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The role of peripheral input and its contribution to phantom limb pain

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LIST OF ABBREVIATIONS

- CN = contralateral intact median nerve
- DRG = dorsal root ganglia
- EEG = electroencephalography
- MRI = magnetic resonance imaging
- PLP = phantom limb pain
- PLS = phantom limb sensation
- PNS = percutaneous nerve stimulation
- RLP = residual limb pain
- S1 = primary somatosensory cortex
- SEP = somatosensory evoked potentials
- SS = skin site of the residual limb
- TENS = transcutaneous electrical nerve stimulation
- TN = truncated median nerve
- VAS = visual analog scale

1. INTRODUCTION

Phantom limb phenomena are very common following traumatic or surgical extremity amputation. Nearly all amputees feel that the missing limb is still present after amputation (i.e. phantom limb awareness), with vivid non-painful sensory or kinesthetic sensations (i.e. phantom limb sensations, PLS). Such phantom phenomena have also been reported in patients who lost other body parts such as teeth (Marbach & Raphael, 2000), internal organs and penis (Wade & Finger, 2010). Non-painful phantom phenomena usually do not pose a clinical problem, however, up to 85% of amputees also complain about spontaneous pain in the missing limb (i.e. phantom limb pain, PLP), which has a devastating impact on their life (Carlen, Wall, Nadvorna, & Steinbach, 1978; Ehde et al., 2000; Kooijman, Dijkstra, Geertzen, Elzinga, & Schans, 2000; Nikolajsen, 2012). Although phantom phenomena have been known since antiquity in medicine and folklores, the etiology of PLP still remains elusive.

Ambroise Paré has been believed to firstly differentiate PLP from residual limb pain (RLP, i.e. the pain perceived in the residual limb, also named ‘stump pain’ in some early literatures) and non-painful phantom and residual limb phenomena in the 16th century (1552). He proposed a comprehensive model of PLP postulating that PLP was due to the peripheral truncated nerves and the memory trace of pain (Keil, 1990). Chronic neuropathic pain such as PLP is still believed to relate to both peripheral and central changes (Flor et al., 1995; Flor, 2002; Hanley et al., 2009; Jensen, Krebs, Nielsen, & Rasmussen, 1985; Kuner & Flor, 2016). However, there is an ongoing debate about the main cause of PLP, if it is primarily caused by peripheral or by central mechanisms. For instance, PLP intensity has been associated with the magnitude of cortical reorganization in the deafferented cortex, possibly resulting from general sensory loss (Flor et al., 1995). Despite some evidence for a central origin of PLP, this type of pain has been assumed to be caused by the peripheral nervous system (Vaso et al., 2014). For instance, anesthesia of inputs in the dorsal root ganglia (DRG), using lidocaine, has been reported to relieve or even abolish PLP. However, controlled studies are lacking. Both mechanisms (i.e. central and peripheral) might not be mutually exclusive. Central plastic changes in PLP may also be driven by peripheral generators, as shown by a

computational model (Boström, de Lussanet, Weiss, Puta, & Wagner, 2015; Spitzer, 1997). The interaction between peripheral input and central processes for PLP generation has not yet been investigated in detail.

The present thesis sought to assess the role of peripheral afferent input for PLP and how it interacts with central activities at the spinal, subcortical and cortical levels, along the ascending somatosensory neuraxis in amputees with PLP compared to those without PLP. The following sections provide: (1) an overview of the current understanding of PLP (section 1.1), including clinical characteristics, central and peripheral mechanisms, and PLP modulation using peripheral nerve stimulation; (2) a brief technical overview of somatosensory evoked potentials (SEP) (section 1.2), including common definitions, anatomy of pathways, and the nomenclature, recording method and generators of human SEP components.

1.1 Current understanding of phantom limb pain

PLP has been classified as a type of neuropathic pain, which occurs following changes in both the peripheral and central nervous systems (see **Figure 1**) (Blume et al., 2014; Draganski et al., 2006; Economides, DeFazio, Attinger, & Barbour, 2016; Hamzei et al., 2001; Jain, Florence, Qi, & Kaas, 2000; Makin, Filippini, et al., 2015; Moseley & Flor, 2012; Serra et al., 2012). PLP proves to be intractable in clinical practice because it does normally not respond to conventional algesic therapies (Flor, 2002; Nikolajsen, 2012). This type of pain may also vary depending on the cause of amputation, e.g. traumatic event (accident) or chronic diseases (diabetes, vascular complications, tumors). Most individuals with acquired amputation have suffered from immediate pain during the traumatic procedure, or pre-amputation pain as a complication in the limb prior to surgery. Pre-amputation pain experience has been suggested to be a risk factor for PLP generation (Larbig et al., 2019; Jensen et al., 1985; Kooijman et al., 2000; Ramachandran & Hirstein, 1998). PLP occurs frequently together with RLP and a positive relationship has been reported between PLP and RLP (Montoya et al., 1997). Moreover, psychological variables such as stress, depression and anxiety (Larbig et al., 2019) can also contribute but may not be the direct cause. The etiology of PLP is not fully understood (Andoh, Milde, Tsao, & Flor, 2018; Flor,

2002; Flor, Diers, & Andoh, 2013; Vaso et al., 2014) and mechanism-based effective therapies for PLP are still lacking (Flor, Nikolajsen, & Jensen, 2006; Kuffler, 2018).

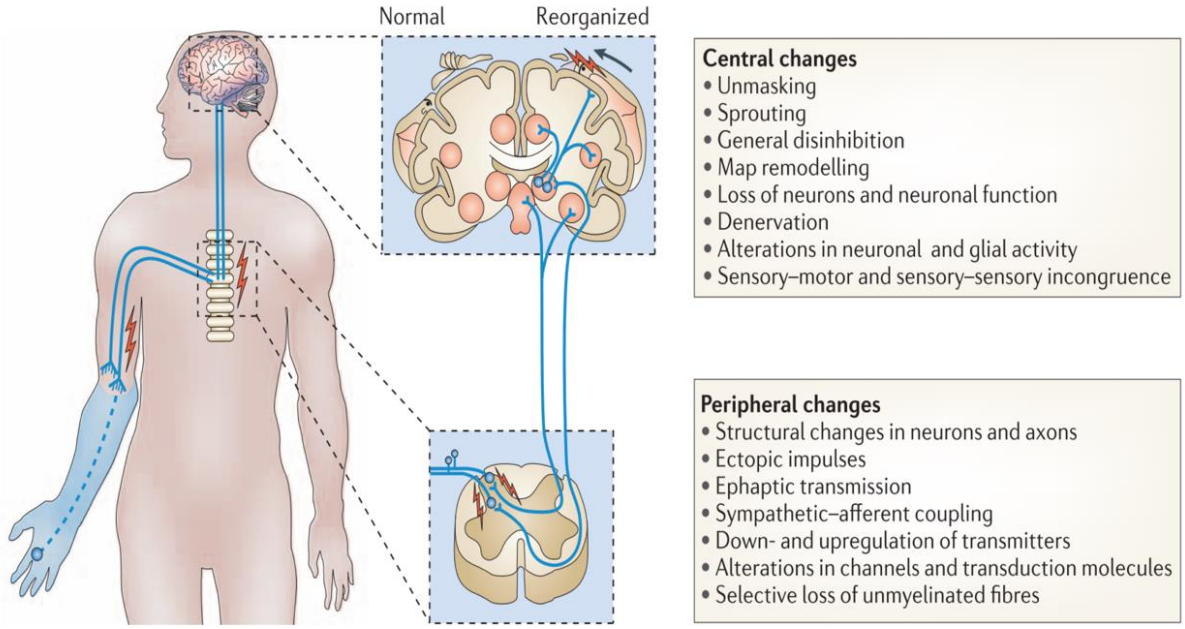


Figure 1 Illustration of peripheral and central pathways and changes possibly leading to the generation of phantom limb pain in an upper-limb limb amputee. Figure reprinted from Flor et al. (2006), *Nature Reviews Neuroscience, 7*(11), 876.

1.1.1 Clinical characteristics of phantom limb pain

Some amputees report to have phantom limb awareness without specific PLS or PLP, while some can have more than one quality of abnormal non-painful or painful phenomena. Non-painful PLS include exteroceptive perception (such as itching, warmth, cold, touch or electrical sensation), kinetic phenomena (including spontaneous or voluntary phantom limb movements) and kinesthetic components (such as abnormal size, shape and position of the phantom limb) (Kooijman et al., 2000; Weeks, Anderson-Barnes, & Tsao, 2010). The kinesthetic components of the phantom limb are not always in accordance with the previous complete body or the intact limb. Nearly 30% of amputees describe the retraction or shrinking of the phantom limb towards the residual limb, also known as telescoping, which has been reported to correlate positively with

cortical organization and even PLP in upper-limb amputees (Flor, 2002; Grüsser et al., 2001; Montoya et al., 1997).

The prevalence rate of PLP ranges from about 60% to 85%. The variability of this prevalence is possibly due to the reasons or level of amputation, or to assessments at different time points after amputation. Prospective studies have shown a PLP incidence of 72% in the immediate postoperative, 67% at six months and 59% at two years after amputation (Carlen et al., 1978; Jensen, Krebs, Nielsen, & Rasmussen, 1983; Jensen et al., 1985). The short-term (mean 5.2 ± 1.1 weeks) incidence of PLP has been reported to be more common in upper-limb (82%) than in lower-limb (54%) amputees (Shukla, Sahu, Tripathi, & Gupta, 1982). The long-term (more than at least 5 years) incidence of PLP has been reported up in 78% of 2750 amputees (Sherman, Sherman, & Parker, 1984), 73% of 43 amputees (Steinbach, Nadvorna, & Arazi, 1982) and 51% of 72 amputees (Kooijman et al., 2000), and 82.7% of 44 amputees (1 year follow-up) (Larbig et al., 2019). Most PLP tends to be chronic and there is no significant decrease over time after amputation (Sherman et al., 1984). PLP occurs rarely in individuals with congenital limb absence and is more common in traumatic amputees, especially in those with chronic pre-amputation limb pain or acute pain prior to amputation (Larbig et al., 2019; Jensen et al., 1985; Kooijman et al., 2000; Ramachandran & Hirstein, 1998). This finding has been interpreted that memories for pain may be generated and that may facilitate the generation of PLP. In fact, the amount of remembered pain episodes was also predictive of PLP in one study (Larbig et al., 2019). In addition, females seem to be more affected by PLP compared with males, indicating that gender could also be a contributing factor, although this was not found significantly in other studies (Ehde et al., 2000; Kooijman et al., 2000). The onset of PLP varies individually from the immediate period to even decades after amputation, but most amputees report PLP occurrence in the first three years (Nikolajsen, 2001). A few cases showed that PLP could also occur much later after amputation. For instance, a man who underwent left-lower-limb amputation at the age of 13 years reported PLP in his missing leg at the age of 58, following diabetic neuropathy (Rajbhandari, Jarratt, Griffiths, & Ward, 1999).

PLP is often described as stabbing, throbbing, burning, cramping, tingling, sharp, aching or resembling electric shock (Weeks et al., 2010). Although it can be perceived

over the entire phantom limb regardless of the amputation level, PLP tends to occur more frequently and intensely in the distal part of the extremity, such as fingers and palm in upper-limb amputees and the toes in lower-limb amputees. These body parts are richly innervated and are represented by large cortical areas. The distribution and characteristics of PLP may change in the first few months but then remain stable with rare further alterations (Jensen et al., 1985). PLP intensity may be constant or vary depending on internal and external factors. In some amputees, PLP can be elicited or increased by a specific position or movement of the phantom and can return to baseline when another gesture is performed. Psychological factors (such as anxiety and depressive symptoms) are also believed to have a strong influence on PLP (Ephraim, Wegener, MacKenzie, Dillingham, & Pezzin, 2005; Hill, 1999; Nikolajsen, 2012). In addition, mechanical stimulation or pressure, changes of weather, and blood flow variation in the residual limb may alter PLP intensity.

1.1.2 Central neural correlates of phantom limb pain

Cortical mechanisms

Post-amputation reorganization in somatosensory and motor cortices was found to be positively correlated with PLP (Grüsser et al., 2001; Halligan, Marshall, Wade, Davey, & Morrison, 1993; Karl, Birbaumer, Lutzenberger, Cohen, & Flor, 2001; Weiss et al., 1998, 2000). In S1, the amount of topographic expansions and shifts from neighboring representations (such as mouth and face) towards the deafferented hand cortex has been reported to be strongly positively correlated with PLP intensity, but neither with non-painful PLS nor RLP (Flor et al., 1995). In addition, more cortical reorganization has been found in amputees with PLP than those without PLP (Flor et al., 1998; Grüsser et al., 2001). Functional maladaptive cortical changes are therefore strongly linked with PLP, and may be triggered by sensory deprivation. This possible neurophysiological basis has been supported by latter therapies to relieve PLP using regional anesthesia (Birbaumer et al., 1997), mirror therapy (Chan et al., 2007; J. Foell, Bekrater-Bodmann, Diers, & Flor, 2014), motor imagery (Gagné, Reilly, Hétu, & Mercier, 2009; MacIver, Lloyd, Kelly, Roberts, & Nurmikko, 2008) or sensory discrimination training (Flor, Denke, Schaefer, & Grüsser, 2001). These therapies have shown a correspondence between a

reversal of cortical topographic changes and a reduction of PLP. For instance, reorganization in somatosensory cortex has been assessed after axillary brachial plexus anesthesia of the residual limb in six amputees with PLP and four PLP-free amputees (Birbaumer et al., 1997). They found three amputees with PLP who experienced a reduction of PLP, which was accompanied by a rapid reduction of cortical reorganization in somatosensory cortex, while in the other three PLP amputees and four PLP-free amputees the pain and cortical reorganization both remained unchanged. Moreover, using a four-week daily mirror training in eleven upper-limb amputees with PLP, Foell et al. (2014) found a significant reduction of PLP which positively correlated with reversed cortical reorganization in S1 and decreased activity in the inferior parietal cortex (Foell et al., 2014). Flor (2002) summarized the main relevant factors, which may facilitate cortical reorganization processes and proposed a model (see **Figure 2**). In this model, peripheral factors have been proposed to enhance the cortical changes associated with PLP.

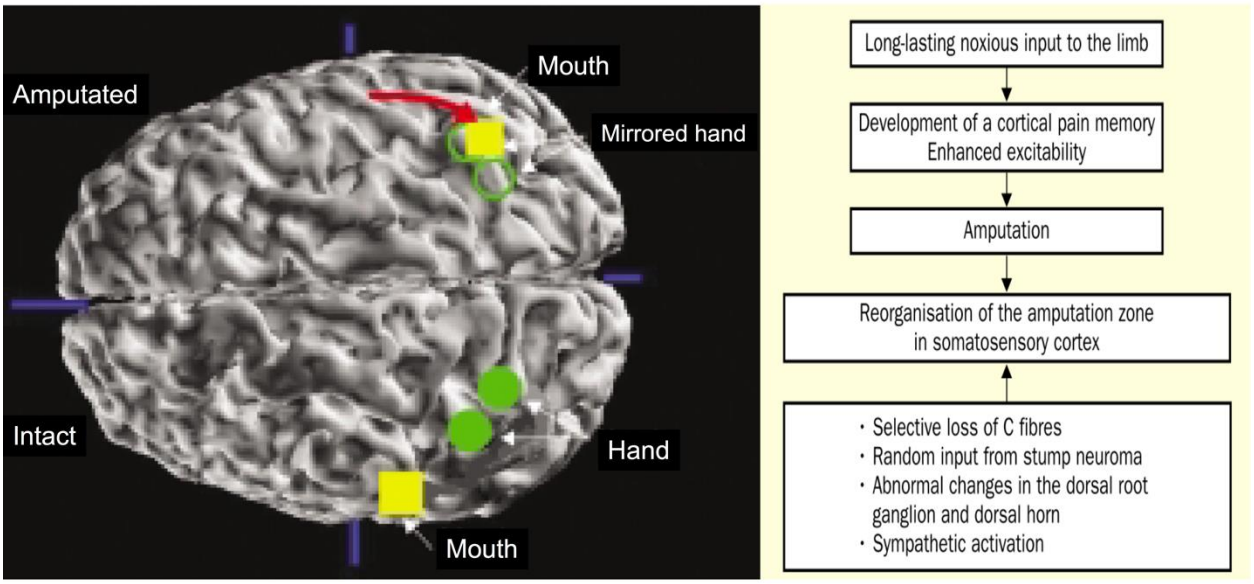


Figure 2 Reorganization in the primary somatosensory cortex in a unilateral upper-limb amputee with phantom limb pain. Topographic expansions and shifts from neighboring representations (mouth) towards the deafferented hand region on the contralateral hemisphere to the amputation side localized by neuromagnetic source imaging technology. The amount of

cortical reorganization has been reported to be strongly positively correlated with PLP magnitude. Peripheral factors have been proposed to contribute to the cortical reorganization and phantom limb pain in a model that summarizes the possible relevant changes demonstrated in a schematic diagram (right). Figures reprinted from Flor (2002), *The Lancet. Neurology, 1*(3), 183 and 186.

However, this statistical relationship between the magnitude of functional cortical remapping from neighboring representations and PLP intensity was challenged in another group, who used phantom movement rather than sensory stimulation of the lip and found a positive relationship between activity in the phantom hand cortex and PLP (Makin, Scholz, Slater, Johansen-Berg, & Tracey, 2015). Another cortical theory was proposed that associated PLP with neural activity in the ‘‘preserved’’ hand representation and the connectivity of the ‘‘intact’’ hand representation (Makin et al., 2013). The amount of activation in the amputated hand representation in primary sensorimotor cortex was positively associated with PLP intensity. Moreover, this study also showed that PLP intensity was related to altered inter-regional functional connectivity between bilateral hand cortices. The authors therefore proposed that cortical plastic changes associated with PLP might be rather driven by strong peripheral input or top-down input to the sensorimotor cortex, but not the sensory loss. Yanagisawa et al. (2016) used magnetoencephalography (MEG) and showed that brain-machine interface training could enhance the representation of phantom hand, and the latter was associated with increased PLP (Yanagisawa et al., 2016). Moreover, the training to dissociate the prosthetic and the phantom hand involved a reduction of PLP. Altogether, these results suggest that PLP can be modulated (increase and decrease) by induced sensorimotor cortical plasticity, supporting a possible causative relationship.

Role of subcortical and spinal structures

Subcortical structures such as thalamus, brainstem and spinal cord have also been reported to play a role in PLP and cortical neuronal alterations. For instance, Davis et al. (1998) conducted thalamic microstimulation and stereotactic recording in human

amputees and found a large thalamic residual limb representation (Davis et al., 1998). PLS and even PLP could be generated by thalamic electrical stimulation, after activation of the neurons in the ‘large’ residual limb representation. These results imply that the neurons in the ‘original’ representation of the amputated limb in the thalamus may functionally serve other body regions (such as the residual limb) and may generate phantoms and PLP. In addition, Yarnitzki et al (1988) reported an amputee whose PLP disappeared after a local stroke localized to the posterior internal capsule as demonstrated by computed tomography (Yarnitsky, Barron, & Bental, 1988). This finding suggests a potential subcortical source in or near the internal capsule for PLP. Reorganization of somatosensory afferents occurring in the brainstem after limb amputation that may also contribute to phantom sensations. It has been proposed that decreased tonic inhibitory influence of the brainstem reticulate formation after complete loss of peripheral sensory input may increase self-sustaining neuronal activity and result in PLP (Melzack, 1971). In addition, white matter changes following limb amputation, such as reorganization of the corpus callosum, may also facilitate the generation of evoked phantom sensations and even PLP (Andoh et al., 2017; Simoes et al., 2012). However, more direct evidence for subcortical mechanisms in human amputees related to the generation of PLP is still needed.

Several clinical observations indicated that spinal circuits may also play a role in PLP. During spinal anesthesia in amputees without obvious PLP prior to anesthesia, PLP can appear transiently or become even excruciating despite complete analgesia (Harrison, 1951; Leatherdale, 1956; Mackenzie, 1983; Murphy & Anandaciva, 1984). Such unexpected PLP after anesthesia cannot be explained due to inputs from the residual limb and apparently spinal or supraspinal mechanisms may contribute to the appearance of PLP. After spinal anesthesia, complete loss of sensory input may decrease the level of inhibition in the spinal cord or brainstem reticular formation, which might unmask self-sustaining neural activity and result in PLP (Mackenzie, 1983). In addition, spinal pathological changes have been reported to suppress chronic PLP or generate new PLP. Aydin et al. (2005) reported a 65-year-old female lower-limb amputee with a history of chronic PLP for 60 years, whose PLP decreased progressively during a cauda equine compression, arising from an intraspinal

meningioma (Aydin, Cesur, Aydin, & Alici, 2005). However, PLP reappeared gradually after surgical removal of the spinal tumor. In this case, the spinal ischemic change and damage induced by the tumor mass might explain the reduction of PLP. After the operation, the decompression might improve spinal electrophysiological function and reactivate the sensory processing of PLP. Another similar case of spinal decompression has been reported by Lida et al. (2004), who observed a reappearance of PLP in a 65-year-old man with non-painful phantom phenomena but without PLP for almost 15 years (Lida, Munakata, Suzuki, Saeki, & Ogawa, 2004). In this case, PLP occurred immediately after cervical myelopathy surgery for the treatment of spondylotic canal stenosis. These findings suggest that spinal decompression after pathological changes can reactivate PLP. However, Cruz et al. (2013) reported one patient with malignant sarcoma and prior hip disarticulation, who experienced severe PLP following a metastatic spinal mass at the lumbar vertebra (L3), which disappeared after resection of the spinal mass (Cruz & Dangaria, 2013). These alterations in the spinal cord involving metastatic pathologies and PLP are complex and need to further investigated.

Although a large number of studies have demonstrated close relationships between central, peripheral changes and PLP, the nature of these interactions is not yet well understood.

1.1.3 Peripheral factors associated with phantom limb pain

Post-amputation pain such as PLP and RLP may result from peripheral damage and nociceptive input from the peripheral nerves or neurons. Amputees suffering from chronic RLP have a higher prevalence of PLP (Carlen et al., 1978; Desmond & Maclachlan, 2010; Gallagher, Allen & Mac, 2001; Kooijman et al., 2000). Most amputees can clearly distinguish the two types of chronic pain. PLP cannot be triggered completely by RLP. PLP and RLP may have different etiologies and need to be differentiated (Hill, 1999; Sherman & Sherman, 1983). The close relationship between these types of pain has not been completely understood. Some investigators assume that PLP and RLP may both be maintained by peripheral afferent input and centrally amplified (Vase et al., 2011). Peripheral input has been shown to play an important role in maintaining or increasing neuropathic pain (Haroutounian et al., 2014). But whether

the afferent input can generate PLP is still under debate (Foell et al., 2014). Mackert et al. (2003) measured the afferent input along the ascending neuraxis at the brachial plexus and S1 in upper-limb amputees with non-painful PLS (Mackert, Sappok, Grüsser, Flor, & Curio, 2003). The results showed that afferent input from the truncated nerves could evoke more activation in S1 than from the skin of residual limb, showing the remaining response from the ‘‘deafferented’’ hand cortex. This finding suggests a possible neural substrate for spontaneous phantom phenomena and even PLP. However, in this study, amputees had no PLP, thus suggesting that these residual responses are also present when no PLP is present. Peripheral input may drive or influence the PLP-associated central reorganization (Birbaumer et al., 1997; Boström et al., 2015; Spitzer, 1997), but may not cause PLP directly.

Repetitive tapping neuromas in the residual limb was shown to increase PLP, accompanied with increased amplitude and discharge rate of the peripheral input recorded by microelectrodes (Nyström & Hagbarth, 1981). The increase of PLP induced by taps could be abolished after anesthesia of the neuroma using lidocaine, however, the PLP baseline was remaining. Neuroma may change the properties of afferent nerve impulses and induce spontaneous activity (Wall & Gutnick, 1974). The spontaneous input is “normally” not painful and discharged at a low frequency as recorded in animal models of neuropathic pain (Devor, 2009; Djouhri, Koutsikou, Fang, McMullan, & Lawson, 2006; Ørstavik et al., 2003; Serra et al., 2012). Resolution of residual-limb-end pathology and removal of neuromas by surgery was shown to reduce PLP (Baron & Maier, 1995). However, not all amputees with neuromas report PLP. PLP has been reported to be fully relived (Sehirlioglu et al., 2009), unchanged or even became worse (Nikolajsen, Black, Kroner, Jensen, & Waxman, 2010), after the surgical excision of painful neuromas. These findings suggest therefore that peripheral changes only at the level of the residual limb or neuroma are not sufficient to entirely determine PLP. A higher site in DRG cells has been proposed as a possible source for neuropathic pain (Devor, 2009; Liu et al., 2000; Vaso et al., 2014). Vaso et al. (2014) showed a temporary but total relief from PLP in lower-limb amputees after selective DRG anesthesia intraforaminally or spinal intrathecal block using lidocaine (Vaso et al., 2014). They proposed that the DRG is critical for PLP generation and argue for a peripheral

origin of PLP. However, this study had several limitations such as the absence of a full placebo condition, no blinded randomized controlled design and the changes in PLP were quantified using verbal ratings only. Recently, Buch et al. (2019) carried out peripheral nerve block using lidocaine and placebo in amputees with constant postamputation pain and found a general reduction of both PLP and RLP after lidocaine injection compared with placebo (Buch et al., 2019). These studies suggest that a decrease in peripheral afferent input can indeed relieve PLP and are in line with a role of peripheral input in PLP. However, other studies showed that either local blockade of neuroma, the brachial plexus or spinal anesthesia could not abolish PLP effectively and sometimes even increased PLP (Birbaumer et al., 1997; Chabal, Jacobson, Russell, & Burchiel, 1992; Mackenzie, 1983; Martin, Grant, Macleod, Breslin, & Brewer, 2003; Paqueron, Lauwick, Guen, & Coriat, 2004). More studies with blinded randomized clinical trials, full placebo and larger sample sizes are needed to thoroughly examine the role of peripheral input. Reduced input from the periphery during anesthesia of the brachial plexus could decrease PLP in half of six amputees and reverse the reorganization in S1, implying that peripheral input has an impact for both PLP and cortical reorganization (Birbaumer et al., 1997).

Blood blow or muscle activity of the residual limb can also influence PLP intensity. Skin temperature at the residual limb has been found to be significantly lower than the intact limb in amputees with PLP and PLS, but not in the group without phantom phenomena (Katz & Katz, 1992). This suggests a role of peripheral sympathetic activity for the phantom phenomena. Amputees with lower near-surface blood flow had more pain (Sherman & Bruno, 1987). In addition, a decrease in blood flow induced by tensing the residual limb induced an increase of PLP and RLP (Sherman & Bruno, 1987). The type of PLP and residual limb muscle tension may play a role. An electromyography study showed a significant positive correlation between changes in activities of the major muscles of the residual limb and the intensity of cramping PLP, but was not significant for other PLP symptoms such as shocking-shooting or burning PLP (Sherman et al., 1992).

These findings indicate a role of the peripheral nervous system for PLP. It remains, however, still unclear whether peripheral afferent input is sufficient to create and maintain PLP and how the input interacts with central processing and PLP.

1.1.4 Modulation of phantom limb pain using peripheral stimulation

PLP is commonly classified as neuropathic pain, however, it usually does not respond to conventional analgesic drugs (Weeks et al., 2010). Various strategies including pharmacological therapies (such as opioids (Huse, Larbig, Flor, & Birbaumer, 2001; Wu et al., 2008, 2002), gabapentin (Bone, Critchley, & Buggy, 2002; Smith et al., 2006), tricyclic antidepressants (Robinson et al., 2004; Wilder-Smith, Hill, & Laurent, 2005) and NADA receptor antagonists (Eichenberger et al., 2008; Maier et al., 2003; Wiech et al., 2004), visual feedback therapy (Barbin, Seetha, Casillas, Paysant, & Pérennou, 2016; Chan et al., 2007; J. Foell et al., 2014), motor imagery or sensory discrimination training (Diers, Christmann, Koeppe, Ruf, & Flor, 2010; Yanagisawa et al., 2016), anesthetic nerve blocks (Birbaumer et al., 1997; Buch et al., 2019; Vaso et al., 2014), electrical neuromodulation (Petersen, Nanivadekar, Chandrasekaran, & Fisher, 2019), surgical destructive procedures (Sehirlioglu et al., 2009) and psychological interventions have been used to treat PLP. So far, there is no effective approach that can abolish PLP (Flor, 2002; Knotkova, Cruciani, Tronnier, & Rasche, 2012; Weeks et al., 2010). A combination of some of these approaches might be beneficial for PLP management.

Peripheral neuromodulation using transcutaneous electrical nerve stimulation (TENS) has been applied to modulate chronic post-amputation pain symptoms (Johnson, Mulvey, & Bagnall, 2015; Petersen et al., 2019; Tashani & Johnson, 2008; Tilak et al., 2016). TENS and TENS-like devices can selectively activate peripheral A fibers to manage peripheral pain syndromes. Melzack and Wall were the first to propose that there may exist a neurological ‘gate’ of pain processing pathway and the ascending passage of nociceptive signals from noxious C or Aδ fibers may be modulated by simultaneous afferences from Aß fibers or top-down signals (Melzack & Wall, 1965). The pain control theory had numerous practical consequences in the past years although it cannot explain all cases. For instance, Mulvey et al. (2013) applied fast-

frequency (100Hz) TENS at the distal site of the residual limb in transtibial amputees with PLP, RLP or both for 60 minutes and they showed a decreased general pain intensity in both rest and movement conditions (Mulvey et al., 2013). In addition, electrical stimulation over the truncated nerves of the residual limb could elicit sensations in the phantom limb, i.e. phantom input, providing a potential approach to modulate PLP and facilitate perceptual embodiment of prosthesis (Mackert et al., 2003; Mulvey et al., 2013; Tashani & Johnson, 2008). PLP has been decreased by electrical stimulation at the contralateral extremity (Carabelli & Kellerman, 1985; Tilak et al., 2016; Yamamoto et al., 1997). A single-blinded randomized controlled trial using mirror therapy or TENS at the contralateral extremity showed no significant difference in PLP change after 4 days of treatment between the two therapies (Tilak et al., 2016). Finsen et al. (2015) applied TENS, sham TENS and chlorpromazine in 26 amputees and found that the prevalence rate of PLP decreased significantly after short-term TENS therapy, but not significantly in the long-term (1 year) follow-up (Finsen et al., 1988). The use of TENS for treating PLP and RLP deserves further investigation with randomized controlled trials (Johnson et al., 2015).

Similar to TENS, minimally invasive percutaneous nerve stimulation (PNS) using implanted microelectrodes or fine-wire electrode has been used in patients with severe post-amputation pain and chronic back pain. Under ultrasound guidance, the electrode can be fixed proximal (≤2mm) to the truncated nerves and one can conduct stimulation of the nerve axons without subcuticular variables. Rauck et al (2014) applied two-week PNS stimulation at a frequency of 50-100Hz on the truncated sciatic or femoral nerves in nine lower-limb amputees with moderate-to-severe postamputation pain (Rauck et al., 2014). A significant relief of PLP was reported during the second week of the PNS stimulation and in the following four weeks after stimulation. Cornish et al. (2015) reported a case of complete and consistent PLP relief in a lower-limb amputee after six-months of PNS therapy, consisting in applying two 8-contact electrical leads in the subcutaneous fat tissues of the residual limb, but not near the nerves (Cornish & Wall, 2015). In addition, mechanical vibratory stimulation at the residual limb has also been reported to alleviate PLP (Lundeberg, 1985).

The mechanisms by which peripheral input can modulate PLP are unclear and the effective period varies between individuals. However, evidence suggests that peripheral input can influence the subjective pain experience.

1.2 Human somatosensory evoked potentials

Somatosensory evoked potentials (SEP) reflect the electrophysiological activity produced by a large population of neurons and synapses and can be recorded noninvasively by placing multiple wire electrodes over the scalp and spine. SEPs have a good temporal resolution (in the order of milliseconds) and can provide information of somatosensory activities. In 1951, George Dawson first recorded cortical SEPs in patients with myoclonus (Dawson, 1951). Spinal and subcortical SEPs were subsequently developed for clinical use for diagnostic assistance and neurosurgical monitoring (Nash, Lorig, Schatzinger, & Brown, 1977), although the precise source of spinal and far-field SEPs in subcortical structures is under debate. Combining SEP recordings at different segmental levels can help to evaluate somatosensory functions. The amplitude and latency enable to assess the relay of body sensations following the afferent pathways and the response of the brain.

1.2.1 Anatomy and physiology of the somatosensory system

Multiple conscious sensory perceptions from the skin, muscles, joints and fascia are conveyed by the somatosensory system, including touch, pressure, temperature, pain, position, movement and vibration. Signals detected from the peripheral sensory receptor cells go through a complex three-neuron system to the cortex and there are two major pathways, i.e. the dorsal column-medial lemniscal system and the spinothalamic tract system (also named anterolateral system), ending in the neurons in the postcentral gyrus of the parental lobe. The low-threshold myelinated Aa/ß fibers project into the dorsal column-medial lemniscal system and high-threshold small diameter myelinated Aδ and unmyelinated C fibers project into the spinothalamic tract system. Normally, mechanoreception and proprioception are subserved by the dorsal column-medial lemniscal system, and thermoreception, nociception and visceroception are facilitated by spinothalamic tract system. However, in patients with chronic

neuropathic pain or allodynia, activities from A group fibers may also play a role in conveying nociception (Devor, 2009; Djouhri & Lawson, 2004; Woolf, 2011). The somata of the fist-order neuron lie in the DRG of the spinal nerve (when sensations come from body parts lower than the head or neck), the trigeminal nerve ganglia or the ganglia of other sensory cranial nerves. The second-order neuron population is located either at the spinal dorsal horn in the spinothalamic tract system, or in the dorsal column nuclei of the lower brainstem serving the dorsal column-medial lemniscal system. Then the ascending axons decussate across the midline to the contralateral side and project to the neurons in the ventroposterior nuclei of the thalamus and are then conveyed to the somatosensory cortical networks.

Large-scale topographic representations of the body have long been established in the somatosensory and motor cortices. Penfield et al. (1937) used intra-cortical stimulation and found that the body map had a distorted cortical representation, i.e. cortical homunculus (Penfield & Boldrey, 1937). The cortical homunculus was also shown to change topographically following stroke, nerve injures and amputation. In amputees with PLP, these changes have been shown to be related to pain and peripheral activation (Birbaumer et al., 1997; Flor et al., 1995).

1.2.2 Method of recording, nomenclature and component sources

SEPs are commonly stimulated by two electrodes, a proximal cathode and a distal anode, separated by a distance of 2.5 cm, and are located on the trajectory of peripheral nerves of the limb. The most common site for peripheral stimulation is either on the median nerve at the wrist crease (i.e. median nerve SEP) or on the posterior tibial nerve placed between the medial border of the Achilles tendon and the posterior border of the medial malleolus (i.e. tibial nerve SEP). A constant current stimulator has been recommended, which applies bipolar transcutaneous electrical stimuli with a frequency of 3 - 5 Hz and monophasic square-wave pulses of 0.1 - 0.2 ms (Cruccu et al., 2008). To obtain evoked potentials with a relevant amplitude, electric stimuli are commonly delivered at a high but tolerable intensity. This intensity is usually defined in relation to 2 or 3 times the sensory threshold (i.e. the lowest electrical intensity necessary to induce a conscious sensation at the stimulation site based on self-report)

or to the sum of the sensory and the motor thresholds (i.e. the lowest electrical intensity necessary to elicit a reproductive muscle switch, such as thumb movement for the median nerve SEP). After delivering electrical input, evoked potentials can be recorded by electrodes (the skin-electrode impedance < 5 kΩ), with an optimal bandwidth from less than 3 Hz to over 2000 Hz (Rossini, Cracco, Cracco, & House, 1981). More than 500 trials are recommended for the cortical component and at least 1000-2000 repetitions for the spinal and subcortical far-field components, with preferably two blocks for each component (Cruccu et al., 2008).

Time-locked somatosensory changes of evoked potentials at each segmental level from the periphery to the cortex can be shown as a series of positive ('P') or negative ('N') waveforms after averaging the single trial values. The latency (ms) of each wave peak can be depicted by a numeral (such as N '9'). Close correlates between the lesion site and abnormal SEPs have been well established in clinical observations and studies (Stöhr, Buettner, Riffel, & Koletzki, 1982; Synek, 1987). Localization using magnetic resonance imaging (MRI) and multichannel SEP recordings coupled with source modeling have brought the most recent advances for the origins of far-field SEPs. Later-latency cortical SEPs of more than 40ms are usually less reliable because there may involve more complex cognitive factors and the sources are under debate.

Multichannel recordings are designed to highlight SEP components during the same run. For instance, when the sensory afferent volley goes through the brachial plexus to the spine, a sharp positive component 'N9' can be obtained at the ipsilateral (to the stimulation site) Erb's point referenced to the contralateral Erb's point or a frontal channel such as Fz (Gobbelé, Buchner, & Curio, 1998). Erb' s point is located approximately 2-3 cm above the clavicle at the posterior border of the clavicular head of the sternomastoid. After transmissions from the first-order neurons in the DRG, the signals go through the posterior spinal cord and a tiny negative peak 'N11' arising from the root entry zone and a subsequent post-synaptic potential 'N13' triggered segmentally in the dorsal horn can be recorded over the cervical spinous process (Sonoo, Kobayashi, Genba-Shimizu, Mannen, & Shimizu, 1996). Using a far-field reference electrode, not located on the head, a positive valley 'P9' from the plexus and 'P11' from the dossal root entry zone can be obtained, but is usually very small. At the

foramen magnum level, ‘P14’ or ‘P13’ - ‘P14’ complexes have been recorded that are generated in the lower brainstem or close to the cervico-medullary junction. The broad ‘N18’ can sometimes be obtained, possibly generated near the thalamus (Desmedt & Cheron, 1981). However, this wave is easily confounded with the following cortical potentials ‘N20’ representing the ascending thalamocortical input into S1 (Mauguière, Desmedt, & Courjon, 1983). The first cortical response ‘N20 - P25’ complexes arises from the contralateral posterior wall of the central fissure in S1 (Cruccu et al., 2008). As far-field channels can include activities from subcortical structures, a purer cortical component can be recorded using a parietal electrode (such as CP3/4) referenced by a scalp electrode in the frontal region (such as Fz or F3/4). **Figure 3** demonstrates the distribution of recommended montages for SEPs in response to median and tibial nerve stimulation (Morizot-Koutlidis et al., 2015) and **Table 1** summarizes the useable recording and reference channels and the putative generators of human median or tibial nerve SEPs (Cruccu et al., 2008; Desmedt & Cheron, 1981; Lee & Seyal, 1998; Mauguière et al., 1983; Morizot-Koutlidis et al., 2015; Restuccia et al., 1995; Sonoo, Genba-Shimizu, Mannen, & Shimizu, 1997; Sonoo et al., 1996). **Figure 4** demonstrates the normal waveforms of human SEPs induced by electrical median or tibial nerve stimulation (Cruccu et al., 2008).

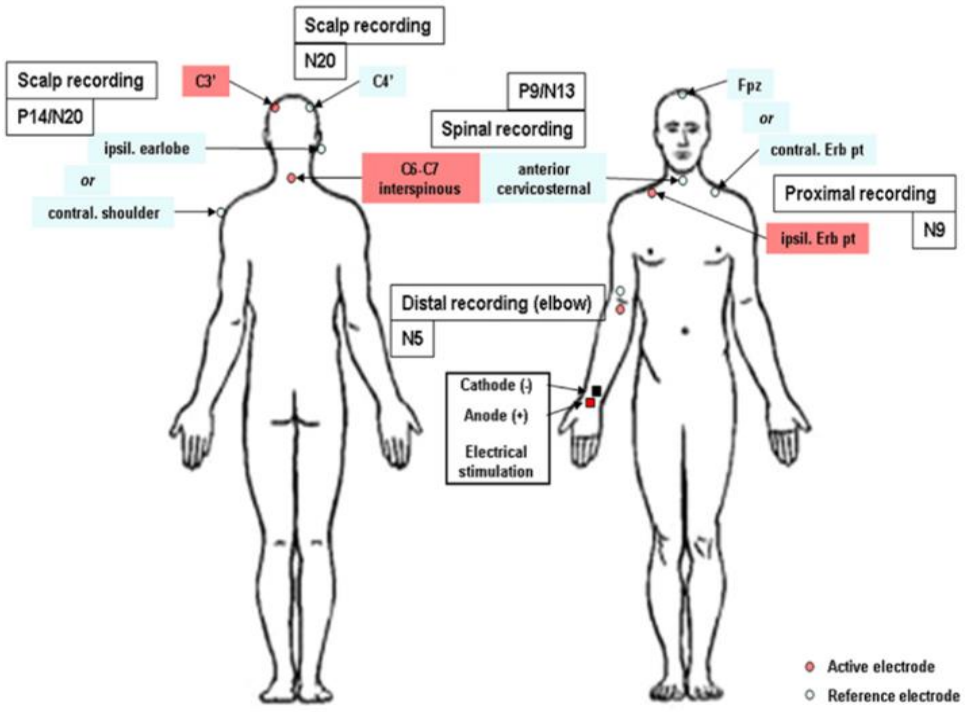
Except for somatosensory lesions, several factors may influence the amplitude and latency of short-latency SEPs. These include the age and the frequency of peripheral stimulation. The amplitude has been shown to increase with age and decrease for high frequency stimulation. For instance, Manzano et al. (1995) studied 10 healthy participants (5 females) with the age range 18-37 years and used electrical stimulation of the median nerve at 3 and 30 Hz (Manzano, De Navarro, Nóbrega, Novo, & Juliano, 1995). They found that the 30Hz stimulation frequency compared with the 3Hz frequency significantly reduced SEP amplitudes and prolonged the latencies of the components N9, N13 and N20 as well as the inter-peak intervals.

Table 1 Recommended recording locations, the reference and the putative generators of human somatosensory evoked potentials (SEP).

SEP Component	Recording Location	Acceptable Reference	Putative generators
Median Nerve SEP			
N9	Ipsilateral Erb's point (EPi)	Contralateral Erb's point (EPc), F(p)z, or ipsilateral earlobe (Ei)	Peripheral ascending volley from the brachial plexus
P9	Fz, or Contralateral CP3/4 (Pc)	Non-cephalic (EPc or shoulder)	Proximal brachial plexus
N13	6th or 7th cervical spinous process (Cv6/7)	Anterior neck (AC), or F(p)z	Cervical potential triggered in the dorsal horn, dorsal column or supra-spinal structures.
P14	CP3/4, Fz	Non-cephalic, or Ei	Lower brainstem or near cervico-medullary junction, likely relative to the nucleus cuneatus
N20	Contralateral CP3/4	F3/4, Fz, or Non-cephalic	the primary somatosensory cortex (S1), the first cortical response (N20/P25) in the posterior wall of the central fissure.
P25	Contralateral CP3/4	F3/4, Fz, or Non-cephalic	
N30	Fz	Non-cephalic, or Ei	Precentral activation
Tibial Nerve SEP			
N8	Popliteal fossa (PF)	Knee (K), or 3cm above PF	Peripheral ascending volley from the tibial nerve or sciatic nerve
N22	L1 spinous process	the supra-umbilical region (Um), L3 spinous process, or the contralateral iliac crest (Ic)	Post-synaptic response in the dorsal grey matter of the lumbosacral cord
	T12	T10	Spinal volley
P30	Fz, or CPz	Cv6/7, or Ei	Supraspinal-subcortical responses, or at cervico-medullary junction
P39	CPz	F(p)z, Ei, or CP3/4	Post-central somatosensory cortex

Scalp electrodes based on the 10–20 international system of EEG electrode placement.
Putative generators were based on previous literature (Cruccu et al., 2008; Desmedt & Cheron, 1981; Lee & Seyal, 1998; Mauguière et al., 1983; Restuccia et al., 1995; Sonoo, Genba-Shimizu, Mannen, & Shimizu, 1997; Sonoo et al., 1996).

A



B

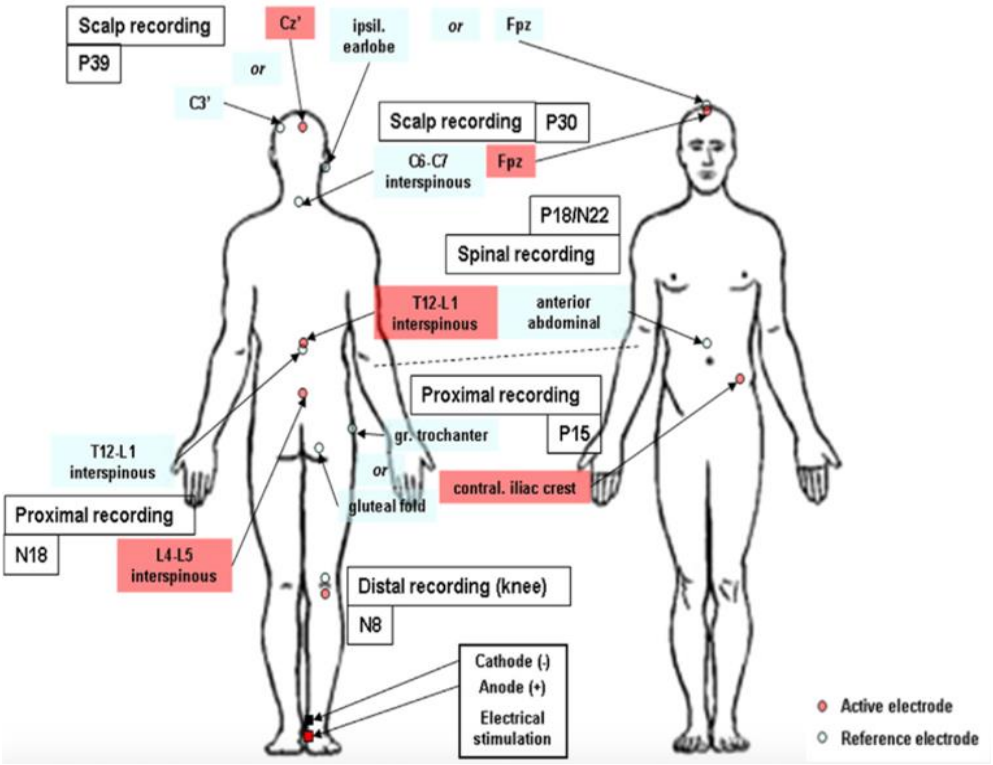


Figure 3 The distribution of recommended montages for SEP in response to median (**A**) and tibial nerve (**B**) electrical stimulation. Figures reprinted from Morizot-Koutlidis et al (2015), *Neurophysiologie Clinique/Clinical Neurophysiology, 45*(2), 136.

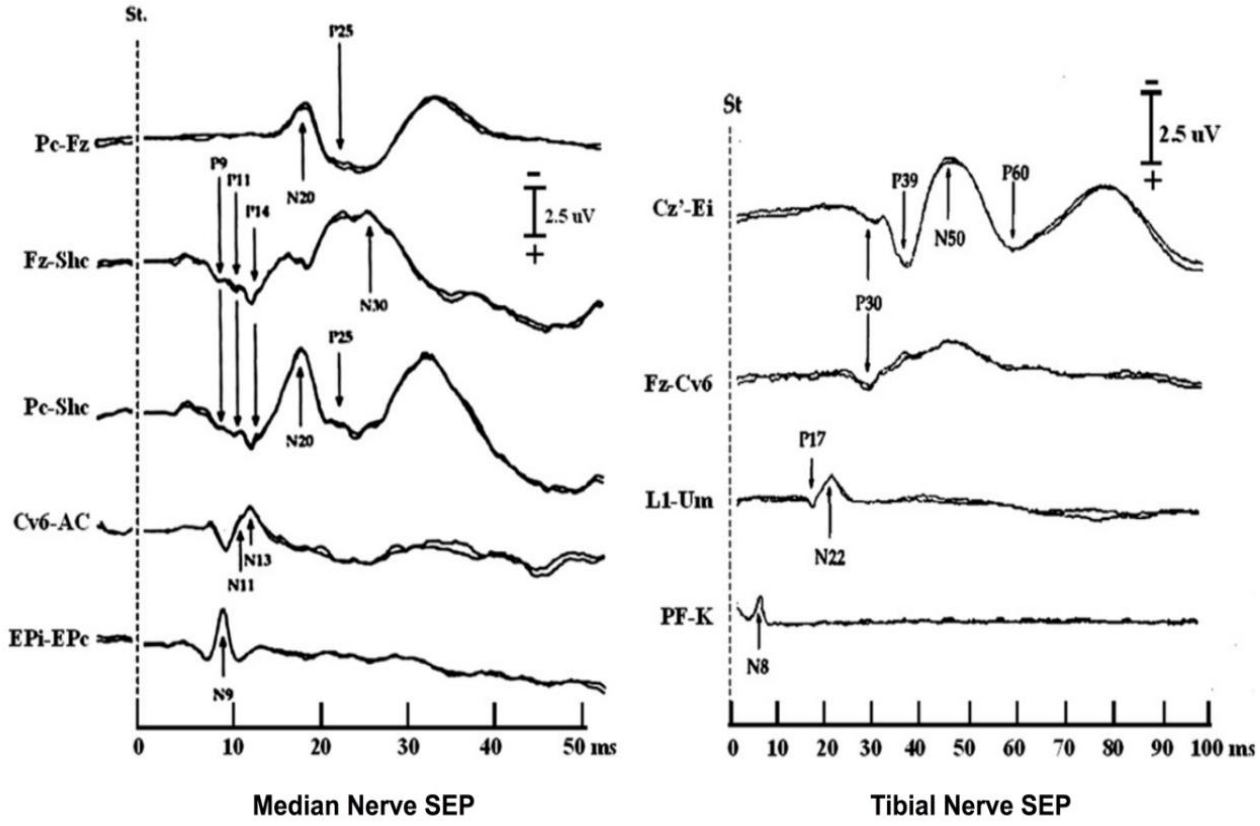


Figure 4 Representative time courses and waveforms of standard median and tibial nerve SEP recordings in a healthy adult. Surface potentials were recorded by bipolar channels on the scalp and body skin (EPi/c: ipsilateral/contralateral Erb's point, Cv6: 6th cervical spinous process, AC: anterior neck, Shc: contralateral shoulder, PF: popliteal fossa, K: knee, L1: 1st lumbar spinous process, Um: the supra-umbilical region). Figures reprinted from Cruccu et al. (2008), *Clinical Neurophysiology*, 119, 1709.

2. Goals and hypotheses

The etiology of PLP remains elusive. The present study was motivated by the ongoing debate about the role of peripheral and central mechanisms in the generation of PLP (Buch et al., 2019; Ringkamp & Raja, 2014; Vaso et al., 2014). Post-amputation plastic changes and especially cortical reorganization, have been reported to be specifically correlated with PLP. However, these findings do not preclude the contribution of peripheral input in the maintenance of PLP or to the PLP-associated cortical reorganization. Buch et al. (2019) and Vaso et el. (2014) showed that PLP could be decreased by blocking peripheral nerves or the DRG (or the spinal cord) (Buch et al., 2019; Vasa et al., 2014), but it is unclear how peripheral input contributes to PLP and its interactions with central activities. This thesis sought to investigate the contribution of peripheral input for PLP. Using peripheral electrical stimulation of the truncated nerves in the residual limb and somatosensory event-related potentials, the ascending neuronal barrages from the periphery, the spinal cord, the brainstem and the cortex were monitored. The somatosensory functions were measured in amputees with and without PLP and the corresponding changes in PLP and RLP before, during and after the afferent input were also assessed.

The main hypotheses in this study were as follows:

Hypothesis 1: PLP and RLP intensities are significantly increased after peripheral input and decrease immediately after the cessation of peripheral input.

Hypothesis 2: The SEP amplitudes in amputees with PLP are significantly higher than in those without PLP, suggesting functional alterations of somatosensory ascending pathways between amputee groups with and without PLP perception.

Hypothesis 3: The increased in PLP intensity induced by peripheral afferent input is associated with corresponding changes in SEP amplitudes, specifically at S1.

3. An empirical study

Peripheral input and phantom limb pain: a somatosensory event-related potential study [1]

[1] Liu, H., Andoh, J., Lyu, Y., Milde, C., Desch, S., Zidda, F., Schmelz, M., Curio, G., & Flor, H. (2019). Peripheral input and phantom limb pain: a somatosensory event-related potential study. *Submitted for publication.*

Abstract

Following amputation, nearly all amputees report non-painful phantom phenomena and many of them suffer from chronic phantom limb pain (PLP) and residual limb pain (RLP). The etiology of PLP remains elusive and there is an ongoing debate on the role of peripheral and central mechanisms. Few studies have examined the entire somatosensory pathway from the truncated nerves to the cortex in amputees with PLP compared to those without PLP. The relationship between afferent input, somatosensory responses and the change in PLP remains unclear. We applied transcutaneous electrical nerve stimulation on the truncated median nerve, the skin of the residual limb and the contralateral homologous nerve in twenty-two traumatic upper-limb amputees (12 with and 10 without PLP). Using somatosensory event-related potentials, the ascending volley was monitored through the brachial plexus, the spinal cord, the brainstem and the thalamus to the primary somatosensory cortex. Peripheral input could evoke PLP in amputees with chronic PLP (7/12), but not in amputees without a history of PLP (0/10), while the amplitudes of the somatosensory components were comparable between amputees with and without PLP. In addition, peripherally induced potentials through the spinal segment were significantly positively associated with evoked residual limb pain (RLP), but not PLP. Peripheral input can modulate PLP but seems insufficient to cause PLP. These findings also suggest different mechanisms for PLP and RLP.

Perspective

In this study we found no significant differences in the electrical potentials generated by stimulation from the nerve and the skin of the residual limb in amputees with and without PLP. Peripheral input enhanced existing PLP but could not elicit it. This suggests an important role of central processes in PLP. These findings indicate the multifactorial complexity of PLP.

Keywords: Phantom limb pain, residual limb pain, somatosensory event-related potentials, peripheral input.

1. Introduction

Peripheral injury and deprivation drive plastic changes in both the peripheral and central nervous system (Draganski et al., 2006; Elbert et al., 1994; Hamzei et al., 2001; Makin, Filippini, et al., 2015; Merzenich et al., 1984; Yang et al., 1994). After amputation, such changes have been related to chronic neuropathic pain, including phantom limb pain (PLP) (Flor et al., 1995; Flor, 2002; Kuner & Flor, 2016). Residual limb pain (RLP) has mostly been attributed to peripheral pathological alterations (such as neuromas, bone spurs, ischemia and infection) and prosthesis use (Guo et al., 2019; Yazicioglu, Tugcu, Yilmaz, Goktepe, & Mohur, 2008). PLP has specifically been found to be associated with central plastic changes, especially in the sensorimotor cortices (Flor, Nikolajsen, & Staehelin Jensen, 2006; Kikkert et al., 2017; Lotze, Flor, Grodd, Larbig, & Birbaumer, 2001; Makin et al., 2013; Reilly & Sirigu, 2008). A strong positive correlation has repeatedly been found between PLP intensity and expansions and/or shifts of neighboring cortical representations in relation to the deafferented hand area, believed to be triggered by sensory deprivation (Flor et al., 1995). Makin et al. (2013) reported a positive relationship between increased activation in the representation of the amputated limb and PLP (Makin et al., 2013). Another group related cortical reorganization to phantom sensations and showed that pain might be not critical in functional sensorimotor changes (Bramati et al., 2019; Simoes et al., 2012). In addition, therapies that relieve PLP such as mirror therapy, motor imagery or sensory discrimination training showed a correspondence between a reversal of maladaptive cortical reorganization and a reduction of PLP (Flor, Denke, Schaefer, & Grüsser, 2001; Foell, Bekrater-Bodmann, Diers, & Flor, 2014; Gagné, Reilly, Hétu, & Mercier, 2009; MacIver, Lloyd, Kelly, Roberts, & Nurmikko, 2008). In addition to cortical changes, subcortical processes have also been related to PLP. PLP has been shown to be generated by thalamic microstimulation (Davis et al., 1998), spinal metastatic pathologies (Cruz & Dangaria, 2013), spinal anesthesia (Harrison, 1951; Mackenzie, 1983; Murphy & Anandaciva, 1984) and has been connected to alterations in the brainstem, possibly because of reorganized somatosensory afferents and decreased tonic inhibition. These findings suggest a preponderance of central mechanisms in PLP.

PLP is highly correlated with RLP (Carlen, Wall, Nadvorna, & Steinbach, 1978; Desmond & Maclachlan, 2010; Gallagher, David Allen, Malcolm Mac, 2001; Kooijman, Dijkstra, Geertzen, Elzinga, & van der Schans, 2000) and both might be maintained by peripheral input and amplified centrally (Ringkamp & Raja, 2014; Vase et al., 2011; Weeks, Anderson-Barnes, & Tsao, 2010). Ectopic generators have been proposed including neuromas, truncated afferent axons and abnormal activity in the dorsal root ganglia (DRG) (Devor, 2009). For instance, peripheral discharges triggered by repetitive taps on neuromas were recorded from the truncated nerves using microelectrode recordings and were related to PLP (Nyström & Hagbarth, 1981). After anesthetizing the DRG and the spine by injecting lidocaine in lower-limb amputees, PLP has been observed to be temporarily eliminated (Vaso et al., 2014). However, placebo-controlled and blinded randomized controlled clinical trials are lacking and further studies are needed to confirm the effectiveness of intervening at the DRG in PLP. Recently, Buch et al. (2019) carried out a randomized, double-blind and placebo-controlled study and showed that PLP could be significantly reduced after peripheral nerve block using lidocaine (Buch et al., 2019), suggesting a role of peripheral input in postamputation pain including PLP. A few studies have measured the somatosensory neuraxis from the truncated nerve fibers to the cortex in upper-limb amputees using somatosensory evoked potentials (SEP) (Mackert, Sappok, Grüsser, Flor, & Curio, 2003; Schwenkreis et al., 2001). Peripheral input from the truncated nerves was found to reach the deafferented cortex in upper-limb amputees with phantom sensations but without PLP, which could provide a possible neural substrate for spontaneous phantom sensations and PLP (Mackert et al., 2003). However, this study did not include amputees with PLP and another study (Schwenkreis et al., 2001) only examined at the cortical level. In addition, computational models of central changes in PLP assume that peripheral generators can drive central reorganizational processes (Boström, de Lussanet, Weiss, Puta, & Wagner, 2015; Spitzer, 1997). The role of peripheral input for PLP and how it interacts with central processes is therefore not yet completely understood.

Here, we aimed to compare somatosensory function in traumatic upper-limb amputees with and without PLP and sought to determine how peripheral input is associated with evoked responses and PLP as well as RLP. Specifically, we examined

the somatosensory pathways through the brachial plexus, the spinal cord, the brainstem and the primary somatosensory cortex (S1) using SEPs. We expected PLP and RLP to be modulated by peripheral afferent input and corresponding changes in SEP amplitudes.

2. Materials and Methods

2.1 Participants

Twelve unilateral traumatic upper-limb amputees with chronic PLP (PLP group, mean age: 55 years, range: 37-76 years) and ten sex- and age-matched unilateral traumatic upper-limb amputated controls, who had never experienced PLP (Non-PLP group, mean age: 55 years, range 42-68 years), were recruited based on an existing database and collaborating pain clinics (Streit et al., 2015). Independent t-tests showed no significant group differences in age, age at time of amputation, time since amputation, or duration/frequency of prosthesis use (see **Table 1** for demographic and clinical details). The study was approved by the Ethics Committee of the Medical Faculty Mannheim, Heidelberg University, and written informed consent was obtained from all participants prior to the study. Each amputee participated in a comprehensive psychometric evaluation including a structured interview about the amputation and its consequences, a detailed assessment of painful and non-painful phantom phenomena, prosthesis use, a psychological evaluation and several pain measures including the German version of the West Haven-Yale Multidimensional Pain Inventory (MPI) (Flor, Rudy, Birbaumer, Streit, & Schugens, 1990) modified to separately measure PLP and RLP (Flor et al., 1995; Lotze et al., 1999).

2.2 Experimental procedure

The participants were comfortably seated on a chair with a soft backrest in a sound-attenuated and illuminated room. They were asked to keep their eyes open and fixate a black cross in the center of a computer monitor in front of them.

Table 1 | Demographic and clinical details.

	Subject	Age/ Sex	Age at Amp.	Amp. site	Pros. use	Habitual PLP intensity (MPI)	Habitual RLP intensity (MPI)	Amplitudes of SEP components (μV)											
								Periphery			Spinal/Supraspinal			Subcortical			S1		
								TN	SS	CN	TN	SS	CN	TN	SS	CN	TN	SS	CN
	PG01	66/F	38	L, 5	0	2	0	1.93	0.36	0.42	1.02	1.06	0.91	1.18	0.88	1.87	1.26	2.00	3.45
	PG02	37/M	20	R, 5	0	2	1	0.52	0.15	0.88	2.19	0.30	0.74	0.38	0.63	1.04	0.92	0.76	1.55
	PG03	48/F	24	L, 3	0	4	3	0.23	NA	0.18	0.54	NA	0.51	0.50	NA	0.86	1.23	NA	1.47
	PG04	76/M	33	R, 5	0	2	2	7.83	0.15	2.18	2.91	0.21	1.62	0.94	0.84	3.23	1.30	0.85	7.00
	PG05	58/F	23	R, 2	2	2	4	0.74	0.33	0.86	1.85	0.81	0.58	1.63	1.11	0.64	2.42	0.97	0.86
PLP	PG06	61/M	23	R, 2	4	1	1	1.21	0.39	3.81	1.47	0.62	2.14	1.87	0.91	3.20	2.58	1.07	4.60
group	PG07	49/F	17	L, 5	0	2	0	1.57	0.21	3.15	0.37	0.15	1.83	0.83	0.55	2.34	0.53	0.49	6.23
	PG08	55/M	53	R, 5	3	1	0	1.73	0.16	11.49	0.39	0.39	5.51	2.90	0.87	1.75	2.06	1.45	1.06
	PG09	50/M	17	L, 3	6	3	2	0.24	0.09	0.39	0.57	0.46	0.60	0.56	0.16	0.38	1.42	1.36	0.83
	PG10	50/M	35	L, 3	4	2	1	1.52	0.24	0.30	1.43	0.55	0.33	1.48	0.51	0.54	2.54	0.87	0.77
	PG11	55/M	18	L, 3	6	3	1	1.24	0.13	1.02	1.23	0.60	1.45	1.33	0.72	2.18	4.34	3.61	3.75
	PG12	58/M	28	R, 5	1	2	1	2.03	0.15	2.54	2.88	0.44	1.27	1.69	1.09	2.16	1.75	0.95	6.65
	Mean ±SD	55.3 ±9.9 8M4F	27.4 ±10.7	6R6L 3.8±1.3	2.2 ±2.4	2.2 ±0.8	1.3 ±1.2	1.73 ±2.02	0.21 ±0.10	2.27 ±3.15	1.40 ±0.90	0.51 ±0.26	1.46 ±1.40	1.27 ±0.71	0.75 ±0.28	1.68 ±0.99	1.86 ±1.02	1.31 ±0.86	3.19 ±2.43
	NG01	60/M	39	R, 5	3	0	0	3.47	0.16	1.59	0.61	0.27	1.04	0.88	0.23	1.34	2.34	0.95	4.09
	NG02	49/M	17	R, 5	0	0	1	4.43	0.28	0.75	0.58	0.32	0.93	0.57	0.70	0.75	0.79	0.45	1.21
	NG03	55/M	20	L, 5	6	0	0	0.71	0.26	0.39	0.83	0.34	0.51	1.53	1.39	0.89	1.03	1.05	2.43
Non-	NG04	68/M	27	R, 3	3	0	0	0.69	0.33	0.45	0.34	0.35	0.81	0.91	1.00	0.88	1.82	0.82	1.86
PLP	NG05	55/M	21	R, 5	0	0	0	0.34	0.30	0.91	1.07	0.47	0.79	0.39	0.27	1.62	1.42	1.66	2.73
group	NG06	58/M	19	L, 5	0	0	0	0.48	0.04	11.07	0.97	0.37	3.34	1.61	0.39	4.72	2.70	0.66	7.19
	NG07	60/M	19	L, 3	6	0	0	1.00	0.12	1.00	1.46	0.37	0.86	1.22	0.80	0.88	1.21	1.95	1.44
	NG08	51/M	18	R, 3	0	0	0	1.07	0.31	0.48	0.41	0.28	0.48	0.91	0.76	0.79	2.97	1.01	1.84
	NG09	56/F	28	L, 5	0	0	1	0.92	0.25	0.53	0.23	0.31	0.41	0.70	0.73	1.29	2.29	2.12	2.61
	NG10	42/M	28	R, 3	0	0	0	0.77	0.36	9.45	1.10	0.42	3.78	0.90	1.08	1.72	1.35	1.50	1.67
	Mean ±SD	55.4 ±7.1 9M1F	23.6 ±6.9	6R4L 4.2 ±1.0	1.8 ±2.5	0 ±0	0.2 ±0.4	1.39 ±1.39	0.24 ±0.10	2.66 ±4.04	0.76 ±0.39	0.35 ±0.06	1.30 ±1.22	0.96 ±0.39	0.74 ±0.37	1.49 ±1.19	1.79 ±0.75	1.22 ±0.56	2.71 ±1.78

PLP = phantom limb pain; RLP = residual limb pain; PLP group: amputees with chronic PLP; Non-PLP group: amputees without PLP; F = female; M = male;
Amp. = amputation; Amp. site: L = left; R= right; 1 = hand, 2 = wrist, 3 = forearm, 4 = elbow, 5 = upper arm, 6 = shoulder;
Pros. = prosthetics; Pros. use: 0 = never, 1 = rarely, 2 = occasionally, 3 = weekly, 4 = daily (less than 4 hours a day), 5 = daily (more than 4 hours a day), 6 = daily (over 8 hours a day).
MPI: German version of the Multidimensional Pain Inventory adjusted to separately measure PLP and RLP, ranging from 0 ('no pain') to 6 ('extreme pain').
TN: truncated median nerve; SS: skin of the residual limb; CN: contralateral intact median nerve. NA: not available for the SS stimulation due to time constraints in one participant (PG03).

Peripheral nerve and skin stimulation

We applied transcutaneous electrical nerve stimulation using a standard bridge electrode (spacing 2.5 cm, cathode proximal) connected to a Digitimer stimulator (Digitimer® DS7A, Hertfordshire, United Kingdom) with monophasic square-wave pulses of 0.2 ms at a 3 Hz frequency. Electrical stimuli were applied over three body sites: the truncated median nerve (TN), the skin of the residual limb (SS), and the contralateral homologous intact median nerve (CN). The order of stimulated body sites was counterbalanced across the participants. To account for differences in distal variables such as different amputation levels, neuromas, scars and sensitive skin in individuals, we set a proximal level at the sulcus bicipitalis medialis in the upper arm for electrical stimulation of each body site (Mackert et al., 2003). To localize the nerve of the CN and TN, we made sure that electrical stimulation elicited thumb movement for the healthy hand and ongoing projected paresthesia towards the elbow, forearm and the territory of the median nerve in the distal intact or phantom hand. In contrast, electrical stimulation of skin site (SS), which was not applied over the peripheral nerve trunk, did not elicit any projected paresthesia and the stimuli were perceived only at the stimulation site.

Sensory and pain thresholds

We determined sensory and pain thresholds for each participant and each body site (TN, SS, CN). For each threshold, we calculated the average value of the intensity (mA) of three consecutive measurements when the participant reported “just perceptible” for the sensory threshold or “it starts to hurt” for the pain threshold. During each measurement, we increased the intensity of electrical stimulation (at the frequency of 3 Hz) starting from 0 mA at the speed of 0.1 mA/5 s with the experimenter outside the room.

Stimulation intensity

We defined the stimulation intensity to be applied on each body site using verbal ratings from 0 (‘just perceptible’) to 10 (‘it starts to hurt’). The intensity of electrical stimulation (at the frequency of 3 Hz) was varied randomly until the participant stably

reported 8 out of 10, which was described as strong but non-painful sensation. This procedure was repeated three times for each body site and the mean intensity was used for electrical stimulation ensuring adequate but non-painful stimulation for the SEPs. Subsequently, ongoing electrical stimulation of the defined strong but non-painful intensity was applied at each body site for SEP recordings.

Due to time constraints, in one participant (PG03) the SS stimulation and in three participants (PG01, PG02, PG03) pain thresholds were not obtained.

2.3 Data acquisition

SEPs

SEP data were acquired using Ag/AgCl electrodes (diameter: 2mm) and an actiCap following the standard 10 - 10 system located at the contralateral CP3/4, F3/4 on the scalp and another six electrodes placed over the ipsilateral Erb's point (Epi), the contralateral Erb's point (Epc), the 2/4/6th cervical spinous process (Cv2/4/6) and a non-cephalic site (NC) at the level of anterior glottis/ thyroid cartilage to obtain median-nerve SEPs (Cruccu et al., 2008; Desmedt, 1985; Seyal & Gabor, 1987). The ground electrode was located at Fpz and the montage was referenced to Fz. Active electrode impedance was monitored and remained below 20 kΩ as suggested by the manufacturer. The signals were recorded with a wide bandwidth from DC to 2470 Hz and a sampling rate of 10 kHz by an actiCHamp amplifier (Brain Products GmbH, Munich, Germany) and BrainVision Recorder software (version 1.21.0102) was employed (Rossini, Cracco, Cracco, & House, 1981). A total of 3000 continuous stimuli lasting approximately 17 min for each body site were applied and recorded (Cruccu et al., 2008).

Ratings

For each participant, the intensity of RLP, PLP and non-painful phantom sensations was assessed before (pre), during (mid) and after (post) the electrical stimulation of each body site, using 25 cm long computer-based horizontal visual analogue scales (VAS) with the endpoints "no pain/ no sensation at all" to "extreme pain/the most vivid sensation". They were then transformed into a scale ranging from 0 to 100. In addition,

immediately after the end of the electrical stimulation, the participants were instructed via computer monitor to rate the maximum intensity (mid) of painful or non-painful sensations that they perceived in the residual or phantom limb during the stimulation using 25 cm long computer-based horizontal VAS with the endpoints “no pain/no sensation at all” to “extreme pain/the most vivid sensation”, which were then transformed into a scale ranging from 0 to 100. Subsequent to the ratings, a detailed questionnaire regarding phantom phenomena perceived during the TN stimulation was carried out, including the temporal pattern of PLP (such as persistent pain with slight or strong variations, persistent pain with pain attacks, or pain attacks with pain-free intervals), quality of PLP (such as warm, cold, sharp, burning, aching, cramping, throbbing or stabbing), spatial distribution of PLP (such as the palm and fingers), phantom movement, and abnormal sensations or pain at the stimulated body site and other body parts (such as face and mouth).

2.4 Data analysis

SEPs

SEP data were preprocessed using EEGLAB v14.1.0 (https://sccn.ucsd.edu/eeglab), an open-source toolbox running under the MATLAB R2017b environment (Delorme & Makeig, 2004). The data were high-pass filtered at 1 Hz and then low-pass filtered at 1500 Hz. Before the low-pass filter, stimulation artefacts within ±3 ms relative to the onset of the electric stimuli were interpolated with a flat line connecting two endpoints at -3ms and 3ms to avoid subsequent ringing after low-pass filtering, which would interfere with subsequent early SEP waves (Waterstraat, Fedele, Burghoff, Scheer, & Curio, 2015). The data were then re-referenced to the appropriate reference for peripheral (Epi-EPc), spinal/supraspinal (Cv6-Fz), subcortical (CP3/4-NC) and S1 components (CP3/4-F3/4) (Cruccu et al., 2008; Morizot-Koutlidis et al., 2015; Sonoo, Kobayashi, Genba-Shimizu, Mannen, & Shimizu, 1996). Data in the time window from -10 ms to 100 ms were epoched and averaged. SEP components were identified according to the standard median-nerve SEPs with specific waveforms and peak directions arising at the appropriate SEP latencies (4-5ms shorter than standard SEPs) (Cruccu et al., 2008). For amputees in conditions without significant SEP waves (such as SS), the peak value

in the time window of 3 ms with the central time point at the appropriate latency was identified as an alternative for the peak amplitudes. **Fig. 1** shows a representative time course and waveforms of the SEPs in one amputee (PG06) who reported an increase of PLP intensity during the TN condition (mid) referred to the baseline (pre).

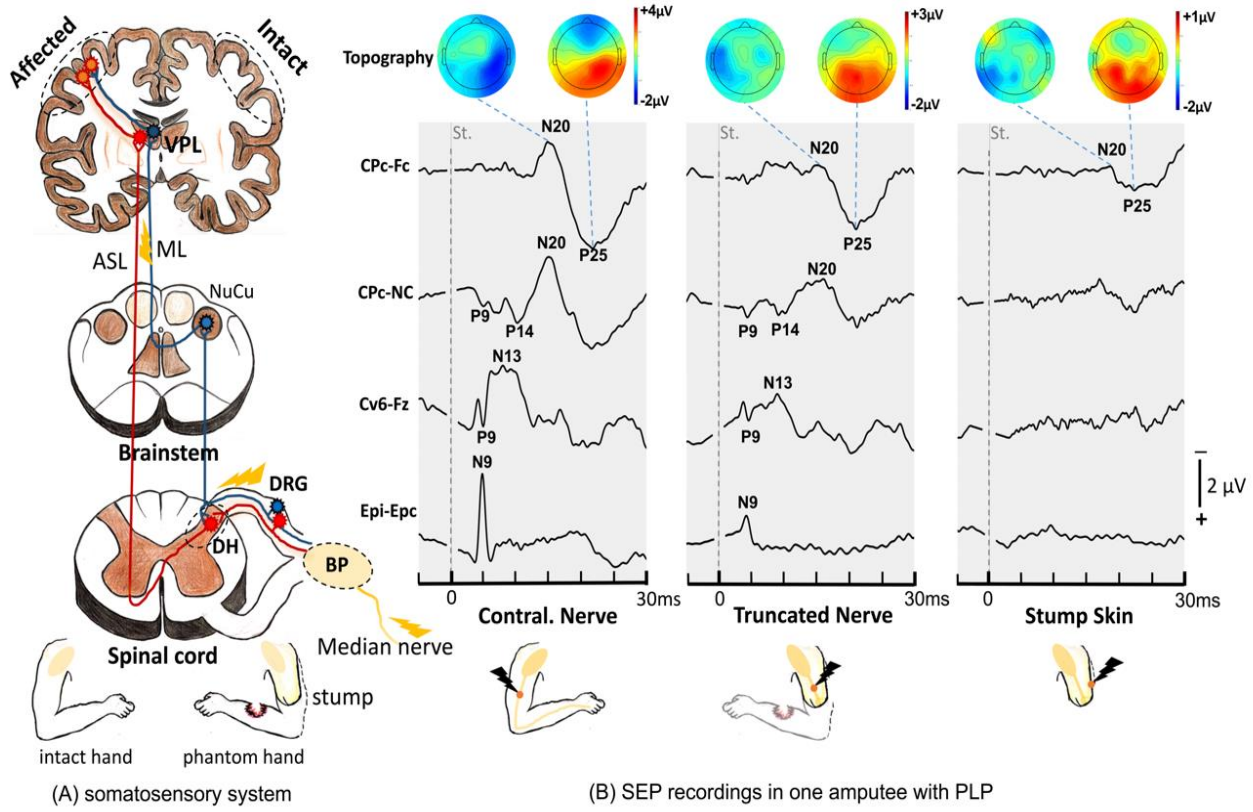


Fig. 1. Schematic drawing of the somatosensory system and representative recordings of somatosensory evoked potentials (SEP). Time courses of SEP recordings in one participant (PG06) who reported a significant increase of phantom limb pain during electrical stimulation (3 Hz) of the truncated median nerve. For each body site, 3000 epochs were superimposed for demonstration. BP: brachial plexus. DRG: dorsal root ganglia. DH: spinal dorsal horn. NuCu: cuneate nucleus. VPL: ventral posterolateral nucleus of thalamus. ML: medial lemniscus. ASL: anterolateral system. Contral.: contralateral.

Statistical analysis

Statistical analysis was carried out using IBM SPSS software (version 25, IBM company, USA). We compared the amplitudes of the SEP components, electric stimulation intensity, the sensory and pain thresholds using 2x3 factorial analyses of variance (ANOVAs) with the between-subjects factor group (PLP, Non-PLP) and the within-subjects factor body site (TN, SS, CN). For PLP and RLP ratings, 2x3x3 mixed ANOVAs with the between-subjects factor group (PLP, Non-PLP) and the within-subjects factors body site (TN, SS, CN) and time point (pre, mid, post) were carried out. For each measure, cases more than three standard deviations from the mean were inspected (one extreme outlier in peripheral SEP was deleted in the ANOVAs, and re-analysis showed that either the presence, absence or the replacement with within-participant means did not affect the results). The sphericity assumption for repeated measures analyses was tested using the Mauchly's test and Greenhouse-Geisser correction ($\varepsilon < 0.75$) of the degrees of freedom was applied in case of a violation. For all multiple comparisons, p values were adjusted using Bonferroni corrections. We also calculated the changes in PLP and RLP intensity from the pre to the mid time point by subtracting the ratings between the two time points (mid-pre). Finally, correlation analyses were carried out to examine associations between the amplitudes of SEP components induced at the residual limb and changes in PLP and RLP intensity as well as the baseline (pre). Correlations were carried out within the amputees with PLP, the amputees with RLP, the amputees with postamputation pain (PLP/RLP), and all amputees with and without pain using two-tailed Spearman rank correlations. Because none of the amputees in the Non-PLP group or pain-free amputees (neither PLP nor RLP) reported a change in PLP intensity (see results), these groups were not analyzed separately. In addition, SEP data in the SS condition were not taken into the correlation analyses separately since the SS stimulation evoked only nonsignificant waves for the peripheral and spinal SEPs.

3. Results

3.1 Amplitudes of SEP components in amputees with and without PLP

The latency of the somatosensory component was approximately 4-5 ms shorter

compared to the standard latency of the median-nerve SEP since the stimulation sites were more proximal at the upper arm compared to the standard site at the wrist. **Fig. 2** shows the mean amplitudes of each SEP component in all conditions (see **Table 1** for more details). There was no significant group difference between amputees with and without PLP for any component.

Peripheral component

For the amplitude of the peripheral component (N9/P9), there was a significant effect for body site ($F(2, 58)= 5.603$, $p=0.006$, $\eta^2 =0.162$), but neither a significant group effect ($F(1, 58)= 0.147$, $p= 0.703$, $\eta^2 = 0.003$) nor a significant group * body site interaction ($F(2, 58)= 0.038$, $p= 0.963$, $\eta^2 =0.001$). The amplitude of the peripheral component in the CN condition was significantly higher compared to the SS condition ($p= 0.005$), however, there was neither a significant difference between the CN and TN conditions ($p= 0.255$), nor between the TN and SS conditions ($p= 0.375$) in the post hoc tests.

Peripheral-spinal/supraspinal component

For the amplitude of the peripheral-spinal/supraspinal component (N13/P9), there was a significant effect for body site ($F(2, 59)=6.419$, $p=0.003$, $\eta^2 =0.179$), but neither a significant group ($F(1, 59)=2.142$, $p=0.149$, $\eta^2 =0.035$) nor group * body site effect ($F(2, 59)=0.542$, $p=0.584$, $\eta^2 =0.018$). The amplitudes in the CN condition ($p=0.002$) and the TN condition ($p=0.043$) were both significantly higher compared to the SS condition, however, there was no significant difference between the TN and CN conditions ($p=0.932$) in the post hoc tests.

Subcortical-cortical component

For the amplitude of the subcortical-cortical component (P14/N20), there was a significant effect for body site ($F(2, 59)=6.923$, $p=0.002$, $\eta^2 =0.190$), but neither a significant group effect ($F(1, 59)=0.893$, $p=0.348$, $\eta^2 =0.015$) nor a significant group * body site interaction ($F(2, 59)=0.214$, $p=0.808$, $\eta^2 =0.007$). The amplitudes in the CN condition were significantly higher compared to the SS condition ($p=0.001$), however,

there was neither a significant difference between the CN and TN conditions (p=0.130), nor between the TN and SS conditions (p=0.274) in the post hoc tests.

S1 component

For the amplitude of the S1 component (N20/P25), there was a significant effect for body site (F(2, 59)=7.734, p=0.001, η^2 =0.208), but neither a significant group effect (F(1,59)=0.359, p=0.552, η^2 =0.006) nor a significant group*site interaction (F(2,59)=0.140, p=0.869, η^2 =0.005). The amplitudes in the CN condition were significantly higher than in the TN condition (p=0.032) and the SS condition (p=0.001), however, there was no significant difference between the TN and SS conditions (p=0.597) in the post hoc tests.

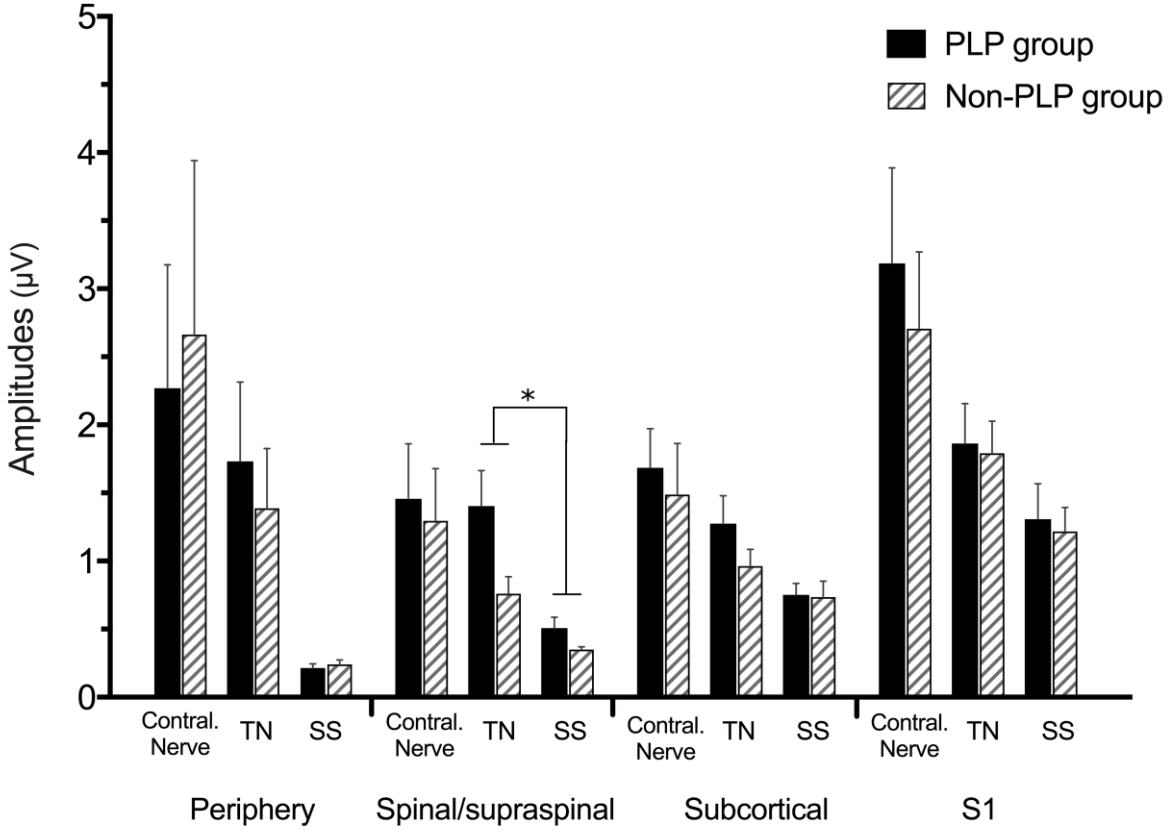


Fig. 2. The amplitudes of peripherally induced somatosensory evoked potentials from the periphery to cortex. Non-painful electrical stimuli (3Hz) were applied respectively at the contralateral intact median nerve, the truncated median nerve (TN) and the skin site of the

residual limb (SS). There was no significant group difference between amputees with and without PLP for the amplitudes of the ascending volleys from the periphery to the primary somatosensory cortex (S1). The TN stimulation induced significantly higher potentials at or near the spinal segment versus the SS stimulation. Contral.: contralateral. Data are presented as mean and standard error of the mean. * p<0.05.

3.2 Stimulation intensity, sensory and pain thresholds in amputees with and without PLP

For the electrical stimulation intensity, there was neither a significant group (F(1, 59) = 1.148, p=0.288) nor body site effect (F(2, 59) = 1.489, p=0.234), nor a significant group* body site interaction (F(2, 59) = 0.719, p=0.491). The same was true for the electrical sensory thresholds (group: F(1, 59) = 3.778, p=0.057, body site: F(2, 59) = 0.159, p=0.853, group * body site: F(2, 59) = 0.056, p=0.945). For the electrical pain thresholds, there was neither a significant group effect (F(1, 51) = 0.912, p=0.344) nor body site effect (F(2, 51) = 1.400, p=0.256), nor a significant group* body site interaction (F(2, 51) = 0.975, p=0.384). **Table 2** shows means of the stimulation intensities, the sensory and pain thresholds at the three body sites in amputees with and without PLP.

Table 2 | Stimulation intensity, sensory and pain thresholds in amputees with and without PLP (mean ± SD).

	Stimulation intensity (mA)			Sensory thresholds (mA)			Pain thresholds (mA)		
	TN	SS	CN	TN	SS	CN	TN	SS	CN
PLP group	14.47 ± 12.26	11.25 ± 5.96	10.90 ± 5.05	3.21 ± 2.13	3.44 ± 1.88	3.12 ± 1.87	16.16 ± 16.06	9.68 ± 5.80	11.57 ± 5.34
Non-PLP group	11.09 ± 3.42	12.35 ± 6.39	7.64 ± 2.76	2.60 ± 1.44	2.50 ± 0.92	2.28 ± 0.98	11.61 ± 4.36	11.75 ± 6.00	8.10 ± 2.79

TN: truncated median nerve; SS: skin of the residual limb; CN: contralateral intact median nerve.
Sensory and pain thresholds were defined by the average of the three intensity values at each body site after increasing the intensity of electrical stimulation (3 Hz) starting from 0 mA until the participant reported "just perceptible" or "it starts to hurt".
Stimulation intensity was defined by the average of the three intensity values at each body site which were described as strong non-painful sensation after the intensity of electrical stimulation (3Hz) varied randomly until the participant reported 8 out of 10 stably using a verbal rating from 0 ('just perceptible') to 10 ('it starts to hurt').

3.3 Ratings

PLP

PLP could be enhanced by peripheral input with an average increase of 5.37 ± 14.41 (TN: 14.58 ± 20.10, SS: 4.09 ± 7.41, CN: -2.67 ± 4.85) on a scale from 0 to 100 in

the amputees in the PLP group, however, PLP could not be elicited at any body site in any amputee in the Non-PLP group. **Table 3** shows changes in PLP and RLP ratings in all conditions. **Fig. 3** depicts the mean PLP and RLP ratings in all conditions. There was a significant group effect for PLP intensity (F(1, 19)=7.427, p=0.013, η^2=0.281), which showed that PLP increased significantly in the PLP group. There was also a significant time point effect (F(1.417, 26.919)=6.118, p=0.012, η^2=0.244) and a significant time point * group interaction (F(1.417, 26.919)=6.118, p=0.012, η^2=0.244) for PLP intensity, indicating that PLP increased significantly during non-painful electrical stimulation (pre to mid and mid to post) and in amputees with PLP only. There was neither a significant body site effect (F(1.371, 26.044)=3.578, p=0.058, η^2=0.158), nor a significant body site * time point * group (F(1.493, 36.918)=3.091, p=0.059, η^2=0.140), body site * group (F(1.371, 26.044)=3.578, p=0.058, η^2=0.158) or body site * time point interaction (F(1.493, 36.918)=3.091, p=0.059, η^2=0.140).

In the PLP group, PLP intensity was significantly higher at the mid versus the pre (adjusted p=0.008) and the post time point (adjusted p=0.001), however, there was no significant difference between the pre and post time point (adjusted p=1.000). In the Non-PLP group, there was neither a significant difference for PLP intensity between the pre and mid time point (adjusted p=1.000), nor between the pre and post time point (adjusted p=1.000), or between the mid and post time point (adjusted p=1.000).

RLP

For RLP intensity, there was a significant effect for body site (F(2, 38)=7.602, p=0.002, η^2=0.286), time point (F(1.161, 22.061)=10.588, p=0.003, η^2 =0.358) and time point * body site (F(2.085, 39.610)=4.774, p=0.013, η^2 =0.201) as well as body site * group (F(2, 38)=3.384, p=0.044, η^2 =0.151). However, there was neither a significant group effect (F(1, 19)=3.819, p=0.066, η^2 =0.167), nor a significant time point * body site * group (F(2.085, 39.610)= 2.409, p=0.101, η^2 =0.113), or time point * group interaction (F(1.161, 22.061)=0.049, p=0.861, η^2 =0.003).

In the TN condition, RLP intensity at the mid time point was significantly higher compared to pre (adjusted p=0.010) and post (adjusted p=0.019). There was no significant difference between the pre and post time point (adjusted p=1.000). In the CN

condition, there was neither a significant difference for RLP intensity between the pre and mid time point (adjusted *p*=1.000), between the pre and post time point (adjusted *p*=1.000), nor between the mid and post (adjusted *p*=1.000). In the SS condition, there was neither a significant difference for RLP intensity between the pre and mid time point (adjusted *p*=0.362), between the pre and post time point (adjusted *p*=0.693), nor between the mid and post time point (adjusted *p*=0.551). At the pre time point, there was neither a significant difference for RLP intensity between the CN and TN condition (adjusted *p*=0.521), between the CN and SS condition (adjusted *p*=1.000), nor between the TN and SS condition (adjusted *p*=0.316). At the mid time point, RLP intensity was significantly higher in the TN condition versus CN condition (adjusted *p*=0.008), but there was neither a significant difference between the TN and SS condition (adjusted *p*=0.139) nor between the CN and SS condition (adjusted *p*=0.312). At the post time point, there was neither a significant difference for RLP intensity between the CN and TN condition (adjusted *p*=0.209), between the CN and SS condition (adjusted *p*=1.000) nor between the TN and SS condition (adjusted *p*=1.000).

In the PLP group, RLP intensity was significantly higher in the TN versus the CN (adjusted *p*=0.006) and the SS condition (adjusted *p*=0.008), however, there was no significant difference between the CN and SS condition (adjusted *p*=1.000). In the Non-PLP group, there was neither a significant difference between the TN and CN condition (adjusted *p*=0.831), nor between the TN and SS condition (adjusted *p*=1.000), nor between the CN and SS condition (adjusted *p*=0.332). In the TN (adjusted *p*=0.038) and CN (adjusted *p*=0.016) conditions, RLP ratings were significantly higher in the PLP versus Non-PLP group. In the SS condition, there was no significant difference between the PLP and Non-PLP group (adjusted *p*=0.825).

Table 3 | Changes in PLP and RLP ratings during electrical stimulation at the three body sites.

	Subject	PLP intensity									RLP intensity								
		TN stimulation			SS stimulation			CN stimulation			TN stimulation			SS stimulation			CN stimulation		
		pre	mid	post	pre	mid	post	pre	mid	post	pre	mid	post	pre	mid	post	pre	mid	post
PLP group	PG01	20	65	20	20	35	20	20	10	20	0	0	0	0	0	0	0	0	0
	PG02	0	11	0	0	0	0	7	0	0	0	49	0	0	11	0	0	0	0
	PG03	17	33	30	NA	NA	NA	8	5	8	24	16	4	NA	NA	NA	0	0	0
	PG04	36	81	73	57	55	51	20	20	20	33	84	0	0	0	0	0	0	0
	PG05	4	9	0	0	5	0	0	0	0	46	66	20	26	17	39	0	12	5
	PG06	8	54	4	5	6	11	7	0	5	0	56	6	7	8	3	10	10	3
	PG07	8	8	10	13	25	13	10	14	12	0	0	0	0	0	0	0	0	0
	PG08	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	PG09	15	5	4	6	5	5	7	5	6	5	7	6	6	5	4	7	11	11
	PG10	5	3	2	1	1	1	2	1	1	3	16	7	1	1	1	2	2	2
	PG11	8	26	6	0	18	10	10	0	0	10	44	31	0	0	8	7	0	0
	PG12	9	9	9	21	18	16	8	12	5	20	33	21	8	13	8	8	6	13
	Mean ±SD	10.8 ±10.1	25.4 ±27.1	13.2 ±20.9	11.2 ±17.2	15.3 ±17.5	11.6 ±14.9	8.3 ±6.5	5.6 ±6.9	6.4 ±7.4	11.8 ±15.6	30.9 ±28.7	7.9 ±10.4	4.4 ±7.9	5.0 ±6.3	5.7 ±11.5	2.8 ±3.9	3.4 ±4.9	2.8 ±4.6
Non-PLP group	NG01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	NG02	0	0	0	0	0	0	0	0	0	0	45	17	0	55	0	0	0	0
	NG03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	NG04	0	0	0	0	0	0	0	0	0	0	65	0	0	0	0	0	0	0
	NG05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	NG06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	NG07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	NG08	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
	NG09	0	0	0	0	0	0	0	0	0	0	0	0	0	64	7	0	0	0
	NG10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mean ±SD	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	11.7 ±23.4	1.7 ±5.4	0±0	11.9 ±25.2	0.7 ±2.2	0±0	0±0	0±0

PLP = phantom limb pain; RLP = residual limb pain; PLP group: amputees with chronic PLP; Non-PLP group: amputees without chronic PLP;
TN: truncated median nerve; SS: skin of the residual limb; CN: contralateral intact median nerve;
Ratings were performed using a visual analogue scale (VAS) and converted to a scale from 0 (‘no pain at all in the residual/phantom limb’) to 100 (‘extreme pain in the residual/phantom limb’); NA: not available for the SS stimulation due to time constraints in one participant (PG03).

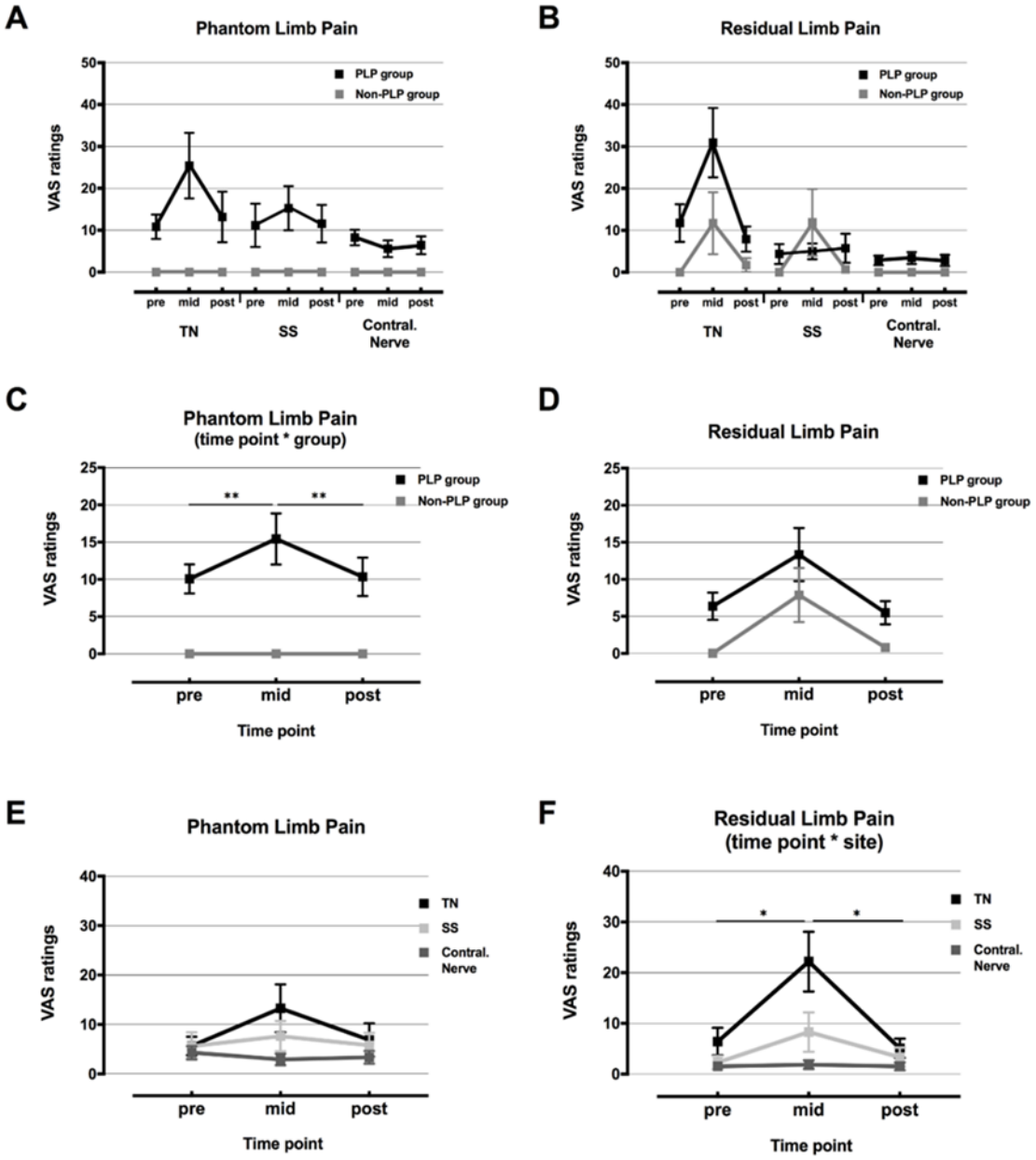


Fig. 3. Phantom limb pain (PLP) and residual limb pain (RLP) intensity before, during and after electrical stimulation. (**A, B**) mean PLP or RLP ratings in all conditions. (**C, D**) Significant time point * group for PLP but not for RLP ratings. Peripheral input increased PLP significantly in amputees with PLP only, but could not elicit any PLP in amputees without a history of PLP, while RLP could be elicited in both groups. (**E, F**) Significant time point * site for RLP but not for PLP ratings. RLP intensity increased significantly during the stimulation of the truncated median

nerve (TN) only, but not of the skin of the residual limb (SS) or the contralateral median nerve (CN). There was no significant change for PLP during the stimulation of each body site in all amputees. pre: before electrical stimulation. mid: during electrical stimulation. post: after electrical stimulation. Contral.: contralateral. Ratings were measured using visual analogue scales (VAS) and converted to a scale ranging from 0 to 100. Data are presented as mean and standard error of the mean. * $p<0.05$, ** $p<0.01$.

3.4 Correlations between SEP amplitudes and pain ratings

During electrical stimulation of the TN, neither the PLP baseline (pre) values nor increases (mid-pre) were significantly correlated with the amplitudes of the peripheral component, the peripheral-spinal/supraspinal component, the subcortical-cortical component, and the S1 component, within the amputees with PLP. This was also true within the amputees with RLP, the amputees with a chronic postamputation pain condition (PLP/RLP), and all amputees (with and without pain). **Table 4** shows the detailed correlation matrix.

However, for RLP changes during electrical stimulation of the TN, there was a significant positive correlation with the amplitudes of the peripheral-spinal/supraspinal-component within the amputees with PLP (*rho*=0.783, *p*=0.003, *n*=12), the amputees with RLP (*rho*=0.656, *p*=0.028, *n*=11), the amputees with chronic postamputation pain (*rho*=0.748, *p*=0.002, *n*=14) and a trend towards significance in all amputees, i.e. those with and without pain (*rho*=0.401, *p*=0.064, *n*=22), suggesting a role of peripherally evoked activity at or near the spinal cord for RLP in chronic pain amputees, including those with PLP, see **Fig. 4**. In addition, the RLP baseline values were significantly correlated with the amplitudes of the spinal component in all amputees, including those with and without pain (*rho*=0.424, *p*=0.049, *n*=22).

Table 4 | Correlations between SEP amplitudes and phantom and residual limb pain

Spearman correlation matrix

		Amputees with PLP (n=12)				Amputees with RLP (n=11)			
		PLP baseline	changes	RLP baseline	changes	PLP baseline	changes	RLP baseline	changes
SEP amplitudes	Periphery	rho= 0.247 p= 0.439	rho= 0.130 p= 0.688	rho= -0.065 p= 0.840	rho= 0.071 p= 0.828	rho= 0.014 p= 0.968	rho= 0.092 p= 0.788	rho= 0.000 p= 1.000	rho= 0.487 p= 0.128
	Spinal/supra spinal	rho= 0.063 p= 0.845	rho= 0.277 p= 0.383	rho= 0.421 p= 0.173	rho= 0.783 ** p= 0.003	rho= 0.184 p= 0.588	rho= 0.349 p= 0.293	rho= 0.330 p= 0.321	rho= 0.656 * p= 0.028
	Subcortical	rho= - 0.307 p=0.332	rho= 0.060 p= 0.854	rho= - 0.051 p= 0.876	rho= 0.176 p= 0.583	rho= 0.092 p= 0.788	rho= 0.183 p= 0.589	rho= 0.191 p= 0.547	rho= 0.282 p= 0.400
	S1	rho= - 0.261 p= 0.413	rho= 0.095 p= 0.770	rho= 0.181 p= 0.573	rho= 0.441 p= 0.151	rho= 0.041 p= 0.904	rho= 0.165 p= 0.627	rho= 0.084 p= 0.807	rho= 0.041 p= 0.905

		Amputees with postamputation pain (PLP/RLP) (n=14)				Amputees with and without pain (n=22)			
		PLP baseline	changes	RLP baseline	changes	PLP baseline	changes	RLP baseline	changes
	Periphery	rho= 0.121 p= 0.681	rho= 0.082 p= 0.780	rho= - 0.120 p= 0.684	rho= 0.218 p= 0.454	rho= 0.206 p= 0.357	rho= 0.189 p= 0.399	rho= 0.016 p= 0.945	rho= 0.233 p= 0.298
	Spinal/supra spinal	rho= 0.252 p= 0.384	rho= 0.365 p= 0.200	rho= 0.472 p= 0.089	rho= 0.748 ** p= 0.002	rho= 0.303 p= 0.170	rho= 0.356 p= 0.104	rho= 0.424 * p= 0.049	rho= 0.401 p= 0.064
	Subcortical	rho= - 0.042 p= 0.886	rho= 0.180 p= 0.538	rho= 0.068 p= 0.817	rho= 0.138 p= 0.638	rho= 0.101 p= 0.654	rho= 0.210 p= 0.347	rho= 0.145 p= 0.518	rho= 0.159 p= 0.480
	S1	rho= - 0.100 p= 0.733	rho= 0.131 p= 0.655	rho= 0.242 p= 0.405	rho= 0.236 p= 0.417	rho= - 0.055 p= 0.807	rho= 0.017 p= 0.940	rho= 0.163 p= 0.470	rho= 0.207 p= 0.356

* $p< 0.05$. ** $p< 0.01$. Application of non-painful electrical stimulation of truncated median nerve at the stump. PLP = phantom limb pain; RLP = residual limb pain.

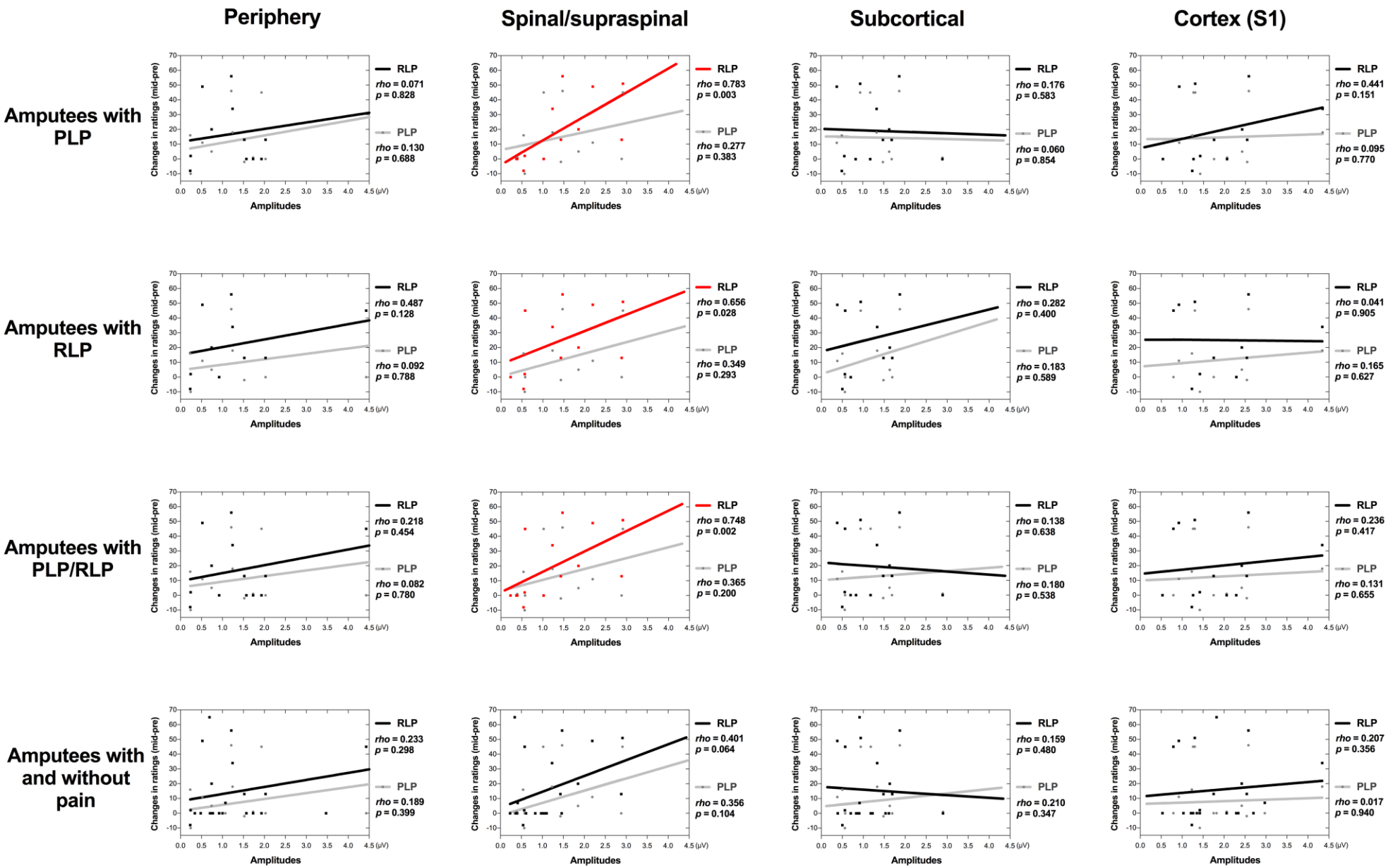

Periphery
Spinal/supraspinal
Subcortical
Cortex (S1)
Amputees with PLP
Amputees with RLP
Amputees with PLP/RLP
Amputees with and without pain
Changes in ratings (mid-pre)
Amplitudes
RLP
PLP
rho = 0.071
p = 0.828
rho = 0.130
p = 0.688
rho = 0.783
p = 0.003
rho = 0.277
p = 0.383
rho = 0.176
p = 0.583
rho = 0.060
p = 0.854
rho = 0.441
p = 0.151
rho = 0.095
p = 0.770
rho = 0.487
p = 0.128
rho = 0.092
p = 0.788
rho = 0.656
p = 0.028
rho = 0.349
p = 0.293
rho = 0.282
p = 0.400
rho = 0.183
p = 0.589
rho = 0.041
p = 0.905
rho = 0.165
p = 0.627
rho = 0.218
p = 0.454
rho = 0.082
p = 0.780
rho = 0.748
p = 0.002
rho = 0.365
p = 0.200
rho = 0.138
p = 0.638
rho = 0.180
p = 0.538
rho = 0.236
p = 0.417
rho = 0.131
p = 0.655
rho = 0.233
p = 0.298
rho = 0.189
p = 0.399
rho = 0.401
p = 0.064
rho = 0.356
p = 0.104
rho = 0.159
p = 0.480
rho = 0.210
p = 0.347
rho = 0.207
p = 0.356
rho = 0.017
p = 0.940

Fig. 4. Correlations between amplitudes of somatosensory evoked potentials (SEPs) and changes in phantom limb pain (PLP) and residual limb pain (RLP). The changes of PLP intensity (grey) induced by peripheral truncated nerve stimulation were not significantly correlated to the amplitudes of ascending barrages from the periphery to the S1 within nor across amputees with chronic PLP. In amputees with PLP, the peripherally evoked potentials at or near the spinal cord were significantly positively associated with evoked RLP (*rho*=0.783, *p*=0.003), but not PLP (*rho*=0.277, *p*=0.383). This was also true for amputees with RLP, as well as all amputees with postamputation pain (PLP/RLP).

4. Discussion

Our results show that non-painful peripheral input can evoke PLP only in amputees with chronic PLP, but not in amputees without a history of PLP. Although we could elicit PLP by peripheral stimulation at the residual limb, we found no significant group differences for the amplitudes of the SEP components from the periphery to the cortex between amputees with and without PLP during stimulation at the truncated nerve, the skin and a contralateral control site. We also did not observe a significant association between the somatosensory potentials and the changes in PLP or the baseline intensities. These data suggest that the increase in PLP during nerve stimulation is related to a referral of sensation rather than increased input to the higher processing areas. In addition, our results showed that the peripherally induced potentials at or near the spinal cord were significantly positively associated with changes in RLP, but not with PLP. Altogether these findings suggest that peripheral input can modulate PLP intensity in amputees who already suffer from PLP but may be insufficient to create PLP in amputees without a history of PLP, implying that sensitized central processing or multifactorial alterations may contribute to PLP generation.

Comparable somatosensory function between amputees with and without PLP

We found no significant difference between amputees with and without PLP for all SEP components from the periphery into the S1, stimulated either at the truncated nerve, the skin or the contralateral control site. Therefore, the absence of evoked PLP in the Non-PLP group cannot be explained by impaired afferent fibers since their SEPs

were not significantly different from those of the PLP group. Neither the magnitude of peripheral input nor the peripherally induced S1 response seems to be crucial for PLP generation. A few studies have monitored the ascending neuronal barrage from the periphery to the cortex in human amputees. Mackert et al. (2003) quantified peripheral and cortical SEPs in twelve upper-limb amputees without painful phantom sensations using transcutaneous electrical nerve stimulation in two sessions of 500 repetitions at 0.9 Hz and 9.1 Hz (Mackert et al., 2003). They showed that cortical responses (N20/P25) during TN stimulation were significantly higher than during SS stimulation at the frequency of 0.9 Hz but not 9.1 Hz, suggesting that the deafferented cortex remains responsive to peripheral input in amputees and allowing for a potential peripheral generator of phantom phenomena including PLP. However, this study did not examine amputees with PLP, and did not assess the intensity of phantom sensations related to the stimulation. Here, we quantified and compared SEP components in amputees with and without PLP. We used strong but non-painful peripheral input at a frequency of 3 Hz and applied 3000 repetitions in each condition for peripheral, peripheral-spinal/supraspinal, subcortical-cortical and cortical components. We did not observe significant higher cortical (S1) potentials during TN stimulation than SS stimulation using a frequency of 3 Hz. This significance at S1 might only be present at very low frequency stimulation (such as 0.9 Hz in Mackert et al. but not 9.1 Hz) because of cortical inhibition/refractoriness with increasing stimulation frequency. Remarkably, we observed a significant increase of potentials for the peripheral-spinal/supraspinal component during the TN stimulation compared to the SS stimulation, which has not been measured in their previous work, showing more input induced by the nerve stimulation than the skin stimulation, but it did also not differ significantly between amputees with and without PLP in each condition.

PLP and RLP induced by non-painful peripheral input

In line with a previous study (Andoh et al., 2017), we could evoke phantom sensations and even PLP by non-painful electrical stimulation at the residual limb in amputees with chronic PLP. Such changes of PLP showed a peripheral contribution to phantom phenomena. PLP has been shown to be temporally reduced after blocking

peripheral inputs by injecting lidocaine in amputees with PLP. However, in these studies it was difficult to determine whether the afferent input can create PLP independently and impossible to disentangle the later involvement of central processing from the input, which might be important for the PLP generation. In this study, we examined the bottom-up processes and used a more homogenous sample of amputees, all with traumatic amputations. The amputees with PLP showed a long history of PLP (28 ± 12 years) and the controls consisted of amputees who never experienced PLP and were matched for time since amputation and age. We also used non-verbal ratings (VAS) to monitor changes in PLP and RLP intensities. This permits a better characterization of changes in PLP without the potential bias from the examiner or confounds from other co-existing painful sensory phenomena, such as RLP (Jensen, Krebs, Nielsen, & Rasmussen, 1985).

Although peripheral input from the truncated nerve could increase PLP intensity temporarily in the majority (seven out of twelve) of the amputees with chronic PLP, it was not sufficient in generating PLP in any amputee without a history of PLP, suggesting that peripheral input itself is not sufficient to induce PLP but can modulate existing PLP. The generation of PLP possibly still requires later sensitized central processing or relevant functional alterations. The absence of PLP in the Non-PLP group cannot be explained by significant differences in peripheral stimulation since there was neither a significant difference in sensory or pain thresholds nor in stimulation intensity or SEP magnitude between both groups.

In addition, we found a significant body site effect for RLP but not for PLP, and RLP intensity increased significantly after the application of non-painful peripheral stimulation at the TN site only and decreased directly after the cessation of the TN stimulation, in all amputees with postamputation pain. In line with this, significantly higher SEPs at or near spinal segment were induced in the TN condition versus the SS condition. Our results are in line with one study (Haroutounian et al., 2014), who reported that a peripheral anesthetic nerve block with lidocaine induced complete pain relief in seven patients with unilateral peripheral nerve injury and seven patients with bilateral pain in the feet with distal symmetric polyneuropathy, while intravenous lidocaine infusion had only a small effect on the latter group. Their findings suggest that spontaneous chronic neuropathic

pain induced by peripheral nerve injury may be maintained by peripheral afferent input. Our findings support a role of peripheral input in generating and maintaining chronic RLP. These data also support the possible putative different etiologies of PLP and RLP, with PLP possibly depending on both peripheral and central processes and RLP mainly depending on peripheral input. This might also explain the limited efficiency in treating and preventing PLP using peripheral nerve block only (Borghi, D'Addabbo & Borghi, 2014; Pinzur, Garla, Pluth & Vrbos, 1996).

Correlations between SEP amplitudes and phantom and residual limb pain

We did not observe a significant relationship between PLP baseline or PLP change and the amplitudes of the SEP components. However, we found that evoked spinal/supraspinal potentials after transmission from the first order neurons in the DRG (Cruccu et al., 2008; Seyal & Gabor, 1987) were significantly positively correlated with RLP, but not with PLP. The significant relationship was stable and remained after re-referencing and re-calculating the cervical electrode to either a scalp or anterior cervical site (see supplementary material). This positive correlation suggests a more important role of peripheral input for RLP than for PLP in amputees with chronic PLP. The spinal/supraspinal excitability evoked by afferent input might play an important role in maintaining spontaneous RLP but not PLP.

Peripheral afferent input can hence modulate PLP, but seems insufficient to create PLP. There might be constant barrages of residual limb muscle spindles, injured touch and warm/cold fibers as well as ectopic spontaneous discharge in A-fiber afferents, which may transmit impulses from the periphery to the 'deafferented' cortex and modulate chronic postamputation PLP. However, such normally not painful afferent inputs might still need some other prerequisites such as sensitized central processing or relevant functional alterations to generate PLP.

Limitations

This study has several limitations. Although we used 3000 repetitions at each body site as recommended in the literature (Cruccu et al., 2008), we did not elicit significant and reliable SEPs in all amputees from all stimulated sites (Mackert et al., 2003). We

were not able to identify the activity triggered by the ascending volley using traditional time-frequency analysis methods such as gamma oscillations because of the fast stimulation rate and short time window, which may possibly relate to PLP. Finally, we did not test for neuromas and could thus not examine if these relationships differ in patients with versus without neuroma.

Conclusion

Primary afferent input can modulate but may not be sufficient to cause PLP, since PLP could be enhanced by peripheral input in amputees with PLP but not in those without a history of PLP. Moreover, there was no significant difference in amplitude of somatosensory input between amputees with and without PLP. Peripherally evoked spinal/supraspinal potentials were positively associated with evoked RLP, but not PLP, in amputees who have postamputation pain including those with PLP. These findings indicate that PLP and RLP may be mediated by different mechanisms, with peripheral input having a dominant role for chronic RLP, but not sufficient for the PLP generation. Longitudinal studies are needed to further disentangle the role of peripheral and central interaction for chronic PLP.

Abbreviations: Dorsal root ganglia (DRG), Phantom limb pain (PLP), Residual limb pain (RLP), Somatosensory evoked potentials (SEP), Visual analog scale (VAS), S1: primary somatosensory cortex, TN: truncated median nerve, CN: contralateral intact median nerve, SS: skin of the residual limb.

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Supplementary material

H. Liu, J. Andoh, Y. Lyu, C. Milde, S. Desch, F. Zidda, M. Schmelz, G. Curio, H. Flor. *"Correlations between spinal SEP amplitudes and phantom and residual limb pain"*

Spinal SEPs

	Amputees with PLP (n=12)				Amputees with RLP (n=11)			
	PLP baseline	**changes**	**RLP baseline**	**changes**	**PLP baseline**	**changes**	**RLP baseline**	**changes**
Cv6-Fz	rho= 0.063 p= 0.845	rho= 0.277 p= 0.383	rho= 0.421 p= 0.173	rho= 0.783 ** p= 0.003	rho= 0.184 p= 0.588	rho= 0.349 p= 0.293	rho= 0.330 p= 0.321	rho= 0.656 * p= 0.028
Cv6-AC	rho= 0.110 p= 0.733	rho= 0.230 p= 0.473	rho= 0.482 p= 0.113	rho= 0.668 * p= 0.018	rho= 0.214 p= 0.527	rho= 0.244 p= 0.470	rho= 0.478 p= 0.137	rho= 0.482 p= 0.134

	Amputees with postamputation pain (PLP/RLP) (n=14)				Amputees with and without pain (n=22)			
	PLP baseline	**changes**	**RLP baseline**	**changes**	**PLP baseline**	**changes**	**RLP baseline**	**changes**
Cv6-Fz	rho= 0.252 p= 0.384	rho= 0.365 p= 0.200	rho= 0.472 p= 0.089	rho= 0.748 ** p= 0.002	rho= 0.303 p= 0.170	rho= 0.356 p= 0.104	rho= 0.424 * p= 0.049	rho= 0.401 p= 0.064
Cv6-AC	rho= 0.316 p= 0.271	rho= 0.284 p= 0.325	rho= 0.502 p= 0.067	rho= 0.606 * p= 0.022	rho= 0.348 p= 0.112	rho= 0.263 p= 0.236	rho= 0.438 * p= 0.041	rho= 0.428 * p= 0.047

* $p< 0.05$. ** $p< 0.01$.

Application of non-painful electrical stimulation of truncated median nerve at the residual limb.

Spinal component was re-referenced and re-calculated to a scalp electrode (Fz) and anterior cervical site (AC) over the larynx/ thyroid cartilage, and both showed the same trend associated with SEP amplitudes with evoked residual limb pain (RLP), but not phantom limb pain (PLP).

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4. General discussion

The present study provides new information about the recurring question on what is the role of peripheral input in PLP, i.e. whether peripheral input can generate PLP independently. Two previous studies by Vaso et al (2014) and Buch et al (2019) have shown that peripheral afferent input plays a role in maintaining PLP, and PLP could be decreased significantly by blocking the DRG or peripheral nerves. However, these studies could not determine whether the peripheral afferent input can generate PLP and may not disentangle the role of peripheral input and the possible involvement of later central processing from the afferent input, which might be an independent and important factor for PLP generation. In line with these findings, the present study showed a significant increase of both PLP and RLP after inducing peripheral input at the residual limb. However, peripheral input could not cause PLP in amputees without a history of PLP, although they had comparable ascending input from the periphery and cortical responses to amputees with PLP. In addition, the significant correlation between the magnitude of the afferent input through spinal cord and changes in RLP was observed, but not with evoked PLP in amputees with chronic postamputation pain. Peripheral afferent input can modulate PLP, but it does not seem to be sufficient to generate PLP.

4.1 The contribution of peripheral afferent fibers to chronic postamputation pain and central sensitization

Peripheral discharge from the ectopic axons or sensory neurons may play an important role in arising neuropathic pain including PLP, and may be a inducer of central sensitization (Haroutounian et al., 2014; Vaso et al., 2014; Woolf, 2011). The role of different types of afferent fibers and how they contribute to chronic pain and central processing and plastic changes is not completely understood (Devor, 2009; Djouhri & Lawson, 2004). Nociceptors include C or Aδ fibers, whereas low-threshold Aα/ß fibers convey non-painful touch and vibration sense but not pain. In line with this, the small bulk of ectopia from hyperexcitable Aδ and C fibers seem to be involved in chronic PLP processing while activities from A group fibers may be relegated to non-painful phantom phenomena. However, it was shown in neuropathy models in animals with a peripheral injury that most ectopic discharges are generated from the large

diameter myelinated Aß afferents. Some investigators proposed that such massive ectopic barrages from A group fibers might be overlooked in patients with chronic pain (Devor, 2009). Ectopic discharge in Aß afferents was supposed to be a primary driver of chronic neuropathic pain as well. After nerve injury and amputation, changes occur in the peripheral fibers (such as neuromas) and neurons (such as sensory DRG). Phenotypic alterations of DRG cells may cause A fiber afferents to provoke painful sensations and activate central network processing (Weissner, Winterson, Stuart-Tilley, Devor & Bove, 2006). Once triggered by early spontaneous activities from the periphery, sensitized central progressing may be activated and possibly become independent and autonomous in neuropathic pain (Devor, 2009; Xie, Strong, Meij, Zhang & Yu, 2005). The development of centralized pain processing may also need many later changes occurring in the both central (brain and spinal cord) and peripheral neurons (DRG). In the present study, A group fibers were activated by powerful but non-painful electrical stimulation and this led to a significant increase in both PLP and RLP intensity. In addition, evoked SEP amplitudes were significantly positively associated with evoked RLP, suggesting ectopic afferents from Aß fibers may also contribute to nociceptive processing. Despite a few Aδ or C fibers could possibly be activated by the strong electrical stimulation in this study and induce pain processing, which was slower than Aß fibers and not be responded accordingly by short-latency SEPs, however, there was still no PLP at all in any amputee without a history of PLP, during or after the entire peripheral electrical stimulation period and the pain thresholds were comparable across groups before the measurements. Multiple lines of evidence imply that the generation of PLP may still be determined by other prerequisites, such as central processing or central network changes and not only by the afferent input itself. Peripheral input from the truncated nerves could induce cortical reorganization and modulate/increase PLP intensity, thus it has a role in PLP, especially for severe PLP episodes. In line with this, our findings suggest that PLP may be related to an interaction between both central and peripheral processes.

4.2 Clinical importance of the present results

A majority of amputees suffer from post-amputation pain including chronic PLP and RLP, even a long period of time after amputation (Carlen et al., 1978; Kooijman et al., 2000). PLP can devastate the life of amputees and mechanism-based treatments for PLP are still lacking. The present study has shown that peripheral input can modulate or enhance PLP intensity in amputees who already have chronic PLP and sensitized central processing might be needed for PLP generation. Although the interaction between peripheral input and central processing in PLP is not completely understood, the results support the application of novel anesthetic modalities, which could be an option to attenuate severe PLP symptoms in which peripheral input may contribute.

In addition, it might be important to investigate how to prevent early peripheral ectopia and the activation or development of sensitized central processing of PLP. Effective pre-emptive analgesia of spontaneous afferent activity may be helpful because the sensitized central processing for chronic neuropathic pain may be triggered by early peripheral activities, perhaps within several days after periphery injury (Xie et al., 2005), or even long before the amputation in patients who already have chronic pain, due to the more promising role of pre-amputation pain experience in postamputation pain (Larbig et al., 2019). Once the interaction between peripheral and central mechanisms and induced central processing has been established, PLP may be more likely to become chronicity and it may be more difficult to abolish using peripheral nerve blockade only. In amputees with chronic PLP, a combination of long-term interventions that target peripheral ectopic input and new drugs or behavioral/stimulation interventions that target the enhanced central activity might be promising approaches. In addition, abnormal DRG and spinal cord activity may play an important role in pain processing and modulation, especially for RLP. Spinal-subcortical mechanisms and peripheral hyperexcitability might be potential targets for RLP attenuation.

4.3 Outlook

Previous studies using peripheral blockade of the afferent input using lidocaine in amputees with PLP showed a reduction of PLP, which highlights that peripheral input can modulate PLP. Our current findings are in accordance with these studies and show that although peripheral input may play an important role for the PLP experience, it is by

itself not sufficient to induce PLP. There might be other prerequisites for the generation of PLP, such as sensitized central processing in PLP. More direct evidence for the role of sensitized central processing for PLP generation is needed.

Current therapeutic approaches for PLP using, for example, DRG or spinal anesthesia need better blinded randomized controlled trials with a full placebo and longer follow-ups. It is, however, still unclear what the short- and long-term influence of ectopic input in central changes and PLP following amputation. Prospective and longitudinal studies are needed to further disentangle the role of peripheral and central mechanisms to prevent and treat PLP. In addition, pre- and post-amputation factors may be worthwhile to examine in amputees with and without PLP to achieve a better understanding of underlying mechanisms. Moreover, this study also shows that PLP and RLP may be mediated by different central processes. Although we tried to disentangle processes underlying PLP and RLP, we cannot rule out that processes might be interacting, since most amputees reported both PLP and RLP. It may be worthwhile to further investigate the ascending pathways and types of peripheral afferent fibers that modulate RLP and PLP. Except for SEPs, laser-evoked potentials (C fibers) and quantitative sensory testing may provide additional reliable neurophysiological markers for these contributing variables.

5. Summary

The precise etiology of PLP remains unknown and both peripheral and central factors have been associated with PLP. The present thesis aimed at identifying whether peripheral afferent input can create PLP and how it interacts with central processing and postamputation pain (RLP/PLP). Using transcutaneous electrical nerve stimulation on the truncated median nerve, the skin of the residual limb and the contralateral homologous nerve in the intact limb, the somatosensory function and volley from the truncated nerves to the S1 were examined in traumatic unilateral upper-limb amputees with PLP (n=12) and without PLP (n=10). Ascending neuronal barrage through the brachial plexus, the spinal cord, the brainstem, and the thalamus up to the primary somatosensory cortex was monitored using somatosensory event-related potentials.

Changes in PLP and RLP were assessed before, during and after non-painful electrical stimulation. The results showed that peripheral afferent input could evoke PLP in most amputees with chronic PLP (7/12), but not in amputees without a history of PLP (0/10). The amplitudes of the somatosensory components were comparable in amputees with and without PLP and both groups reported vivid phantom sensations. In addition, peripherally induced potentials through the spinal segment were significantly positively associated with evoked RLP, but not with PLP, in amputees with postamputation PLP/RLP, indicating that spinal/spraspinal hyperexcitability might be an important mechanism in maintaining RLP but not PLP. This study suggests that PLP and RLP are mediated by different mechanisms. The mechanisms of PLP are multifactorial and complex. Peripheral input can therefore enhance PLP, but may not be sufficient to cause PLP by itself.

6. References (Introduction and General discussion)

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7. Curriculum vitae

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