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SPECIAL TOPIC
Peripheral Nerve

Pathophysiological and Neuroplastic Changes in Postamputation and Neuropathic Pain: Review of the Literature

Christopher J. Issa, BS*
Shelby R. Svientek, MD†
Amir Dehdashtian, MD†
Paul S. Cederna, MD†‡
Stephen W. P. Kemp, PhD†‡

Background: Despite advancements in surgical and rehabilitation strategies, extremity amputations are frequently associated with disability, phantom limb sensations, and chronic pain. Investigation into potential treatment modalities has focused on the pathophysiological changes in both the peripheral and central nervous systems to better understand the underlying mechanism in the development of chronic pain in persons with amputations.

Methods: Presented in this article is a discussion outlining the physiological changes that occur in the peripheral and central nervous systems following amputation. In this review, the authors examine the molecular and neuroplastic changes occurring in the nervous system, as well as the state-of-the-art treatment to help reduce the development of postamputation pain.

Results: This review summarizes the current literature regarding neurological changes following amputation. Development of both central sensitization and neuronal remodeling in the spinal cord and cerebral cortex allows for the development of neuropathic and phantom limb pain postamputation. Recently developed treatments targeting these pathophysiological changes have enabled a reduction in the severity of pain; however, complete resolution remains elusive.

Conclusions: Changes in the peripheral and central nervous systems following amputation should not be viewed as separate pathologies, but rather two interdependent mechanisms that underlie the development of pathological pain. A better understanding of the physiological changes following amputation will allow for improvements in therapeutic treatments to minimize pathological pain caused by amputation. *(Plast Reconstr Surg Glob Open 2022;10:e4549; doi: 10.1097/GOX.0000000000004549; Published online 28 September 2022.)*

INTRODUCTION

Approximately one in 190 Americans are currently living with an amputation, which often leads to debilitating pain and chronic disability.[1,2] Patients with amputations often experience intense, pathological pain that can be neuropathic in nature or can occur secondarily to neuromas and phantom limb pain (pain in the absent extremity).[3–5] The pathophysiology of amputation-induced pain is not yet fully understood; however, multiple factors play a key role in its development that include changes in both the peripheral and central nervous systems. Given the higher risk of anxiety and depression in this population,[6,7] effective treatment is, therefore, imperative for both physical health and mental health. Understanding the pathophysiology underlying chronic pain after amputation will enable clinicians to better tailor more individualized treatments.

The following review summarizes the peripheral molecular changes and the central nervous system adaptations that take place following amputation, leading to the pathological pain commonly experienced by patients with amputations. Furthermore, this review will discuss the current state-of-the-art treatments and the potential for novel treatment strategies to alleviate pain experienced after amputation.

*From the *Oakland University William Beaumont School of Medicine, Auburn Hills, Mich.; †Department of Surgery, Section of Plastic Surgery, University of Michigan, Ann Arbor, Mich.; and ‡Department of Biomedical Engineering, University of Michigan, Ann Arbor, Mich.*

Received for publication February 1, 2022; accepted August 5, 2022.

DOI: 10.1097/GOX.0000000000004549

Disclosure: *The authors have no financial interest to declare in relation to the content of this article.*

PERIPHERAL AND CENTRAL MOLECULAR CHANGES FOLLOWING AMPUTATION

Sodium Channels

Understanding the molecular physiology of neuroplastic changes is the basis for today's pharmaceutical approach to pain management in persons with amputations.[8,9] Medications such as gabapentin and pregabalin—the first-line treatment for neuropathic pain—take advantage of the peripheral and the central molecular physiology responsible for pathological pain states.[10] Specifically, one-mechanism drugs like the gabapentinoids reduce neuropathic pain by decreasing ectopic firing in injured peripheral nerves.[11] This addresses a common pathological change seen in chronic pain development.[11]

Peripheral nervous system changes following amputation play a key role in the development of postamputation pain, specifically through changes in excitability in the dorsal root ganglia (DRG).[12] The DRG transmit sensory afferent signals from the periphery to the central nervous system,[13,14] and following amputation, DRGs can develop continuous ectopic firing due to increased excitability.[15–17] This ectopic firing of the DRG results in activation of the central nociceptive pathway in the absence of stimulus, generating an aberrant spontaneous pain sensation.[18] The increase in neuronal stimulation also triggers the physiological response of central sensitization of the spinal cord,[18] which is the activity-dependent increase in neuronal reactivity due to neuroplastic changes and decreased nociceptive threshold.[19] This results in the feeling of allodynia (the experience of pain from a nonnoxious stimulus[20]) and hyperalgesia (an increased pain response from a normal painful stimulus[20]) that is common among patients who have neuropathic pain.[19] Through similar mechanisms, ectopic firing also contributes to the development of neuroma and phantom limb pain, thus making the pathophysiology behind spontaneous neuronal activity a main target in chronic pain therapy.[15,21] The exact mechanism for development and maintenance of chronic ectopic firing is not yet fully understood, but voltage gated sodium channels have been shown to play a vital role.[15]

Voltage-gated sodium (Na_V) channels are predominantly responsible for maintaining control of both neuronal excitability and ectopic firing.[22] Sensory neurons have varying expressions of sodium channels, with the small diameter c-fiber nociceptive afferents expressing mostly $Na_V1.1$, $Na_V1.6$, $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$.[23] In particular, $Na_V1.7$ has been described as an essential pain receptor, as patients presenting with a loss of function mutation are completely insensitive to pain.[24,25] Experiments blocking $Na_v1.7$ in transected spinal nerves of rats resulted in a significant reduction in allodynia, further supporting their role in pathological pain.[26,27] Additionally, $Na_v1.7$ has been shown to predominate within neuromas,[28] suggesting that increases in this nociceptive receptor could play a role in the development of neuroma-associated pain. One potential mechanism leading to the accumulation of these receptors within neuromas is through membrane remodeling following axotomy.[29] During axotomy, demyelination and membrane remodeling results in a disturbance of sodium channel cluster formation, subsequently leading to a large accumulation of sodium channel receptors in neuroma endbulbs.[16,29] Thus, identification of $Na_v1.7$ within neuroma endbulbs can provide a mechanism for targeted pharmacological therapy.

The changes in specific Na_V channel expression following amputation remain a topic of further investigation; however, thus far, it is clear that sodium channels as a whole play a role in DRG excitability and pathological pain.[30] Application of sodium channel blockers, such as lidocaine and amitriptyline, has been shown to be effective in the suppression of ectopic firing in the DRG.[30–32] In addition, a study performed on patients with chronic pain secondary to diabetic neuropathy showed significant relief of pain for up to 21 days following intravenous lidocaine infusion, further supporting sodium channel antagonization as a potential therapeutic target.[32]

Brain-derived Neurotrophic Factor and N-Methyl-D-Aspartic Receptors

Numerous neurotrophic factors are involved in the development of pathological pain following peripheral nerve injury, but brain-derived neurotrophic factor (BDNF) has been repeatedly identified as a primary pain modulator.[33] Clinical studies have shown that patients with diabetic neuropathy had elevated serum BDNF levels compared with diabetics without neuropathy, with serum levels positively correlating with the severity of pain.[34] The pathological effects of BDNF have also been implicated in the development of postamputation pain, garnering interest into understanding the molecular physiology of this neurotrophic factor to better allow for more targeted therapy for pain relief.[35]

Following axotomy, sensory neurons increase expression of BDNF in the DRG, which subsequently undergoes anterograde transport to the dorsal horn of the spinal cord.[35–37] Once in the dorsal horn, BDNF binds to tropomyosin receptor kinase B receptors on the interneurons in the spinal laminae, leading to the phosphorylation and potentiation of excitatory N-methyl-D-aspartic acid (NMDA) receptors.[35,38] NMDA receptor potentiation permits stronger responses

Takeaways

Question: What are the physiological changes that occur in the peripheral and central nervous system following amputation?

Findings: The development of both central sensitization and maladaptive neuronal remodeling in the spinal cord and cerebral cortex allows for the development of neuropathic and phantom limb pain following amputation.

Meaning: A better understanding of the pathophysiological changes following amputation will allow for improvements in therapeutic treatments to minimize pain in persons with amputation.

from nociceptive c-fibers, leading to the subsequent development of central sensitization following continuous nociceptive stimulation—such as from the ectopic firing discussed previously.[33,39] Moreover, since axotomy induces an increase in tropomyosin receptor kinase B receptors in the dorsal horn, this allows for BDNF excitatory effects to be amplified through a positive feedback loop, further increasing the excitation and potentiation of NMDA receptors along with the sensitization of the spinal cord.[35] The aforementioned excitatory effects of BDNF have been supported by several animal studies; in one study conducted in rats, a spinal infusion of BDNF significantly enhanced nociceptive response and NMDA depolarizations.[40] Respectively, electrophysiological testing also demonstrated that BDNF-induced potentiation was blocked by the NMDA receptor antagonist D-2-amino-5-phosphonovalerate.[41]

BDNF has been shown to play an important role in the development of neuropathic pain in rodents.[42] Following axotomy, adult rats showed a significant increase in BDNF expression and subsequent development of mechanical allodynia.[43] Additionally, intrathecal injection of anti-BDNF antibody in rats with spinal nerve ligation resulted in a reduction in mechanical allodynia.[33] However, although strong evidence supports BDNF's role in pain sensitization in the spinal cord, some studies have shown that BDNF injections in the brainstem of rats have antinociceptive effects through mechanisms related to serotonin release from the raphe nuclei.[44–46] Hence, BDNF possesses location-dependent physiological effects in the peripheral and central nervous systems.

Adenosine Triphosphate and Microglia

Adenosine triphosphate has been shown to contribute to the development of pathological pain through the activation of purinergic receptors on microglia adjacent to the dorsal horn.[47,48] More specifically, P2X4 purinergic receptors have been implicated in this process.[47,48] Following peripheral nerve injury, release of adenosine triphosphate binds to the upregulated P2X4 receptors, leading to the release of BDNF into the dorsal horn causing two main central nervous system effects.[49,50] First, BDNF enhances NMDA activation through mechanisms discussed previously, increasing neuronal hyperexcitability.[49] The second effect is a depolarizing shift in the anion gradient of spinal interneurons causing GABA responses to be depolarizing instead of hyperpolarizing, nullifying the inhibitory responses of the dorsal horn.[50] As a result of the BDNF-induced changes, spinal interneurons gain the ability to transmit low-threshold mechanical stimuli, increasing discharge and spontaneous activity.[50]

Interestingly, experimentation with mice has shown that microglia proliferation occurs in both males and females; however, P2X4 receptor upregulation only occurs in males.[49,51] This sexual dimorphism was further supported by experiments in which blockage of the P2X4 receptor only reversed tactile allodynia in male rats with injured peripheral nerves.[52] In females specifically, the potential mechanism underlying neuropathic pain is through activated T cells.[51] Although the exact mechanism is still unknown, peroxisome proliferator-activated receptor γ on T cells has been shown to play a role.[53] Despite the sexual dimorphism exhibited in the etiology of some pain pathologies, experiments conducted on mice showed an eventual convergence in pain pathways for both sexes at NMDA receptors.[49] Thus, enhancement of NMDA receptors is a common factor seen in both males and females.[49] This was further supported by a study in which antagonizing NMDA receptor activity in mice alleviated pain hypersensitivity in both males and females.[49] Respectively, a clinical study conducted on the effects of ketamine, a NMDA receptor antagonist, showed there was no significant difference in analgesic effects at equivalent doses between male and female patients.[54] Thus, although P2X4 receptor is a potential target for drug development, given the sexual dimorphism that exists, efforts may be better spent targeting NMDA receptors due to the potential to benefit both sexes (Fig. 1).

CENTRAL SENSITIZATION FOLLOWING AMPUTATION

Central sensitization is responsible for many of the pain sensitivity changes seen in persistent pathological pain.[19] The peripheral and central molecular mechanisms discussed previously work synergistically to induce the formation of sensitization.[19] The purpose of nociceptive sensitization is to protect an injured organism from further injury,[55] but with chronic pain, the pain continues long after the initial injury.[56] The pathological process of central sensitization is based on the mechanism of recruiting new inputs to the nociceptive pathway, such as low threshold mechanoreceptor (Aβ) fibers, resulting in hypersensitivity and allodynia.[19] This occurs due to constant peripheral input to the nociceptive c-fibers—such as after amputation—resulting in recurrent activation of NMDA receptors and subsequent spinal long-term potentiation (LTP).[19,57] Spinal LTP is a synaptic strengthening caused by heterosynaptic facilitation (recruitment of nonnociceptive, low-threshold inputs) and homosynaptic facilitation (use-dependent facilitation of a synapse due to stimulation) due to repetitive neuronal stimulation.[19,57] The development of LTP, along with the enhancement of NMDA receptor responses and decreased GABA inhibition, leads to sensitization that is seen in many pathological pain states.[19]

NMDA receptors in the dorsal horn play a fundamental role in the development of central sensitization.[58] The increased activation of NMDA receptors, along with the neuroplastic changes occurring at the spinal cord, is one of the proposed methods for the neuropathic changes that play a pivotal role in chronic and phantom limb pain following amputation.[4] Studies conducted on rats showed that NMDA receptor antagonists dizocilpine and 3((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid were able to prevent and reverse central sensitization, supporting NMDA receptor's role in pain pathogenesis and potential for therapeutic capabilities.[59]

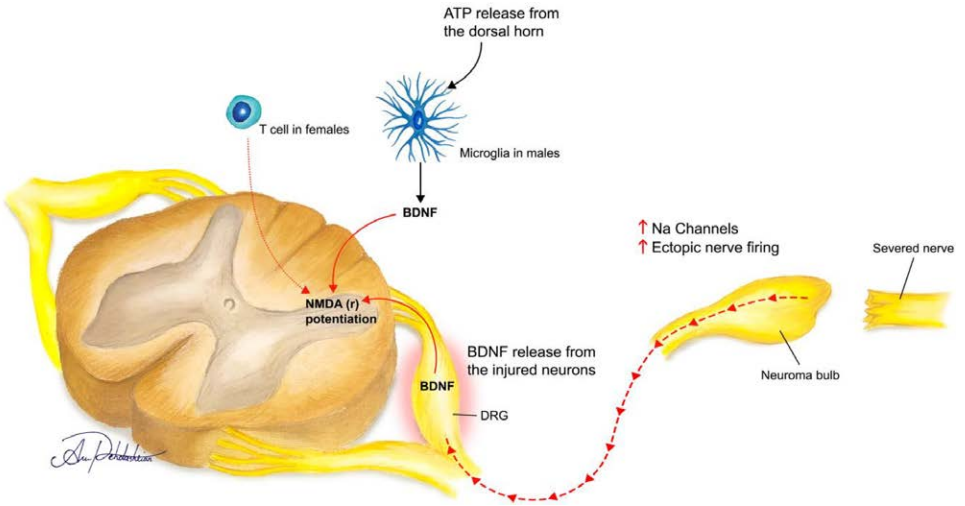

Fig. 1. Schematic illustration of the different mechanisms that occur following nerve amputation that plays a role in the development of pathological pain. The three main pathological changes following nerve amputation include an increase in Na_v channel expression, upregulation of BDNF production in the DRG, and proliferation of microglia and T-cells. The increase in sodium channels allows for the ectopic firing seen in axotomized nerves, whereas the upregulation of BDNF enhances NMDA receptors in the spinal cord. Additionally, the proliferation of microglia and T-cells further potentiate NMDA receptors in the spinal cord through BDNF release (in males) and inflammatory cytokines (in women), respectively.

NONMOLECULAR MECHANISMS OF PAIN

Cerebral Cortex Changes

One of the primary complications of amputation is the reorganization (remapping) of the somatosensory and motor cortices.[60] The principal concept underlying cortical reorganization is the invasion of neighboring neurons into the deafferented cortex, resulting in the deprived sensorimotor modality being replaced by another.[60] For instance, cortical areas of the lip can invade areas previously occupied by the hand in an upper limb amputee.[60] Studies have successfully elicited phantom limb sensations in the now-absent extremity provoked by stimulation of the mouth area.[61] Similarly, functional magnetic resonance imaging (fMRI) studies have also shown an invasion of neighboring neurons in the deafferented cortex of patients with lower limb amputations[60] (Fig. 2).

The relationship between phantom limb pain and cortical reorganization has been well documented[62]; for example, some studies involving upper limb amputees have showed a positive correlation between the magnitude of somatosensory and motor cortex reorganization and phantom limb pain.[61,63,64] Along those lines, a study conducted on a patient with an upper extremity amputation who underwent a slow (1 Hz) repetitive transcranial magnetic stimulation on the primary sensory cortex showed significant reduction in phantom pain.[65] Thus, by attenuating aberrant cortical activity in the somatosensory cortex, treatment for phantom limb pain was achieved.[65] Additionally, a study conducted on patients with upper and lower limb amputations with phantom pain showed significant benefits with transcranial direct current stimulation of the motor cortex.[66] By stimulating the deafferented motor cortex, the reduction in phantom limb pain is believed to be due to the reactivation of the cortical representation of the amputated limb, therefore, reducing the maladaptive plasticity associated with the lack of motor input.[66] However, in contrast to these studies, other studies have also shown that phantom limb pain is associated with activation of preserved cortical structure representing the former amputated limb.[67] Even though these two cortical phenomena are not mutually exclusive, the exact cortex adaptation responsible for phantom limb pain is still in question.

The majority of research regarding phantom limb pain analyzes patients with traumatic amputation, but few focus on the central nervous system changes of congenital amputees (children born without all or part of a limb). Prevailing historical dogma for these patients has denied the existence of phantom limb pain, as consistent peripheral input is necessary for the cortex to develop somatosensory representation of the limb.[68] A major study from 1998 showed that it was rare for congenital amputees to experience phantom limb pain and sensation and that cortical reorganization was low and similar to traumatic amputees without phantom pain.[69] However, several case studies have shown that congenital amputees can have vivid phantom sensations and pain starting at a young age.[70–72] As such, humans may have an innate sensation of limbs that is genetically determined, along with experiential factors that play a part in the development of limb sensation.[73] Thus, the development of phantom limb pain may also possess both a genetic and an environmental component.

Changes in Spinal Cord and Peripheral Neurons following Amputation

Reinnervation of motor neurons following amputation is a fundamental adaptation that occurs in the peripheral

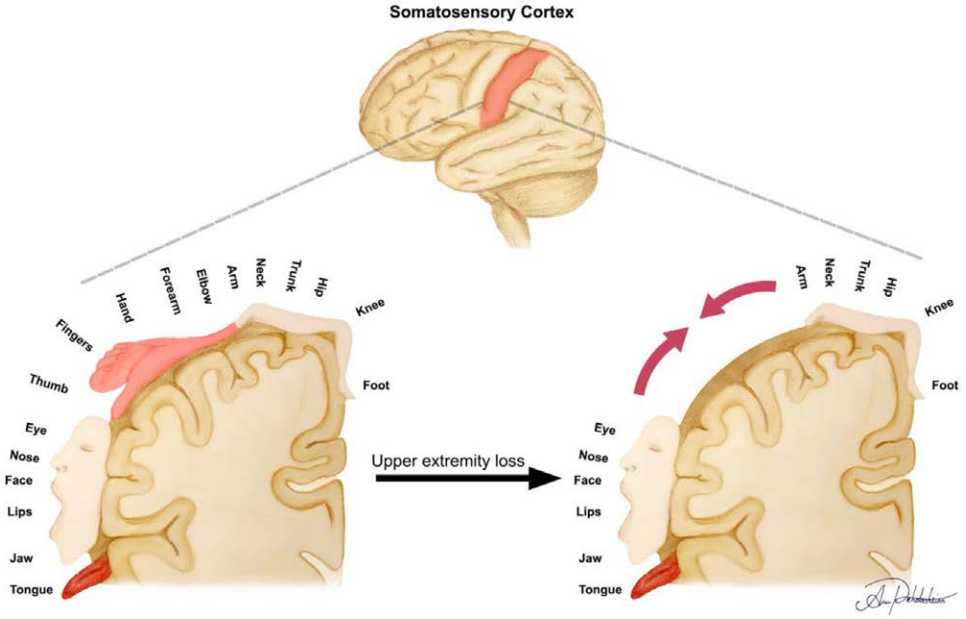

Fig. 2. Schematic illustration of cortical reorganization following amputation. Following amputation, the deafferented cortical representation of the former limb is replaced by neighboring cortical areas. With an amputated upper limb, the deafferented somatosensory cortex is being replaced by the cortical area representing the lip.

nervous system.[74] Studies have shown that motor neurons to the former limb can reinnervate residual muscles proximal to the amputation site.[75] Specifically, in monkeys, residual muscles were found to gain additional innervation from those motor neurons formerly supplying distal muscle targets.[75] This was also supported by experimentation on rats, which showed that stimulation of amputation-deprived areas of the primary motor cortex resulted in movements of the residual muscles proximal to the stump.[74] The reinnervation of motor neurons could be a possible mechanism responsible for the reorganization of the primary motor cortex commonly seen in persons with amputations, and it could potentially play a role in the development of phantom limb movement sensation.[75,76] Additionally, since motor cortex reorganization is correlated with phantom limb pain,[62] reinnervation of motor neurons could be an underlying influence in the pathogenesis of this neuropathic syndrome.

Sensory afferents from both the residual stump and skin have also been shown to reinnervate postamputation-deprived territories in the cuneate nucleus and external cuneate nucleus.[77] The cuneate nucleus relays information to the somatosensory cortex and, therefore, provides a potential mechanism in which stimulation of stump muscles and skin can result in phantom limb sensations.[77] Additionally, the reorganization of the neurons in the cuneate nucleus could provide an underlying mechanism responsible for the cortical reorganization seen in the somatosensory cortex,[77] which has also been shown to be correlated with phantom limb pain.[62]

TREATMENTS

Treatment for pathologic pain following amputation most commonly focuses on centrally mediated mechanisms, employing various strategies including medications, cognitive therapy, and spinal cord stimulation.[4,78] Medications such as nortriptyline, pregabalin (generic version of Lyrica), opiates, and ketamine can be effective at treating this pain through blockage of receptors associated with central sensitization as well as increasing inhibition at the nerve to reduce pathologic stimulation.[19,79,80] Despite many studies demonstrating "statistically significant" improvement in phantom limb pain with pharmacological treatment, this relief is oftentimes not clinically relevant from the patient's perspective.[81,82]

In contrast, mirror therapy has demonstrated success in achieving a significant reduction in phantom limb pain for these patients.[83,84] Mirror therapy utilizes a flat mirror to project the image of the intact limb onto the absent limb, providing the illusion of movement.[4] Although the patient is aware of this illusion, it is thought that mirror therapy results in stimulation of quiescent areas of the cortex previously associated with the amputated extremity, thereby reducing resultant phantom limb sensations.[79] Mental imagery therapy has also shown efficacy in reducing phantom limb pain by encouraging the patient to imagine movements in the phantom limb, similarly stimulating these deprived neurons in the cortex.[79] A study involving patients with upper limb amputations who partook in the mental imagery program demonstrated significant reduction in phantom limb pain.[79,85] Furthermore, studies have also shown that spinal cord stimulation reduces phantom limb pain through a mechanism referred to as gate-control theory.[78,86] This theory employs that the electrical stimulation of nonpainful Aβ fibers in the dorsal column of the spinal cord blocks transmission of pain from neighboring nociceptive nerve fibers; thus, the nonpainful Aβ fibers act as a "gate" blocking neighboring pain pathways.[86]

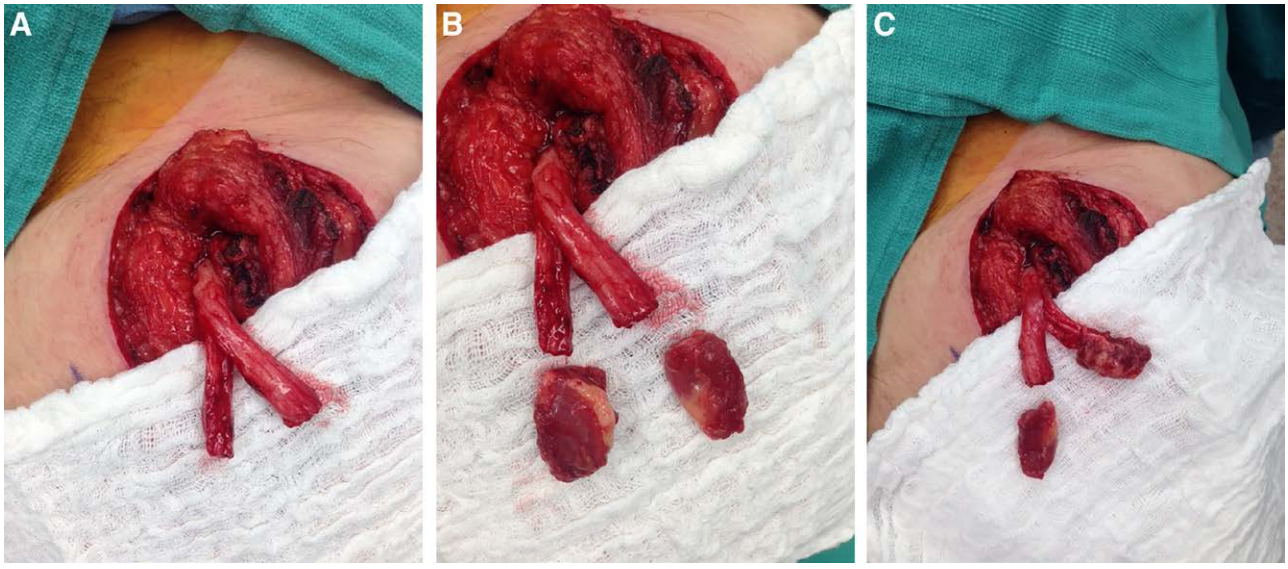

Fig. 3. Surgical treatment of symptomatic neuroma using RPNIs. A, Following the resection of a sciatic neuroma in a patient with a transfemoral amputation, the nerve was divided into two fascicles. Two autologous free muscle grafts (3 x 1.5 x 1 cm) were harvested (B) and, subsequently, were neurotized using each of the nerve fascicles to create the RPNIs (C).

In contrast to centrally mediated treatment strategies, peripherally based treatments are far more targeted, largely focusing on management of symptomatic terminal neuromas and their associated allodynia. Although no standard of treatment has been accepted for neuromas, common treatments can include neurolytic and/or steroid injections, chemical ablation, cryotherapy, and surgical resection.[87] The primary goal of surgical resection is removal of pathologic, disorganized swellings of terminal nerve axons, with various methods employed to reduce recurrence.[3,87,88] Methods utilized can vary from ligation, relocation into bone or muscle, and nerve capping, for example, but all have notable issues with recurrence as well as clinically significant resolution of pain in the majority of study participants long term.[3,87,88] These shortcomings could be theorized to be secondary to a failure to address the underlying cause of the pain, namely neuronal hyperexcitability and aberrant signaling, resulting from a loss of end-organ innervation following amputation.

Two promising strategies in particular to provide neuronal end-organ targets to transected nerves include targeted muscle reinnervation (TMR)[89,90] and the regenerative peripheral nerve interface (RPNI).[91–94] TMR relies on nerve transfer to provide these end-organ targets, anastomosing amputated nerve endings to intact motor nerve branches in nearby residual muscle.[89,90] Although this method facilitates muscle reinnervation by the previously transected nerve, it involves sacrifice of an intact motor nerve, replacing one transected nerve for another. In comparison, the RPNI entails implantation of a transected peripheral nerve into a segment of autologous muscle graft, avoiding any additional nerve injury. Over time, the RPNI revascularizes, regenerates, and becomes reinnervated by its contained nerve.[92,95,96] As the muscle graft is denervated as a result of the fabrication process for both TMR and RPNI, these now-denervated motor endplates provide functional innervation targets for these formerly "purposeless" transected axons. The prevailing hypothesis underlying these approaches is that through providing end-organ targets for these nerves, it is possible to decrease the aberrant signaling and hypersensitivity often associated with residual nerves postamputation (Fig. 3).

A prospective study conducted in 2019 utilizing TMR as a treatment arm indicated overall absolute reductions in the prevalence of phantom (–32%) and residual limb pain (–40%) compared with controls at long-term follow-up.[90] The RPNI's efficacy has also been demonstrated in prior retrospective review, as patients undergoing RPNI treatment had a 71% reduction in stump pain and a 53% reduction in phantom limb pain posttreatment.[97] These results were consistent with a retrospective review utilizing the construct as a prophylactic measure, demonstrating that 51% of interventional patients developed phantom limb pain compared with 91% in the control, non-RPNI treatment group.[98] Clinical prospective studies are currently ongoing, with preliminary results indicating that the RPNI is a promising treatment strategy to address transected nerve pain pathology.

CONCLUSIONS

Following amputation, a complex combination of molecular changes, spinal adaptations, and cerebral cortex reorganization contributes significantly to the development and maintenance of pathological pain. Recent research has indicated that some degree of interdependence exists between postamputation changes in the peripheral and central nervous systems; however, the exact mechanisms underlying this relationship are largely unknown. The ideal treatment strategy would likely require addressing changes at both the peripheral and central levels. For example, an ideal treatment could involve provision of endpoint targets for transected neurons, thereby preventing the downstream cascade of peripheral and spinal cord changes, thereby reestablishing physiologic cerebral mapping. By

establishing effective treatment for chronic pain following amputation, there exists significant potential to reduce disability, facilitate prosthetic use, and regain quality of life in this patient population.

Stephen W. P. Kemp, PhD
Department of Biomedical Engineering
Section of Plastic Surgery
University of Michigan
1150 W Medical Center Drive, MSRB II, A570
Ann Arbor, MI 48109-5456
E-mail: swpkemp@med.umich.edu

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