author suggested that sensory input and proprioceptive feedback drive neural representation (Price, 2006). This in in line with several studies that showed that neural plasticity is influenced by peripheral input (Molina-Luna et al., 2008; Recanzone et al., 1992; Walz et al., 2015). In utero these this sensory input and proprioceptive feedback are provoked by spontaneous muscle activity (Price, 2006). Price suggested a bilateral neural representation since body parts are either

directly or via cross-cortical connections presented in both hemispheres. Therefore, sensory input from the intact limb could lead to neural activity in both hemisphere (Price, 2006). It is already a common phenomenon in amputees that the percept of the missing limb originating from different body parts. For example, a sensory input like touch on the intact arm can provoke sensations in the missing limb and leads also to bilateral neural activity in amputees (Andoh et al., 2017; Hunter et al., 2005; Ramachandran et al., 2010; Ramachandran & Hirstein, 1998). However, this phenomenon has not yet been studied in amelics. Another point of Price's theory refers to external factors such as the environment which might influence the perception one has of his own body. The body image is consolidated by sensations in the remaining limbs, visual input, mirror neurons or prosthesis usage (Price, 2006). It was shown that the same neurons are activated when observing a movement or when performing the movement actively (Dushanova & Donoghue, 2010; Gallese et al., 1996). Mirror neurons are discussed as a factor that drives the development of phantom experiences later in life of amelics, also called "somatic empathy" (Price, 2006).

Price offered an explanation only for non-painful phantom sensations but not for painful phantom sensations. The presence of PLP has however been shown in congenital limb deficiency, although less commonly than for non-painful sensations (Melzack et al., 1997). The idea of a multifactorial development of phantom limbs in congenital limb deficiency deserves further investigation.

1.3 Neural correlates in amelics and amputees

While amputees and amelics have both missing limbs, they report different phantom phenomenons, with amelics rarely reporting PLP. One could therefore reasonably presume that amputation and amelia could be related to different mechanisms and might be related to different brain reorganization patterns.

When comparing amelics with amputees, one has to consider that they use different type of motor behaviours. Amelics use in daily life several body parts such as the intact hand, lips or feets (Makin, Cramer, et al., 2013; Striem-Amit et al., 2018). For example, Makin et al. reported that amelics use their residual arm more often for daily tasks and have a more bimanual arm usage compared with amputees (Makin, Cramer, et al., 2013). Such differences in motor behaviours can also be seen when examining neural activity: compared with amputees, amelics showed increased

activation in the hemisphere contralateral to amputation when moving the residual limb. However, amelics showed less neural activity in the hemisphere ipsilateral to amputation when moving the intact hand compared with amputees. The authors concluded that the area of the missing limb is not only invaded by the adjacent body part, it even is invaded by the over-used (compensatory) arm (Makin, Cramer, et al., 2013). Furthermore, movements of other body parts, that have a compensatory role (e.g. feet, lips, lower face) in daily life, led to activation in the hemisphere contralateral to the missing limb (Hahamy et al., 2017). Hahamy et al. suggested that the hand territory may not represent the hand but rather the body part which overtook the function of the missing hand. This assumption was not shared by Striem-Amit et al., who investigated amelics with bilateral absent upper limbs who used their feet compensatorily (Striem-Amit et al., 2018). The primary sensorimotor hand area showed neural activity not only during foot movement but also during movement with the shoulder. In contrast to Hahamy et. al. (2017), Striem-Amit and colleagues suggested that neural activity in the cortex area contralateral to the missing limb was not selective for compensatory use but was also activated by movements of proximal body parts (Striem-Amit et al., 2018).

When comparing amelics and amputees, it is important to differentiate amputees with and without PLP. Indeed, reorganization patterns have been shown to be related to the presence or absence of PLP (Andoh et al., 2020), and also to the intensity of PLP (Flor et al., 1995; Raffin et al., 2016). Movements of the intact limb led to similar neural activity in amelics and controls (Cruz et al., 2003). Lip movements in amputees led to activation in the former hand area and showed an enlarged mouth representation with a shift towards to the missing hand area (Lotze et al., 2001). This aberration was only shown in amputees with PLP, and not in patients with non-painful phantom phenomena (Birbaumer et al., 1997; Lotze et al., 2001). In amputees, somatosensory stimulation showed an invasion of the chin and lips into the former hand area (Birbaumer et al., 1997; Elbert et al., 1994; Flor et al., 1995). These findings demonstrate neural differences in specific brain areas between amputees and amelics, especially when taking into account the role of PLP in amputees.

Regarding differences in functional connections, it was shown that amelics and amputees do not differ but amputees with PLP had a reduced resting-state functional connectivity compared with amputees without PLP (Makin, Scholz, et al., 2013). The

authors however did compare amelics with amputees but did not differentiate amputees with and without PLP.

Amputees and amelics do however have a reduced connectivity compared with two-handed controls. This reduced connectivity in amelics was negatively correlated with the usage of the residual arm (Hahamy et al., 2017; Hahamy et al., 2015).

Interestingly, bimanual hand usage led to a higher inter-hemispheric connectivity between the left and right hand region (Hahamy et al., 2015). Amelics had an increased coupling between the former hand area and both the lip and foot regions (Hahamy et al., 2017). The authors thus found higher connectivity between the cortex areal for the hand and body parts that are used compensatorily in everyday life. Hahamy et al. discussed that congenital limb deficiency might led to an input loss resulting in reduced inhibitory connections and therefore to a decrease in the concentration of GABA. This is supported by the finding of decreased concentrations of GABA in the missing-hand area of amelics compared with controls. Reduced inhibitory connections and lower GABA levels may result in an increased connectivity (Hahamy et al., 2017). However, the relationship between motor behaviour and functional connectivity in amputees and amelics is not fully understood and requires further research.

Reorganization after amputation is not restricted to the cortex but was also shown to occur in other brain areas as the cerebellum or the putamen. Movements of the lips, feet or the residual arm showed neural activity in an area of the cerebellum that represented the former hand region. In addition, amelics showed activation in the putamen contralateral to amputation during movements of the residual arm, lips and feet but not while moving the intact hand (Hahamy & Makin, 2019).

In conclusion, amelics seem to show several differences but also similarities in neural activity and functional connections with amputees. Both amelics and amputees show reduced connectivity between the cortical hand regions but reduced connectivity is greater in amputees with PLP. Amelics showed an increased coupling between the hemisphere contralateral to the missing hand and body parts which are used in a compensatory manner. Lip movements led to activation in the former hand territory in both groups. It could be speculated that in amputees this phenomenon could be due to PLP and in amelics it could be due to compensatory usage in daily tasks. In the literature, no study at our knowledge compared amelics to amputees with and without PLP. Based on previous findings (Andoh et al., 2020; Raffin et al., 2016) different

neural reorganisation patterns and motor behaviours interact with the presence and intensity of PLP and should be taken into account.

2 AIMS AND HYPOTHESES

Previous studies showed that amelics have a similar cortical representation, neural activity, connectivity and cortical volume of the missing limb compared with two-handed controls (Makin, Cramer, et al., 2013; Montoya et al., 1998). Amelics are a special group of one-handers, which are known to rarely experience PLP (Melzack et al., 1997; Weinstein & Sersen, 1961) and are therefore an interesting group to test cortical representation related to a missing limb. Moreover, amelics are usually in a good health condition, and show less comorbidities compared with the amputee population for example. Although very interesting research questions can be answered by investigating amelics, studies are still very limited. Compared with traumatic amputees, amelics have been shown to have decreased neural activity in hemisphere contralateral to the missing hand during intact hand movement (Makin, Cramer, et al., 2013). The area where the missing hand should be represented is activated by other body parts (e.g. feet or lips) that are partly used for compensatory usage (Hahamy et al., 2017; Striem-Amit et al., 2018). However, no study compared the cortical representation of the missing limb in amelics to the one of amputees with and without PLP. Such information would further our knowledge on neuroplasticity, adaptive or maladaptive, the latest probably leading to PLP.

A virtual mirror motor task was carried out in an MRI scanner in amelics, amputees with and without PLP and controls. The current thesis investigated the neural activity and connectivity in primary motor cortices associated with a virtual reality movement task. The cortical representation of the missing hand and the intact hand were examined in amelics and in traumatic amputees with and without PLP and compared to two-handed controls.

<u>The main hypothesis of the current thesis are as follows:</u>

Hypothesis 1: Amelics have similar compensatory behaviours (use of a prosthesis or intact hand) compared with amputees without PLP, which would lead to comparable neural activity and connectivity in M1/S1 cortices.

Hypothesis 2: Amelics expected to have less neural activity in the hemisphere ipsilateral to movement compared with amputees with PLP while moving their intact hand since amelics do not experience PLP.

Hypothesis 3: Amputees with and without PLP have a different neural activity, which is related to PLP intensity.

3 MATERIAL AND METHODS

3.1 Participants

We selected data of 40 participants (14 females, 26 males) from the projects SFB1158-B07[1] and Phantommind[2]. The studies were approved by the Ethics Committee of the Medical Faculty Mannheim, Heidelberg University, and every participant gave written informed consent prior to the study.

Subjects were divided into four groups: a two-handed group (controls; n = 10, mean age ± SD 49.6 ± 8.29); an upper congenital limb deficiency group (amelics; n = 10, mean age ± SD 50.3 ± 15.56), and an acquired upper limb amputation group including amputees with PLP (n = 10, mean age ± SD 51.1 ± 11.85) and amputees without PLP (n = 10, mean age ± SD 51.1 ± 7.52). PLP criteria was defined based on the German version of the Multidimensional Pain Inventory (MPI-D), a common and reliable tool to quantify chronic pain (Flor et al., 1990; Kerns et al., 1985), using the mean of the score of the questions 1, 7, and 12[3]. Participants with mean scores equal or bigger than 1 were classified as subjects with PLP (PLPamp), participants with scores lower than 1 were classified as subjects without PLP (nonPLP).

Groups were matched by mean age per group and side of amputation (amputees) or missing arm (amelics). Psychometric data like time since missing limb/ amputation, age at missing limb/ amputation, stump usage and depression were examined through questionnaires and compared between amelics, amputees with and without PLP. To quantify potential depressive symptoms, a shortened version of the ADS (Allgemeine Depressionsskala, ADS-K) questionnaire was performed by all participants. The ADS questionnaire is the German version of the Centre for Epidemiologic Studies Depression Scale (CES-D scale) and is a reliable and valid screening for depressive disorders (Radloff, 1977; Stein et al., 2014; Stieglitz, 2008).

[1] Deutsche Forschungsgemeinschaft SFB 1158: B 07 - Neural circuits involved in Phantom Limb Pain.
[2] Europäische Union 230249: ERC PHANTOMMIND: Phantom phenomena: a window to the mind and the brain
[3] Question 1: Rate the level of your pain at the present moment (exact wording: Schätzen Sie das Ausmaß Ihrer derzeitigen Schmerzen ein (jetzt im Moment))
Question 7: On the average, how severe has your pain been during the last week? (exact wording: Wie stark waren Ihre Schmerzen in der letzten Woche (im Durchschnitt)?)
Question 12: How much suffering do you experience because of your pain? (exact wording: Wie sehr leiden Sie unter Ihren Schmerzen?)
Every question has a scale from 0 up to 6, thereby 0 is no pain/no suffering, 6 means extremely sever pain/extreme suffering.

The cut-off value for depression was set at higher or equal 18 points, with higher scores indicating more severe depression (Lehr et al., 2008). For demographic details see Table 1.

ID	Group	Sex	Age	Amputation side/ Missing limb side	Age at missing limb/ amputation (years old)	Time since missing limb/ amputation (years)	Phantom limb pain (MPI)	Stump usage	Depression score
A01	Amelic	f	64	left	0	64	0.00	1	0
A02	Amelic	f	72	right	0	72	0.00	-	5
A03	Amelic	m	56	right	0	56	0.00	1	5
A04	Amelic	m	35	right	0	35	0.00	-	6
A05	Amelic	f	25	left	0	25	0.00	-	5
A06	Amelic	m	33	left	0	33	0.00	1	8
A07	Amelic	m	47	left	0	47	0.00	1	1
A08	Amelic	f	65	left	0	65	0.00	1	1
A09	Amelic	m	47	right	0	47	0.00	1	5
A10	Amelic	m	59	left	0	59	0.00	1	12
B01	PLPamp	f	53	left	27	27	3.33	0	6
B02	PLPamp	f	57	right	23	34	1.33	0	11
B03	PLPamp	m	57	right	25	31	1.67	0	14
B04	PLPamp	m	33	right	17	15	1.67	0	7
B05	PLPamp	f	47	left	25	21	2.00	-	21
B06	PLPamp	m	40	left	26	13	2.33	-	8
B07	PLPamp	m	55	left	18	37	3.00	1	16
B08	PLPamp	f	65	left	37	27	2.67	-	9
B09	PLPamp	m	36	right	19	16	1.00	1	8
B10	PLPamp	m	68	left	45	24	3.33	1	5
B11	NonPLP	f	57	left	29	28	0.00	1	2
B12	NonPLP	m	60	right	23	37	0.67	1	4
B13	NonPLP	m	42	right	23	19	0.67	1	10
B14	NonPLP	m	50	right	17	32	0.00	1	2
B15	NonPLP	m	47	left	22	25	0.00	0	3
B16	NonPLP	m	51	left	15	32	0.00	1	4
B17	NonPLP	m	55	left	20	35	0.00	0	14
B18	NonPLP	f	52	left	18	10	0.00	0	10
B19	NonPLP	m	60	right	39	21	0.00	0	15
B20	NonPLP	m	37	left	13	24	0.33	0	4
C01	Control	f	54	left	-	-	-	-	5
C02	Control	f	59	right	-	-	-	-	5
C03	Control	m	34	right	-	-	-	-	5
C04	Control	m	49	right	-	-	-	-	2
C05	Control	f	45	left	-	-	-	-	6
C06	Control	m	50	left	-	-	-	-	6
C07	Control	m	42	left	-	-	-	-	5
C08	Control	f	63	left	-	-	-	-	1
C09	Control	m	52	right	-	-	-	-	4
C10	Control	m	48	left	-	-	-	-	2

Table 1 Demographic and clinical details. PLPamp = amputees with PLP, nonPLP = amputees without PLP, f = female, m = male, 1 = yes, 0 = no, - = data is missing or was not collected, Phantom limb pain (MPI) ratings ≥1 are classified as PLPamp, <1 are classified as NonPLP

3.2 MRI data acquisition

3.2.1 Experimental protocol

Functional magnetic resonance imaging (fMRI) was carried out during a virtual reality movement task. The participants wore a glove on their intact hand which transformed their movement to movements of a synchronous avatar hand. The avatar hand was mirrored, therefore a left and right hand were presented to the participants (similar to the classical mirror box). Participants were instructed to open and close their intact hand (matched side for two-handed controls) with a frequency of 0.5 Hz, paced by an auditory signal with 0.5 Hz, delivered through headphones. While moving their hand, participants were instructed to observe the movement of the virtual hand and to perceive that this movement as movements with their phantom hand (in case of acquired or congenital limb deficiency) or rather as their own hand (controls) (Figure 4).

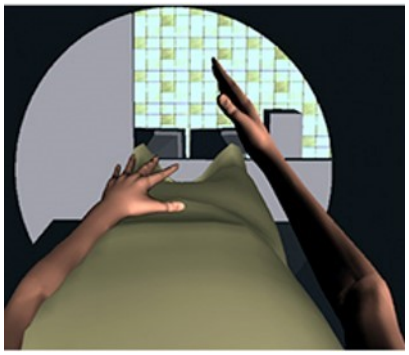

Figure 4 Virtual reality movement task. The participants wore a glove on their intact hand that transformed their movements to movements of a synchronous avatar hand. The avatar hand was mirrored and participants were able to observe a right and left hand. They were instructed to open and close their hand with a frequency of 0.5 Hz, paced by an auditory signal presented via earphones. While moving their hand, participants were instructed to observe the movement of the virtual hand and to perceive this movements as movements of their own/ phantom hand.

The task consisted of alternating 19.8 sec periods of movements and rest. This was repeated eight times for subjects of study B07 and six times for subjects of Phantommind. Some of the participants from the study of Andoh et al. were used in this dissertation, for further information see Andoh et al., 2020. We tested that the number of task repetitions (eight for B07 and six times for Phantommind) was not a confound in our analyses.

3.2.2 MRI data acquisition

MRI data were acquired on a 3 Tesla TRIO whole body scanner (Siemens AG, Erlangen, Germany) with a 32-channel head coil. Parameters of functional MRI data are: 40 slices, voxel size = 2.3 mm isotropic, TE 45ms, TR 3.3s, matrix size 64 x 64. Structural images were taken using T1-weighted images (TR = 2.3s, TE = 2.98, voxel size = 1mm isotropic). We used 30 datasets of B07 and 10 datasets of Phantommind.

3.3 Data Analysis

3.3.1 Preprocessing and Functional Analysis

All imaging data were processed using FSL Software Version 5.0.9 and 6.0.4 (software switch due to technical updates) (Jenkinson et al., 2012; Smith et al., 2004; Woolrich et al., 2009) [4]. First of all, non-brain structures were removed with the Brain Extraction Tool (BET) (Smith, 2002). Since some participants had his/her right and some had his/her left arm missing, the data of participants with missing right arms were mirrored across the mid-sagittal plane to align the hemispheres. The left hemisphere therefore is always the hemisphere contralateral to movement. Functional MRI analysis (Woolrich et al., 2001) was carried out with fMRI Expert Analysis Tool (FEAT, version 6.0.0). Preprocessing included motion correction with MCFLIRT (Jenkinson et al., 2002), high-pass-temporal filtering to remove low frequency drifts (cut-off = 100) and spatial smoothing with an isotropic Gaussian kernel of 5 mm (full width at half maximum) and FILM (FMRIB's Improved Linear Model) prewhitening was performed. Based on visual inspection, standard or extended head motion parameters were added as needed to achieve better registration. In addition, confounding explanatory variables (EVs) were added to further control for head motion when needed. First-level of analysis was performed for each subject. For group comparison, a higher-level of analysis was carried out with the FMRIB's Local Analysis of Mixed Effects (FLAME) (Woolrich et al., 2004). For first-level and second-level analysis, z-threshold was set at 2.3, cluster p-threshold was defined at 0.05 and was corrected for multiple comparisons. MRI data from Phantommind participants included 80 volumes, whereas MRI data from B07 participants included 120 volumes. We compared five datasets with 80 volumes and

[4] www.fmrib.ox.ac.uk/fsl

five datasets with 120 volumes in an unpaired t-test and found no significant differences in neural activity.

3.3.2 Region of Interest (ROI)

Different regions of interest (ROI) were defined. A literature-based ROI was created based on the coordinates of the peak activity induced by intact hand movements resulting from neural activity from both amelics and controls from a study of Hahamy and colleagues (left hemisphere x = -38, y =-26, z = 58; coordinates are given in MNI 152 standard space) (Hahamy et al., 2015). For the ROI on the right hemisphere, the above mentioned were mirrored on the mid-sagittal plane (right hemisphere: x = +38, y = -26, z = 58). Each literature-based coordinate (left, right) was used to define the center of a spherical ROI with a radius of 5 mm.

We chose coordinates from the literature to obtain an ROI which is independent from our sample and is therefore unbiased in terms of sample or number of participants. Moreover, the choice of this ROI enables us to relate our findings to other studies in a similar field.

Additionally, a ROI was defined for each subject based on the individual neural activity (“individual ROI”) in the primary motor cortices. For this purpose, individual neural activity was masked by probabilistic maps Brodmann areas BA4a and BA4p (Geyer et al., 1996) from the Juelich atlas (Eickhoff et al., 2005) and thresholded at 0.3. We then extracted the peak neural activity from the masked motor area and used it to define the center of spherical ROIs with 5mm radius.

Furthermore, a conjunction mask was calculated based on the overlapped activation of amelics, amputees (with and without PLP) and controls following the procedure used by Makin, Cramer, et al., 2013. For this purpose, we calculated the conjunction of activation from the three groups, resulting in a conjunction ROI.

For each ROI we extracted the percent bold signal change (%BSC), which was then compared between hemispheres using paired t-tests and between groups using unpaired t-tests.

3.3.3 Statistical Analysis

Statistical analysis was performed with RStudio Team (2020), Version 1.2.5042[5]. Normality distribution was tested with Shapiro-Wilk-test. If normal distribution was violated, parametric tests were still used because they are known to be more robust than non-parametric tests especially for relatively small sample sizes (Janusonis, 2009). The homogeneities of variances were tested with Levene's test. For each ROI, a two-way analysis of variance (ANOVA) was carried to test for differences in hemispheres and groups. One-way ANOVA was carried out for comparisons between more than two groups in each hemisphere. Student's t-test were performed for comparisons between two groups. The threshold for significance was set at $p < 0.05$ two-sided and corrected for multiple testing by controlling the false discovery rate (FDR) (Benjamini & Hochberg, 1995).

We also calculated indices of functional connectivity between the left and right motor cortices. For each participant and for each scan, we calculated Pearson correlation coefficients between the fMRI time courses from the right and the left motor cortices using FSL. These coefficients were then converted to Z values through Fisher's r-to-z transformation to obtain a normal distribution (Fisher, 1915). The results provide a specific quantitative index of the degree of interhemispheric functional interaction between left and right motor cortices during the virtual reality movement task. Two-way ANOVA, one-way ANOVA and Student's t-test were carried out to test for significant differences in connectivity indices between the groups.

Cortical distances were calculated between the individual peak coordinates in the literature-based ROI and the individual peak coordinates in the individual ROIs using euclidean distances.

Correlation analyses between MPI-pain values and %BSC, functional connectivity or cortical distances were performed with Pearson correlation coefficient. A multiple linear regression was carried out to evaluate the effect of stump usage and depression on neural activity in the hemisphere ipsilateral and contralateral and on indices of functional connectivity in amelics and amputees.

[5] RStudio Team (2020). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.

4 RESULTS

Every participant was able to perform the instructed movements of the hand. None of the participants with congenital limb deficiency experienced phantom limb sensations or phantom limb pain during the experiment. Based on the MPI-D, PLP intensity in the amputee group with PLP was 2.23 ± 0.83 (mean ± SD).

4.1 Psychometric data and motor behaviour

Amputees with PLP were slightly older compared with amputees without PLP when the amputation occurred (mean age ± SD in amputees with PLP 26.2 ± 8.75; amputees without PLP 21.9 ± 7.56). However, age at amputation did not significantly differ between groups ($t(18) = 1.17$, $p = 0.25$), nor did time since amputation relative to the date of measurement (mean ± SD in amputees with PLP 24.5 ± 8.22; amputees without PLP 26.3 ± 8.25; $t(18) = -0.49$, $p = 0.63$).

Regarding psychological factors, depression scores measured using the ADS questionnaire, were statistically different between the four groups (one-way anova $F(3, 36) = 4.96$, $p = 0.01$), such that amputees with PLP showed significantly higher depression scores compared with amelics ($t(18) = -2.91$, $p = 0.01$, $p_{FDR} = 0.03$) and compared with controls ($t(18) = -3.77$, $p = 0.001$, $p_{FDR} = 0.01$). Depression scores in amputees without PLP did not significantly differ from the ones in amputees with PLP ($t(18) = 1.64$, $p = 0.12$, $p_{FDR} = 0.2$) or from the ones in amelics $t(18) = -1.03$, $p = 0.32$, $p_{FDR} = 0.38$) or from the ones in controls ($t(18) = -1.61$, $p = 0.12$, $p_{FDR} = 0.19$). Depression scores were also not statistically different between amelics and controls ($t(18) = 0.55$, $p = 0.59$, $p_{FDR} = 0.59$).

Regarding stump usage, six data out of 30 were missing because the participants did not answer the respective questions (three amelics and three amputees with PLP). All remaining data showed that all amelics used their stump when they did not wear a prosthesis. Similarly, half of the amputees without PLP used their stump as well when they did not wear a prosthesis and the other did not use their stump. In contrary, only three amputees with PLP used their stump when they did not wear a prosthesis compared with four amputees with PLP who did not use their stump. Linear simple regression was carried out to examine the effect of pain on stump usage, but the correlation did not reach significance (adjusted $R^2 = -0.02$, $F(1, 22) = 0.54$, $p = 0.47$).

4.2 Whole Brain analyses: mean neural activity during hand movement

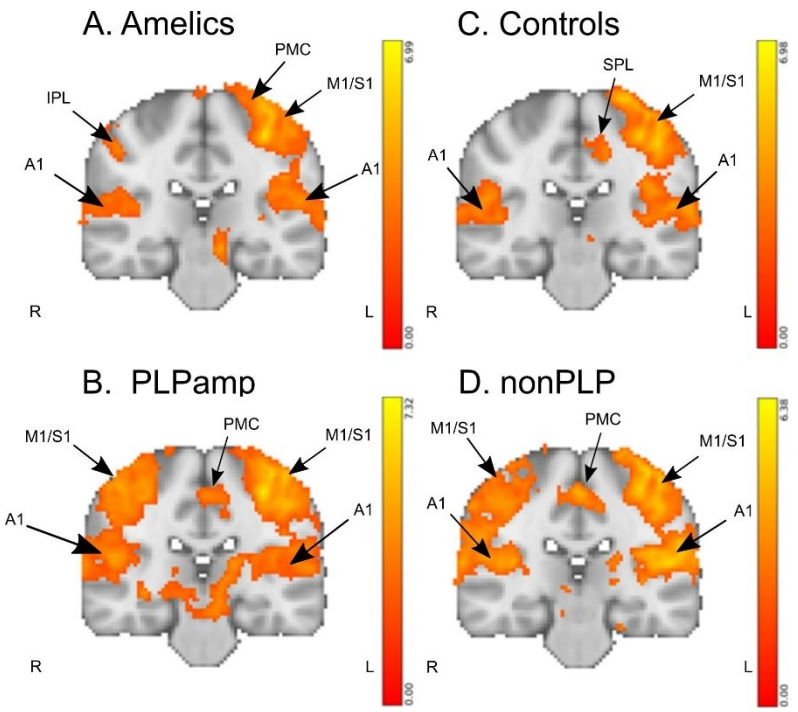

Figure 5 Whole brain mean task-related neural activity during hand movements in **A.** Amelics, **B**. Amputees with PLP ("PLPamp"), **C.** Controls, **D.** Amputees without PLP ("nonPLP"). Activations are mapped on the MNI152 template provided by FSL (images are shown at slice coordinates in standard space: x = -40, y = -26, z = 58). Abbreviations: M1 = primary motor cortex, S1 = primary somatosensory cortex, A1 = primary auditory cortex, PMC = premotor cortex, SPL = superior parietal lobule, IPL = inferior parietal lobule

Mean neural activity during the virtual reality movement task was calculated for each group at a whole brain level. All groups showed neural activation in the primary motor cortex contralateral to the movement (left hemisphere) and as well in the bilateral auditory cortex (Figure 5A-D*)* [6]. In amputees (with and without PLP) additional neural activity was found in M1/S1 in the hemisphere ipsilateral to the hand movements (right hemisphere). (Figure 5B, D*)*. Unpaired t-test was then carried out to compare mean neural activity during the virtual reality movement task between groups. Compared with amelics, amputees with and without PLP showed increased neural activity in M1/S1 ipsilateral to hand movement, right superior and inferior parietal lobules, and right premotor cortex (Figure 6A, B). Compared with controls, amputees with and without PLP showed increased neural activity in M1/S1 ipsilateral to hand movement (Figure 6C, D). There was no significant differences in task-related activity between amputees with and amputees without PLP and between amelics and controls (data not shown).

[6] Labels of the functional brain areas based on the Juelich histological atlas (Eickhoff et al., 2005)

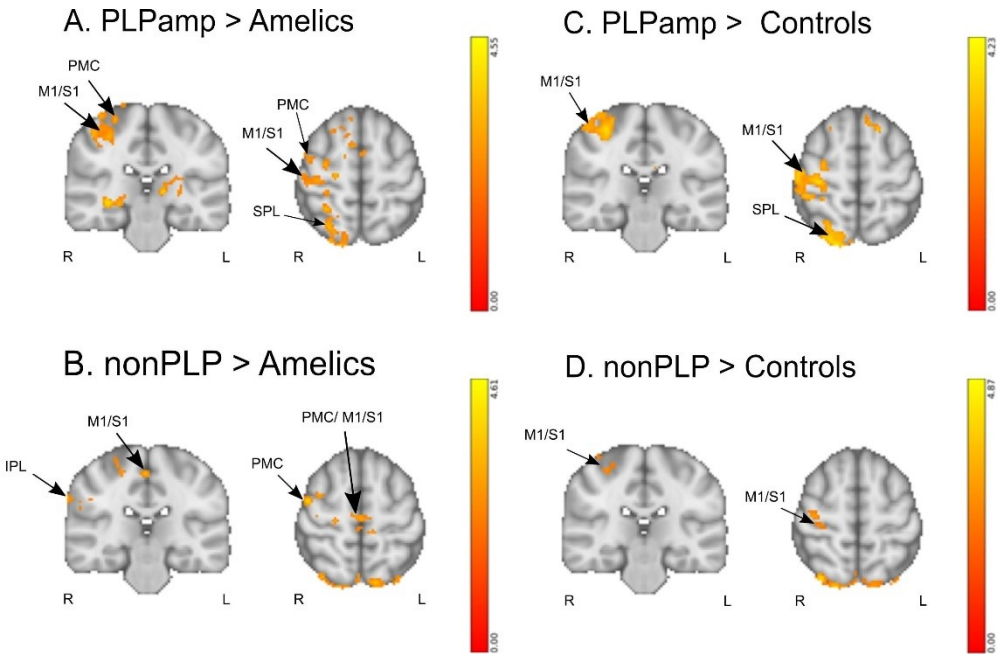

Figure 6 Whole brain comparisons of task-related neural activity between groups. Amputees showed higher neural activity in the hemisphere ipsilateral to hand movement. **A.** Amputees with PLP showed greater activation than amelics in the M1/S1 and PMC in the hemisphere ipsilateral to movement. **B.** Amputees without PLP showed greater activation than amelics in M1/S1 and IPL. **C.** Amputees with PLP showed higher neural activity in M1/S1 and SPL than controls **D.** Amputees without PLP showed higher activation in M1/S1 in the hemisphere ipsilateral to movement compared with controls. Amputees with and without PLP and as well as amelics and controls did not show significant differences (data not shown). Activations are mapped on the MNI152 template provided by FSL (images are shown at slice coordinates in standard space: x = -40, y = -26, z = 58). Abbreviations: M1 = primary motor cortex, S1 = primary somatosensory cortex, PMC = premotor cortex, SPL = superior parietal lobule, IPL = inferior parietal lobule

4.3 Conjunction ROI

To examine the overlapping task-related neural activity in amelics, amputees (with and without PLP) and controls, a conjunction ROI approach was used. As shown in Figure 7A, the three groups showed overlapping activation in S1/M1 in the left hemisphere, contralateral to hand movement.

As a comparison, I also mapped the literature-based ROI from Hahamy et al. (2015), which is shown as a white circle in Figure 7B. The literature-based ROI overlapped nicely with our conjunction ROI.

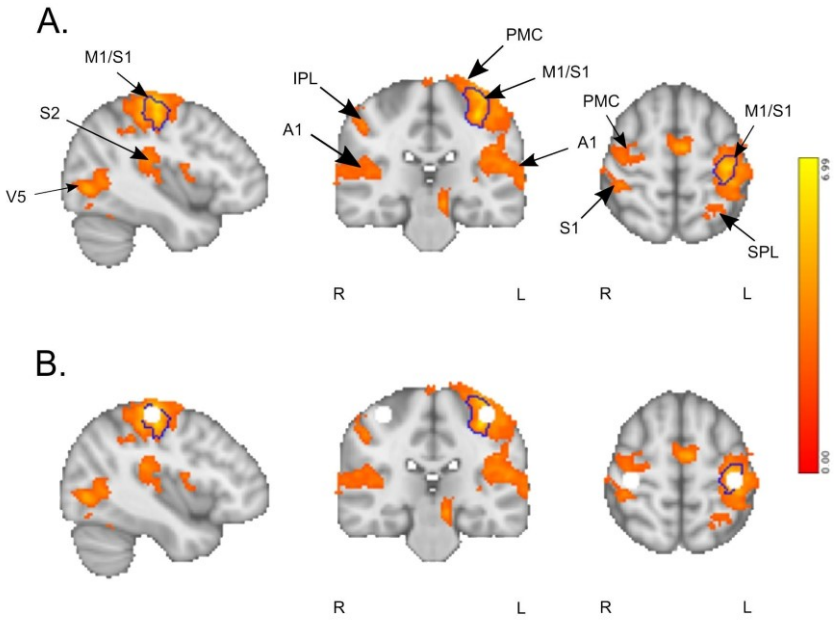

Figure 7 Conjunction ROI of task-related neural activity in amelics, amputees and controls. **A.** The conjunction ROI is shown in blue, red-orange indicates the task-related neural activity of amelics (Figure 5A). **B.** Literature-based ROI is indicated in white and overlaps with the conjunction ROI. Activations are mapped on the MNI152 template provided by FSL (coordinates in standard space: x = -40, y = -26, z = 58). Abbreviations: M1 = primary motor cortex, S1 = primary somatosensory cortex, S2 = secondary somatosensory cortex, PMC = premotor cortex, SPL = superior parietal lobule, IPL = inferior parietal lobule, A1 = primary auditory cortex, V5 = middle temporal visual cortex

4.4 Literature-based ROI: mean neural activity in M1/S1 during hand movement

There was no significant difference in bold signal change (%BSC) in the literature-based ROI between amputees with and without PLP, neither in the hemisphere contralateral to movement (one-way ANOVA, $F(1,18) = 2.26$, $p = 0.15$) nor in the hemisphere ipsilateral to movement (one-way ANOVA, $F(1,18) = 0.83$, $p = 0.38$). Neural activity could therefore be averaged between the two groups and combined into one, that will be named “amputee group”.

To examine differences in neural activity in the literature-based ROI, a two-way anova was performed with the variables group (amelics, amputees and controls) and side of hemisphere (ipsilateral and contralateral to movement). I did not find a significant interaction between the two variables on neural activity ($F(2,74) = 0.95$, $p = 0.39$). The main effect for group did not reach significance ($F(2,74) = 2.48$, $p = 0.09$), but the main effect for side of hemisphere reached significance ($F(1,74) = 77.12$, $p < 0.001$). All groups showed increased task-related neural activity in the hand area contralateral to movement compared with the hemisphere ipsilateral to movement (paired t-test: amelics $t(9) = 6.17$, $p = 0.0002$; amputees $t(19) = 6.08$, $p < 0.0001$; controls $t(9) = 7.79$, $p < 0.0001$). Significant differences between groups

were found in the hemisphere ipsilateral to hand movements (one-way ANOVA, F(2, 37) = 6.54, p = 0.004). Neural activity in the hemisphere ipsilateral to movement was significant decreased in amelics compared with amputees (t(28) = -2.35, p = 0.03, p_{FDR} = 0.04). Moreover, amputees showed significantly increased neural activity in the ipsilateral hemisphere compared with controls (t(28) = -3.00, p = 0.01, p_{FDR} = 0.02). Amelics and controls had similar neural activity in the hemisphere ipsilateral to movement (t(18) = 0.78, p = 0.45, p_{FDR} = 0.45) (Figure 8A).

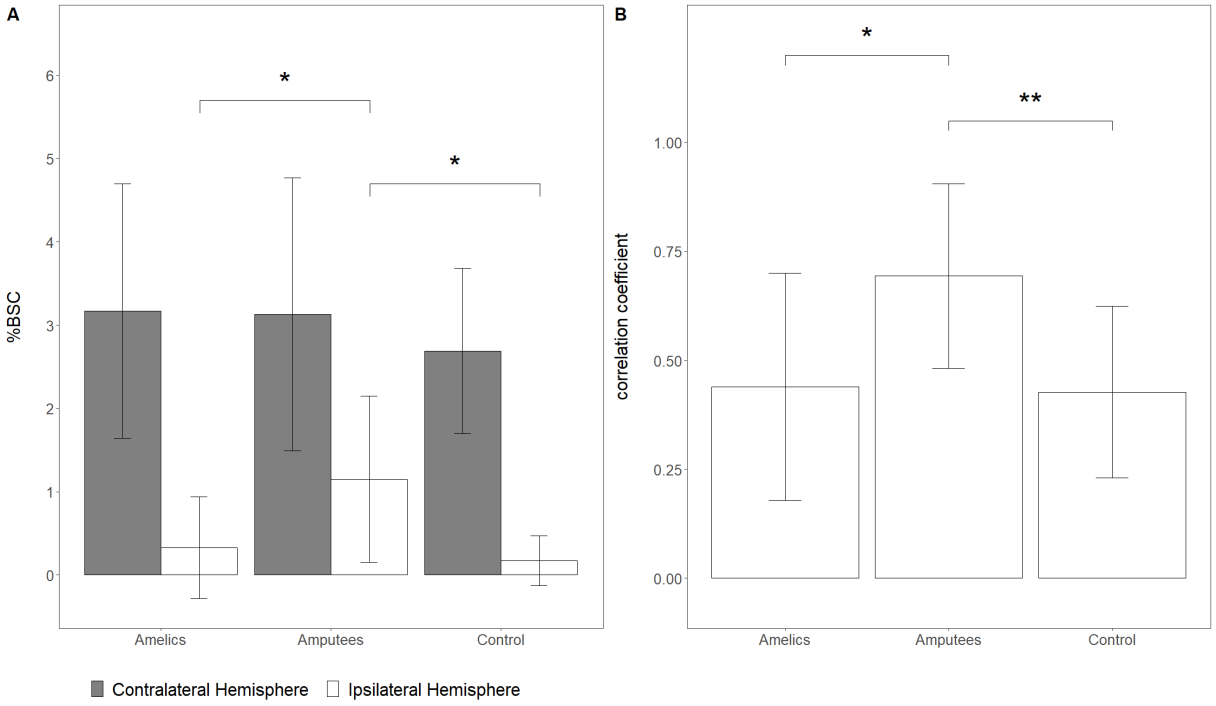

Figure 8 Comparison of task-related neural activity and functional connectivity in the literature based ROIs between groups in the hemisphere contralateral and ipsilateral to hand movements. **A. Task-related neural activity (%BSC) in the literature-based ROI across groups in the hemisphere contralateral and ipsilateral to intact hand movements**: increased neural activity in amputees compared with both amelics and controls in hemisphere ipsilateral to movement. **B. Mean indices of functional connectivity between left and right hemispheres in the literature-based ROI**: increased functional connectivity in amputees compared with both amelics and controls. In figures A and B errors bars indicate standard deviation of the mean. * p< 0.05, ** p< 0.01, *** p< 0.001

In contrast, no significant difference in %BSC was found between amelics, amputees and controls in the M1/S1 contralateral to hand movement (one-way ANOVA, (F(2, 37) = 0.34, p = 0.71) (mean %BSC ± SD in amelics 3.17 ± 1.53, in amputees 3.13 ± 1.64, in controls 2.69 ± 0.99). For detailed information about %BSC see Table 2.

Functional connectivity

Indices of functional connectivity between left and right M1/S1 were comparable between amputees with and without PLP (one-way ANOVA, $F(1, 18) = 0.03$, $p = 0.86$) and were therefore averaged together into one group (namely amputees).

Amelics, amputees and two-handed controls had statistically significant differences in functional connectivity between left and right M1/S1 (one-way ANOVA, $F(2, 37) = 6.89$, $p = 0.003$). Amelics had a significant reduced functional connectivity compared with amputees ($t(28) = -2.87$, $p = 0.01$, $p_{FDR} = 0.01$), but showed no significant difference compared with controls ($t(18) = 0.12$, $p = 0.91$, $p_{FDR} = 0.91$). Amputees showed statistically significant higher indices of functional connectivity compared with controls ($t(28) = -3.31$, $p = 0.003$, $p_{FDR} = 0.01$) (Figure 8B). For detailed information about functional connectivity see Table 2.

Relationship between PLP, motor behaviour and psychological factors

In the hemisphere ipsilateral to movement, %BSC was not significant correlated with PLP intensity using the entire sample of amputees ($rho = 0.17$, $p = 0.46$) (Figure 9A). Considering only amputees with PLP, the correlation did not become significant ($rho = -0.05$, $p = 0.88$) (Figure 9B). As one amputee with PLP showed extremely high values of %BSC, I excluded the participant from this correlation. Without the outlier, the correlation in the entire sample of amputees remained non-significant ($rho = -0.28$, $p = 0.24$) (Figure 9A). Amputees with PLP excluding the outlier showed a significant negative correlation between neural activity and PLP intensity ($rho = -0.81$, $p = 0.0076$) (Figure 9B).

Furthermore, correlation analyses between neural activity in the hemisphere contralateral to movement and PLP intensity were statistically not significant, when considering in the entire amputee sample ($rho = 0.22$, $p = 0.35$) or amputees with PLP only ($rho = -0.15$, $p = 0.68$).

Indices of functional connectivity were not significant correlated with PLP intensity, when considering the entire amputee sample ($rho = -0.13$, $p = 0.57$) (Figure 9C) or amputees with PLP only ($rho = -0.4$, $p = 0.25$) (Figure 9D).

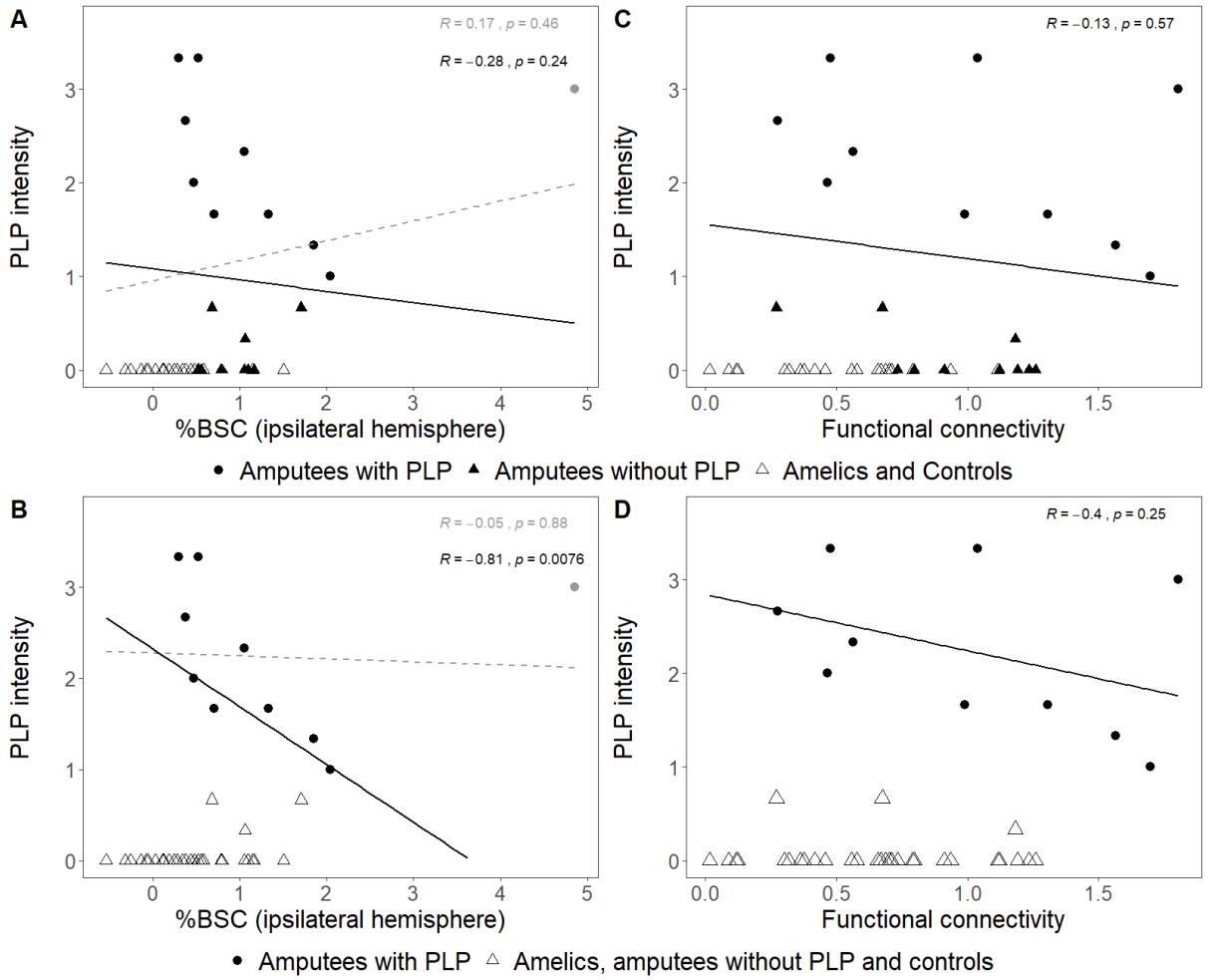

Figure 9 Correlation between PLP intensity and measures of brain activity in the literature-based ROIs. A. Correlation between PLP intensity and %BSC in the hemisphere ipsilateral to movement in the entire amputees sample. **B** Correlation between %BSC in the hemisphere ipsilateral to movement and PLP intensity in amputees with PLP. **C.** Correlation between PLP intensity and indices of functional connectivity in the entire sample of amputees. **D.** Correlation between functional connectivity and PLP intensity in amputees with PLP. In A,B, the grey dashed line shows the non-significant correlation between PLP intensity in the entire amputee group with the outlier. The black correlation line shows the correlation between PLP intensity and %BSC in amputees without the outlier. The outlier is indicated by the grey dot. In A,B,C,D black-filled shapes indicate participants that were included in the respective correlation, white-filled shapes indicate participants that were not included.

A regression analysis did not show a significant effect of depression and stump usage on neural activity in the hemisphere ipsilateral to movement in amelics and amputees (adjusted R^2 = 0.09, F(2,21) = 2.074, p = 0.15). However, depression showed a trend towards a significant effect on neural activity in the hemisphere ipsilateral to movement (β = 0.44, p = 0.06), indicating that higher depression scores lead to higher neural activity. Stump usage did not reach significance (β = 0.16, p = 0.47).

Regarding the hemisphere contralateral to movement in amelics and amputees, a regression analysis did not show a significant effect of depression and stump usage on neural activity (adjusted R^2 = -0.04, F(2,21) = 0.56, p = 0.58). Both variables

stump usage (β = 0.17, p = 0.46) and depression (β = 0.23, p = 0.34) did not show a significant effect.

A regression analysis revealed no significant effect of depression and stump usage on indices of functional connectivity of amelics and amputees (adjusted R^2 = 0.09, F(2,21) = 2.15, p = 0.14) but showed a positive trend towards significance for depression (β = 0.34, p = 0.13). Again, stump usage did not reach significance (β = -0.14, p = 0.53).

4.5 Individual ROIs: mean neural activity in M1/S1 during hand movement

Using individual ROIs, there was no significant differences in %BSC between amputees with and without PLP, neither in the hemisphere contralateral to movement (one-way ANOVA (F(1, 18) = 1.12, p = 0.30) nor in the ipsilateral hemisphere (one-way ANOVA (F(1, 18) = 1.14, p = 0.30). Therefore, neural activity could be averaged and combined into one amputee group.

There was no significant interaction between groups (amelics, amputees, controls) and side of hemisphere on neural activity (two-way ANOVA F(2,74) = 0.99, p = 0.37). The main effect for the variable group was not significant (F(2, 74) = 0.46, p = 0.63), but the main effect for the variable side of hemisphere became significant (F(1, 74) = 44.11, p < 0.001). All groups showed increased task-related neural activity in the hemisphere contralateral to movement compared with the hemisphere ipsilateral to movement (paired t-test: amelics t(9) = 4.8, p = 0.001; amputees t(19) = 5.1, p < 0.0001; controls t(9) = 4.94, p = 0.001) (Figure 10A). Neural activity was not significantly different between the groups, neither in the hemisphere ipsilateral to movement (one-way ANOVA (F(2, 37) = 2.65, p = 0.08), or in the hemisphere contralateral to movement (one-way ANOVA (F(2, 37) = 0.07, p = 0.94). For detailed information about %BSC see Table 2.

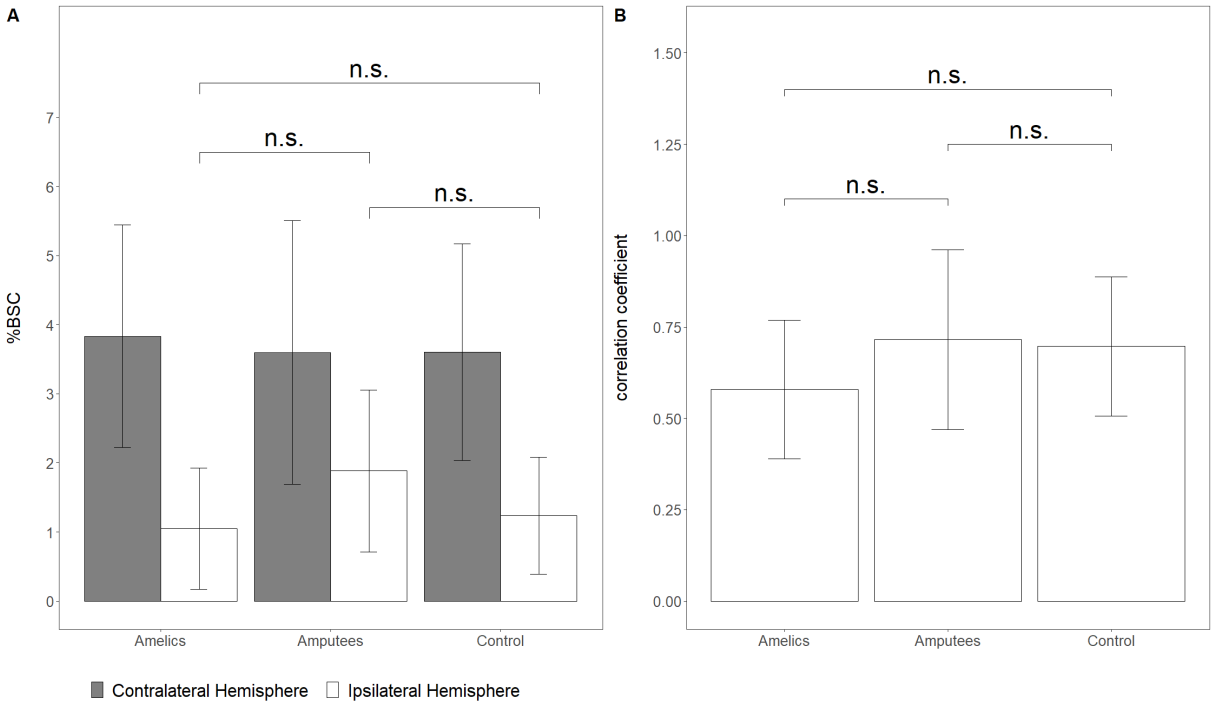

Figure 10 Comparison of task-related neural activity and functional connectivity in the individual ROIs between groups in the hemisphere contralateral and ipsilateral to hand movements. **A.** Mean percent of bold signal change (%BSC) in the individual ROI for the left and right hemispheres. **B.** Comparison of mean indices of functional connectivity between left and right individual ROIs between the groups. In figures A and B errors bars indicate standard deviation. * p< 0.05, ** p< 0.01, *** p< 0.001, n.s. = not significant

Functional connectivity

Using the individual ROIs, indices for functional connectivity between left and right M1/S1 did not differ between amputees with and without PLP (one-way ANOVA, F(1, 18) = 1.24, p = 0.28) and were averaged together into one group (amputees). Indices of functional connectivity were not statistically different between amelics, amputees and controls (one-way ANOVA, F(2, 37) = 1.32, p= 0.28; mean indices of functional connectivity ± SD in amelics 0.58 ± 0.19, in amputees 0.72 ± 0.25, in controls 0.7 ± 0.19) (Figure 10B). For detailed information about indices of functional connectivity see Table 2.

Relationship between PLP, motor behaviour and psychological factors

Using individual ROI, neural activity in the hemisphere ipsilateral to movement did not show a significant correlation with PLP intensity in amputees (rho = 0.22, p = 0.35) (Figure 11A). In amputees with PLP, %BSC in the hemisphere ipsilateral to movement was not statistically significant correlated to PLP intensity (rh = -0.14, p = 0.70) (Figure 11B). In the hemisphere contralateral to movement, correlation between neural activity and PLP intensity in the entire amputee sample did not reach significance (rho = 0.11, p = 0.65). Furthermore, considering only amputees with

PLP, there was no significant correlation between %BSC in the hemisphere contralateral to movement and PLP intensity (rho = -0.25, p = 0.49). There was no significant correlation between indices of functional connectivity and PLP intensity in the entire sample of amputees (rho = 0.05, p = 0.83) (Figure 11C). Considering only amputees with PLP, indices of functional connectivity were not significantly correlated to PLP (rho = -0.53, p = 0.11) (Figure 11D).

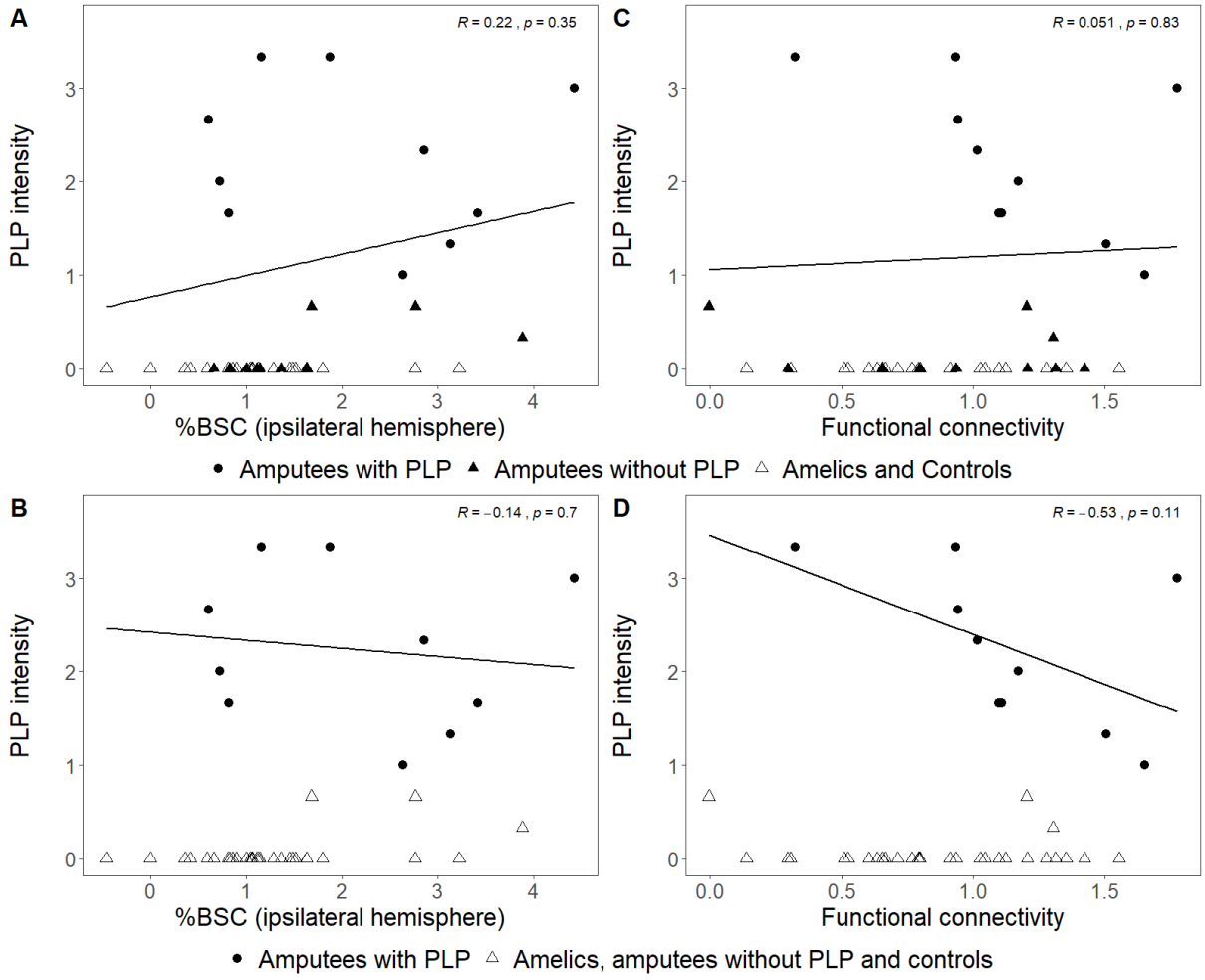

Figure 11 Correlation between PLP intensity and measures of brain activity in the individual ROIs A. Correlation between PLP intensity and %BSC in the hemisphere ipsilateral to movement in the entire amputee sample. **B.** Correlation between PLP intensity and neural activity in amputees with PLP. **C.** Correlation between PLP intensity and indices of functional connectivity in amputees. **D.** Correlation between PLP intensity and indices of functional connectivity in amputees with PLP. In A,B,C,D black-filled shapes indicate participants that were included in the respective correlation, white-filled shapes indicate participants that were not included.

A regression analysis showed a significant effect of stump usage and depression on neural activity in the hemisphere contralateral to movement in amelics and amputees (adjusted R^2 = 0.25, F(2,21) = 4.78, p = 0.02). Both depression (β = 0.57, p = 0.01) and stump usage (β = 0.41, p = 0.048) had a significant effect on neural activity in the individual ROI in the hemisphere contralateral to movement. Higher depression

scores and more frequent stump usage were associated with higher neural activity in M1/S1 contralateral to movement.

A regression analysis did not show a significant effect of depression and stump usage on neural activity in the hemisphere ipsilateral to movement in amelics and amputees (adjusted R^2 = 0.05, F(2,21) = 1.66, p = 0.21). However, depression showed a trend towards significance (β = 0.37, p = 0.11). Stump usage did not reach significance (β = -8.61, p = 1.00).

A regression analysis with the factors depression and stump usage showed no statistically significant effect on indices of functional connectivity (adjusted R^2 = -0.04, F(2,21) = 0.52, p = 0.6). Both depression (β = 0.14, p = 0.56) and stump usage (β = -0.12, p = 0.6) did not become significant and thus showed therefore no significant effect on functional connectivity.

ID	Group	Literature-based ROI			Individual ROI		
		% BSC (left hemisphere)	% BSC (right hemisphere)	Functional connectivity Index	% BSC (left hemisphere)	% BSC (right hemisphere)	Functional connectivity Index
A01	Amelic	3.24	0.28	0.52	3.61	1.06	0.54
A02	Amelic	2.35	1.15	0.81	2.27	1.62	0.80
A03	Amelic	2.22	0.44	0.66	2.33	1.45	0.47
A04	Amelic	5.64	0.38	0.35	4.80	0.81	0.66
A05	Amelic	2.81	-0.06	0.09	5.45	1.06	0.66
A06	Amelic	4.99	-0.26	0.02	5.25	0.00	0.14
A07	Amelic	0.81	-0.54	0.73	1.68	-0.47	0.78
A08	Amelic	4.88	1.50	0.43	4.09	2.76	0.61
A09	Amelic	2.35	0.25	0.29	2.48	1.28	0.48
A10	Amelic	2.36	0.19	0.51	6.39	0.89	0.65
B01	PLPamp	1.96	0.52	0.44	2.09	1.15	0.73
B02	PLPamp	2.38	1.85	0.92	2.98	3.13	0.91
B03	PLPamp	4.42	1.33	0.86	4.25	3.41	0.80
B04	PLPamp	3.10	0.70	0.76	2.94	0.82	0.80
B05	PLPamp	3.85	0.47	0.43	3.85	0.72	0.82
B06	PLPamp	8.31	1.06	0.51	8.52	2.86	0.77
B07	PLPamp	5.86	4.85	0.95	7.55	4.42	0.94
B08	PLPamp	1.55	0.38	0.27	1.62	0.60	0.74
B09	PLPamp	3.85	2.05	0.27	6.07	2.63	0.93
B10	PLPamp	1.33	0.30	0.78	0.61	1.87	0.31
B11	NonPLP	3.34	0.56	0.83	3.44	1.14	0.89
B12	NonPLP	3.10	1.71	0.26	2.86	1.68	0.00
B13	NonPLP	1.51	0.68	0.59	3.26	2.76	0.83
B14	NonPLP	2.16	1.06	0.84	2.34	0.99	0.84
B15	NonPLP	3.21	1.17	0.72	2.99	1.11	0.86
B16	NonPLP	2.73	1.09	0.62	3.82	0.83	0.66
B17	NonPLP	2.08	0.78	0.85	2.20	0.66	0.58
B18	NonPLP	2.35	0.80	0.66	2.39	1.36	0.29
B19	NonPLP	2.09	0.52	0.81	3.76	1.63	0.73
B20	NonPLP	3.34	1.06	0.83	4.41	3.88	0.86
C01	Control	2.14	0.03	0.31	1.94	1.04	0.58
C02	Control	2.76	0.58	0.58	3.45	1.12	0.86
C03	Control	3.02	0.12	0.61	6.57	1.51	0.77
C04	Control	2.87	0.48	0.39	4.03	1.48	0.72
C05	Control	3.48	-0.32	0.36	3.95	0.85	0.81
C06	Control	2.37	-0.14	0.12	3.90	1.79	0.87
C07	Control	3.26	0.34	0.58	3.36	0.41	0.30
C08	Control	3.62	-0.07	0.31	4.48	0.59	0.56
C09	Control	3.21	0.52	0.60	3.81	3.22	0.91
C10	Control	0.22	0.11	0.61	0.54	0.36	0.58

Table 2 %BSC and indices of functional connectivity for each participant. %BSC shows task-related neural activity in the literature-based ROI and in the individually-defined ROI. Functional connectivity index refers to the time course of fMRI signal. %BSC= percent of bold signal change.

4.6 Cortical distances

Cortical distances calculated between the literature-based ROI and the individual ROI, were statistically not different between amputees with PLP and without PLP, neither in the hemisphere contralateral to movement (one-way ANOVA, $F(1,18) = 0.01$, $p = 0.94$), nor in the hemisphere ipsilateral to movement (one-way ANOVA, $F(1,18) = 0.03$, $p = 0.86$). Therefore, cortical distances were averaged across amputees with and without PLP and combined into one group (namely “amputees”).
A two-way anova was performed and showed a significant main effect for side of hemisphere on cortical distances ($F(1,74) = 11.66$, $p = 0.001$). The main effect for group did not become significant ($F(2,74) = 0.43$, $p = 0.65$), as well as the interaction of the variables group and side of hemisphere on cortical distance was not significant ($F(2,74) = 1.00$, $p = 0.37$). Cortical distances were statistically not different between the three groups, neither in the hemisphere ipsilateral to movement (one-way ANOVA, $F(2,37) = 0.701$, $p = 0.50$), nor in the hemisphere contralateral to movement (one-way ANOVA, $F(2,37) = 0.75$, $p = 0.48$) (Figure 12A).

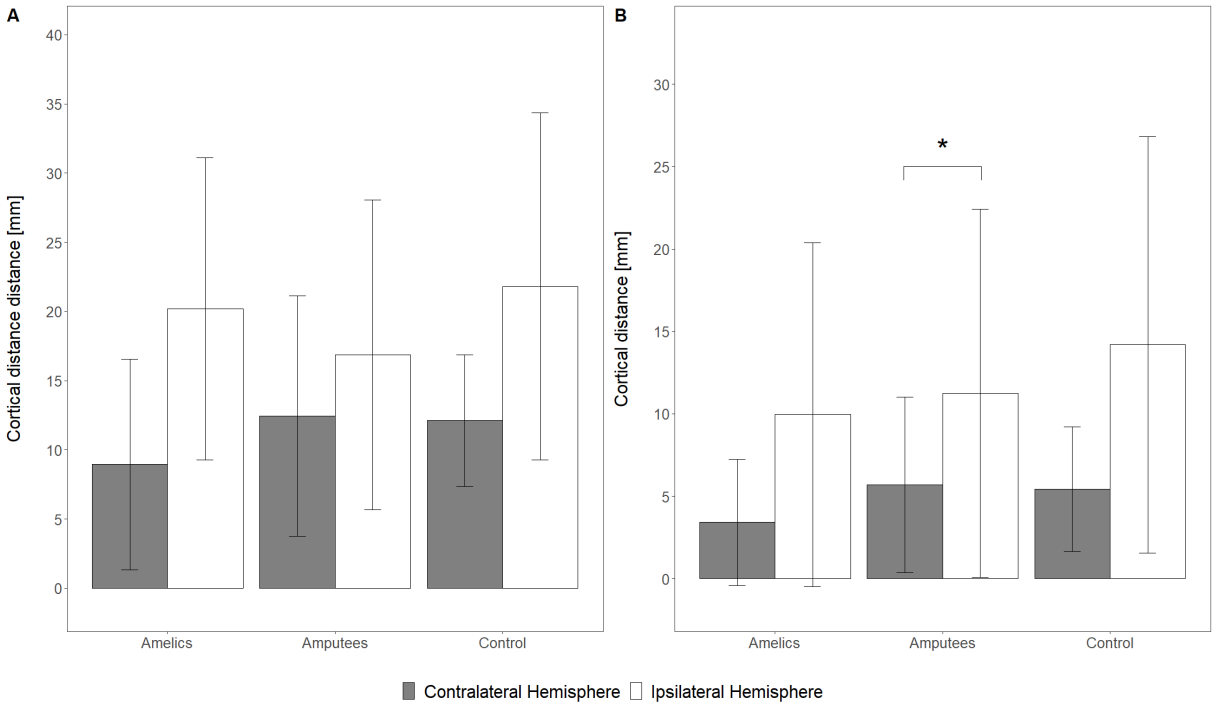

Figure 12 Cortical distances between the literature-based ROI and individual ROI in the hemisphere contralateral (grey) and ipsilateral (white) to movement using using **(A)** the spatial x,y,z coordinates and **(B)** the mediolateral axis. Errors bars indicate standard deviation of the mean. * $p < 0.05$

In order to examine potential cortical reorganisation in the mediolateral axis (frontal plane), I calculated cortical distances between the literature-based ROI and the individual ROI in the mediolateral axis. Amputees with and without PLP did not show significant differences, neither in cortical distance in the hemisphere contralateral (one-way ANOVA, $F(1,18) = 0.02$, $p = 0.88$), nor in the hemisphere ipsilateral to movement (one-way ANOVA, $F(1,18) = 0.004$, $p = 0.95$). Cortical distances in the mediolateral axis could therefore be averaged across the amputees with and without PLP and combined into one group (namely “amputee group”).

A two-way anova showed no significant interaction between group and hemispheres ($F(2,74) = 0.23$, $p = 0.80$). There was no main effect of group ($F(2,74) = 0.65$, $p = 0.52$) but a main effect for hemisphere ($F(1, 74) = 11.54$, $p = 0.001$).

Comparing the cortical distances in the mediolateral axis per group, amputees showed a significant greater cortical distance in the hemisphere ipsilateral compared with the hemisphere contralateral to movement (paired t-test $t(19) = -2.22$, $p = 0.04$). Amelics and controls did not show significant differences in cortical distances between left and right hemispheres (paired t-test: amelics: $t(9) = -1.61$, $p = 0.14$; controls: $t(9) = -1.95$, $p = 0.08$) (Figure 12B). Comparing the cortical distances in the hemisphere ipsilateral to movement between amelics, amputees and controls, no significant differences were found (one-way ANOVA, $F(2,37) = 0.37$, $p = 0.69$). Likewise, the groups showed similar cortical distances in the hemisphere contralateral to movement (one-way ANOVA, $F(2,37) = 0.84$, $p = 0.44$). For detailed information about the coordinates of %BSC and cortical distances in the hemisphere ipsilateral and contralateral see Table 3, Table 4 and Table 5.

Relationship with PLP

In the hemisphere ipsilateral to movement, PLP intensity was not significantly correlated with cortical distance in amputees ($rho = 0.17$, $p = 0.48$) (Figure 13A). Considering only amputees with PLP, the correlation remained non-significant ($rho = 0.28$, $p = 0.43$) (Figure 13B). In the hemisphere contralateral to movement, PLP intensity did not show a significant correlation with cortical distance in the entire sample of amputees ($rho = 0.18$, $p = 0.44$) as well as when only amputees with PLP were considered ($rho = 0.36$, $p = 0.31$).

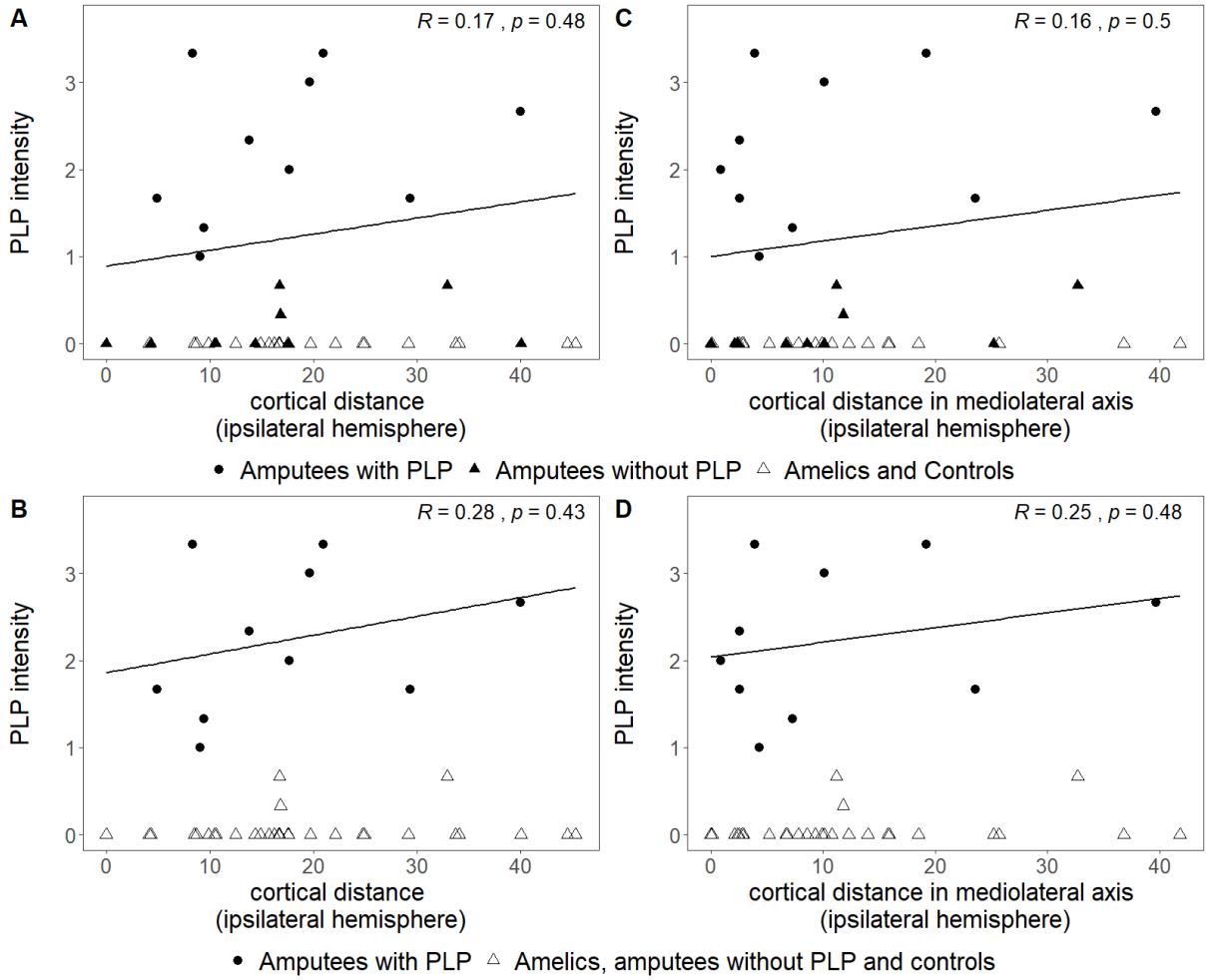

Figure 13 Correlation between PLP intensity and cortical distances (**A**) in the entire sample of amputees and in (**B**) only amputees with PLP using the spatial x,y,z coordinates, (**C**) in the entire sample of amputees and in (**D**) amputees with PLP in the mediolateral axis

In the mediolateral axis, no significant correlation between cortical distances in the hemisphere ipsilateral to movement and PLP intensity in amputees was found (rho = 0.16, p = 0.5) (Figure 13C). The correlation did not become significant when amputees with PLP were included (rho = 0.25, p = 0.48) (Figure 13D). Using 3D euclidean distances in the hemisphere contralateral to movement, there was no significant correlation between cortical distances and PLP intensity in amputees (rho = 0.2, p = 0.41). Considering only amputees with PLP, no significant correlation between euclidean distances in the hemisphere contralateral to movement and PLP intensity was found (rho = 35, p = 0.32).

		Literature-based ROI			Individual ROI			Cortical distance	
ID	Group	x	y	z	x	y	z	spatial (x, y, z)	medio-lateral axis
A01	Amelic	-43.40	-22.70	60.00	-43.40	-23.50	53.20	6.85	0.00
A02	Amelic	-39.00	-21.80	62.90	-27.00	-25.70	78.80	20.30	12.00
A03	Amelic	-39.90	-22.70	55.00	-39.90	-22.70	55.00	0.00	0.00
A04	Amelic	-37.40	-27.10	59.70	-34.80	-25.20	69.30	10.13	2.60
A05	Amelic	-37.50	-21.00	63.60	-35.50	-16.70	73.70	11.16	2.00
A06	Amelic	-37.40	-24.50	53.30	-35.10	-22.00	53.20	3.40	2.30
A07	Amelic	-38.80	-20.30	59.50	-38.80	-20.30	59.50	0.00	0.00
A08	Amelic	-33.50	-28.60	58.70	-35.90	-29.40	59.00	2.55	2.40
A09	Amelic	-38.30	-19.90	62.70	-31.80	-29.10	50.40	16.68	6.50
A10	Amelic	-36.60	-21.10	55.00	-43.00	-23.60	72.00	18.34	6.40
B01	PLPamp	-34.60	-29.10	57.20	-42.50	-13.60	65.70	19.36	7.90
B02	PLPamp	-40.30	-19.50	58.20	-29.00	-28.70	73.90	21.42	11.30
B03	PLPamp	-36.80	-26.60	63.90	-36.80	-26.60	63.90	0.00	0.00
B04	PLPamp	-37.10	-27.10	57.50	-44.20	-22.00	70.20	15.42	7.10
B05	PLPamp	-40.10	-21.80	64.80	-45.00	-17.50	60.50	7.81	4.90
B06	PLPamp	-41.80	-25.30	60.00	-41.80	-25.30	60.00	0.00	0.00
B07	PLPamp	-43.90	-21.90	55.40	-46.40	-21.40	65.60	10.51	2.50
B08	PLPamp	-39.40	-24.60	58.10	-34.10	-27.50	48.00	11.77	5.30
B09	PLPamp	-36.70	-25.90	62.50	-34.60	-21.80	71.40	10.02	2.10
B10	PLPamp	-44.20	-23.10	62.40	-61.90	-11.30	41.50	29.82	17.70
B11	nonPLP	-42.40	-21.20	63.70	-42.80	-20.60	67.10	3.48	0.40
B12	nonPLP	-42.70	-27.10	58.60	-60.70	-14.80	38.40	29.72	18.00
B13	nonPLP	-34.90	-20.80	58.40	-37.20	-18.90	68.00	10.05	2.30
B14	nonPLP	-35.70	-25.80	60.50	-37.20	-15.60	70.20	14.16	1.50
B15	nonPLP	-36.30	-27.30	63.30	-36.30	-27.30	63.30	0.00	0.00
B16	nonPLP	-44.80	-24.10	61.20	-40.60	-14.50	45.70	18.71	4.20
B17	nonPLP	-37.30	-26.40	57.10	-47.40	-11.40	58.30	18.12	10.10
B18	nonPLP	-33.40	-27.70	58.30	-40.90	-22.20	52.30	11.07	7.50
B19	nonPLP	-41.90	-23.40	64.50	-35.40	-18.00	71.20	10.78	6.50
B20	nonPLP	-42.60	-22.00	63.80	-47.20	-17.30	62.40	6.72	4.60
C01	control	-44.20	-23.20	61.50	-38.70	-27.10	48.20	14.91	5.50
C02	control	-42.60	-21.80	55.90	-47.70	-14.00	62.10	11.19	5.10
C03	control	-40.50	-21.50	63.20	-28.60	-21.80	73.30	15.61	11.90
C04	control	-43.30	-25.90	57.80	-42.60	-17.60	69.30	14.20	0.70
C05	control	-38.30	-21.70	56.20	-43.50	-15.90	61.70	9.54	5.20
C06	control	-43.80	-26.00	62.90	-46.30	-19.20	60.70	7.57	2.50
C07	control	-43.10	-22.70	63.40	-45.70	-20.40	62.70	3.54	2.60
C08	control	-44.50	-22.90	58.90	-32.80	-27.40	72.80	18.72	11.70
C09	control	-40.60	-20.50	62.40	-37.80	-16.80	54.90	8.82	2.80
C10	control	-38.00	-23.60	53.50	-44.30	-19.70	38.20	17.00	6.30

Table 3 Coordinates for peak of %BSC and cortical distance for the hemisphere contralateral to the moving hand. Coordinates are given millimetres in MNI 152 standard space

		Literature-based ROI			Individual ROI			Cortical distance	
ID	Group	x	y	z	x	y	z	spatial (x, y, z)	medio-lateral axis
A01	Amelic	33.80	-19.90	59.40	47.80	-22.10	42.40	22.13	14.00
A02	Amelic	35.20	-26.00	65.00	38.00	-19.50	75.30	12.50	2.80
A03	Amelic	38.40	-30.30	59.20	1.60	-23.50	83.40	44.57	36.80
A04	Amelic	36.60	-19.40	62.10	36.50	-35.90	59.80	16.66	0.10
A05	Amelic	41.90	-21.50	62.40	52.70	2.10	40.30	34.09	10.80
A06	Amelic	35.50	-20.20	57.50	43.30	-33.70	59.40	15.71	7.80
A07	Amelic	32.90	-24.80	60.10	45.20	-15.70	66.90	16.74	12.30
A08	Amelic	40.20	-23.00	53.50	43.10	-32.80	66.10	16.22	2.90
A09	Amelic	34.80	-26.70	62.30	40.00	-15.80	71.00	14.88	5.20
A10	Amelic	32.50	-21.30	62.40	39.30	-18.30	66.50	8.49	6.80
B01	PLPamp	40.10	-25.30	56.20	59.30	-17.70	52.70	20.94	19.20
B02	PLPamp	36.00	-23.10	62.90	43.30	-17.30	64.30	9.43	7.30
B03	PLPamp	34.20	-19.50	62.40	10.60	-20.70	79.80	29.35	23.60
B04	PLPamp	40.90	-22.50	62.00	43.50	-19.60	65.00	4.92	2.60
B05	PLPamp	36.70	-21.80	62.60	37.60	-37.20	54.00	17.66	0.90
B06	PLPamp	37.50	-20.90	58.70	40.10	-10.00	66.80	13.83	2.60
B07	PLPamp	43.00	-25.80	58.80	32.90	-20.70	74.90	19.68	10.10
B08	PLPamp	34.40	-24.30	54.40	-5.30	-20.00	52.10	40.00	39.70
B09	PLPamp	36.80	-20.90	60.90	41.10	-14.80	66.10	9.10	4.30
B10	PLPamp	34.70	-19.40	59.40	38.60	-15.40	65.60	8.35	3.90
B11	nonPLP	33.40	-20.50	55.80	31.30	-21.10	52.10	4.30	2.10
B12	nonPLP	39.30	-19.90	60.90	6.60	-16.50	58.80	32.94	32.70
B13	nonPLP	38.00	-22.60	63.70	49.20	-10.80	59.80	16.73	11.20
B14	nonPLP	35.60	-24.60	59.80	38.00	-34.40	57.10	10.44	2.40
B15	nonPLP	31.20	-27.70	56.70	41.30	-17.50	56.10	14.37	10.10
B16	nonPLP	34.50	-21.20	58.10	27.80	-27.00	52.30	10.59	6.70
B17	nonPLP	34.20	-30.00	61.20	34.20	-30.00	61.20	0.00	0.00
B18	nonPLP	38.50	-26.10	63.90	63.70	-2.80	43.20	40.08	25.20
B19	nonPLP	38.60	-22.60	58.80	47.20	-7.50	61.50	17.59	8.60
B20	nonPLP	36.60	-20.30	62.60	48.40	-8.40	61.10	16.83	11.80
C01	control	38.70	-23.00	57.90	54.60	-1.80	37.00	33.75	15.90
C02	control	34.80	-21.00	63.60	44.70	-9.30	55.00	17.57	9.90
C03	control	38.80	-30.00	57.80	48.10	-29.20	61.00	9.87	9.30
C04	control	34.00	-21.10	62.40	52.50	-6.00	55.70	24.80	18.50
C05	control	38.80	-19.30	61.60	38.70	-7.60	45.70	19.74	0.10
C06	control	43.00	-23.00	61.40	1.20	-5.50	60.80	45.32	41.80
C07	control	39.10	-24.20	64.20	41.50	-23.40	67.50	4.16	2.40
C08	control	38.10	-19.80	57.90	53.90	-4.60	46.00	24.95	15.80
C09	control	33.70	-22.00	55.30	59.40	-11.50	46.30	29.18	25.70
C10	control	35.50	-22.80	61.30	38.00	-15.50	57.30	8.69	2.50

Table 4 Coordinates for peak of %BSC and cortical distance for the hemisphere ipsilateral to the moving hand. Coordinates are given millimetres in MNI 152 standard space

A. **Hemisphere contralateral to movement**

	Literature-based ROI			Individual ROI		
	x	**y**	**z**	**x**	**y**	**z**
Amelics	-38.18 ± 2.52	-22.97 ± 2.92	59.04 ± 3.60	-36.52 ± 9.08	-23.82 ± 3.83	62.41 ± 10.13
Amputees	-39.35 ± 3.57	-24.54 ± 2.70	60.47 ± 2.94	-42.1 ± 8.19	-19.82 ± 5.41	60.88 ± 10.44
Controls	-41.89 ± 2.39	-22.98 ± 1.81	59.57 ± 3.59	-40.8 ± 6.24	-19.99 ± 4.45	60.39 ± 10.96

B. **Hemisphere ipsilateral to movement**

	Literature-based ROI			Individual ROI		
	x	**y**	**z**	**x**	**y**	**z**
Amelics	36.18 ± 3.11	-23.31 ± 3.56	60.39 ± 3.24	38.75 ± 13.93	-21.52 ± 11.83	63.11 ± 13.46
Amputees	36.71 ± 2.85	-22.95 ± 2.91	59.99 ± 2.78	36.47 ± 16.65	-18.47 ± 8.76	60.23 ± 8.45
Controls	37.45 ± 2.90	-22.62 ± 3.01	60.34 ± 2.93	43.26 ± 16.44	-11.44 ± 8.81	53.23 ± 9.23

Table 5 Mean coordinates (± SD) of peak neural activity during the virtual reality movement task for the **(A)** hemisphere contralateral to movement and for the (**B**) hemisphere ipsilateral to movement. Coordinates are given millimetres in MNI 152 standard space

5 GENERAL DISCUSSION

The aim of this dissertation was to investigate cortical plasticity related to a missing limb and its relationship with motor behaviours and sensory perception like PLP. By using a group of amelics who rarely perceive PLP, and by comparing it to amputees with and without PLP, I wanted to further the knowledge on neuroplasticity and how it may lead to PLP. In this work, I showed that acquired amputation and congenital limb-deficiency have different motor behaviours and different neural reorganization patterns, such that amelics show reduced neural activity and reduced functional synchronization compared with amputees. Such findings indicate that congenital limb deficiency and acquired amputation might have different underlying mechanisms, which may be the cause of differences in sensory perception (e.g. phantoms sensations or PLP). Furthermore, amelics showed similar neural activity and connectivity patterns compared with controls, suggesting possible adaptive mechanisms that are set up early in life.

Regarding psychometric data, amputees with and without PLP did not differ with regards to time since amputation, age at amputation or depression scores. However, amputees with PLP had higher depression scores compared with amelics and controls.

Using whole brain analyses, amelics, amputees and controls showed different neural activity in primary somatosensory and primary motor cortices (M1/S1) ipsilateral to movement and in secondary motor regions. Neural activity was however similar between the three groups in M1/S1 contralateral to movement and in auditory cortices bilaterally.

Using ROIs-based analyses, amputees showed higher neural activity and functional connectivity in M1/S1 in the hemisphere ipsilateral to movement compared with amelics and controls. In the hemisphere contralateral to movement, amputees, amelics and controls showed similar neural activity, functional connectivity and measures of cortical reorganisation. Amputees showed higher cortical distances in mediolateral axis in the hemisphere ipsilateral to movement compared with the hemisphere contralateral to movement. In terms of neural activity, functional connectivity or cortical distances, amputees with and without PLP showed similar

patterns. Furthermore, amelics and controls had similar neural activity, functional connectivity and measures of cortical reorganisation.
I found a trend towards a negative correlation between neural activity and PLP intensity, as well as between functional connectivity and PLP intensity, but not between cortical distances and PLP intensity. Moreover, depression led to higher neural activity in M1/S1 contralateral to movement. Additionally, a trend was visible for a positive relationship between depression scores and neural activity in M1/S1 ipsilateral to movement and increased functional connectivity in all groups. Frequency of stump usage did not have a significant effect on neural activity or functional connectivity.

5.1 Psychological factors and motor behaviour

I tested the role of various psychological factors and motor behaviours on the prevalence of PLP. In my data, time since amputation did not differ between amputees with and without PLP and seems therefore not to play a role in my sample. Some studies showed however that the prevalence of PLP decreases with time since amputation (Jensen et al., 1985; Sherman et al., 1984) but this finding is not consistently reproduced (Flor et al., 1995; Larbig et al., 2019). Furthermore, amputees with and without PLP had a similar age at time of amputation in my data. This leads to the assumption that age at time of amputation might not have an influence on the prevalence of PLP (Flor et al., 1995).
Considering depression scores, I showed that amputees with PLP had higher mean depression scores than amelics and controls. Amputees with and without PLP did not differ in depression scores. However, it should be noted that in all but one participant, all were still below the cut-off for depression disorders. Larbig et. al reported that higher depression or anxiety scores before the amputation could be a predictor for PLP (Larbig et al., 2019). Fuchs et al. reported in their review that several previous studies found higher depression scores in amputees with PLP (Fuchs et al., 2018) and amputees who suffered from worse pain had higher depression scores (Darnall et al., 2005). It is important to point out, that questionnaires about depression, e.g. the Beck Depression Inventory (BDI) can overestimate depression in chronic pain patients because consequences of pain could be misinterpreted as somatic symptoms of depression (Peck et al., 1989). However, I used the ADS questionnaire, for which to my knowledge, such overestimation issues have not been not reported

(Lehr et al., 2008). It is known that depression and anxiety occur frequently in the first year following the amputation (Fuchs et al., 2018). Fuchs et al. suggested that this phenomenon might not be due to PLP but rather to concerns about adapting to the new situation like new motor behaviours or disability than to PLP itself. At later stages of the amputation, higher depression rates could be linked to PLP (Fuchs et al., 2018). In my sample, approximately 24.5 years elapsed since the amputation occurred in amputees with PLP, therefore the higher depression scores in amputees with PLP could be rather linked to PLP and not to concerns about adapting to the new situation. Additionally, in the review, Fuchs et al. reported that patients who experienced both PLP and residual limb pain (RLP), showed higher affective distress than patients who experienced either PLP or RLP. Interestingly, patients with both pain types (PLP or RLP) had less depression rates than patients with other types of chronic pain, such as chronic back pain or musculoskeletal pain. The authors suggested that “pain predicted depression, but depression did not predict pain” (Fuchs et al., 2018). Although psychological factors like depression may not be the main cause for PLP, they may have an influence on the prevalence and severity of pain (Flor et al., 2006; Larbig et al., 2019). I found that amelics had similar depression scores compared with controls. This is in line with previous work showing that persons with congenital limb-deficiency seem to have comparable psychosocial functioning compared with two-handed controls (Michielsen et al., 2010). However, very little research has been done about the role of depression in congenital limb-deficiency.

I found different motor behaviours in amelics and amputees. More amelics use their stump when they do not wear a prosthesis compared with amputees with and without PLP. This is in line with a previous study showing that amelics used their residual limb more frequently in daily life compared with amputees (Makin, Cramer, et al., 2013). This could be related to the fact that the amelics acquired “adaptive” motor behaviours and are therefore more familiar with the bimanual usage of hand and stump. Amputees may use their intact hand more due to phantom limb pain on the amputated side. However, this assumption remains hypothetical, as I did not find a significant effect of PLP on motor behaviour, such as residual limb use, in my sample.

5.2 Neural activity, functional connectivity and cortical distance

5.2.1 Amputees

In this work, amputees with and without PLP showed similar neural activity and functional synchronisation in both the literature-based and in the individual ROIs which is in line with Andoh et al. (2020). The authors also reported increased task-related neural activity in amputees with and without PLP compared with controls in the hemisphere ipsilateral to movement, but this was not related to PLP (Andoh et al., 2020). However, when considering only amputees with PLP, the authors found a positive relationship between PLP intensity and task-related neural activity in the hemisphere ipsilateral to movement. This led the authors to conclude that activation in the hemisphere ipsilateral to movement seemed to be related to the magnitude of PLP rather than the presence versus absence of PLP.

In my sample, amputees had higher neural activity in the hemisphere ipsilateral to movement using the literature-based ROIs compared with amelics and controls. These results agree only partly with Makin and colleagues (2013) who similarly reported increased neural activity in amputees versus amelics, but no significant differences between amputees and two-handed controls. Such discrepancy with my results could be related to the task being performed with mirrored intact hand movements in my work and a combination of phantom/ non-dominant and imagery of hand movements in the work of Makin, Scholz, et al. (2013). There was no imagery of motor movement carried out in our study and all participants carried the same task. This allowed us to perform homogeneous comparison of neural activity across groups.

I found differences in neural activity between amputees and amelics and two-handed controls during a virtual hand motor task. Such findings could be related to use-dependent plasticity processes (Elbert et al., 1997; Hahamy et al., 2017; Lotze et al., 2001; Makin, Cramer, et al., 2013). Indeed, a previous study showed that amelics and amputees might have different motor behaviours, such that amelics over-use their residual limb while amputees over-use their intact hand (Makin, Cramer, et al., 2013). This may lead to different neural patterns in both hemispheres. My results showed that amputees had a higher neural activity M1/S1 in the hemisphere ipsilateral to the moving hand compared with amelics and controls. A previous study showed comparable results and showed also that amelics had a higher neural activity in M1/S1 ipsilateral to residual arm movements compared with amputees and

controls (Makin, Cramer, et al., 2013). Such findings reinforce the idea that amputees and amelics have different compensatory motor behaviours because amputees use their intact hand more often compared with amelics while amelics rely more on their stump. Such motor behaviours may lead to an increased representation of the intact hand in amputees and therefore to higher task-related neural activity in amputees. This hypothesis is supported by previous findings showing that movement of limbs which overtook the function of the missing hand (like the feet), led to increased neural activity in the missing hand area (Hahamy et al., 2015; Stoeckel et al., 2009).

There was no statistical difference in task-related neural activity between amputees with and without PLP, which may be related to similar motor behaviours between the two groups, that is over-use of the intact hand. However, since no study so far compared motor behaviour between amputees with and without PLP, this hypothesis remains speculative.

I showed that amputees had a significantly higher functional connectivity between left and right motor cortices compared with amelics and controls. Previous studies reported however a decreased functional connectivity in amputees compared with controls (Bramati et al., 2019; Makin, Scholz, et al., 2013), but no significant differences between amputees and amelics. In these studies, the results were however not based on active movements but at resting state (i.e. baseline neural activity). These authors suggested that the reduced connectivity between the left and right somatosensory cortices could be due to reduced input from the amputated limb (Makin, Scholz, et al., 2013).

Reduced inter-hemispheric connectivity in amputees compared with controls was also reported in lower-limb amputees without PLP using tactile stimulation. These studies also reported increased intra-hemispheric connectivity in the hemisphere contralateral to the amputation (Andoh et al., 2017; Bramati et al., 2019). The authors suggested that reorganization of adjacent areas into the missing limb area could lead to an increased intra-hemispheric functional connectivity. In contrast to the results of Bramati and colleagues, Makin et al. showed reduced functional connectivity between the missing hand area and the sensorimotor network. Makin et al. interpreted their findings by a mixture of sensory deprivation, adaptive plasticity and increased aberrant peripheral input, which led to the observed decoupling between the missing hand area and the sensorimotor network (Makin, Filippini, et al., 2015) . It is important to note that I used active hand movements of the intact (matched) hand

and that the previously mentioned studies examined the functional connectivity during resting-state or using tactile stimulation. This could be one possible explanation for the discrepancy between my work and previous studies. Another possible explanation refers to possible different compensatory processes in amelics and amputees. In amputees, the intact hand may have overtaken the function of the missing hand and led to increased representation of the intact hand in the missing hand area. This may have led to increased functional connectivity between the hand representation in both hemispheres. But this assumption requires further investigation.

It remains debatable whether the hemisphere contralateral to the intact hand plays a role in PLP. I showed that amputees had similar neural activity in the hemisphere contralateral to movement compared with amelics and controls. This is in line with previous work by Lotze et al., showing that movements of the intact hand in amputees and movements of the dominant hand in controls led to similar task-related neural activity in the hemisphere contralateral to movement. The authors therefore suggested that reorganization in M1/S1 contralateral to the amputation was the main driver for the development of PLP (Lotze et al., 2001). Another theory suggest that reorganization may take place in the hemisphere contralateral to the intact hand. Using tactile stimulation, Elbert and colleagues showed that amputees had higher cortical distances of the digits of the intact hand in amputees and suggested an increased representation of the intact hand due to increased dependence on the intact hand (Elbert et al., 1997). My findings support the idea of Lotze et al. (2001) that acquired amputation does not lead to significant reorganization in the hemisphere contralateral to the intact hand. Because amputees and amelics have similar neuronal activity in the hemisphere contralateral to the intact hand, this hemisphere does not play a significant role in the pathophysiology of PLP.

5.2.2 Amelics and controls

In the present dissertation, amelics and controls showed similar neural activity in both hemispheres. This may indicate that amelics undergo early reorganization processes, which lead to comparable neural patterns to controls. Similar neural patterns between amelics and controls were also reported in previous work (Hahamy et al., 2017; Montoya et al., 1998; Wesselink et al., 2019). Hahamy et al. found similar task-related neural activity in M1/S1 ipsilateral to movement comparing movements of the intact hand in amelics and movements of the non-dominant hand in controls

(Hahamy et al., 2017). Wesselink et al. investigated the missing hand representation in amputees, amelics and controls. Similar to my results, amelics showed a significant different hand representation in the missing hand area compared with amputees and showed similar representation compared with controls (Wesselink et al., 2019).

It is important to point out that in my work movements of the intact hand in amelics and amputees was not matched to dominant hand movements of the control group. Because of the matching procedure, controls had to move their left or right hand, independently of their handedness. Lotze et al. showed that it is important whether movements of the dominant or non-dominant hand of controls are compared to intact hand movements of amputees (Lotze et al., 2001). However, previous work controlling for handedness did not find significant difference in neural activity between two-handers and amelics (Hahamy et al., 2017). Therefore, a possible explanation for similar neural activity in amelics and controls could be, that regardless of the handedness, the brain reorganises at an early stage in amelics and develops in a similar manner as for two-handed controls.

I found similar functional connectivity between the hand area of the left and right hemispheres in amelics and controls. This might be related to similar motor behaviours in amelics and controls such as both groups have a bimanual arm usage in daily life. All amelics used their stump when they did not wear a prosthesis, suggesting that they have a relatively bimanual arm/limb usage. This hypothesis is supported by Hahamy et al., who found that bilateral arm/limb usage led to similar functional connectivity in amelics and controls. On average, amelics had reduced resting-state connectivity compared with controls but amelics who used their residual arm more often, had higher inter-hemispheric connectivity, which was comparable to the one of controls (Hahamy et al., 2015). A possible explanation for the discrepancy between Hahamy et al. (2015) and my findings are that the authors assessed functional connectivity at rest, whereas I investigated functional connectivity during motor-related neural activity. In a later study, the same authors showed that in amelics, a significant reduced connectivity was found between both hand areas but they also reported an increased coupling to areas that overtook the function of the missing hand (e.g. lips, feet). The authors suggested that the hand region may not represent the hand itself but rather other body parts that have the same function as the hand (Hahamy et al., 2017). This idea is supported by the findings of Yu and

colleagues who showed that toe tapping led to neural activity in the missing hand area in bilateral arm amputees (Yu et al., 2014). Similar results are reported in a study with individuals with congenital upper limb malformations. Toe movements showed neural activity in the medial M1, representing the foot area, and additionally in the lateral M1 close to the location activated by finger movements. Interestingly, this was not the case for a participant with largely preserved hand function who did not use his feet for daily living. This leads to the assumption that reorganization does not require a whole limb to be absent but occurs when a hand function is strongly deprived (Stoeckel et al., 2009). To conclude, my findings suggest that amelics and controls have similar task-related neural activity and functional connectivity in primary M1/S1 cortices. Amelics may have early compensatory mechanisms, such as bimanual motor behaviour, which may lead to similar neural patterns in amelics and controls.

5.2.3 Cortical distances

Amelics, amputees and controls showed similar cortical distances in M1/S1 in both hemispheres. Cortical distances are an index of functional reorganisation and show therefore that in terms of 3d Euclidean distances (x, y, z) there were no significant differences between the location of the M1/S1 in the left and right hemispheres between groups. However, when considering the mediolateral direction, amputees showed significant higher cortical distances in the hemisphere ipsilateral to movement compared with the hemisphere contralateral to movement. Amelics and controls showed similar cortical distances in M1/S1 in the mediolateral direction between the left and right hemispheres. Previous studies showed a cortical shift of the lip or the face area into the missing hand area in amputees such that the amount of the cortical shift was correlated with PLP intensity (Birbaumer et al., 1997; Elbert et al., 1994; Lotze et al., 2001; Montoya et al., 1998; Raffin et al., 2016). Makin et al. used the centre of gravity (CoG) and calculated the distances between the location of neural activity provoked by hand and feet movements. In amputees, the authors did not find a shift of the hand representation but observed a shift of the lips towards the missing hand (Makin, Scholz, et al., 2015). Andoh et al., who also calculated cortical distances between individual ROI and conjunction ROI, showed that cortical distances differ significantly in amputees with and without PLP, but only in S1 and not in M1 or M1/S1, indicating the importance of the ROI being examined (i.e. M1, S1 or M1/S1) (Andoh et al., 2020). However, Andoh et al. did not include persons with

congenital limb deficiency and therefore did not examine cortical reorganization in amelics. Here, I was able to show a shift in the hand representation of the missing hand in M1/S1 for amputees and not for amelics. My findings lead to the assumption that reorganization takes place in the missing hand area in amputees but not in amelics.

5.2.4 Relationship between neural activity, PLP, motor behaviours and psychological factors

I did not find a significant correlation between neural activity and PLP intensity in the entire amputee sample or when considering only amputees with PLP. But a trend towards a negative correlation between PLP intensity and neural activity emerged in most of the correlation analysis. In previous studies, a positive correlation between neural activity in M1/S1 and PLP intensity was shown (Andoh et al., 2020; Makin, Scholz, et al., 2013). However, such relationship outcomes may depend strongly on which groups are included, which tasks are being performed and how ROIs are defined. Makin and colleagues showed a positive correlation between PLP intensity and neural activity in the missing hand (Makin, Scholz, et al., 2013). Their sample included two-handed controls, amelics, amputees with and without PLP and they used a conjunction ROI (overlap of neural activity between the 3 groups). It is important to note, that Makin et al. used phantom hand movement and I used mirrored hand movements to examine neural activity which could explain the discrepancy with my results. Similarly to my results, Andoh and colleagues showed different results depending on whether amputees with and without PLP were examined. When including all amputees, no significant correlation between PLP intensity and %BSC was found, whereby considering only amputees with PLP, a positive significant correlation in M1/S1 was reported. Additionally, this positive correlation was only found using conjunction ROIs, but not using individual ROIs (Andoh et al., 2020).

As a preliminary conclusion, I showed that neural activity might be negative correlated with PLP intensity in amputees with PLP using the literature-based ROI. Similar to the suggestions of Andoh et al. (2020), the reported results led to the conclusion that it is important to differentiate which groups are included in the correlations of PLP. I did not find a significant correlation in the literature-based ROI between PLP intensity and neural activity when all amputees were included, but the

correlation became significant when amputees with PLP without the outlier were considered.

It is important to point out that in my data, an outlier was obvious in the correlation analyses. Excluding this outlier, neural activity was significantly negatively correlated with PLP intensity in amputees with PLP, suggesting that higher PLP intensity led to lower neural activity. Correlation with the outlier should be considered as the participant did not show differences in time since amputation, age at amputation and prosthesis usage to other amputees with PLP in the data. Additionally, the participant's depression score was below the cut-off for depression disorders in the ADS questionnaire. However, the participant reported a current depression episode during the measurement and took antidepressant drugs. So the depression symptoms might be alleviated, and the neuronal activity is altered by the drugs. It is known that depression leads to altered neural activity (Doan et al., 2015). The neural activity of this participant was not noise related.

Functional connectivity did not show a significant correlation with PLP intensity but showed a trend towards a negative correlation suggesting that higher PLP led to lower indices of functional connectivity. Makin et al. reported a significant negative correlation between PLP intensity and resting-state functional connectivity between bilateral hand regions (Makin, Scholz, et al., 2013). This led to the assumption that higher PLP intensity is associated with a decoupling of the missing and intact hand region. However, my findings showed only a trend towards significance for the correlation between PLP intensity and functional connectivity. More research with bigger sample sizes should be carried out in order to examine the relationship between functional connectivity and PLP.

Correlations between cortical distances and PLP intensity were not significant. This is in line with previous studies that did not find a significant relationship between cortical distance and PLP intensity (Andoh et al., 2020; Makin, Scholz, et al., 2015). The results suggest that although lip reorganization into the missing hand area was correlated with PLP (Birbaumer et al., 1997; Lotze et al., 2001; Raffin et al., 2016), this is not the case for shifts of the hand representation. However, more studies need to be done since the studies differ in terms of tasks, sample composition (e.g. participants with congenital limb deficiency, amputees with and without PLP) and different methods to evaluate cortical reorganization (e.g. Euclidean distance, surface- based methods (freesurfer)).

I found that depression had a significant effect on neural activity in the hemisphere contralateral to movement using individually-defined ROIs. In the hemisphere ipsilateral to movement, a trend towards a positive effect emerged suggesting that higher depression scores led to higher neural activity. Previous studies reported that psychological factors like depression can significantly influence PLP but the question if pain predicts depression or vice-versa is yet to be answered (Darnall et al., 2005; Fuchs et al., 2018; Larbig et al., 2019). It is important to point out, that depression before the amputation (and not depression after amputation) was significantly correlated with PLP (Larbig et al., 2019). Additionally, it was reported that depression led to abnormal neural activity in several brain regions, e.g. the prefrontal cortex or the amygdala (Doan et al., 2015). My results showed that higher depression scores were linked to higher neural activity. To my knowledge, no study examined the effect of depression on neural activity in M1/S1 in amputees. Interestingly, both patients with chronic pain and depressed patients showed dysregulation in similar brain regions (Doan et al., 2015). However, it remains unclear how depression and PLP might have altered the neural activity I observed in the amputee group.

Furthermore, I did not find a significant effect of depression on functional connectivity in the individual ROI, but a positive trend was visible using the literature-based ROI. In the review of Doan et al., the authors reported an altered functional connectivity between the default mode network (DMN) and the affective network (e.g. amygdala, orbitofrontal cortex, insular cortex) in depressed patients (Doan et al., 2015). However, it has yet to be demonstrated whether depression could alter functional connectivity in the primary motor cortices.

Stump usage showed a significant positive correlation with neural activity in the hemisphere contralateral to movement using individually-defined ROIs but not in the hemisphere ipsilateral to movement. Makin et al. found an effect of stump usage on neural activity ipsilateral to movement and reported that less frequent stump usage led to higher neural activity in the cortex ipsilateral to movement during intact hand movement (Makin, Cramer, et al., 2013). More research should be done to further the knowledge on how stump usage might alter neural activity.

5.2.5 Choice of ROIs

The literature-based ROI was based on a study by Hahamy et al. (2015). The ROI was built on neural activity resulting from intact hand movements in amelics and controls and overlapped with our conjunction ROI. I was therefore able to reproduce

the location of intact hand movements reported by Hahamy et al. (2015). Therefore, the literature-based ROI likely reflects location of the intact hand movement in our participants and suggests that it represents the former hand area in amputees and amelics.

After amputation, the cortical representation of the missing hand might be altered and might not overlap with the “intact hand area” representation anymore. Such changes in cortical representation might not be visible when one uses a ROI based on intact hand movements. The individual ROI was based on individual neural activity and built using M1/S1 probabilistic maps by the Juelich histological atlas. Such individual definition of ROI should detect possible change in body representation. The mean coordinates for the mediolateral (x-axis) and for the proximodistal (y-axis) axes of the peak %BSC in the individual ROIs showed a shift for amelics and amputees since they showed slightly different coordinates compared with controls. I found similar neural activity between amelics, amputees and controls using the individual ROIs. This might indicate that the individual ROI might capture the position of the intact in the three groups.

Interestingly, using the literature-based ROIs (and not the individual ROI), I found significant differences in neural activity and functional connectivity in amelics, amputees and controls. However, Andoh et al. examined also the relationship between neural activity and PLP using different ROIs (i.e. individual and conjunction ROIs). Contrary to my findings, the authors found significant differences in task-related neural activity between amputees and controls using individual ROIs, but not using conjunction ROIs. Significant correlation between PLP intensity and %BSC was reported only using the conjunction ROI and not using the individual ROI (Andoh et al., 2020). The authors however did not include an amelic group, therefore the conjunction ROI might be different from the one used in this work. Additionally, Andoh et al. examined S1 and M1 separately. More research needs therefore to be done that takes the locations of neural activity into account to examine differences between amelics, amputees and controls.

5.3 Whole brain analyses

Amelics, amputees and controls showed different neural patterns in the hemisphere ipsilateral to movement and in secondary motor regions like premotor cortex (PMC) or superior and inferior parietal lobule. It is known that reorganization does not only

lead to changes in the primary motor cortex but also in associated cortex areas such as the presupplementary motor area and to changes in functional connectivity (Andoh et al., 2018; Bogdanov et al., 2012; Bramati et al., 2019; Cruz et al., 2003). Compared with amelics, amputees had higher neural activity in the premotor cortex. Previous work showed that the premotor cortex was activated in motor imagery in amputees but not in amelics or in controls (Bogdanov et al., 2012; Cruz et al., 2003). The function of the premotor cortex is not fully understood. In monkeys the premotor cortex was shown to include mirror neurons, which were activated during observation of hand grasping (Gallese et al., 1996). Additionally, the premotor cortex was shown to play an important role in the feeling of ownership of a seen hand (Ehrsson et al., 2004). In the current work, the participants were instructed to imagine the observed hands as their own hands. Therefore, the observation of the virtual hands may have led to a stronger feeling of ownership in amputees compared with amelics and controls, possibly leading to an increased neural activity in the premotor cortex in amputees.

Amputees showed higher neural activity in the right superior and inferior parietal lobules compared with amelics and higher neural activity in the superior parietal lobules compared with controls. Previous studies reported that inferior and superior parietal lobules showed neural activity during motor imagery (Andoh et al., 2018; Raffin, Mattout, et al., 2012). Interestingly, Raffin et al. showed that parts of the parietal lobules are only activated during motor imagery but not during motor execution (Raffin, Mattout, et al., 2012). The superior parietal lobule is known to be responsible for the discrimination of qualities in sensation like size and texture. Additionally, this area is responsible for awareness of the contralateral body side and proprioception. Here, I found higher neural activity in the parietal lobule in amputees compared with amelics and compared with controls during active hand movement. Due to amputation, amputees might have an altered awareness and proprioception leading to different neural activity in the parietal lobules compared with amelics and with controls.

It was shown that amputation led to reorganization not only in the primary sensorimotor cortex but also in secondary motor areas (Andoh et al., 2018; Bogdanov et al., 2012; Cruz et al., 2003). However, primary and secondary cortex regions might play different roles in phantom sensations and phantom limb pain (Bolognini et al., 2013). Bolognini and colleagues reported that excitatory and not

inhibitory stimulation of M1 using transcranial direct current stimulation (tDCS) led to a decrease of PLP intensity. Application of inhibitory stimulation of the posterior parietal cortex had no effect on PLP but modulated nonpainful phantom sensations. The authors suggested that M1 was specific to PLP intensity whereas the parietal cortex was specific to nonpainful phantom sensations. (Bolognini et al., 2013). I found different neural activity in premotor cortex and in the parietal lobules in amputees compared with amelics and controls. Based on the findings of Bolognini et al. (2013), the increased neural activity in secondary motor areas in amputees compared with amelics and controls are maybe consequences of amputation but might be not linked with PLP. However, this deserves further investigations.

5.4 Limitations

I included 40 participants in this work, which were separated into four groups. Although our sample size is relatively small, it remains comparable with previous studies (Lotze et al., 2001; Makin, Cramer, et al., 2013; Simoes et al., 2012).

Whether mirrored movements can selectively show neural activity in the phantom hand representation can be subject to discussion. Previous studies using a similar task as in our work showed however that mirrored movement led to activation of phantom hand representation and that this neural activity was independent from intact hand movements (Andoh et al., 2020; Diers et al., 2010).

My sample included a matched number of left and right missing arms across amputees and amelics. All amputees were right-handed before the amputation, however, the controls were not matched for handedness to amelics and to amputees. A previous study showed that it does make significant differences if the intact hand movement in amputees is compared with dominant or non-dominant hand movements of controls (Lotze et al., 2001). However, I found similar results to Hahamy et al. who matched their participants by handedness in terms of neural activity based on intact hand movements (Hahamy et al., 2017).

To align the hemispheres consistently across left and right missing limbs, the data from participants with missing right arms (acquired amputation or congenital) were mirrored across the mid-sagittal plane in the same manner as in previous studies (Andoh et al., 2020; Makin, Cramer, et al., 2013). Whole brain analyses comparing neural activity between flipped and non-flipped data from amelics, amputees and

controls did not show significant differences in primary motor and somatosensory cortices (data not shown).

5.5 Outlook

Previous studies showed differences in neural activity and functional connectivity in amputees compared with amelics and controls. My data are in line with these studies and underlines that the development of PLP may be multifactorial.

My data indicate that the choice of ROIs and task which is used to examine neural activity (active hand movements, resting-state, phantom hand movements) plays a central role in the investigation of neural changes. I found different results using active hand movements compared with studies who used resting-state or phantom hand movements. Additionally, I found different results using the literature-based and individually-defined ROIs. The role of the different ROIs may be underestimated and more methodical research comparing different methods to define ROIs are needed (based on intact hand movements, individually defined or conjunction ROI).

Psychological factors like depression have been shown to influence the prevalence and intensity of PLP. In amelics, psychological factors have rarely been examined. Further research on the psychological state of amelics and amputees is needed, and how it might relate to PLP.

The cortical representation of the body is still unclear in amelics, especially the cortex area of the missing hand. In terms of bimanual arm usage, amelics seem to have similar motor behaviour compared with controls, leading to similar neural pattern. Longitudinal studies are needed how the cortex and the representation of the body develops in individuals with congenital limb deficiency. Potential different motor behaviours in amelics, amputees with and without PLP need to be considered when neural activity is examined. Longitudinal studies are needed to understand the influence of motor behaviour on cortical representation and how this might relate to PLP.

6 SUMMARY

Phantom limb pain (PLP) is very common after amputation and has been associated with neural reorganization. The aetiology of PLP and its underlying mechanisms remain however unclear. Amelics are born with a missing limb and suffer from PLP extremely rarely, which makes them an interesting group to investigate cortical reorganization without the influence of chronic pain. Therefore, we decided to study amelics. The aim of this dissertation was to investigate cortical plasticity related to a missing limb and its relationship with motor behaviours and psychological factors. We also compared cortical plasticity between amelics, amputees with and without PLP and two-handed controls.

We used a virtual reality movement task with functional magnetic resonance imaging in amelics (n = 10), amputees with (n = 10) and without PLP (n = 10) and in two-handed matched controls (n = 10). Amelics had similar motor behaviour compared with controls in terms of bimanual arm usage. Amputees showed higher depression scores compared with amelics and controls.

There was no significant difference between amelics and two-handed controls in task-related activity and connectivity in primary somatomotor cortices. However, amelics showed decreased neural activity, functional connectivity and cortical distances compared with amputees. There was no significant difference between amputees with and without PLP. We found a trend towards a negative correlation between PLP intensity and neural activity, functional connectivity but not between PLP intensity and cortical distances in amputees. In amelics and amputees, depression scores were positively correlated with neural activity in the hemisphere contralateral to movement.

This work highlights that congenital limb deficiency and acquired amputation have different neural correlates, which may be the cause for differences in sensory perception (e.g. PLP). Amelics showed similar neural patterns to two-handed controls, possibly indicating early adaptive behaviours. The dissertation offers new insights into the understanding of the cortical representation related to a missing limb, and how it interacts with additional factors (hand usage, depression).

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