LITERATURE REVIEW: APPLICATIONS FOR

Traumatic brain injury

F. Marsili

3. CORTICAL RECOVERY

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Author's choice

Traumatic brain injury (TBI) is a lesion of the brain which occurs as a consequence of trauma following falls (40.5%) or car/motor accidents (14.3%) [1]. Birth brain injuries are a sub-category of TBI with a yearly prevalence of 26.46 per 1000 hospital births [2]. Generally, TBI is associated with older individuals, aged 75 or above. Though children with birth brain damage (birth related or otherwise) cover a relatively small percentage of the total TBI population, the significant impact of TBI on the quality of life of children, of their parents and their extended families, makes the research on the improvement of TBI symptomatology especially relevant [2], [3].

The first few weeks or months of an infant are the most critical: children are born with around 100 billion neurons, which are yet to be connected. Neuroplastic events occur continuously during the first developing phases of a newborn, where connections are build and wired experientially [4]. This fact makes early detection and intervention on newborns with TBI essential.

Electrical stimulation therapies have been demonstrated to have significant effects on recovery from brain injuries, such as stroke, ischaemic events, brain and spinal cord trauma, and TBI [5], [6], [7]. Even though the exact underlying mechanisms of electrical stimulation are yet to be understood, clinical evidence shows its efficacy on neurophysiological reorganisation of cortical areas as well as functional recovery including facilitation of movements and pain relief [8], [9]. It can be concluded that electrical stimulation takes advantage of the neuroplastic ability of peripheral nerves and central neurons to trigger adaptive cascades to counteract the maladaptation occurring as a consequence of injuries or disease.

In this collection of papers, we first explore the concept of neuroplasticity, with particular focus on the significance of cortical organisation in developing brains and the role that electrical stimulation plays in triggering reorganisation of cortical areas in developing as well as adult brains. Following, we focus on the clinical evidence of electrical stimulation in enhancing both functional peripheral recovery (e.g., motor and sensory functions) and cortical adjustments (e.g., plastic changes on sensorimotor cortex).

In summary, TBI is a condition significantly affecting the quality of life of the individuals affected by it. In the case of birth brain injuries, children and their families experience significant and long-term impact on their daily lives. Being able to leverage on the brain's ability to reorganise after maladaptation using neuroplastic processes could have an essential role in the treatment of TBI in infants and children. The following papers explore the role that electrical stimulation could have in enhancing adaptive, neuroplastic responses in TBI: a potential therapeutic application for children with brain injuries.

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3. Cortical recovery

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PLOS ONE

Primary Sensory and Motor Cortex Excitability Are Co-Modulated in Response to Peripheral Electrical Nerve Stimulation

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Abstract

Peripheral electrical stimulation (PES) is a common clinical technique known to induce changes in corticomotor excitability; PES applied to induce a tetanic motor contraction increases, and PES at sub-motor threshold (sensory) intensities decreases, corticomotor excitability. Understanding of the mechanisms underlying these opposite changes in corticomotor excitability remains elusive. Modulation of primary sensory cortex (S1) excitability could underlie altered corticomotor excitability with PES. Here we examined whether changes in primary sensory (S1) and motor (M1) cortex excitability follow the same time-course when PES is applied using identical stimulus parameters. Corticomotor excitability was measured using transcranial magnetic stimulation (TMS) and sensory cortex excitability using somatosensory evoked potentials (SEPs) before and after 30 min of PES to right abductor pollicis brevis (APB). Two PES paradigms were tested in separate sessions; PES sufficient to induce a tetanic motor contraction (30–50 Hz; strong motor intensity) and PES at sub motor-threshold intensity (100 Hz). PES applied to induce strong activation of APB increased the size of the N_{20}-P_{25} component, thought to reflect sensory processing at cortical level, and increased corticomotor excitability. PES at sensory intensity decreased the size of the P25-N33 component and reduced corticomotor excitability. A positive correlation was observed between the changes in amplitude of the cortical SEP components and corticomotor excitability following sensory and motor PES. Sensory PES also increased the sub-cortical P_{14}-N_{20} SEP component. These findings provide evidence that PES results in co-modulation of S1 and M1 excitability, possibly due to cortico-cortical projections between S1 and M1. This mechanism may underpin changes in corticomotor excitability in response to afferent input generated by PES.

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Introduction

Peripheral electrical stimulation (PES) is used in clinical settings for a diverse range of applications from facilitation of voluntary muscle contraction to management of pain in neurological and musculoskeletal conditions. Although evidence for clinical effectiveness is growing, the physiological bases for such effects are not completely understood. In terms of PES interventions that change muscle activation, most investigations have focussed on changes at the muscle or spinal motoneurones. For instance, PES-induced muscle contractions enhance oxidative capacity, increase number of capillaries and transform muscle fibre type within a muscle [1,2]. Yet, PES can also induce plastic change in motor regions of the human cortex (for review see [3]). Corticomotor excitability, assessed by transcranial magnetic stimulation (TMS), is increased following PES at intensities sufficient to produce muscle contraction, but decreased when PES is applied at lower intensities that are sufficient to evoke sensation without muscle contraction [4]. The mechanisms responsible for these intensity-dependent differences in the direction of the changes in excitability are not known.

Afferent input is a powerful driver of plastic change in M1. Functional and anatomical interactions exist between primary sensory (S1) and primary motor (M1) cortical areas. For example, long term potentiation (LTP) is evident in neurons of the motor cortex following tetanic stimulation of S1 [5], and ablation of S1 impairs learning, but not retention, of new motor skills [6]. These findings suggest an important role of input from S1 to M1 in modulation of M1 excitability and motor learning. Such a mechanism may underlie altered M1 excitability with PES. Specifically, excitability changes in M1 with PES may be secondary to activation of, or changes in, S1.

Previous studies have examined the effect of PES using a range of stimulus parameters on excitability of *either* M1 or S1. In relation to S1, the amplitude of short-latency components of the somatosensory evoked potential (SEP), thought to be related to cortical processing in S1 (e.g. N_{20}-P_{25}-N_{33}), is decreased in response to high frequency PES (100–200 Hz) at intensities ranging from below motor threshold to that sufficient to induce a muscle twitch [7–9]. The amplitude of motor evoked potentials (MEPs) from TMS applied to M1 are decreased following PES at

similar frequencies (100 Hz), but with weaker stimulation intensity [4]. No study has investigated the effect of PES applied at an intensity and frequency sufficient to induce a tetanic motor response (strong motor intensity; 30–50 Hz) on responses related to function of the primary sensory cortex (S1), despite use of this paradigm in clinical settings. The heterogeneous approach to experimental study of stimulus parameters, and failure to examine both S1 and M1 concurrently, mean it is not yet possible to conclude whether changes at S1 present a possible candidate mechanism underpinning changes in motor output following PES.

Here we compared the response of S1 and M1 to PES paradigms applied either at an intensity sufficient to evoke a contraction of the stimulated muscle or at an intensity sufficient to induce sensory stimulation, but below motor threshold.

Materials and Methods

Ethics Statement

All procedures were approved by the Human Research Ethics Committee at The University of Queensland and conformed to the Declaration of Helsinki.

Participants

Thirteen healthy individuals (nine female, four male; age 27±9 years; mean ± standard deviation) gave informed and written consent to participate in the study. Participants had no history of neurological or upper limb conditions and completed a TMS safety screen prior to commencement [10].

Electromyography (EMG)

EMG activity was recorded using disposable silver/silver chloride surface electrodes from the right abductor pollicis brevis muscle (APB). The reference electrode was placed over the metacarpophalangeal joint and the active electrode over the muscle motor point. EMG signals were amplified 1000 x, filtered between 20–1000 Hz and sampled at 2000 Hz using Signal3 software and a Micro1401 data acquisition system (Cambridge Electronic Design, Cambridge, UK).

TMS of the Primary Motor Cortex

TMS was applied using a Magstim 200 stimulator (Magstim Co. Ltd, Dyfed, UK) with a figure-of-eight shaped coil (external wing diameter, 7 cm). The coil was held over the left hemisphere at an angle of 45° to the sagittal with the handle posterior. This coil orientation is optimal for stimulation of the hand region of the motor cortex. The optimal scalp site to evoke motor evoked potentials (MEPs) in right APB was established and marked on the scalp. Resting motor threshold (rMT) was identified as the minimum stimulator intensity at which 5 out of 10 stimuli applied at the optimal scalp site evoked a response with a peak-to-peak amplitude of at least 50 μV in the target muscle. MEPs were recorded from right ABP with stimulator output at 120% rMT. All TMS procedures adhered to the TMS checklist for methodological quality [11].

Brachial Plexus Stimulation

Electrical stimuli of 200 μs duration were applied with a constant current stimulator (DS7A, Digitimer Ltd, Welwyn Garden City, UK) applied to the brachial plexus to evaluate changes in excitability at the muscle and neuromuscular junction. The active electrode was positioned in the supraclavicular fossa (Erb's point) and the reference electrode over the acromion. Stimulus intensity was set 50% above the intensity required to elicit a maximal compound muscle action potential (M_{max}) in the APB muscle at rest.

Electroencephalography (EEG) Recordings - SEP

SEPs were obtained by stimulation of the median nerve at the wrist. EEG was recorded over the approximate location of the hand area of the primary sensory cortex using gold plated cup electrodes (C3' [2 cm posterior to C3] and referenced to Fz) [12]. Electrode impedance was maintained below 5 kΩ. Additional recording electrodes were placed over the cervical spine (C7) and Erb's point (supraclavicular fossa and acromion) in order to track the afferent volley in the spine and periphery. EEG signals were amplified 50000x, filtered 5–500 Hz and sampled at 1000 Hz using the Micro1401 data acquisition system.

A constant current stimulator was used to deliver electrical stimuli of 1-ms duration to the median nerve at a rate of 2 Hz (maximum current of 1 A). A 20% variance was incorporated into the stimulus frequency to avoid accommodation. Stimulus intensity was set at 3× perceptual threshold. This intensity was considered comfortable by all participants and was sufficient to evoke a visible muscle twitch in APB. Where necessary, the stimulus intensity was adjusted to ensure the size of the peripheral volley (recording at Erb's point) remained constant throughout the experiment. Two blocks of 500 stimuli were recorded and averaged off line for analysis.

PES Interventions

Each subject participated in two sessions separated by at least 72 hours. On each occasion, a different electrical stimulation intervention was administered to the right APB. The order in which participants received the two electrical stimulation paradigms was randomised. Each intervention lasted for 30 min and was delivered using a monophasic waveform with a pulse duration of 0.1 ms (Chattanooga Intelect Advanced therapy system, OPC Health, Melbourne, Australia). Habituation to the stimulus was monitored and, where necessary, the intensity adjusted to maintain a consistent motor or sensory response. To control for attention participants were directed to focus on the stimulation and verbal reminders were given at 5 min intervals.

The two interventions were:

1. *Motor Movement:* To mimic a voluntary contraction in the APB muscle, current was delivered at 30 Hz with a ramped intensity with six periods of stimuli applied per minute (4 s on: 6 s off periods). Stimulus intensity set at that sufficient to induce a mid-range thumb abduction.
2. *Sensory 100 Hz:* Intensity of electrical stimulation was set at that where the subject first reported perception of the stimulus, and delivered at a frequency of 100 Hz. This intensity was sufficient to produce a mild cutaneous tingling over the APB muscle, but without muscle contraction.

Experimental Protocol

Participants were positioned comfortably in an armchair with their right arm relaxed and supported on an arm rest for the duration of the experiment. Fifteen baseline MEPs, 4 M_{max} measures and 2 blocks of SEP measures (500 stimuli each) were recorded. Following this, one of the PES paradigms was applied to the right APB. After completion of the stimulation period, measures of MEPs, M_{max} and SEPs were repeated.

Data and Statistical Analyses

MEPs and M_{max} were analysed as peak-to-peak amplitudes. Each parameter was assessed with a separate two-way repeated measures analysis of variance (ANOVA) with factors TIME (pre/post PES) and CONDITION (sensory PES/motor PES). To account for any activity-dependent changes in muscle fibre action potentials resulting from the PES interventions, statistical analysis was also performed with MEP amplitudes expressed as a proportion of M_{max} amplitude.

SEP parameters were analysed as peak-to-peak amplitudes for the components: P_{14}-N_{20}, N_{20}-P_{25}, P_{25}-N_{33} and the spinal (N_{13}) and peripheral (N_9) volley. Latencies were calculated as the time from stimulus onset to N_{20}, N_9 and N_{13}. An example of the SEP components is presented in Figure 1. Amplitudes and latencies were analysed using separate two-way repeated measures ANOVA with factors TIME (pre/post PES) and CONDITION (sensory PES/motor PES) for each parameter.

Linear regression analyses were performed to determine whether peripheral electrical stimulation induced changes in corticomotor excitability (increased/decreased MEP amplitude) were associated with changes in the amplitude of cortical (N_{20}-P_{25} and P_{25}-N_{33}) components of the SEP. A linear regression was calculated using the pre-post change scores, calculated as 100–(MEP or SEP pre/MEP or SEP post * 100) for each measure. As findings from the repeated measures ANOVA indicated that M1 and S1 co-modulate in response to both motor and sensory PES, linear regression was calculated with data averaged over PES conditions.

Where appropriate, post-hoc tests were performed using Holm-Sidak pair-wise comparisons. Significance was set at 5%.

Results

There was no change in M_{max} across time with either PES intervention (TIME $p = 0.94$, CONDITION $p = 0.26$, Interaction TIME × CONDITION $p = 0.47$). As M_{max} did not change, results obtained using raw MEP amplitudes and those normalised to M_{max} were comparable and as such, data are presented as absolute MEP

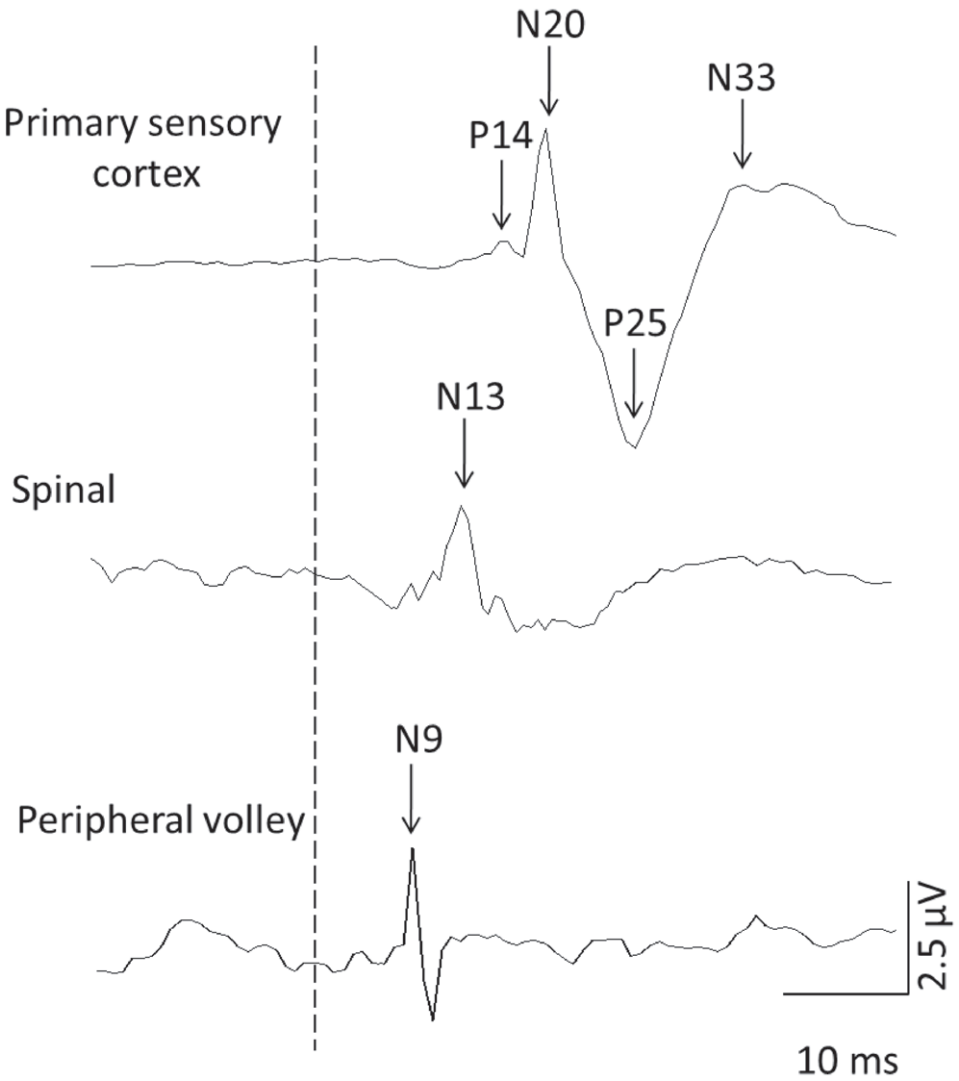

Figure 1. Raw data from a representative subject demonstrating the SEP components used for analysis of conduction and processing of the afferent volley at the primary sensory cortex, brainstem and the peripheral volley recorded at Erb's point. The dotted line represents the time of stimulation.
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amplitudes in the text and figures to facilitate comparison with other published research.

Effect of PES on Corticomotor Excitability

Motor and sensory PES paradigms induced different effects on corticomotor excitability (Interaction TIME × CONDITION $p<0.001$). Motor PES applied to right APB increased MEP amplitudes (post-hoc pre vs. post $p<0.001$), whereas sensory PES suppressed MEP amplitudes (post-hoc pre vs. post $p = 0.019$; Figure 2). There was no difference in MEP amplitude between the two interventions at baseline (post-hoc sensory PES vs. motor PES pre intervention $p = 0.24$). However, the two interventions induced effects on corticomotor excitability that differed from each other following the 30-min stimulation period (post-hoc sensory PES vs. motor PES post intervention $p<0.001$).

Effect of PES on Sensory Cortex Excitability

There was no effect of either intervention on the spinal (N_{13}; main effect of TIME $p = 0.32$; Interaction TIME × CONDITION $p = 0.66$) or peripheral (N_9; main effect of TIME $p = 0.40$; Interaction TIME × CONDITION $p = 0.67$) volley. Neither motor nor sensory PES induced a change in the latency of the N_{20} (main effect of TIME $p = 0.74$; Interaction TIME × CONDITION $p = 0.68$), N_{13} (main effect of TIME $p = 0.78$; Interaction TIME × CONDITION $p = 0.48$) or N_9 (main effect of TIME $p = 0.51$; Interaction TIME × CONDITION $p = 0.53$) components. Differential effects of *motor* and *sensory* PES on SEPs were observed for the P_{14}-N_{20} (Interaction TIME × CONDITION $p = 0.039$), N_{20}-P_{25} (Interaction TIME × CONDITION $p = 0.032$) and P_{25}-N_{33} (Interaction TIME × CONDITION $p = 0.023$) components. Following *motor* PES the N_{20}-P_{25} increased (post-hoc pre vs. post $p = 0.007$, Figure 3b) but there was no change in the P_{14}-N_{20} (post-hoc pre vs. post $p = 0.34$) or P_{25}-N_{33} (post-hoc pre vs. post $p = 0.77$) components. Conversely, *sensory* PES increased the amplitude of P_{14}-N_{20} (post-hoc pre vs. post $p = 0.01$, Figure 3a) and reduced P_{25}-N_{33} (post-hoc pre vs. post $p<0.001$, Figure 3c). The N_{20}-P_{25} component was unchanged by sensory PES (post-hoc pre vs. post $p = 0.34$).

The magnitude and direction (increase or decrease) of the change in corticomotor excitability induced by sensory and motor PES was positively correlated with the change in the cortical SEP components ($r = 0.71$, $p<0.001$, Figure 4).

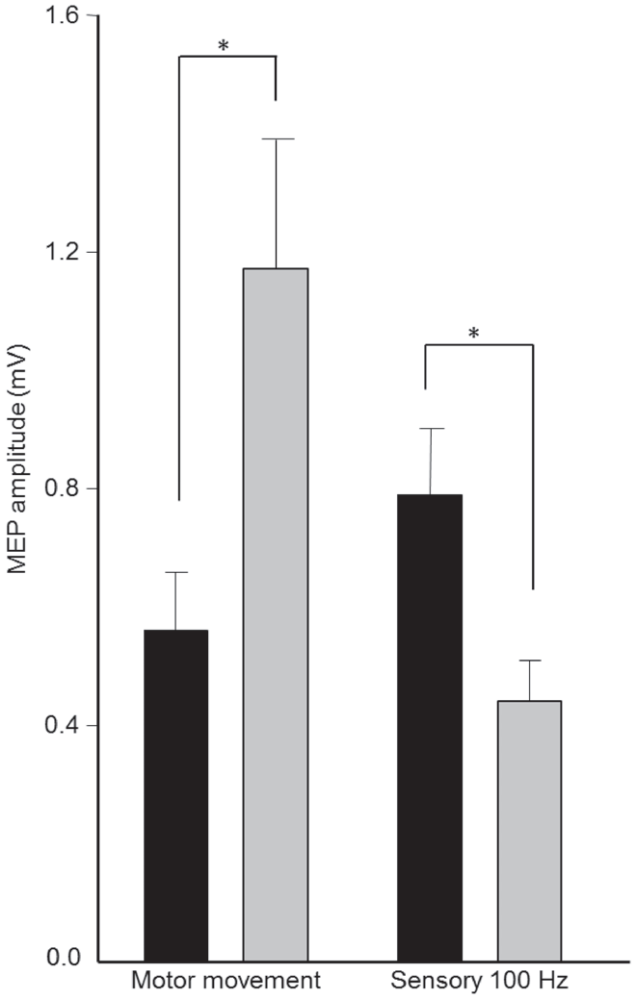

Figure 2. Group data (mean ± standard error) of amplitudes motor evoked potentials (MEP) before (black bars) and after (grey bars) ''Motor Movement'' and ''Sensory 100 Hz'' peripheral electrical stimulation (PES) to right abductor pollicis brevis muscle (APB). MEP amplitude increased following Motor Movement PES and reduced following Sensory 100 Hz PES. * $p<0.05$.
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Discussion

This study is the first to concurrently examine the influence of two PES paradigms on S1 and M1 excitability. Our data demonstrate increased excitability of the corticomotor pathway and increased amplitude of S1 responses, specifically of the early N_{20}-P_{25} component, with PES at intensities sufficient to induce the movement of thumb abduction. Decreased excitability of the corticomotor pathway with PES applied at sub motor threshold (sensory) intensities was mirrored by a decrease in the N_{25}-P_{33} component and an increase in subcortical processing, as evidenced by an increase in the P_{14}-N_{20} component. These novel findings indicate that the excitability of S1 and M1 are co-modulated following PES and the direction of effect appears dependent on the combination of stimulus intensity and frequency.

PES at motor intensities is used to facilitate movement and improve function in a variety of pathologies including stroke and spinal cord injury [13–17]. Conversely, PES at sensory intensities (without muscle contraction), commonly termed ''transcutaneous electrical nerve stimulation'' (TENS), is used for pain relief and is effective for management of pain associated with rheumatoid arthritis, surgery and labour [18,19]. We recently demonstrated increased corticomotor excitability when PES is applied at motor intensities but decreased when PES is applied at sub-motor threshold sensory intensities [4]; effects confirmed in the current study. The observed changes in corticomotor excitability likely occur at the motor cortex as both peripheral M-waves, indicative of excitability changes occurring at the neuromuscular junction and muscle, and measures of spinal/motoneurone excitability (H-reflex and F-waves) are unchanged following motor [20] and sensory PES [21–23]. Changes in motor cortex excitability following PES have been attributed to altered synaptic efficacy and associated long-term potentiation (LTP) or depression (LTD)-like mechanisms [24]. However, no study has attempted to examine how afferent input in the form of PES (in the absence of contraction) may drive reorganization in M1.

Afferent input plays a vital role in motor learning and its manipulation induces organisational changes in M1 [25]. For instance, removal of sensory input can change the cortical motor representation in a manner that is reversed when sensation is restored [26]. The presence of structural and functional connections between S1 and M1 suggests modulation of S1 excitability

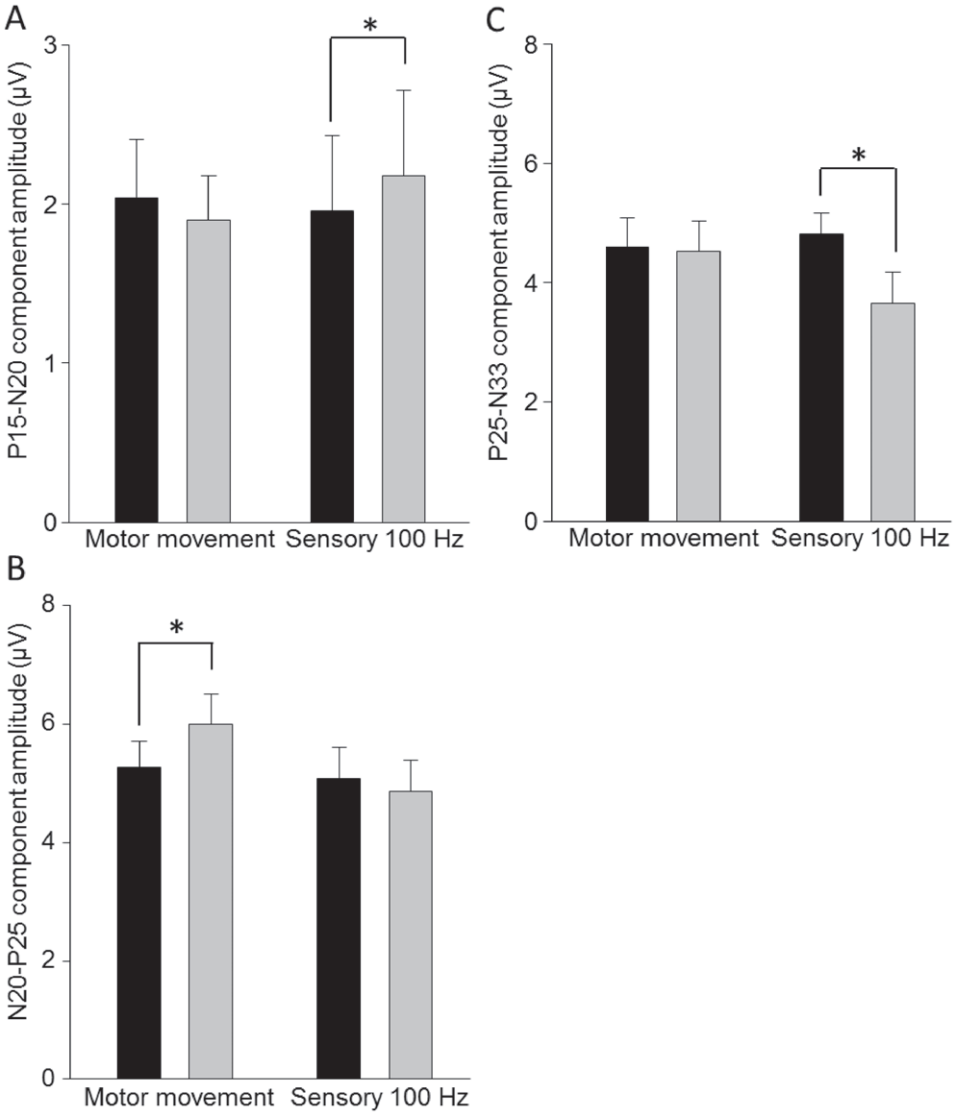

Figure 3. Group data (mean ± standard error) before (black bars) and after (grey bars) Motor Movement and Sensory 100 Hz peripheral electrical stimulation (PES) to the right abductor pollicis brevis muscle (APB) for the SEP components (a) P_{14}-N_{20}, (b) N_{20}-P_{25} and (c) P_{25}-N_{33}. Motor Movement PES increased the amplitude of the N_{20}-P_{25} component. Sensory 100 Hz PES increased the amplitude of the sub-cortical P_{14}-N_{20}, and reduced the size of the P_{25}-N_{33} component. * $p<0.05$.
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might result in similar changes in M1 excitability following PES. In support of this, in the current study changes in M1 excitability mirrored the changes in SEP components that relate to S1 function; motor PES increased, and sensory PES decreased both S1 and M1 excitability. Further, the magnitude and direction of the PES induced effects on corticomotor excitability were positively correlated with changes in S1 excitability. One explanation for our findings is that afferent information from PES is relayed to S1 via thalamo-cortical projections, activating or inducing a change in sensory processing and this provides the signal for LTP or LTD-like changes in M1. Cortico-cortical projections between S1 and M1 have been identified in animals and humans [27,28] and these projections are topographically specific. Evidence from animal studies demonstrates that stimulation of S1 can induce LTP of motor cortical synapses probably through altered discharge of intracortical interneurons [5]. This mechanism may underpin the co-modulation of S1 and M1 observed here. To further clarify this mechanism, future studies should seek to examine intracortical inhibitory and facilitatory networks in response to PES at various stimulus intensities. However, direct connections also exist between the thalamic nucleus and M1 [29–31]. Thus, we cannot dismiss the possibility that afferent input from PES may relay directly to S1 and M1 via the thalamus, providing a stimulus for LTP or LTD-like changes in synaptic efficacy in both regions within a similar timeframe.

There is good evidence that the N_9 component of the SEP represents conduction of the potential along the peripheral nerve, N_{13} in the cervical dorsal horn and P_{14}-N_{20} in the cervicomedullary junction near the cuneate nucleus [7–9,32–34]. The N_{20}-P_{25} component represents arrival of the afferent volley in S1 and the P_{25}-N_{33} is thought to represent processing of the afferent volley in S1 [7–9,32–34]. Traditionally, the spinal cord has been considered an important site affected by sensory PES [35]. Yet, spinal N_{13} was unchanged by sensory PES in the current study. Consistent with

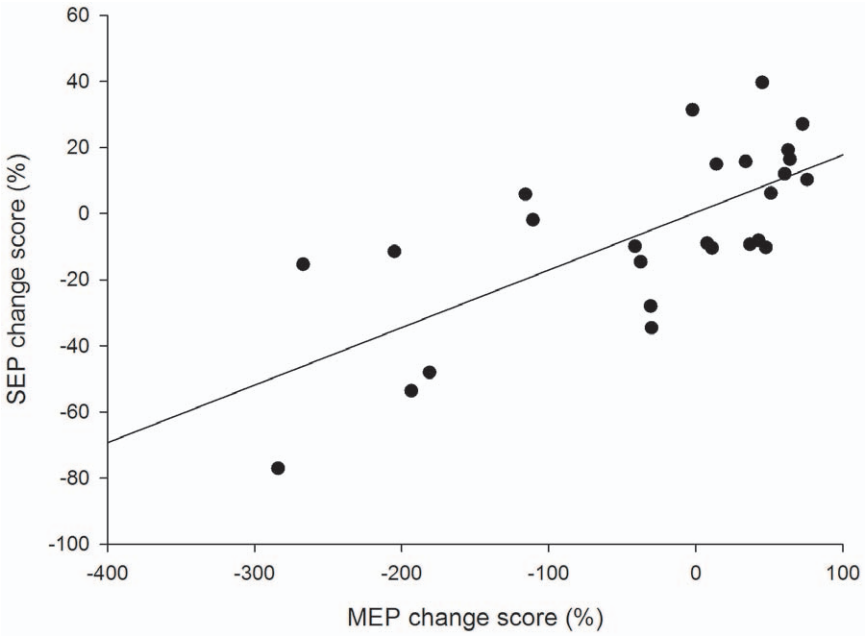

Figure 4. Linear regression between cortical SEP components (N_{20}-P_{25} and P_{25}-N_{33}) and corticomotor excitability (MEP amplitude). Note the significant positive correlation ($r = 0.71$, $p < 0.001$) between these parameters.
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previous studies, this suggests sensory PES does not inhibit electrically evoked spinal N_{13} activity [9,34]. Further, consistent N_9 and N_{13} amplitudes, regardless of stimulation type, indicate that altered SEP excitability in response to PES occurred at supraspinal levels, and these could be either sub-cortical or cortical.

Single electrical stimuli of increasing intensity have been shown to amplify afferent signals in the central nervous system (CNS) [32,34]. This amplification occurs primarily at the level of the cuneate nucleus (measured as an increase in P_{14}-N_{20}) and is maintained at the level of S1. Application of sensory PES in the current study produced an increase in the size of the P_{14}-N_{20} component, suggesting sensory PES as applied here did not alter expected amplification at the cuneate nucleus. However, consistent with previous reports [34], our findings indicate that amplification is suppressed at S1 (N_{20}-P_{25} and P_{25}-N_{33}). The magnitude of the N_{20}-P_{25} and P_{25}-N_{33} SEP components reflect the size of the arriving synaptic input and responsiveness of the post-synaptic cell respectively [36]. As the size of the input arriving at S1 remained stable with sensory PES, suppression of S1 excitability is most likely explained by activation of post-synaptic inhibitory mechanisms [34]. This inhibitory response may drive reduced corticospinal output via S1-M1 cortico-cortical circuitry in response to sensory PES.

Several possibilities may explain the differential effect of sensory and motor PES on S1 and M1. First, corticomotor excitability is increased when motor PES is applied to a mixed nerve or over the muscle motor point, but identical PES protocols administered to digital nerves (consisting primarily of cutaneous afferents) fail to alter M1 excitability [37,38]. These findings, in conjunction with those of the present study, suggest input from large-diameter afferents from *muscle* may be an important factor driving enhanced S1 excitability and subsequent LTP-like changes in M1 with motor PES. Second, a key feature of sensory PES is the bombardment of S1 with consistent afferent stimuli that presumably provide little or no useful information regarding sensory or motor function. It is possible that repeated, functionally irrelevant activation of S1 'gates' or suppresses S1 excitability during sensory PES [36]. On the other hand, motor PES generates afferent input both from electrical stimulation of the afferent neurons and the "natural" input from the evoked movement, providing potentially "useful" information relating to movement. The N_{20}-P_{25} and P_{25}-N_{33} SEP components are thought to reflect processing related to kinaesthesia and position sense [39,40]. Therefore, their enhancement (and the associated increase in corticomotor excitability) following motor PES may be important for modulating motor output.

Conclusion

Excitability of primary sensory and motor cortical areas is co-modulated in response to PES, regardless of stimulus intensity and frequency. PES applied in a manner that induced strong thumb abduction increased S1 and M1 excitability, whereas PES at sensory intensities (below motor threshold) reduced S1 and M1 activity. These findings appear consistent with the hypothesis that reorganisation of M1 in response to PES is influenced by cortico-cortical projections between S1 and M1, a circuit that has been previously implicated in motor learning.

Author Contributions

Conceived and designed the experiments: SMS LSC MCR PWH MPG. Performed the experiments: SMS LSC. Analyzed the data: SMS LSC. Wrote the paper: SMS LSC MCR PWH MPG.

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Inhibitory Mechanisms in Primary Somatosensory Cortex Mediate the Effects of Peripheral Electrical Stimulation on Tactile Spatial Discrimination

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Abstract—Selective afferent activation can be used to improve somatosensory function, possibly by altering cortical inhibitory circuit activity. Peripheral electrical stimulation (PES) is widely used to induce selective afferent activation, and its effect may depend on PES intensity. Therefore, we investigated the effects of high- and low-intensity PES applied to the right index finger on tactile discrimination performance and cortical somatosensory-evoked potential paired-pulse depression (SEP-PPD) in 25 neurologically healthy subjects. In Experiment 1, a grating orientation task (GOT) was performed before and immediately after local high- and low-intensity PES (both delivered as 1-s, 20-Hz trains of 0.2-ms electrical pulses at 5-s intervals). In Experiment 2, PPD of SEP components N20/P25_SEP-PPD, N20_SEP-PPD and P25_SEP-PPD, respectively, were assessed before and immediately after high- and low-intensity PES. Improved GOT discrimination performance after high-intensity PES (reduced discrimination threshold) was associated with lower baseline performance (higher baseline discrimination threshold). Subjects were classified into low and high (baseline) GOT performance groups. Improved GOT discrimination performance in the low GOT performance group was significantly associated with a greater N20_SEP-PPD decrease (weaker PPD). Subjects were also classified into GOT improvement and GOT decrement groups. High-intensity PES decreased N20_SEP-PPD in the GOT improvement group but increased N20_SEP-PPD in the GOT decrement group. Furthermore, a greater decrease in GOT discrimination threshold was significantly associated with a greater N20_SEP-PPD decrease in the GOT improvement group. These results suggest that high-intensity PES can improve somatosensory perception in subjects with low baseline function by modulating cortical inhibitory circuits in primary somatosensory cortex.

Key words: peripheral electrical stimulation, tactile orientation discrimination, paired-pulse depression.

INTRODUCTION

Afferent input induced by repeated peripheral somatosensory stimulation is highly effective for improving somatosensory function. For example, several studies have shown that high-frequency repetitive tactile stimulation improves tactile two-point spatial discrimination of the stimulated finger (Godde et al., 2000; Pleger et al., 2003; Dinse et al., 2006; Höffken et al., 2007; Ragert et al., 2008a,b; Kowalewski et al., 2012). It is widely accepted that the repetitive nature of peripheral somatosensory stimulation is critical for modulating perceptual performance in tactile spatial discrimination tasks. Repeated peripheral electrical stimulation (PES) has been shown to have effects similar to repeated tactile stimulation. Indeed, PES applied to the index finger improved the tactile two-point discrimination of that finger (Schlieper and Dinse, 2012) as well as the somatosensory temporal discrimination threshold, the smallest time interval between two tactile or electrical stimuli still detected as separate (Erro et al., 2016; Rocchi et al., 2017).

In contrast, PES had no effect on tactile orientation discrimination, a perceptual task similar to tactile spatial two-point discrimination (Rocchi et al., 2017), suggesting that the effect of PES may differ between discrimination tasks. Alternatively, it is possible that this difference is due to PES intensity. Schlieper and Dinse (2012) reported that both high- and intermediate-intensity PES improved tactile spatial two-point discrimination, while low-

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Abbreviations: ANOVA, analysis of variance; GOT, grating orientation task; IPI, interpulse interval; PES, peripheral electrical stimulation; PPD, paired-pulse depression; SEP, somatosensory-evoked potential.

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intensity PES did not. On the other hand, Rocchi et al. (2017) have found that high-intensity PES had no effect on tactile orientation discrimination. However, to our knowledge little is known regarding the effect of low-intensity PES on tactile orientation discrimination. Moreover, the effects of PES may be obscured by subject heterogeneity. A previous study using transcranial magnetic stimulation has reported that PES effectively decreased corticospinal excitability (Fernandez-Del-Olmo et al., 2008), while another neurophysiological study has found that PES using stimulus parameters similar to Fernandez-Del-Olmo et al. (2008) had no effect on corticospinal excitability (Tinazzi et al., 2005). Although high-intensity PES did not appear to influence orientation discrimination, the effects of both high- and low-intensity PES on tactile orientation discrimination may be obscured by subject heterogeneity.

It is well known that perceptual improvement induced by PES is related to changes in excitability and functional circuit organization in primary somatosensory cortex. Godde et al. (1996) have reported that repetitive somatosensory stimulations enlarged cutaneous receptive fields in primary somatosensory cortex, and Pleger et al. (2003) have found that improved tactile spatial two-point discrimination induced by repetitive somatosensory stimulation was related to the enlargement of stimulated digit representation in primary somatosensory cortex.

Recently, it is shown that perceptual improvement induced by PES is related to the activity of inhibitory circuits in primary somatosensory cortex. Rocchi et al. (2017) have shown that PES decreased the paired-pulse somatosensory-evoked potential (SEP) amplitude ratio (somatosensory-evoked potential paired-pulse depression, SEP-PPD), suggesting a contribution from inhibitory circuits in primary somatosensory cortex and that increased SEP-PPD induced by PES can improve performance in a somatosensory temporal discrimination task. Conversely, Höffken et al. (2007) have found that repeated tactile stimulation decreased SEP-PPD, indicating a decrease in inhibitory circuit activity in primary somatosensory cortex. Thus, it is currently unclear how changes in inhibitory circuit activity within primary somatosensory cortex are related to perceptual improvement or disruption. However, considering that decreased SEP-PPD was associated with the worsening of tactile spatial two-point discrimination in elderly subjects (Lenz et al., 2012), increased SEP-PPD may be related to the perceptual improvement in tactile orientation as well as spatial two-point discrimination tasks following PES.

The SEP N20/P25 component has been used to investigate the neurophysiological mechanism underlying perceptual improvement induced by PES and repeated tactile stimulation (Höffken et al., 2007; Rocchi et al., 2017). Considering that N20 is generated by area 3b (Allison et al., 1989) and P25 by areas 1 and 2 (Allison et al., 1991) and 4 (Desmedt and Bourguet, 1985), separate analyses of these two SEP components may help reveal the neurophysiological mechanisms underlying perceptual improvement induced by PES.

The purpose of this study was to investigate the effects of PES on performance of a tactile orientation discrimination task, and the relationship between changes in tactile orientation discrimination and SEP-PPD by individual analyses of N20 and P25 components.

EXPERIMENTAL PROCEDURES

Subjects

Twenty-five neurological normal subjects (age range, 20–33 years; mean ± standard deviation, 22.0 ± 2.5 years; 12 females) participated in this study. Twenty-four subjects were right handed, and one subject was left handed. All subjects provided written informed consent before entering this study. This study was performed in accordance with the Declarations of Helsinki and approved by the ethics committee of Niigata University of Health and Welfare.

PES

PES was applied to the right index finger pad for 30 min using a bipolar electrode connected to an electrical generator (SEN-7203; Nihon Kohden Co., Tokyo, Japan) through an isolator (SS-104; Nihon Kohden Co.). The stimulus intensity was set to either (i) the highest intensity endured without pain minus 0.1 mA (high-intensity PES) or (ii) the lowest intensity that the subjects could perceive plus 0.1 mA (low-intensity PES). A previous study reported that high-intensity PES was effective for improving tactile discrimination performance, while low-intensity PES did not affect performance (Schlieper and Dinse, 2012). Stimulation was delivered in 1-s trains of 20 single electrical pulses (20 Hz) with a pulse width of 0.2 ms and inter-train interval of 5 s based on previous studies (Schlieper and Dinse, 2012; Freyer et al., 2013; Rocchi et al., 2017).

Grating orientation task

Tactile spatial discrimination performance was assessed at the tip of the right index finger by a grating orientation task (GOT) widely accepted as a measurement of somatosensory spacing discrimination performance (Sathian et al., 1997; Goldreich and Kanics, 2003; Ragert et al., 2008a,b). The subjects were comfortably seated blindfolded on a reclining chair and received tactile stimulation from eight hemispherical domes with grooves of different width (3.0, 2.0, 1.5, 1.2, 1.0, 0.75, 0.5, and 0.35 mm). We used a custom-made device that automatically controls up–down movements of the domes for high accuracy and reproducibility of tactile stimulation (S-16026; Takei Scientific Instruments Co. Ltd., Niigata, Japan). The subject's right finger was laid on a hard surface with a 20-mm-diameter hole to allow the stimulation of the index finger by the dome. Elevation speed of the hemispherical dome was set to 20 mm/s, and tactile stimulation duration was set to 1 s. The applied force was set to 1.5 N. The subjects were instructed to place their finger on the device and maintain the initial position for constant stimulation across trials.

Measurement of PPD

Inhibitory mechanisms in primary somatosensory cortex were evaluated by a paired-pulse protocol. Paired-pulse stimuli with an interpulse interval (IPI) of 100 ms were delivered to the median nerve at the right wrist using a bipolar electrode connected to an electrical generator through an isolator. The interstimulus interval between each stimulus was 3 s. The stimulus intensity was set to 120% of the motor threshold, which was defined as the lowest stimulation that induced a visible muscular twitch in the thenar muscle. The pulse duration was set to 0.2 ms.

The somatosensory-evoked potential (SEP) signals were recorded using EPLYZER II (KISSEI COMTEC Co. Ltd., Nagano, Japan). The active electrode was located 2 cm posterior to C3 (C3′), and the reference electrode was located at the midfront (Fz) position according to the international 10–20 system (Klem et al., 1999). During SEP signal recording, the subjects were comfortably seated on a reclining chair in a shield room. The SEP signals were recorded from 50 ms before to 200 ms after the stimuli at a sampling rate of 5 kHz.

Experimental procedure

The subjects received the following stimulus conditions: (i) high-intensity PES and (ii) low-intensity PES (Fig. 1). The PES sessions were separated by at least 3 days, and the order was counterbalanced among the subjects.

In Experiment 1, GOT was performed before and immediately after PES. Each GOT consisted of 160 trials (20 trials for two orientations × 8 different groove widths). The domes were presented in following order: 3.0, 2.0, 1.5, 1.2, 1.0, 0.75, 0.5, and 0.35 mm. During trials, domes were randomly oriented orthogonal or parallel to the long axis of the index finger. Each dome was presented 10 times in both orientations. After the dome touched the index finger pad, the subjects were required to judge the dome orientation and press one button to indicate that they perceived the orthogonal direction or another when they perceived the parallel direction.

In Experiment 2, PPD was measured before and immediately after PES. In each measurement block, single- and paired-pulse stimuli were delivered 200 times individually. The stimulus order was counterbalanced among subjects.

Data analysis

In Experiment 1, we analyzed the percentage of correct responses at each grating width. Grating width was also plotted against the percentage of correct responses and fitted by logistic regression based on a generalized linear model. The linear regression coefficient was calculated using the following equation:

$$K_1 + K_2X = \log(Y/1 - Y)$$

K: linear regression coefficient;
X: grating width;
Y: correct response rate.

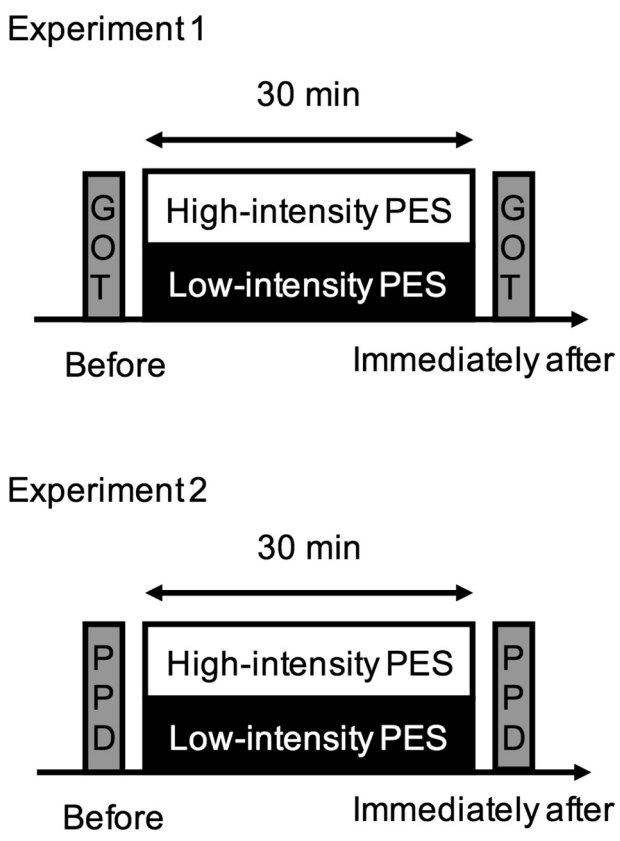

Fig. 1. Schemata of the two experiments. Experiments were conducted under the following stimulus conditions: (i) high-intensity PES and (ii) low-intensity PES. The order of PES sessions was counterbalanced among the subjects. In Experiment 1, GOT was performed before and immediately after PES. In Experiment 2, PPD of the somatosensory-evoked potential was measured before and immediately after PES. PES, peripheral electrical stimulation; GOT, grating orientation task; PPD, paired-pulse depression.

In this study, the values of the linear regression coefficients (K_2) were considered with tactile sensitivity. In addition, the grating orientation discrimination threshold was calculated using the following equation:

$$\text{Threshold} = (\log(0.75/1 - 0.75) - K_1)/K_2$$

We first examined whether the percentage of correct responses, grating orientation discrimination threshold, the value of linear regression coefficient, and changes in grating orientation discrimination threshold and regression coefficients (e.g., following PES) fit a normal distribution using the Shapiro–Wilk test. Using the Smirnov–Grubbs test, we examined whether the percentage of correct responses, grating orientation discrimination threshold, and linear regression coefficient value were outliers; subsequently, we eliminated the outliers. The percentage of correct responses was analyzed by a three-way repeated measures analysis of variance (ANOVA), with the main factors being stimulus intensity (high- or low-intensity PES), grating width (3.0, 2.0, 1.5, 1.2, 1.0, 0.75, 0.5, or 0.35 mm), and time (before or immediately after PES). We compared the effect of PES on the grating orientation discrimination threshold and linear regression

coefficient value using analysis of covariance (ANCOVA), with each data immediately after and before PES as dependent variable and covariate, respectively. In addition, we tested the correlation between the value of linear regression coefficient before PES and changes in the value of the linear regression coefficient induced by PES as well as the correlation between the grating orientation discrimination threshold before PES and the changes in grating orientation discrimination threshold after PES employing Spearman's rank correlation coefficients. The 25 subjects were divided into two groups according to the change in grating orientation discrimination threshold induced by PES: a GOT improvement group exhibiting decreased grating orientation discrimination threshold and a GOT decrement group exhibiting increased grating orientation discrimination threshold. The changes in the percentage of correct response at all grating widths in the GOT improvement and decrement groups were analyzed using the Friedman test. Further, we compared the changes in the percentage of correct response among each grating width using the Friedman test. In addition, the changes in the percentage of correct responses at each grating width were compared between the GOT improvement and decrement groups using the Mann–Whitney test. The grating orientation discrimination threshold before and immediately after high-intensity PES was analyzed in GOT improvement and decrement groups using the Friedman test. Further, we compared the grating orientation discrimination threshold between before and immediately after high-intensity PES using the Mann–Whitney test. In addition, the 25 subjects were divided into two groups according to GOT discrimination threshold before PES: a low performance group exhibiting a GOT discrimination threshold larger than median value and high performance group exhibiting a threshold smaller than the median value.

In Experiment 2, we analyzed the peak-to-peak amplitude of N20/P25 and the peak amplitudes of N20 and P25 in response to the first pulse of the paired-pulse stimulus (A1) directly from the SEP waveform. The peak-to-peak amplitude of N20/P25 and peak amplitudes of N20 and P25 in response to the second pulse of the paired-pulse stimulus (A2) were acquired from the SEP waveform of the paired-pulse minus that recorded for a single-pulse paradigm. The values for N20/P25_SEP-PPD, N20_SEP-PPD and P25_SEP-PPD are expressed as the ratios of the SEP amplitude of the second to the first response (A2/A1). We examined whether each SEP-PPD, the peak-to-peak amplitude of N20/P25, and the peak amplitudes of N20 and P25 in response to the first and second pulses of the paired-pulse stimulus were normally distributed using the Shapiro–Wilk test and whether these in response to the first and second pulses of the paired-pulse stimulus were outliers using Smirnov–Grubbs test, and we subsequently eliminated the outliers. We compared the effect of PES on N20/P25_SEP-PPD, N20_SEP-PPD, and P25_SEP-PPD using ANCOVA, with each SEP-PPD data immediately after and before PES as dependent variable and covariates, respectively. The peak-to-peak amplitude of N20/P25 and the peak amplitudes of N20 and P25 in response to the first and second pulses of the paired-pulse stimulus were analyzed by a two-way ANOVA, with the main factors being stimulus intensity (high- or low-intensity PES) and time (before or immediately after PES). In addition, we examined whether N20/P25_SEP-PPD, N20_SEP-PPD, and P25_SEP-PPD in the GOT improvement and decrement groups were normally distributed using the Shapiro–Wilk test. We analyzed the baseline N20/P25_SEP-PPD, N20_SEP-PPD, and P25_SEP-PPD using a two-way ANOVA with main factors stimulation intensity (high-intensity PES or low-intensity PES) and group (GOT improvement group or GOT decrement group). We then compared N20/P25_SEP-PPD, N20_SEP-PPD, and P25_SEP-PPD before and immediately after PES in both the GOT improvement group and GOT decrement groups using Mann–Whitney test. We examined whether the peak amplitudes of N20 in response to both the first and second pulse of the paired-pulse stimulus were normally distributed using the Shapiro–Wilk test. To analyze changes in N20_SEP-PPD induced by high-intensity PES in both GOT improvement and GOT decrement groups, we compared the peak amplitudes of N20 in response to the first pulse of the paired-pulse stimulus (A1) and the second N20 response of the paired-pulse minus single-pulse stimulus (A2) before and immediately after PES using the Wilcoxon signed-rank test. In addition, using the Shapiro–Wilk test, we examined whether the changes in the grating orientation discrimination threshold and linear regression coefficients (e.g., following PES) and those in N20_SEP-PPD in the low and high GOT performance groups fit a normal distribution. Further, we analyzed the correlation between the PES-induced changes in grating orientation discrimination threshold and N20_SEP-PPD in GOT improvement and decrement groups and between the changes in the linear regression coefficient values after PES and those in N20_SEP-PPD after PES using Pearson correlation coefficients or Spearman's rank correlation coefficient. We also tested the correlation between the change in grating discrimination threshold and that in the coefficient of linear regression induced by PES and the change in N20_SEP-PPD induced by PES using the Pearson correlation coefficients or Spearman's rank correlation coefficient in low performance and high performance groups. Finally, we examined whether PES intensity was normally distributed in each condition using the Shapiro–Wilk test and compared high-intensity to low-intensity PES in each experiment using the Wilcoxon signed-rank test.

All statistical analyses were performed using SPSS ver25 for Mac. Statistical significance was defined as $P < 0.05$.

RESULTS

Stimulus intensity

In Experiment 1, average high-intensity PES was significantly greater than the average low-intensity PES

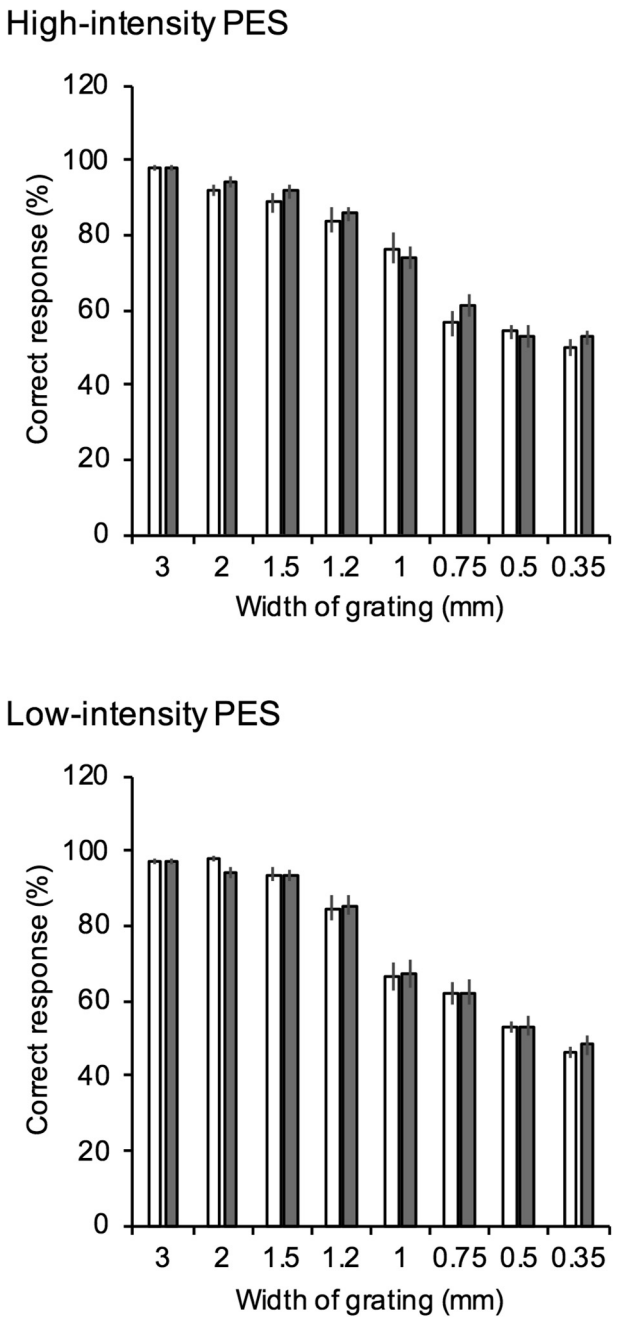

Fig. 2. Comparison of correct response rate (%) to grating orientation (parallel or orthogonal) at all grating widths before (baseline) and immediately after high-intensity PES (upper) and low-intensity PES (lower). For the entire cohort, correct response rate was not altered after PES at any grating width. PES, peripheral electrical stimulation.

(13.6 ± 2.4 mA vs. 3.3 ± 1.1 mA; $t_{(25)} = -4.373$, $P < 0.001$, $r = 0.88$). Similarly in Experiment 2, high-intensity PES was significantly greater than low-intensity PES (14.4 ± 2.1 mA vs. 3.8 ± 1.0 mA; $t_{(25)} = -4.374$, $P < 0.001$, $r = 0.88$).

Effect of PES on perceptual performance in the GOT (Experiment 1)

The effects of PES on correct response rate (%) at all grating widths are shown in Fig. 2 for the entire subject cohort. As expected, the three-way repeated measures ANOVA indicated a significant effect of grating width ($F_{(7,\ 105)} = 155.565$, $P = 1.94e{-}52$) on the correct response rate but no significant effect of stimulus intensity ($F_{(1,\ 15)} = 0.060$, $P = 0.809$) or time ($F_{(1,\ 15)} = 0.590$, $P = 0.454$); in addition, although it showed significant interactions between stimulus intensity and time ($F_{(1,\ 15)} = 7.039$, $P = 0.018$) and between stimulus intensity and grating width ($F_{(7,\ 105)} = 2.724$, $P = 0.012$), it showed no significant interaction between time and grating width ($F_{(7,\ 105)} = 0.477$, $P = 0.849$) and among stimulus intensity, grating width, and time ($F_{(7,\ 105)} = 0.348$, $P = 0.930$). Neither high- nor low-intensity PES showed significant effects on correct response rate at any width for the entire cohort. Table 1 summarizes the effects of PES on grating orientation discrimination threshold and linear regression coefficient for the entire subject cohort. Notably, ANCOVA revealed no significant effect of PES on the grating orientation discrimination threshold ($F_{(1,\ 43)} = 0.002$, $P = 0.963$) and linear regression coefficient value ($F_{(1,\ 44)} = 1.380$, $P = 0.246$), indicating no significant difference in terms of high- and low-intensity PES effects on the grating orientation discrimination threshold and linear regression coefficient.

It is possible, however, that effects of PES are obscured by subject heterogeneity. Therefore, we analyzed the association between baseline orientation discrimination threshold and the change induced by PES (i.e., the differential effect of PES according to individual perceptual performance ability in the GOT) (Fig. 3). A negative correlation was observed between the change in grating orientation discrimination threshold induced by high-intensity PES and grating orientation discrimination threshold at baseline (Spearman's $R = -0.604$, $P = 0.001$). In contrast, we found no significant correlation between change in grating orientation discrimination threshold induced by low-intensity PES and grating orientation discrimination threshold at baseline (Spearman's $R = -0.180$, $P = 0.389$).

Table 1. Effect of PES on grating orientation discrimination threshold and linear regression coefficient

		GOT discrimination threshold (mm)	Linear regression coefficient
High-intensity PES	Before	1.04 ± 0.05	1.77 ± 0.16
	Immediately after	1.01 ± 0.04	1.88 ± 0.16
Low-intensity PES	Before	1.00 ± 0.04	2.04 ± 0.16
	Immediately after	1.00 ± 0.04	1.76 ± 0.15
			Average ± SEM

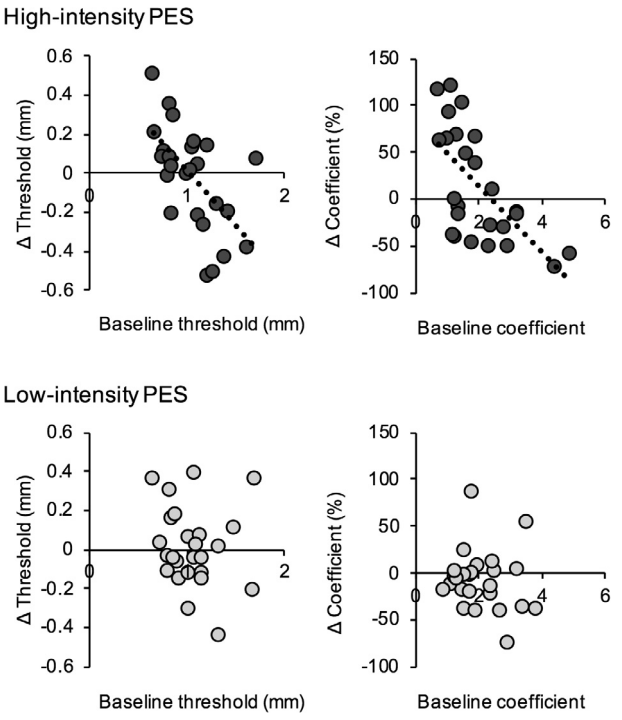

Fig. 3. Correlation between the change in grating orientation discrimination threshold induced by PES and baseline grating orientation discrimination threshold for high-intensity PES (upper) and low-intensity PES conditions (lower) in each subject (n = 25). A negative correlation was observed between the change in grating orientation performance (grating orientation discrimination threshold and linear regression coefficient) and grating orientation performance at baseline in the high-intensity PES condition. PES, peripheral electrical stimulation.

Furthermore, we found a negative correlation between the change in linear regression coefficient induced by high-intensity PES and the linear regression coefficient at baseline (Spearman's $R = -0.626$, $P = 0.001$). In contrast, we found no significant correlation between changes in linear regression coefficient induced by low-intensity PES and linear regression coefficient at baseline (Spearman's $R = -0.078$, $P = 0.709$). These results indicated that high-intensity PES was effective in improving perceptual performance in GOT (i.e., lowering the orientation discrimination threshold), but only in subjects with high baseline grating orientation discrimination threshold (i.e., low baseline GOT performance). In contrast, the effect of low-intensity PES on GOT performance was not associated with baseline GOT performance.

To analyze the differential effect of PES intensity on perceptual performance in greater detail, we classified the subjects into two groups: those showing a decrease in grating orientation discrimination threshold following PES (GOT improvement group) and those showing an increase in grating orientation discrimination threshold following PES (GOT decrement group). In the high-intensity PES condition, 11 subjects were included in the GOT improvement group and the remaining 14 subjects in the GOT decrement group. In the low-intensity PES condition, 13 subjects were included in the GOT improvement group and the remaining 12 subjects were included in the GOT decrement group.

We then compared the effects of PES on GOT performance between the GOT improvement group and GOT decrement group. Table 2 summarizes the effects of high-intensity PES on the grating orientation discrimination threshold for the complete subject cohort. We found significant differences in terms of the grating discrimination threshold between the values before and immediately after high-intensity PES and/or between the GOT improvement and decrement groups ($F_{(11)} = 19.691$, $P \leq 0.01$). In the GOT improvement group, the grating discrimination threshold immediately after high-intensity PES was significantly lower than the baseline threshold ($t_{(11)} = -2.934$, $P \leq 0.01$, $r = 0.89$). On the other hand, the grating discrimination threshold immediately after high-intensity PES was significantly higher than baseline threshold in the GOT decrement group ($t_{(14)} = 3.296$, $P \leq 0.01$, $r = 0.88$). Changes in correct response rate induced by PES at all grating widths are shown in Fig. 4. The Friedman test revealed significant difference in terms of the changes in the correct response rate following high-intensity PES at each grating width between the GOT improvement and decrement groups and/or the changes in the correct response rate among each grating width ($\chi^2_{(11)} = 29.046$, $P \leq 0.05$). Notably, we found no significant differences in terms of the changes in the correct response rate following high-intensity PES among each grating width (GOT improvement group: $\chi^2_{(11)} = 3.882$, $P = 0.793$; GOT decrement group: $\chi^2_{(14)} = 11.389$, $P = 0.123$). The change in the correct response rate induced by high-intensity PES significantly differed between the GOT improvement and decrement groups when the grating width was 1.2 or 1.5 mm (1.2 mm: $U_{(11,\ 14)} = -2.309$, $P \leq 0.05$, $r = 0.44$; 1.5 mm: $U_{(11,\ 14)} = -2.180$, $P \leq 0.05$, $r = 0.46$; Mann–Whitney test). On the other hand, the Friedman test revealed significant differences in terms of the changes in the correct response rate following low-intensity PES at each grating width between the GOT improvement and decrement groups and/or the changes in the correct response rate among each grating width ($\chi^2_{(12)} = 25.823$, $P \leq 0.05$). We found significant effect of

Table 2. Effect of high-intensity PES on grating orientation threshold in GOT improvement group and GOT decrement group

		Before (mm)	Immediately after (mm)
High-intensity PES	GOT improvement group	1.22 (1.06–1.35)	0.91 (0.78–1.07)
	GOT decrement group	0.86 (0.78–1.09)	1.16 (0.89–1.20)
			Median (interquartile range)

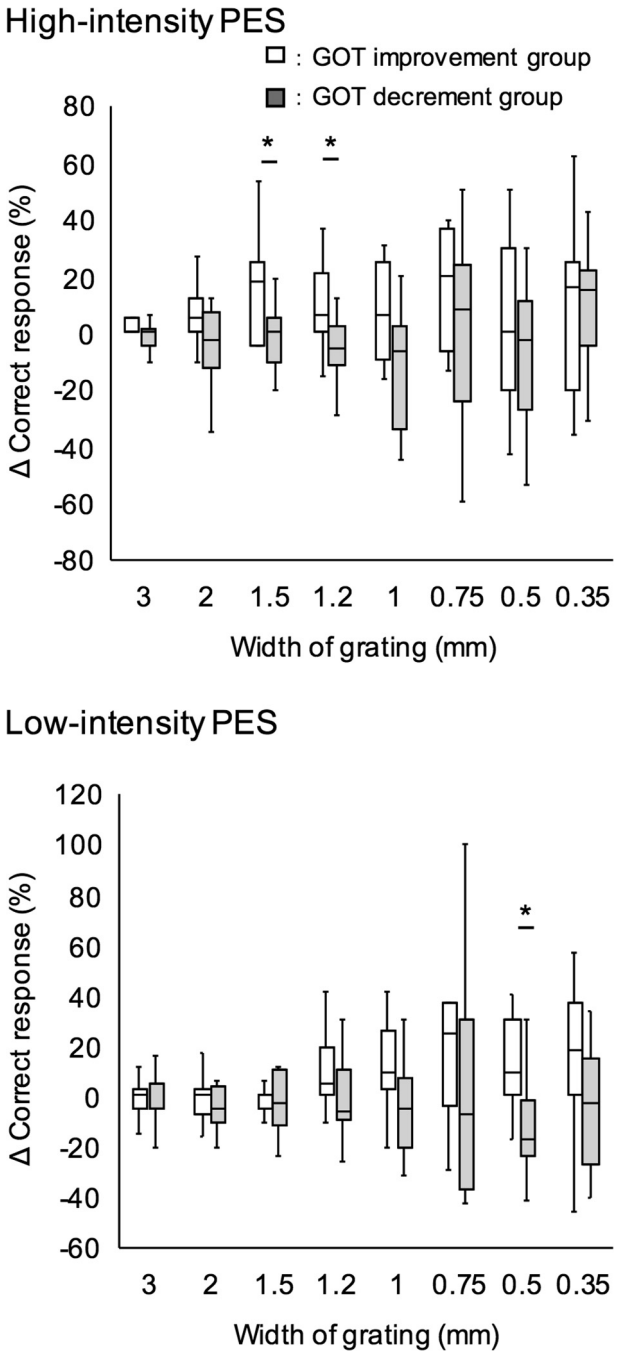

Fig. 4. Comparison of changes in correct response rate (%) induced by high-intensity PES (upper) and low-intensity PES (lower) at all grating widths between the GOT improvement group (white boxes) and GOT decrement group (gray boxes). Changes in correct response rate induced by high-intensity PES differed significantly between GOT improvement and GOT decrement groups at grating widths of 1.5 and 1.2 mm. On the other hand, changes in correct response rate induced by low-intensity PES differed significantly between GOT improvement and GOT decrement groups only at a grating width of 0.5 mm. $^{*}P < 0.05$. PES, peripheral electrical stimulation; GOT, grating orientation task.

grating width on the changes in the correct response rate following low-intensity PES in the GOT improvement group ($\chi^2_{(13)} = 20.248$, $P \leq 0.01$; Friedman test) but no significant differences in terms of the changes in the correct response rate following low-intensity PES in the GOT improvement group among each grating width (all $P > 0.05$; Wilcoxon signed-rank test with Bonferroni correction). In addition, we found no significant effect of grating width on the changes in the correct response rate following low-intensity PES in the GOT decrement group ($\chi^2_{(12)} = 3.933$, $P = 0.787$). The changes in the correct response rate induced by low-intensity PES significantly differed between the GOT improvement and decrement groups when the grating width was 0.5 mm ($U_{(13,\ 12)} = -2.620$, $P \leq 0.01$, $r = 0.52$; Mann–Whitney test). These results indicate that high-intensity PES is more effective at improving GOT performance at grating widths of 1.2 and 1.5 mm in the GOT improvement group than in the GOT decrement group.

Effect of PES on N20/P25_PPD and N20_SEP-PPD (Experiment 2)

The effects of PES on N20/P25_SEP-PPD, N20_SEP-PPD, and P25_SEP-PPD are summarized in Table 3. ANCOVA also did not reveal a significant effect of PES on each SEP-PPD (N20/P25_SEP-PPD: $F_{(1,\ 47)} = 0.191$, $P = 0.664$; N20_SEP-PPD: $F_{(1,\ 47)} = 0.475$, $P = 0.494$; and P25_SEP-PPD: $F_{(1,\ 47)} = 0.047$, $P = 0.829$, respectively). The effects of PES on the peak-to-peak amplitude of N20/P25 and the peak amplitudes of N20 and P25 in response to the first and second pulses of the paired-pulse stimulus are summarized in Table 4. Two-way ANOVA revealed no significant effect of stimulus intensity on the peak-to-peak amplitude of N20/P25 (first SEP, $F_{(1,\ 96)} = 0.002$, $P = 0.968$; second SEP, $F_{(1,\ 96)} = 0.306$, $P = 0.581$), the peak amplitudes of N20 (first SEP, $F_{(1,\ 96)} = 0.054$, $P = 0.816$; second SEP, $F_{(1,\ 96)} = 0.005$, $P = 0.946$), or the peak amplitudes of P25 (first SEP, $F_{(1,\ 96)} = 0.039$, $P = 0.845$; second SEP, $F_{(1,\ 96)} = 0.537$, $P = 0.465$) and no significant effect of time on the peak-to-peak amplitude of N20/P25 (first SEP, $F_{(1,\ 96)} = 0.179$, $P = 0.673$; second SEP, $F_{(1,\ 96)} = 0.387$, $P = 0.535$), the peak amplitude of N20 (first SEP, $F_{(1,\ 96)} = 0.056$, $P = 0.813$; second SEP, $F_{(1,\ 96)} = 6.0e^{-6}$, $P = 0.998$), or the peak amplitude of P25 (first SEP, $F_{(1,\ 96)} = 0.183$, $P = 0.669$; second SEP, $F_{(1,\ 96)} = 0.671$, $P = 0.415$). In addition, we found no significant correlation between stimulus intensity and time on the peak-to-peak amplitude of N20/P25 (first SEP, $F_{(1,\ 96)} = 0.351$, $P = 0.555$; second SEP, $F_{(1,\ 96)} = 0.068$, $P = 0.795$), the peak amplitude of N20 (first SEP, $F_{(1,\ 96)} = 0.012$, $P = 0.915$; second SEP, $F_{(1,\ 96)} = 0.001$, $P = 0.977$), or the peak amplitude of P25 (first SEP, $F_{(1,\ 96)} = 0.526$, $P = 0.470$; second SEP, $F_{(1,\ 96)} = 0.142$, $P = 0.707$).

To examine the mechanism underlying changes in perceptual performance induced by high-intensity PES, we separately analyzed the effects of PES on N20/P25_SEP-PPD, N20_SEP-PPD, and P25_SEP-PPD in the GOT improvement and GOT decrement groups (Figs. 5 and 6). As expected, the two-way ANOVA revealed no significant effect of stimulus intensity on baseline N20/P25_SEP-PPD ($F_{(1,\ 10)} = 0.006$, $P = 0.938$), at baseline N20_SEP-PPD ($F_{(1,\ 10)} = 2.968$, $P = 0.116$), or baseline P25_SEP-PPD ($F_{(1,\ 10)} = 0.157$,

Table 3. Effect of PES on N20/P25_SEP-PPD, N20_SEP-PPD and P25_SEP-PPD

	High-intensity PES			Low-intensity PES		
	N20/P25	N20	P25	N20/P25	N20	P25
Before	0.72 ± 0.04	0.69 ± 0.04	0.74 ± 0.06	0.71 ± 0.03	0.74 ± 0.05	0.70 ± 0.05
Immediately after	0.72 ± 0.04	0.65 ± 0.05	0.77 ± 0.06	0.73 ± 0.03	0.70 ± 0.06	0.73 ± 0.05
					Mean ± SEM (ratio)	

Table 4. Effect of PES on the peak-to-peak amplitude of N20/P25 and peak amplitudes of N20 and P25 in response to both first and second pulses of the paired-pulse stimulus

		N20		P25		N20/P25	
		First SEP	Second SEP	First SEP	Second SEP	First SEP	Second SEP
High-intensity PES	Before	2.97 ± 0.23	2.11 ± 0.23	5.49 ± 0.43	3.99 ± 0.43	8.47 ± 0.56	6.12 ± 0.50
	Immediately after	3.07 ± 0.29	2.11 ± 0.28	6.04 ± 0.47	4.45 ± 0.41	9.11 ± 0.61	6.55 ± 0.54
Low-intensity PES	Before	2.94 ± 0.29	2.12 ± 0.21	5.93 ± 0.53	3.86 ± 0.34	8.87 ± 0.66	8.76 ± 0.68
	Immediately after	2.97 ± 0.30	2.13 ± 0.27	5.79 ± 0.48	4.03 ± 0.33	8.76 ± 0.68	6.16 ± 0.47
						Mean ± SEM (μV)	

$P = 0.700$) and no significant effect of group on baseline N20/P25_SEP-PPD ($F_{(1,\ 10)} = 0.011$, $P = 0.919$), baseline N20_SEP-PPD ($F_{(1,\ 10)} = 1.994$, $P = 0.188$), or baseline P25_SEP-PPD ($F_{(1,\ 10)} = 0.052$, $P = 0.825$). In addition, we found no significant correlation between PES intensity and group (baseline N20/P25_SEP-PPD, $F_{(1,\ 10)} = 0.653$, $P = 0.438$; baseline N20_SEP-PPD, $F_{(1,\ 10)} = 0.621$, $P = 0.449$; baseline P25_SEP-PPD, $F_{(1,\ 10)} = 0.375$, $P = 0.554$). In separate group analyses, we found no effect of PES on N20/P25_SEP-PPD or P25_SEP-PPD in either the GOT improvement or GOT decrement group (all $P > 0.05$, Wilcoxon signed-rank test). However, high-intensity PES significantly reduced N20_SEP-PPD in the GOT improvement group ($t_{(11)} = 2.401$, $P \leq 0.05$, $r = 0.72$, Wilcoxon signed-rank test) and significantly increased N20_SEP-PPD in the GOT decrement group ($t_{(14)} = -2.794$, $P \leq 0.01$, $r = 0.75$, Wilcoxon signed-rank test). Conversely, low-intensity PES has no effect on any SEP-PPD value in the GOT improvement group (all $P > 0.05$, Wilcoxon signed-rank test). While low-intensity PES had no effect on N20/P25_SEP-PPD and N20_SEP-PPD in the GOT decrement group (all $P > 0.05$, Wilcoxon signed-rank test), P25_SEP-PPD after low-intensity PES was higher than that at the baseline ($t_{(12)} = 2.040$, $P \leq 0.05$, $r = 0.59$, Wilcoxon signed-rank test). These results indicate that high-intensity PES differentially modulates N20_SEP-PPD and that the direction of change is associated with the effect of PES on perceptual performance (reduced N20_SEP-PPD associated with improved GOT performance and increased N20_SEP-PPD associated with reduced GOT performance).

SEP-PPD can be altered by changes in either the first response amplitude (A1) or the second (A2). To reveal the specific cortical response altered by high-intensity PES, we analyzed the effects on the first and second N20 responses. Grand averaged SEP waveforms induced by single- and paired-pulse stimuli applied to the median nerve in each PES condition are presented in Fig. 7, and the effects of high- and low-intensity PES on the first (A1) and second response (A2) for N20 the component are shown in Fig. 8. High-intensity PES had no effect on the first N20 response in either GOT performance group (GOT improvement group: $t_{(11)} = 0.889$, $P = 0.374$, GOT decrement group: $t_{(14)} = 0.031$, $P = 0.975$; Wilcoxon signed-rank test). However, high-intensity PES significantly increased the second N20 component in the GOT improvement group ($t_{(11)} = 2.934$, $P \leq 0.01$, $r = 0.89$; Wilcoxon signed-rank test), thereby reducing N20_SEP-PPD, and decreased the second N20 response in the GOT decrement group ($t_{(14)} = -2.103$, $P \leq 0.05$, $r = 0.56$; Wilcoxon signed-rank test), thereby increasing N20_SEP-PPD. Alternatively, there was no effect of low-intensity PES on N20 components in response to the first or second pulse in either group (all $P > 0.05$; Wilcoxon signed-rank test). These results suggest that a differential change in the second N20 response may account for the unique perceptual responses of the GOT decrement and GOT improvement groups.

Correlation between GOT performance and N20_SEP-PPD (Experiment 1 and 2)

To provide further evidence that distinct changes in N20_SEP-PPD account for the difference in perceptual response between GOT performance groups following PES, we analyzed the association between the change in GOT performance and the change in N20_SEP-PPD induced by high-intensity PES in both GOT improvement and GOT decrement groups (Fig. 9). In the GOT improvement group, a negative correlation was observed between changes in discrimination threshold and N20_SEP-PPD induced by high-intensity PES (Spearman's $R = -0.627$, $P \leq 0.05$). Furthermore, a

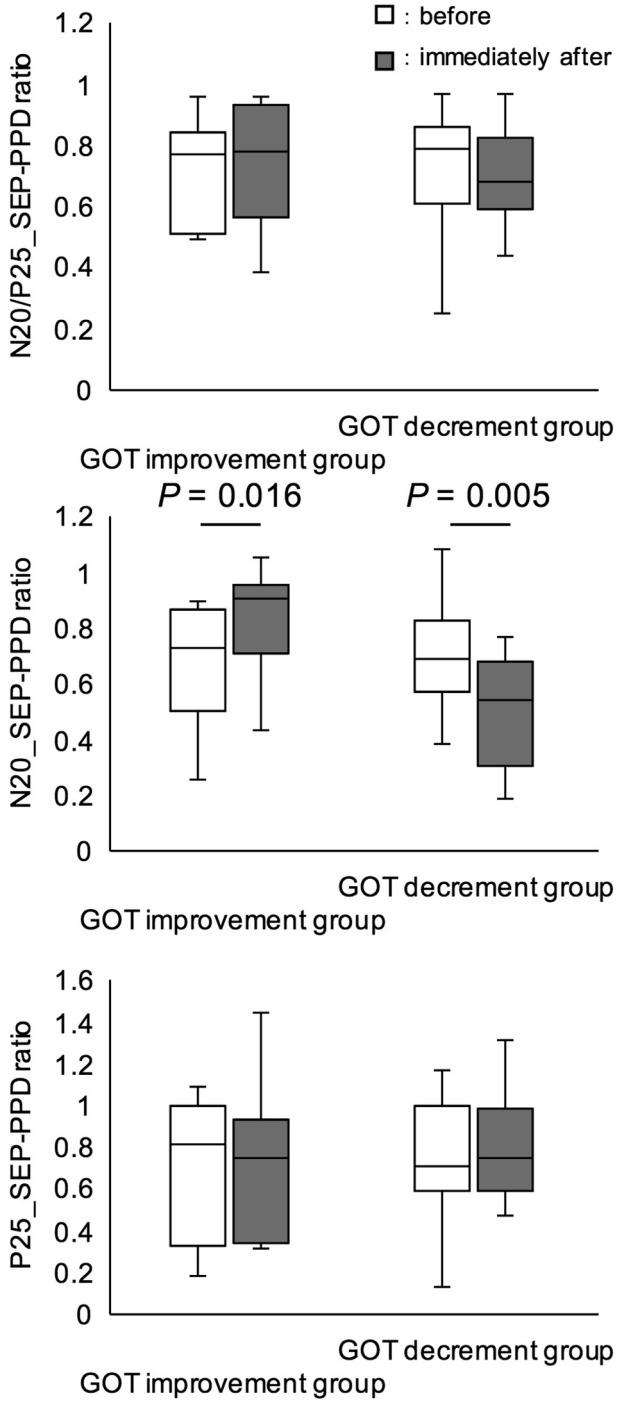

Fig. 5. Effect of high-intensity PES on N20/P25_SEP-PPD (upper), N20_SEP-PPD (bottom), and P25_SEP-PPD (lower) in each group (white boxes: before PES, gray box: immediately after PES). High-intensity PES significantly decreased N20_SEP-PPD in the GOT improvement group, and significantly increased N20_SEP-PPD in the GOT decrement group. PES, peripheral electrical stimulation; GOT, grating orientation task; SEP-PPD, somatosensory-evoked potential paired-pulse depression.

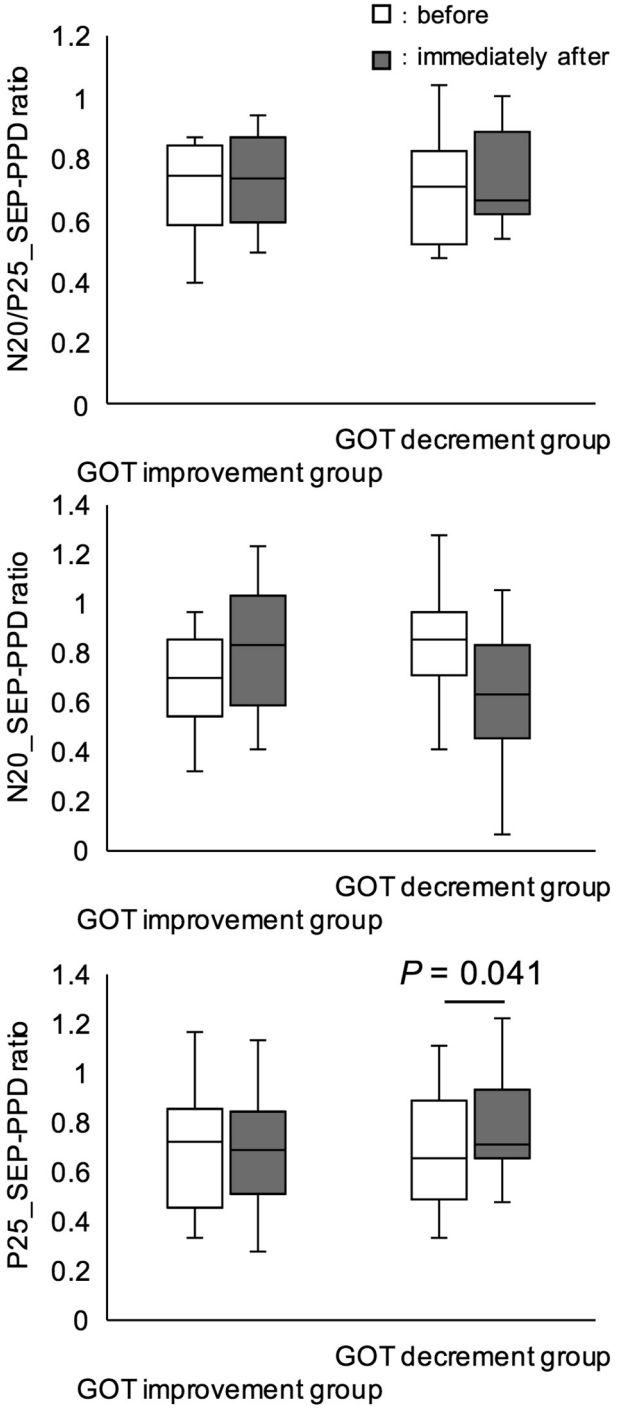

Fig. 6. Effect of low-intensity PES on N20/P25_SEP-PPD (upper), N20_SEP-PPD (bottom), and P25_SEP-PPD (lower) in each group (white box: before PES, gray box: immediately after PES). Low-intensity PES had no effect on each SEP-PPD in each group. PES, peripheral electrical stimulation; GOT, grating orientation task; SEP-PPD, somatosensory-evoked potential paired-pulse depression.

positive correlation was observed between the change in linear regression coefficient induced by high-intensity PES and the change in N20_SEP-PPD induced by high-intensity PES (Spearman's $R = 0.882$, $P \leq 0.01$). These results indicate that a larger decrease in SEP-PPD (higher A2/A1 ratio) led to improved perceptual performance. On the other hand, we found no significant correlation between grating orientation discrimination threshold and N20_SEP-PPD (Spearman's $R = 0.086$, $P = 0.771$), and no significant correlation between the change in linear regression

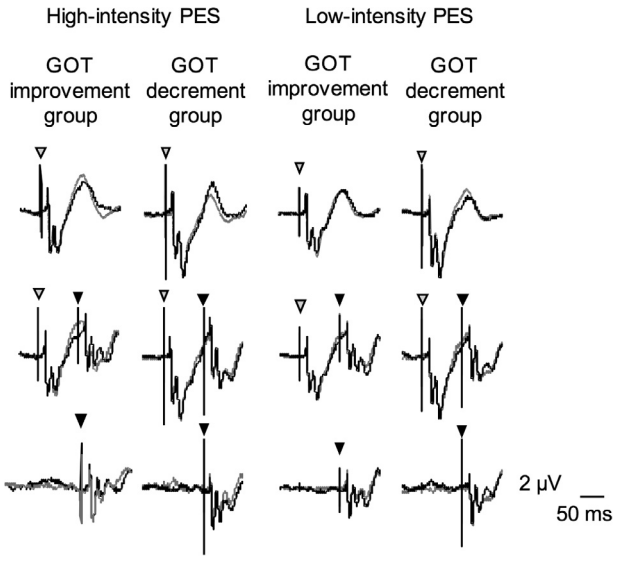

Fig. 7. Grand averaged SEP waveforms induced by single- and paired-pulse stimuli applied to the median nerve under the high-intensity PES condition (left) and the low-intensity PES condition (right) (gray line: before PES, black line: immediately after PES). The gray triangles indicate the first stimulus onset and the black triangles indicate the second stimulus onset. PES, peripheral electrical stimulation; SEP, somatosensory-evoked potential.

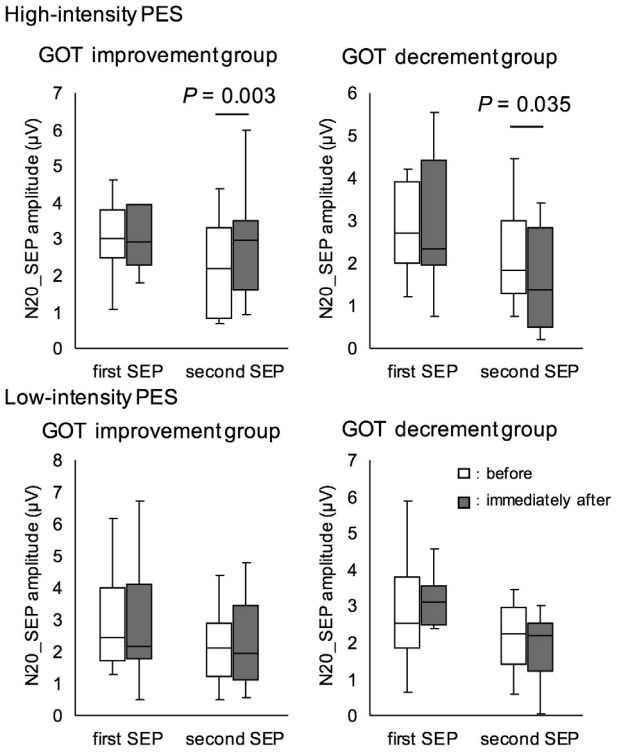

Fig. 8. Effect of high-intensity PES (upper) and low-intensity PES (lower) on the SEP N20 component evoked by the first and second pulses of the paired-pulse stimulus in the GOT improvement group (left) and GOT decrement group (right) (white boxes: before PES, gray box: immediately after PES). High-intensity PES significantly increased the N20 component in response to the second pulse in the GOT improvement group, but significantly decreased the N20 component in response to the second pulse in the GOT decrement group. PES, peripheral electrical stimulation; GOT, grating orientation task.

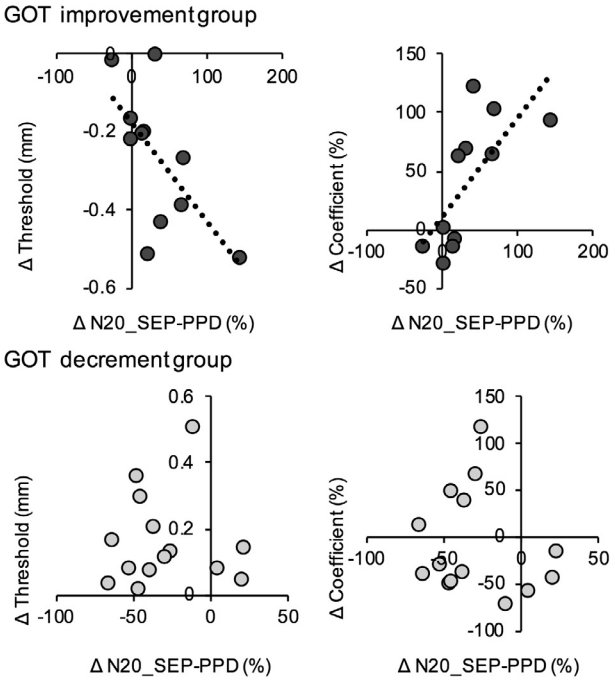

Fig. 9. Correlation between change in grating orientation performance and change in N20_SEP-PPD induced by high-intensity PES for each subject (n = 11) in the GOT improvement group (upper) and for each subject (n = 14) in the GOT decrement group (lower). A negative correlation was observed between the change of grating orientation discrimination threshold and the N20_SEP-PPD change in the GOT improvement group. Furthermore, a positive correlation was observed between the change of linear regression coefficient and the N20_SEP-PPD change in the GOT improvement group. PES, peripheral electrical stimulation; GOT, grating orientation task; SEP-PPD, somatosensory-evoked potential paired-pulse depression.

coefficient induced by high-intensity PES and the change in N20_SEP-PPD induced by high-intensity PES (Pearson's $R = -0.113$, $P = 0.703$) in the GOT decrement group. These results indicate that the improved tactile performance after high-intensity PES in the GOT improvement group is strongly related to a reduction in N20_SEP-PPD.

To provide further evidence that high-intensity PES differentially modulates perceptual performance and N20_SEP-PPD depending on baseline perceptual performance, we analyzed the association between the change in GOT performance and the change in N20_SEP-PPD induced by high-intensity PES in each baseline performance group (Fig. 10). A negative correlation was revealed between the change in grating orientation discrimination threshold induced by high-intensity PES and the change in N20_SEP-PPD induced by high-intensity PES in the low baseline performance group (Pearson's $R = -0.738$, $P \leq 0.01$). In addition, a positive correlation was revealed between the change in the linear regression coefficient induced by high-intensity PES and the change in N20_SEP-PPD induced by high-intensity PES in the low performance group (Spearman's $R = 0.555$, $P \leq 0.05$). Conversely, we found no significant correlation between the change in grating orientation discrimination threshold induced by high-intensity PES and change in N20_SEP-PPD

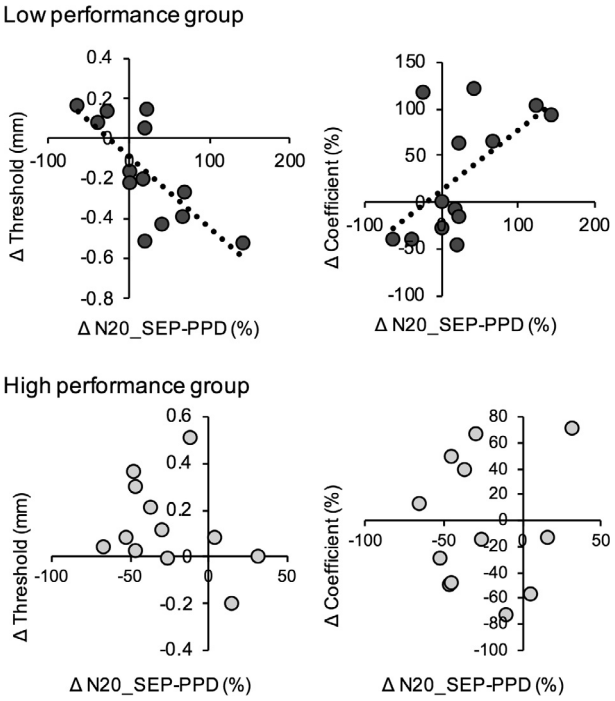

Fig. 10. Correlation between change in grating orientation performance and change in N20_SEP-PPD induced by high-intensity PES in the baseline low performance group (upper) observed for each subject (n = 13) and baseline high performance group (lower) observed for each subject (n = 12). A negative correlation was observed between the change of grating orientation discrimination threshold and the N20_SEP-PPD change in the low performance group. Furthermore, a positive correlation was observed between the change of linear regression coefficient and the N20_SEP-PPD change in the low performance group. PES, peripheral electrical stimulation; SEP-PPD, somatosensory-evoked potential paired-pulse depression.

induced by high-intensity PES (Pearson's $R = -0.294$, $P = 0.353$) or between the change in linear regression coefficient induced by high-intensity PES and the change in N20_SEP-PPD induced by high-intensity PES (Spearman's $R = 0.081$, $P = 0.803$) in the high performance group. These results indicate that the improved tactile performance after high-intensity PES in the low baseline performance group is strongly related to the reduction in N20_SEP-PPD.

DISCUSSION

There are three important findings from this study. First, high-intensity PES effectively improved GOT performance in subjects with low GOT performance at baseline. Second, high-intensity PES effectively decreased N20_SEP-PPD in the GOT improvement group, but increased N20_SEP-PPD in the GOT decrement group. Third, improved GOT performance following high-intensity PES was correlated with the magnitude of N20_SEP-PPD reduction in the GOT improvement group and low baseline performance group. Thus, appropriate PES can improve GOT performance in individuals with low baseline ability, possibly by suppressing SEP-PPD in specific regions of somatosensory cortex.

Effect of PES on perceptual performance in a GOT

High-intensity PES improved perceptual performance in subjects with low GOT performance at baseline. Similarly, Dinse et al. (2006) reported that repeated tactile stimulation (analogous to PES) improved tactile spatial two-point discrimination of the stimulated finger when discrimination performance was low at baseline. Collectively, these results indicate that the differential effect of high-intensity PES on perceptual performance is related to individual baseline perceptual performance. In contrast, a previous study has found no effect of high-intensity PES on GOT performance (Rocchi et al., 2017). This discrepancy may be related to the greater absolute magnitude of PES used as high-intensity in the current study (14 mA in the current study vs. 5 mA in Rocchi et al., 2017). Our results, however, are consistent with those reported by Schlieper and Dinse (2012), who found that high-intensity PES effectively improved tactile two-point discrimination performance, while low-intensity PES had no effect on performance. Collectively, these results suggest that the effect of PES on perceptual performance depends on PES intensity and that the higher PES intensity in the present study may have been the primary factor in improved GOT discrimination performance.

Furthermore, in present study, we noted a regression at approximately 1 mm in the grating discrimination threshold regardless of the group. A previous study reported that the mean value of the grating discrimination threshold at the finger was 0.94 mm (Van Boven and Johnson, 1994). Thus, high-intensity PES possibly plays an important role in converging the grating discrimination threshold on optimal threshold; nonetheless, the reasons for this convergence following high-intensity PES are yet to be investigated. However, it might relate to homeostatic plasticity in the primary somatosensory cortex. Perhaps, equivalent synaptoplastic changes in the high baseline GOT performance group would induce hypersensitivity. Additional studies are warranted to reveal the reason behind high-intensity PES converging the grating discrimination threshold on optimal threshold.

Effect of PES on PPD

High-intensity PES had no effect on N20/P25_SEP-PPD or P25_SEP-PPD in either group, but significantly decreased N20_SEP-PPD in the GOT improvement group and increased N20_SEP-PPD in the GOT decrement group. In contrast, a previous study found that N20/P25_SEP-PPD was increased following PES to the fingers (Rocchi et al., 2017). However, the properties of SEP-PPD appear to depend on the IPI. For instance, repetitive tactile stimulation was reported to decrease N20/P25_SEP-PPD at an IPI of 30 ms, but did not affect N20/P25_SEP-PPD at an IPI of 100 ms (Höffken et al., 2007). As in Höffken et al. (2007), we employed a paired-pulse protocol with IPI of 100 ms, so the absence of an effect on N20/P25_SEP-PPD is consistent with their

findings. The specific inhibitory circuits activated by the paired-pulse paradigm may differ depending on IPI. Therefore, it is possible that changes in N20/P25_SEP-PPD could be induced by PES at other IPIs. Nonetheless, high-intensity PES significantly affected N20_SEP-PPD at ISI = 100 ms in both groups, which suggests that distinct circuits in different cortical areas underlie N20/P25_SEP-PPD and N20_SEP-PPD. The N20 at position C3′ appears to originate from area 3b (Allison et al., 1989), while P25 at C3′ arises from areas 1 and 2 (Allison et al., 1991) and 4 (Desmedt and Bourguet, 1985). Therefore, high-intensity PES to the right index finger may differentially influence circuits mediating N20_SEP-PPD. Furthermore, Chowdhury and Rasmusson (2003) have reported that $GABA_A$ receptor activity is predominant in PPD at ISI = 5 ms, while $GABA_B$ receptor activity is predominant in PPD at ISI = 100 ms. Thus, different GABA-mediated inhibitory circuits in primary somatosensory cortex may mediate SEP-PPD depending on ISI. High-intensity PES may more effectively influence the GABAergic circuits in primary somatosensory cortex that impact GOT discrimination performance. However, Rocchi and colleagues found that improvement in somatosensory temporal discrimination performance was significantly related to increasing SEP-PPD at ISI = 5 ms (Rocchi et al., 2017) and decreasing SEP-PPD at ISI = 5 ms (Rocchi et al., 2016). Thus, additional studies under constant conditions are necessary to assess whether high-intensity PES influences SEP-PPD depending on ISI.

Correlation between GOT performance and N20_SEP-PPD

Improved GOT performance following high-intensity PES was correlated with the magnitude of N20_SEP-PPD reduction in the GOT improvement group and low performance group. Similarly, Höffken et al. (2007) reported that improved spatial two-point discrimination of the stimulated finger after repeated tactile stimulation was related to reduction in N20/P25_SEP-PPD at ISI = 30 ms. Further, Rocchi et al. (2016) observed that improvement in a somatosensory temporal discrimination task following cTBS to primary somatosensory cortex was associated with N20_SEP-PPD at ISI = 5 ms. In direct contrast to our findings, however, Rocchi et al. (2017) have reported that the mechanism underlying improved somatosensory temporal discrimination after high-intensity PES was related to increased N20/P25_SEP-PPD at ISI = 5 ms. The reasons for these apparent differences in inhibitory mechanism underlying improving perceptual performance remain to be investigated. Perhaps distinct GABAergic inhibitory circuits in primary somatosensory cortex are recruited and altered by specific PES protocols and differentially regulate discrimination depending on somatosensory stimulus characteristics.

CONCLUSION

High-intensity PES differentially affected perceptual performance depending on individual baseline ability. High-intensity PES improved perceptual discrimination in subjects with low baseline performance, and this improvement was associated with reduced SEP-PPD of the stimulus evoked potential N20 waveform. It might be possible to influence somatosensory function in patients with central nervous system injuries by specific application of PES.

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DECLARATIONS OF INTEREST

None.

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OPEN

Enhancing rehabilitation and functional recovery after brain and spinal cord trauma with electrical neuromodulation

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Purpose of review
This review discusses recent advances in the rehabilitation of motor deficits after traumatic brain injury (TBI) and spinal cord injury (SCI) using neuromodulatory techniques.

Recent findings
Neurorehabilitation is currently the only treatment option for long-term improvement of motor functions that can be offered to patients with TBI or SCI. Major advances have been made in recent years in both preclinical and clinical rehabilitation. Activity-dependent plasticity of neuronal connections and circuits is considered key for successful recovery of motor functions, and great therapeutic potential is attributed to the combination of high-intensity training with electrical neuromodulation. First clinical case reports have demonstrated that repetitive training enabled or enhanced by electrical spinal cord stimulation can yield substantial improvements in motor function. Described achievements include regaining of overground walking capacity, independent standing and stepping, and improved pinch strength that recovered even years after injury.

Summary
Promising treatment options have emerged from research in recent years using neurostimulation to enable or enhance intense training. However, characterizing long-term benefits and side-effects in clinical trials and identifying patient subsets who can benefit are crucial. Regaining lost motor function remains challenging.

Keywords
electrical neuromodulation, motor recovery, rehabilitative training, spinal cord injury, traumatic brain injury

INTRODUCTION

A trauma to the central nervous system (CNS), that is, spinal cord injury (SCI) and traumatic brain injury (TBI), is a devastating event and an important global cause of morbidity and mortality exhibiting an upward trend in frequency [1,2]. Directed interventions during the acute injury period are designed to limit secondary damage [3,4], but effective therapeutic strategies to manage the neurological sequelae and to promote axon regeneration are yet beyond reach [5,6]. Rehabilitative training is currently the only treatment option for injured patients that bears the potential to improve short and long-term recovery of motor function [6,7]. The large number of patients who are dependent on a wheelchair or suffer from lifelong disabilities and impairments implies that reparative effects are highly limited. In recent years, the combination of rehabilitative training with neuromodulation of the brain or the spinal cord has been investigated as means to enhance the excitability of motor circuits and to increase training efficacy promoting motor recovery [8,9]. Latest findings are promising and might open up possibilities even for patients with severe spinal cord or traumatic brain injury.

The article mainly focuses on the recovery of motor function after CNS injury. It addresses the growing field of neurorehabilitation augmented by electrical neuromodulation and highlights some of the recent advances in both basic and clinical science. The fast-growing field of robotic and

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KEY POINTS

- Activity-dependent functional and anatomical plasticity on all levels of the CNS is an important basis for functional recovery with rehabilitative training after an injury to the CNS, which can be enhanced with electrical stimulation of the brain or spinal cord.
- Electrical stimulation seems to mainly enable or enhance the effects of intense rehabilitative training, especially after large lesions.
- Although evidence from animal studies and the first clinical trials came from several recent studies in SCI, preclinical and clinical studies investigating the synergistic effects of repetitive training and electrical neuromodulation on long-term recovery of motor function after TBI are urgently needed.
- Several case reports demonstrated the therapeutic potential mainly of epidural spinal cord stimulation in combination with intensive training to enable and improve gait and also upper extremity motor function in chronic SCI.
- More, larger and well-controlled clinical trials are required to define target patient populations and elucidate possible adverse effects and physical consequences of high-intensity training and neuromodulation after CNS trauma.

exoskeleton assisted training [10–12] is of great interest but lies beyond the scope of the present review.

Injury-induced neuronal plasticity promotes motor recovery

Contrary to previous assumptions, the central nervous system has a substantial potential for structural and functional adaptations after injury. In the spinal cord, for example, various descending systems have been shown to exhibit pronounced spontaneous circuit reorganization of partially spared tracts after an SCI. A correlation and temporal overlap between recovery of function and injury-induced anatomical plasticity has been observed, and these plastic processes may be an important element and basis for spontaneous and training-enhanced recovery of motor function after neurotrauma.

Spinal cord injury

After sustaining an injury to the spinal cord, most patients experience some degree of spontaneous functional recovery within the first year, but improvement of motor function greatly decreases thereafter [13]. In the last few years, both projections descending from the motor cortex [14,15] or the brainstem [16,17▪▪] and the intraspinal circuits [18,19] (central pattern generators, CPGs) have been shown to reorganize following an injury. Using a dual viral silencing approach in rodents, Hilton *et al.* [14] demonstrated that spared corticospinal fibers play a pivotal role in spontaneous recovery after cervical SCI. Transient silencing of uninjured corticospinal neurons temporarily eliminated motor function that had recovered after injury. In another study in rodents with severe incomplete SCI (iSCI), Asboth *et al.* [17▪▪] showed that the cortex mediates recovery of hindlimb function via the brainstem by activating spared reticulospinal axons. However, spontaneous cortico-reticulospinal plasticity alone is insufficient to form sufficient relay connections between cortex and brainstem and to warrant substantial recovery. Changes in the excitability of motor neuron and interneuron circuits between acute and chronic SCI have been reported by Bellardita *et al.* [19]. Such changes may also play a crucial role for the development of spasms in SCI patients. Züchner *et al.* [20] demonstrated rewiring of spared serotonergic axons in the neonatal, injured rodent spinal cord paralleled by functional recovery and thus suggest modulatory changes within the CPG after SCI.

Taken together, these recent studies, among many others, suggest that a number of reorganizational processes are initiated by an SCI, leading to sprouting of surviving sensory and motor tract fibers as an adaptive mechanism that facilitates motor output. However, the CNS's innate repair mechanisms and growth capacity are insufficient for higher levels of recovery of motor function after large lesions.

Traumatic brain injury

A traumatic brain injury initiates a cascade of insufficiently studied pathological processes that can ultimately result in substantial sensori-motor as well as cognitive dysfunction, depending on the severity and location of the trauma. Even though motor dysfunction including gait disturbances or limb paralysis and spasticity is less frequent compared with neurocognitive and behavioral impairments [21] after a TBI, 30% of TBI survivors exhibit disabling motor deficits [22]. Motor recovery is largely restricted to a short-time window of approximately 3 months following the primary injury and starts to stagnate thereafter [23]. Even though the age-standardized incidence of TBI is 30 times higher than that of SCI [2], fundamental knowledge about neuroanatomical correlates of the observed behavioral changes and the dynamic circuit changes that follow a traumatic impact to the brain is scarce. It has been hypothesized that serotonergic axons bear potential for regrowth after TBI [24,25]. Kajstura *et al.* [24] demonstrated that a significant acute

loss of serotonergic fibers was followed by substantial axonal outgrowth between 1 and 3 months postinjury in the neocortex of adult mice. However, no causal link or temporal correlation to functional recovery has been established. Interestingly, neuroplastic responses (c-Fos, Tgfb1) to a distant trauma have been pointed out by Kononenko *et al.* [26▪], suggesting a systemic upregulation of the regenerative capacity in the CNS. The reported findings indicate that a focal TBI can initiate plastic processes in distant spinal circuits and highlight that injury-induced plasticity could be a synergistic process taking place throughout the CNS. Whether the suggested interactions between TBI and spinal circuitry contribute to motor recovery remains to be seen.

Activity-dependent plasticity – the basis for rehabilitation

The vast majority of spinal cord injuries is anatomically incomplete [27,28] and thus do not entirely disconnect the sublesional spinal cord from the brain and brainstem [29]. In patients with a clinically complete injury (ASIA A) as well as in ASIA B and C patients, spared fibers at the lesion site are insufficient to transmit functionally meaningful signals for volitional motor control to the lower spinal cord [30]. Despite this deprivation of supraspinal input, locomotor circuits (CPGs) located below the injury remain functional and able to process information [31]. Furthermore, propriospinal circuits, which interconnect spinal segments over short or long distances, have been shown to be crucial for motor recovery after partial SCI [32,33]. A certain number of spared descending fibers, propriospinal fibers, and local interneuron and motoneuron circuits are the basis for use-dependent recovery of functions after an incomplete injury to the spinal cord [34]. Importantly, although by themselves insufficient for a functionally relevant recovery, they can be modulated and reintegrated into a functional state by intense activation, for example, during repetitive training of defined functional tasks [35▪▪,36]. The current concept of rehabilitation thus suggests that repetitive use leads to strengthening of spared projections as well as stabilization and strengthening of newly sprouted fibers and connections both between cortex and brainstem, between brainstem and spinal cord, and within the spinal cord [37]. Literature on activity-induced plasticity and circuit reorganization following TBI is scarce. However, it is hypothesized that compensatory anatomical plasticity occurs in large parts of the CNS. Spared and new fibers and connections are then integrated into functional circuits by intense rehabilitative training, in this way restoring a certain degree of both structural connectivity and motor function [38–40].

Electrical neuromodulation to enhance the efficacy of rehabilitative training

Rehabilitative training alone often does not yield sufficient recovery of motor functions, especially in patients with severe lesions and impairments. Over the last few years, translation of stimulation enhanced activity-based rehabilitation from the preclinical to a clinical setting has been carried out successfully, yielding substantial improvements in motor functionality [30,34]. The data published so far point out that the combination of intense rehabilitative training with neuromodulation by electrical stimulation might be a very promising treatment option for the recovery of motor function after SCI and TBI, at least in a subpopulation of patients [30,34]. Based on their anatomical target, current approaches of electrical neuromodulation can be roughly subdivided into cortical, deep brain, and spinal cord stimulation.

Cortical neuromodulation

Current electrical neuromodulation techniques after brain injury include epidural electrical cortical stimulation (eECS) and transcranial direct current stimulation (tDCS) [21,41]. eECS is a minimally invasive technique that involves the insertion of small electrodes into the epidural space and allows the selective stimulation of specific cortical areas. tDCS is a noninvasive method for brain stimulation, which uses directed current flow to activate restricted cortical areas. Yu *et al.* [42] recently compared the effects of eECS and tDCS on motor and cognitive recovery in rats with acute, focal TBI. After 4 weeks of either subthreshold eECS or tDCS during rehabilitation, rats outperformed their unstimulated controls in a motor cortex dependent skilled reaching movement task (single-pellet grasping) and locomotor task (the rotarod test), with a slight superiority of tDCS effects. A reduction of motor impulsivity with tDCS after bilateral frontal TBI was reported by Martens *et al.* [43] However, the only study that has tried to correlate both motor recovery and structural reorganization with motor training augmented by cortical stimulation after TBI is, to our knowledge, a study by Jefferson *et al.* [44] published in 2016. In this study, rats with an impact lesion to the caudal forelimb area underwent 9 weeks of rehabilitative training with or without subthreshold eECS of the injured motor cortex. Neuromodulation assisted rehabilitation led to significantly larger improvements over time, and intracortical microstimulation mapping revealed a structural

reorganization of the wrist representation in the injured cortex upon long-term eECS. These results encourage further research on neuromodulation-assisted training for recovery of deficient motor function after TBI. Schönfeld *et al.* [45], who demonstrated that standalone cortical stimulation is insufficient for significant motor improvements in rats with severe TBI, outlined the importance of combining stimulation with training.

In a small clinical study, Middleton *et al.* [46] reported an improved upper extremity Fugl–Meyer score with upper-extremity physiotherapy augmented by bihemispheric tDCS in two TBI patients. However, most clinical studies focus on the effect of cortical stimulation on the nonmotor impairments in patients with TBI [21], and reports on motor recovery are scarce.

Deep brain stimulation

The application of deep brain stimulation (DBS) is routine in the treatment of pharmacotherapy-resistant movement disorders. DBS of the subthalamic nucleus and the internal globus pallidus is a highly effective treatment for drug-resistant Parkinson's disease, especially for patients with marked dyskinesia or motor fluctuation [47]. However, literature on the use of DBS to improve motor function in the context of neurotrauma is scarce. Chan *et al.* [48] showed that DBS of the lateral cerebellar nucleus contralateral to a unilateral fluid percussion injury of the motor cortex promotes motor recovery in rats. Additionally, DBS of the midbrain locomotor center (mesencephalic locomotor region [MLR]) has been proposed as a treatment strategy for locomotor recovery after SCI and stroke [49,50]. Highly promising results were achieved in a rodent model with more than 80% spinal cord transection where MLR-DBS acutely led to functional hindlimb walking and swimming movements [50]. A clinical study to investigate DBS of the MLR for its potential to enhance training and improve gait in nonambulatory patients with chronic iSCI (DBS-SCI, ClinicalTrials.gov identifier: NCT03053791) is currently recruiting patients.

Spinal cord stimulation

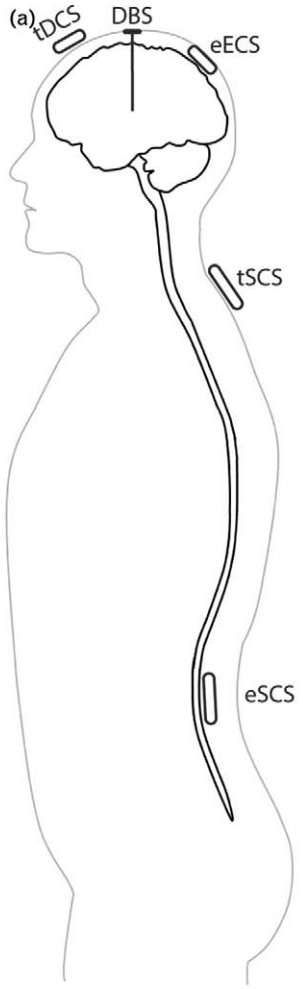
Spinal cord stimulation (SCS) is currently the most frequently investigated type of electrical circuit modulation and comprises intraspinal, transcutaneous, and epidural stimulation. In the past years, both preclinical and clinical literature have focused primarily on epidural SCS (eSCS), whose combination with rehabilitative training was suggested as a promising treatment strategy for deficient motor function after severe SCI [17▪▪,29,51▪▪,52▪▪,53,54,55▪]. Preclinically, a recent study by Gerasimenko *et al.* [55▪] highlighted the capability of eSCS to initiate hindlimb stepping in rats with complete SCI. The authors additionally observed that the more caudal spinal networks are insufficient to control locomotion in the absence of more rostral, upper lumbar and lower thoracic segments, a criterion that should be considered when recruiting patients for clinical testing. The therapeutic potential of eSCS was also emphasized by Asboth *et al.* [17▪▪], who additionally demonstrated that stimulated rats were capable of engaging context-specific locomotor behavior. Further, Capogrosso *et al.* [56] showed that antigravitational strength could be improved with eSCS during overground locomotion in the nonhuman primate with acute, incomplete SCI.

eSCS is currently the clinically most studied neuromodulatory technique in the context of neurotrauma [35▪▪,51▪▪,52▪▪,57,58]. Gill *et al.* [35▪▪] published the first report on a chronic, clinically motor complete SCI patient that regained independent stepping ability with task-specific training supported by eSCS 3 years after injury. In contrast to bilateral stepping on the treadmill, walker and trainer assistance was required during overground stepping. Angeli *et al.* [51▪▪] tested the effects of intense locomotor treadmill training with weight support accompanied by eSCS in four patients that had failed to improve with training alone. Although all four patients recovered independent standing and trunk stability, two patients even regained overground walking capability. Wagner *et al.* [57] and Calvert *et al.* [58] demonstrated improved voluntary control during walking or cycling and rhythmic motor activity, respectively. The 'Epidural Stimulation After Neurologic Damage clinical trial' (E-STAND, Trial Number: NCT03026816) is currently ongoing and has been designed to investigate the generalizability of eSCS in a greater population with, for example, differences in age, sex, time post-injury, and lesion size. Darrow *et al.* [52▪▪] published preliminary findings proposing that eSCS might be beneficial for a greater variety of patients than previously thought, without requiring preimplantation training in contrast to previous studies. Their preliminary data further indicate beneficial effects of eSCS beyond motor function. Inanici *et al.* [59▪▪] reported improved long-term recovery of upper extremity function with noninvasive transcutaneous electrical stimulation (tSCS) and physical therapy in a patient with chronic iSCI. In all these patients who developed certain degrees of volitional motor control after combined eSCS and rehabilitation therapy, spared fibers must have been present in their spinal cords in spite of an initial clinical complete ASIA A diagnosis. Additionally, all these patients were younger patients, often former

athletes, in very good physical condition and able to go through a physically very demanding training over many weeks and months. Overall, there is great variability in stimulation parameters used in both preclinical and clinical studies. At a given frequency and pulse width, each individual has a certain threshold intensity eliciting, for example, rhythmic muscle activity. As the SCI population is highly heterogeneous, future research should focus on the establishment of stimulation parameters that are effective and safe according to patient subgroup, for example, depending on lesion level, lesion extent, or time that has passed since injury, and specific stimulation sites, for example, with electrode arrays targeting different segments of the lumbar spinal cord. This would increase comparability among individuals and between studies, which is required to ultimately draw conclusions on the effectiveness of neuromodulation. The E-STAND trial (Trial Number: NCT03026816) has taken the first step in this direction.

CONCLUSION

Despite major advances in the field of neurorehabilitation, the management of severe motor impairments resulting from TBI and SCI continues to challenge both basic scientists and clinicians. Figure 1 schematically illustrates electrical neuromodulation approaches and main clinical



(b)

Publications					
Publication	Study type	Injury	Intervention	Phase	Promoted functions
Yu et al., 2018	pre-clinical	TBI	eECS	acute	balance, reaching
Yu et al., 2018	pre-clinical	TBI	tDCS	acute	balance, reaching
Martens et al., 2019	pre-clinical	TBI	tDCS	chronic	motor impulsivity
Jefferson et al., 2018	pre-clinical	TBI	eECS	acute	reaching
Chan et al., 2018	pre-clinical	TBI	DBS	chronic	forepaw dexterity, motor coordination
Middleton et al., 2014	clinical	TBI	tDCS	chronic	upper extremity Fugl-Meyer score
Bachmann et al., 2013	pre-clinical	SCI	DBS	chronic	hindlimb stepping
Asboth et al., 2018	pre-clinical	SCI	eSCS	chronic	bipedal stepping
Gerasimenko et al., 2019	pre-clinical	SCI	eSCS	chronic	bipedal stepping
Capogrosso et al., 2016	pre-clinical	SCI	eSCS	acute	over-ground locomotion
Gill et al., 2018	clinical	SCI	eSCS	chronic	independent over-ground stepping, treadmill stepping, standing
Angeli et al., 2018	clinical	SCI	eSCS	chronic	independent standing, trunk stability, over-ground stepping
Wagner et al., 2018	clinical	SCI	eSCS	chronic	voluntary control during standing, walking, cycling
Calvert et al., 2018	clinical	SCI	eSCS	chronic	intentional control of step-like activity
Darrow et al., 2019	clinical	SCI	eSCS	chronic	volitional muscle activity
Inanici et al., 2018	clinical	SCI	tSCS	chronic	improved GRASSP score

Ongoing Trials					
Principle investigator	Identifier	Injury	Intervention	Phase	Primary outcome
Lennart H Stieglitz, MD	NCT03053791	SCI	DBS	chronic	improvement of gait measured by 6 minute walk test
David Darrow, MD	NCT03026816	SCI	eSCS	chronic	change in volitional response index magnitude

FIGURE 1. Summary of electrical neuromodulatory approaches, publications, and ongoing clinical trials discussed in this review. (a) Schematic illustration of different neuromodulatory approaches. (b) List of publications and ongoing trials by study type, injury type, intervention, and postinjury phase with the observed facilitated or enhanced functions. eECS, Epidural electrical cortical stimulation; tDCS, transcranial direct current stimulation; DBS, deep brain stimulation; eSCS, epidural spinal cord stimulation, tSCS, transcutaneous spinal cord stimulation; TBI, traumatic brain injury; SCI, spinal cord injury; GRASSP, Graded and Redefined Assessment of Strength, Sensibility and Prehension. Phase refers to the postinjury phase. Identifier refers to ongoing studies' ClinicalTrials.gov identifier.

implications of the literature discussed in this review. Preclinical and clinical literature on electrical neuromodulatory approaches to regain motor function after TBI is still scarce and has focused on different postinjury phases. This is different for the field of SCI where neuromodulatory interventions to enable or enhance intense locomotor training are currently well studied in animal models and the first clinical trials. First case reports of patients with chronic SCI have shown that electrical neuromodulation of the spinal cord bears promising therapeutic potential to enable a different form and intensity of training which can lead to a significantly higher degree of recovery of lost motor functions. However, considering the heterogeneity of the TBI and SCI patient population, well-controlled clinical trials with larger numbers of participants are required to define the specific effects of the treatment and to identify the subsets of patients that can benefit. Identifying potential long-term adverse effects of electrical stimulation and the physical consequences of high-intensity training on the organism is also key. Furthermore, the optimal temporal relationship between neuromodulation and rehabilitative training needs to be identified in both preclinical and clinical studies to maximize therapeutic efficacy.

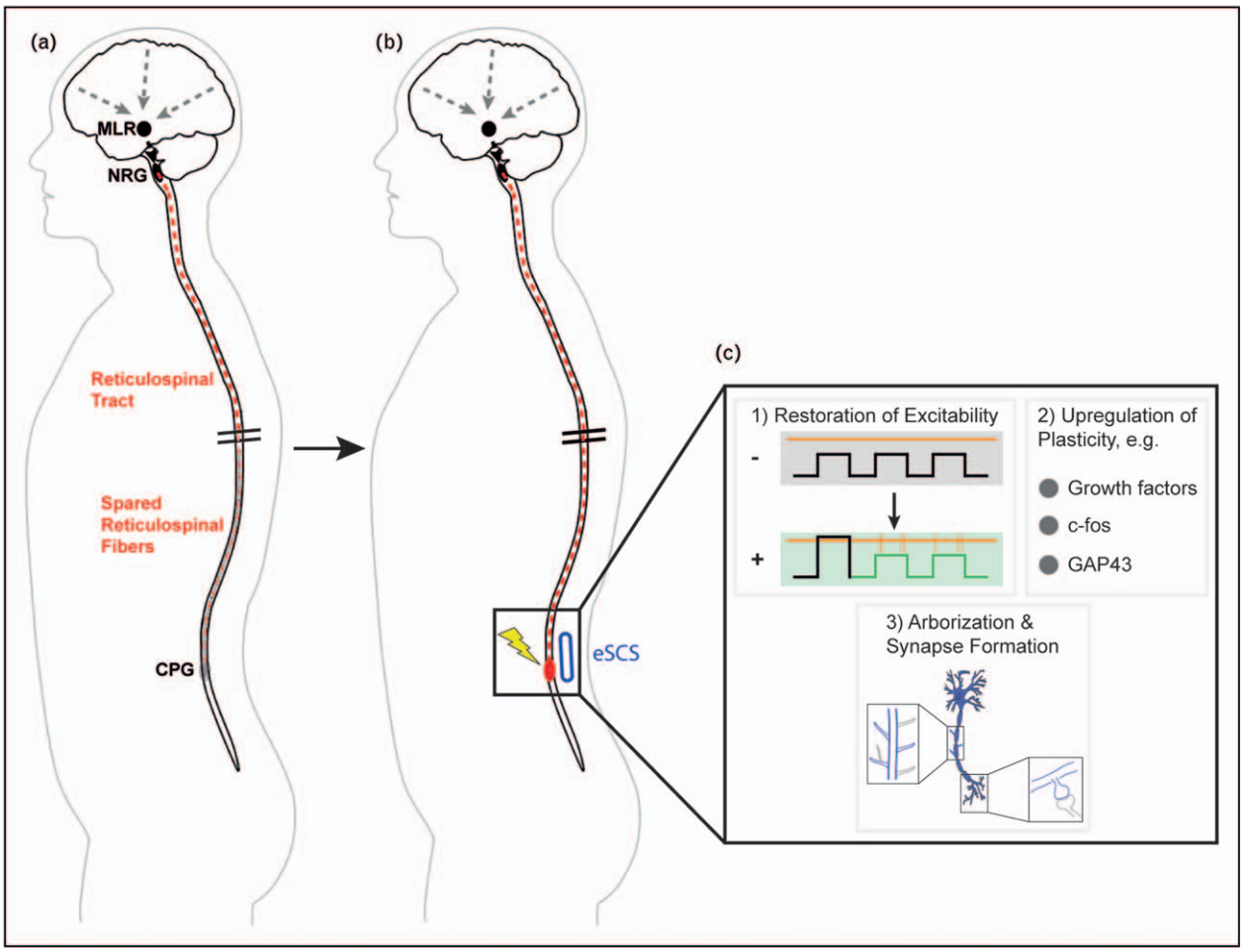


FIGURE 2. Putative biological effects of epidural spinal cord stimulation on neuronal structures. (a) After large, incomplete spinal cord injury, spared reticulospinal fibers are incapable to sufficiently activate the sublesional CPGs to generate rhythmic muscle activity and locomotion. (b) With epidural stimulation of the lumbar spinal cord, the local neurons including the CPGs regain a certain level of background activity, which makes them excitable by spared reticulospinal fibers. (c) Inset summarizing putative mechanisms. (1) Stimulation changes the resting membrane potential of CPGs, either directly or by enhancing input from propriospinal sensory fibers, thereby restoring excitability (− = no stimulation; + = stimulation; orange horizontal line = threshold potential; black and green squares = membrane potential; orange vertical lines = spikes of muscle activity). (2) Plasticity markers are upregulated by electrical activity, including, for example, growth factors, c-fos, and the growth-associated protein GAP43. (3) Neurons start to sprout, to reorganize, and to adapt the local circuits to the decreased descending input of spared fibers. CPG, Central pattern generator; eSCS, epidural spinal cord stimulation; MLR, mesencephalic locomotor region; NRG, gigantocellular reticular nucleus; GAP, growth-associated protein.

The majority of current and recent SCI studies hypothesize that electrical stimulation restores the excitability of sublesional neurons, which can then be reintegrated into functional circuits by repetitive use, and these studies, thus, focus on neuromodulation applied during training (stimulation-enabled training). However, subthreshold stimulation over prolonged time periods has been shown to induce neuronal growth [60], and it has been suggested previously that the effects of a sequential application of a growth-promoting treatment followed by training might be superior [61,62]. Therefore, both preclinical and clinical studies investigating the implementation of neuromodulation prior to training to promote the expression of plasticity genes are required, as not only the absolute time point of treatment start (acute versus chronic SCI state) but also the relative timing of treatment options (sequential versus parallel) are essential for an optimal therapeutic schedule. The many ways by which electrical stimulation can affect neurons and how electrical stimulation positively influences functional recovery remain to be analyzed in detail. Figure 2 illustrates putative mechanisms of action at the example of epidural spinal cord stimulation after incomplete spinal cord injury. Preclinical and clinical studies applying high-precision stimulation are required to determine which subsets of neuronal populations and structures (soma versus axon) respond most strongly to stimulation and whether acute or long-term responses are more crucial. The combination of multiple approaches, including multilevel neuromodulation [60,63], should be pursued in the long run to meet the wide range of needs that arise from a trauma to the CNS and go far beyond motor dysfunction.

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Conflicts of interest

There are no conflicts of interest.

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- ■ of special interest
- ■■ of outstanding interest

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Large-scale changes in cortical dynamics triggered by repetitive somatosensory electrical stimulation

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Abstract

Background: Repetitive somatosensory electrical stimulation (SES) of forelimb peripheral nerves is a promising therapy; studies have shown that SES can improve motor function in stroke subjects with chronic deficits. However, little is known about how SES can directly modulate neural dynamics. Past studies using SES have primarily used noninvasive methods in human subjects. Here we used electrophysiological recordings from the rodent primary motor cortex (M1) to assess how SES affects neural dynamics at the level of single neurons as well as at the level of mesoscale dynamics.

Methods: We performed acute extracellular recordings in 7 intact adult Long Evans rats under ketamine-xylazine anesthesia while they received transcutaneous SES. We recorded single unit spiking and local field potentials (LFP) in the M1 contralateral to the stimulated arm. We then compared neural firing rate, spike-field coherence (SFC), and power spectral density (PSD) before and after stimulation.

Results: Following SES, the firing rate of a majority of neurons changed significantly from their respective baseline values. There was, however, a diversity of responses; some neurons increased while others decreased their firing rates. Interestingly, SFC, a measure of how a neuron's firing is coupled to mesoscale oscillatory dynamics, increased specifically in the δ-band, also known as the low frequency band (0.3- 4 Hz). This increase appeared to be driven by a change in the phase-locking of broad-spiking, putative pyramidal neurons. These changes in the low frequency range occurred without a significant change in the overall PSD.

Conclusions: Repetitive SES significantly and persistently altered the local cortical dynamics of M1 neurons, changing both firing rates as well as the SFC magnitude in the δ-band. Thus, SES altered the neural firing and coupling to ongoing mesoscale dynamics. Our study provides evidence that SES can directly modulate cortical dynamics.

Keywords: Somatosensory electrical stimulation (SES), Peripheral nerve, Spiking dynamics, Motor cortex, Low frequency oscillations

Background

Somatosensory input is essential for skilled movements [1–3]; this is particularly true for dexterous movements [1, 4–6]. Interestingly, the somatosensory system has been shown to experience relatively rapid bidirectional changes in organization as a result of repetitive manipulations of peripheral inputs. Consistent with this notion are seminal studies in both animals and humans which demonstrated that reductions in sensory feedback, either by denervation or ischemic nerve block, induced changes in motor representations [7, 8].

Studies have also shown that increases in afferent input by stimulating peripheral pathways (i.e. repetitive somatosensory electrical stimulation or SES) can alter sensorimotor representations of the stimulated body part [9, 10]. One of the first studies examining this neuromodulation method found that sensory stimulation of oral structures resulted in prolonged changes in excitability as well as an increase in the area of representation

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determined using functional imaging [11]. Consistent with these results are studies demonstrating that altered patterns of physical contacts to the fingers can also persistently reorganize sensory maps [12, 13]. Importantly, repetitive SES has also proven to be a promising therapeutic tool for motor rehabilitation [10, 14–16].

In both humans and rodents, SES can increase excitability as measured by responses to transcranial magnetic stimulation (TMS) pulses [9, 17]. Past studies have used non-invasive measures to examine cortical excitability such as motor evoked potentials (MEPs) with TMS [9, 17] and cortical reorganization using blood oxygenation signals [11]. It remains unclear what are the precise mechanisms underlying these changes. For example, the observed change in the evoked MEPs following SES may occur without changes in brainstem electrical stimulation-evoked potentials or spinal reflexes [9, 18, 19]. This suggests the possibility that the cortex may be an important site of plasticity. While our recent study showed that SES can also modify low-frequency dynamics as measured using electroencephalogram (EEG) [20], it remains unclear if these changes are local to cortex. Invasive electrophysiology offers one method to assess if SES can directly alter local motor cortical dynamics.

While the body of literature summarized above has provided important mechanistic insight, little is known about how SES interacts with ongoing cortical dynamics at the level of single neurons and groups of neurons, or neural ensembles. Single neurons are a fundamental unit of the nervous system. The coordinated firing of neural ensembles, e.g. co-firing of neurons in a temporally coupled manner, is now also recognized as an important module for information processing [21–26]. In addition, oscillations may provide a mechanism for dynamic coordination of ensembles across motor and sensory areas [21–25, 27]. Oscillations likely reflect synchronized rhythmic excitability linked to coordinated firing of neurons [28]. Our collective understanding of both single neuron and ensemble firing patterns has greatly improved our understanding of how neural activity patterns underlie complex sensory and motor behaviors. Similarly, it is likely that such activity may play an important role in driving neural plasticity after injury and during neuromodulation using methods such as SES.

The goal of this study was to develop a model of the cortical effects of SES using high-resolution, invasive recording of neurons. We were particularly interested in understanding the diversity of single neuron responses to SES. It is unlikely that all neurons respond identically to a given perturbation. This may be, in part, the result of the multiple cell-types in a given region and the diversity of network connectivity for single neurons [29]. We also wanted to compare changes in neural activity related to larger scale network oscillatory activity. More specifically, we examined the effects of SES on primary motor cortex (M1) at the level of single neuron firing rates as well as the neural coupling to ongoing spontaneous oscillations. We found that SES could independently change both the firing rate and the phase locking, i.e. the consistency of the neural firing relative to oscillatory dynamics. Together, our results provide evidence that SES can directly modulate neural dynamics in M1.

Methods

Animal and surgery preparation

All animal procedures were in accordance with protocols approved by the Institutional Animal Care and Use Committee at the San Francisco Veterans Affairs Medical Center. Adult male Long Evans rats ($n = 8$, 250-400 g, ~ 8 weeks old, Charles River Laboratories) were housed in a 12 h light:12 h dark cycle with lights out at 6:00 AM and were kept under controlled temperature. One animal was excluded from the study due to significant recording drift and electrical noise in the recording, thus $n = 7$ animals were used for the analysis shown. Animals were initially anesthetized using a ketamine/xylazine cocktail (85 mg/kg ketamine, and 10 mg/kg xylazine), with supplemental ketamine (at half of the induction dose) given every 40–60 min as needed to maintain a stable anesthetic level, and also to maintain anesthesia at stage III characterized by predominantly slow oscillations. Moreover, 0.05 mg/kg of atropine was given separately to counter respiratory and cardiac depression, and decrease secretion. Animals were sacrificed at the end of the recordings.

Somatosensory electrical stimulation and electrophysiology

After anesthesia induction, transcutaneous stimulation electrodes were clipped near forelimb peripheral nerves (medial, ulnar, and radial nerve), in the configuration noted in Fig. 1a. These copper metal clips were wrapped around the forelimb and then connected to a Multi-Channel Systems Stimulus Generator (MCS STG4000 series) to deliver transcutaneous stimulation. SES current parameters were set by determining the maximum amount of current where no evoked movement in the forelimb was seen (typically 300–750 μA currents).

Following a craniotomy and a durectomy procedure, either 64-channel custom probes in a tetrode configuration ($n = 5$, 1 X 4/8, Neuronexus, MI) or 32 channel tungsten microwire arrays ($n = 2$, MEAs, Tucker-Davis Technologies or TDT, FL) were implanted using precise stereotactic measurements into layer 5 of motor cortex (1200–1500 μm deep; + 1.5 to + 2.0 anterior to bregma and + 2 to + 3.5 lateral from midline) to record extracellular neural activity. In general, tetrodes allow better isolation of single neurons. However, as our microwire

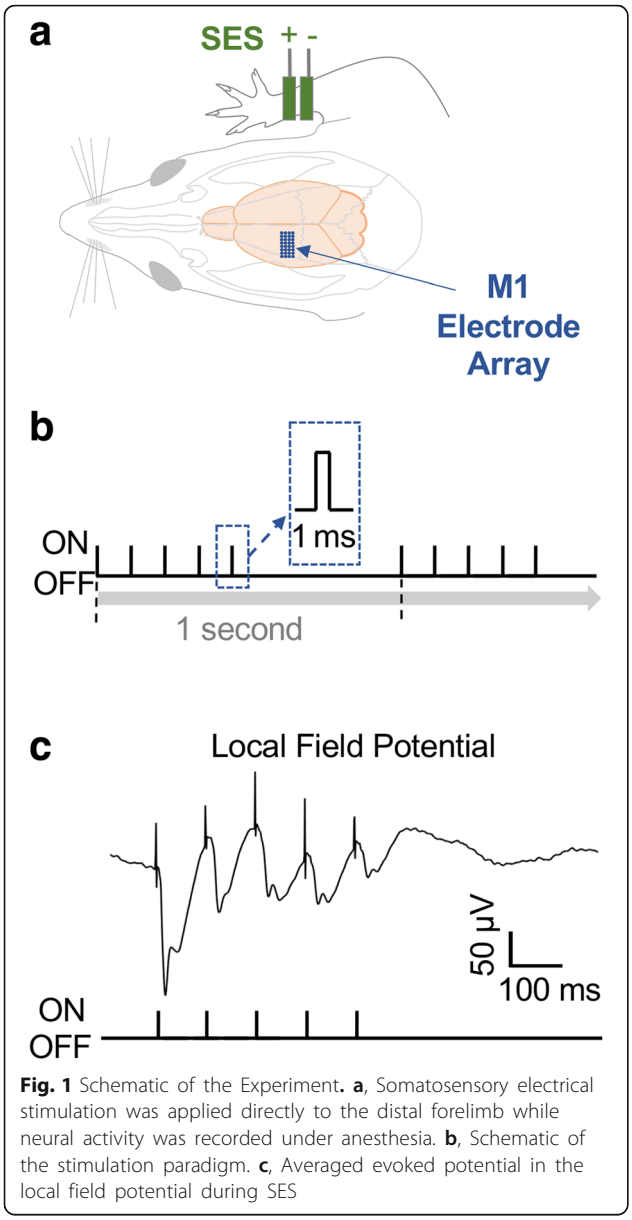

Fig. 1 Schematic of the Experiment. **a**, Somatosensory electrical stimulation was applied directly to the distal forelimb while neural activity was recorded under anesthesia. **b**, Schematic of the stimulation paradigm. **c**, Averaged evoked potential in the local field potential during SES

recordings also demonstrated identical findings, we have grouped the results together.

Spike data was sampled at 24414 Hz and LFP data at 1018 Hz. ZIF–clip based analog headstages with a unity gain and high impedance (~ 1 MΩ) were used. Unsorted multi-unit, single-unit, and LFP data were then recorded from 30 min to 1 h to ensure stability of recordings and to minimize drift during stimulation experiments. Then a baseline period of neural activity (~ 30–60 min) was recorded, followed by a recording of neural activity during SES. The stimulation paradigm was 5 single pulses (square pulse width, 1 ms) at 10 Hz over 500 ms, i.e. with a 1% duty cycle. This was immediately followed by 500 ms of no stimulation. This pattern of 10 Hz stimulation and no stimulation was repeated on a 1 Hz pattern (30 min for $n = 4$, or 60 min for $n = 3$ animals, current magnitude: 564.29 ± 57.46 μA, Fig. 1b). After SES stimulation was finished, post recording of neural activity was used to assess the effects of stimulation lasting ~ 30–60 min.

Data analysis

LFP and single-unit analyses

Analyses were conducted using a combination of custom-written routines in Matlab 2015a/2017b (MathWorks, Natick, MA), along with functions and routines from the Chronux toolbox (http://chronux.org/). Preprocessing steps for LFP involved: removing periods of artifacts (removing broken channels, and noisy segments of LFPs based on offline visual inspection); taking the median signal (at every time point the median signal across electrodes was calculated); and z-scoring this signal (i.e. removal of the mean value, μ, of the signal, X, and dividing by the standard deviation, σ, z-scored LFP = [X−μ]/σ). Median referencing was used to remove any volume conducted signals and to thereby focus on signals local to M1.

Single units were sorted using Plexon Offline Sorter (Plexon, Dallas, TX). Single units and LFPs were used to calculate spike-field coherence (SFC) using chronux functions. SFC measures phase synchronization between the LFP and spike times as a function of frequency; its magnitude is a function of frequency and has a value between 0 and 1 [22]. For its calculation, the pre- and post-stimulation time segments were first time matched to the shortest recording period, then segmented into 10 s segments, and then the coherency measured was averaged across segments. The average time series used for analysis was 46.8157 ± 6.5765 min. For the multitaper analysis, we used a time-bandwidth (TW) product of 10 with 19 tapers. To compare coherences across groups, a *z*-score was calculated using the programs available in the Chronux Toolkit. Coherence between activity in two regions was calculated and defined as

$$C_{xy} = \frac{|R_{xy}|}{\sqrt{|R_{xx}|}\sqrt{|R_{yy|}}}$$

where R_{xx} and R_{yy} are the power spectra and R_{xy} is the cross-spectrum. Spectral analysis was calculated in segmented time periods pre- and post-stimulation and averaged across these epochs. Mean coherence was calculated across the δ-band (0.3–4 Hz, i.e. all values in the range were averaged together), θ-band (6–10 Hz), α-band (8–15 Hz), β-band (18–25 Hz), γ-band (30–60 Hz). For the frequency band analysis, statistical analysis was performed on the average coherence estimates of each frequency band's respective pre-SFC and post-SFC values (see section below). We also equaled the number of

spikes in the pre- and post-stimulation period to account for the changes in firing rates [30]. The power spectrum of the LFP channels used in the coherence calculation, as well as for overall LFP power change in pre- and post-stimulation, was also determined using the multitaper method. For spiking analyses, sorted spikes were binned at 50 ms. A significant change in firing was estimated by calculating the mean post-stimulation firing rate and checking if it was outside of the 95% distribution of pre-stimulation firing rate distribution. Some analyses were further filtered down by choosing high signal-to-noise ratio (SNR) units. To clearly identify units with stable waveforms and high amplitudes, we measured SNR using the following equation:

$$SNR = \frac{A}{2 * SD_{noise}}$$

Where A is the peak-to-peak voltage of the averaged spike waveform and *SDnoise* is the standard deviation of the "noise", or the baseline fluctuations in the voltage during the first 245 microseconds of the saved waveform snippet [31].

Spike width analysis

We grouped neurons based on the width of the recorded spikes. Spike width was calculated by finding the distance between the peak of the waveform and its valley. Past studies have demonstrated that spike width can distinguish putative fast spiking interneurons and pyramidal neurons [27, 31]. To specify a cutoff, we applied k-means to the entire neuronal population. In general, our results were concordant with this previous literature. We thus used values of 100–400 μs for narrow-width, putative interneurons and 500–1000 μs for broad-width, putative pyramidal neurons.

Statistical analysis

Parametric statistics were used in this study, and each test was implemented within MATLAB. We used *t*-tests for comparison of power between pre- and post- SES sessions, as well as *t*-tests for the comparison of SFC pre and post-SES averaged across each common frequency band used in previous literature (δ-band, θ-band, α-band, β-band, γ-band) [31]; we used a Bonferroni correction for multiple comparisons. We used Pearson's correlation and linear regression to evaluate trends between changes in firing rate and SFC after SES. The linear mixed-effects model (implemented using MATLAB fitlme) was used to compare the differences in SFC and firing rate in all units in Fig. 3f/g, and for the broad and narrow-width neurons in Fig. 4b. This model accounts for the fact that units, channels, or trials from the same animal are more correlated than those from different animals and is more stringent than computing statistical significance over all units, channels, and trials.

Results

Long Evans rats ($n = 7$) were implanted with either microwire ($n = 2$) or tetrode ($n = 5$) arrays in M1 (Fig. 1a). Stimulation was then applied to the distal forearm peripheral nerves (30 min for $n = 4$ animals, 60 min for $n = 3$ animals, current magnitude: 564.29 ± 57.46 μA). We found that the motor evoked response was clearly visible in the LFP and showed a large deflection during the train of pulses at 10 Hz that lasted 500 ms, i.e. with a 1% duty cycle (Fig. 1c). As expected, there was a decrement in the response within each train [32].

Firing rate changes

We first examined if SES altered the firing rate of neurons in M1 (Fig. 2) and compared changes in firing rate relative to a pre-stimulation baseline period. The overall population was widely distributed and the mean change (1.791 Hz) and median change (− 0.2338 Hz) were close to a baseline value of 0. Examples of both a significant increase (mean pre = 2.603 Hz, mean post = 5.472 Hz, $p < 0.05$) and a decrease (mean pre = 14.198 Hz, mean post = 7.603 Hz, $p < 0.05$) in firing rate are shown. In general, all animals exhibited a firing rate change in the majority of the recorded neurons after SES (i.e. > greater than 50% with a net change in firing rate at 30 min post stimulation). In an example animal T54, 56% of its units decreased their firing rate, while 18% increased their firing rates (Fig. 2b). At a population level ($n = 214$ neurons), we found that while 36% of neurons exhibited an increase in firing (mean pre = 5.93 Hz, mean post = 14.93 Hz), 36% experienced a reduction in firing rate (mean pre = 8.63 Hz, mean post = 4.64 Hz), and 28% showed no change (mean pre = 6.77 Hz, mean post = 6.52 Hz) (Fig. 2c). Regardless of the length of the time period recorded and analyzed (30–60 min), we saw a significant change relative to the baseline across all animals in neurons that either significantly increased ($p < 10^{-04}$) or decreased ($p < 10^{-19}$) their firing rates. Together, these results indicate that SES can have persistent, but diverse effects on single neuron firing rates within M1.

Spike-field coherence changes

We also investigated whether SES persistently modulated the synchronization between LFP and spike times as a function of frequency, i.e. spike-field coherence or SFC (Fig. 3) [25, 33]. We recorded both single unit spiking and LFP from the population of M1 units (Fig. 3a). SFC is a measure of how consistently a given unit fires relative to the phase of the median LFP (Fig. 3b). The only frequency band that showed a significant change after SES was the δ-band (Fig. 3c, mean change for 0.3–4 Hz δ-band

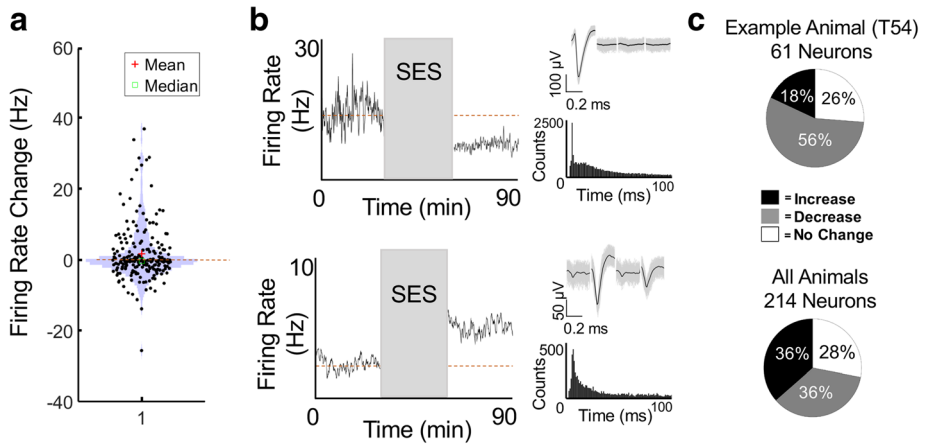

Fig. 2 Changes in Firing Rate after SES. **a**, Violin plot of the firing rate changes for all neurons. The red cross represents the mean (1.7918); green triangle is median (− 0.2338). **b**, Example of either a significant decrease ($p < 0.05$; top) or increase ($p < 0.05$; bottom) in firing rate after SES. Also shown are tetrode waveforms and the interspike intervals. The dotted lines represent the mean during the pre-stimulation period. **c**, Percentage of neurons which significantly increased, decreased, or had no change for one animal (top) and for all animals ($n = 7$; bottom)

pre- vs post-stimulation, t-test with Bonferroni correction, $p < 10^{-09}$). The θ-band (6–10 Hz), α-band (8–15 Hz), β-band (18–25 Hz), and γ-band (30–60 Hz) did not show any significant changes ($p > 0.05$).

At a single neuron level, 64% of the units increased, 26.4% decreased, and 9.6% had no change in the δ-band SFC (Fig. 3d). At a population level, the majority of neurons demonstrated an increase in the δ-band SFC relative to the baseline period (Fig. 3e). Figure 3f shows a representative change in the SFC in the low frequency, δ-band (0.3–4 Hz) of a single neuron; this was also evident on average for all neurons recorded in that animal. When also examining all units ($n = 214$) from all seven animals, we again found evidence for a significant SFC increase in the lower frequency band (mixed-effects model which takes into account that multiple neurons were recorded from the same animal, Fig. 3g, $p < 10^{-05}$) [34]. This indicates that after SES, neural firing was significantly more likely to be phase-locked to low-frequency oscillatory dynamics.

Narrow and broad spiking neurons

We further investigated the differences in firing rate and SFC by classifying neurons into two distinct groups: narrow-spiking, putative interneurons (100–400 μs), and the broad-spiking, putative pyramidal neurons (500–1000 μs) [27, 31]. Figure 4a shows an example animal's distribution of neuron spike widths; the color labels are based on a k-means classification. Interestingly, broad-spiking neurons demonstrated a robust increase in the SFC after SES (mixed linear model, $p < 10^{-06}$); there was no change in firing based on this classification. In contrast, narrow-spiking neurons did not show significant changes in either firing rate or SFC after SES. This implies that putative pyramidal neurons might be a main driver of the increase in SFC in the δ-band after SES.

Power spectral density

We also examined if global changes to the LFP were also evident. The LFP is widely believed to represent an aggregate mesoscale measurement of activity [21]. There was not a significant change in the LFP power (Fig. 5).

Firing rate and SFC changes are independent

As shown above, SES significantly modulated both the firing rates and the δ-band SFC. While we used methods to account for changes in firing rates (see Methods), it is possible that the SFC changes were co-regulated with the change in firing rate. We thus examined the relationship between the two variables. Interestingly, the firing rate and δ-band SFC were not significantly correlated with one another (Fig. 6, $r = 0.1300$, $p > 0.05$). This suggested that the effects of SES on the firing rate and the SFC were independent of each other.

Discussion

We found that SES can induce persistent M1 plasticity lasting at least 30–60 min after the end of stimulation; over half of the neural population significantly changed its firing rate in response to SES. Moreover, phase locking of firing to mesoscale oscillatory dynamics was significantly modulated in a manner that was independent of the direction of change in firing rate. The most prominent SFC increase occurred in the low frequency range; there was not a concomitant change in LFP power. Together, these finding suggests that SES can directly modulate M1 dynamics.

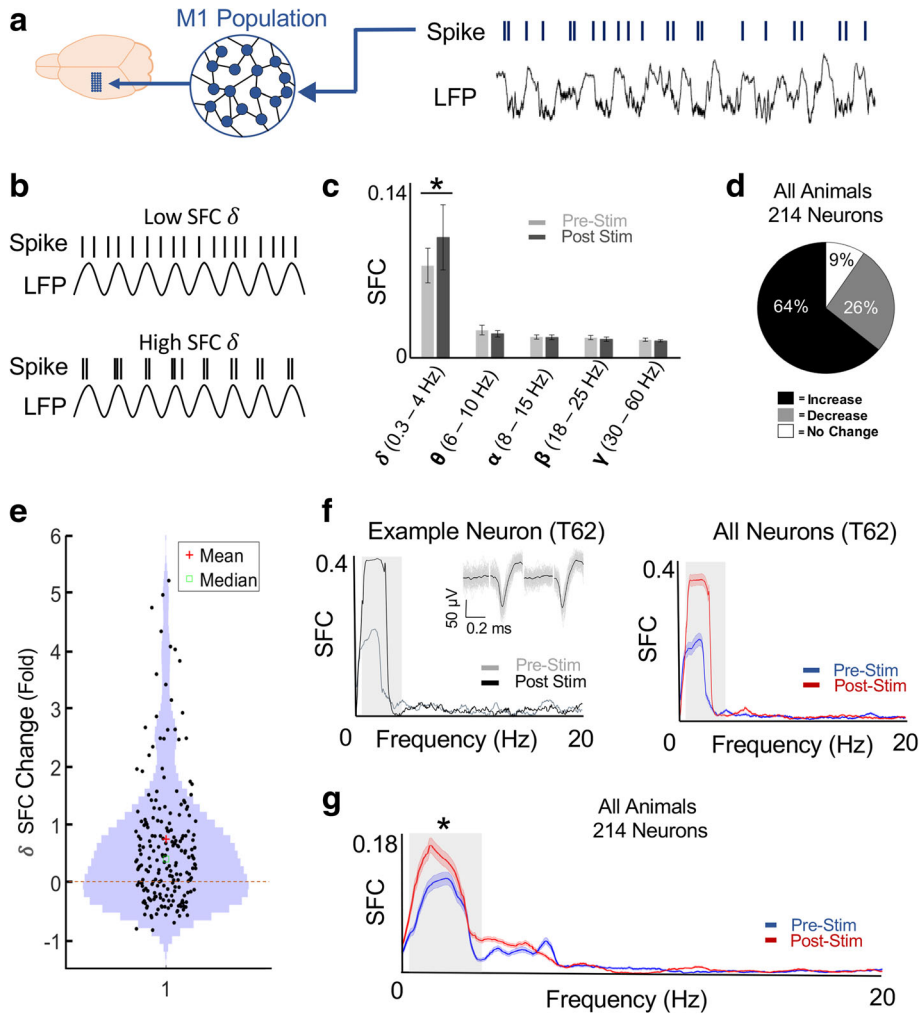

Fig. 3 Changes in Spike Field Coherence (SFC) after SES. **a**, Schematic depicting neural spikes relative to LFP recordings from M1. **b**, Schematic of the relation of spiking to LFP for variations in the SFC. **c**, Comparison of the averaged SFC across each frequency band (see Methods) for all units before and after SES. (*$p < 0.001$). Error bars represent the standard error of the mean or SEM. **d**, Percentage of neurons which significantly increased, decreased, or had no change for all animals ($n = 7$). **e**, Violin plot of the SFC fold change relative to baseline for all neurons. A value of 1 represents a doubling of the SFC. **f**, Example single neuron and all neuron SFC plot for one animal. The grey box highlights 0.3–4 Hz band. Error bars are SEM. **g**, Mean SFC plot for all animal including all neurons ($n = 214$, *p < 0.001). Follows convention from **f**

Relation to previous models of SES

Studies have previously shown that SES can apparently alter both the sensorimotor representations of the stimulated body part as well as excitability [9, 10, 17]. Changes in sensorimotor representations have been primarily examined using functional imaging [11], which is an indirect measure of neural activity. Moreover, in both humans and rodents, SES has also been shown to increase excitability as measured by responses to TMS pulses [9, 17]. The main uncertainty was whether M1 is directly affected by SES.

Our results add to this body of literature by demonstrating three main points. First, SES can directly modulate the activity patterns of M1; this is demonstrated by the changes in firing rates of single neurons. Second, our findings of a diversity of neural firing changes suggest a more complex neural response to SES. A better understanding of the diversity of responses and their underlying neural basis (e.g. neural connectivity, cell-types) might help improve the efficacy of SES. Third, our results suggest two possible mechanisms of SES. Namely, there was a change in spontaneous firing rate as well as coupling to mesoscale dynamics.

Somatosensory electrical stimulation and neural plasticity

SES induced plasticity appears to be experienced differentially by the large sets of M1 neurons recorded; while a majority of the neurons experienced a change in firing rate, the extent and the direction of change was variable. Moreover, the changes in firing rate appears to equally

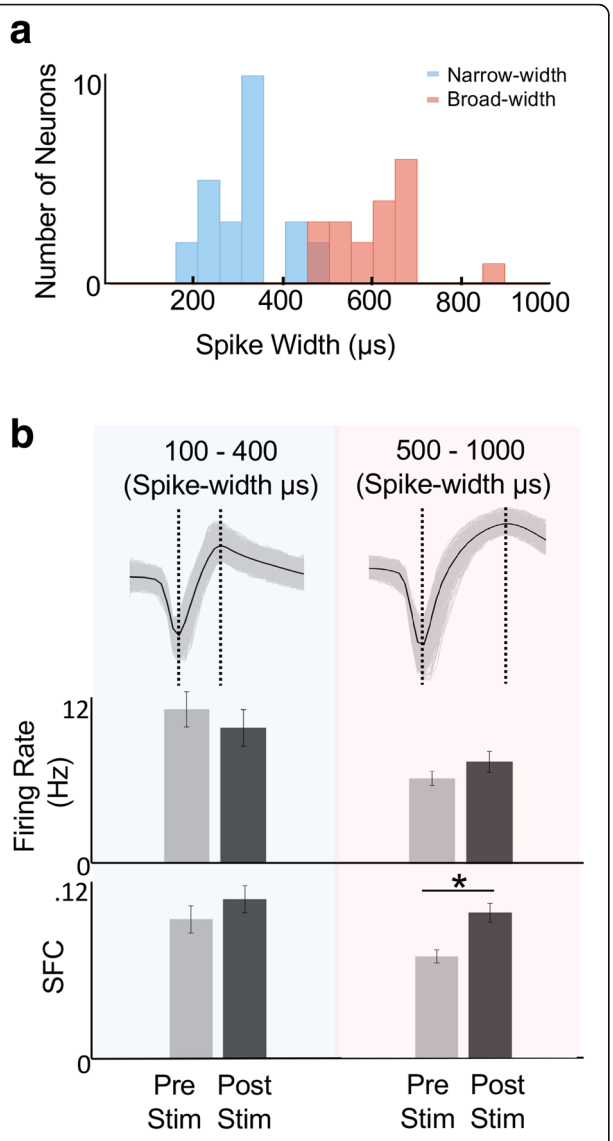

Fig. 4 Comparison of Broad and Narrow-Width Spiking Units. **a**, Example animal's distribution of neurons classified by spike widths ($n = 46$). The color coding is based on k-means clustering. **b**, Differences in the spiking activity and SFC for narrow-width (left blue column) and broad-width (right red column) (*$p < 0.001$)

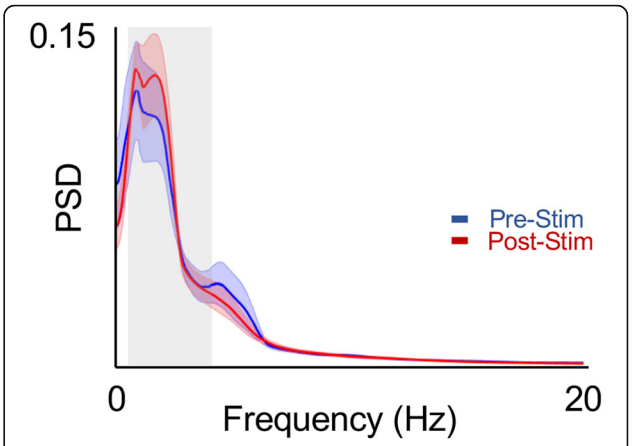

Fig. 5 LFP Power Before and After SES. Shows the power spectrum of the LFP prior to and after SES. There was no significant relationship observed

affect both putative interneurons and pyramidal neurons. What are the potential mechanisms that can account for the diversity of changes in neural firing? On a macroscopic level, SES evoked deflections in the M1 LFP during stimulation (Fig. 1c). This is consistent with past work showing that sensory inputs can directly influence motor areas [35–37]. The reduction in response with each pulse is also consistent with the adaptation evident during sensory stimulation [32]. It is quite likely that the observed input also triggered synchronous spiking in M1. Thus, it is possible that the extent that a single neuron participated in the synchronous spiking during SES could account for the observed direction of change. It is possible that repetitive stimulation of sensory inputs to an area can result in short-term homeostatic regulation of network dynamics [38–40].

SES could also trigger activity-dependent synaptic plasticity [41, 42]. In general, brief periods of activity can trigger long-term potentiation and long-term depression that depends on the specific patterns of activation [38, 43]. Such activity can also increase or decrease the intrinsic excitability of presynaptic neurons [38, 44]. This mechanism might explain the diversity of plasticity evident at the level of single neurons. It is also worth noting that emerging computational methods to quantify functional network connectivity [23] might eventually be used to predict the specific plasticity effects at a single neuron level.

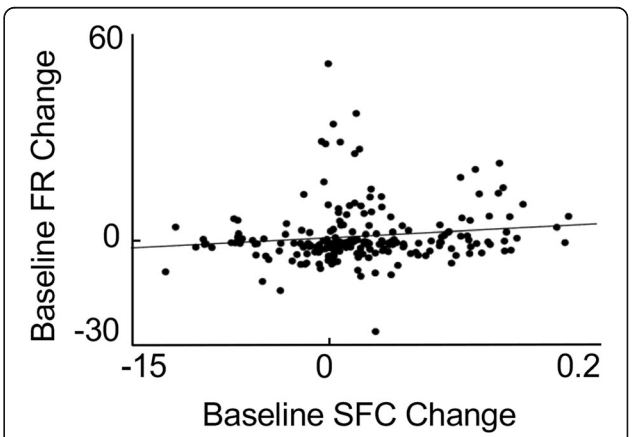

Fig. 6 Comparison of Changes in Firing Rate versus SFC. Plot shows correlation of single neuron changes in firing rate versus the corresponding SFC change. There was not a significant relationship between the two ($r = 0.13$, $p > 0.05$). Line was generated using linear regression

Another possibility is that the observed changes in M1 firing are the result of network plasticity in the sensorimotor system. Electrical stimulation of peripheral nerves causes synchronous activation of muscle spindles and cutaneous afferents that appear to target area specific activation and reorganization in primary somatosensory areas [14, 45–47]. Moreover, SES can trigger changes in TMS-evoked MEPs [9, 17, 18]. While past work has suggested that mechanisms of plasticity below the brainstem may not account for excitability changes [9, 18, 19], it is reasonable to suppose that larger scale network dynamics are modulated [20]. In this scenario, the observed changes in M1 could be the result of plasticity at other cortical sites. For example, given the known strong connections between sensory and motor areas [3], changes at a primary sensory area could result in spontaneous firing changes at a connected site.

Spike coupling to low frequency oscillations

The greatest change in the coupling of neural spiking to oscillatory LFP dynamics was in the δ-band, also known as low frequency oscillations (LFO) [22, 48]. Our results further suggest that the change in coupling or phase-locking to mesoscale dynamics is independent from the changes in firing rate. For example, at a single neuron level, changes in firing rate did not predict changes in SFC. Moreover, we observed a change in SFC for putative pyramidal neurons without a concomitant change in firing rate. It is unclear what might drive this change. The lack of a change in LFP power in the LFO range suggests that changes in input to M1 are not a main driver; LFP is widely believed to be a measure of synaptic inputs [21, 28, 29]. Changes in intrinsic excitability is certainly a possible mechanism through which neurons can be more coupled to population dynamics [38]. This might also explain the previously observed changes in M1 evoked potentials after SES [9, 17]. Alternatively, changes in local synaptic connectivity [29], i.e. as distinct from synchronous inputs to M1, could be a driver of the changes in neural coupling to population dynamics.

What might be the broader physiological consequences of SES induced changes in LFO dynamics? In general, ketamine anesthesia is known to result in such low-frequency oscillatory activity [22, 48]. However, in rodents, non-human primates and humans, LFOs have been observed at the level of spiking and LFP in the motor cortex during reaching tasks [22, 24, 48, 49]. It has been postulated that LFOs represent an intrinsic property of motor circuits that are involved in the production of fast and accurate movements. Stroke disrupts these movement related potentials in humans, which are highly correlated with motor impairments [22, 49]. LFOs are therefore a potential biomarker of restored circuit dynamics after stroke as it relates to fast and accurate skilled reaching [20, 22]. Interestingly, our recent study also found that parameters for modulation of LFOs in anesthesia also generalized to the awake state [22]. It is thus possible that the locking of spiking to LFOs is a general principle for the cortical effects of SES. In other words, SES might be particularly suited for modulating the neural dynamics linked to cortical slow oscillations. Future work can examine if SES also similarly modulates movement-related spiking in the healthy or perilesional cortex; this might be one mechanism through which SES improves function in stroke patients [20, 50].

Conclusions

In summary, brief periods of SES induced long-lasting cortical plasticity in M1. We identified significant changes in firing rate and spike coupling to low frequency oscillations in the majority of recorded neurons. Further tailoring of these processes to identified cortical dynamics might further improve the efficacy of SES in those with motor disabilities after stroke or other acquired brain injuries [22, 50].

Abbreviations

EEG: Electroencephalogram; LFO: Low frequency oscillation; LFP: Local field potential; M1: Primary motor cortex; MEP: Mean evoked potential; PSD: Power spectral density; SD*noise*: Standard deviation of noise; SEM: Standard error of the mean; SES: Somatosensory electrical stimulation; SFC: Spike field coherence; SNR: Signal-to-noise ratio; TMS: Transcranial magnetic stimulation; TW: Time-bandwidth; α-band: Alpha-band; β-band: Beta-band; γ-band: Gamma-band; θ-band: Theta-band; δ-band: Delta-band

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

AH analyzed the data. MB conducted the experiments. TG provided code and assisted with analysis. KG supervised all aspects of the experiments. AH and KG wrote and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

KG has submitted a provisional patent application for closed-loop SES. The results presented in this manuscript are not a part of the provisional patent application. AH, MB and TG do not have any competing interests.

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Functional Spectroscopy Mapping of Pain Processing Cortical Areas During Non-painful Peripheral Electrical Stimulation of the Accessory Spinal Nerve

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Peripheral electrical stimulation (PES), which encompasses several techniques with heterogeneous physiological responses, has shown in some cases remarkable outcomes for pain treatment and clinical rehabilitation. However, results are still mixed, mainly because there is a lack of understanding regarding its neural mechanisms of action. In this study, we aimed to assess its effects by measuring cortical activation as indexed by functional near infrared spectroscopy (fNIRS). fNIRS is a functional optical imaging method to evaluate hemodynamic changes in oxygenated (HbO) and de-oxygenated (HbR) blood hemoglobin concentrations in cortical capillary networks that can be related to cortical activity. We hypothesized that non-painful PES of accessory spinal nerve (ASN) can promote cortical activation of sensorimotor cortex (SMC) and dorsolateral prefrontal cortex (DLPFC) pain processing cortical areas. Fifteen healthy volunteers received both active and sham ASN electrical stimulation in a crossover study. The hemodynamic cortical response to unilateral right ASN burst electrical stimulation with 10 Hz was measured by a 40-channel fNIRS system.

Abbreviations: ACC, Anterior Cingulate Cortex; ASN, Accessory Spinal Nerve; CA, Cortical Activation; CAI, Cortical Area of Interest; DLPFC, Dorsolateral Prefrontal Cortex; EA, Electroacupuncture; fNIRS, Functional Near Infrared Spectroscopy; fMRI, Functional Magnetic Ressonance Imaging; HbO, Oxygenated Hemoglobin; HbR, Deoxygenated Hemoglobin; HRF, Hemodynamic Response Function; IMS, Intramuscular Stimulation; M1, Primary Motor Cortex; MC, Motor Cortex; NIBS, Non-invasive Brain Stimulation; NMES, Neuromuscular Electrical Stimulation; PAG, Periaqueductal Gray; PES, Peripheral Electrical Stimulation; PFC, Prefrontal Cortex; PMC, Premotor Cortex; S1/SI, Primary Somatosensory Cortex; S2/SII, Secondary Somatosensory Cortex; SMA, Supplementary Motor Area; SMC, Sensorimotor Cortex; SSC, Somatosensory Cortex; VN, Vagus Nerve.

The effect of ASN electrical stimulation over HbO concentration in cortical areas of interest (CAI) was observed through the activation of right-DLPFC (p = 0.025) and left-SMC (p = 0.042) in the active group but not in sham group. Regarding left-DLPFC (p = 0.610) and right-SMC (p = 0.174) there was no statistical difference between groups. As in non-invasive brain stimulation (NIBS) top-down modulation, bottom-up electrical stimulation to the ASN seems to activate the same critical cortical areas on pain pathways related to sensory-discriminative and affective-motivational pain dimensions. These results provide additional mechanistic evidence to develop and optimize the use of peripheral nerve electrical stimulation as a neuromodulatory tool (NCT 03295370—www.clinicaltrials.gov).

Keywords: cortical activation, near infrared spectroscopy, peripheral nerve stimulation, electrical nerve stimulation, electroacupuncture, accessory spinal nerve

INTRODUCTION

Pain processing physiology involves inter-related individual systems, with discriminative, affective, cognitive and social domains, leading to a magnitude of physical and emotional expressions (Melzack, 2001; Chapman et al., 2008). Advances in neuroscience attempted to map brain areas and pathways involved in this neural network, bringing a better understanding of structural and functional brain connectivity. The prefrontal cortex (PFC) has been increasingly associated with pain processing because of its interconnections, including efferent signals to periaqueductal gray (PAG) and dorsal horn neurons (Ong et al., 2019). As an associative cortex, the dorsolateral prefrontal cortex (DLPFC) mediates appraisal to a rewarding stimulus, regulation of emotion and behavior and "keeping pain out of mind" function, that is, moving attention to other things rather than nociception (Wiech et al., 2008). DLPFC is also related to depression and emotional pain aspects related to anxiety (O'Connell et al., 2010). Still, musculoskeletal and neuropathic pain are strongly correlated to motor cortex (MC) and its connections and has been related to pain and cognitive dysfunction by cortico-striatal-thalamo-cortical loops (CSTC; Leite et al., 2017). Afferent nociceptive information that crosses mediodorsal thalamus and anterior cingulate cortex (ACC) reaches DLPFC, which is related to affective-motivational aspects of pain. In turn, the sensory-discriminative dimension of pain involves spinothalamic tract pathway to ventrobasal lateral thalamus and then to sensorimotor cortex (SMC), which in turn anatomically and functionally involves MC, premotor cortex (PMC), supplementary motor area (SMA) and primary somatosensory cortex (S1; Ohara et al., 2005; Hadjipavlou et al., 2006; Yaksh and Luo, 2007). The importance to study the cortical processing of pain in these two target areas, nominally DLPFC and SMC, is to extend data upon the therapeutic approaches effects at the cortical level.

Peripheral electrical stimulation (PES) is being used as a non-pharmacological tool for clinical rehabilitation and treatment of pain presumably by an upward effect inducing reorganization of segmental and central networks (bottom-up outcomes; Chipchase et al., 2011a; Rossini et al., 2015; Chakravarthy et al., 2016). The postulated mechanisms include modulation of the descending modulatory system, release of peptides and endorphins at central and peripheral levels, improvement in motor recruitment, local anti-inflammatory effects, regulation of autonomic activity and changes in long-term depression (LTD)/long-term potentiation (LTP) at synaptic sites (Sandkühler, 2000; Jiang et al., 2013; Zhang et al., 2014). Neurophysiological and neuroimaging studies with PES has shown cortical hemodynamic outcomes in contralateral somatosensory cortex (SSC) and SMC to painful/non-painful type of stimulus, dependent on intensity, in the upper body (median nerve, hand or head) towards activation, using functional near infrared spectroscopy (fNIRS) devices (Tanosaki et al., 2001, 2003; Franceschini et al., 2003; Niederhauser et al., 2008; Takeuchi et al., 2009; Hu et al., 2014; Muthalib et al., 2018) and functional magnetic resonance imaging (fMRI; Blickenstorfer et al., 2009). Lee et al. (2013) correlated the changes in the amplitude of the oxygenated and de-oxygenated hemoglobin with fNIRS with the pain scores on the visual analog scale (VAS) reported by volunteers after applying pain stimulus to the right thumb. Using fNIRS, neuromuscular electrical stimulation (NMES) above motor threshold with evoked pain activated contralateral SMC and bilateral PFC (Muthalib et al., 2015). Aasted et al. (2016) found deactivation of frontal lobe with fNIRS after applying a painful stimulus. Subsequent studies have found different patterns of activation/deactivation comparing painful to non-painful and even paresthetic stimuli using diffuse optical tomography (Becerra et al., 2008, 2009) and fNIRS (Yücel et al., 2015).

Different PES techniques are being studied to improve understanding the mechanism of action and potential indications to pain treatment. Electroacupuncture (EA) can help to treat chronic neck pain (Seo et al., 2017), chronic back pain (Lam et al., 2013) and fibromyalgia (Salazar et al., 2017). Intramuscular electrical stimulation (IMS) with needles improved pain and disability in patients with osteoarthritis (de Graca-Tarragó et al., 2016) and chronic miofascial pain (Couto et al., 2014; Botelho et al., 2018). In previous studies using transcranial magnetic stimulation (TMS), IMS reduced the excitability of the cortical spinal pathway, decreased motor evoked potential (MEP) and

intracortical facilitation (ICF) and increased current silent period (CSP; Botelho et al., 2016; Tarragó et al., 2016). NMES studies have demonstrated peripheral neuromuscular adaptations such as increased muscle strength and metabolism, as well as spinal and supraspinal responses (Blickenstorfer et al., 2009; Chipchase et al., 2011a,b; Muthalib et al., 2015). PES can also generate afferent signals for nerve-machine interfaces, that can be used in amputated members rehabilitation, for example (Tan et al., 2015; Ghafoor et al., 2017). Complementary, top-down techniques such as non-invasive brain stimulation (NIBS) are being strongly studied to successfully treat chronic pain by the application of an electrical field on central neural tissue (Castillo Saavedra et al., 2014; Jensen et al., 2014).

Likewise, there is consistent evidence upon vagus nerve (VN) stimulation with an implantable device to aim epilepsy treatment, including potential to help to treat some neuropsychiatric conditions (Hachem et al., 2018). Using fMRI, VN transcutaneous stimulation *via* cervical and auricular sites demonstrated widespread activity in the nucleus of the solitary tract, spinal trigeminal nucleus (TN), locus coeruleus and cortical areas (Frangos et al., 2015; Yakunina et al., 2017; Frangos and Komisaruk, 2017). Still, occipital and trigeminal nerve are being studied and seem to have a role on pain autonomic response and headache treatment (Rigo et al., 2014; Chassot et al., 2015; Chou et al., 2017; Waki et al., 2017). Another peripheral nerve with a close connection with the VN is the accessory spinal nerve (ASN). It is the eleventh cranial nerve formed by a spinal portion from C1 to C4, and a cranial portion from nucleus ambiguous, which also forms VN (Sarrazin et al., 2013; Liu et al., 2014; Shoja et al., 2014). At the level of jugular foramen, the ASN is connected to VN *via* internal ramus or *pars vagalis*. The ASN has a superficial landmark in the posterior cervical triangle and innervates the sternocleidomastoid and trapezius muscles where it receives sensory, proprioceptive and autonomic fibers *via* vagal anastomoses (Benninger and McNeil, 2010; Mitsuoka et al., 2017). In this way, ASN can be an interesting target for its anatomical characteristics and technical facility, accessible to needles and electrodes, regarding new targets for non-invasive therapeutic interventions.

To assess cortical activation, we choose Functional Near Infrared Spectroscopy (fNIRS). It is a non-invasive neuroimaging method used to evaluate cortical function by calculating relative concentrations of oxygenated hemoglobin (HbO), de-oxygenated hemoglobin (HbR) and total hemoglobin (Total-Hb) in cortical capillary networks. Brain activity produces increased oxygen consumption, which is accompanied by increased cerebral blood flow due to neurovascular coupling, that reflects changes in HbO and HbR measurements in the observed region (Ferrari and Quaresima, 2012; Scholkmann et al., 2014; Phillips et al., 2016). This can be interpreted as a change in tonic neural activity within that region (Owen et al., 2010). This activity can be measured with fMRI or electroencephalography (EEG), among other techniques. FMRI has high spatial and low temporal resolution, and it is expensive; on the other hand, EEG has low spatial and high temporal resolution. The advantages of fNIRS are its low cost, portability and possibility of use during daily activities, with a plausible spatial and temporal resolution (Nguyen and Hong, 2016; Hong and Zafar, 2018). The main disadvantage is that it does not evaluate infracortical layers, because light has a optimal penetration-scattering rate of 2 cm deep, suffering influence of the extracerebral superficial layers (Hoshi, 2016; Nguyen et al., 2016). Some authors postulate that fNIRS is a preferable tool to evaluate cortical activation induced by any type of electrical stimulation because it is less sensitive to electrical interference when compared to other neuroimaging techniques (Jang et al., 2014). fNIRS evaluating SSC can also be used to discriminate different stimulations, like handshake and cold temperature, as it presents different patterns of hemodynamic responses (Hong et al., 2017). Besides that, it is being used for the development of brain-computer interfaces (BCIs; Strait and Scheutz, 2014; Naseer and Hong, 2015), alone or together with others techniques as EEG (Khan et al., 2014; Hong and Khan, 2017).

Thus, to advance in the comprehension of the relationship between PES and the neural substrates at cortical areas involved in pain processing and understand possible therapeutic effects observed in clinical settings, this study assessed the changes on the concentration of HbO at DLPFC and SMC using fNIRS in healthy subjects that received accessory spinal nerve-peripheral electrical stimulation (ASN-PES). We tested the hypothesis that ASN-PES can promote cortical activation *via* bottom-up pathway on pain processing cortical areas modulated by top-down NIBS. Hence, this result can help to understand the clinical impact of PES on pain treatment and rehabilitation.

METHODS

The study protocol was approved by Hospital de Clínicas de Porto Alegre Ethics Committee Board (Institutional Review Board IRB 0000921), according to the Declaration of Helsinki. All subjects provided their written informed consent. The protocol was developed in accordance with the Consolidated Standards of Reporting Trials—CONSORT, and registered at ClinicalTrials.gov (NCT 03295370).

Design Overview, Setting and Randomization

This crossover, sham-controlled clinical trial was carried out at Clinical Research Center of Hospital de Clínicas de Porto Alegre, Brazil. Healthy male volunteers, aged between 20 and 55 years, were recruited from the local community to undergo unilateral ASN-PES to evaluate cortical activation with fNIRS. Twenty-one right-handed, healthy male volunteers were eligible and agreed to participate. A standard screening questionnaire and a written consent was applied. Subjects could not have clinical co-morbidity, chronic pain, cerebral implants, history of neurologic or psychiatric disorders, BDI-II depression scale 12 or more and no drugs or alcohol abuse. Participants were instructed not to take analgesics, anti-inflammatory drugs, caffeine or any stimulant drinks at least 6 h prior to the intervention. The randomization plan to initiate the experiment in active or sham intervention was generated by specific software[1].

[1]www.randomization.com

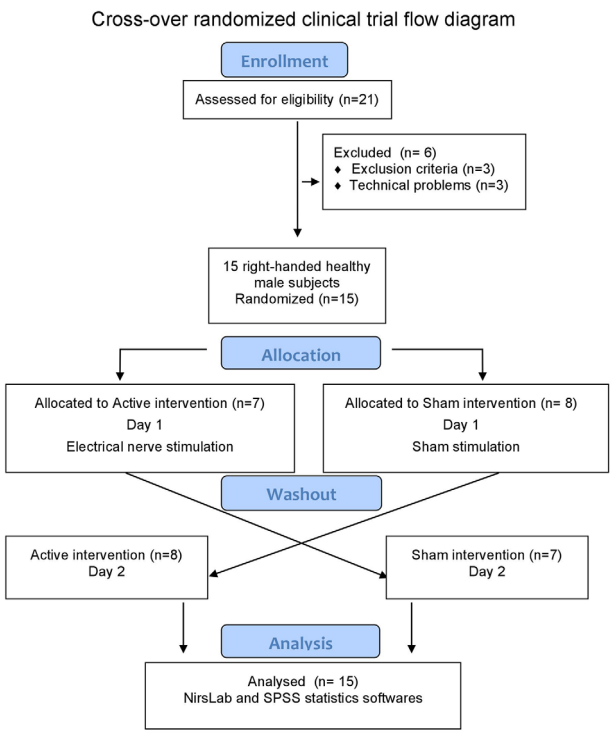


FIGURE 1 | Flow diagram.

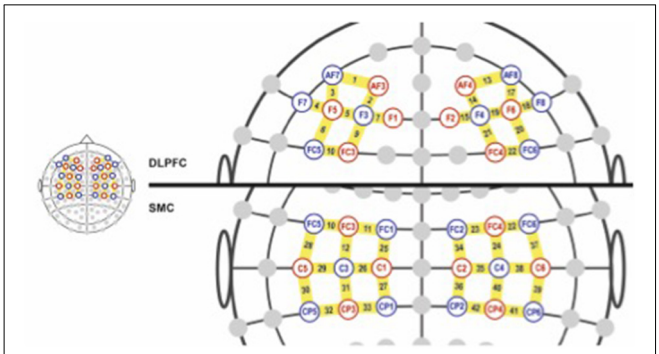


FIGURE 2 | Cap montage. Sources (red), detectors (blue), in correspondence with 10/10 electroencephalography (EEG) system. Channels formed are in yellow bar. Dorsolateral prefrontal cortex (DLPFC) and sensorimotor cortex (SMC) areas are shown separately.

Six participants were excluded, three because of exclusion criteria application and three because they did not complete recording data due to technical problems with quality of signal on fNIRS calibration before starting the procedure. After a minimum interval of 6 days, participants were crossed-over to the second intervention. The study flow is represented in **Figure 1**.

For sample size estimation (minimum 12 subjects), we performed a internal pilot study with five subjects considering an effect size on changes on the concentration of HbO related to ASN stimulation equal to 0.8 for a standard deviation equal to 6.2 (error type II of 80% and error type I lower than 5%; Birkett and Day, 1994). The power of the initial estimative was confirmed at study end.

Assessment of Demographic and Clinical Variables

Demographic data were assessed by a standard questionnaire. Beck II Depression Inventory (BDI-II) and Strait-Trait Anxiety Inventory (STAI) evaluated depressive and anxiety symptoms, respectively. The Pittsburgh Sleep Quality Index (PSQI) assessed sleep pattern.

Assessment of Cortical Activation

Cortical activation was assessed by fNIRS. We used a NIRx® continuous waveform NIRScout 16 × 24 device, sampling rate of 3.91 Hz, dual-wavelength LED sources (760 nm and 850 nm), differential pathlength factor (DPF) of 7.25 for WL1 and 6.38 for WL2, for a distance between sources and detectors of 3 cm, as suggested by literature to evaluate cortical layers (Kohl et al., 1998; Zhao et al., 2002). Software equipment used was NIRStar 14.2 and nirsLAB 2017[2]. The montage intended to use as many channels (source-detector combination) as possible to cover motor and dorsolateral pre-frontal cortical bilateral areas, with a total of 40 measurement channels (**Figure 2**).

Intervention

Subjects were seated on a comfortable reclining chair and asked to avoid any unnecessary movements. After the placement of the cap and software calibration checks, the signal was recorded for 10 min in resting state to surrounding accommodation. The right ASN was needled subcutaneously, at the right lateral cervical region, and the 0.25 × 40 mm sterilized acupuncture needle was fixed to the stimulator by a cable. A 12-min active or sham stimulation period was undertaken (720 s), followed by another 10 min resting-state period (**Figure 3**).

Electrical stimulation was undertaken with an EA stimulator (NKL 608®, made in Brazil) configured to apply a burst rectangular 200 μs-width current with maximum 5 mA of intensity on the needle. A special trigger marker device was developed to mark in registered data the exact moment the electrical current was discharged to the subject.

The active intervention consisted of 10 Hz electrical non-painful stimulus in burst current, 10 s ON and 20 s OFF, for 12 min, generating 24 blocks of hemodynamic curves in response to electrical current on unilateral right ASN. The intensity was determined during the first 2 min according to subject tolerance, in order to get mild or moderate muscular contraction of the right superior trapezius muscle for 10 s, followed by its relaxation for 20 s. In sham procedure, the intensity button was fixed on zero and there was no muscle contraction over the 12-min period, although it was previously provoked for the localization of ASN on needling phase. Thus, sham intervention had a very small electrical stimulation period (3–5 s).

A physician researcher with more than 10 years of needling experience conducted the study. The participants were not informed of intervention type on either day. At the end of

[2]www.nirx.net

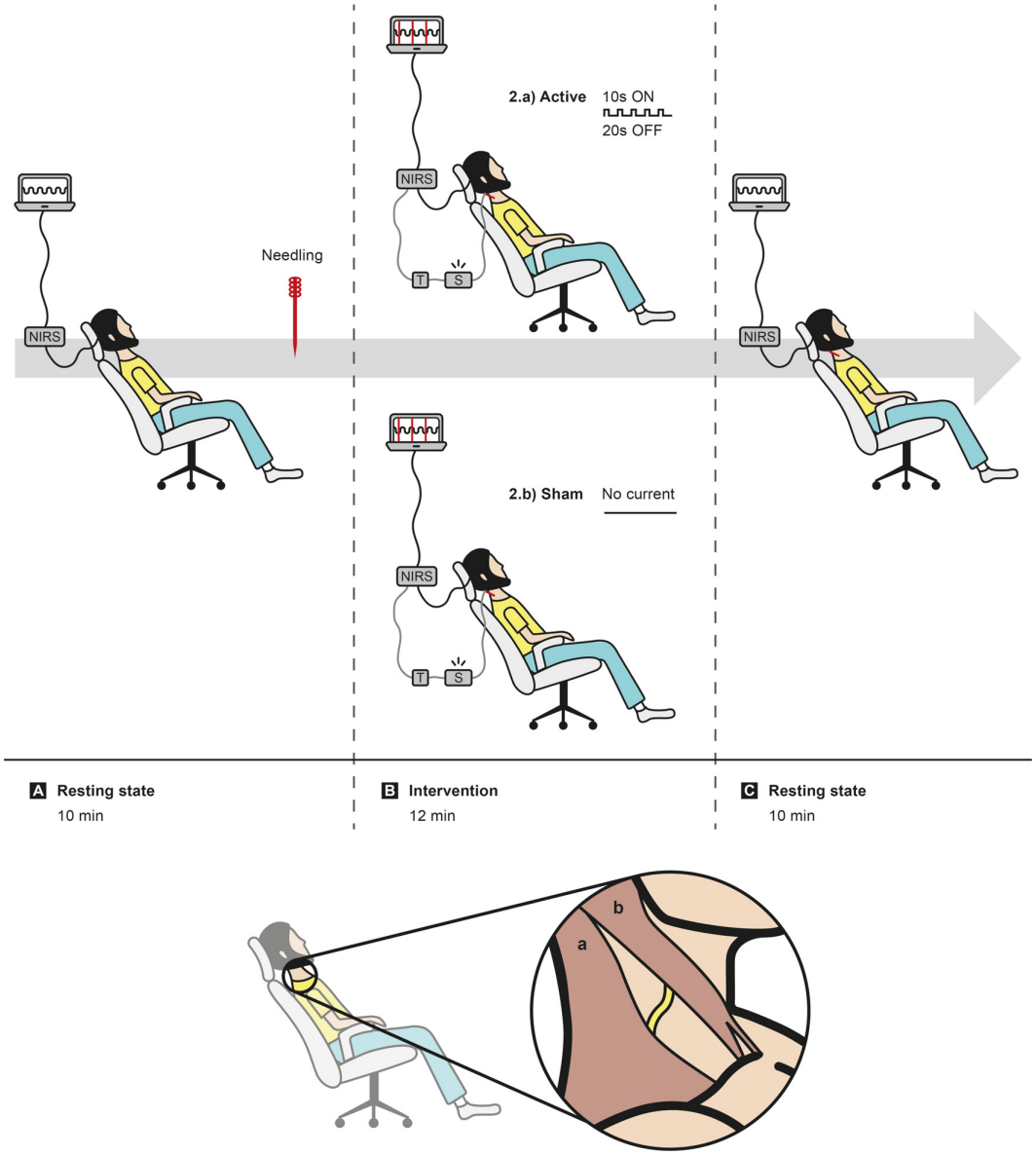


FIGURE 3 | Experiment layout. Sequence of events: **(A)** 10 min of data acquisition in resting state, followed by needling right accessory spinal nerve (ASN); **(B)** randomization in active or sham intervention; **(C)** 10 min of data acquisition in resting state. In caption, representation of the subcutaneous location of the ASN (yellow trace) between trapezius muscle (a) and sternocleidomastoid muscle (b).

each day of intervention, the subject filled a standard adverse effects questionnaire, adapted to the particularities of EA and fNIRS devices.

Based on Jurcak et al. (2007) and Koessler et al. (2009), validation of spatial resolution of scalp surface and its correlation with 10/10-system EEG parameters and Brodmann's area, channels were grouped into four cortical areas of interest (CAI): left DLPFC, right DLPFC, left SMC and right SMC. **Table 1** shows an approximate correlation of 10/10-system and cortical gyrus below, according to these authors. Note that the area called MOTOR includes sensory cortical zone, so it refers to SMC.

Data Processing and Statistical Analysis

While filtering and preparing the raw data, only the 12-min stimulation period was analyzed to observe the acute effects of electrical nerve stimulation on cortical hemodynamic response. Optical density changes recorded by the software was checked for quality and continuity; channels were considered adequate in a gain setting of 7 or less and coefficient of variation of 7.5% or less to improve the signal-to-noise ratio. To calculate HbO/HbR concentration changes using modified Beer-Lambert law, data were pre-processed with default band pass filters (low cut-off 0.01 Hz; high cut-off 0.2 Hz; Scholkmann et al., 2014). For each channel, the software computed the mean amplitude

TABLE 1 | Approximate anatomical correlation of international 10/10 EEG system and cortical gyrus (n = 40 channels).

DLPFC		
10/10 system	*10/10 system*	*Cortical lobe (gyrus)–Brodmann area*
AF3–AF7	AF4–AF8	Superior frontal BA 9–middle frontal BA 10
AF3–F3	AF4–F4	Superior frontal BA 9–middle frontal BA 8
F5–AF7	F6–AF8	Middle frontal BA 46–middle frontal BA 10
F5–F7	F6–F8	Middle frontal BA 46–inferior frontal BA 45
F5–F3	F6–F4	Middle frontal BA 46–middle frontal BA 8
F5–FC5	F6–FC6	Middle frontal BA 46–precentral frontal BA 6
F1–F3	F2–F4	Superior frontal BA 6–middle frontal BA 8
FC3–F3	FC4–F4	Middle frontal BA 6–middle frontal BA 8
FC3–FC5	FC4–FC6	Middle frontal BA 6–precentral frontal BA 6
SMC		
10/10 system	*10/10 system*	*Cortical lobe (gyrus)–Brodmann area*
FC3–FC5	FC4–FC6	Middle frontal BA 6–precentral frontal BA 6
FC3–FC1	FC4–FC2	Middle frontal BA 6–superior frontal BA 6
FC3–C3	FC4–C4	Middle frontal BA 6–postcentral parietal BA 123
C1–FC1	C2–FC2	Precentral frontal BA 4–superior frontal BA 6
C1–C3	C2–C4	Precentral frontal BA 4–postcentral parietal BA 123
C1–CP1	C2–CP2	Precentral frontal BA 4–postcentral parietal BA 7
C5–FC5	C6–FC6	Postcentral parietal BA 123–precentral frontal BA 6
C5–C3	C6–C4	Postcentral parietal BA 123–postcentral parietal BA 123
C5–CP5	C6–CP6	Postcentral parietal BA 123–supramarginal parietal BA 40
CP3–C3	CP4–C4	Inferior parietal BA 40–postcentral parietal BA 123
CP3–CP5	CP4–CP6	Inferior parietal BA 40–supramarginal parietal BA 40
CP3–CP1	CP4–CP2	Inferior parietal BA 40–postcentral parietal BA 7

Adapted from Koessler et al. (2009).

for hemodynamic response averaging the measurements of 10 s of stimulation from the baseline period, that is, before stimulus.

As fNIRS devices calculate the concentration changes of HbO/HbR in millimoles per liter (mmol/l or mM) in relative proportion related to a measured baseline, the synchronization of the electrical stimulation made by the trigger marker in recorded signals was essential to correct interpretation of data, since the peak of the standard hemodynamic response function (HRF) curve is 2–6 s from the stimulus onset. In our analysis, we used HbO relative concentration changes, since it is the most sensitive parameter of activity-dependent changes in optical measurements, compared to HbR and total hemoglobin (Tanosaki et al., 2001).

Data analysis was made by nirsLAB software by NIRx® Technologies, using a general linear model (GLM) with the standard canonical HRF pattern, and statistical parametric mapping (SPM) Student's t-test corrected for multiple comparisons, for the single subject level and for the group level. GLM coefficients were estimated by equation $Y = X\beta + E$, where Y is the matrix of hemodynamic data; X is the design matrix; β is the GLM-coefficient matrix and E is the residual term. We used GLM parameters with no pre-whitening type of analysis, where the designed matrix used rest/stimulus to generate contrast 0/1 (nirsLAB 2017 manual[3]; Tak and Chul Ye, 2014).

TABLE 2 | Demographic characteristics between groups at baseline (n = 15).

	Active (n = 7)	**Sham (n = 8)**	***p*-value**
Age (years)	36 (2.64)	32.75 (3.23)	0.458
Education (years)	19.43 (1.92)	19.5 (0.96)	0.973
Body Mass Index—BMI	26.6 (1.28)	24.1 (1.26)	0.186
Alcohol consumption (≤1 week)	6/7	6/8	–
Caffeine intake before intervention (h)	>6	>6	–
State-Anxiety Inventory (STAI)	22 (2.49)	19.75 (1.28)	0.420
Trait-Anxiety Inventory (STAI)	18.14 (1.45)	17.87 (1.29)	0.892
Beck Depression Inventory (BDI-II)	4.86 (1.62)	2.25 (1.05)	0.190
Pittsburgh Sleep Quality Index (PSQI)	4.29 (0.86)	3 (0.75)	0.281

Comparisons using Student's t-test for independent samples. Results are presented in mean and standard error.

Shapiro-Wilk test was used to evaluate normal distribution of the variables, and Student's t-test was applied to evaluate differences between groups in parametric data. Multivariate analysis of covariance (MANCOVA) was used to assess statistical differences on multiple continuous dependent variables to verify differences regarding the activation of right and left DLPFC and right and left SMC areas. Comparisons were performed using a generalized estimating equation (GEE) model, followed by the Bonferroni correction for *post hoc* multiple comparisons. We analyzed the differences in HbO concentration changes by linear regression coefficients (Tak and Chul Ye, 2014), using SPSS version 22.0 (SPSS, Chicago, IL, USA). For all statistical analysis, the significance was set at $p < 0.05$.

RESULTS

Fifteen healthy right-handed male volunteers, mean 34.27 years old (±8.09), completed the 2-day study protocol. Demographic characteristics at baseline are shown in **Table 2**. No significant difference was found between groups that started with active or sham procedure on Day 1.

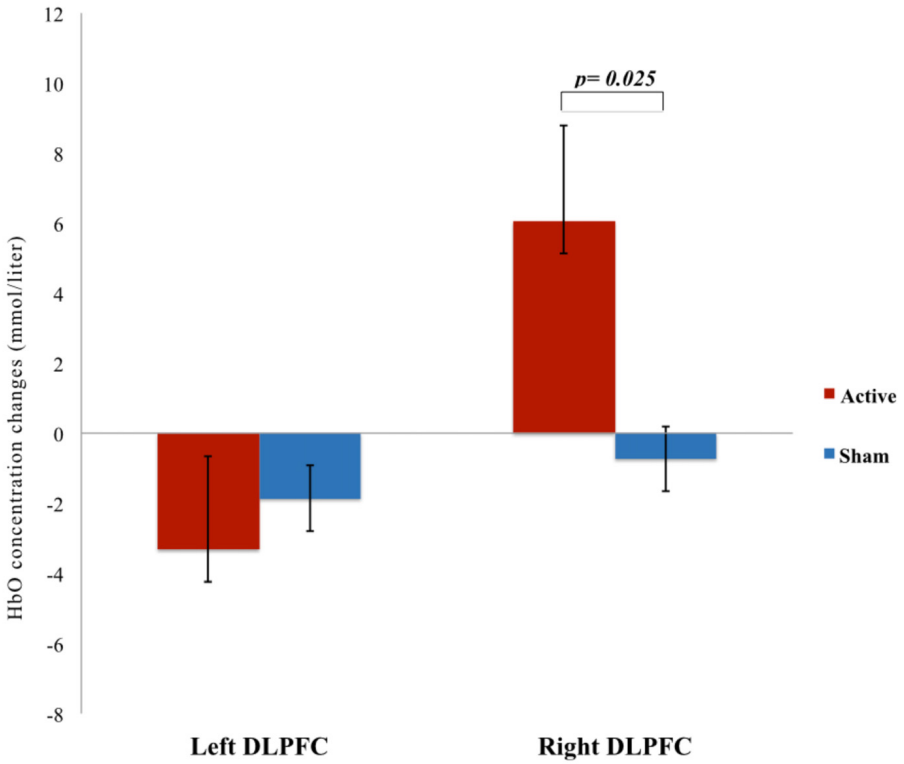
Minimal stress and/or mild muscular tension were reported before the experiment in some subjects (n = 5 in active and n = 8 in sham), without any major clinical manifestation. Four subjects complained of minimal to mild headache or cervical pain in both active and sham procedure, however, they were not able to distinguish if it was related to the fNIRS equipment (cap and optodes contact) or to the electrical stimulation *per se*. Prickling, itching, burning and/or heat sensation was mentioned by three subjects, related to the cap and optodes. The major discomfort mentioned was pain in the scalp, due to the tight cap and the pressure exerted by the optodes (n = 9 in active and n = 10 in sham). Somnolence was the most commonly reported symptom (24/30) in both active (n = 14) and sham (n = 10) procedures. The intensity of electrical current during active intervention required to get non-painful muscle contractions

[3]www.nirx.net

TABLE 3 | Oxygenated Hemoglobin (HbO) concentration changes on Cortical Area of Interest (CAI) between groups (n = 15).

Dependent variable		Type III Sum of Squares	*df*	Mean Square	*F*	*p*	Partial Eta Squared
Left DLPFC	Corrected Model	$1.587\ 10^{-9(a)}$	1	$1.587\ 10^{-9}$	0.266	0.610	0.009
Right DLPFC		$3.455\ 10^{-8(b)}$	1	$3.455\ 10^{-8}$	5.572	0.025	0.166
Left SMC		$5.001\ 10^{-8(c)}$	1	$5.001\ 10^{-8}$	4.542	0.042	0.140
Right SMC		$1.076\ 10^{-8(d)}$	1	$1.076\ 10^{-8}$	1.943	0.174	0.065
Left DLPFC	Intercept	$2.018\ 10^{-8}$	1	$2.018\ 10^{-8}$	3.382	0.077	0.108
Right DLPFC		$2.131\ 10^{-8}$	1	$2.131\ 10^{-8}$	3.437	0.074	0.109
Left SMC		$2.510\ 10^{-8}$	1	$2.510\ 10^{-8}$	2.280	0.142	0.075
Right SMC		$3.190\ 10^{-8}$	1	$3.190\ 10^{-8}$	5.763	0.023	0.171

Univariate Tests: F tests the effect based on linearly independent pairwise comparisons among the estimated marginal means. Test of Between-Subjects Effects; Multivariate Tests Observed Power = 1, 0. [(a)] *R Squared = 0.009 (Adjusted R Squared = −0.026);* [(b)] *R Squared = 0.166 (Adjusted R Squared = 0.136);* [(c)] *R Squared = 0.140 (Adjusted R Squared = 0.109);* [(d)] *R Squared = 0.065 (Adjusted R Squared = 0.031).*



	Mean		SEM		*p*
	Active	Sham	Active	Sham	
Left DLPFC	-3.321	-1.866	2.662	0.932	0.610
Right DLPFC	6.059	-0.728	2.721	0.928	0.025

Bars indicate the mean and the standard error of the mean (SEM). Comparisons were performed using a generalized estimating equation (GEE) model, followed by the Bonferroni correction for post hoc multiple comparisons.

FIGURE 4 | Comparison of DLPFC activation between active and sham groups (n = 15). The figure shows a representation of the mean oxygenated hemoglobin (HbO) concentration changes, measured in millimoles per liter (mmol/l) with correspondent p-value, indicating the difference of right DLPFC activation during accessory spinal nerve-peripheral electrical stimulation (ASN-PES).

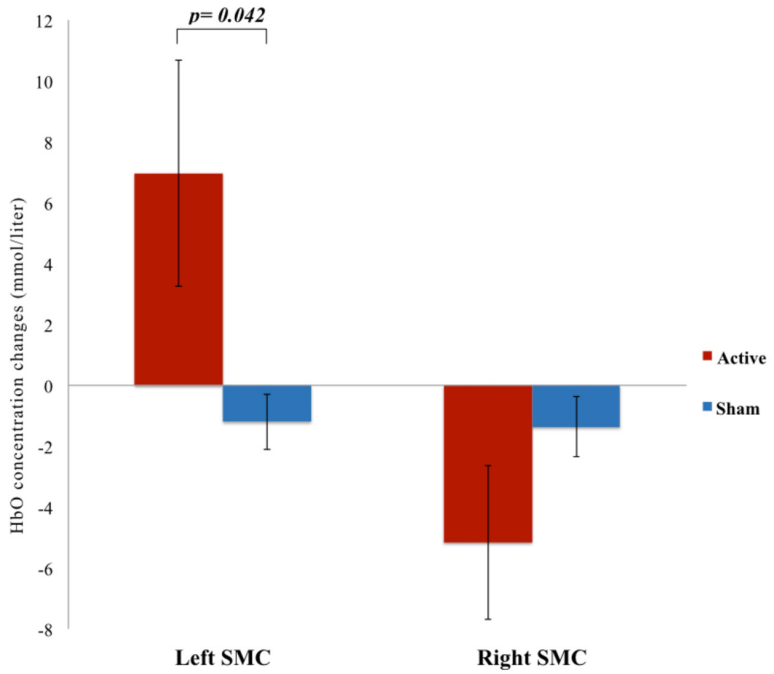
were minimal, as nerves need less electrical current to depolarize (1.133 mA ± 0.86). The electrical stimulation was well tolerated and asserted as non-painful by the participants. No relevant clinical complaint was observed.

We analyzed HbO concentration changes obtained in 30 experiments, 40 channels each, divided into active and sham group and into four CAI: left DLPFC, right DLPFC, left SMC and right SMC. The multiple dependent variables on MANCOVA model on CAI in active and sham groups are shown in **Table 3**. The effect of ASN electrical stimulation on HbO concentration changes was observed through the activation of right DLPFC ($F = 5.572$; $p = 0.025$) and left SMC ($F = 4.542$; $p = 0.042$) during the 10 s period of stimulation, compared to the 20 s period of rest, in active group but not in sham group. Regarding the activation of left DLPFC ($F = 0.266$; $p = 0.610$) and right SMC ($F = 1.943$; $p = 0.174$), there was no statistical difference between groups.

The representation of DLPFC and SMC activation between active and sham groups during ASN-PES are showed in **Figures 4**, **5**, respectively, with mean HbO concentration changes in millimoles per liter (mmol/l), standard error of the mean (SEM) and correspondent *p*-value. **Figures 6–8** show different representations of the same results found in statistical analysis. Additional data from each channel are available at **Supplementary Material** section. In HbO mean curves for each cortical area of interest shown in **Figure 7**, note that the 10 s stimulation time has a different pattern than the subsequent rest period.

DISCUSSION

This study confirms our hypothesis that the ASN-PES can promote cortical activation on areas involved in pain and emotion, nominally SMC and DLPFC. Our findings showed that unilateral right ASN electrical burst stimulation with 10 Hz 10 s ON and 20 s OFF was able to activate ipsilateral dorsolateral prefrontal (DLPFC) and contralateral sensorimotor



	Mean		SEM		*p*
	Active	Sham	Active	Sham	
Left SMC	6.976	-1.190	3.721	0.914	0.042
Right SMC	-5.154	-1.367	2.532	0.984	0.174

Bars indicate the mean and the standard error of the mean (SEM). Comparisons were performed using a generalized estimating equation (GEE) model, followed by the Bonferroni correction for post hoc multiple comparisons.

FIGURE 5 | Comparison of SMC activation between active and sham groups ($n = 15$). The figure shows a representation of the mean HbO concentration changes, measured in millimoles per liter (mmol/l) with correspondent *p*-value, indicating the difference of left SMC activation during accessory spinal nerve-peripheral electrical stimulation (ASN-PES).

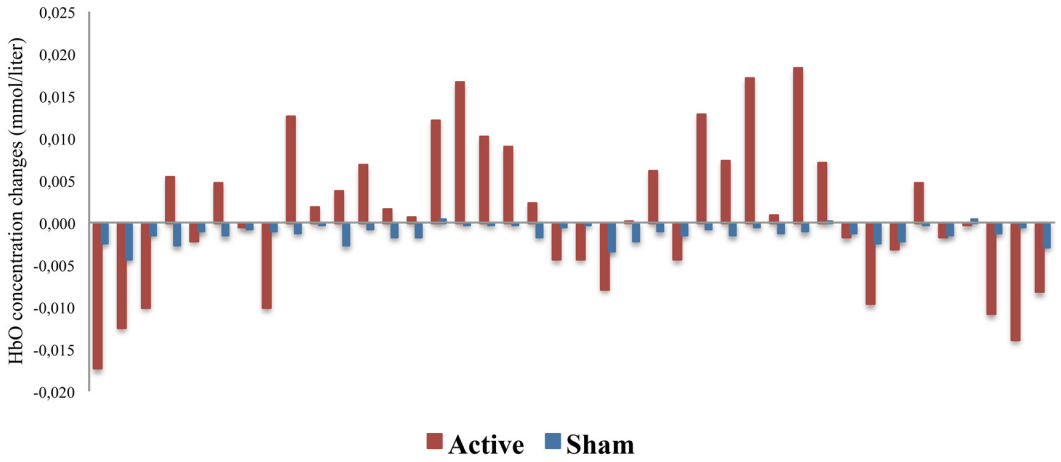


FIGURE 6 | HbO concentration changes in each channel between active and sham groups (n = 40). The figure shows a representation of the mean HbO concentration changes, measured in millimoles per liter (mmol/l), in each channel, demonstrating subtle changes in oxy-hemoglobin between active and sham groups in almost all of 40 channels.

(SMC) cortical areas during stimulation. ASN-PES induced changes in regional cerebral blood flow in central pain-related regions, significantly increasing the perfusion in those areas in active but not in sham stimulation. Thus, it was able to produce bottom-up activation to central brain regions of pain processing.

The relevance of these results is to extend literature upon PES effects to modulate cortical areas involved in pain processing and help to investigate neurobiological mechanisms of peripheral neuromodulatory techniques. Furthermore, it helps to understand systemic effects observed in clinical practice and supports the possibility of using this type of non-painful peripheral stimulation as a therapeutic approach in pain treatment, including the possibility to use combined methods to induce a top-down (e.g., NIBS or behavioral therapies) and bottom-up modulation (e.g., dry-needling). It also allows more understanding on pain mechanisms considering its dimensions, which comprises sensory-discriminative, affective-motivational and cognitive-behavioral aspects (Melzack, 2001), as these manifestations are linked to neural networks of SMC and DLPFC.

Regarding the activation of SMC, our work is lined up to previous results in the literature and suggest that the temporal resolution of fNIRS offers an efficient technical solution to study the cortical areas activated by PES. However, in left DLPFC and right SMC, we did not find statistical difference between baseline and 10 s stimulation, but we observe that there is a subtle rise in HbO concentration towards activation on subsequent 10 s of rest, as shown in **Figure 7**. Although it can be associated to an error type, another hypothesis is that these areas are also activated, with a temporal delay, in active but not in sham group. Another hypothesis is that some targets areas are activated in detriment of deactivation of others. Indeed, temporal changes were found by others authors, as decrease of cortical activation during execution of hand movements using fNIRS after 5 min of electrical stimulation (Jang et al., 2014). Besides that, parts of activated circuits and subsequent temporal responses seem to be enrolled by inter-hemispheric functional connections (Sankarasubramanian et al., 2017). Furthermore, different functions of the right and left hemispheres, right and left DLPFC and medial and lateral PFC sub-regions in pain processing and in unpleasant sensations are involved in neural networks not yet clarified (Lorenz et al., 2003; Cieslik et al., 2013; Brasil-Neto, 2016).

This study added value to the fact that ASN-PES is **non-painful** and utilize intensities above motor threshold. The goal of ASN needling is not to cause pain in the subcutaneous insertion of the needle in the cervical region, tangentiating the nerve to get its depolarization. The electrical current must flow through the perinervous layer, without hurting the nerve tissue. This causes mild to moderate movement of the muscles under ASN domain, i.e., trapezius muscle and sometimes sternocleidomastoid muscle, without pain. It has the same goal as functional electrical stimulation (FES), where a non-painful electrode stimulus generates action potentials resulting in contraction of the target muscles. In Blickenstorfer et al.'s (2009) study with FES, fMRI showed activation pattern in the contralateral M1, S1, PMC and the ipsilateral cerebellum, as well as bilateral S2, SMA and ACC.

It is conceivable that the bottom-up activation of DLPFC induced by ASN-PES may trigger top-down responses, since ACC is implicated in the elicitation and control of sympathetic autonomic arousal. Therefore, the activation of right DLPFC by ASN may culminate in nucleous accumbens (NAc) activation in order to activate pain descending modulatory system together with PAG and rostroventral medulla (RVM; Navratilova and Porreca, 2014; Elman and Borsook, 2016). This pathway could explain the sense of relaxation and well being reported by subjects following the active intervention.

We observed that stimulation of right ASN produced similar results seen during VN stimulation with electrodes and implanted devices (Frangos and Komisaruk, 2017). During that study, fMRI images showed ipsilateral activation of nucleus

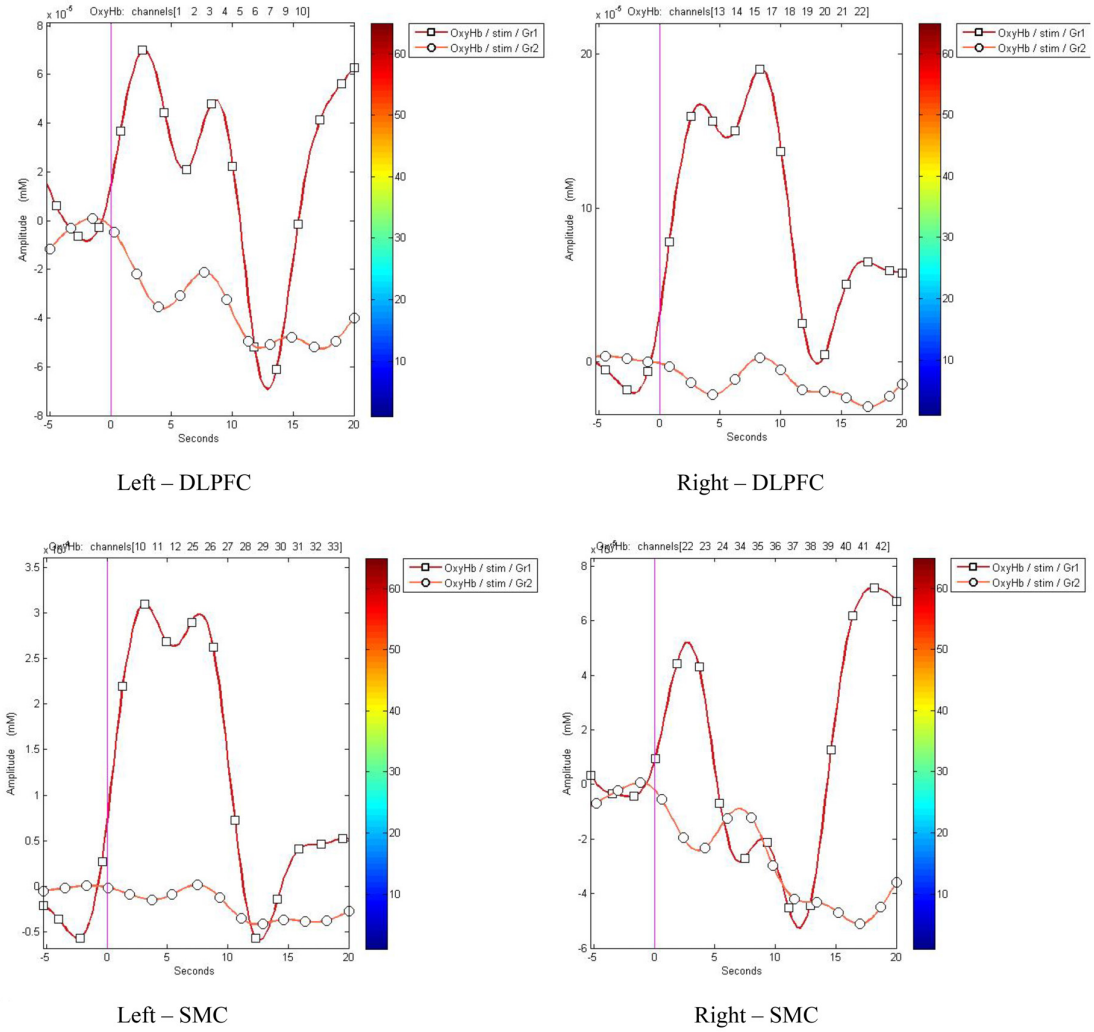


FIGURE 7 | HbO mean curves for each cortical area of interest between groups. Channels were gathered to display the oxy-hemoglobin changes for active (Gr1) and sham (Gr2) groups, from 5 s before (baseline) to 20 s after stimulation onset.

of solitary tract (NST), which is the primary central relay of vagal afferents, insula, thalamus, caudate nucleus and SSC; deactivation occurred in hippocampus, contralateral NST and ipsilateral spinal TN. In a subsequent period, activation was observed in substantia nigra, ventral tegmental area (VTA), dorsal raphe nuclei (DRN) and PAG. Based on our findings, we cannot affirm that the ASN-PES involves the activation of subcortical areas, but the anatomical correlation of ASN and VN raises an intriguing question to be explored in future studies. The anatomical structure of ASN gives us biological support to investigate the ASN-PES as a more accessible alternative for routine clinical use when therapeutic approach is to target the VN.

Also, a better comprehension of ASN-PES effect as a bottom-up neuromodulatory approach is its potential to be combined with other top-down NIBS techniques, such as transcranial direct current stimulation (tDCS) and TMS. The argument to support this question is a potential additive effect and, consequently, a better clinical response. A previous study that applied tDCS together with PES over the median nerve found increase in MEP compared to baseline in TMS parameters (Rizzo et al., 2014). Other study showed frequency-dependent motor cortex response with combined TMS and PES to test bi-directional plasticity (Pitcher et al., 2003). Combined PES and tDCS intervention on patients with chronic low back pain improved symptoms than either intervention alone or sham in another trial (Schabrun et al., 2014). In addition, a systematic review on stimulus parameters of PES in healthy subjects demonstrated that higher intensities of stimulation produced more consistent effects on the increase in excitability of the corticomotor pathway (Chipchase et al., 2011a). In another study, IMS [which appears to encompass the same type of stimulus as EA (Kim et al., 2012)] enhanced inhibitory modulation in cortical and infracortical pain processing systems when applied

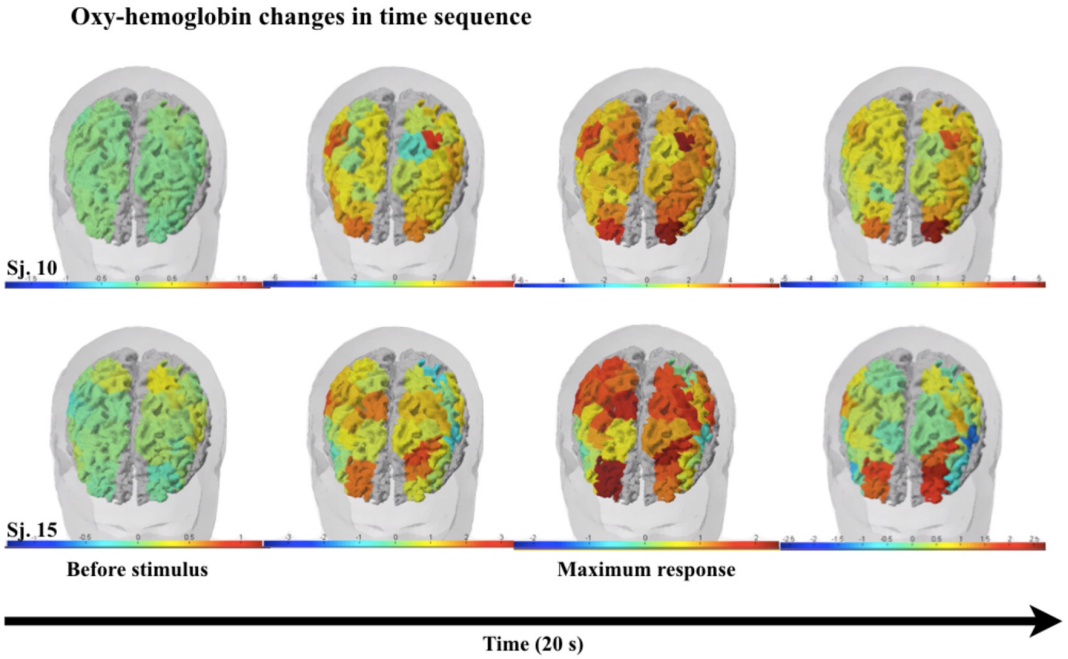


FIGURE 8 | Graphic representation frames on a 3D brain surface model in two subjects, showing oxy-hemoglobin changes in time sequence. Color bars represent activation (red) or deactivation (blue) response. The first frame represents oxy-hemoglobin before the electrical stimulus, followed by frames in time sequence.

to women with knee osteoarthritis undergoing tDCS (Tarragó et al., 2016). Possibly, modulatory techniques such as NIBS and PES attempt to re-reorganize neural circuits, improving malfunction of the whole system on cortical, infracortical, spinal and local sites.

Study Limitations

It is necessary to point out some limitations concerning this study. We did not have a 3D device to confirm the probe location to relate it to Brodmann's areas. Instead, we used the 10/10 International System, as shown in **Table 1**. Likewise, we did not have short distance inter-probes, which would have helped to control noise data from skin blood flow, although we did not place probes in the forehead (Takahashi et al., 2011). Scalp hemodynamics often contaminates fNIRS signals, and standard source-detector distance channels tend to over-estimate the artifacts (Sato et al., 2016). These limitations interfere with the evaluation of cortical activation. Actually, fNIRS technical limitations include superficial depth cortical evaluation, cardiovascular frequency noise, environmental light noise and motion artifacts (Ferrari and Quaresima, 2012; Scholkmann et al., 2014; Tak and Chul Ye, 2014). Furthermore, as it was already pointed out, a single-session of unilateral electrical stimulation of a craniocervical nerve can tell us about its acute manifestations without temporal changes, that can be different in subsequent measurements, as pointed out by other authors (Tanosaki et al., 2001; Jang et al., 2014; Frangos and Komisaruk, 2017).

While a physiological basis study on cortical responses, we must consider the amostral design that included only right ASN stimulation in healthy, right-handed males in a controlled environment. As expected, we observed large inter-individual responses, which might be due to a particular cortical organization or anatomical features, such as skull and subcutaneous tissues thickness, head format and skin or hair pigmentation (Niederhauser et al., 2008). Variables such as tiredness, stress, muscular tension, anxiety, expectancy, fear of pain, discomfort due to sitting still or cap pressure can change mental status, which can activate unexpected areas; this may be the reason why sham procedure data showed more variability than active stimulation data. Females were not included in our study to avoid hormonal influences on results, as women are more susceptible to negative emotional responses such as fear of pain, stress and anxiety (da Silva et al., 2015). The exclusion of females may generate either better or worse cortical responses to stimulation. Response patterns may also be different with bilateral stimulation in healthy vs. chronic pain patients. Other variables, such as age, lifestyle, education level, genetics and even recent news about chronobiology may play a fundamental role on response patterns in other subgroups that experience top-down or bottom-up modulations (Cummings and Baldry, 2007; Ridding and Ziemann, 2010).

Moreover, we observed that studies related to PES are very heterogeneous with unstandardized nomenclature, protocols, electrical features, duration and type of stimulus (Chipchase et al., 2011a; Rossini et al., 2015; Chakravarthy et al., 2016). It is necessary to develop an academic consensus aiming to standardize research and clinical protocols since PES techniques seem to be a promising therapeutic tool for pain management and neuro-rehabilitation.

Conclusions

In conclusion, cortical activation of sensorimotor and DLPFC induced by non-painful ASN-PES seems to activate the same crucial pain cortical related areas, acting on bottom-up modulation pathway. Also, it opens a novel window of research into the possibilities of ASN-PES on modulation for treatment purposes. Further studies are needed in order to explore this technique as a potential therapeutic tool and its impact in clinical settings.

ETHICS STATEMENT

The study protocol was approved by Hospital de Clínicas de Porto Alegre Ethics Committee Board (Institutional Review Board IRB 0000921), according to the Declaration of Helsinki. All subjects provided their written informed consent. The protocol was developed in accordance with the Consolidated Standards of Reporting Trials—CONSORT, and registered at ClinicalTrials.gov (NCT 03295370).

AUTHOR CONTRIBUTIONS

All authors made a significant contribution to: (a) the study concept and design, acquisition of data, or analysis and interpretation of data; (b) drafting/revising the manuscript for important intellectual content; and (c) approval of the final version to be published.

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SUPPLEMENTARY MATERIAL

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Review

Rewiring the Lesioned Brain: Electrical Stimulation for Post-Stroke Motor Restoration

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Electrical stimulation has been extensively applied in post-stroke motor restoration, but its treatment mechanisms are not fully understood. Stimulation of neuromotor control system at multiple levels manipulates the corresponding neuronal circuits and results in neuroplasticity changes of stroke survivors. This rewires the lesioned brain and advances functional improvement. This review addresses the therapeutic mechanisms of different stimulation modalities, such as noninvasive brain stimulation, peripheral electrical stimulation, and other emerging techniques. The existing applications, the latest progress, and future directions are discussed. The use of electrical stimulation to facilitate post-stroke motor recovery presents great opportunities in terms of targeted intervention and easy applicability. Further technical improvements and clinical studies are required to reveal the neuromodulatory mechanisms and to enhance rehabilitation therapy efficiency in stroke survivors and people with other movement disorders.

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Introduction

Stroke is the second leading cause of death and the leading cause of disability worldwide, recent study showed that its disability-adjusted life year is nearly 113 million globally.[1] Stroke incidence and mortality increases with age, and for the coming aging population, more stroke cases are expected which would induce a severe burden on the society.[2] About 20% of stroke patients die, whereas 80% of stroke survivors experience motor impairments contralateral to the lesioned hemisphere.[3] Typical stroke symptoms include unilateral motor weakness, limb hemiparesis, spasticity, gait disturbance, and loss of coordination.[4] More than half of stroke patients cannot fully recover from motor impairments, and the quality of their life is substantially affected.[5]

Motor control is the ability to regulate mechanisms requisite to locomotion.[6] The hierarchical motor control process involves multiple brain structures, as illustrated in Figure 1. Both the central nervous system (including the cerebral cortex, cerebellum, brain stem, and spinal cord) and peripheral extremities are involved in the motor control process.[7,8] Corticospinal tract (CST) derives from the sensorimotor cortex, projecting its output to spinal interneuron or motoneuron circuits. It is essentially the dominant descending pathway for the motor control process in primates.[9] The proprioceptor and other sensory inputs may transmit back to the sensorimotor cortex through the spinal tracts.[10] Motor commands are transferred from the cortex to the reticular formation in the brainstem, and further transmitted to spinal interneuron or motoneuron circuits and peripheral extremities via the reticulospinal tract.[11] The reticulospinal tract is crucial for human locomotion, balance, and coordination. Integrity of the motor control system is pivotal

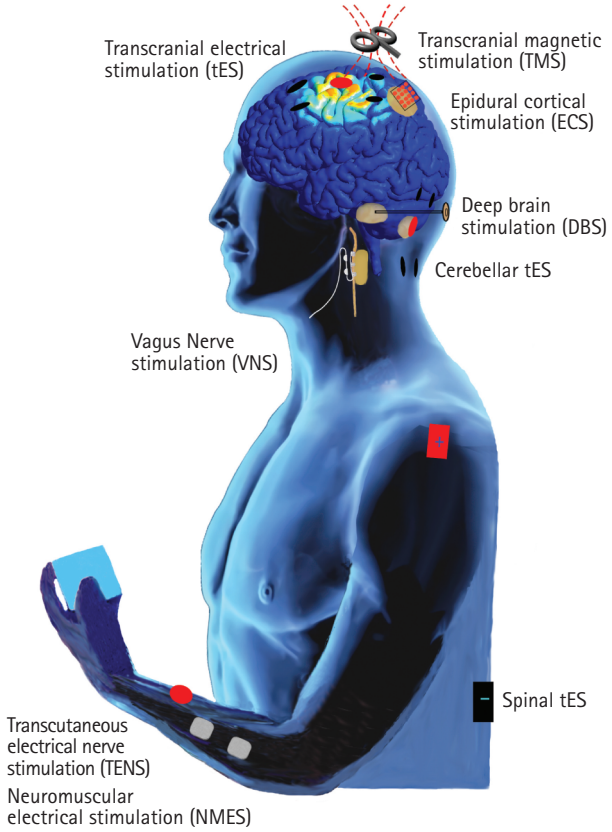

Figure 1. Typical electrical stimulation modalities for post-stroke motor restoration. Finite element modeling result of transcranial electrical stimulation (tES)-induced electrical field is illustrated.

for human locomotion. Therefore, damage to sensorimotor or higher-order brain regions results in motor impairment.[12] In such a case, rehabilitation is essential in regaining functional improvement or restoration. Post-stroke motor restoration is challenging due to genetic, pathophysiologic, sociodemographic, and other clinical factors.[13] Hence, manipulating related neural circuits and rewiring the lesioned brain might be critical factors for post-stroke motor recovery.

In 1949, neuropsychologist Donald Hebb[14] proposed the rule that "Neurons that fire together, wire together." Hebb's rule provided the theoretical foundations that homosynaptic and heterosynaptic activities facilitate synaptic formation and consolidation during motor rehabilitation.[14] Neuroplasticity could be augmented through rehabilitation strategies,[15,16] such as basic task-oriented training (TOT). However, TOT alone cannot effectively alleviate motor impairment and restore motor functions.[17] Unlike pharmacological therapy, electrical stimulation provides a more targeted intervention to damaged motor neural circuits, resulting in better functional recovery for patients with motor impairments.[18,19]

Various electrical stimulation modalities have been used to promote neuroplasticity and facilitate post-stroke motor recovery in different levels of the neuromotor control system. Tentative stimulation targets include the motor cortex, peripheral extremities, cerebellum, deep brain, vagus nerve, and other related areas, as shown in Figure 1. Table 1 summarizes the milestones of various electrical stimulation modalities in post-stroke motor recovery, with classification, main findings, and references listed in a chronological order. Among these protocols, noninvasive brain stimulation (NIBS) and peripheral electrical stimulation protocols have been extensively employed. Other related stimulation protocols are still in the prefatory laboratory or preclinical exploration stages. Figure 2 presents an intuitive summary to Table 1, highlighting the timeline and a brief history of typical electrical stimulation techniques in post-stroke motor recovery. Although neural electrical stimulation was proposed nearly 60 years ago, its application in post-stroke motor recovery was not actualized until the end of the twentieth century. Markedly, the past 5 years witnessed progressive developments in electrical stimulation techniques and availability of new interventions in post-stroke motor recovery. Here, we provide a detailed review of multi-level electrical stimulation-based post-stroke motor recovery summarizing the published studies and future trends in this field.

Noninvasive brain stimulation

NIBS has been utilized as a stand-alone or supplementary rehabilitation tool in stroke related motor recovery.[19] NIBS modulates neural synaptic plasticity and motor skill acquisition beyond the stimulation period. Such modulatory effects facilitate motor learning and neurorehabilitation process, and further enhance paretic limb motor function.[20] NIBS modalities reviewed here include transcranial electrical stimulation (tES) or transcranial magnetic stimulation (TMS), TMS is also included as it induced electric currents through electromagnetic induction. Cerebellum and spinal cord stimulation protocols are also discussed.

Transcranial electrical stimulation

As a representative NIBS protocol, tES modulates cortical excitability and induces CST changes lasting beyond stimulation periods.[21-23] Pioneer tES applications date back 2,000 years ago during the Greco-Roman period. Electricity from organs of electric fish was used to treat pain, limb paresis, and other symptoms.[24] Earlier studies with rat models demonstrated neuronal depolarization after anode electrical stimulation.[25] Modern noninvasive transcranial direct current stimulation (tDCS) studies began at the end of the 20th century.[26,27]

Noninvasive tES is powered by battery-based electrical cir-

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Table 1. Milestones of various electrical stimulation modalities in post-stroke motor recovery

Stimulation modalities	Representative studies	
	Key findings	Reference
Noninvasive brain stimulation		
tES	ES induced neuronal depolarization in rats	Bindman et al. (1962)[25]
	Conventional tDCS modulation effects of motor excitability in healthy subjects	Priori et al. (1998)[26], Nitsche et al. (2000)[27]
	tDCS facilitates post-stroke motor recovery	Hummel et al. (2005)[49]
	High definition tDCS with increase focality	Borckardt et al. (2012)[28]
	Network-based tDCS targeting multiple-area	Fischer et al. (2017)[57]
	Online closed-loop EEG-tDCS	Leite et al. (2017)[59]
	In vivo neuronal circuits modulated by tDCS for human and rats	Vöröslakos et al. (2018)[44]
	Gait-synchronized tACS	Koganemaru et al. (2019)[61]
TMS	TMS influence on healthy motor cortex	Barker et al. (1985)[63]
	rTMS cortical excitability effects in healthy	Maeda et al. (2000)[67]
	rTMS in post-stroke motor recovery	Takeuchi et al. (2005)[68]
	PAS increase MEP in healthy subjects	Fratello et al. (2006)[74]
	TBS applications in healthy subjects	Huang et al. (2007)[72]
	Unknown rTMS parameters in stroke, review	Hao et al. (2013)[76]
	Multi-locus TMS to increase targeting	Koponen et al. (2018)[82]
Cerebellar and spinal cord stimulation	Cerebellar tDCS influence CBI in healthy	Galea et al. (2009)[88]
	Cerebellar tDCS to improve motor skill learning and adaptation in healthy	Doppelmayr et al. (2016)[90], Erfmann (2018)[89]
	Cerebellar tACS and stroke neuroplasticity	Naro et al. (2016)[93]
	Cerebellar tDCS in stroke standing balance	Zandvliet et al. (2018)[91], (2019)[92]
	Combined effect of spinal tDCS, robot training, and cerebellar/cortical tDCS	Picelli et al. (2015)[97], (2018)[98], (2019)[99]
Peripheral electrical stimulation		
NMES	FES in post-stroke hemiplegic gaiting	Liberson et al. (1961)[105]
	Implanted NMES system	Peckham et al. (1988)[106]
	Myoelectric control of NMES	Cauraugh et al. (2000)[110]
	BCI control of NMES	Meng et al. (2008)[109]
	Invasive BCI-NMES with fine movement	Bouton et al. (2016)[119]
	High density NMES to allow fine control	Annetta et al. (2019)[120]
TENS	TENS for pain relief	Augustinsson et al. (1977)[122]
	TENS for stroke sensorimotor functions	Peurala et al. (2002)[124]
	TENS in post-stroke motor recovery, review	Grant et al. (2018)[129]
Emerging electrical stimulation techniques		
DBS	DBS in limb paresis after stroke	Phillips et al. (2000)[138]
	DBS for DTC pathway in stroke	Machado et al. (2012)[143]
	Noninvasive interference DBS	Grossman et al. (2017)[145]
	Cerebellar DBS-based post-stroke motor recovery, review	Wathen et al. (2018)[144]
ECS	ECS in rat stroke model	Brown et al. (2006)[147]
	Phase I, II clinical trials in stroke patients	Levy et al. (2008)[149], (2016)[150]
	Array focal cortical stimulation	Yang et al. (2017)[155]

Table 1. Continued

Stimulation modalities	Representative studies	
	Key findings	Reference
VNS	Invasive VNS in stroke rat model	Khodaparast et al. (2013)[164]
	Noninvasive VNS in stroke rat model	Ay et al. (2016)[167]

tES, transcranial electrical stimulation; ES, electrical stimulation; tDCS, transcranial direct current stimulation; EEG, electroencephalogram; tACS, transcranial alternating current stimulation; TMS, transcranial magnetic stimulation; rTMS, repetitive TMS; PAS, paired associative stimulation; MEP, motor evoked potential; TBS, theta burst stimulation; CBI, cerebellar brain inhibition; NMES, neuromuscular electrical stimulation; FES, functional electrical stimulation; BCI, brain computer interface; TENS, transcutaneous electrical nerve stimulation; DBS, deep brain stimulation; DTC, dentatothalamocortical; ECS, epidural cortical stimulation; VNS, vagus nerve stimulation.

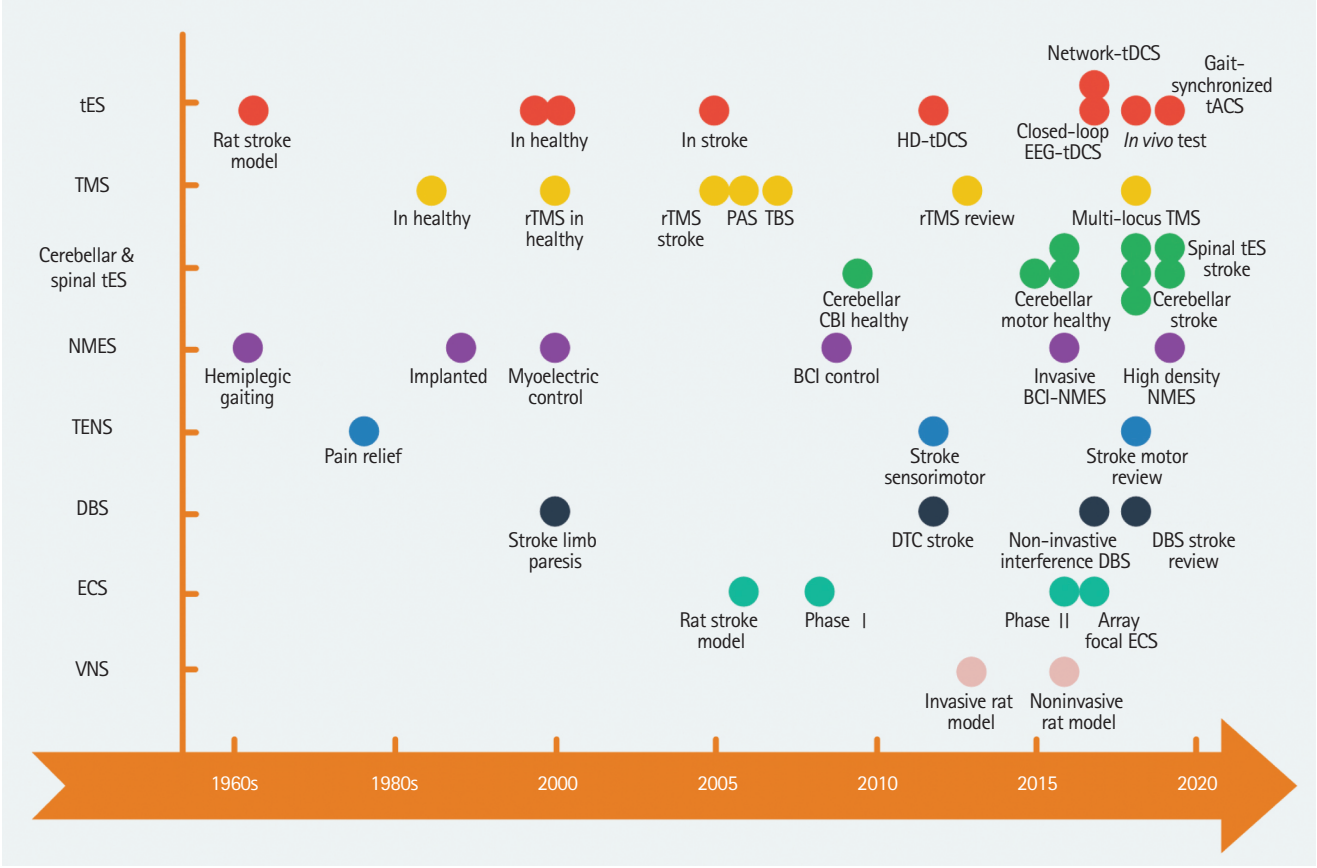

Figure 2. Timeline and brief history of representative electrical stimulation techniques in post-stroke motor recovery. Each dot represents one typical finding as shown in Table 1, different color indicates different stimulation modality. x-axis, year in sequence, before 2000, each tick means 20 years, after 2000, each tick means 5 years; y-axis, different electrical stimulation techniques. tES, transcranial electrical stimulation; TMS, transcranial magnetic stimulation; NMES, neuromuscular electrical stimulation; TENS, transcutaneous electrical nerve stimulation; DBS, deep brain stimulation; ECS, epidural cortical stimulation; VNS, vagus nerve stimulation; tDCS, transcranial direct current stimulation; tACS, transcranial alternating current stimulation; EEG, electroencephalogram; rTMS, repetitive TMS; PAS, paired associative stimulation; TBS, theta burst stimulation; CBI, cerebellar brain inhibition; BCI, brain computer interface; DTC, dentatothalamocortical.

cuits, the generated low-amplitude currents penetrate the skull and influence the brain area underneath stimulation sites. tES modifies transmembrane neuronal potential and further modulates cortical excitability. Essentially, tES with different parameter settings induces different modulation effects.[24] A conventional tES system comprises a conductive rubber pad-based tES (5×7 cm, for example), while the newer high definition (HD) tES with small ring-based electrodes has better focality and outperforms the conventional settings.[28,29] Typical tES comprises tDCS and transcranial alternating current stimulation (tACS). tACS utilizes sinusoidal current with different stimulation frequencies and evokes cortical activations. Studies have reported that different stimulation frequencies lead to different modulatory effects. For instance, 10 Hz tACS enhanc-

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es motor learning significantly,[30] 20 Hz tACS decreases beta band cortico-muscular coupling in finger tapping tasks,[31] while 1 to 5 kHz range tACS increases motor cortex excitability.[32] Further studies are needed to translate tACS into clinical applications. Other versions of tES including transcranial random noise stimulation and transcranial pulsed current stimulation are not commonly used in stroke rehabilitation yet.

As the most frequently used tES modality, tDCS employs weak direct electrical current stimulation (around 0.5 to 2 mA) with two or more electrodes placed on the primary motor cortex (M1) or its neighboring area for post-stroke motor recovery. Modulation after-effects of 10 to 20 minutes stimulation could last for about 30 to 40 minutes depending on the stimulation settings.[24] Such stimulation induces polarity-dependent neural modulatory effects. Anode and cathode stimulation enhances and inhibits motor excitability, respectively.[33] Particularly, tDCS induced persistent bidirectional modification of post-synaptic connections is similar to long-term potentiation (LTP, anode) and long-term depression (LTD, cathode).[24] At the neuron level, tDCS generates glutamatergic plasticity with a modulatory effect on neurotransmitters and ion channels, including N-methyl-D-aspartate glutamate, and brain-derived neurotrophic factor (BDNF).

Pioneer study found that tDCS induced motor evoked potentials (MEPs) changes with TMS, which allows for reproducible measurement of cortical excitability.[27] Other electrophysiological, hemodynamic, and neurophysiological measurement tools including functional magnetic resonance imaging (MRI),[34] and functional near-infrared spectroscopy (fNIRS)[35] have been utilized to scrutinize modulatory effects of tDCS. Additionally, the immediate modulation effects of tDCS on task-specific brain oscillation have been explored using electroencephalogram (EEG), electromyogram (EMG), and local field potential.[36-38] These studies reported that tDCS modulates motor control process and induces cortical excitability changes. Moreover, tDCS fosters external limb properties of leg tibialis anterior muscle pinching, voluntary paretic ankle control, and isometric contraction myoelectric control.[39-41] In addition to local modulatory effects underneath the stimulation area, tDCS also modulates regions distant from stimulation sites by influencing motor-related neural synchrony, including cortical connectivity, corticospinal excitability, and cortico-muscular coupling. For instance, anode tDCS over left M1 facilitated cortical synchronization in the alpha and lower bands of the frontal and parieto-occipital cortex, the high gamma frequency bands of the motor cortex,[42] and increased functional coupling of EEG rhythms in the sensorimotor cortex.[43] *In vivo* intracellular and extracellular measurements illustrated that neuronal circuits are instantaneously influenced by electrical stimulation in rats and human cadaver brains *in situ*.[44] An recent study reported that anode HD-tDCS could promote cortico-muscular coherence in chronic stroke subjects,[45] suggesting an enhanced cortico-muscular communication after HD-tDCS. Using diffusion MRI, tDCS strengthens the descending corticospinal pathway from M1 to target muscles during brain computer interface (BCI)-based stroke rehabilitation with increased CST integrity.[46] In addition to modulating functional plasticity, recent evidence suggested that tDCS induces structural plasticity and physiological BDNF expressions.[47]

Post-stroke motor recovery relies on neuroplasticity and brain reorganization. Such reorganization appears in the ipsilesional motor cortex, contralesional area, or deep brain regions.[48] tDCS was first applied in post-stroke motor recovery in 2005,[49] and has thereafter been extensively utilized.[22,50,51] These bench-to-bedside studies have provided evidences that tDCS and task-specific motor training contributed to long-term post-stroke motor learning and recovery. Stroke focal lesion disrupts the balanced interhemispheric inhibition, with the over-inhibition of ipsilesional hemisphere preventing paretic limbs from acquiring better recovery.[52] However, such interhemispheric competition models are still under scrutiny, a bimodal balance-recovery model was proposed for guiding neurorehabilitation in 2014.[53] tDCS is currently employed in either inhibiting the contralesional hemisphere or exciting the lesioned hemisphere, simultaneous stimulation of bilateral hemispheres has also been attempted.[54] Such tDCS induced neuroplasticity could induce a long-lasting motor enhancement and recovery. Although several clinical experiments assessing the functional role of tDCS in post-stroke motor recovery have concluded with promising results, no consensus has been reached on its therapeutic efficacy from randomized controlled trials.[22,23,55] Intra-subject and inter-subject variability of response might limit the wide application of tDCS in stroke subjects. Large-scale, well-designed Phase III clinical trials and in-depth understanding of tDCS modulatory mechanisms will enhance tDCS-based rehabilitation efficiency. In addition, biophysical models could predict treatment efficacy, elucidating the underlying mechanisms in different levels of post-stroke recovery. Several preliminary theoretical frameworks have been proposed towards understanding the effects of tES on neurorehabilitation.[56] Future studies should provide more personalized and reliable models of tDCS-based neurorehabilitation.

tES electrode placement and the corresponding electric fields could also influence modulation results. Recent advancements in multichannel network-based tDCS showed better modulatory effects as compared to 2 to 5 channel tDCS in healthy subjects, indicating better tools for future stroke rehabilitation studies.[57] In addition, it is imperative to employ computational

modeling in obtaining optimized stimulation settings for individual stroke subjects, factoring in the impact of stroke lesion and the heterogeneity of brain anatomy.[58] A recent closed-loop EEG-tDCS system introduced online control of electrical stimulation with promising clinical applications.[59] To enable simultaneous stimulation and artifact-free recordings, advanced artifact removal strategies are required.[60] Moreover, a recent pilot study reported that gait-synchronized tACS could facilitate gait recovery in stroke patients.[61] When developing and advancing these state-of-art techniques, it will be vital to evaluate their reliability with large sample size randomized controlled clinical trials. Such systems will provide a blueprint on future rehabilitation applications.

Transcranial magnetic stimulation

TMS induces a transient time-varying magnetic field perpendicular to the stimulation coil, which further produces electric currents parallel to the coil underneath the cortical tissues. Electromagnetic induction results in focused electrical currents, further inducing neuronal depolarization and propagation of action potentials.[62] Barker et al.[63] introduced TMS as a potential neuromodulation tool on the human motor cortex, TMS has since then been utilized for either physiological measurement or neuromodulation depending on stimulation settings.[64] Single-pulse and paired-pulse TMS could measure the neurophysiological properties like MEPs and intracortical excitability.[65,66] Repetitive TMS (rTMS) and patterned TMS modulate cortical excitability beyond stimulation period depending on stimulation settings.[52,67] High-frequency rTMS (usually ≥5 Hz) excites the brain, while low-frequency rTMS (≤1 Hz) inhibits cortical excitability. Modulation of stroke related neural circuitry and cortical substrates implies potential applications of rTMS in post-stroke rehabilitation.

Several studies have investigated the functional role of rTMS in motor recovery in stroke subjects.[68,69] Like tES, rTMS influences neuroplasticity from synaptic connections similar to LTP and LTD process. It modulates the imbalanced interhemispheric inhibition between hemispheres, by either inhibiting the contralesional hemisphere or exciting the lesioned hemisphere.[54] Excitatory rTMS could facilitate synchronicity of neural firing of ipsilesional cortical regions and further harness neuroplasticity following a stroke. Additional corticospinal pathways could also be activated and adjacent lesion areas could be recruited.[70] Due to potential risk of rTMS-induced seizure in stroke patients,[71] it is necessary to follow a strict screening process before conducting rTMS-based clinical trials. Simple rTMS induces modulatory effects for a few minutes, while theta burst stimulation (TBS) with subthreshold high-frequency stimulation (for example, 50 Hz) induce modulation for about 30 to 60 minutes, the intermittent TBS promotes while continuous pattern inhibits cortical activity, respectively.[72] When magnetic stimulus on the contralateral M1 was paired with peripheral nerve stimulus, it presented as a potential therapeutic intervention tool for post-stroke recovery.[73] For such paired associative stimulation (PAS), M1 corticospinal excitability was modulated by the repeated pairing of the two stimuli, and the modulatory effects were linked to the interstimulus interval.[74]

Though numerous rTMS-based clinical trials have been conducted, there is no consensus on the adjunct therapeutic effects of rTMS. Therefore, the clinical applications of rTMS in post-stroke motor recovery are limited.[75,76] Moreover, randomized controlled trials on stroke subjects were still lacking, and adjuvant use of rTMS with constraint-induced therapy showed no significant enhancement in an exploratory randomized clinical trial.[77] Optimal protocols and stimulation parameter settings differ across subjects.[78] Randomized controlled clinical trials with large sample size are required to determine the long-term and therapeutic effects of rTMS. Combination of rTMS with other intervention techniques could enhance post-stroke motor recovery. Nevertheless, many neurophysiological processes following a stroke are involved in rTMS-based rehabilitation. In addition, the underlying mechanisms of rTMS neural circuit modulation remain only partially understood, greatly limiting the wide application of rTMS in post-stroke motor recovery. Computational modeling could be valuable in providing insights on fundamental cause and effect principles. Cortical networks and corticospinal changes following rTMS could be investigated by multimodal neurophysiological measures in animal models and a wide variety of stroke patients.[79-81] Recent advancements in multi-locus TMS could contribute to individualized multiple-region stimulation therapy and further enhance neuroplasticity.[82]

Cerebellar and spinal cord stimulation

The cerebellum is a vital structure in movement control and coordination, including balance maintenance, gait, and fine motor skills.[83] It is connected to M1, premotor, prefrontal, and other cerebral regions. The cerebellum is an essential part of error-based motor learning process, and the LTD-like plasticity of Purkinje cells in the cerebellum is associated with Hebbian learning.[84] Cerebellar activities depend on the descending inputs from the contralateral cerebrum, and the ascending inputs from the cerebellum provide feedback to M1. Therefore, the cerebellum is involved in synchronization of both sensory input and motor output.[85] Additionally, cerebellar excitability is correlated to motor adaptation in healthy and stroke subjects, im-

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plying that its neuroplasticity in sensorimotor learning could boost motor recovery.[86] Not all stroke subjects can acquire motor recovery with noninvasive cortical stimulation. Alternatively, cerebellar tES shows promise in motor rehabilitation in stroke patients with a lesion in the cerebellum and other related regions.[87]

Pioneer cerebellar tDCS study investigated polarity-dependent modulation effects of cerebello-brain connectivity (cerebellar brain inhibition [CBI]) on healthy subjects, both anode and cathode stimulation protocols were effective in changing motor performance. Cathode stimulation results in decreased CBI by enhanced LTD of Purkinje cells, but did not induce M1 or corticospinal changes.[88] A single cerebellar tDCS training session for swallowing skill was sufficient to improve swallowing performance in healthy subjects, but it was not enough for stroke patients with dysphagia.[89] Furthermore, cerebellar HD-tDCS facilitated motor adaptation in healthy subjects, whereas HD-tDCS on M1 could not have such effects.[90] In another proof-of-concept study, short-term contralesional cerebellar tDCS promoted standing balance performance in chronic stroke patients.[91,92] Nevertheless, randomized controlled trials with a larger sample size are necessary to resolve inter-individual differences in the therapeutic interventions. To achieve qualitative functional improvements, optimal timing and dosage should also be determined. Further studies employing neuroimaging techniques are necessary to unravel the underlying neuromodulation effects following a stroke. In a study by Naro et al.,[93] different cerebellar tACS protocols resulted in different CBI-sustaining Purkinje cell responses, affecting neuroplasticity of specific cerebellar pathways. Although several studies have been conducted, therapeutic applications of cerebellar stimulation are still in preliminary stages. Future studies should investigate the functional role of cerebellar stimulation in the corticospinal and corticobulbar motor control process. Moreover, cerebellar stimulation electrical flow, its corresponding modulatory effects and long-term impacts should be thoroughly evaluated.

The spinal cord contains neuronal circuits, mediating locomotion activities and segmental spinal reflexes. It is a bidirectional integration center for descending motor and ascending sensory feedback signals.[94] Unlike tES, investigation on spinal cord stimulation in post-stroke motor recovery began in the recent decade. Spinal cord stimulation can modulate both the local and distal neural circuits, and induce neurophysiological and behavioral changes.[95,96] To investigate the combined effects of transcutaneous spinal direct current stimulation (tsDCS) on cortical tDCS or cerebellar tDCS, Picelli et al.[97-99] conducted several double-blinded, randomized controlled gait training clinical trials. Anode tDCS combined with cathode tsDCS enhanced the effect of robot-assisted gait training (RAGT) in chronic stroke patients, larger enhancement in gait cadence was identified with anode tDCS+cathode thoracic tsDCS as compared to after tDCS or tsDCS alone.[97] Similarly, cathode cerebellar tDCS+tsDCS+RAGT resulted in higher improvements in walking capacity and gait cadence in chronic ischemic stroke and supratentorial stroke patients.[98,99] Though several clinical trials have been conducted, the rationale of tsDCS in stroke patients and the mechanism of spinal locomotion control remain unclear. Future studies should elucidate the mechanism of tsDCS modulation effects on the local spinal, supra-spinal, and intracortical motor control process. tsDCS could provide a potential therapeutic tool in various movement disorders.

Peripheral electrical stimulation

Peripheral electrical stimulation has been investigated for more than half of a century to activate bladder voiding, to relieve pelvic pain and other symptoms. Neuromuscular electrical stimulation (NMES) has mainly two forms in motor rehabilitation after stroke, particularly, functional electrical stimulation (FES) has been used to facilitate voluntary movement, while therapeutic electrical stimulation was used for strengthening muscle, reducing spasticity, and inducing motor recovery in paralyzed stroke patients.[13,19] Transcutaneous electrical nerve stimulation (TENS) on the nerves also enhanced neural motor control and paretic limb functions in stroke subjects.[100]

Neuromuscular electrical stimulation

NMES utilizes short external electrical pulses to excite the peripheral nerves by modulating neuron hyperpolarization or depolarization. It generates muscle contractions through the skin surface, percutaneous or implanted electrodes.[13] Typical NMES parameters include the pulse frequency (10 to 100 Hz), amplitude (10 to 120 ms), and pulse width (200 μs to 1 ms). NMES of higher frequencies generates larger forces, but quickly leads to muscle fatigue and fast reduction of contraction force.[101] Wider pulse widths induces more pronounced cortical and muscular responses.[102]

Although stroke subjects cannot voluntarily move their affected limbs or generate muscle contractions similar to healthy subjects, their spinal motor neurons are intact and excitable.[103] NMES intervention provides a supplementary or replacement tool for stroke patients to move paretic limbs.[104] Pioneering study in 1961 demonstrated the feasibility of FES applications in hemiplegic gait performance.[105] Moreover, implanted NMES hand neuroprosthesis was invented in 1988 for quadriplegic

patients.[106] NMES is effective in increasing muscle strength, relieving pain, decreasing muscle spasticity, and promoting post-stroke motor control and physical rehabilitation.[101,107,108] A closed-loop NMES system could also contribute to the motor recovery process. Here, cortical or muscular signals were used as control signals for either motor intention decoding or triggers.[107,109-113] However, previous studies showed heterogeneous rehabilitation results with not enough subjects. Translation of the available research findings into clinical practice is still at its infancy. Several clinical trials have been conducted to examine the supplemental rehabilitation effects of NMES. For instance, systematic reviews and meta-analyses have concluded that EMG-NMES on upper limbs could promote functional recovery following chronic stroke and can readily be integrated into clinical practice.[113] Future studies should conduct randomized control trials with larger sample size and with different patient characteristics to examine the rehabilitation efficiency of NMES in lower limb applications. More efficient stimulation protocols and rehabilitation strategies for individual subjects may further increase NMES therapeutic effects.

The underlying mechanisms of NMES in post-stroke motor recovery are only partially understood. Previously, motor stimulation has been focused to the muscle and motoneuron of the paretic limbs, and there is evidence that it could induce plasticity at the spinal levels.[114] It is proved recently that peripheral stimulation has central modulation effects. NMES also induces cortical plasticity by modulating the ascending pathways through the Ia muscle fiber afferents.[102,115,116] Additionally, somatosensory inputs to the motor cortex are essential for motor learning and control, and play critical roles in the motor recovery process.[100,117] NMES above the motor threshold increases excitability of corticomotor pathway by activating sensory axons and recruiting synaptic motoneurons and motor reflex.[115] In a previous study, the cortico-muscular coherence in the NMES group was significantly higher in stroke patients when compared with the control group after 8 weeks NMES and motor training.[100] Moreover, interaction of NMES in dynamic movements could facilitate understanding of post-stroke motor rehabilitation mechanisms in the physical world, and foster to its wide applications in stroke survivors.[118]

Previous studies primarily used standardized stimulation settings. It is necessary to investigate more optimized NMES paradigms considering muscle/cortical responses in different motor tasks and subjects. Recent progress in BCI could also assist NMES-based prosthetic systems through the brain.[119] Additionally, advancements of HD noninvasive NMES system in tetraplegia could allow for precise motor control of hand movement, and further benefit stroke patients.[120] The latest inventions in electrical muscle stimulation including the self-powered triboelectric nanogenerator could facilitate deployment of sustainable therapeutic interventions.[121] However, randomized controlled trials are needed to evaluate the clinical reliability of such therapeutic interventions. Through deliberate efforts, these techniques could be translated into practical clinical applications.

Transcutaneous electrical nerve stimulation

From the early 1970s, TENS has been extensively used for pain relief by modulating the descending pain inhibitory systems.[122] In addition, TENS could effectively facilitate functional performance in hemiplegic patients[123] and sensorimotor function restoration in chronic stroke patients.[124] When TENS was combined with TOT in a randomized clinical trial, it enhanced voluntary lower limb movement for chronic stroke subjects.[125] Moreover, home-based TENS with trunk training increased trunk muscle strength and motor control after stroke.[126] Sensory stimulation with TENS promoted motor recovery therapeutic effects when combined with active rehabilitation training, the force production of ankle dorsiflexors was enhanced.[127] Stimulation over peripheral nerves induced sensation along nerves and activated the related cortical area. Furthermore, a recent study showed that bilateral TENS applied over common peroneal nerve combined with TOT was superior to unilateral TENS with TOT in stroke paretic ankle dorsiflexion tasks.[128] However, no consensus was reached owing to contradictory rehabilitation results across different TENS intensity. This necessitates evaluation of the underlying therapeutic mechanisms and optimization of efficient stimulus settings.[129]

A previous study manifested that cortical neuroplasticity could be induced by sensory input of TENS, which further influenced functional reorganization in brain regions adjacent to the stroke lesion.[130] Decreased hyperexcitability of alpha motor neurons producing spastic ankle plantarflexor movement resulted from enhanced presynaptic inhibition after TENS.[131] Similarly, reduction of intracortical inhibition was reported after TENS, with significant enhancement in upper limb functional score.[132] Additionally, a 40-minute TENS over paretic median nerve can enhance gamma-band cortico-muscular coupling strength and modulate the CST.[133] A recent fNIRS study showed that median nerve electrical stimulation induced ipsilesional prefrontal functional network changes and enhanced residual functions of paretic hands.[134] Nevertheless, the detailed TENS modulatory mechanisms in motor recovery are still limited. This calls for further studies to elucidate the modulation mechanisms of neuroplasticity. In addition, randomized controlled trials with larger sample sizes should be conducted to assess the therapeutic effects of TENS.

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Emerging electrical stimulation techniques

In addition to NIBS and peripheral electrical stimulation protocols, invasive neurostimulation techniques emerged as potential rehabilitation strategies in the recent decade.[135] The current invasive neurostimulation strategies for improving post-stroke motor recovery are mainly based on preliminary animal models. Thus, further research is necessary to test the clinical performance of such invasive neurostimulation strategies. Similar to noninvasive neurostimulation modalities, invasive stimulation tools also harness neuroplasticity, facilitate functional reorganization of brain regions, and ultimately promote clinical improvements of contralateral paretic limbs. Representative invasive neurostimulation modalities are summarized in the following section, including deep brain stimulation (DBS), epidural electrical stimulation, and vagus nerve stimulation (VNS).

Deep brain stimulation

DBS utilizes stimulating electrodes implanted deep into the brain. DBS was previously used to treat various movement disorders including essential tremor, Parkinson's disease, dystonia, and other related symptoms.[136] It modulates local or remote brain regions depending on the parameter and target settings. DBS stimulates impaired neural circuits, thereby enhancing cortical network plasticity and facilitating functional reorganization of the perilesional cortex.[137]

As for post-stroke related motor deficits, DBS-based post-stroke rehabilitation directly modulates deep brain regions which has shown great promise in resolving the limitations of previous noninvasive electrical stimulation settings. Though lacking systematic randomized clinical trials and conclusive explanation, for a stroke patient with motor weakness and spasticity, voluntary upper limb movement was improved following DBS intervention.[138] Targeted stimulation was applied at posterior limb of the internal capsule (PLIC) or its neighboring area, where somatotopically organized CST fibers descend. PLIC transfers cortical information from M1 to motor neurons in the spinal cord.[139] Therefore, DBS at PLIC possibly activated the descending neurons and further facilitate motor rehabilitation. Medial interhemispheric fissure area is responsible for lower limb cortical control, some of lower limb related cortical regions are deep inside and cannot be easily stimulated using noninvasive stimulation modalities. Invasive DBS could therefore be useful in recovering motor functions in stroke patients with lower limb impairment.[140]

The cerebral cortex and cerebellum are connected through the cerebro-ponto-cerebellar (CPC) and dentatothalamocortical (DTC) pathways. Cross cerebellar diaschisis results from CPC tract disruption following a stroke, with an impact on residual motor functions.[141] Dentate nucleus, the largest deep cerebellar nuclei, receives input from the lateral cerebellar hemisphere and CPC tract. Its primary outputs are transferred to the thalamus and the motor regions through the DTC tract.[142] Cerebellar DBS at the dentate nucleus manipulates the DTC pathway, thereby facilitating motor recovery following ischemic stroke.[143] Wathen et al.[144] reviewed the latest advancements, theoretical foundations, rodent preclinical experiments, and current clinical trials in cerebellar DBS-based motor recovery following ischemic stroke. Results from preclinical and Phase I clinical trial underscored a therapeutic role of cerebellar DBS. Advanced Phase II and Phase III human clinical trials are needed to validate this effect. Moreover, the underlying modulation mechanisms of DBS should be illustrated exhaustively. A closed-loop DBS system could allow for real-time measurement of neurophysiological properties with enhanced precision of electrical stimulation.

Despite demonstrated benefit of DBS therapy, its application in post-stroke motor recovery is still limited owing to the risk involved in invasive surgery. Recent advancements in noninvasive DBS via temporally interfering electric fields stimulate deep neurons in the brain of a living mouse.[145] Such noninvasive DBS could pave the way for potential treatment of post-stroke motor recovery and other movement disorders.[146] Future studies should explore new techniques and translate them into practical applications.

Epidural cortical stimulation

One of the main limitations of NIBS is that only about 25% of current penetrates deep into the brain to induce cortical excitability changes. The rest of the current is attenuated by the skin, skull, and subcutaneous tissues.[44] This reduces the resolution and efficiency of stimulation. Invasive stimulation addresses this challenge by delivering currents directly to the ipsilesional peri-infarct cortices like the M1, with modulatory effects similar to that of noninvasive tES modalities. Neuroplasticity could be enhanced through electrical neurostimulation, further inducing neuronal reorganization and functional improvements. Epidural cortical stimulation (ECS) has been paired concurrently with physical rehabilitation training to foster stroke functional recovery.[135] ECS applied on ipsilesional brain in rodent models, Phase I and II clinical trials have demonstrated its safety and efficacy in motor recovery.[147-149] However, Phase III clinical trials did not show significant functional score improvement.[150] This should be attributed to the diverse stimulation site, lesion geometry, the inherent differences between animal and human experiments,

and inviable descending motor pathways.[151] Intact corticothalamic or CST fibers are essential factors influencing stimulation efficacy. Future studies should optimize electrical distribution and timing of stimulation.

There is no consensus on the placement of epidural stimulation. In rats with CST lesion, epidural stimulation on contralesional M1 restored motor functions by promoting CST sprouting based ipsilateral control.[152] Moreover, premotor stimulation could be an alternative target for impaired M1.[153] In an ischemic rodent model, distributed stimulation showed better motor recovery compared to focal M1 stimulation.[154] Such inconsistent stimulation placement settings might relate to the unclear rationale behind. Progress in microelectrode arrays underpins the prospective application in motor recovery with precise stimulation and real-time neurophysiological monitoring.[155,156] This highlights crucial factors influencing post-stroke rehabilitation. Subsequent studies should employ the new techniques in post-stroke motor restoration.

Vagus nerve stimulation

Vagus nerve regulates different physiological functions and pathways, including inflammation, cerebral blood flow, glutamate excitotoxicity, and other neurotrophic processes.[157] The vagus nerve comprises 80% sensory afferent fibers that carry information from the peripheral system to the brain, and 20% motor efferent fibers that perform autonomous functions.[158] Several complex cascades of processes in early stroke are influenced by afferent and efferent pathways of the vagus nerve.[159] VNS is a potential tool for subacute stroke recovery owing to its anti-inflammatory and neuromodulators releasing properties.[160,161]

Invasive VNS is normally 0.25 to 3 mA, equipped with bipolar electrodes placed underneath the chest skin and the left vagus nerve.[162] Studies with ischemic rat models have demonstrated safety and feasibility of using VNS in post-stroke motor recovery.[163,164] Coupled with rehabilitation training, VNS significantly promoted forelimb functional movement.[164,165] VNS was also effective in facilitating long-lasting recovery and structural plasticity in corticospinal motor networks in rat models, with a resultant increased connectivity to forelimb muscles.[166] Moreover, noninvasive VNS on cervical vagus nerve significantly decreased infarct volume, enhanced clinical scores and strength of forelimb grip following middle cerebral artery occlusion in rat models.[167] The aforementioned VNS studies were still in preliminary stages and focused on animal models with small sample sizes. Further studies are required to investigate the functional role of VNS in motor restoration and validate its therapeutic effects in human.

Summary and future directions

Electrical stimulation has been widely applied to facilitate post-stroke motor recovery, but the modulatory mechanisms are not fully understood yet. Electrical stimulation manipulates corresponding neuronal circuits, which induces neuroplasticity changes that correlate with functional motor improvement. This nascent review lays the foundation for harnessing neuroplasticity of prospective electrical stimulation techniques to restore motor functions in stroke patients. NIBS and peripheral electrical stimulation are most frequently applied. Among the central-oriented approaches, NIBS protocols are the most convenient cortical stimulation, but their applications are limited by low stimulation resolution, non-optimized stimulation settings, and inter-subject variability. Peripheral electrical stimulation on the muscles and nerves induces corticospinal neuroplasticity, which influences cortical reorganization and functional recovery. Going forward, the performance of these two stimulation protocols requires further development. Specifically, optimized stimulation settings should be explored to enhance the motor recovery efficiency. Other emerging techniques, such as invasive brain stimulation tools for DBS and epidural stimulation, are limited by the high surgical risks to human stroke subjects, but its development would indeed address the limitation of noninvasive settings. Currently, protocols that accelerate post-stroke motor recovery by vagus stimulation are still in the elementary preclinical studies and worth efforts subsequently.

The recovery of motor function after stroke is influenced by the timing, targeting, stimulation intensity, other stimulation parameter settings, suitable experimental designs, and task-specificity. Rational combination of different stimulation protocols may yield better clinical outcomes, such as PAS integrating cortical and peripheral stimulation. Further, precision medicine incorporating patient-tailored stimulation and rehabilitation training might be more effective in motor rehabilitation. The development of precise and flexible computational models of electrical stimulation modalities can facilitate understanding of current flow and refining electrotherapy designs. The latest technological advancements, such as self-powered, high density microelectrodes, and the minimally invasive electrical stimulation tools, indicate more precise and localized stimulation modalities. Additionally, measurements with adequate spatial and temporal resolution may reveal the neurophysiological properties during/following electrical stimulation and the underlying motor control and recovery mechanisms. Thus, closed-loop electrical stimulation with neural feedback provides higher temporal resolution and real-time control, whereas optimal artifact removal algo-

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rithms might be pivotal for such systems. Lastly, all the stimulation devices and protocols should be guaranteed safe and well-tolerated in practical applications.

Conclusions

Electrical stimulation protocols have shown great clinical potential in post-stroke motor recovery. More precise and effective motor restoration strategies may further benefit individual stroke subjects. Subsequent studies should expand the detailed modulatory mechanisms of the existing modalities and translate the state-of-art techniques to improve the treatment of stroke survivors and people with other movement disorders.

Disclosure

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TOPICAL REVIEW

Neuromuscular electrical stimulation-promoted plasticity of the human brain

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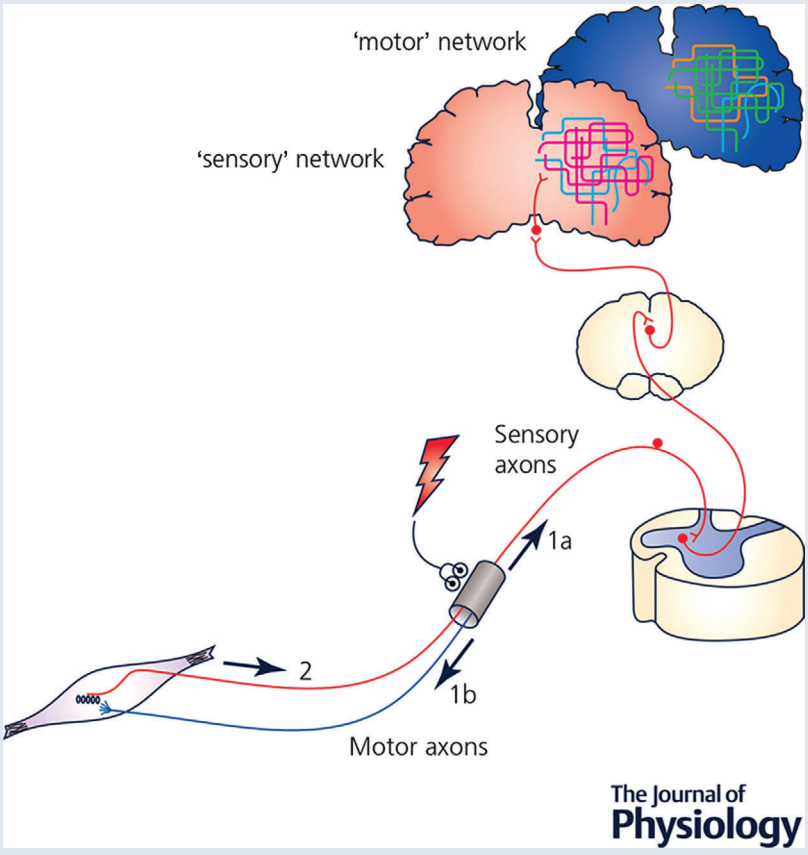

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Abstract The application of neuromuscular electrical stimulation (NMES) to paretic limbs has demonstrated utility for motor rehabilitation following brain injury. When NMES is delivered to a mixed peripheral nerve, typically both efferent and afferent fibres are recruited. Muscle contractions brought about by the excitation of motor neurons are often used to compensate for disability by assisting actions such as the formation of hand aperture, or by preventing others including foot drop. In this context, exogenous stimulation provides a direct substitute for endogenous neural drive. The goal of the present narrative review is to describe the means through which NMES may also promote sustained adaptations within central motor pathways, leading ultimately to increases in (intrinsic) functional capacity. There is an obvious practical motivation, in that detailed knowledge concerning the mechanisms of adaptation has the potential to inform neurorehabilitation practice. In addition, responses to NMES provide a means of studying CNS plasticity at a systems level in humans. We summarize the fundamental aspects of NMES, focusing on the forms that are employed most commonly in clinical and experimental practice. Specific attention is devoted to adjuvant techniques that further promote adaptive responses to NMES thereby offering the prospect of increased therapeutic potential. The emergent theme is that an association with centrally initiated neural activity, whether this is generated in the context of NMES triggered by efferent drive or via indirect methods such as mental imagery, may in some circumstances promote the physiological changes that can be induced through peripheral electrical stimulation.

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Abstract figure legend The delivery of electrical current via a peripheral nerve (or across a muscle belly) activates contractile muscle fibres indirectly by depolarizing motor axons (1b). As the sensory axons in the same mixed nerve bundle have lower activation thresholds, ascending afferent volleys are also generated at intensities of electrical stimulation that exceed themotor threshold (1a). These volleys are followed by (secondary) reafference arising from the invoked muscle contraction (2). The goal of this review is to address the means through which the sensory-mediated consequences of the stimulation alter the state of 'sensory' networks, and induce sustained 'neuroplastic' modifications within central 'motor' networks. Figure redrawn and adapted from the author's original artwork, which is available at: https://commons.wikimedia.org/wiki/File:Neuromuscular_electrical_stimulation_promoted_brain_plasticity.jpg (original figure published under a Creative Commons Attribution-Share Alike 4.0 International license).

Background

Although historical antecedents are often ascribed to Galvani's *Commentarius*, published in the late 18th century, the practice of employing electricity to stimulate human nerves can be traced to ancient times (Finger & Piccolino, 2011). Murals depicting the Nile catfish, *Malopterurus electricus*, have been discovered in Egyptian tombs dating from the Fifth Dynasty (around 2400 BCE). In extant records, however, it is not until 46 BCE that the utilization of the (saltwater) torpedo ray's electric discharge for electrotherapy is noted by the Roman physician Scribonius Largus (Cambiaghi & Sconocchia, 2018). Writing some 30 years later, Dioscorides (see Gunther, 1934) provided perhaps the first explicit reference to the use of the torpedo's electric discharge for artificial muscle stimulation in relating a remedy for propalsus ani (Kellaway, 1946). The introduction of the Leyden jar in 1746 provided a platform for the modern progression of electrotherapy, with Benjamin Franklin observing in 1774 that muscle contractions could be brought about by exposure to static electricity (Isaacson, 2003). Subsequently, Faraday's application of the principle of magnetic induction provided a means of delivering electric current to the human body in a controlled fashion – for which the term 'Faradization' was coined. Prominent among 19th century practitioners investigating the physiology of 'localized electrization', Duchenne de Boulogne employed a Faradic stimulating machine to stimulate a wide range of muscles trans-cutaneously via a pair of 'humid rheophore' electrodes (e.g. Clarac *et al.* 2009). Performing his studies in cat and monkey, Sherrington (1894) observed that a third to one-half of the myelinated fibres of peripheral nerves failed to degenerate following section of their ventral (motor) spinal roots. As the application of maximal Faradic currents to these remaining fibres failed to elicit 'motor reactions', he concluded that they must provide sensory innervation. The presence of both sensory and

motor axons in the same ('mixed') nerve bundle, as revealed by Sherrington, is a key factor determining the physiological effects of contemporary forms of neuromuscular electrical stimulation (NMES).

The Greek name for the torpedo ray, *narkè*, meaning numbness, suggests the nature of the initial therapeutic applications of electrotherapy (Debru, 2006). Scribonius Largus, for example, records use of the torpedo's electric discharge as a treatment for the pain associated with intractable headache and gout (Kellaway, 1946). In the guise of 'transcutaneous electrical nerve stimulation' (TENS), modern devices designed to achieve the same analgesic goals are now widely available. These typically generate high frequency (>50 Hz) trains of electrical stimulation, at current intensities that are insufficient to evoke overt motor responses. A contemporaneous historical lineage for the therapeutic application of electrotherapy in motor rehabilitation can also be traced – from Dioscorides through Duchenne de Boulogne to the present day. Modes of electrical nerve stimulation used for this purpose (which tend to differ from those employed typically for pain relief – by using lower frequencies and higher intensities of stimulation) constitute the subject matter of the present review (Table 1).

In a contemporary therapeutic context, applications of NMES in motor rehabilitation can be conceived of as being adaptive or restorative (Pomeroy *et al.* 2011). The term functional electrical stimulation (FES) refers typically to instances in which tetanic muscle contractions are induced to assist or reinstate movement, thereby enabling an otherwise quiescent limb to be engaged in goal-directed actions. This form of stimulation is deemed to be adaptive, as it provides direct compensation for the motor disability. In the period since Liberson and colleagues (1961) demonstrated that stimulation delivered to the common peroneal nerve reduced the degree of foot-drop during the swing phase of gait, numerous applications of FES have been developed successfully to assist movement of the upper and lower extremities (Prochazka, 2018). Yet NMES may also be used restoratively, with a view to promoting neural changes that lead ultimately to increased (intrinsic) functional capacity. This is the primary focus of the current review.

The delivery of electrical current to neuromuscular tissue (i.e. via a peripheral nerve or across a muscle belly) activates contractile muscle fibres indirectly by first depolarizing motor axons. As the sensory axons in the same mixed nerve bundle have lower activation thresholds, ascending afferent volleys are also generated at intensities of electrical stimulation that exceed the motor threshold (MT) (Dawson, 1956). These are followed by (secondary) reafference arising from the invoked muscle contraction. While the capacity of NMES to provide a direct substitute for (descending) endogenous neural drive to muscles in circumstances of CNS injury or disease can be readily appreciated, our goal is to address means through which the sensory-mediated consequences of NMES induce sustained 'neuroplastic' modifications within central motor pathways.

Given an empirical literature that is characterized by extraordinary diversity with respect to the stimulation protocols that are employed (varying in relation to such features as stimulation frequency, intensity, duration and temporal pattern), there is little consensus with respect to the cellular mechanisms engaged by NMES. Beyond providing insights in relation to the expression of CNS plasticity at a systems level in humans, there is an obvious practical motivation for seeking the elucidation of these processes. Detailed knowledge concerning the mechanisms of adaptation clearly has the potential to inform the development of neurorehabilitation practice.

Scope of the review

While the intent of this narrative review is to examine general principles, the scope of the analysis is necessarily restricted – for the most part to the effects of transcutaneous (surface) electrical stimulation delivered using intensities at or above the threshold for a motor response. The emphasis is largely upon the upper limb, and upon supraspinal adaptations (cf. Bergquist *et al.* 2011). To the extent that specific clinical applications are considered, these will generally be drawn from the domain of stroke rehabilitation.

Evidently NMES exhibits the capacity to generate changes in the excitability of descending (e.g. corticospinal) projections from the cortex to the spinal cord (Chipchase *et al.* 2011*a*). It has generally been assumed that such changes in excitability reflect, at least in part, modifications in the organization of the same brain networks that serve ultimately as a basis for the improvements in functional capacity that may be brought about by neuromuscular stimulation (Traversa *et al.* 1997; Vang *et al.* 1999; Barker *et al.* 2012). Although, as we shall see, there are grounds to be cautious about such assumptions (Carson *et al.* 2016), we include a survey of studies that have characterized the neurophysiological effects of NMES in terms of corticospinal excitability. Most often these have been assessed through muscle responses evoked by transcranial magnetic stimulation (TMS) delivered over primary motor cortex (M1). We also consider instances in which the effects of NMES have been registered using various brain imaging methodologies. In the closing sections, we return to the issue of whether the neural pathways upon which NMES has the most readily detectable effects are necessarily also those that play an instrumental role in mediating changes in functional capacity.

In the course of the review, specific attention is devoted to adjuvant techniques that further promote restorative

Table 1. Common variants of peripheral electrical stimulation

Type of stimulation	Typical intent	Typical frequency range	Typical intensity
NMES	Activation of sensory and motor axons for diverse purposes	1–100 Hz	At or above motor threshold
FES	Activation of both sensory and motor axons with the specific goal of assisting motor function	20–60 Hz	Above motor threshold
EST	Activation of both sensory and motor axons with the specific goal of preventing muscle weakness	35–100 Hz	Above motor threshold
TENS	Activation of sensory axons for the goal of pain relief.	>50 Hz	Below motor threshold

EST, electrostimulation strength training; FES, functional electrical stimulation; NMES, neuromuscular electrical stimulation; TENS, transcutaneous electrical nerve.

responses to NMES. The emergent theme is that an association with centrally initiated neural activity, whether this is generated in the context of NMES triggered by efferent drive or via indirect methods such as mental imagery, can in some circumstances be efficacious in promoting neural adaptations upon which changes in functional capacity may be based.

Exemplars

We do not seek to be comprehensive with respect to the characteristics of NMES that can be altered in either an experimental or a clinical context. Rather, the empirical literature is circumscribed with a view to emphasizing a limited number of key concepts. It being evident that the 'dose' of NMES has a significant bearing on the changes in brain activity thus invoked, we consider both protocols in which the level of stimulation is just above motor threshold and those in which it is of sufficient magnitude to elicit overt movement.

Stimulation at motor threshold intensity. Sensory axons in a mixed nerve bundle innervating skeletal muscle are typically depolarized at levels of electrical stimulation below those which are necessary to recruit motor axons (Panizza *et al.* 1989, 1992; Veale *et al.* 1973). At intensities of NMES at or above MT, therefore, ascending afferent volleys will be generated directly by the depolarization of sensory axons (e.g. Collins, 2007). Some degree of secondary reafference arising (indirectly) from the invoked muscle contraction will follow. While the nature and the extent of the reafference will in turn be determined by the characteristics of the joint movement thus induced (which will itself be influenced by the posture of the limb, degree of restraint and so on), a more general point is that the relationships between the intensity of stimulation and the level (and distribution) of brain activity arising from (1) the direct sensory afference and (2) the indirect secondary reafference are unlikely to be the same. Indeed, both are context dependent and must be determined empirically. Their relative contributions notwithstanding, it is the sensory corollaries of NMES that provide the principal means by which sustained (central) neuroplastic adaptations are induced (Bergquist *et al.* 2011).

If the magnitude of a single electrical stimulus delivered transcutaneously to a peripheral nerve is set to approximately three times perceptual threshold, direct motor responses in the innervated muscles are typically observed (e.g. Ridding *et al.* 2001; McKay *et al.* 2002; Litvak *et al.* 2007). At such intensities, extended (up to 2 h) sequences of stimulation are necessary to bring about sustained increases in the excitability of corticospinal projections to the muscles in which the responses are evoked (see also Luft *et al.* 2002). For example, Ridding *et al.* (2000) delivered trains of pulses (10 Hz, 1 ms pulse width) to the ulnar nerve at the wrist, at a rate of one train per second, using a 50% duty cycle (i.e. 1 s on, 1 s off), for a period of 2 h. The area of the scalp over which TMS elicited MEPs in the ulnar nerve-innervated first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles increased as a consequence of the intervention. Using precisely the same protocol, Kaelin-Lang *et al.* (2002) obtained increases in the amplitude of MEPs elicited in ADM (but not in FDI). As these were not accompanied by corresponding changes in the size of potentials evoked by stimulation by corticospinal axons at the level of the cervicomedullary junction, a cortical locus for the adaptation was inferred (see also Ridding *et al.* 2000). The capricious nature of the changes in corticospinal excitability induced using these stimulation durations and intensities is emphasized by the wide variation in response across individuals reported by Charlton *et al.* (2003), when FDI afferents were stimulated via the skin overlying the muscle, rather than via the nerve trunk at the wrist (using a protocol that was otherwise equivalent). Furthermore, if the frequency at which the trains are delivered and the total duration of the intervention is reduced, reliable elevations of MEP amplitude are not obtained (Uy & Ridding, 2003).

If, however, the effective dose (if not the specificity) of NMES is increased by delivering pulses simultaneously to

both the radial and ulnar nerves, a progressive increase in the amplitude of potentials evoked in FDI occurs over the time course of the intervention (McKay *et al.* 2002). Furthermore, this dual stimulation technique increases reliably both the area of the scalp over which TMS-elicited MEPs can be obtained in FDI (and other hand muscles) and the amplitude of the MEPs recorded following the cessation of NMES (Ridding *et al.* 2001). Indeed, when motor point stimulation is delivered simultaneously to FDI and ADM via the skin overlying the muscles, an intervention of 1 h duration is sufficient to induce reliable increases in corticospinal excitability (Schabrun & Ridding, 2007; cf. Charlton *et al.* 2003). As there are no accompanying changes in the size of responses elicited by cervicomedullary stimulation, a spinal locus for the adaptation appears to be precluded (Ridding *et al.* 2001).

Stimulation at supra-motor threshold intensities. FES typically comprises short bursts of electrical pulses delivered at a frequency above that necessary to yield a fused contraction (~12 Hz) (Peckham & Knutson, 2005; Sheffler & Chae, 2007). The assumption that given an adequate dose of NMES persistent elevations in the excitability of corticospinal projections can be induced is supported by studies that have employed stimulation at an intensity and frequency sufficient to induce tetanic motor responses (see Chipchase *et al.* 2011*a* for a review). While it is not possible to exclude the possibility that such supra-threshold intensity stimulation generates antidromic impulses that modify synapses in the ventral horn (Rushton, 2003), the consensus view is that the observed changes in corticospinal excitability are driven primarily by cortical reorganization (e.g. Luft *et al.* 2005).

For example, Schabrun *et al.* (2012) applied 30 min of NMES to the skin overlying the abductor pollicis brevis (APB) muscle at 30 Hz (4 s on, 6 s off) with six periods of stimulation being applied every minute. The intensity of stimulation was that which produced a mid-range abduction of the thumb. The amplitudes of MEPs evoked in APB following the intervention were substantially greater than those obtained prior to the stimulation. Corresponding effects have been reported when biceps brachii is the target of stimulation (Chipchase *et al.* 2011*b*). When NMES is applied to APB in this manner for periods of 20 or 40 min, the induced changes in corticospinal excitability are maintained for at least 20 min following the cessation of the intervention (Andrews *et al.* 2013).

While it is clear that increases in the dose of stimulation that is administered may be achieved by increases in the current/voltage of individual shocks, and/or by a higher frequency of delivery, it has been proposed (Chipchase *et al.* 2011*b*) that increases in corticospinal reactivity are generated reliably only by those forms of NMES giving rise to a motor response that mimics a voluntary muscle contraction. As noted previously, in addition to the initial ascending afferent volley induced directly by electrical stimulation of the nerve, such protocols encapsulate secondary reafference arising from the muscle contractions (Schabrun *et al.* 2012). The extent of the neural activity induced in M1 by such reafference can be substantially greater than that brought about directly by the ES-mediated depolarization of the sensory axons (Shitara *et al.* 2013). De Kroon and colleagues (2005) in their review of the relationships between electrical stimulation characteristics and clinical outcomes hypothesized that supra-motor stimulation is more likely than sub-motor stimulation to lead to improvements in motor control, as a consequence of muscle and joint afferent feedback, i.e. in addition to that derived from cutaneous afferents, which are also engaged at lower intensities of stimulation.

Indeed, repeated changes in muscle length brought about passively by mechanical joint rotation also induce both acute (Lewis *et al.* 2001) and chronic (Macé *et al.* 2008) increases in corticospinal excitability. Collectively, these observations suggest that the secondary mediation of Ia (muscle spindle) afferent projections to higher brain centres is instrumental in augmenting the direct depolarizing effects of NMES. Although it has been proposed that cutaneous afferents make a greater contribution than muscle spindle afferents to cortical potentials produced by electrical stimulation of mixed nerves in the upper limb (e.g. Halonen *et al.* 1988; Allison *et al.* 1991), it is the precise brain circuits that exhibit a change in state as a result of peripheral stimulation which is likely to assume particular functional significance. It is believed that Ia afferent input has its most direct effects upon both area 4 (primary motor cortex) (Jones & Porter, 1980) and area 3a (in primary somatosensory cortex) (Heath *et al.* 1976; Hore *et al.* 1976), whereas, input from cutaneous receptors and low threshold mechanoreceptors first alters the excitability of neurons in areas 3b and 1 (Kaas & Pons, 1988). We thus turn our attention to the brain circuitry that is engaged by NMES, and to the impact of its parametric variation.

Brain circuitry engaged by NMES

Somatosensory cortex. On the basis of findings derived using a variety of neuroimaging techniques, it has been surmised that electrical stimulation of peripheral afferents engages circuits in the primary somatosensory cortex (S1 – including Brodmann areas 3, 1 and 2) within the postcentral gyrus, the second somatosensory area (S2 – including parts of Brodmann areas 40 and 43) within the parietal operculum on the ceiling of the lateral sulcus, and the posterior parietal cortex (Korvenoja *et al.* 1999; Boakye *et al.* 2000; Nihashi *et al.* 2005). In relation to the

complex cortical responses that are extracted from electroencephalographic (EEG) and magnetoencephalographic (MEG) recordings, there is consensus that short-latency potentials occurring within the first 40 ms following stimulation of the median nerve (e.g. at the wrist) at intensities sufficient to elicit a muscle twitch arise principally from contralateral S1 (Allison *et al.* 1991). The presence of synchronized neuronal population activity in S2 (registered by MEG) during this period, while consistent with an influence of cortical afferents from S1, does not, however, preclude the possibility of mediation via additional parallel thalamocortical projections to S2 (Karhu & Tesche, 1999). With respect to the medium latency (>40 ms) components, there is a distributed pattern of activation that includes not only S1, but also S2 bilaterally and contralateral posterior parietal cortex (Hari *et al.* 1984; Allison *et al.* 1989*a*, 1989*b*, 1992; Forss *et al.* 1994). It is currently believed that cortico-cortical connections mediated by transcallosal projections play a major role in shaping the bilateral character of the S2 response profile (Del Vecchio *et al.* 2019). These sources continue to be active simultaneously during a period 70–140 ms following the onset of stimulation (Mauguière *et al.* 1997). When a sequence of stimuli is administered, the offset of the sequence gives rise to a (P100 and N140) stimulus evoked potential (SEP) signature distinct from that associated with the individual stimuli (Yamashiro *et al.* 2008, 2009).

The functional magnetic resonance imaging (fMRI)-derived blood oxygenation level-dependent (BOLD) response measured in contralateral S1 scales with the intensity of ES (at least up to MT) (Krause *et al.* 2001, see also Nelson *et al.* 2004). In contrast, bilateral activity evident in S2 and posterior parietal cortex does not appear to vary in this manner. A BOLD signal is, however, registered in S2 at lower levels of stimulation than in S1. This is augmented when attention is directed explicitly to the stimulation (Backes *et al.* 2000). In circumstances in which ES is applied in a range between the sensory threshold (ST) and 1.2 × MT, the amplitude of the N9, N20 and N20-P25 SEP components derived from EEG recordings increases in proportion to stimulation intensity (Gatica Tossi *et al.* 2013; cf. Lakhani *et al.* 2012). This effect remains present at 2.5 × MT (Urasaki *et al.* 1998). Components of the SEP recorded in S1 saturate at a level below the pain threshold (Parain & Delapierre, 1991), while the asymptote of the S2 response occurs at lower stimulation intensities than for the S1 response (Lin *et al.* 2003).

It is now broadly accepted that the initial (i.e. N20) EEG responses to NMES are dominated by cutaneous afferent input (Gandevia & Burke, 1990; Kunesch *et al.* 1995). The origin of the N20 response to cutaneous inputs is considered to be a deep tangential generator in area 3b (e.g. Desmedt & Ozaki, 1991; McLaughlin & Kelly, 1993), whereas, it is probable that the source generator for cortical potentials invoked by muscle spindle afference is principally area 3a, although additional contributions from area 2 cannot be excluded (Mima *et al.* 1996; MacKinnon *et al.* 2000). This is consonant with evidence drawn from comparative studies that that the most significant input to area 3a is from muscle spindle afferents (Kaas, 1983). Thus surface electrical stimulation at intensities above motor threshold will give rise to cutaneous afferent-mediated activity in area 3b of primary somatosensory cortex (S1), and also to activity in area 3a and area 2 (Wiesendanger & Miles, 1982), including that arising by virtue of muscle contraction-induced reafference.

Cortico-cortical connections from somatosensory cortex to M1. Studies in cat indicate that stimulation of sensory cortex can induce long-lasting potentiation of synaptic potentials evoked in the motor cortex (Sakamoto *et al.* 1987). Detailed investigations in non-human primates (e.g. Jones *et al.* 1978; Pons and Kaas, 1986; Ghosh *et al.* 1987; Huerta and Pons, 1990) and in cat (Grant *et al.* 1975; Zarzecki *et al.* 1978; Waters *et al.* 1982; Burton and Kopf, 1984; Yumiya and Ghez, 1984; Porter and Sakamoto, 1988; Avendaño *et al.* 1992; Schwark *et al.* 1992) have revealed extensive networks of cortico-cortical connections between SI and primary motor cortex (M1) (Burton & Fabri, 1995). Neurons that exhibit short-latency excitatory postsynaptic potentials (EPSPs), indicative of direct input, in response to microstimulation of area 3a, are found in all laminae of the motor cortex, with the exception of layer I (Herman *et al.* 1985; Huerta & Pons, 1990; Porter *et al.* 1990). By comparison, only cells in the superficial layers of M1 (II and III) respond in this fashion to stimulation of area 2 (Kosar *et al.* 1985; Porter *et al.* 1990). It has thus been proposed that area 3a should be viewed as a relay to motor cortex (Jones & Porter, 1980), or even as a part of area 4 (Jones *et al.* 1978, cf. Kuehn *et al.* 2017). This intimacy of association provides a means through which muscle spindle input that is relayed through area 3a can exert a direct influence on pyramidal and multipolar neurons in deep (V and VI) layers of M1 (Porter *et al.* 1990). In contrast, while there are reciprocal connections between area 3b and area 1 in particular, and further projections to area 2 (which are ostensibly not reciprocated), projections from area 3b to M1 are sparse (Darian-Smith *et al.* 1993; Burton & Fabri, 1995), if indeed detectable (Jones *et al.* 1978).

Cerebello-thalamo-cortical and thalmo-cortical connections. Although the possibility of direct activation of the primary motor cortex via sensory afferents from the periphery (Padel & Relova, 1991) cannot be excluded, studies in non-human primates indicate that the ventral

posterior complex of the thalamus, the major sensory thalamic relay, has relatively few direct projections to M1 (Darian-Smith & Darian- Smith, 1993; Huffman & Krubitzer, 2001*a*). In this regard, it is worth noting that while S1 areas 1, 2 and 3b are represented across the ventrobasal complex of the thalamus, area 3a has connectional relationships similar to those for area 4 (Jones *et al.* 1979). For example, area 3a receives projections from nuclei of the thalamus classically associated with the motor system, including indirect input from the cerebellum and basal ganglia via the ventral lateral (VL) nucleus (Huffman & Krubitzer, 2001*b*). Thalamic processing of somatosensory input extends beyond the relaying of primary afferent signals to the cortex. For example, at levels of ES above perceptual threshold, thalamic SEPs can be elicited over intervals greater than 75 ms following the peripheral shock, with the duration extending to 150 ms when the intensity is set to MT (Klostermann *et al.* 2009).

Through receipt of convergent inputs from both the sensorimotor cortex and the spinal cord, the interpositus nucleus of the cerebellum also exerts a modulating influence upon motor network responses to sensory stimulation via thalamic projections to premotor and primary motor cortices (Luft *et al.* 2005). Hemicerebellectomy blocks the modulation of cortical motor output associated with repetitive ES of the sciatic nerve in the rat (Ben Taib *et al.* 2005). It has also been proposed that the state of the motor cortex itself, acting via the intermediate cerebellum, may further serve to tune the gain of polysynaptic responses to peripheral stimulation (Manto *et al.* 2006). This is a possibility to which will return in the sections that follow.

Motor network. In view of the patterns of connectivity outlined above, one might surmise that the electrical stimulation of peripheral afferents has clear potential to alter the state of circuits not only within somatosensory cortex, but also within the (classically defined) motor network. Although it does not provide a basis upon which to resolve the specific mediating pathways that are engaged, empirical support can now be drawn from human neuroimaging data. For the present purposes it will suffice to provide a brief, and necessarily partial, representation of the relevant findings. The picture that emerges is of a multi-stage hierarchical process in which various elements of the cortical motor network are consistently engaged (Avanzini *et al.* 2018).

When median nerve stimulation at motor threshold intensity (0.5–2.7 Hz; 0.2–0.3 ms pulse duration) is employed, elevated activity registered concurrently by fMRI (Spiegel *et al.* 1999) and by MEG (Kawamura *et al.* 1996) is evident in both contralateral S1 and M1. Similar protocols also yield an elevated BOLD response in supplementary motor area (SMA) (Manganotti *et al.* 2012). Notwithstanding the likelihood of prior disease- and drug treatment-related adaptations in brain organization, recent reports of intracerebral recordings from epilepsy patients have provided hitherto unanticipated opportunities to resolve the spatiotemporal characteristics of motor network responses to peripheral nerve stimulation. These recordings indicate that in addition to enhanced gamma band power in areas 3a and 3b (exceeding that of areas 1 and 2), 1 Hz median nerve stimulation (0.2 ms pulse duration) at MT (and 20% below MT) gives rise to elevated activity in M1, and in large sectors of dorsal and ventral premotor cortex, and SMA (Avanzini *et al.* 2016). Further detailed analysis of the time course of these responses (Avanzini *et al.* 2018) indicates that M1 (BA4) exhibits an initial (peaks $\approx$ 30–40 ms) phasic response to median (and tibial) nerve stimulation that closely resembles those registered for areas 3a and 3b, whereas the responses recorded from premotor areas occur somewhat later. It is also notable that while median nerve stimulation just above MT gives rise to elevated gamma band activity (50–150 Hz) in ipsilateral dorsal premotor cortex (PMd), no such response has been detected in ipsilateral M1 (Del Vecchio *et al.* 2019; see also Klingner *et al.* 2011).

There is an apparent dose-dependent character to the BOLD response to NMES observed for M1. For example, it appears to increase monotonically as the level of stimulation applied over the motor point of the quadriceps muscle is increased from sensory threshold to that eliciting a maximum motor response (Smith *et al.* 2003). Using functional levels of stimulation sufficient to bring about alternating flexion and extension of the wrist, Blickenstorfer *et al.* (2009) reported simultaneously registered BOLD activation peaks in regions defined as contralateral primary motor cortex, primary somatosensory cortex and premotor cortex, the ipsilateral cerebellum, bilateral secondary somatosensory cortex, supplementary motor area and anterior cingulate cortex (see also Del Gratta *et al.* 2000; Arienzo *et al.* 2006; Joa *et al.* 2012). Patterned NMES (50 Hz with 200 [s pulses) sufficient to invoke finger flexion elevates the BOLD response in contralateral M1 and S1 and bilaterally in S2 (Iftime-Nielsen *et al.* 2012). A recent report suggests that 100 s of 30 Hz stimulation at intensities sufficient to generate wrist flexion (against gravity), gives rise to subsequent changes in EEG/EMG-registered corticomuscular coherence (Xu *et al.* 2018).

It has also been shown that in some instances the physiological changes reflected in the BOLD response may be sustained. Two hours of median nerve stimulation (10 Hz trains, 50% duty cycle at 1 Hz, intensity just above MT) applied at the wrist was observed (in the context of a thumb movement task) to bring about an increase in signal intensity and number of voxels activated in M1, S1 and PMd, which persisted for

up to 60 min after the stimulation had ended (Wu *et al.* 2005). Employing a protocol in which mesh-glove stimulation was applied at a level below sensory threshold for 30 min, Golaszewski *et al.* (2004) observed that the magnitude of the BOLD response registered in primary motor and primary somatosensory regions of both hemispheres during a finger-to-thumb tapping task was greater than when the task was performed in the absence of prior stimulation. The elevated activity registered for the contralateral primary motor region remained present 2 h following the cessation of stimulation.

In general the spatial extent of the BOLD registered response (i.e. number of voxels) and the magnitude of the signal change (i.e. relative to rest) are larger for voluntary movement than those brought about by FES (Francis *et al.* 2009; Joa *et al.* 2012; Wegrzyk *et al.* 2017), although the particular regions of interest for which the greatest differences are obtained tend to vary somewhat across studies. In addition, S2 activation that is greater during FES than during voluntary contractions has been reported (Iftime-Nielsen *et al.* 2012; Christensen & Grey, 2013). At least with respect to ankle dorsiflexion, the spatial extent of the BOLD-registered activity in M1, S1, S2, SMA, cingulate motor area (CMA), bilateral dorsal and ventral premotor areas, and cerebellum VI is greater during FES-generated movements than during passive movements (Francis *et al.* 2009; see also Gandolla *et al.* 2014). The nature of the brain activation that characterizes combined NMES and voluntary or imagined movement is a matter to which we will return in the sections that follow.

Corticospinal projections. In circumstances in which the expressed intent has been to bring about changes in the state of the CNS (rather than produce overt movements) (see Bergquist *et al.* 2011), the effects of parametric variations in NMES upon the state of corticospinal projections have been investigated. When delivered in a 4 s on and 6 s off cycle for 20 min at 30 Hz, median nerve stimulation applied at the wrist gave rise to increases in the amplitude of MEPs recorded in APB when the intensity was 110% of MT, but not when it was 90% of MT (Sasaki *et al.* 2017). Applying 30 min of mesh-glove whole-hand stimulation, Golaszewski *et al.* (2012) noted that 50 Hz stimulation at sensory threshold, and 2 Hz stimulation at motor threshold, gave rise to increases in corticospinal excitability extending to 1 h following. Such changes were not obtained when 50 Hz stimulation at a level below the sensory threshold or 2 Hz stimulation at sensory threshold was used. The outcomes of this specific form of intervention (i.e. using mesh glove stimulation), in which afferent fibres of multiple types, with widespread innervation zones, are likely to be involved, are not necessarily emblematic of those obtained when a single nerve is stimulated. Specifically, the magnitude of the change in corticospinal excitability depends on the stimulation frequency (for intensity $\approx$ MT). When applied at 100 Hz and in the range of 20–50 Hz, increases in corticospinal excitability (CSE) in excess of 50% are routinely observed. This is not generally the case for stimulation applied at 10 Hz or less (Jaberzadeh *et al.* 2017).

If the intensity of peripheral nerve stimulation applied in humans is between 30% and 50% of that required to produce a maximum compound muscle action potential (M-max), MEPs evoked subsequently by TMS over M1 are facilitated at inter-stimulus intervals (ISIs) from 25 to 60 ms in abductor pollicis brevis (APB) following median nerve stimulation at the wrist (Deletis *et al.* 1992). A similar outcome was noted (Komori *et al.* 1992) for the thenar muscle at ISIs between 50 and 80 ms when the peripheral shock was set to 10% of M-max. Devanne *et al.* (2009) reported than even when stimulation intensity is set just above motor threshold, median nerve stimulation (at the wrist) gives rise to marked facilitation of MEPs recorded in the APB, FDI and extensor carpi radialis (ECR) muscles when ISIs ranging from 40 to 80 ms are employed. At ISIs extending beyond 200 ms (and below 25 ms – around the latency of the N20 component of the somatosensory evoked potential), a diminution of MEP amplitude is generally obtained (e.g. Turco *et al.* 2018). It is of particular interest in the present context that after NMES is delivered over the ulnar nerve (100 Hz in a 20s on, 20s off duty cycle; intensity $\sim$15% of that to elicit a maximum m-wave) for 40 min, short-latency afferent inhibition (SAI: ISI 18–25 ms) is markedly diminished, whereas for those ISIs (28–35 ms) at which there occurred potentiation of MEP amplitudes following a (single) conditioning peripheral nerve stimulus, the NMES intervention served to further increase the amplitude of the TMS-evoked response (Mang *et al.* 2012). These findings are consistent with the possibility highlighted above, that the state of M1 (potentially acting via the intermediate cerebellum) may influence the gain of polysynaptic circuits that modulate the effects of peripheral stimulation (Manto *et al.* 2006).

It remains unclear at present whether sustained changes in corticospinal excitability brought about by prolonged NMES interventions are instrumentally related to changes in behaviour. Veldman *et al.* (2016) applied trains to the radial and median nerves (proximal to the elbow) consisting of five square wave pulses at 10 Hz (pulse width, 1 ms) 50% duty cycle, at intensities just below MT. In three separate interventions the stimulation was applied for 20, 40 or 60 min. Changes in the performance of a visuomotor tracking task (post-intervention relative to baseline) were compared to a fourth group of participants who did not receive stimulation. Although some improvements in task completion and in measures of CSE were observed over the course of the following week, there was no evidence

that these outcomes were related. A more general issue (to which we will return) is thereby illustrated. Variations in CSE, as revealed by TMS, are not necessarily indicative of the functional adaptations (in this case brought about by NMES) that mediate improvements in performance (Carson *et al.* 2016).

In light of the assumption that contractions of an intensity sufficient to mimic some features of those brought about by voluntary activation are necessary to cause reliable changes in CSE (Chipchase *et al.* 2011*a*), it may appear paradoxical that FES (primarily lower limb) protocols bring about immediate effects that are of lesser magnitude than those associated with 20–50 or 100 Hz stimulation delivered closer to MT (Jaberzadeh *et al.* 2017). Nonetheless, it is also the case (i.e. as with intensity ≈ MT) that supra-motor threshold stimulation is more effective at increasing CSE when delivered at 30 Hz than at 10 Hz (Chipchase *et al.* 2011*b*). It has been reported that while 20 and 40 min of stimulation (30 Hz) at intensities sufficient to generate a 'voluntary-like' contraction in APB increased CSE, this was not the case for 60 min of stimulation (Andrews *et al.* 2013). Although perhaps counterintuitive, a similar but less pronounced non-monotonic effect of duration is, however, also present for MT level stimulation (Jaberzadeh *et al.* 2017). In other words, there comes a point at which increasing the intensity or duration of stimulation brings about no further gains, at least in terms of the excitability of corticospinal projections to the target muscles.

There exist forms of NMES (typically delivered over the muscle belly) that have been developed with the express aim of preventing skeletal-muscle weakness, for example during acute critical illness. They are sufficient to generate high levels of force (and thus sometimes designated electrostimulation strength training). Usually utilizing frequencies between 35 and 100 Hz, the stimulation can be applied for up to an hour daily, over periods ranging between 1 and 6 weeks (Maffiuletti *et al.* 2011, 2013). There are comprehensive reviews dealing with the nature of the central and peripheral adaptations that may mediate the observed increases in functional capacity that can be accrued by these methods (e.g. Hortobágyi & Maffiuletti, 2011). The present aim is not to recapitulate these analyses. It is, however, pertinent to highlight one of the key observations to emerge in the course of this research. As noted in preceding sections, bilateral alterations in the state of brain circuits that constitute the classical motor network in both hemispheres are frequently observed following unilateral NMES. It is therefore particularly salient that these NMES variants can increase the force-generating capacity of homologous muscles in the limb opposite to the one in receipt of stimulation (Cabric and Appell, 1987; Hortobágyi *et al.* 1999; Zhou *et al.* 2002; Huang *et al.* 2007; Kadri *et al.* 2017).

In recent studies conducted with the aim of determining the mechanistic basis of such effects, there has been an understandable initial focus upon the degree to which less 'intense' forms of unilateral NMES might bring about bilateral changes in CSE. Veldman *et al.* (2015) applied trains consisting of five square wave pulses delivered to the radial and median nerves of the right arm (above the elbow) at 10 Hz (pulse width, 1 ms) 50% duty cycle, using an intensity equal to twice the perceptual threshold (i.e. presumed to be below MT) in five blocks of 5 min duration. They noted increases in the amplitude of MEPs recorded in both right and left ECR following the intervention, which were accompanied by improvements in the performance of a visuomotor tracking task (i.e. for both limbs). There was, however, no evidence of a statistical association between these measures (see also Summers *et al.* 2017). Using a largely equivalent stimulation protocol, Veldman *et al.* (2018) also observed improvements in the performance of the opposite limb, in this case during a retention test conducted 2 days following the intervention. And as in the preceding study, electrophysiological measures (in this case EEG derived) of directional oscillatory coupling (representing 'cortico-cortical connectivity'), between posterior parietal and primary somatosensory cortex to the primary motor cortex, did not vary in accordance with the changes in behaviour.

A reflection on the brain circuitry engaged by NMES. It is evident that there exist variants of NMES that provide a means of altering the state of elements within an extended brain network (encompassing not only classically defined somatosensory and motor areas), and the excitability of circuits with projections to the spinal cord (e.g. Schabrun *et al.* 2012). What remains to be determined are the causal relations between the changes in brain state that can be registered by modern neuroimaging and electrophysiological techniques, and alterations in functional capacity that can in some circumstances be brought about by NMES. In recent years there has perhaps been an undue haste to infer that intervention-induced changes in corticospinal excitability are indicative of the neural adaptations that mediate sustained changes in behaviour (Carson *et al.* 2016). Indeed, well-powered individual studies (e.g. Ruddy *et al.* 2016) and several meta-analyses (e.g. Veldman *et al.* 2014; Berghuis *et al.* 2017; Manca *et al.* 2018) have failed to demonstrate an association between changes in CSE and improvements in motor performance. There is consequently a growing recognition that in our empirical investigations we must devote greater attention to paths and structures other than the ones that can be assayed easily by such techniques as TMS (Veldman *et al.* 2016) or conventional brain imaging analysis approaches. For example, a case can be made for considering the

individual differences in functional or structural brain connectivity associated with variations in the expression of performance changes (e.g. Ruddy *et al.* 2017) that follow the administration of NMES. This would be in contrast to simply registering brain regions or pathways that exhibit a change in state following stimulation.

Adjuvant techniques

The production of voluntary movement has two essential components: central efferent drive that is initiated at the level of the cortex and consequentially muscle contractions that displace joints and thus give rise to afference. Electrical stimulation of peripheral nerves provides a means of producing muscular contractions without the initial central drive by direct depolarization of motor axons located below the stimulating electrodes. It has been noted previously that the effectiveness (both adaptive and restorative) of NMES may be enhanced through the use of specific protocols (e.g. pulse width/frequency combinations) that promote synaptic recruitment of spinal motoneurons by the electrically evoked sensory volley (e.g. Collins, 2007). The adaptive benefits are readily appreciated. For example, afference-mediated (i.e. synaptic) recruitment of spinal motoneurons is likely to occur in normal physiological order, and thus preferentially include fatigue-resistant motor units. The restorative benefits, while perhaps less obvious, are, however, also potentially significant. In this regard, emphasis has been placed on a capacity for the repeated evocation of sensory volleys by NMES to induce increased activity in spinal and supraspinal circuits, and in turn bring about acute and chronic neuroplastic adaptations that are sufficient to enhance function (e.g. Bergquist *et al.* 2011). While in this scheme the accent is on the cumulative effects of stimulus repetition *per se*, there are further possibilities.

In recent years, there has been particular interest in associative forms of neural plasticity, such as those in which the repeated coincidence of experimentally induced activity in both sensory circuits (by peripheral nerve stimulation) and motor circuits (by TMS applied over M1) gives rise to sustained changes in corticospinal excitability (e.g. Stefan *et al.* 2000). In terms of the phenomenology of the induced effects, there is notionally a resemblance to Hebbian plasticity (Hebb, 1949), whereby a presynaptic input onto a postsynaptic neuron is strengthened as a consequence of both the pre- and postsynaptic neurons being active simultaneously. In seeking to provide a more mechanistic account of this paired associative stimulation (PAS), it has been proposed that it shares key features with spike timing-dependent plasticity (STDP) (Müller-Dahlhaus *et al.* 2010) – as this has been elaborated in animal models and reduced (e.g. slice) preparations. In STDP, the polarity of the induced change in synaptic efficacy is determined by the sequence of pre- and postsynaptic neuronal activity (for reviews see Dan & Poo, 2006; Markram *et al.* 2011). In prototypical representations of STDP (e.g. Song *et al.* 2000), potentiation occurs if a presynaptic neuron fires no more than 50 ms in advance of the postsynaptic neuron (Feldman, 2000). Depression arises if postsynaptic spikes precede presynaptic action potentials (or transpire without activity in the presynaptic neuron) (Levy & Steward, 1983; Bi and Poo 1998; Cooke & Bliss, 2006). There is also held to be a sharp transition from a weakening of synaptic efficacy (long term depression) to strengthening of synaptic efficacy (long term potentiation) at time differences in the vicinity (within 5 ms) of zero (Feldman, 2012).

In the sections that follow, we use the conceptual framework of associative plasticity to consider the impact of adjuvant techniques upon responses to NMES. The argument is made that an association of NMES-generated afference with centrally initiated neural activity, such as that which occurs if the stimulation is triggered by efferent drive, or is delivered following instructions to engage in mental imagery, may promote neural adaptations upon which changes in functional capacity may be based. In doing so, we first make the critical point that the induction of associative effects that can be observed at a systems level in humans does not require adherence to the defining characteristics of STDP. In particular, associative effects are expressed when the relative timing of the activity induced in sensory and motor circuits is not precisely circumscribed.

Extending the concept of associative stimulation. There are a number of recent and comprehensive reviews of paired associative stimulation (e.g. Carson & Kennedy, 2013; Suppa *et al.* 2017). It is not our intent to reprise their contents. There are nonetheless important points that can be gleaned from these reviews, and from empirical findings that have appeared subsequently. Foremost among these is the observation variants of PAS in which the timing of the contributory elements is not strictly confined, for example when extended trains of peripheral nerve stimuli are used (e.g. Ridding & Taylor, 2001; Carson *et al.* 2013; McNickle & Carson, 2015; Shulga *et al.* 2016; Carson & Rankin, 2018; Tolmacheva *et al.* 2019), produce elevations in CSE that are comparable to, if not greater than, those obtained when the ISI separating the peripheral and cortical events is precisely circumscribed. The associative nature of the effects are, however, emphasized by the fact that in these studies the NMES alone (typically at an intensity $\approx$ MT) does not bring about changes in CSE. The conclusion that the relative timing need not be either precise or restricted is further emphasized by reports that the nerve stimulation component of PAS can be replaced by movement-generated afference, without loss of generality

(Edwards *et al.* 2014; see also McNickle & Carson, 2015). In this vein, cortical microstimulation experiments in freely behaving non-human primates reveal that changes in synaptic strength between stimulated sites in precentral and/or postcentral cortex can be brought about without adherence to STDP rules (Seeman *et al.* 2017). These recent findings also serve to emphasize what should perhaps be apparent on *a priori* grounds alone, that when applied *in vivo*, there are multiple pathways via which the corollaries of (i.e. peripheral) stimulation may reach and influence the cortex (Carson & Kennedy, 2013), and as a consequence relative timing is likely to be only one of many factors that govern the induction of neuroplastic adaptations (Feldman, 2012).

It is in this light that the outcomes yielded by associative stimulation protocols can be more easily reconciled with the results of studies demonstrating that the combined effects of NMES and forms of exogenous cortical stimulation other than TMS are greater than those of each stimulation modality alone. Rizzo *et al.* (2014) described a protocol in which NMES (500 [s pulse duration, at 5 Hz for 5 min, 1500 stimuli, intensity $\approx 2 \times$ ST) was delivered to the median nerve simultaneously with transcranial direct current stimulation (tDCS). When the cortical electrode montage was such that the anode was positioned on the scalp over M1 contralateral to the site of peripheral nerve stimulation, the elevation in CSE recorded following the cessation of the intervention was markedly greater than that induced by tDCS alone (NMES + sham tDCS did not alter CSE). In addition, the duration of the elevation in CSE brought about by the combined stimulation persisted for at least 1 h (considerably longer than following anodal tDCS alone). Employing an NMES variant in which 1 ms pulses (intensity $\approx$ MT) were applied simultaneously to the FDI and ABP motor points (at frequencies between 0.35 and 6.7 Hz $\approx$ 6345 pairs) for a period of 30 min, and anodal tDCS delivered for the final 25 min, Hoseini *et al.* (2016) observed subsequent improvements in performance of the Purdue pegboard test (used to assess dexterity) that were not seen following either NMES + sham tDCS or anodal tDCS + sham NMES. For cases in which tDCS is applied (i.e. continuously) over an extended period during which NMES is also delivered at various intervals, there exists no discrete timing relationship between peripheral and cortical stimulation events. Yet associative effects are nonetheless obtained. Although not in accordance with STDP-based models of associative plasticity, this general pattern of findings is, however, consistent with recent analyses showing that not only the phase, but also the power of the cortical oscillatory beta cycle (e.g 16–17 Hz) at the moment stimulation is delivered influences the increase in CSE caused by TMS (Khademi *et al.* 2019). There is a more general point. Since a single relative timing relationship between the corollaries of cortical and peripheral stimulation is not a prerequisite for the induction of associative effects, when NMES is paired with endogenously generated elevations in motor network excitability, similar neuroplastic adaptations are likely to occur. There is now a considerable body of evidence to support this conjecture, and to suggest that the adaptations may be functionally significant.

Augmenting NMES at motor threshold intensity. For the present purposes, we consider two endogenous means of altering the state of the motor network: voluntary contractions and mental imagery. With a view to confining the limits of the discussion, 'cognitive' factors such as the focus of attention, which are believed to have an influence on the efficacy of associative stimulation protocols (e.g. Stefan *et al.* 2004), will not be treated in any detail.

It has for some time been appreciated that when NMES is applied in the context of voluntary contractions, the consequential changes in the state of efferent projections from the brain to the spinal cord are greater than those achieved through NMES alone (de Kroon *et al.* 2005). Although the majority of empirical studies conducted in this domain have employed levels of stimulation sufficient to evoke overt motor responses, it can also be shown that these features emerge when much lower intensities of NMES are used. For example, Taylor and colleagues (2012) delivered biphasic pulses (50 Hz; 200 [s pulse duration; intensity $\approx$ MT; 50% duty cycle for 6 s) over the wrist extensors (ECR and extensor carpi ulnaris) at the onset of 60 isometric wrist extension contractions (to 15% MVC) – triggered when the surface EMG recorded from the target muscles exceeded 25 [V. In a control condition NMES was delivered in isolation. An elevation of CSE was observed following EMG-triggered delivery of NMES, but not following NMES alone. Similar findings have been obtained for the lower limb, when NMES is delivered either over the tibialis anterior (TA) muscle or to the (common peroneal) nerve during ballistic dorsiflexions of the ankle (Jochumsen *et al.* 2016). In this regard, it is notable that the acute augmentation of CSE appears to be greater when NMES is combined with shortening contractions than with isometric contractions (Saito *et al.* 2014).

Of greater practical relevance are the changes in functional capacity that arise from the combination of NMES and voluntary contractions. Carvalho *et al.* (2018) conducted a double-blind, sham-controlled, randomized trial engaging healthy adults, in which median nerve stimulation (random frequency ranges (1–4, 8–12 and 60–90 Hz) and intensity levels (2–6 mA)) at the wrist was applied during 20 min practice of a serial reaction time task (SRTT) requiring keypress responses. This was followed by a similar 'consolidation' session of 30 min duration. It was noted that explicit recall of the learned

sequence improved following both initial training and consolidation. No such improvements were obtained for either a group that received 'off line' NMES, or a group that was given sham stimulation.

That the origin of the neuroplastic effects of combined voluntary contraction and NMES is likely to be predominantly supraspinal rather than spinal, at least when relatively low levels of electrical stimulation are employed, is indicated by a series of studies in which the delivery of NMES has been in the context of motor imagery tasks performed by the recipient. Employing a task in which the participants were asked to imagine that they were squeezing and relaxing a ball (motor imagery), while watching a video of the action (observation) (during which time the ball was held 'passively'), Yasui *et al.* (2019) applied NMES (trains of 20 pulses at 10 Hz; 1 ms pulse duration; intensity ≈ 90% MT; 50% duty cycle of 2 s on, 3 s off) during four blocks of 5 min duration. A cumulative increase in the amplitude of MEPs recorded from FDI was obtained in this condition, but not for NMES alone (or imagery/observation alone). Corresponding effects that are sustained for at least 30 min following cessation of combined NMES/motor imagery have also been reported for the lower limb (Takahashi *et al.* 2019). In a small-scale study (without a control group), Okuyama *et al.* (2018) observed increases in upper extremity function in 10 chronic stroke survivors, following an intervention (10 trials per day for 10 days) in which stimulation (≈ MT of the extensor digitorum communis) of the radial nerve, innervating wrist and finger extensors, was combined with motor imagery/observation.

A compelling case that these effects are associative in nature can be made on the basis of reports that they can be obtained when the delivery of NMES is triggered by EEG-registered movement-related cortical potentials (MRCPs) – generated when individuals follow an instruction to imagine the 'kinaesthetics' of ballistic movements. Deploying an intervention of this type, Niazi *et al.* (2012) triggered stimulation (1 ms pulse duration; intensity ≈ MT) of the common peroneal nerve upon detection of the initial negative phase of the MRCP, as 50 self-paced imagined movements were performed. The intervention gave rise to increases in the excitability of corticospinal projections to TA. No such changes were induced by NMES alone or by motor imagery alone (see also Mrachacz-Kersting *et al.* 2017). Comparable results are obtained if the timing of the NMES is yoked (using an estimate of the contingent negative variation) to the onset of a cued imagined movement (Mrachacz-Kersting *et al.* 2012). In a recent investigation using MRCP-triggered NMES (equivalent to the protocol of Niazi *et al.* 2012), increases in CSE persisting for one hour were registered (Olsen *et al.* 2018; see also Jochumsen *et al.* 2018). In addition to giving rise to increases in CSE in both chronic (Mrachacz-Kersting *et al.* 2016) and sub-acute (Mrachacz-Kersting *et al.* 2019) stroke survivors, imagery-related MRCP triggered NMES appears capable of promoting positive changes in motor function. As far as we are aware, however, it has not been established that any changes in CSE brought about by these techniques are instrumentally related to improvements in performance.

Given the very large number of brain imaging studies that have been conducted, there are several meta-analyses (e.g. Grezes and Decety, 2001; Caspers *et al.* 2010; Molenberghs *et al.* 2012; Hétu *et al.* 2013; Hardwick *et al.* 2018) that provide a basis upon which to survey the brain regions engaged during voluntary movement, action observation and motor imagery. As has been highlighted recently, however (Savaki & Raos, 2019), by and large these meta-analyses are based upon studies in which the three task contexts have been investigated independently of one another. On the basis of these analyses it appears reasonable to draw the conclusion that voluntary movement, action observation and motor imagery all give rise to consistent activation of a brain network encompassing premotor, parietal and somatosensory areas (e.g. Hardwick *et al.* 2018). In the present context, we follow the lead of Savaki and Raos (2019) in suggesting that there is additional information to be gained by giving particular weight to the small number of studies in which fMRI has been used to assay the whole brain when all three variants of the same 'motor' task have been performed by the same group of participants. In a recent study in which there were no *a priori* constraints upon regions of interest (ROIs) deemed to be of interest, Simos *et al.* (2017) determined that during both motor imagery and execution of a geometric tracing task performed by the *right* index finger, BOLD activity in the following regions surpassed the assigned threshold: bilateral dorsal and ventral premotor cortex, left supplementary motor cortex (SMA proper), bilateral BA 7 in the superior and BA 40 in the inferior parietal cortex, bilateral BA 8 in the middle frontal gyrus, bilateral BA 22 in the posterior part of superior temporal gyrus including the temporo-parietal junction, bilateral BA 37 in the posterior part of the middle temporal gyrus including the extrastriate body area, the left extrastriate visual BA 19 in the cuneus, the right lingual gyrus (LG) and the left middle occipital gyrus (MOG), left BA 7 in the posterior precuneus and right BA 37 in the fusiform gyrus. The left secondary somatosensory cortex (SII) was also deemed engaged in both tasks. As might be anticipated, while the upper limb representations of the primary motor and somatosensory cortical areas (2/3) exhibited bilateral activity, the magnitude of the BOLD response was larger during execution than during imagery. In contrast, during motor imagery there was relatively greater BOLD response magnitude bilaterally in prefrontal, premotor and parieto-temporal cortices. In a related investigation in which the technique of multi-voxel

pattern analysis was used in conjunction with *a priori* selection of ROIs (excluding MI and SI), Filimon *et al.* (2015) reported that during both execution and motor imagery of reaching to visual targets, the BOLD response is registered across both ventral and dorsal premotor, and parietal areas.

It is readily apparent therefore that during both the execution of (upper limb) movements and motor imagery there is a high degree of overlap with those brain regions that are believed to exhibit increased activity in response to NMES (see preceding sections). As such, and the consequential changes in CSE that have been observed in some cases notwithstanding, it cannot be assumed that the M1 or S1 is the principal locus of the associative interactions that occur when NMES is delivered during either motor imagery tasks or voluntary contractions. Indeed, it is clear that there are many potential loci. At present there is no empirical basis upon which to resolve the various possibilities. It is important to emphasize that during all motor tasks, the notionally 'active' (i.e. in a BOLD registration context) brain regions constitute a network of functional connections (e.g. Simos *et al.* 2017), such that the task-relevant contribution of any specific region of interest cannot sensibly be considered in isolation (e.g. Anderson, 2008). In closing this section, it should also be noted that there have been very few randomized clinical trials (with appropriate blinding) in which the combined effects on function of either voluntary contractions or motor imagery and NMES at motor threshold intensity have been evaluated.

Augmenting NMES at supra-motor threshold intensities. Empirical studies, in which the focus has been upon the combined effects of voluntary contractions and NMES delivered at intensities sufficient to generate functional levels of muscle tension (i.e. FES), have typically been undertaken in a clinical context. In many such instances the focus has been upon the promotion of movement capacity in stroke survivors. In light of the relatively large number of investigations of this kind that have been undertaken, several systematic reviews have been compiled. Although initial summaries of this nature (e.g. de Kroon *et al.* 2005) tended to suggest that clinical outcomes obtained for FES triggered by voluntary contraction (e.g. via EMG registration) were superior to those following FES alone, it was not generally the case that cumulative effect size estimates were obtained. In a more recent analysis that was restricted to the outcomes of randomized controlled trials (RCTs) engaging chronic stroke survivors, Yang *et al.* (2019) reported that the changes in function (as assessed by the Fugl–Meyer test) and activity (e.g. as assessed by the Action Research Arm Test) arising from 'cyclic' FES (not triggered by voluntary contraction) and EMG-triggered FES could not be distinguished in terms of their quantified effects (although both were superior to control). In their systematic reviews, both Monte-Silva *et al.* (2019) and Nascimento *et al.* (2014) arrived at a same conclusion. In the single RCT of which we are aware (Wilson *et al.* 2016) that compared their relative efficacy in acute stroke survivors (<6 months post-stroke), the improvements in Fugl–Meyer scores and the Arm Motor Ability Test, registered following an 8-week intervention period, did not differ between administrations of 'cyclic' FES and EMG-triggered FES.

It is particularly notable, therefore, that when the delivery of stimulation at levels sufficient to produce joint displacement is triggered by contractions of the *opposite* (i.e. non-impaired) limb, improvements in clinical outcomes greater than those induced by NMES alone have been obtained in several trials. Knutson *et al.* (2016) employed with chronic stroke survivors a method whereby opening of the ipsilesional hand (monitored using an instrumented glove) modulated the intensity of stimulation applied to the finger (and wrist) extensors of the paretic hand, such that both hands opened synchronously. Fugl–Meyer scores and performance of the Arm Motor Ability Test exhibited by following a 12-week intervention ($\approx$ 10 h of stimulation per week) were greater than those exhibited by patients who received cyclic FES (see also Knutson *et al.* 2012). In the context of a trial of 3 weeks duration (5 sessions per week; 20 min per session) engaging acute ($\leq$3 post) stroke survivors, Shen *et al.* (2015) implemented a protocol whereby a wrist extension movement executed by the non-impaired limb triggered the delivery of stimulation (50 Hz; 200 [s pulse duration; intensity up to that sufficient to produce full range wrist extension) to the impaired limb. In the NMES group, matched levels of stimulation were applied. Although both groups exhibited clinically relevant improvements in capacity (Fugl–Meyer assessment, the Hong Kong version of functional test for the hemiplegic upper extremity (FTHUE-HK) and active range of motion), the magnitude of these changes was substantially greater in the group for whom NMES was triggered by movement of the opposite limb. In a more recent trial using the same methodology that engaged individuals within 15 days of stroke, the combination of routine rehabilitation with NMES triggered by movement of the ipsilesional limb gave rise to better outcomes than routine rehabilitation combined with matched levels of electrical stimulation (Zheng *et al.* 2019).

The contrasting effects (relative to FES alone) of FES triggered by voluntary engagement of the same limb and of FES triggered by movement of the opposite limb might also be considered in light of the following. Systematic reviews of randomized or quasi-randomized controlled trials examining the effects of electrical stimulation delivered at intensities close to sensory threshold (e.g. TENS) on motor recovery following a stroke suggest that

clinical outcomes are superior when it is combined with voluntary movement (e.g. Ikuno *et al.* 2012; Laufer & Elboim-Gabyzon, 2011). Taken together, these findings suggest that the functional impact of combining NMES with voluntary contraction depends on the intensity of the electrical stimulation. When it is insufficient to generate muscle contractions, additive effects are obtained. In contrast, when FES intensities are employed, the combined effects are comparable to those induced by FES alone. There are at least two possible accounts of this phenomenon. The first is that there is a ceiling effect. That is, if the effects of FES alone on the state of the motor network approach asymptotic levels, there may be little scope for endogenous activity generated in the context of voluntary contractions to promote additional restorative changes. The additive effects of contractions performed by the opposite limb, however, suggest that this explanation is insufficient. As described above, in both acute and chronic stroke survivors, when the delivery of FES is triggered by contractions of the ipsilesional limb, the benefits in term of clinical outcomes are greater than those brought about by FES alone. Similarly, when NMES at functional intensities is delivered during mental imagery (triggered by very low 'incidental' levels of EMG), improvements in function achieved by chronic stroke survivors are greater than those achieved using FES alone (Hong *et al.* 2012; You & Lee, 2013; cf. Park, 2019). There is no ceiling effect. An alternative possibility is that when voluntary contractions are combined with, or initiate (e.g. EMG-triggered FES), NMES delivered at intensities sufficient to produce joint displacement, there is a mismatch between the anticipated consequences of the efferent drive and the afferent feedback that arises from the combined effects of the voluntary contraction and stimulation-driven recruitment of motoneurons (e.g. Iftime-Nielsen *et al.* 2012). As a corollary, the degree of any such 'mismatch' is likely to depend not only on the intensity of the stimulation, but also on the degree to which the pattern of its application mimics natural muscle synergies. For example, it is known that in the context of tasks in which a large number of degrees of freedom (muscular and biomechnical) must be coordinated such as the formation of a grasp, if electrical stimulation (of the intrinsic and extrinsic flexor muscles) is imposed upon a voluntary contraction, maximal grip force diminishes (Boisgontier *et al.* 2010). In other tasks in which a relatively small number of muscles actuate a single joint (e.g. Barker *et al.* 2008, 2017), the discrepancy may be smaller. The assumption is that, to the degree to which a mismatch is present, further augmentation of the effects of NMES through associative mechanisms is precluded.

There have been relatively few studies in which imaging techniques have been used to compare patterns of brain activity arising when FES is delivered both in isolation and in combination with voluntary contractions. Employing the method of near-infrared spectroscopy with healthy adults, Lin *et al.* (2016) reported that when NMES was delivered at a level sufficient to augment force output during isometric knee extension contractions, the O_2 demand in the contralateral premotor cortices and SMA was greater than the sum of that observed during NMES alone and during voluntary movement alone. Oxy-Hb increases in 'sensory-motor cortex' (relative to rest) of greater magnitude during EMG-triggered FES than for voluntary contractions alone (and FES alone) have also been reported for chronic stroke survivors (Hara *et al.* 2013). There are two further studies (of which we are aware) in which fMRI has been employed during upper limb movements (for the lower limb see Gandolla *et al.* 2014). Joa *et al.* (2012) reported that FES combined with voluntary wrist extension gave rise to a greater BOLD signal in ipsilateral cerebellum, contralateral MI ('primary central gyrus') and SI ('post central gyrus') than during FES alone. Christensen and Grey (2013) noted that a larger BOLD response was registered during combined FES and voluntary (finger flexion–extension) movements than during voluntary movements alone in the following brain regions: superior temporal gyrus, supramarginal gyrus, insula, rolandic operculum and angular gyrus. There were no regions for which a larger BOLD response was obtained during voluntary movements alone. Of particular interest in the present context is the observation that following administration of an ischaemic nerve block that removed sensory feedback (but preserved the capacity for voluntary movement), there were no differences in BOLD response between the two conditions (i.e. voluntary movement with and without FES). This pattern of outcomes supports the conjecture that the additional brain activity otherwise evident during combined voluntary movement and FES (i.e. compared to voluntary movement) is related to the integration of afferent feedback (i.e. relative to that anticipated on the basis of the efferent command). Gandolla *et al.* (2014) present a somewhat similar line of argument.

For completeness, we highlight briefly the finding that for both healthy adults and survivors of stroke, the excitability of corticospinal projections to muscles in receipt of FES is greater when it is combined with, or triggered by, voluntary contraction than when it is delivered in isolation (Khaslavskaia & Sinkjaer, 2005; Barsi *et al.* 2008; Stein *et al.* 2013; McGie *et al.* 2015). Although these data were not obtained in the context of the clinical trials described above, they do serve to emphasize an important point. Two variants of an intervention that can be distinguished clearly in terms of the changes in corticospinal excitability to which they give rise do not necessarily lead to different treatment outcomes when they are deployed over multiple sessions in a rehabilitation setting.

A reflection on the augmentation of NMES at supra-motor threshold intensities. There was a period during which it was widely assumed that NMES at supra-motor threshold intensities in combination with voluntary contractions, particularly when there was a contingent relation (as in EMG-triggered FES), gave rise to outcomes superior to those that could be achieved by NMES alone. In such circumstances it was natural to seek explanatory constructs. For example, De Kroon *et al.* (2005), in what was then a comprehensive review of the available data, hypothesized that there may be an additional cognitive element present in EMG-triggered NMES that is not a feature of NMES alone. In was suggested that an additional investment of mental resources and attention improves performance. Any explanation of this type should apply in equal measure to instances in which the effects of NMES delivered at lower (e.g. ∼MT) intensities are accentuated by simultaneous voluntary contractions. The inconvenient truth is, however, that there is currently little by way of systematic evidence to indicate that EMG (or movement)-triggered FES is more efficacious than cyclic FES (which is not yoked to voluntary movement).

Clarac *et al.* (2009, page 367) remark that Wundt (1863) was among the first to note explicitly that passive movements and active movements differ in respect of their perceptual consequences. More particularly, they are distinguished by the relationship between efferent impulses and the referent response. Duchenne de Boulogne also promoted the concept of an 'efferent sense' of central origin, which precedes a muscle contraction and is necessarily distinguishable from the sensation that arises as a result of the contraction (Clarac *et al.* 2009). On the basis of electrophysiological recordings obtained using modern methodologies, Lebedev *et al.* (1994) established that during self-initiated movement, activity in the primary somatosensory cortex becomes evident before the initiation of motor output. This was interpreted as preparation for receipt of the imminent changes in afferent inflow that will result from the movement (see also Nelson *et al.* 1991; Nelson, 1996). fMRI-based investigations in healthy volunteers further reveal that during active but not passive movement, a BOLD response in the Brodmann area 2 subregion of S1 is closely associated with that registered in premotor and supplementary motor areas, the parietal cortex and the cerebellum, in the absence of common mediation by area 3b (Cui *et al.* 2014). These and similar observations have been taken as evidence in support of the construct of efference copy – conceived of by von Holst and Mittelstaedt (1950) as the internal copy of an outgoing, action-producing 'command' generated by the motor system. In an extension of the concept, it is proposed that the CNS instantiates forward internal models that utilize efference copy in order to anticipate the sensory consequences of an action (e.g. Miall & Wolpert, 1996).

The conventional contemporary line of thinking is that brain computer interfaces (BCI) that instantiate closed-loop control (i.e. brain–efference–change in muscle length/joint displacement–afference–brain) offer concordance between the efference copy and sensory consequences of an action. It is furthermore assumed that (repeated) concomitance of voluntarily generated brain activity, and movement-related afference (even if generated by artificial means) can promote neuroplastic adaptation and in some cases restoration of function (e.g. Jackson & Zimmermann, 2012). A key requirement in this regard is that there is a persistent causal relationship between the initiating endogenous neural activity (e.g. descending drive leading to recruitment of motoneurons – as registered by EMG) and the consequential endogenous neural activity (e.g. afference generated by EMG-triggered FES). A further necessity is temporal congruency. That is, the delay between the initiating and consequential neural activity must be consistent with the natural latency between the efference copy of a motor command and the reafferent sensory feedback (e.g. Leube *et al.* 2003). It has been highlighted recently that, even in circumstances in which the afference generated by EMG-triggered NMES is dominated by that which arises from direct activation of sensory axons (i.e. for intensities ≈ MT), conduction delays within the central and peripheral nervous systems dictate that stimulus-evoked activity is unlikely to be able alter the state of circuits in M1 sooner than 60 ms following the voluntary activity that generated the triggering EMG (Brown *et al.* 2016). In the event that the afference generated by NMES is dominated by reafference produced by the resulting contraction (i.e. such as with FES), and given electromechanical delays in the order of 40 ms (Cavanagh & Komi, 1979), the latency will be very much greater. If the FES is triggered by joint displacement (rather than by EMG), it will be longer still. We have emphasized in preceding sections that the induction of associative effects that can be observed at a systems level in humans does not require adherence to the defining characteristics of STDP (i.e. precisely circumscribed relative timing, with presynaptic firing occurring no more than 50 ms in advance of postsynaptic firing; Feldman, 2000). Nonetheless, if the interval over which the contingent relationship is defined exceeds certain bounds, the effects of the association are likely to be diminished (Carson & Rankin, 2018). Indeed, even in STDP schemes, it is predicted that the magnitude of potentiation is inversely related to the delay between pre- and postsynaptic activity (Markram *et al.* 1997).

Such considerations raise the possibility that the failure of EMG triggered FES to bring about functional adaptations that are greater than those achieved by cyclic FES is attributable to the extended delay between initiation of the voluntary command (that generates the EMG), and the reafference produced by the resulting contraction. In the case of EMG-triggered NMES delivered at intensities

sufficient to activate only a relatively small proportion of motor axons (i.e. around motor threshold), in which the resulting afference is dominated by that which arises from direct activation of sensory axons, the delay following the voluntary command will be shorter. It is notable therefore that such protocols appear (at least based on evidence currently available) to more consistently yield positive changes in functional capacity that exceed those brought about by NMES alone.

General conclusions

It is widely held that the application of NMES in a rehabilitation setting can bring about effects that are both adaptive and restorative. Direct compensation for motor disability (i.e. the 'adaptive' response) aside, assessment of the evidence gathered in contemporary systematic reviews and meta-analyses suggests that NMES delivered at levels sufficient to generate fused contractions (Nascimento *et al.* 2014; Howlett *et al.* 2015; Monte-Silva *et al.* 2019; Yang *et al.* 2019) is capable of promoting restorative changes in a number of neurological disorders that are at least equivalent to those brought about by conventional therapy. It also appears to have a positive effect on the functional status of older adults who do not have neurological conditions (Langeard *et al.* 2017). There is preliminary evidence that it may elevate serum levels of brain-derived neurotrophic factor (BDNF) – a neurotrophin that plays a well-documented role in the expression of neural plasticity (Kimura *et al.* 2019).

With respect to lower levels of stimulation (e.g. using intensities in the vicinity of motor threshold), the picture is less clear. This is partly due to the fact that the widely heterogeneous (in terms of stimulation parameters and target muscles) nature of the studies that have been conducted, generally precludes their combination in meta-analyses (Chipchase *et al.* 2011*a*; Wattchow *et al.* 2018). Given conflicting evidence concerning the efficacy of stimulation delivered at intensities that evoke paresthesia but generally no motor response (Grant *et al.* 2018), a restorative effect of low-level NMES cannot necessarily be assumed. Nonetheless, on the basis of a small scale meta-analysis of studies restricted to those that adopted a variant of the stimulation protocol described by Ridding *et al.* (2000) (i.e. ulnar, median or radial nerve stimulation (10 Hz, 1 ms pulse width, duty cycle 1 s, 500 ms on–500 ms off) for period of 2 h), it can be inferred that NMES at an intensity close to MT may improve upper limb motor function in (chronic) stroke survivors (Conforto *et al.* 2018). Although not yet supported by sufficient evidence derived from RCTs, there are some indications that adjuvant techniques, such as voluntary contractions, and mental imagery may further promote restorative responses to NMES delivered at around motor threshold.

What is common to all forms of NMES is the absence of a clear understanding of the mechanisms that mediate its influence on motor function. Evidence derived using a range of methodologies both in humans and non-human primates indicates clearly that NMES alters the state of circuits in many parts of the brain, often extending beyond the classical sensory and motor networks. An increase in the excitability of corticospinal projections from primary motor cortex (generally assayed using TMS) is a pervasive feature of the immediate physiological response to NMES. Nonetheless, there is presently no indication of which we are aware that the increases in CSE brought about by NMES are instrumentally related to any improvements in function. We base this conclusion on the fact that there have been no reports of statistical associations between alterations in CSE and motor function following the administration of NMES. In addition, there are interventions that can be distinguished in terms of the changes in CSE to which they give rise that do not differ with respect to the changes in movement function that they bring about. This analysis highlights the more general concern (e.g. Carson *et al.* 2016) that TMS is perhaps not the best tool for the purpose of discriminating neural mechanisms that mediate the restorative effects of NMES (Veldman *et al.* 2016).

The augmentation of the effects of NMES that occurs when it is combined with adjuvant techniques such as voluntary contractions and mental imagery bears the hallmarks of associative plasticity. As we have noted elsewhere (Carson & Kennedy, 2013) the induction of associative effects that can be observed at a systems level in humans does not necessarily require protocols that adhere to the defining characteristics of STDP. The appeal to constructs that have been elaborated in the context of reduced slice or animal preparations is, however, seductive. It can also be reinforced (perhaps inadvertently) by the identification at a systems level of features that bear a resemblance to those that have been studied and manipulated *in vitro*. It appears that in closed loop control, such as EMG-triggered FES, temporal congruency of the initiating (i.e. efferent) and consequential (i.e. afferent) endogenous neural activity is critical for the induction of restorative effects. This should not, however, be taken as reflecting adherence to STDP rules as they apply to individual presynaptic and postsynaptic neurons. In seeking to provide a deeper understanding of the mechanisms that mediate the effects of NMES, it might also be useful to consider the influence of the integrative properties of the brain (e.g. cortical 'rhythms') that only emerge as a consequence of its topological network properties, and the coupling of individual neural (and non-neural) elements to which this architecture gives rise (e.g. Guggenberger *et al.* 2018; Kraus *et al.* 2018). There is certainly also scope for greater consideration of the potential role of subcortical structures such as the

thalamus in mediating the changes in functional capacity that can be induced by NMES (e.g. Kimura *et al.* 1999; see also Veldman *et al.* 2018).

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Additional information

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There are no competing interests of which the authors are aware.

Author contributions

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Cortical Re-organization After Traumatic Brain Injury Elicited Using Functional Electrical Stimulation Therapy: A Case Report

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Functional electrical stimulation therapy (FEST) can improve motor function after neurological injuries. However, little is known about cortical changes after FEST and weather it can improve motor function after traumatic brain injury (TBI). Our study examined cortical changes and motor improvements in one male participant with chronic TBI suffering from mild motor impairment affecting the right upper-limb during 3-months of FEST and during 3-months follow-up. In total, 36 sessions of FEST were applied to enable upper-limb grasping and reaching movements. Short-term assessments carried out using transcranial magnetic stimulation (TMS) showed reduced cortical silent period (CSP), indicating cortical and/or subcortical inhibition after each intervention. At the same time, no changes in motor evoked potentials (MEPs) were observed. Long-term assessments showed increased MEP corticospinal excitability after 12-weeks of FEST, which seemed to remain during both follow-ups, while no changes in CSP were observed. Similarly, long-term assessments using TMS mapping showed larger hand MEP area in the primary motor cortex (M1) after 12-weeks of FEST as well as during both follow-ups. Corroborating TMS results, functional magnetic resonance imaging (fMRI) data showed M1 activations increased during hand grip and finger pinch tasks after 12-weeks of FEST, while gradual reduction of activity compared to after the intervention was seen during follow-ups. Widespread changes were seen not only in the M1, but also sensory, parietal rostroventral, supplementary motor, and premotor areas in both contralateral and ipsilateral hemispheres, especially during the finger pinch task. Drawing test performance showed improvements after the intervention and during follow-ups. Our findings suggest that task-specific and repetitive FEST can effectively increase cortical activations by integrating voluntary motor commands and sensorimotor network through functional electrical stimulation (FES). Overall, our results demonstrated cortical re-organization in an individual with chronic TBI after FEST.

Keywords: brain injury, functional electrical stimulation, functional electrical stimulation therapy, neuroplasticity, rehabilitation

INTRODUCTION

Acquired brain injuries, such as stroke or traumatic brain injury (TBI), can cause large portions of the frontal and parietal cortex and/or subcortical structures such as the striatum and thalamus to be affected, which can induce sensorimotor impairment in the contralateral limbs (Nudo, 2013). Neurological injuries resulting from trauma are typically diffuse and affect widespread cortical activation changes associated with movement of the paretic limbs. Even in case of focal brain injuries, disruption of sensorimotor networks can trigger reassembly of inter- and intra-cortical networks, resulting in loss of fine motor control (Nudo, 2013). Excitability of the motor cortex can be considerably reduced near the injury site, resulting in decreased cortical motor map representations of the affected muscles (Traversa et al., 1997; Butefisch et al., 2006). Spontaneous (natural) recovery can occur even in absence of rehabilitative intervention in the acute stages (Nudo, 2013). Compensating behaviors and learned non-use can also arise if unsuccessful attempts to use affected limbs persist (Taub et al., 1998). By restraining use of the non-affected limb, constraint-induced movement therapy has been shown to improve use of the affected limb (Wolf et al., 2006). Intact motor areas adjacent to the injury site and areas outside of the motor cortex or ipsilateral cortical areas may contribute to recovery via intracortical connectivity networks (Weiller et al., 1992; Seitz et al., 2005; Nudo, 2013). However, enabling successful movement execution of the affected limbs is still challenging.

Functional electrical stimulation (FES) is a neurorehabilitation approach that can be used to apply short electric impulses on the muscles to generate muscle contractions in otherwise impaired limbs with the goal of assisting motor function (Popovic et al., 2002; Quandt and Hummel, 2014; Carson and Buick, 2019). When stimulation is sequenced over the appropriate muscles, FES can generate functional movements, including grasping and reaching (Popovic et al., 2001, 2002). Applications of FES include improving voluntary limb movements in individuals such as stroke and incomplete spinal cord injury (SCI). Specifically, using FES therapy or functional electrical stimulation therapy (FEST) (Popovic et al., 2002), we have previously demonstrated recovery of upper-limb function in a randomized control trial with stroke patients (Thrasher et al., 2008). FEST was delivered along with conventional therapy in the intervention group, while the control group received 45 min of conventional therapy for 3–5 days per week for a total of 12–16 weeks (40 sessions in total). Compared to the control group, the stroke FEST group improved in terms of object manipulation, palmar grip torque, and pinch grip force (Thrasher et al., 2008). Another randomized trial with cervical incomplete SCI individuals tested short- and long-term efficacy of 60 min of FEST applied for 5 days per week for 8 weeks (40 sessions), over conventional occupational therapy for improving voluntary upper-limb function (Kapadia et al., 2011). Participants receiving FEST showed greater improvements in hand function at discharge, as well as at 6-month follow-up, compared to the control group (Kapadia et al., 2011). Therefore, FEST was shown as an effective treatment to improve voluntary upper-limb motor function in individuals with both acute and chronic neurological injuries. Despite the clinical evidence, little is known about cortical changes after FEST and whether it can be effective for treating motor dysfunction after TBI.

Repetition, temporal coincidence, and context-specific reinforcement during motor task performance can help induce experience-dependant cortical plasticity after TBI (Nudo, 2013). During FEST, task-specific and repeated training is delivered with the assistance of a therapist. Specifically, participants are first asked to attempt to perform a motor task, while the therapist provides reinforcement by triggering appropriate muscles using FES to assist completion of attempted tasks (Popovic et al., 2002). FEST can therefore deliver sensorimotor integration-based training which can help guide experience-dependant cortical plasticity after TBI. Nonetheless, reports on FEST after TBI are relatively few and far between. While some studies showed possible effectiveness of FES for motor recovery after TBI (Oostra et al., 1997; McCain and Shearin, 2017), conflicting results have also been shown in a randomized trial (de Sousa et al., 2016). Therefore, the objective of the current study was to investigate the efficacy of the FEST using protocols developed by our team (Thrasher et al., 2008; Kapadia et al., 2011) on improving upper-limb motor function and cortical re-organization in a clinical case study with an individual suffering from mild upper-limb motor impairment after chronic TBI. Specifically, the objectives were to understand cortical changes using neuroimaging and neurophysiological evaluations as well as to examine motor function changes during FEST. Based on our results in stroke (Thrasher et al., 2008) and incomplete SCI (Kapadia et al., 2011), we hypothesized that FEST would be effective to improve upper-limb motor function, which would be accompanied by cortical changes after the therapy.

MATERIALS AND METHODS

Clinical Presentation

The participant was a 39-year old male who suffered a diffuse TBI in the frontal lobe region resulting from a motor vehicle accident. The accident occurred 7 years prior to start of the study. At the onset of the study, the participant was diagnosed by his medical team with symptoms of mild motor impairment affecting the right upper- and lower-limbs and higher brain dysfunction, which were the results of the TBI (see **Supplementary Materials**: Participant history). The participant was enrolled in the study aiming to improve upper-limb function using FEST. The participant was informed about the study objectives and signed a written informed consent in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the local institutional research ethics committee at the University of Tokyo.

Functional Electrical Stimulation Therapy

Functional electrical stimulation was delivered using the Compex Motion system (Compex, Switzerland). Electrical stimulation was used to activate the muscles by applying a rectangular, biphasic, and asymmetric charge balanced stimulation pulses at a frequency of 40 Hz and 300 μs pulse width (Popovic et al., 2001, 2002). Electrical stimulation was applied on the

muscles using surface electrodes (5 × 5 cm square electrodes on larger muscles and 2 cm diameter circular electrodes on smaller muscles). During each FEST session, the therapist determined the stimulation levels for each muscle by gradually increasing the FES amplitude in 1 mA increments until they identified palpable contractions. The stimulation amplitude was then set to 150% of the amplitude that evoked palpable contractions, and adjusted if necessary, to produce smooth muscle contractions (for average amplitudes, see **Supplementary Materials**: FES).

The FEST training protocol is summarized in **Figure 1**. Training was delivered over the course of 3-months (12-weeks), with 3 sessions per week, each lasting 45–60 min (**Figure 1A**). Each FEST session consisted of three functional training protocols, consistent to previous FEST protocols (Thrasher et al., 2008 and Kapadia et al., 2011), which are illustrated in **Figure 1B** (see **Supplementary Materials**: FES). In each protocol, participant performed a specific functional task, including grasping a water bottle (palmar grasp), bringing an object to his mouth (hand-mouth), and pointing toward a target (pointing forward). For each trial, the participant was first asked to attempt to perform the task, while the therapist triggered a pre-programmed FES sequence to assist voluntary efforts.

Assessment Protocols

Timeline of assessments is summarized in **Figure 1A**. Assessments were carried out to evaluate cortical and corticospinal circuits associated with upper-limbs as well as upper-limb functional performance and clinical scores. Short-term cortical changes were assessed once per week over the course of 12-weeks of training immediately before and after each FEST session using transcranial magnetic stimulation (TMS). Long-term assessments were carried out every 6-weeks over the course of the 12-weeks of FEST and during the 12-weeks follow-up after the intervention was complete. Specifically, long-term changes were assessed before the training at baseline (Pre), after 6-weeks of the training (During), and immediately after 12-weeks of FEST (Post0), as well as 6-weeks after FEST was completed (Post1), and 12-weeks after FEST was completed (Post2). Long-term cortical changes and corticospinal excitability were evaluated using TMS and functional magnetic resonance imaging (fMRI), while functional performance was assessed using an instrumented drawing test and clinical scores.

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation sessions were carried out during both short-term and long-term assessments. During the assessments, participant remained seated comfortably on the chair with the right hand supported on the table. Electromyographic (EMG) activities were recorded using bipolar Ag/AgCl surface electrodes (Vitrode F-150S, Nihon Kohden, Tokyo, Japan) from the right (intervention) hand: (i) first dorsal interosseous (FDI) and (ii) abductor pollicis brevis (APB) muscles. A ground electrode was placed on the elbow of the right arm. It was ensured that the EMG electrodes were placed roughly on the same locations of the muscle between assessment days. EMG signals were band-pass filtered (15–1,000 Hz), amplified (1,000×; MEG-6108, Nihon Kohden, Tokyo, Japan) and sampled at 4,000 Hz using an analog-to-digital converter (Powerlab/16SP, AD Instruments, Castle Hill, Australia).

Using a mono-phasic magnetic stimulator (Magstim 200, Magstim Co., Whitland, United Kingdom) through a figure-of-eight coil, single-pulse TMS was delivered over the left primary motor cortex (M1) area that was optimal for inducing motor evoked potentials (MEPs) in the right FDI. The "hot spot" location was determined by detecting the point with the highest MEPs from the FDI (target) muscle and defined with respect to cranial landmarks as references during the baseline assessment (Pre). The same "hot spot" location was used to center the grid for all TMS map assessments (Pre, During, Post0, Post1, and Post2), while the exact location was confirmed on each day for single-location MEP assessments. The MEPs were always evoked with the participant keeping voluntary contraction at 10% maximal voluntary contraction (MVC) effort of the FDI muscle during the finger pinch task since there were no visible MEP responses at rest during baseline assessments (Pre). Contractions were maintained by holding a force sensor (OKLU-100K-S1-H18, Frontier Medic, Hokkaido, Japan) with his right thumb and index fingers, while the force level was shown on a visual display. The motor threshold (MT) for evoking MEPs was the minimum TMS intensity to elicit peak-to-peak amplitudes of at least 50 μV from the FDI muscle in five of ten consecutive trials (Groppa et al., 2012). It was ensured that the MEPs of the APB muscle could also be evoked and recorded simultaneously.

During short-term and long-term assessments, the input–output relationship between TMS stimulation intensity and MEP responses amplitude was obtained by applying TMS at 60, 70, 80, 90, and 100% of the TMS stimulator intensity. The exact "hot spot" location was confirmed on each assessment day with the starting point as the location defined during the baseline (Pre) assessment. Three trials were performed at each TMS intensity and the responses obtained for each muscle (FDI and APB) at each intensity (Ridding et al., 2001). Since MEPs were recorded during active contractions at 10% MVC, it was also possible to record the cortical silent period (CSP) of the MEPs from the same trials. Three CSP trials were also calculated from the responses evoked at 70% of the stimulator output (Farzan, 2014). Post processing evaluation revealed that it was not possible to elicit clear APB (non-target muscle) CSP response during the Pre assessment, resulting in removal of data from long-term assessment analysis. Moreover, APB response during the short-term assessment day 11 were unclear, also resulting in removal of CSP and input–output data for that assessment day.

During long-term assessments, MEP maps of corticospinal responses of each muscle were recorded by applying TMS at 70% of the stimulation output, which was determined to be the 120% MT stimulation intensity during the baseline (Pre) assessment and remained unchanged. During each assessment, the participant was asked to keep voluntary contractions at 10% of MVC of the FDI muscle. The MEP map was centered at the FDI "hot spot" location, which was defined with respect to cranial landmark during the baseline (Pre) assessment and remained unchanged. The MEP map was then expanded to the surrounding points on the 10 × 10 cm grid with a 1 cm resolution (100 cm^2 area) around the "hot spot" location using

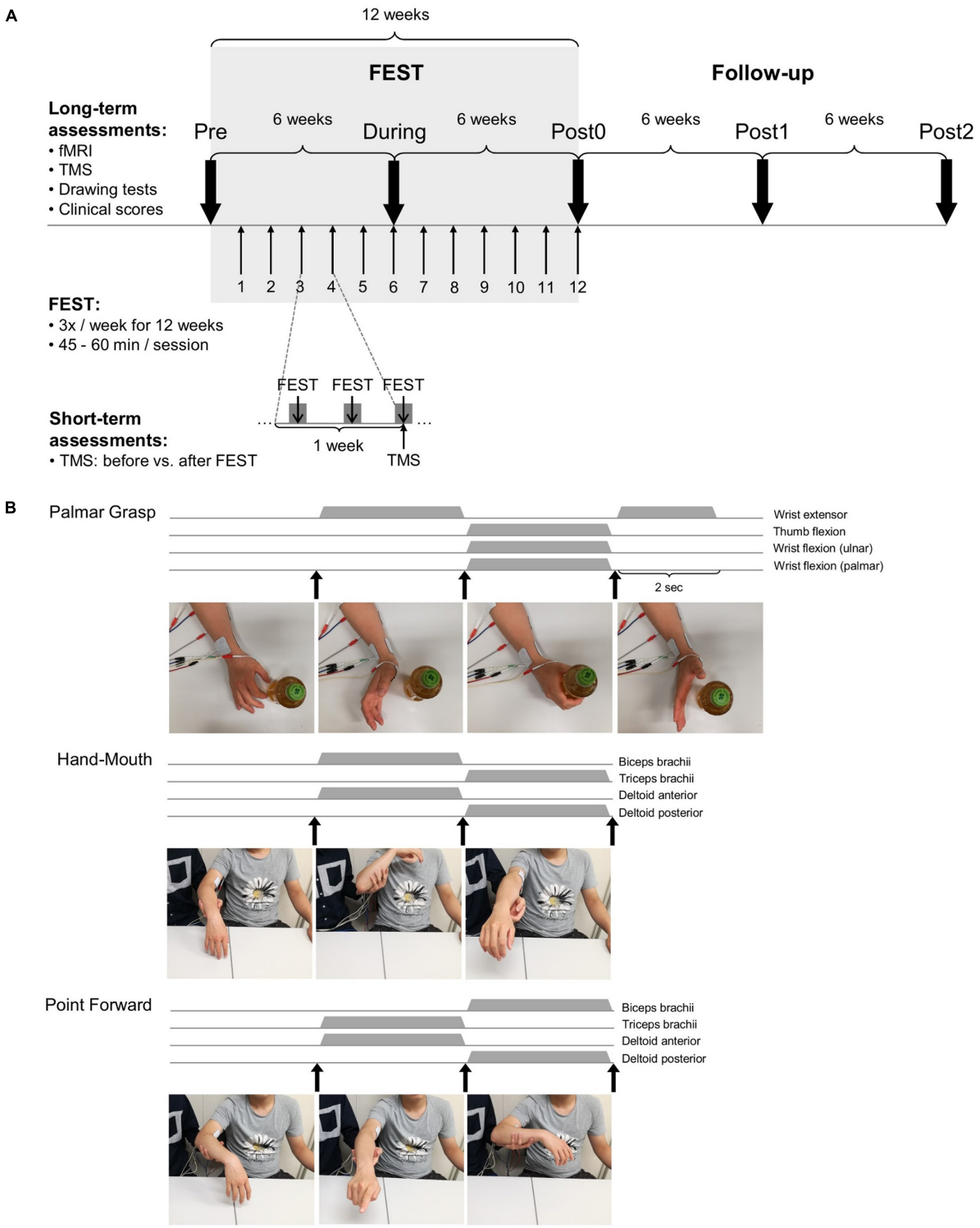

FIGURE 1 | Experimental setup. **(A)** Experimental protocol – functional electrical stimulation therapy (FEST) was delivered over the course of 12-weeks with three sessions per week and each session lasting 45–60 min. Long-term assessments were carried out at baseline (Pre), after 6- and 12-weeks of FEST (During and Post0), as well as during follow-up 6- and 12-weeks after FEST (Post1 and Post2) and they included: functional magnetic resonance imaging (fMRI), transcranial magnetic stimulation (TMS), drawing tests, and clinical test evaluations. Short-term assessments were carried out once per week over the course of 12-weeks to compare before and after each FEST session using TMS assessments. **(B)** Each FEST training session consisted of three functional training protocols including the palmar grasp – to generate hand opening, hand-mouth – to generate elbow and shoulder flexion, and point forward – to generate hand pointing forward, by activating a sequence of muscles activations.

pre-determined markings on a tight-fitting cap. Three stimuli were delivered at each location in a semi-randomized order at a rate of approximately every 6 s and averaged to obtain a peak-to-peak amplitude response for each location (Mortifee et al., 1994; Ridding et al., 2001).

Functional Magnetic Resonance Imaging

During fMRI sessions, which were carried out during long-term assessments, the participant remained in the supine position in an MRI scanner (MAGNETOM Prisma, Siemens, Germany) and was asked to perform: (i) hand grip and (ii) finger pinch force matching tasks with the right (intervention) hand, while holding a force sensor (OKLU-100K-S1-H18, Frontier Medic, Hokkaido, Japan). The force matching tasks was a trapezoidal pursuit consisting of four phases: rest, ascending, keep, and descending, each lasting 10 s. The target force level (keep phase) was set to 20% of the MVC effort (Ward et al., 2003), while the ascending and descending phase linearly increased and decreased to the target force over the course of 10 s. The participant could see the target force on the visual display, which they attempted to match during the experimental trials. A total of four force matching tasks were repeated within each session with a rest period of 20 s between tasks. One hand grip task session and one finger pinch task session were performed on each assessment day, which were conducted in separate scans. The MVC levels were determined prior to the experiment for the hand grip and finger pinch tasks. During fMRI assessments, the participant was asked to follow the target force trajectories as precisely as possible. All MRI images were acquired using a 3T MRI scanner (MAGNETOM Prisma, Siemens, Germany). Functional T2*-weighted echo-planar images that reflect blood oxygenation level-dependent (BOLD) responses (Ogawa et al., 1990) as well as high-resolution T1-weighted structural images were collected (see **Supplementary Materials**: fMRI data acquisition).

Drawing Tests

To evaluate upper-limb fine motor function, which was carried out during long-term assessments, the participant was asked to perform: (i) tracing and (ii) target tracking tasks of a sine wave (wavelength: 50 mm, amplitude: 25 mm, and distance: 150 mm) using an instrumented tablet system (TraceCoder® Version 1.0.8, Surface Pro4, SystemNetwork, Osaka, Japan) (Itotani et al., 2016). During the assessments, the participant was comfortably seated in a chair with his elbow on the table and flexed at 90°. During the tracing task, the participant was instructed to follow the outline of a sine wave at his preferred speed without a moving target, while during the target tracking task, the participant was instructed to follow the moving target on the tablet screen which moved on a sine wave at 12 mm/s. For both tasks, the participant was asked to draw as precisely as possible. Two trials, each consisting of three sine waves, were recorded for each of the tracing and tacking tasks. Before each assessment day, a practice period of approximately 1 min was allowed to prevent any learning effects and to allow the participant to assume a comfortable position for the assessments.

Clinical Assessments

Clinical scores, which were evaluated during long-term assessments, included functional independence measure (FIM; Granger and Hamilton, 1992), Fugl-Meyer assessment (FMA; Fugl-Meyer, 1980), and Motor Activity Log (MAL; van der Lee et al., 2004). All tests were performed by the same trained therapist.

Data Analysis

Motor Evoked Potentials

All MEP analysis was performed using a custom program written in MATLAB (The MathWorks Inc., United States). To evaluate the input–output curve relationship between the TMS stimulation intensity and the MEP responses for the FDI and APB muscles, MEP peak-to-peak amplitudes of each muscle for each of the three repeated trials at each stimulation intensity (60, 70, 80, 90, and 100% of the TMS stimulator output) were first calculated. The MEP amplitudes were plotted relative to the TMS stimulation intensity and a linear fit line was obtained using simple linear regression. The slope of the linear regression line was used to define the three repeated trial gain parameters of the input–output relationship curve (Farzan, 2014).

The CSP duration was defined for each muscle for three repeated trials as the time between the end of the MEP (i.e., where EMG activity was below 3SD of mean pre-stimulus activity) and the time at which the post-stimulus EMG returned to the pre-stimulus EMG activity (i.e., where EMG activity exceeded 3SD of the mean pre-stimulus activity) (Farzan, 2014).

Corticospinal representation MEP maps were calculated from the MEP peak-to-peak amplitudes of each point on the 100 cm^2 area (10 × 10 cm map with 1 cm resolution). The three repeated trials for each point were first averaged and normalized with the peak MEP amplitude on the map for each assessment day. The MEP map was then constructed from the average MEP amplitudes from each point on 10 × 10 cm grid using MATLAB's "gridfit" function to define 2,500 partitions within 100 cm^2 area (D'Errico, 2005). Finally, activated area on the 100 cm^2 map was calculated by taking the ratio of the number of partitions where the approximated MEP exceeded 50% of maximum MEP ($aMEP_{50\%}$) relative to all partitions (N_{total} = 2,500): $\text{area} = \frac{N\ (\text{aMEP}_{50\%})}{N_{\text{total}}} \times \text{area}_{\text{map}}$, where area_{map} is 100 cm^2 (Uy et al., 2002; van de Ruit et al., 2015; Tazoe and Perez, 2021).

Functional Magnetic Resonance Imaging

All fMRI data analysis was performed using Statistical Parametric Mapping (SPM12, Wellcome Trust Center for Neuroimaging, London, United Kingdom) software implemented in MATLAB (The MathWorks Inc., United States). First, data preprocessing procedures were applied (see **Supplementary Materials**: fMRI data processing). If the head motion remained over 2 mm, the scans would be considered for removal from subsequent analysis. However, the participant's head motion always remained within 2 mm during all scan, thus no trials were removed. After the preprocessing, the general linear model regression to the time course data was obtained to estimate the amount of neural activation (Friston et al., 1994, 1995). Whole brain analysis was

performed to depict the general features of brain activations during the hand grip and finger pinch tasks. First, the brain regions where the BOLD signals increased during the hand grip and finger pinch were depicted by evaluating the T-values obtained from each session to contrast a task specific voxel by voxel activation map. The threshold was set at voxel level $p < 0.001$ (uncorrected) and cluster level $p < 0.050$ family-wise error correction (FWE; Woo et al., 2014).

Next, the region of interest (ROI) was set in six anatomical hand areas defined bilaterally: primary motor cortex (M1: $x = \pm 37$, $y = -21$, and $z = 58$) (Mayka et al., 2006), sensory cortex (S1: $x = \pm 40$, $y = -24$, and $z = 50$) (Mayka et al., 2006), secondary somatosensory cortex (S2: $x = \pm 58$, $y = -27$, and $z = 30$) (Iftime-Nielsen et al., 2012), parietal rostroventral area (PR: $x = \pm 54$, $y = -13$, and $z = 19$) (Hinkley et al., 2007), supplementary motor area (SMA: $x = \pm 20$, $y = -8$, and $z = 64$) (Ciccarelli et al., 2006), premotor cortex (PM: $x = \pm 8$, $y = -6$, and $z = 64$) (Ciccarelli et al., 2006). These ROI regions were chosen based on the previous studies that investigated cortical effects of FES (Blickenstorfer et al., 2009; Gandolla et al., 2016) and implemented as 10 mm diameter spheres centered around each defined coordinate. In addition, the most activated voxel (peak voxel) in the contralateral M1 region was calculated to define the most active ROI location (Verstynen et al., 2005). A control region was defined as the hippocampus gyrus (HC: $x = -22$, $y = -34$, and $z = -8$ for contralateral and $x = 32$, $y = -30$, and $z = -8$ for ipsilateral) (Hayes et al., 2011), which was not associated with hand movements. Significant activation maps during both finger pinch and hand grip tasks for all assessment points were also computed to compare the ROI results (see **Supplementary Table 1**). The BOLD signal time-series data from all ROIs was extracted and calculated as the percent signal change for each force matching phase volume (ascending, keep, and descending) relative to the mean BOLD signal in the rest phase volume (Uehara et al., 2019). The task was repeated four times, resulting in 12 measurements for each assessment point.

Drawing Tests

Tracing and target tracking tasks were evaluated using the following parameters to assess performance: (i) sum of error – difference between the target coordinates of the sine wave and participant's pen in the x direction (medio-lateral), y direction (antero-posterior), and xy direction (sum of squared error); (ii) velocity – mean velocity during the tasks; (iii) acceleration – mean acceleration during the tasks; and (iv) pressure – mean pressure exerted during the tasks. The parameters were calculated for each full sine wave and the task was repeated two times, resulting in six measurements for the tracing and sine wave tracking tasks for each assessment point. All parameters were calculated using a custom program written in MATLAB (The MathWorks Inc., United States).

Clinical Assessments

Clinical scores for the FIM, FMA, and MAL tests were tabulated and evaluated by a trained occupational therapist and compared between different assessment points.

Statistics

Short-term TMS assessments were analyzed using paired samples t-test to compare the input–output curve slope and CSP between assessment points (before and after). Long-term TMS assessments were analyzed using the one-way repeated measures analysis of variance (ANOVA) to compare the input–output curve slope and CSP between assessment points (Pre, During, Post0, Post1, and Post2). Same statistical procedures were applied to compare long-term fMRI cortical activations during hand grip and finger pinch tasks in the peak activated voxel in M1 as well as in the contralateral and ipsilateral hemisphere in each ROI (M1, S1, S2, PR, SMA, PM, and HC), as well as drawing task error (x, y, and xy directions), velocity, acceleration, and pressure between assessment points. For long-term assessments, when significant results were found on the ANOVA, *post hoc* multiple comparisons with Holm adjustment to correct for comparison between assessment time points were conducted to compare Pre to other assessment points. Parametric tests were chosen since the Shapiro–Wilk test was used to confirm that most data were normally distributed. Short-term assessments were performed before and after each FEST session over the 12-weeks, while long-term assessments were performed on repeated trials on each assessment point. Statistical comparisons were performed using SPSS Statistics (IBM Corp., Armonk, NY, United States). Significance level for all tests was set to $p < 0.050$.

RESULTS

Short-Term Effects

Short-term TMS assessment comparisons are summarized in **Figures 2A,B**. Input–output curve showed no statistically significant differences between slopes of FDI ($t_{(11)} = -2.137$, $p = 0.056$) and APB ($t_{(10)} = 0.226$, $p = 0.830$) muscles after each FEST session, compared to before the session (**Figure 2A**). However, CSP showed statistically significant decrease in the silent period in both FDI ($t_{(11)} = 2.503$, $p = 0.029$) and APB ($t_{(10)} = 4.000$, $p = 0.002$) muscles after each FEST session, compared to before the session (**Figure 2B**).

Long-Term Effects

Transcranial Magnetic Stimulation

Long-term TMS assessment comparisons are summarized in **Figures 2C–E**. Input–output curve showed statistically significant differences between assessment points in both FDI ($F_{(4,8)} = 147.678$, $p < 0.001$) and APB ($F_{(4,8)} = 31.790$, $p < 0.001$) muscles. *Post hoc* comparisons (**Figure 2C**) showed that the slope increased significantly after 12-weeks of FEST (Post0) in the APB muscle and that it remained for at least another 12-weeks after the FEST intervention was completed (Post1 and Post2) in both FDI and APB muscles. CSP showed that there were no statistically significant differences between assessment points in both FDI ($F_{(4,8)} = 3.001$, $p = 0.086$) and APB ($F_{(3,6)} = 2.261$, $p = 0.182$) muscles (**Figure 2D**). Finally, descriptive comparisons of MEP maps suggest that the area in the motor cortex in both FDI and APB muscles increased after 12-weeks of FEST (Post0) and that it remained for at least another 12-weeks after the FEST

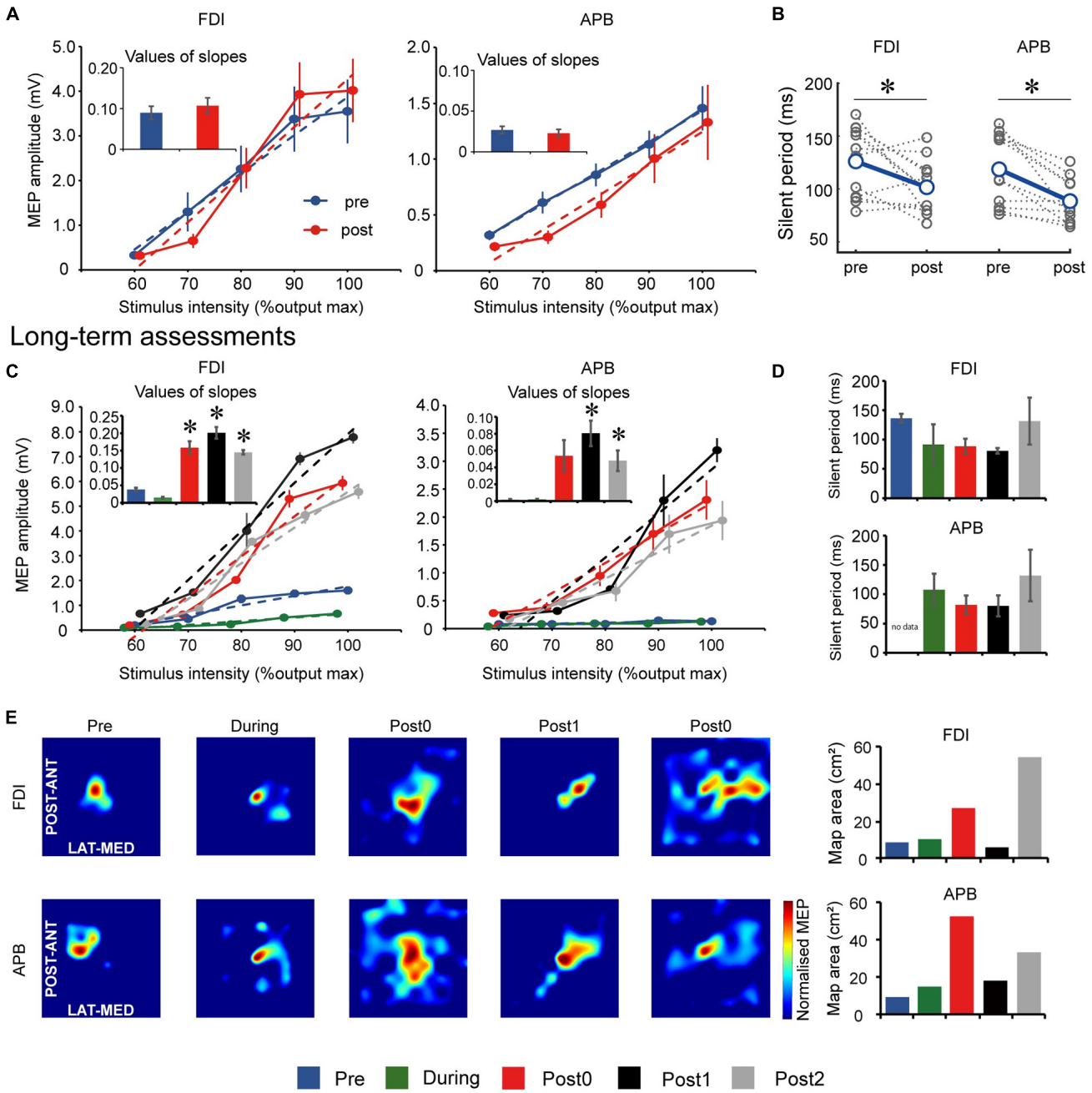

FIGURE 2 | Motor evoked potential (MEP) results for the short-term assessments. **(A)** Input–output relationship curve for the first dorsal interosseous (FDI) and abductor pollicis brevis (APB) muscles. Dotted lines indicate simple linear regression lines of the curves before and after one FEST session. Bar graphs indicate values of regression line slope and standard error. **(B)** Cortical silent period (CSP) for the FDI and APB muscles before and after one FEST session. Gray dotted lines indicate data of each day. MEP results for the long-term assessments. **(C)** Input–output relationship curve for the FDI and APB muscles. Dotted lines indicate simple linear regression lines of the curves at baseline (Pre), after 6- and 12-weeks of FEST (During and Post0) as we as during follow-up assessments 6- and 12-weeks after FEST (Post1 and Post2). Bar graphs indicate values of regression line slope and standard error. **(D)** CSP for the FDI and APB muscles during Pre, During, Post0, Post1, and Post2 assessments. Bar graphs indicate values of regression line slope and standard error. **(E)** MEP maps before and after FEST for the FDI and APB muscles. The size of the MEP activated is approximated by the heatmap color scale, which denotes amplitudes normalized to the maximum value in assessment. Bar graphs indicate the calculated area of the MEP map. *$p < 0.050$.

intervention was completed (Post1 and Post2) in both FDI and APB muscles (**Figure 2E**).

Functional Magnetic Resonance Imaging

Long-term assessment fMRI activations of the whole brain during the hand grip task are summarized in **Figure 3A**. Peak activated voxel in M1 showed statistically significant differences between assessment points for the hand grip task ($F_{(4,44)} = 5.814$, $p = 0.001$). *Post hoc* comparisons (**Figure 3A**) showed that activation significantly increased after 12-weeks of FEST (Post0) but returned to baseline after the FEST intervention was completed (Post1 and Post2). ROI analysis for the hand grip task is summarized in **Figure 3B**. Contralateral hemisphere comparisons showed that activations in M1 ($F_{(4,44)} = 6.070$, $p = 0.001$), PR ($F_{(4,44)} = 7.113$, $p < 0.001$), SMA ($F_{(4,44)} = 7.064$, $p < 0.001$), and PM ($F_{(4,44)} = 144.163$, $p < 0.001$) had statistically significant differences, while S1 ($F_{(4,44)} = 3.781$, $p = 0.010$; note: no statistically significant *post hoc* comparisons were shown), S2 ($F_{(4,44)} = 2.485$, $p = 0.057$), and HC ($F_{(4,44)} = 0.256$, $p = 0.905$) had no significant differences between assessment points. *Post hoc* comparisons (**Figure 3B**, top) indicate that contralateral motor related areas (M1, PR, SMA, and PM) primarily increased activations after 12-weeks of FEST (Post0) during the hand grip task. Ipsilateral hemisphere comparisons showed that activations in M1 ($F_{(4,44)} = 6.538$, $p = 0.001$) and S1 ($F_{(4,44)} = 3.925$, $p = 0.008$) had small statistically significant differences, while S2 ($F_{(4,44)} = 0.835$, $p = 0.510$), PR ($F_{(4,44)} = 0.224$, $p = 0.925$), SMA ($F_{(4,44)} = 1.275$, $p = 0.294$), PM ($F_{(4,44)} = 1.029$, $p = 0.403$), and HC ($F_{(4,44)} = 0.545$, $p = 0.704$) had no significant differences between assessment points. *Post hoc* comparisons (**Figure 3B**, bottom) indicate little or not ipsilateral activations during the hand grip task.

Long-term assessment fMRI activations of the whole brain during the finger pinch task are summarized in **Figure 3C**. Peak activated voxel in M1 showed statistically significant differences between assessment points for the finger pinch task ($F_{(4,44)} = 13.319$, $p < 0.001$). *Post hoc* comparisons (**Figure 3C**) showed that activation significantly increased after 6 and 12-weeks of FEST (During and Post0) as well as in the 6-week and 12-week follow-up period (Post 1 and Post2). ROI analysis for the finger pinch task is summarized in **Figure 3D**. Contralateral hemisphere comparisons showed that activations in M1 ($F_{(4,44)} = 21.505$, $p < 0.001$), S1 ($F_{(4,44)} = 10.306$, $p < 0.001$), S2 ($F_{(4,44)} = 19.246$, $p < 0.001$), PR ($F_{(4,44)} = 4.471$, $p = 0.004$), SMA ($F_{(4,44)} = 29.309$, $p < 0.001$), PM ($F_{(4,44)} = 24.644$, $p < 0.001$), as well as HC ($F_{(4,44)} = 3.308$, $p = 0.019$) all had statistically significant differences between assessment points. *Post hoc* comparisons (**Figure 3D**, top) indicate contralateral motor cortex activations (M1) increased after 12-weeks of FEST (Post0) as well as widespread changes in all other areas after 6-weeks of FEST (During) which persisted in follow-up (Post1 and Post2) during the finger pinch task. Ipsilateral hemisphere comparisons showed that activations in M1 ($F_{(4,44)} = 9.227$, $p < 0.001$), S1 ($F_{(4,44)} = 3.925$, $p = 0.008$), S2 ($F_{(4,44)} = 17.585$, $p < 0.001$), PR ($F_{(4,44)} = 11.634$, $p < 0.001$), SMA ($F_{(4,44)} = 11.516$, $p < 0.001$), PM ($F_{(4,44)} = 11.587$, $p < 0.001$), as well as HC ($F_{(4,44)} = 9.004$, $p < 0.001$) all had statistically significant differences between assessment points. *Post hoc* comparisons (**Figure 3D**, bottom) indicate widespread ipsilateral changes in all areas after 6-weeks of FEST (During) which persisted in follow-up (Post1 and Post2) during the finger pinch task.

Drawing Tests

Long-term assessment drawing test comparisons are summarized in **Figure 4**. Tracing task comparisons showed that velocity ($F_{(4,20)} = 5.219$, $p = 0.005$), acceleration ($F_{(4,20)} = 4.333$, $p = 0.011$), and pressure ($F_{(4,20)} = 10.361$, $p < 0.001$) had statistically significant differences, while sum of x errors ($F_{(4,20)} = 1.710$, $p = 0.187$), sum of y errors ($F_{(4,20)} = 2.432$, $p = 0.081$), and sum of xy errors ($F_{(4,20)} = 1.885$, $p = 0.152$) had no significant differences between assessment points. *Post hoc* comparisons (**Figure 4C**, top) indicate decreased velocity and acceleration after 12-weeks of FEST (Post0) which persisted in follow-up (Post1 and Post2) during the tracing task (note: pressure also seemed to decrease in all time points except Post2), as well as a similar trend in error reduction, although not statistically significant.

Target tracking task comparisons showed that sum of x errors ($F_{(4,20)} = 3.887$, $p = 0.017$), sum of xy errors ($F_{(4,20)} = 4.570$, $p = 0.009$), and pressure ($F_{(4,20)} = 5.727$, $p < 0.001$) had statistically significant differences, while sum of y errors ($F_{(4,20)} = 2.290$, $p = 0.095$), velocity ($F_{(4,20)} = 1.232$, $p = 0.329$), and acceleration ($F_{(4,20)} = 2.106$, $p = 0.118$) had no significant differences between assessment points. *Post hoc* comparisons (**Figure 4C**, bottom) indicate decreased error predominantly in the medio-lateral x-direction (note: pressure also seemed to decrease in all time points except Post2).

Clinical Assessments

Long-term clinical score results are summarized in **Table 1**. The FIM and FMA scores were not different after 6-weeks (During) and 12-weeks (Post0) of FEST, as well as during the follow-up assessments at 6-weeks (Post1) and 12-weeks (Post2) after the FEST intervention was completed, compared to baseline (Pre). However, the MAL score increased by 1 point after 6-weeks of FEST (During) and remained after 12-weeks of FEST (Post0) and for at least another 12-weeks after the FEST intervention was completed (Post 1 and Post 2) (**Table 1**).

DISCUSSION

Evidence of Cortical Re-organization After FEST

Our results showed the time course of cortical re-organization elicited by a FEST intervention in an individual with chronic TBI. Specifically, short-term assessment results showed reduced CSP (**Figure 2B**). CSP refers to an interruption of voluntary muscle activity by TMS applied over the contralateral motor cortex (Wolters et al., 2008; Farzan, 2014). It is generally agreed that spinal inhibitory mechanisms contribute to the silent period up to its first 50 ms, while the later part is generated exclusively by inhibition within the motor cortex (Wolters et al., 2008). It

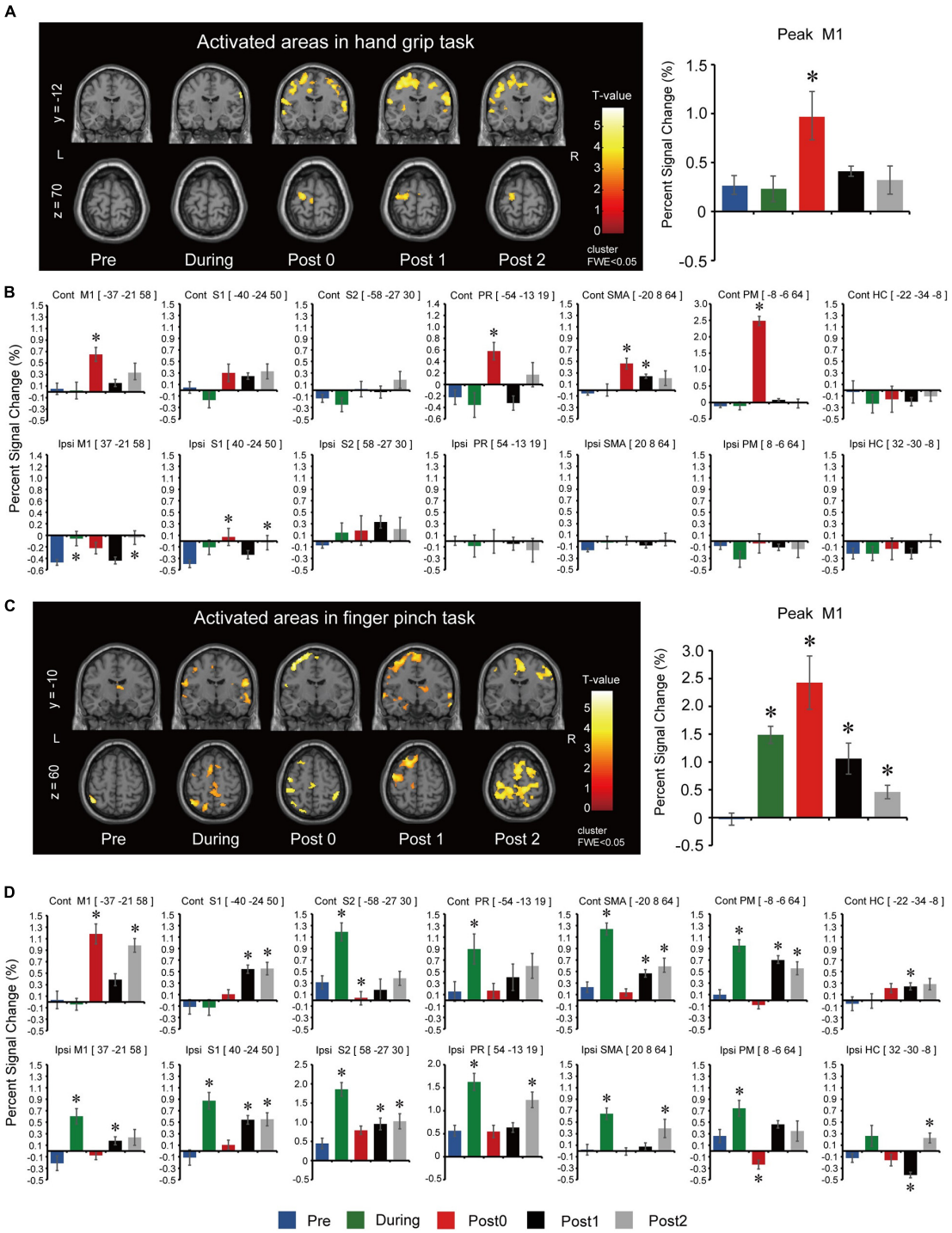

FIGURE 3 | Functional magnetic resonance imaging results for the long-term assessments during the hand grip task. **(A)** Activated regions in the left (L) and right (R) hemisphere during right (intervention) hand grip task. To observe the whole brain activity, the coordinates of $y = -12$ and $z = 70$ planes were used. *T*-values are plotted, and the threshold was set at voxel level $p < 0.001$ (uncorrected) and cluster level $p < 0.050$ [family-wise error correction (FWE)]. Assessments were carried out at baseline (Pre), after 6- and 12-weeks of FEST (During and Post0), as well as during follow-up assessments 6- and 12-weeks after FEST (Post1 and Post2). Region of interest (ROI) results of the most activated voxel in the primary motor cortex (M1) for each assessment are shown next to the activated regions. **(B)** ROI results based on anatomical regions in the M1 as well as the sensory cortex (S1), secondary somatosensory cortex (S2), parietal rostroventral area (PR), supplementary motor area (SMA), premotor cortex (PM), and the hippocampus gyrus (HC). The upper bar graphs show activity of the contralateral hemisphere (Contra) and the lower bar graphs shows activity of the ipsilateral hemisphere (Ipsi). fMRI during the finger pinch task.

(Continued)

FIGURE 3 | Continued
(C) Activated regions during right (intervention) finger pinch task. To observe the whole brain activity, the coordinates of $y = -10$ and $z = 60$ planes were used. T-values are plotted and the threshold was set at voxel level $p < 0.001$ (uncorrected) and cluster level $p < 0.05$ (FWE). Assessments were carried out at Pre, During, Post0, as well as Post1 and Post2. ROI results of the most activated voxel in the primary motor cortex (M1) for each assessment were shown next to the activated regions. **(D)** ROI results based on anatomical regions in the M1 as well as S1, S2, PR, SMA, PM, and HC. The upper bar graphs show activity of the contralateral hemisphere (Contra) and the lower bar graphs shows activity of the ipsilateral hemisphere (Ipsi).

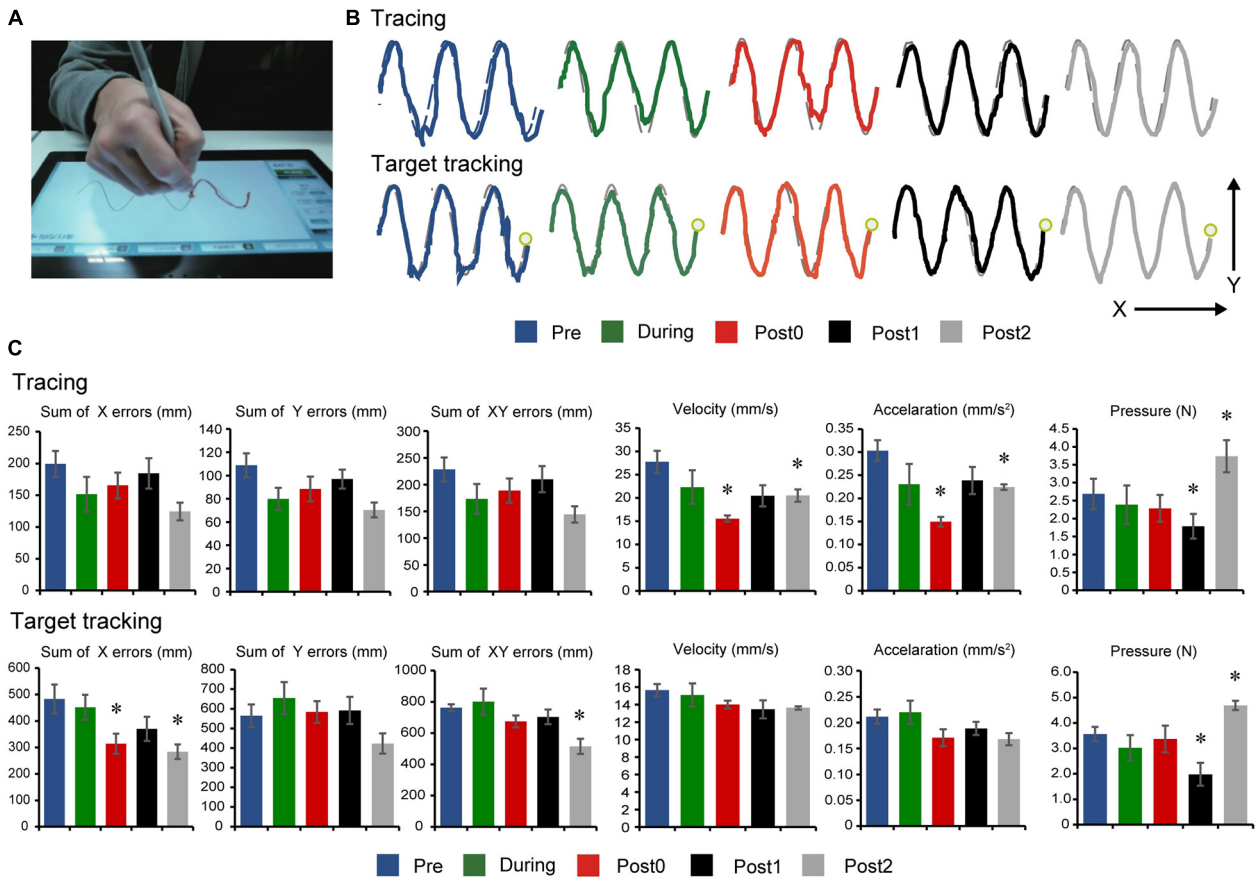

FIGURE 4 | Drawing test results. **(A)** Experimental setup showing the instrumented tablet with the participant. **(B)** Representations of the participant's performances on the drawing tests at baseline (Pre), after 6- and 12-weeks of FEST (During and Post0), as we as during follow-up assessments 6- and 12-weeks after FEST (Post1 and Post2) are shown. Tracing performance is shown in the upper graphs, when the participant was required to follow the outline of a sine wave at a self-selected speed. Target tracking performance is shown in the lower traces, when the participant was required to follow a moving target on the screen-. **(C)** The sum of error (x, y, and xy directions), velocity, acceleration, and pressure performance during the tracing task are shown in the upper graphs and the target tracking task in the lower graphs.

TABLE 1 | Clinical measurements scores, including the functional independence measure (FIM) self-care, Fugl-Meyer assessment (FMA) of the upper-limb (U/L) function, and Motor Activity Log (MAL), amount of use score (AS) and how well score (HW).

	Pre	During	Post0	Post1	Post2
FIM self-care (max score: 42)	42	42	42	42	42
FMA U/L (max score: 66)	63	63	63	63	63
MAL AS and HW (max score: 150/150)	78/92	79/92	79/92	79/92	79/92

has previously been shown that FES can inhibit spinal reflex excitability (Kawashima et al., 2013). Moreover, consistent to our results, electrical stimulation of cutaneous nerves in the upper-limbs was also shown to shorten the CSP (Hess et al., 1999; Classen et al., 2000), which suggests involvement of cortical-level sensorimotor integration (Wolters et al., 2008). Cutaneous and afferent feedback from FEST may activate the somatosensory cortex, which may affect cortico-cortical connections (Carson and Buick, 2019). It has previously been demonstrated that somatosensory cortices are activated during electrical stimulation

of muscles (Korvenoja et al., 1999; Boakye et al., 2000; Nihashi et al., 2005). In fact, our fMRI results also showed an increase in signal intensity not only in M1 but also in S1 and S2 during long-term assessments after FEST, which supports these considerations (**Figures 3B,D**). Therefore, short-term effects of FEST are likely related to sensorimotor integration through intracortical inhibition or possibly spinal reflex inhibition after each FEST session.

Our long-term assessment results indicate that the slope of MEP input–output curve was not facilitated after 6-weeks of FEST, while there was significant facilitation after 12-weeks, which remained even during follow-up (**Figure 2C**). The slope of the MEP input–output curve reflects the strength of corticospinal projections to the target muscles (Farzan, 2014) and can become less steep with $GABA_A$ (inhibitory) receptor agonist (lorazepam), while administration of an indirect dopaminergic-adrenergic (excitatory) agonist (D-amphetamine) increased the slope (Boroojerdi et al., 2001). Taken together, our results indicate considerable long-term facilitation of corticospinal excitability after 12-weeks of FEST which may persist for another 12-weeks even in the absence of any intervention in an individual with TBI, possibly via upregulation of dopaminergic excitatory receptors and/or downregulation of GABAergic inhibitory receptors.

Increased corticospinal excitability can likely be explained by larger area over which MEPs can be obtained in the hand muscles, which were shown in our study. Specifically, MEP map results indicate enlarged hand muscle representations within the M1 after 12-weeks of FEST and during follow-up (**Figure 2E**). Motor maps obtained using TMS-evoked MEPs are reliable for extracting useful somatotopic information from the primary motor cortex (Wassermann et al., 1992; Wilson et al., 1993). It was previously shown that 2-h of electrical nerve stimulation can produce larger areas over which MEPs can be evoked (Ridding et al., 2001). We confirmed considerable expansion of the motor areas which are consistent with the time-course of changes of MEP facilitation evoked over a single "hot spot" location during long-term follow-ups. While motor evoked responses could reflect cortical and/or spinal excitability, increased motor map area and subsequent MEP amplitude facilitation (Ridding and Rothwell, 1997) confirm cortical re-organization after FEST in an individual with chronic TBI in our study.

Cortical re-organization was further corroborated by our fMRI data, which showed larger BOLD responses after 12-weeks of FEST compared to baseline assessments during both hand grip and finger pinch tasks (**Figures 3A,C**). Peak signal intensity within the M1 during the hand grip task was significantly increased after 12-weeks of FEST, while it returned to baseline during follow-up (**Figure 3A**). On the other hand, during the finger pinch task, the peak M1 signal was significantly increased after 6 and 12-weeks of FEST as well as during follow-up assessments, while a gradual reduction of signal compared to after the intervention was observed when FEST was completed (**Figure 3C**). Changes in M1 can also be confirmed using significant activation maps (see **Supplementary Table 1**). Moreover, the time course of cortical changes obtained using fMRI in the contralateral M1 ROI (**Figures 3B,D**) is consistent to the MEP results obtained using TMS. Analysis of other ROI voxels indicates widespread changes not only in the M1, but also in the PR, SMA, and PM area during both hand grip and finger pinch tasks. Since the participant in our study had difficulty performing fine motor tasks, widespread activations during the finger pinch task may have been affected by the task difficulty (trapezoidal pursuit at the 20% MVC target level), which may have caused hyperactivity in various cortical regions. Widespread activations can be confirmed from significant activation maps in both motor and non-motor areas (see **Supplementary Table 1**). Moreover, during the finger pinch task, which required fine motor skills that were most notably impaired in our participant, the primary (S1) and secondary somatosensory cortex (S2) changes were also shown, as well as overall earlier activations (i.e., 6-weeks after FEST) and more widespread changes in both contralateral and ipsilateral hemispheres which included the control region (HC) that was not expected to change. Evidence from various neuroimaging studies has previously shown that somatosensory cortices, including both S1 and S2 areas, are activated during electrical stimulation of muscles (Korvenoja et al., 1999; Boakye et al., 2000; Nihashi et al., 2005). When FES is applied at MT intensity to generate flexion and extension wrist movements, cortical activations in the contralateral M1, S1, and PM areas, as well as bilateral S2 and SMA activation were shown to be activated (Blickenstorfer et al., 2009). During FEST, the participant was asked to attempt each movement before the therapist applied FES to activate the appropriate muscles. Long-term repeated sensorimotor integration facilitated by FES during task-specific upper-limb training that includes voluntary engagement may therefore elicit cortical re-organization. Specifically, integration of motor commands during voluntary movement attempt and sensorimotor network activation through FES are the candidate mechanisms of long-term cortical changes after FEST. Intact motor areas topologically adjacent to the damaged site within the M1 and areas outside of motor cortex may assume control over the affected muscles via intracortical connectivity networks after task-specific repetitive training by Hebbian synaptic strengthening (Weiller et al., 1992; Seitz et al., 2005; Nudo, 2013). Our findings therefore indicate that widespread cortical re-organization caused by FEST can elicit neuroplasticity after chronic TBI in cortical areas related to fine motor function.

Carry-Over Effects After FEST

Consistent to our results that demonstrated carry-over effects during follow-up assessments (**Figures 2**, **3**), other evidence also points that sustained cortical changes can outlast the intervention period. Therapeutic applications of FES delivered over longer periods indicated long-term cortical re-organization after the intervention (Shin et al., 2008; Sasaki et al., 2012). Specifically, 30 min of FES-assisted finger flexion and extension applied once per day for a total of 12-weeks was shown to elicit cortical changes in the somatosensory cortex after the intervention, which were correlated to the improvements in the motor function in chronic hemiplegia patients (Sasaki et al., 2012). Similarly, 60 min of FES wrist extension applied 5 days per week for a total of 10-weeks resulted in shifting of the somatosensory area activations from ipsilateral to the contralateral hemisphere after the intervention,

which was related to significant improvements in the motor function in chronic stroke patients (Shin et al., 2008). Taken together, our results suggest that approximately 40-h of task-specific and repetitive FEST are required to induce cortical re-organization associated with the upper-limb control (Shin et al., 2008; Sasaki et al., 2012), while only some changes were observed with less training after 6-weeks of FEST (**Figures 3C,D**). Importantly, our current study also demonstrated that long-term cortical re-organization could persist for several months (i.e., for as long as 12-weeks) after FEST, which is consistent with clinical recovery profiles shown by our group (Kapadia et al., 2011). Considering that the individual in our current study was in the chronic stage (>7 years) after the injury, spontaneous recovery can be ruled out. Evidence therefore suggests that cortical re-organization after TBI can be elicited using FEST and that carry-over effects may outlast the intervention period. However, it must be noted that clinical scores were not affected in our current study as our participant had a relatively low level of impairment, which led to ceiling effects in clinical evaluations. Future studies should therefore confirm the link between cortical re-organization and clinical improvements.

Fine Motor Function Improvements After FEST

Clinical scores suggest that the individual in our study had a relatively high level of motor function at the onset of the FEST intervention. Specifically, our participant had a FIM score of 42/42 (**Table 1**), which indicates complete independence in activities of daily living, including motor scores, communication, and social cognition (Granger and Hamilton, 1992). Similarly, the upper-limb portion of the FMA was 63/66 (**Table 1**), indicating high level of upper-limb function. As expected, neither the FIM nor the FMA scores were changes after FEST. While the MAL score increase from 78 to 79/92 after 6-weeks of FEST (**Table 1**) may indicate minimal clinically important improvements (Simpson and Eng, 2013), no major changes in gross motor function were shown due to ceiling effects.

However, drawing test results, which may be more sensitive to assess fine motor function, were affected after FEST (**Figure 4C**). Specifically, the tracing task, which required following the outline of a sine wave at a self-selected speed, showed significantly decreased mean velocity and acceleration after 12-weeks of FEST and during follow-up, which may suggest less abrupt and smoother movements during the target tracing task (**Figure 4C**, top). Decreased velocity may imply better performance because of a trade-off between speed and accuracy (Fitts, 1992), which was also reported during handwriting tasks on an instrumented tablet (Dui et al., 2020). Specifically, after the intervention, the participant was able to better control his fine motor performance and tremor, which resulted in ability to follow the target more accurately by decreasing the speed. While the error seemed to decrease during both tasks, significant reduction during the target tracking task, which required following a moving target on the screen, was shown after 12-weeks of FEST and during follow-up, indicating improved fine motor function performance (**Figure 4C**, bottom). It has been suggested that cortical changes resulting from FES interventions or other rehabilitation programs are not always correlated to improvements in motors function (Quandt and Hummel, 2014), or that motor function can event initially deteriorate (Murata et al., 2008). Nonetheless, our results showed changes on the drawing tests after FEST. Improved tracing task performance was shown after 4-weeks of upper-limb FEST in a clinical randomized trial in individuals with hemiplegia (Popovic et al., 2003). More intense FEST protocols also improved drawing performance and were associated with reduced spasticity after stroke (Kawashima et al., 2013). Similarly, improvements in drawing accuracy were also reported in individuals with chronic stroke after 10-weeks of FES upper-limb therapy, consistent to increased cortical activations, while the control group which did not display altered cortical activations also did not improve on the drawing test (Shin et al., 2008). Electrical stimulation may therefore elicit cortical re-organization, which can ultimately serve as a basis for improved functional capacity (Traversa et al., 1997; Fraser et al., 2002; Carson and Buick, 2019). Our current study utilized the FEST protocols developed by our group, which were shown in randomized clinical trials to improve gross motor function after neurological injuries (Thrasher et al., 2008; Kapadia et al., 2011). Using these protocols, we demonstrated considerable cortical re-organization after FEST in an individual with chronic TBI, which may be related to fine motor function although further work is warranted to fully prove this.

Limitations

The main limitation of this study is the small sample size and lack of a control group to examine benefits of equivalent conventional therapy. Moreover, the individual in our study had limited motor impairment, which also limits generalizability of our results. Our team has previously demonstrated in randomized controlled clinical trials that upper-limb FEST intervention is superior for improving hand motor function compared to conventional therapy after stroke and incomplete SCI in individuals with more severe impairments (Thrasher et al., 2008; Kapadia et al., 2011). Therefore, superiority of FEST has previously been shown in larger studies, while cortical mechanism remained unclear. Our study utilized detailed assessments with an individual suffering mild upper-limb motor impairment after chronic TBI to understand mechanisms of recovery and time course of cortical changes after FEST. While case study results may be prone to some aberration, interpretations should be drawn based on multiple assessment variables as well as together with other literature. For instance, a limitation of our study is that we did not use a navigation system to track the TMS coil location between assessments. However, the cortical re-organization implications based on TMS assessments are corroborated by fMRI data, providing more confidence in these findings. Moreover, as recently pointed out, case study observations utilizing detailed aspects of intervention can serve as a basis for future studies targeting larger populations (Bloem et al., 2020). Therefore, our current study results should be used to develop specific hypotheses for the future studies related to cortical mechanisms

of motor improvement using FEST after TBI. Specifically, future studies with a larger cohort of patients should quantify other regions and clusters based on anatomical ROIs and adapted using independent functional localizer tasks to test hypotheses from results obtained herein.

CONCLUSION

Our clinical case study results showed that an upper-limb FEST intervention can be effective for eliciting cortical re-organization of an individual suffering from mild motor impairment resulting from chronic TBI. Our study showed that motor changes were related to cortical re-organization, consistent to previously shown clinical carry-over effects (Kapadia et al., 2011). Specifically, we showed that 12-weeks of FEST, which included 36 sessions lasting 45–60 min of task-specific and repetitive FES-assisted reaching and grasping, can elicit long-term facilitation of corticospinal excitability, likely due to larger motor map representations in and around the primary motor cortex. Increased activations after FEST were also shown in the somatosensory areas, as well as other areas related to voluntary motor control and sensorimotor integration, suggesting widespread cortical re-organization. Assessments also suggested that cortical changes may persist after the intervention. The mechanism of long-term FEST elicited cortical re-organization likely involve integration of voluntary motor commands and sensorimotor network engagement through FES. Overall, our study showed evidence that FEST can be applied in chronic stage TBI to elicit cortical re-organization.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the data that support the findings of this study are available from the corresponding author upon reasonable request. Requests to access the datasets should be directed to corresponding author.

ETHICS STATEMENT

This study protocol was approved by the local institutional research ethics committee at the Graduate School of Arts and Sciences at the University of Tokyo (No. 558). The participant provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MM conceived the study, interpreted the data, and wrote the manuscript. TNa conducted the interventions, collected and analyzed the data, and contributed to writing the manuscript. AS and AY conducted the intervention and collected and analyzed the data. KN, MRP, and TNo supervised the study and interpreted the data. All authors contributed to the article and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2021.693861/full#supplementary-material

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REVIEW

Peripherally Induced Reconditioning of the Central Nervous System: A Proposed Mechanistic Theory for Sustained Relief of Chronic Pain with Percutaneous Peripheral Nerve Stimulation

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Abstract: Peripheral nerve stimulation (PNS) is an effective tool for the treatment of chronic pain, although its efficacy and utilization have previously been significantly limited by technology. In recent years, purpose-built percutaneous PNS devices have been developed to overcome the limitations of conventional permanently implanted neurostimulation devices. Recent clinical evidence suggests clinically significant and sustained reductions in pain can persist well beyond the PNS treatment period, outcomes that have not previously been observed with conventional permanently implanted neurostimulation devices. This narrative review summarizes mechanistic processes that contribute to chronic pain, and the potential mechanisms by which selective large diameter afferent fiber activation may reverse these changes to induce a prolonged reduction in pain. The interplay of these mechanisms, supported by data in chronic pain states that have been effectively treated with percutaneous PNS, will also be discussed in support of a new theory of pain management in neuromodulation: Peripherally Induced Reconditioning of the Central Nervous System (CNS).

Keywords: chronic pain, neuromodulation, peripheral nerve stimulation, cortical plasticity, peripherally induced reconditioning, mechanism of action

Introduction

Modern understanding of the relationship between electrical stimulation and pain dates back to 1965 with Melzack and Wall's seminal paper outlining their theory of the "gate control" system of pain.[1] It proposed that there is a gating mechanism in the spinal cord that relies on the relative firing of small (nociceptive) and large (sensory) diameter neurons. Increased firing of the large diameter neurons would "close" the gate, reducing transmission of painful stimuli to the brain, while firing of small diameter neurons would "open" it. Although the first therapeutic application of this theory involved stimulation of peripheral nerves following neurosurgical lead implantation,[2] the field was quickly dominated by widespread adoption of implanted leads delivering dorsal column or spinal cord stimulation (SCS) for the treatment of chronic pain.

SCS has been the leading force in the neuromodulation market for the last 50 years, with many advances in device technology during that time.[3–7] Due to the market dominance of SCS, the electrode technology available for researchers in

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PNS has historically been limited to adaptation of SCS devices for use in the periphery.[8–10] Nonetheless, continued research over the decades has shown that PNS can be successfully delivered by conventional (ie, permanently implanted) PNS systems with a variety of nerve targets, lead designs, waveforms, frequencies, and stimulation paradigms to treat a wide range of chronic pain conditions, such as intractable neuropathic pain, post-traumatic nerve pain, causalgia, chronic axial back pain, post-operative pain, joint pain, postherpetic neuralgia, chronic migraine, and orofacial pain (available PNS systems are not currently approved for use in the craniofacial region).[11–32]

Despite this efficacy, PNS has historically been conceptualized as a treatment of last resort.[33] One major limitation has been the lack of systems specifically designed for use in the periphery. Physicians often used devices designed for SCS, including percutaneous cylindrical leads or surgically placed paddle-type leads, both placed immediately adjacent to or in contact with the targeted nerve.[8,9,13–17,19,34,35] However, the periphery induces greater mechanical stresses on the lead than those experienced in the epidural space,[10] historically resulting in frequent lead migration (9–25% of PNS cases[36]), and limiting placement to locations that did not require the tunneled leads to cross joints with high degrees of flexion or extension that could cause stress-related lead migration or fracture.[37]

Recent years have seen the advancement of various PNS features and techniques intended to enable the development and adoption of improved neurostimulation systems designed specifically for use in the periphery, including: 1) The development of methods for the minimally invasive, percutaneous implantation of conventional PNS leads,[35] eliminating the need for invasive techniques to expose the nerve; 2) Advancements in and growing prevalence of ultrasound imaging to guide lead placement,[17,23] enabling visualization and targeting of an increasing number of peripheral nerves;[38] 3) Increases in the number and quality of interventionally trained pain physicians, especially with regard to ultrasound guided procedures; 4) Improvement in reimbursement for PNS; 5) Renewed focus on the development and implementation of non-opioid treatment alternatives for acute and chronic pain; 6) Improvement in long-term efficacy when a percutaneously implanted lead is employed without an implanted pulse generator or receiver;[39,40] 7) The incorporation of open coil leads with axial flexibility designed to enable tissue ingrowth within the coils to secure the electrode in place with lower rates of infection, as seen throughout a long history of use in electrical stimulation applications.[41–52]

Recently, based on these advancements, percutaneous PNS with temporary (eg, up to 60 days) treatment through open coil leads has been used to treat a wide variety of pain conditions via two different implementations. The first method (Figure 1A) has demonstrated effectiveness in acute and chronic pain conditions such as neuropathic and non-neuropathic pain following amputation,[39,53–56] post-surgical pain following total knee arthroplasty,[57,58] and ambulatory foot, knee, and rotator cuff surgeries.[59–61] This method targets mixed or sensory nerve(s) innervating the painful region with the goal of activating large diameter primary afferent sensory fibers at frequencies (eg, ~100 Hz) that induce comfortable sensations in the region of pain. In the second method (Figure 1B), efferent fibers are targeted at a lower frequency (eg, ~12 Hz) and an intensity that induces comfortable contractions in muscle(s) in the region of pain innervated by the targeted nerve, as demonstrated for chronic musculoskeletal pain including chronic shoulder pain,[32,62–66] and axial low back pain.[40,67,68] Recent studies using these two implementations reported that 77% (75/98) of subjects experienced substantial (≥50%) reductions in pain intensity and/or pain interference during treatment, with 90% (88/98) of patients experiencing clinically meaningful (≥30%) reductions.[32,53–55,57,62–69] Of note, many of those studies reported significant pain relief that may be maintained long after the end of the short-term PNS treatment, with some reports of sustained pain relief through one year of follow-up.[39,40]

Conventional forms of neuromodulation for chronic pain, such as PNS, SCS, and dorsal root ganglion stimulation (DRGS), have not typically provided prolonged pain relief after cessation of stimulation, with preclinical studies reporting a short-term carryover effect on the order of minutes to a few days and very little clinical data on the matter.[2,70–74] Reports of sustained analgesia across multiple pain indications following a short-term PNS treatment are therefore a unique observation that merits further examination from a mechanistic perspective. While the clinical evidence for PNS has been reviewed elsewhere,[75,76] the primary goal of this narrative review is to explore potential theories and mechanisms by which percutaneous PNS may produce sustained pain relief. A secondary goal is to generate discussion in the clinical and scientific communities that may lead to studies that further explore the possibility of modulating

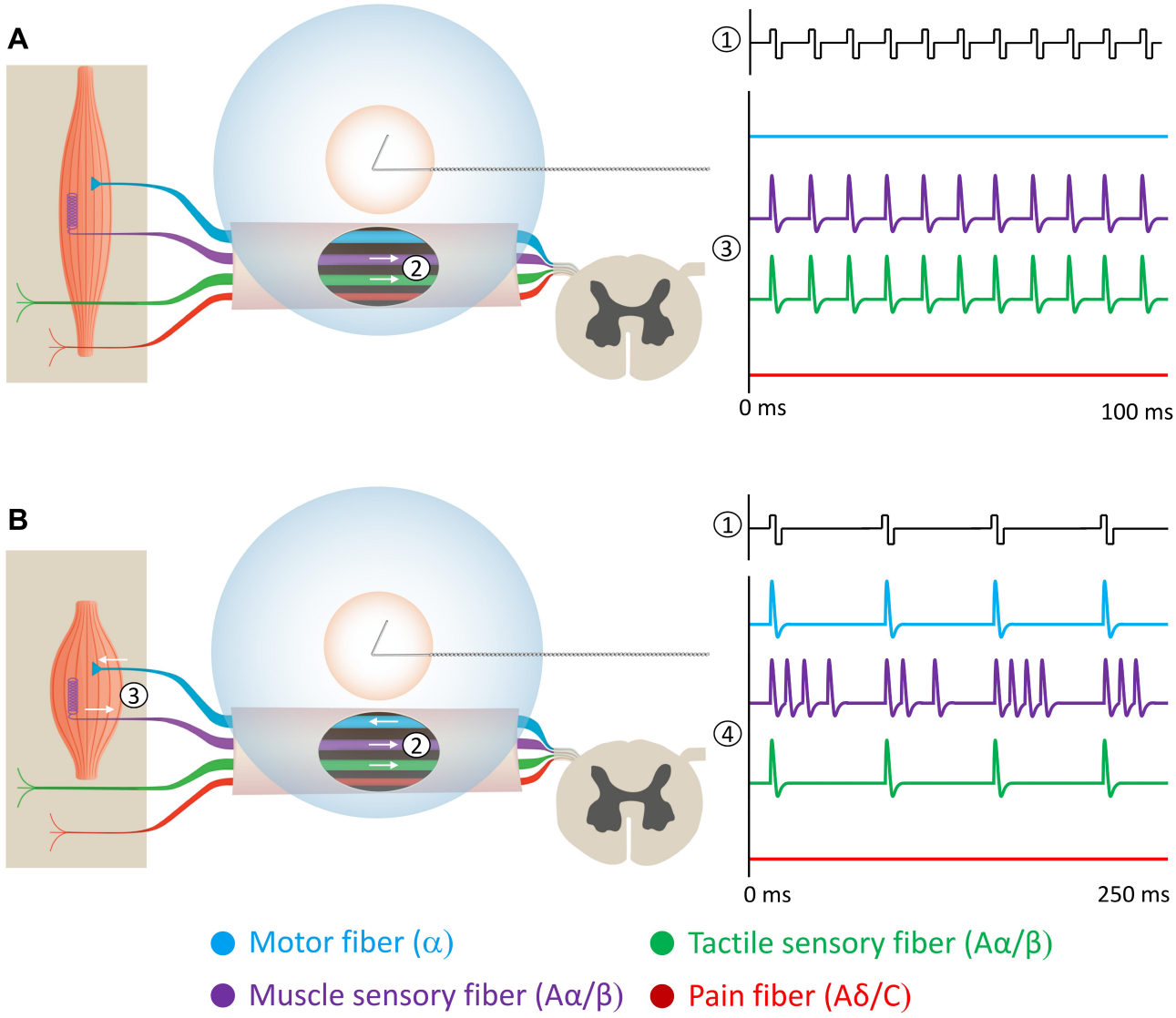

Figure 1 Two percutaneous PNS approaches have demonstrated sustained relief of chronic pain. Stimulation is delivered from a system with open-coiled leads designed to be placed remote from the nerve to selectively activate Aα/β fibers while avoiding Aδ/C fiber activation (ie, remote selective targeting). The activation zones are shown for Aα/β fibers (blue) and Aδ/C fibers (orange). (**A**) Stimulation of mixed nerves at 100 Hz (1) can selectively activate the largest sensory afferents (many of which are larger than muscle efferents[147]). (2) Stimulation activates the large diameter muscle and tactile afferents while avoiding activation of muscle efferents and nociceptive afferents. (3) Directly induced large diameter afferent action potentials enter the spinal dorsal horn at the rate of the stimulation frequency (100 Hz) to engage the gating mechanism, typically producing comfortable sensations in the innervated region. (**B**) Stimulation of mixed nerves at 12 Hz (1) at a sufficient intensity can also activate muscle efferent fibers. (2) Stimulation activates large diameter fibers, including cutaneous afferents, muscle afferents, and muscle efferents while avoiding nociceptive afferents. (3) Orthodromic firing of muscle efferents causes muscle contraction, generating physiological activation of muscle afferent fibers. (4) Large diameter afferent action potentials (directly induced by stimulation and indirectly through muscle contraction) enter the spinal dorsal horn to collectively engage the gating mechanism.

a centrally maintained pain state by providing peripheral input through PNS.

Chronic Pain is Associated with Peripheral and Central Sensitization

Under basal conditions, noxious thermal, chemical, and mechanical stimuli activate nociceptive receptors in the skin. These noxious signals are then carried to the spinal cord by small diameter first order afferents with slower conduction velocities, typically unmyelinated C or myelinated Aδ fibers. The Aδ fibers are thought to carry the sharp, "first pain," while C fibers carry "second pain" signals, characterized by more prolonged aching or burning.[77] In the spinal cord, nociceptive fibers generally synapse in the dorsal horn with nociceptive-specific (NS) or wide dynamic range (WDR) second-order neurons (Figure 2A) that then project to the brainstem or thalamus.[77] Within the brain, pain signals are processed in a number of different regions collectively referred to as

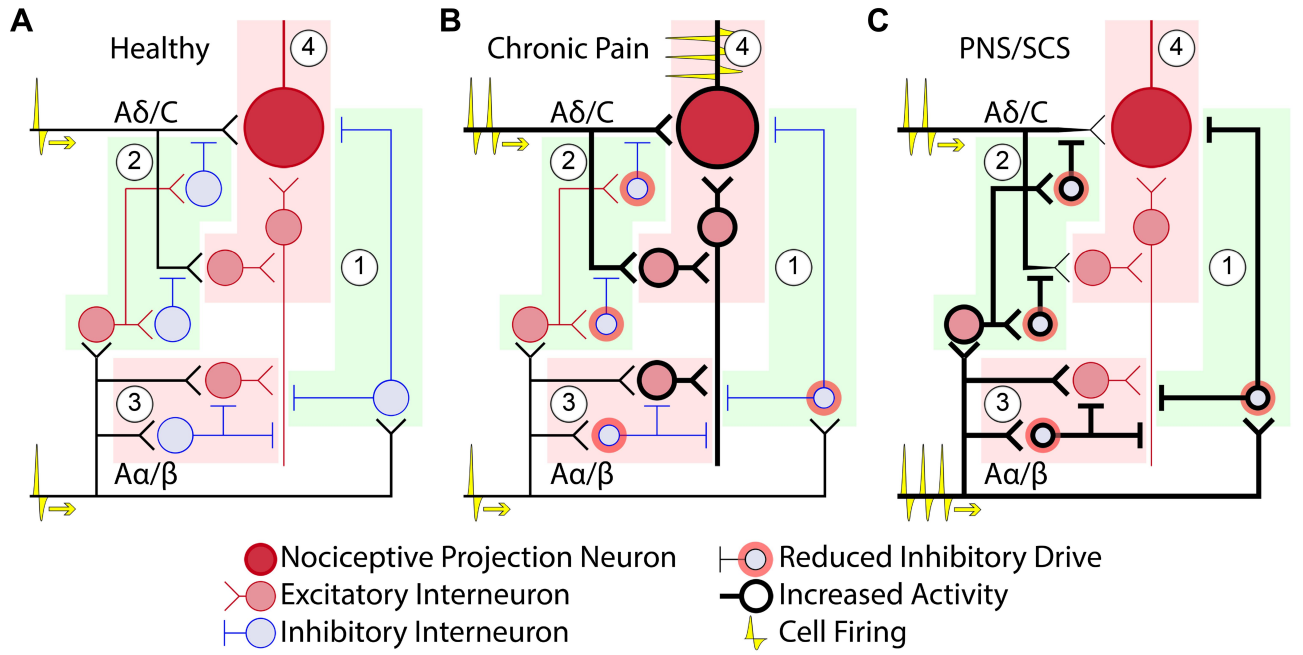

Figure 2 Pain circuitry in the spinal dorsal horn. Four primary sub-circuits are represented: (1) post-synaptic inhibition of nociceptive projection neurons, (2) pre-synaptic inhibition of nociceptive projection neurons, (3) basally inhibited PKCγ excitatory interneurons, and (4) polysynaptically excited nociceptive projection neurons. (**A**) In a healthy case there is a balance between nociceptive and non-nociceptive afferent input and dorsal horn circuit strengths, resulting in minimal activation of nociceptive projection neurons. (**B**) In the case of chronic pain, peripheral nerve damage/inflammation elevates firing of nociceptive afferent fibers. Additionally, GABAergic and glycinergic drive from inhibitory interneurons are reduced, resulting in: (1) reduction in post-synaptic inhibition, (2) reduction in pre-synaptic inhibition, (3) disinhibition of PKCγ interneurons, enabling allodynia-producing circuits, and (4) sensitization of nociceptive projection neurons, characterized by increased excitability and decreased inhibition. (**C**) Neurostimulation is believed to cause elevated firing of Aα/β afferent fibers, counteracting many of the circuit-level effects of chronic pain. Specifically, high rates of Aα/β firing induce: (1) elevated post-synaptic inhibition, (2) elevated pre-synaptic inhibition (3) return of inhibition to the PKCγ cells, reducing allodynia, and (4) elevated inhibition and reduction of nociceptive drive to the nociceptive projection neurons.

the "pain matrix," including the thalamus, somatosensory cortex, insular cortex, anterior cingulate cortex (ACC), prefrontal cortex, amygdala, and hippocampus.[77–79] In the case of chronic pain, persistent nociceptive input induces multilevel changes from the periphery to the brain that result in abnormal pain processing and hypersensitivity, including hyperalgesia (increased sensitivity to noxious stimuli), secondary hyperalgesia (painful sensitivity at sites adjacent to or removed from the injured site), allodynia (painful sensitivity to non-noxious stimuli), and spontaneous pain.[78,80]

In the periphery, damage to nerves can induce peripheral sensitization. Peripheral sensitization is mediated by the release of a wide variety of pro-inflammatory cytokines and neuropeptides whose net result is a drastic reduction in nociceptive thresholds that causes hyperexcitability of nociceptive afferents, spontaneous discharge, and plays a crucial role in the onset and maintenance of hyperalgesia and spontaneous pain.[81–84]

Increased nociceptive activity due to injury and/or sensitization in the periphery also triggers a complex series of changes in the central nervous system collectively referred to as central sensitization.[85,86] Sustained firing of nociceptive afferents leads to sensitization of NS and WDR neurons in the dorsal horn.[79,86] The influx of Ca^{2+} triggered by persistent nociceptive input causes phosphorylation of ion channels and receptors, trafficking of more excitatory channels to the surface, increases in dendritic spine density, and transcriptional changes, all of which promote and maintain a state of increased excitability and decreased inhibition in the dorsal horn (Figure 2B).[79,80,86,87] Neuronal hyperexcitability is further exacerbated by the activation of glial cells and their subsequent release of pro-inflammatory signaling molecules. The role of glial activation in chronic pain is reviewed elsewhere,[79,80,88,89] and spinal glial involvement in neurostimulation-induced analgesia is only recently being explored.[90,91]

In addition to the increased excitability of nociceptive pathways in the spinal cord, nerve injury typically results in a reduction in inhibitory GABAergic and glycinergic drive in the spinal dorsal horn (Figure 2B).[78–80,86,87,92] This disinhibition further amplifies nociceptive signaling directly and also engages excitatory PKCγ interneurons that are driven by activity in large diameter Aβ fibers

and are typically held in check by glycinergic inhibition (Figure 2B).[79,92] Loss of GABAergic and glycinergic inhibition, therefore, perpetuates pain hypersensitivity and causes tactile sensations, which are not typically perceived as painful, to activate nociceptive pathways (one of the key mechanisms believed to contribute to allodynia).[93–96]

Supraspinal circuits also play a major role in the processing of pain and have been implicated in centrally mediated chronic pain, including changes in descending modulation from the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM)[78,97,98] and major structural and functional cortical changes such as alterations in cell spiking dynamics, microglia activation, brain connectivity, gray matter volume, and cortical representation (see[87,99] for review). Specifically, in the somatosensory cortex, which encodes the sensory-discriminative aspects of pain,[100] the nociceptive representational zones exhibit a sensitized state characterized by expansion and/or shifting of pain representations, reduced GABAergic inhibition, and stronger response to activation, while non-nociceptive representational zones may diminish in size and response to activation.[101–106] These maladaptive shifts in the balance of sensory processing are likely due to activity-dependent cortical remapping caused by the increase in nociceptive and relative decrease in non-nociceptive information coming from the region of pain.[104,107] Maladaptive cortical plasticity, coupled with spinal and peripheral sensitization, presents a challenge to treatments' intent on producing long-term pain relief. It is theorized, therefore, that sustained analgesia may be produced by neurostimulation that acts at multiple levels, beginning with spinal modulation of the nociceptive barrage from the periphery.

Activation of Large Diameter Fibers Has the Potential to Attenuate Nociceptive Signaling in the Spinal Dorsal Horn

Neurostimulation systems delivering stimulation at conventional frequencies (eg, 5–150 Hz), including conventional SCS, DRGS, PNS, and even peripheral nerve field stimulation (PNFS), have long been theorized to produce analgesia by modulating pain signals in the spinal dorsal horn via spinal segmental mechanisms that were first described in the well-known gate control theory.[1] Spinal segmental mechanisms of analgesia, including the putative gating mechanism, rely on the activation of large diameter fibers, which are typically myelinated Aα and Aβ fibers (often either grouped together as Aα/β or simply referred to as Aβ due to their highly overlapping morphologies and fiber diameters).[108–116] Since Aα/β fibers generally transmit signals from low-threshold mechanoreceptors and proprioceptors, successful activation often elicits non-painful sensations in the innervated region. The colocalization of these sensations with the region of pain can be used as a marker for focal (ie, specifically targeting the region of pain) activation of large diameter fibers.[54,117] Experimental studies have demonstrated the profound control that Aα/β fibers exert over the transmission of nociceptive signals in the spinal dorsal horn (Figure 2C). Conventional PNS, DRGS, and dorsal column stimulation of large diameter fibers can inhibit the firing of WDR neurons in response to painful stimuli through the inhibition of long-term potentiation and induction of long-term depression of C fiber activity.[70,118–123]

These effects are mediated by a variety of post-synaptic and pre-synaptic circuits in the dorsal horn. Post-synaptically, Aα/β fibers play a primary role in the activation of GABA- and glycinergic inhibitory interneurons in the dorsal horn, which polysynaptically reduce the firing of both superficial and deep dorsal horn projection neurons, subsequently reducing the transmission of nociceptive signals through the spinal dorsal horn (Figure 2C).[92,124,125] Pre-synaptically, early recordings of extracellular potentials in the dorsal root (the dorsal root potential, DRP) found that activation of Aα/β fibers induces widespread subthreshold depolarization of primary afferents (primary afferent depolarization, PAD) in the dorsal root.[126,127] This depolarization is mediated by GABAergic interneurons that synapse on the pre-synaptic terminals of primary afferents and can cause pre-synaptic inhibition.[128] Although fibers most commonly inhibit others of the same type (eg, Aα/β fibers inhibit other Aα/β fibers),[129] some studies have shown that activation of Aα/β fibers can cause pre-synaptic inhibition of nociceptive primary afferents,[124,128,130,131] suggesting a pre-synaptic gating mechanism by Aα/β fiber activation (Figure 2C).

Although gate control has provided the long-standing framework for how many neurostimulation systems may modulate pain, the original 1965 theory has been critically reviewed and supplemented over time to better explain phenomena observed experimentally.[92,125] For example, additional proposed mechanisms of action for conventional stimulation include both peripheral (eg, altering nerve fiber excitability or conduction), and central factors (eg, inducing or depleting excitatory and/or inhibitory neurotransmitters, modulating expression of neuronal signaling proteins, altering activity in central pain matrix regions or descending inhibitory

pathways).[9,132–134] These additional mechanisms highlight the overall complexity of the chronic pain state, though spinal segmental mechanisms remain the predominate mechanistic theory for pain relief with conventional neurostimulation.

Novel Approaches to Selective Activation of Large Diameter Fibers

Nerve fibers with larger diameters are activated by electrical stimulation at a lower intensity compared to smaller diameter fibers,[135,136] so the gating mechanism may be engaged by titrating stimulation intensities to maximally activate large diameter Aα/β fibers while avoiding activation of small diameter nociceptive fibers. Preclinical and clinical evoked compound action potential (eCAP) recordings and computational modeling indicate that conventional SCS at therapeutic intensities activates only a small proportion of the Aα/β fibers in the dorsal columns (estimates range from 0.25% to 8.7% of targeted fibers[137,138]) before reaching discomfort thresholds, purportedly due to activation of the adjacent dorsal roots.[137,139–141] Meanwhile, PNS and DRGS have the potential to activate Aα/β fibers in a more focal, targeted fashion by stimulating the specific nerve(s) or ganglia innervating the region of pain. However, conventional PNS and DRGS utilize small electrodes placed on or immediately adjacent to a nerve that are likely to produce intense electric fields that rapidly decay across short distances such that fibers nearer the electrode may be activated (including small diameter fibers) while fibers slightly more distant from the electrode (eg, deeper in or across the nerve) may experience little to no stimulation (Figure 3).

In contrast to conventional "intimate" electrode placement, it has been hypothesized that percutaneous PNS systems designed to enable remote selective targeting may activate a greater proportion of large diameter fibers while avoiding the unwanted activation of nociceptive afferents (Figure 3).[54] Remote selective targeting describes a PNS system and leads designed to optimize the relationships between stimulation strength, electrode characteristics, electrode-fiber distance, and fiber diameter to create a greater separation of activation thresholds between large and small diameter fibers and enable stimulation from electrodes placed up to several centimeters away (eg, 0.5–3 cm) at therapeutic intensities more selective for large diameter fibers.[54,135,136,142–144] Leads designed for remote selective targeting have multiple features that may enable activation of larger-diameter fibers and avoidance of smaller-diameter fibers while delivering stimulation from such distances. For example, these leads have large monopolar electrodes such that the generated electric fields, which decay exponentially across distance, are broad and relatively homogeneous at remote distances and have the potential to activate large diameter fibers throughout the entire cross-section of a nerve before reaching activation thresholds of smaller fibers. Remote selective targeting may therefore enable more robust activation of large diameter fibers (ie, a larger proportion of targeted fibers) while avoiding unintended discomfort by optimizing the strength-distance and strength-diameter

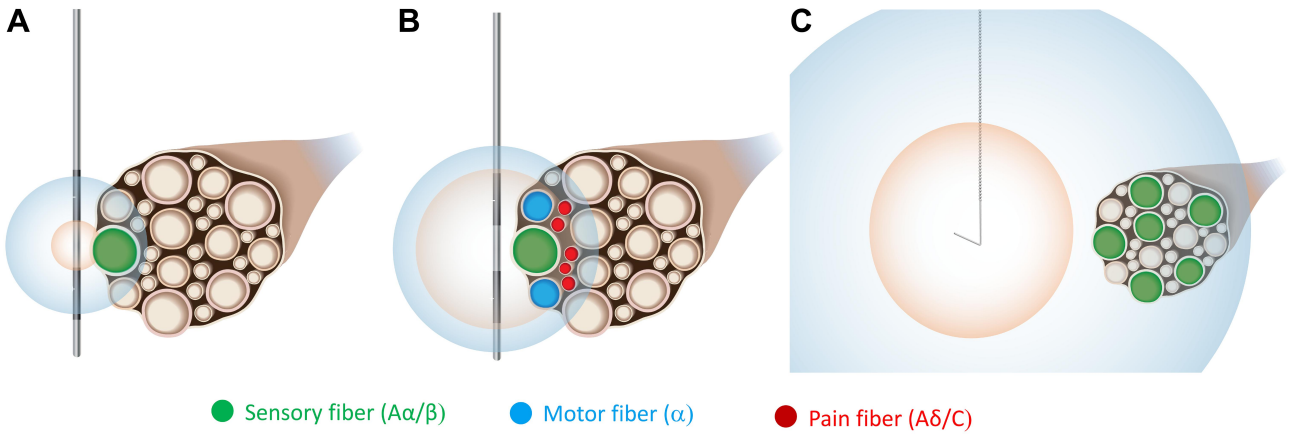

Figure 3 Remote selective targeting promotes activation of large diameter fibers while avoiding activation of small diameter fibers using PNS systems and open coil leads designed for placement distant to the nerve. Large diameter fibers have lower activation thresholds than smaller diameter fibers, and thresholds also increase with electrode-to-fiber distance. The activation zones are shown for Aα/β fibers (blue) and Aδ/C fibers (orange). (**A**) For a conventional PNS electrode placed intimate to the nerve, a limited number of Aα/β fibers may be activated. (**B**) Increasing the intensity to activate a larger proportion of Aα/β fibers begins to concurrently activate Aδ/C fibers or motor fibers, causing unintended discomfort. (**C**) A system using a percutaneous open-coil electrode placed remotely from the nerve (eg, 0.5–3 cm) is designed to selectively activate a larger proportion of Aα/β fibers without concomitant activation of Aδ/C fibers.

relationships that govern the activation of nerve fibers by electrical stimulation (Figure 3).[54,69,135,136,142]

In addition to activation of Aα/β fibers, percutaneous PNS studies have demonstrated prolonged pain relief using stimulation parameters and electrode locations specifically targeting the activation of efferent fibers in mixed nerves that result in strong, physiological muscle contractions without discomfort (Figure 1B).[64,67,145] Remote selective targeting can enable a wider therapeutic window that aids in the activation of motor efferent fibers while avoiding activation of small nociceptive fibers (Figure 1B). Muscle afferents, including proprioceptive Aα/β fibers linked to muscle spindles and Golgi tendon organs, have similar diameter, morphology, and functional connections in the dorsal horn compared to tactile Aα/β fibers.[129,146] Proprioceptive afferents secondarily activated by physiological muscle contractions therefore likely contribute to the gate control mechanism of pain relief in the same way as tactile afferent fibers that innervate the skin.[124] In addition to secondary activation of proprioceptive afferents, the stimulation approach that activates efferent fibers in mixed nerves also likely produces primary activation of Aα/β sensory afferents, which tend to be larger in diameter[147] and are recruited at lower stimulation intensities than efferent fibers (Figure 1B).[142] Notably, this strategy contrasts with conventional stimulation therapies for the treatment of chronic pain, which have historically attempted to avoid efferent activation and consequent motor activity.[14,140,148,149]

Improving the selectivity and robustness of large diameter afferent fiber activation may enhance the transient reduction in pain via spinal segmental mechanisms, such as the gating mechanism. However, these mechanisms rely on active stimulation and are likely insufficient to produce sustained analgesia following the end of treatment, as evidenced by the lack of significant sustained relief following the cessation of stimulation provided by conventional approaches. As the next section will explore, sustained pain relief is theorized instead to be enabled by reconditioning of the central nervous system by robust activation of large diameter fibers in the periphery.

Stimulation of Afferents Can Result in Peripherally Induced Plasticity to Reverse Central Features of Chronic Pain

In addition to spinal segmental mechanisms of pain relief, stimulation of large diameter fibers is believed to induce supraspinal analgesic effects. On a macro scale, studies have identified changes in the magnitude and latency of cortical evoked potentials during PNS, which may relate to changes in the sensory and affective components of pain processing.[150–152] Additionally, electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) studies have revealed that dorsal column stimulation induces changes in cortical activation throughout many of the regions making up the pain matrix, and is hypothesized to activate the descending pain inhibitory system through modulation of the pregenual ACC.[153,154] Given the significant role that cortical processes play in producing, and potentially reducing, chronic pain,[87,99,155] the new theory of Peripherally Induced Reconditioning of the Central Nervous System may help to explain sustained relief following PNS.

The somatotopic representation map in the primary somatosensory cortex (S1) is dynamic and can substantially change as a result of shifts in afferent input, with expansion of regions that experience stronger and more frequent input than those around them and contraction of regions that have reduced inputs.[156,157] In cases of chronic pain, sensory imbalance in the form of elevated nociceptive input from the painful region and/or, importantly, decreased non-nociceptive input can result in drastic shifts in cortical organization and function. Magnetoencephalography (MEG) studies show a correlation between severity of pain and sensitization of the nociceptive region in S1, characterized by reduced intracortical inhibition, expansion and/or shifting of the representational zone, and stronger response to nociceptive stimuli.[101–104] Expansion of the nociceptive response may be coupled with a decrease in the non-nociceptive representational zone and an attenuated response to non-nociceptive stimuli.[102,105,106,158–160] For some patients, blocking nociceptive afferent input is sufficient to transiently alter the cortical reorganization and reduce chronic pain.[103,161] However, for others, nerve block has no effect or only a transient effect,[103] indicating that although some cases of cortical sensitization rely on continued peripheral input, others appear to be centrally maintained.

Robust non-nociceptive afferent input to the cortical areas representing the focal painful region may reduce the severity of pain by actively reconditioning the CNS from the periphery (Figure 4), as opposed to the passive deprivation of nociceptive input that may occur as the result of nerve blocks or ablation.[104,107,162,163] This process has been termed "reconditioning" because it remains unclear whether

the cortex reverses or returns to its exact pre-injury architecture as opposed to achieving a new homeostatic state.

Activity-dependent cortical remapping requires that the peripheral conditioning input to the cortex be robust, since sufficient signal strength is needed to drive the plasticity, and focal from a specific region, since functional plasticity relies on low relative activity in surrounding cortical regions.[107,156,157,162–165] Analysis of conventional stimulation techniques suggests that they are unlikely to achieve these conditions, potentially informing why they can produce excellent pain relief but have not been reported to produce significant sustained outcomes without permanent implantation.[2,70–74] For example, conventional SCS activates only a small proportion of Aα/β fibers before reaching discomfort thresholds, likely spread non-focally across multiple dermatomes due to the lack of somatotopically targeted stimulation (Figure 4A).[139–141] Paresthesia-based DRGS may act via similar mechanisms as other conventional stimulation modalities (ie, activation of large diameter sensory afferents) by placing electrodes in the compact intraforaminal space in close proximity to the DRG to target large diameter axons in the ganglia (Figure 4B).[166–168] Although more focal than SCS, recent computational modeling of DRGS suggests that the percentage of activated Aα/β fibers at clinically relevant stimulation amplitudes varies significantly with lead location, stimulation polarity, and stimulation parameters, indicating that electrode placement in close contact with the DRG may, much like conventional PNS, amplify the deleterious effects of lead migration and limit the scope of activation before discomfort thresholds are reached.[167,169] Lastly, conventional PNS can provide focal stimulation by targeting individual nerve(s) that innervate a region of pain, but a large proportion of Aα/β fibers in the nerve are not typically activated before discomfort thresholds are reached (Figure 4C).[70,112]

Percutaneous PNS with remote selective targeting is theorized to enable more selective activation of non-nociceptive, large diameter afferent fibers, generating peripheral signals that are both focal and robust to optimally recondition the S1 cortex (Figure 4D). Unlike SCS, stimulating individual nerves in a distribution-specific pattern to target a defined region of pain may provide a focal signal from the periphery that is well suited for cortical reconditioning. And, in contrast to conventional DRGS and PNS, remote selective targeting is theorized to widen the gap in activation thresholds between Aα/β and small diameter pain fibers to permit more robust activation of the targeted fiber populations. Furthermore, cortical reorganization can

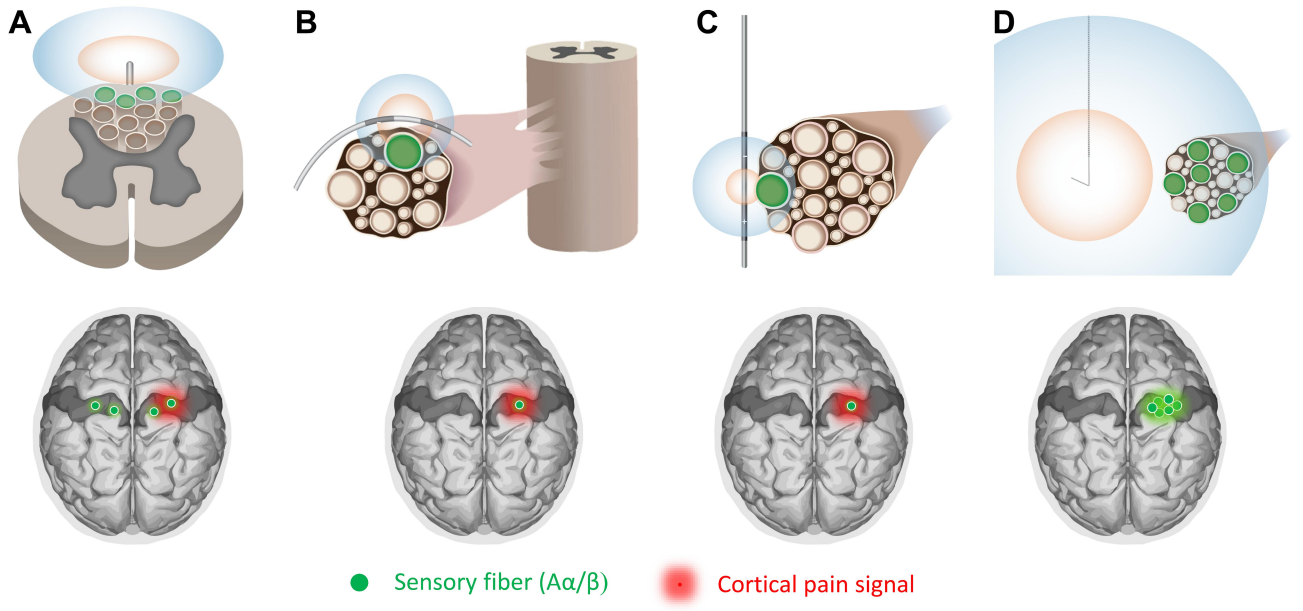

Figure 4 Varying degrees of cortical activation using different stimulation methods. Optimal induction of cortical remapping requires selective activation of a large number of afferent fibers (ie, robust activation) that is generated focally (ie, from the region of pain). The activation zones are shown for Aα/β fibers (blue) and Aδ/C fibers (orange). (**A**) SCS activates a small number of fibers in the superficial dorsal column before reaching discomfort thresholds due to dorsal root activation, and the dorsal column fibers it does activate are commonly spread across multiple dermatomes. The afferent input to S1 is thus neither robust nor focal. Conventional DRGS (**B**) or PNS (**C**) can more focally target the dermatome and/or nerve innervating the specific region of pain, though DRGS often involves multi-level stimulation, but limitations with conventional systems and stimulation strategies curb the degree of large diameter fiber activation before reaching discomfort thresholds due to small diameter nociceptor activation. The afferent input to S1 is thus more focal than SCS but not robust. (**D**) Percutaneous PNS with remote selective targeting enables both focal and robust activation of the target nerves, potentially resulting in optimal cortical input to induce activity-dependent remapping and sustained analgesia, facilitating reconditioning of the CNS.

occur on the time course of weeks,[157,170] suggesting that prolonged pain relief may be produced from short-term (weeks-long) treatments without requiring a permanent implant if the peripheral signals are sufficiently robust and focal to drive beneficial plastic changes.

Applications in Treating Chronic Pain

Percutaneous PNS with remote selective targeting has been successfully used to treat a variety of chronic pain conditions, including chronic pain following amputation, chronic shoulder pain, and axial low back pain. The following section will explore how the proposed mechanisms are theorized to occur in specific cases in which sustained pain relief has been reported following up to 60 days of PNS.

Post-Amputation Pain

Amputation of a limb is incredibly traumatic and induces chronic pain in the residual limb (RLP) and phantom limb (PLP) that can last for many years in up to 80% of patients.[171,172] RLP and PLP have neuropathic features and are associated with peripheral and central sensitization, including functional reorganization of nociceptive pathways in the spinal cord and brain, sensory remapping, expansion of receptive fields, and altered cortical representation of the limb.[172–175] Historically, conventional neurostimulation has been used to treat RLP and PLP with permanently implanted systems that require continuous treatment and tend to lose efficacy over time.[12,176,177] A recent randomized, double-blind, placebo-controlled study delivered percutaneous PNS to the femoral and sciatic nerves for up to 60 days in lower extremity amputees (n=28 total enrollment, n=12 in treatment group). Despite attrition of 25% during follow-up in the treatment group, significant reductions in both RLP and PLP were maintained through 12 months from the start of the 60-day treatment in a majority of subjects (67%, 6/9 at 12 months in treatment group, 70% average pain reduction in responders).[39,55] Activation of large diameter sensory afferents at frequencies that evoke comfortable sensations in the region of pain (eg, 100 Hz) may activate spinal gating mechanisms during the 60-day treatment period to modulate peripheral nociceptive signals (eg, ectopic firing of nociceptive afferents from neuromas or dorsal root ganglia). This attenuated spinal transmission of nociceptive signals, coupled with the robust selective activation of tactile and proprioceptive afferents that innervate the painful region, may also help recondition the maladaptive cortical plasticity that occurs following amputation and restore balance between non-nociceptive and nociceptive representations in S1 to produce the observed sustained pain relief.

Chronic Shoulder Pain

Chronic shoulder pain is a common and complex complication following stroke, with recent studies reporting a prevalence ranging from 19% to 63% in stroke survivors.[178] Shoulder pain may impede rehabilitation from stroke by interfering with self-care activities, reducing ambulation, limiting ability and desire to participate socially, and leading to withdrawal from rehabilitation programs.[179,180] Persistent shoulder pain has characteristics of peripheral and central sensitization, such as allodynia, hyperalgesia, central hypersensitivity, and altered cortical somatosensory processing.[181–185] Multiple randomized controlled trials (RCTs) and case series (n=8–28[32,63,64,66]) using percutaneous PNS with remote selective targeting of the axillary nerve branches innervating the shoulder (Figure 1B) have shown effective long-term pain relief through 6 months in patients with chronic shoulder pain. Stimulation of the terminal branches of the axillary nerve with a lower frequency (eg, 12 Hz) pulse train likely has a dual effect, activating both sensory afferents and muscle efferent fibers. Efferent fiber activation in the terminal branches of the nerve causes contraction of the middle and posterior deltoid muscles,[63] producing proprioceptive signals in large diameter fibers that convergently, along with directly activated sensory afferents, engage the gating mechanism in the spinal cord. Supraspinally, the non-noxious proprioceptive and cutaneous afferent barrage may facilitate cortical neuroplasticity and representational remapping, potentially reversing the cortical contribution to the chronic pain state and enabling patients to achieve sustained relief of their shoulder pain.

Chronic Low Back Pain

Chronic low back pain (LBP) is a leading cause of disability among adults and is both prevalent and challenging to treat.[186,187] In many cases (up to 85%), chronic LBP may be nonspecific or have an unidentified cause.[40] A recent case series (n=9) suggested that low frequency (eg, 12 Hz) stimulation of efferent fibers in the lumbar region may produce sustained relief of chronic low back pain (67%, 6/9 with ≥50% pain relief at 12 months, 80%

average pain reduction in responders).[40] Stimulation of the medial branch nerves of the dorsal ramus in the lumbar region may act by similar mechanisms as described above for chronic shoulder pain, specifically through lower-frequency pulse train activation of efferent fibers, producing secondary isolated contractions of the lumbar multifidus (Figure 1B).[67] A combination of proprioceptive signals from the multifidus and sensory input from direct activation of afferents in the targeted nerve may engage spinal segmental mechanisms of pain relief during stimulation while also providing focal, robust physiological input to drive beneficial central plasticity and produce sustained relief.

Summary and Conclusions

Advancements in imaging and neurostimulation technology have enabled a resurgence of PNS for pain relief in recent years. Studies of percutaneous PNS systems utilizing remote selective targeting have suggested the ability to produce clinically meaningful sustained reductions in pain following temporary (eg, up to 60 days) treatment periods across a variety of chronic pain conditions. Mechanistically, it is theorized that these results may be the result of a widened therapeutic window for stimulation that enables robust and selective activation of Aα/β fibers at frequencies (such as 5–150 Hz) that produce comfortable sensations in the region of pain, leading to multiple analgesic mechanisms from the periphery to the dorsal horn and cortex. These diverse effects may be explained in a new theory of pain management, Peripherally Induced Reconditioning of the CNS, involving stimulation-evoked reversal of the central sensitized state that contributes to chronic pain.

The goal of this narrative review is to propose a mechanism of action theory based on observations in the clinical literature and novel technological advancements in the field of PNS and to generate discussion in the clinical and scientific communicates that may encourage future studies to further explore the observed clinical phenomena. Although the purpose of the present review is not to systematically review the clinical evidence, sustained relief following a short-term percutaneous PNS treatment has emerged in small studies across multiple pain indications, and additional studies that address the limitations of existing evidence would help support the proposed mechanistic theories, including independent investigations, larger cohorts, more active or sham controlled studies, and more consistent periods of long-term follow-up. Direct evidence supporting the mechanistic theory proposed here, such as the reversal of maladaptive cortical plasticity driven by robust and focal inputs from stimulation of peripheral nerves with remote selective targeting, is also needed to confirm the phenomena that may underlie the observed clinical evidence. Future research efforts should therefore endeavor to continue to evaluate this proposed mechanistic theory and explore its clinical utility in a wide range of chronic pain conditions.

The development of neurostimulation systems specifically designed for use in the periphery and the growing volume of clinical data supporting the utilization of PNS across a wide range of pain indications is an encouraging development that offers interventionally trained physicians and neuromodulators new effective tools to treat chronic pain. The demonstrated ability to potentially provide sustained relief from a temporary system that does not require a permanent implant may enable the further adoption of percutaneous PNS earlier in the treatment continuum and avoid the potential costs and/or risks of more invasive or neurodestructive procedures. Future research efforts should continue to evaluate the validity of the theories proposed in the present work, including the role of central plasticity in chronic pain conditions and the potential role for treatments that peripherally target and reverse centrally mediated pain.

Abbreviations

PNS, peripheral nerve stimulation; CNS, central nervous system; SCS, spinal cord stimulation; DRGS, dorsal root ganglion stimulation; NS, nociceptive-specific; WDR, wide dynamic range; ACC, anterior cingulate cortex; PAG, periaqueductal gray; RVM, rostral ventromedial medulla; PNFS, peripheral nerve field stimulation; GABA, gamma aminobutyric acid; DRP, dorsal root potential; PAD, primary afferent depolarization; eCAP, evoked compound action potential; EEG, electroencephalography; fMRI, functional magnetic resonance imaging; MEG, magnetoencephalography; RLP, residual limb pain; PLP, phantom limb pain; RCT, randomized controlled trial; LBP, low back pain.

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Disclosure

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Peripheral Nerve Injury Induces Changes in the Activity of Inhibitory Interneurons as Visualized in Transgenic GAD1-GCaMP6s Rats

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Abstract: Peripheral nerve injury induces cortical remapping that can lead to sensory complications. There is evidence that inhibitory interneurons play a role in this process, but the exact mechanism remains unclear. Glutamate decarboxylase-1 (GAD1) is a protein expressed exclusively in inhibitory interneurons. Transgenic rats encoding GAD1–GCaMP were generated to visualize the activity in GAD1 neurons through genetically encoded calcium indicators (GCaMP6s) in the somatosensory cortex. Forepaw denervation was performed in adult rats, and fluorescent Ca^{2+} imaging on cortical slices was obtained. Local, intrahemispheric stimulation (cortical layers 2/3 and 5) induced a significantly higher fluorescence change of GAD1-expressing neurons, and a significantly higher number of neurons were responsive to stimulation in the denervated rats compared to control rats. However, remote, interhemispheric stimulation of the corpus callosum induced a significantly lower fluorescence change of GAD1-expressing neurons, and significantly fewer neurons were deemed responsive to stimulation within layer 5 in denervated rats compared to control rats. These results suggest that injury impacts interhemispheric communication, leading to an overall decrease in the activity of inhibitory interneurons in layer 5. Overall, our results provide direct evidence that inhibitory interneuron activity in the deprived S1 is altered after injury, a phenomenon likely to affect sensory processing.

Keywords: pain; somatosensory cortex; calcium imaging; corpus callosum; plasticity; rehabilitation

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1. Introduction

Peripheral nerve injury (PNI) is characterized by abnormal pathologies in sensory and motor pathways. It is often accompanied by neuropathic and phantom limb pain, leading to poor prognosis and recovery. Substantial research shows that PNI and sensory deprivation prompt a complex sequence of changes in neural activity that lead to the remapping of cortical representations in humans [1,2], non-human primates [3], and rodent brains [4]. Evidence suggests that this plasticity dictates the degree of sensory complications [5,6]. Therefore, identifying the neural circuits and the plasticity mechanism associated with PNI is essential in developing new and improved treatment strategies to minimize post-injury complications.

Removing peripheral input affects multi–synaptic pathways, including thalamocortical connections, intrahemispheric connections, and interhemispheric connections. Strengthening of thalamocortical synapses after PNI in rats has been documented using functional magnetic resonance imaging (fMRI) and electrophysiology [7]. Plasticity of local circuits after limb and whisker denervation has been shown to occur in the primary somatosensory

cortex (S1) contralateral and ipsilateral to the side of denervation [8–15]. These studies demonstrate that both excitatory neurons and inhibitory interneurons within the deprived S1 (contralateral to the injury) are affected by the loss of input. Inhibitory interneurons are known to shape sensory integration, cortical maps, and sensory processing of stimuli [16]. Nevertheless, it remains unclear how inhibitory interneurons are affected by injury and subsequently lead to abnormal sensory processing.

Several studies suggest that injury leads to upregulation in the activity of inhibitory interneurons [9,15,17]. Possible mechanisms include the decreased activity of excitatory neurons due to a lack of thalamic input to cortical layer 4 (L4) and abnormal interhemispheric, transcallosal communication. Indeed, modulating interhemispheric communication by optogenetics decreased inhibitory activity in the deprived S1 and restored the excitation-inhibition balance [15]. Using non–invasive brain stimulation over the deprived S1 to increase activity has been shown to reduce pain and increase performance after injury [18]. On the other hand, there is evidence suggesting sensory deprivation increases cortical excitability through transcallosal communication, which may suggest downregulation in the activity of inhibitory interneurons [19,20]. Nonetheless, all these studies show that injury induces changes in the balance between excitation and inhibition in the S1 and changes in the communication between neurons in the S1. Together, this leads to abnormal sensory perception.

The goal of the present study was to determine the role of inhibitory interneurons in cortical remapping after injury. Inhibitory interneurons are typically smaller than excitatory neurons and account for only 20% of cortical neurons. Thus, recording and visualizing their activity using electrophysiology and microscopy is often challenging. Advances in transgenic technology now allow the genetic engineering of rats [21] and mice [22] to express genetically encoded calcium–sensitive proteins (GCaMPs) [23] under specific neural promoters [24,25].

Glutamate decarboxylase–1 (GAD1) is a protein expressed in inhibitory interneurons and is responsible for basal GABA production [26]. Transgenic Sprague Dawley rats were generated using the CRISPR/Cas9 system to encode GAD1–GCaMP6s. The CRISPR/Cas9 system is an effective tool for gene editing in various model organisms, including mice and humans [27]. This new transgenic rat allows for the visualization of neuronal activity in GAD1-inhibitory interneurons by measuring calcium changes.

To measure the activity of inhibitory interneurons in the present study, a bipolar tungsten electrode was positioned inside layers 2/3 (L2/3), layer 5 (L5), or in the corpus callosum (CC) of the deprived S1 in denervated and control GAD1–GCaMP6s rats. The effects of intrahemispheric stimulation were analyzed in L2/3 and L5 of the deprived S1, while the effect of interhemispheric stimulation of the CC was analyzed in L5 of the deprived S1. The results suggest that denervation leads to increased activity of inhibitory interneurons in response to local, intrahemispheric stimulation, whereas denervation impacts interhemispheric communication and leads to an overall decrease in the activity of inhibitory interneurons. Overall, our results provide direct evidence that the activity of inhibitory interneurons in the deprived S1 is altered after injury.

2. Materials and Methods

Animal experiments were approved by Michigan State University's Institutional Animal Care and Use Committee and conducted according to the NIH Guide for the Care and Use of Laboratory Animals.

2.1. Generation of Transgenic GAD1-GCaMP6s Knock-In Rat

The rat GAD1 locus (ENSRNOG00000000007) was targeted using CRISPR–Cas9 genome editing and a long single-stranded oligodeoxynucleotides (lssODN) HDR donor template [28,29]. Selection of guide RNAs (gRNAs), locus analysis, construct design, and sequence analysis, and alignments were performed using the Benchling platform and MacVector software. A gRNA targeting exon 2 with a protospacer and protospacer adjacent

motif (PAM) sequence 5′–CGTGGAAGATGCCATCAGCTCGG–3′ was chosen to generate a double–strand break (DSB) 2bp upstream of the translational start site (ATG).

An HDR donor construct was generated to include 5′ and 3′ homology arms flanking the GCaMP6s coding sequence (cds) and a P2A self–cleaving signal peptide, upstream and in–frame with the GAD1 coding region in exon 2. Homology arm (HA) regions were PCR amplified from Sprague Dawley rat genomic DNA with a Q5® High–Fidelity DNA Polymerase (M0491, New England Biolabs, Ipswich, MA, USA) and primers O619F and O620R (Primer Table). The GCaMP6s cds were subcloned from vector pGP–CMV–GCaMP6s, a gift from Douglas Kim & GENIE Project (Addgene plasmid #40753). A GSG–P2A sequence was synthesized, and individual fragments were PCR–amplified with appropriate overlaps for assembly into a pBKSII backbone using the NEBuilder® HiFi Assembly Cloning kit (E5520S, New England Biolabs).

To produce a lssODN donor template, a nickase–based method was employed using the Long ssDNA Preparation Kit (DS620, BioDynamics Laboratory Inc., Hackensack, NJ, USA). The GCaMP6s–P2A insert flanked by 375 bp 5′HA and 343bp 3′HA was amplified (O712F/O713R) and cloned into the nickase vector pLSODN–3. The resulting sequence–verified plasmid was digested with NsiI and the nickase Nb.BbvCI, and the released ssDNA was denatured, gel extracted, and purified using a Clontech NucleoSpin® gel extraction kit (NC923380, Fisher Scientific, Waltham, MA, USA).

Sprague Dawley rats were purchased from Charles River Laboratory (Crl:Sprague Dawley, strain code 400). Ribonucleoprotein (RNP) complexes were prepared by hybridization of synthetic Alt-R® CRISPR crRNA and tracrRNA, which were then complexed in equimolar amounts with [100 ng/µL] Alt-R® S.p. Cas9 Nuclease V3 protein (Integrated DNA Technologies Inc., Coralville, IA, USA). RNP complexes were mixed with the lssODN donor template [10 ng/µL] and delivered into Sprague Dawley rat zygotes by pronuclear microinjection. Microinjected embryos were implanted into pseudo-pregnant recipients using standard approaches.

Founder litters were screened for correct HDR events by PCR with 5′ (O663F/O664R; O753F/756R) and 3′ (O665F/O666R; O757F/O752R) external primers. Founder T1641 was identified as having the correct insertion, and the entire cassette and surrounding genomic regions were amplified, cloned, and verified by Sanger sequencing. One histidine residue was deleted from the His-tag at the N–terminus of the GCaMP6s cds, and the remaining insert sequence and flanking genomic regions were intact.

GAD1–GCaMP6s rats were kept heterozygous and were bred to wild–type Sprague Dawley animals for multiple generations to out–cross any potential off–target mutations. Analysis of the gRNA used for targeting with CRISPR and Benchling prediction algorithms did not identify any significant off–target hits either in exons (all CFD specificity scores <0.27) or on the same chromosome (all CFD specificity scores <0.21).

2.2. Peripheral Nerve Injury

Sprague Dawley adult rats (100–130 g, 5 weeks old, n = 12, (9 male, 3 female)) underwent forepaw denervation by excision of the radial, median, and ulnar nerves [10]. Forepaw denervation was performed by cutting the median nerve below the level of the triceps muscles and cutting the radial and ulnar nerves beneath the area of the bicep muscles. Rodents were under 2% isoflurane anesthesia while denervation was performed. As a result, both sensory and motor fiber pathways were completely severed. The incision was cleaned and closed using silk sutures and tissue glue. Tramadol (0.1 mg/300 mg) was administered orally for 5 days after the injury. For sham controls, rats underwent the entire procedure, including exposure of the nerves, followed by suturing of the skin.

2.3. Immunochemistry of Brain Slices

Rats were transcardially perfused with 0.1 M phosphate buffer saline solution (PBS) at pH 7.4. This was followed by an ice-cold 4% paraformaldehyde solution, and the brains were subsequently removed. Brains were cryoprotected in 30% sucrose overnight. The brain

tissue was then embedded in OCT compound (Tissue–Tek) and sliced on a cryostat (Leica Microsystems GmbH, Wetzlar, Germany) to obtain 20 µm thick coronal sections, which were collected on glass slides. Sections were incubated overnight with primary antibodies to detect GAD1 (1:100; Abcam #ab97739) and GFP (1:500; Invitrogen #ab13970) at 4 °C. After incubation with the primary antibody, sections were washed with PBS (three times, 5 min each) and incubated for 3 h at room temperature with secondary antibodies (Alexa Fluor 555 & Alexa Fluor 488). Sections were washed twice with PBS, and ProLong Gold Antifade Reagent (Thermofisher Scientific, Waltham, MA, USA) on coverslips were used.

2.4. Confocal Imaging

Confocal images were acquired using the Nikon A1–Rsi Confocal Laser Scanning Microscope (Nikon Instruments, Inc., Tokyo, Japan) configured on a Nikon Eclipse Ti inverted microscope. Images were collected using either a Nikon 10× Plan Apo (NA 0.45) objective, a Nikon 20× Plan Apo VC (NA 0.75) objective, a Nikon 40× Plan Fluor (NA 1.30) oil objective, or a Nikon 60× Apo (NA 1.40) oil objective. Image acquisition was performed using Nikon NIS–Elements AR software (version 5.20.02). Green fluorescence was excited using a 488 nm diode laser, and fluorescence emission was detected through a 525/50 nm bandpass emission filter. Red fluorescence was excited using a 561 nm diode laser, and fluorescence emission was detected through a 595/50 nm bandpass emission filter. For each data set, a confocal series through the thickness of the tissue section was collected. For the 20× objectives, confocal images were collected in 1.5 µm increments through an average thickness of 30 µm. For the 40× objectives, confocal images were collected in 1 µm increments through an average thickness of 20 µm. For each confocal series, a Maximum Intensity Projection image was generated, representing the brightest intensity pixels through the Z–depth.

2.5. Calcium Imaging and Stimulation

Cortical coronal brain slices were obtained from rats 2 weeks post-PNI surgery. Rats were euthanized with isoflurane, and the brain was removed and placed in oxygenated (95% O_2/5% CO_2) ice–cold artificial cerebrospinal fluid (ACSF) in mM: NaCl–119, $MgSO_4 \cdot 7H_2O$–1.2, KCl–2.5, NaH_2PO_4–1.15, Glucose–11.0, $NaHCO_3$–26.2, $CaCl_2 \cdot 2H_2O$–2.5. 300 µm slices were obtained using tissue vibratome (Leica Biosystems, Deer Park, IL, USA) in ice–cold ACSF. Slices were then bubbled with 95% O_2/5% CO_2, pH 7.4, at room temperature for 45 min before using them for experimentation. Slices were then loaded on a fixed stage microscope (DM6FS, Leica Biosystems) fitted with a Hamamatsu ORCA-fusion sCMOS camera.

Constant perfusion with ACSF was performed to ensure the physiological health of slices. GCaMP6s positive fluorescent cells in cortical L2/3 and L5 were identified and imaged with a 5x objective (1.25 internal magnification chamber, resulting in a magnification of 6.25). Identified GAD1–GCaMP6s fluorescent cell(s) were imaged as a time series experiment. Regions of interest were drawn around GAD1–GCaMP6s neurons in L2/3 and L5 using LAS X (Leica Biosystems). A bipolar tungsten electrode was positioned inside L2/3, L5, or in the CC in the deprived S1, and 100 Hz stimulation was delivered for 5 s. Fluorescence intensity changes over time were recorded with regions of interest before and after electrical stimulation in the desired cortical region.

2.6. Statistical Analysis

We assumed non-normality based on the Kolmogorov–Smirnov Pearson test and used the Mann–Whitney Wilcoxon test to determine significance.

3. Results

To visualize the activity of inhibitory interneurons, transgenic rats were generated to express GCaMP6s in GAD1+ inhibitory interneurons. Transgenic GAD1–GCaMP6s knock-in rats were generated by CRISPR–Cas9 genome editing using a long single-stranded DNA

repair template. To preserve the expression of the endogenous GAD1 protein, a GCaMP6s cassette was followed by a P2A self–cleaving peptide [30] sequence and was inserted at the translational start site, in–frame with the coding sequence of GAD1.

To validate the expression of GCaMP6s and GAD1, immunohistochemistry was performed with primary antibodies against GFP and GAD1, respectively. Confocal imaging revealed GCaMP6s expression (green) throughout the cortical layers (Figure 1). GAD1 (red) expression was also observed throughout the cortical layers with a sparse labeling pattern. Examination of merged (GCaMP6s + GAD1) images revealed colocalization of GCaMP6s and GAD1 expression. Taken together, these results demonstrate the transgenic rat successfully expresses GCaMP6s in GAD1 neurons.

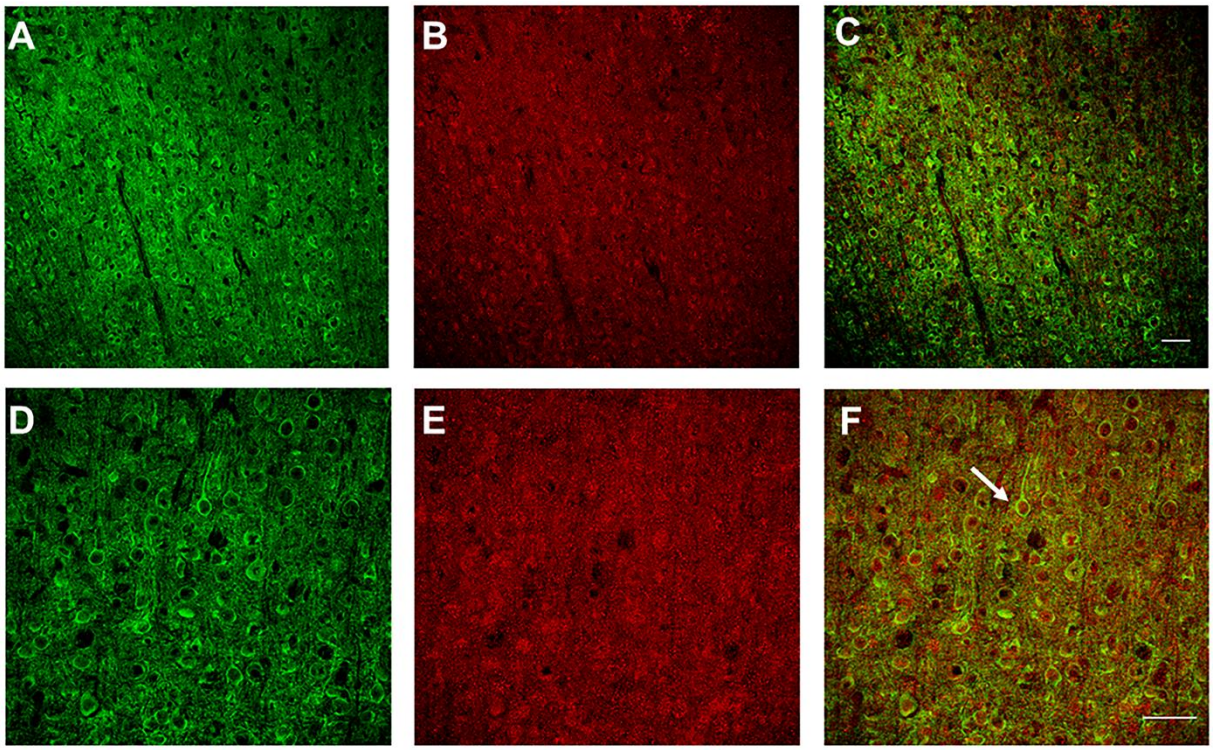

Figure 1. Immunohistochemistry verification of GAD1-GCaMP6s expression in cortical interneurons. Double-labeling immunohistochemistry was performed for GCaMP6s (green) and GAD1 (red) in GAD1–GCaMP6s transgenic rats. The top row shows (20×) magnification of a coronal section labeled with (**A**), GCaMP6s antibody (**B**), GAD1 and (**C**), merged (GAD1–GCaMP6s) image. Bottom row shows 40× coronal sections labeled with (**D**), GCaMP6s (green) (**E**), GAD1 (Red), and (**F**), merged GAD1 + GCaMP6s image. The white arrow highlights an interneuron that shows colocalization between GAD1 and GCaMP6s. Scale bars: 50 μm.

3.1. Intrahemispheric Upregulation of GAD1 Neurons in the Deprived S1

Changes in fluorescence of GCaMP6s from identified GAD1 neurons in L2/3 and L5 of the deprived S1 were collected in response to local stimulation. Representations of identified GAD1 neurons in L2/3 and L5 are demonstrated in Figure 2A,C, respectively. Experimental schematics demonstrating fluorescence intensity changes over time were recorded in L2/3 (Figure 2B) & L5 (Figure 2D) in response to electrical stimulation, 30s post basal activity. For intrahemispheric L2/3 and L5 experiments, we imaged 27 slices from denervated rats (n = 5) and 37 from control rats (n = 6). From these slices, we identified 248 GAD1–GCaMP6s positive neurons in denervated rats and 366 in control rats. The fluorescence change amplitude (ΔA) was calculated by taking the difference between the maximum fluorescence value after stimulation (max value from 0–10 s post-stimulation;

i.e., *MaxF*) and the average fluorescence value prior to stimulation (0–29 s pre-stimulation; i.e., *BaseF*) and dividing it by *BaseF*, as represented in the following:

$$\Delta A = \frac{MaxF - BaseF_{0-29s}}{BaseF_{0-29s}}$$

In denervated rats, local stimulation induced an average fluorescence change of 5.76 ± 6.49% (mean ± standard deviation (*SD*)) in L2/3 and 2.75 ± 2.77% in L5. Additionally, local stimulation in control rats induced an average fluorescence change of 0.24 ± 1.39% in L2/3 and 0.62 ± 1.65% in L5 (Figure 3A,B; Mann–Whitney Wilcoxon test, $p < 0.0001$ and $p < 0.0001$, respectively). GAD1 neurons were considered responsive to stimulation when *MaxF* was 2*SD* above *BaseF*, as indicated below:

$$Responsive \geq 2SD \times BaseF_{0-29s}$$

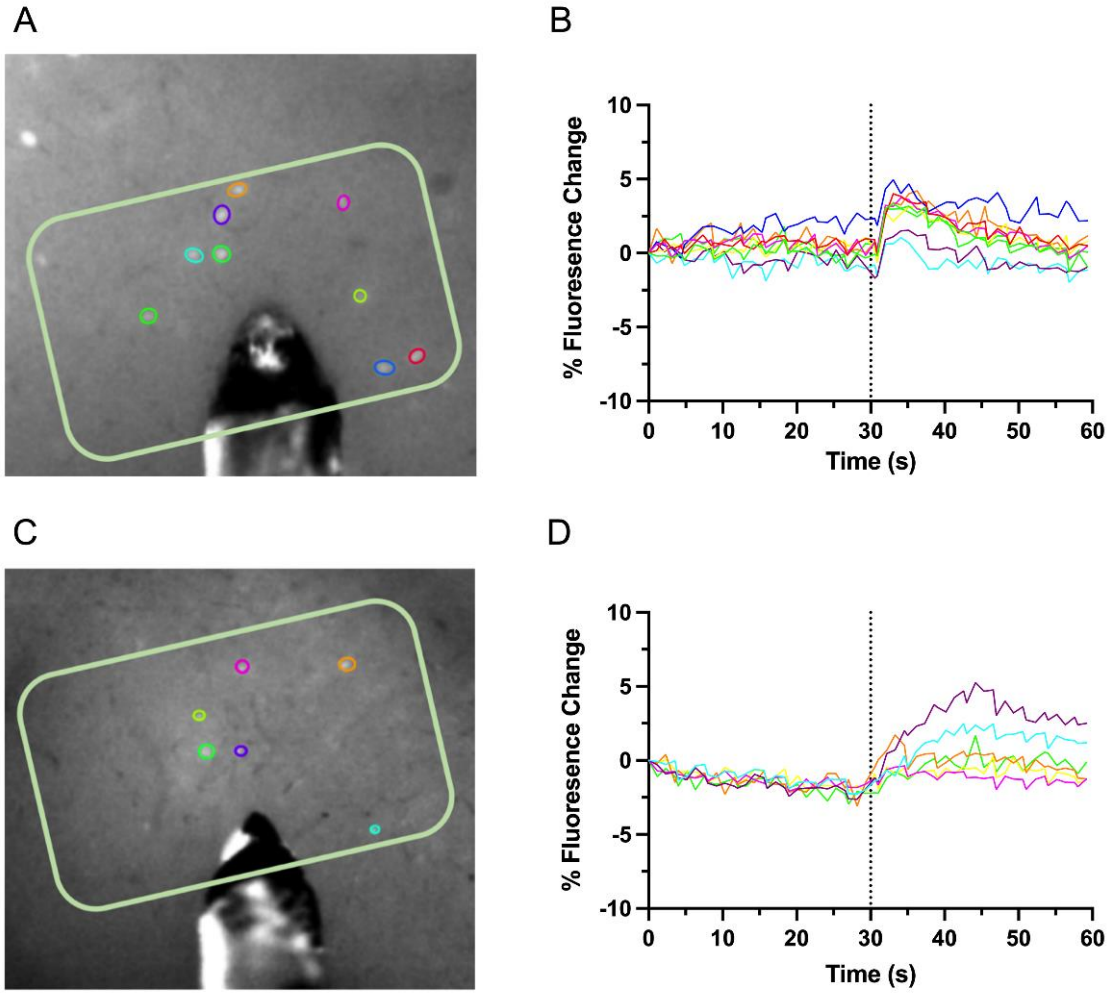

Figure 2. Intrahemispheric connectivity in L2/3 and L5. (**A**,**C**), Representative images of L2/3 and L5, respectively, depicting the identified, fluorescing GAD1 neurons (ROIs shown as color coded circle) and their associated; (**B**,**D**), percent fluorescence change over time, with stimulation via bipolar tungsten electrode in the brain slice (pictured in (**A**,**C**)) occurring at 30 s.

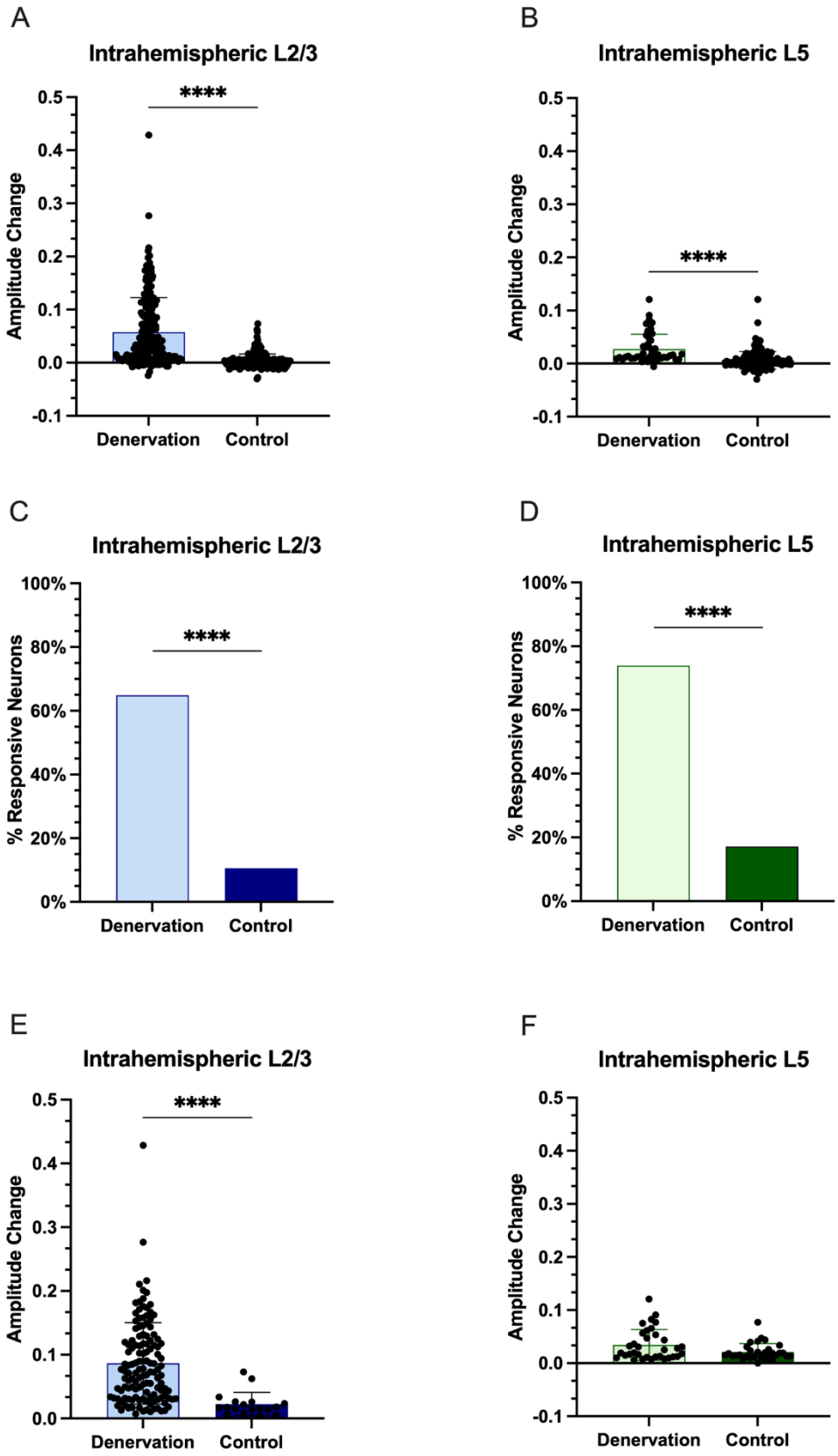

Figure 3. Intrahemispheric upregulation of GAD1 neuron activity in L2/3 and L5 in the deprived S1 after injury. (**A**,**B**), fluorescence change (mean + *SD*) of all identified GAD1 neurons after stimulation. (**C**,**D**), number of GAD1 neurons responsive to stimulation, and (**E**,**F**), the average fluorescence change of the responsive GAD1 neurons. ($p < 0.0001$, ****).

In denervated rats, 131 out of 202 GAD1 neurons (64.85%) in L2/3 were deemed responsive, while 34 of 46 (73.91%) were deemed responsive to stimulation in L5. In control rats, significantly fewer GAD1 neurons were responsive to stimulation: 18 out of 185 (9.73%) in L2/3, and 31 of 181 (17.13%) in L5 (Figure 3C,D; L2/3 Chi–squared = 113.20, $p < 0.0001$; L5 Chi–squared = 56.88, $p < 0.0001$). Additionally, the responsive GAD1 neurons in the denervated rats had a significantly larger average amplitude change in fluorescence in L2/3 (8.64 ± 6.38%) when compared to those of the control rats (2.24 ± 1.83%; Figure 3E, Mann–Whitney Wilcoxon test, $p < 0.0001$). However, no significant difference was found for the responsive GAD1 neurons between denervated (3.45 ± 2.90%) and control rats (2.12 ± 1.56%), as shown in Figure 3F (Mann–Whitney Wilcoxon test, $p = 0.1439$).

3.2. Interhemispheric Downregulation of GAD1 Neurons in the Deprived S1

Changes in fluorescence of GCaMP6s from identified GAD1 neurons in L5 of the deprived S1 were collected in response to stimulation of the CC. Representations of identified L5 GAD1 neurons and their evoked-response activity are demonstrated in Figure 4. For interhemispheric L5 experiments, we imaged 9 slices from denervated rats ($n = 3$) and 15 slices from control rats ($n = 4$). From these slices, we identified 70 GAD1–GCaMP6s positive neurons in denervated rats and 144 in control rats.

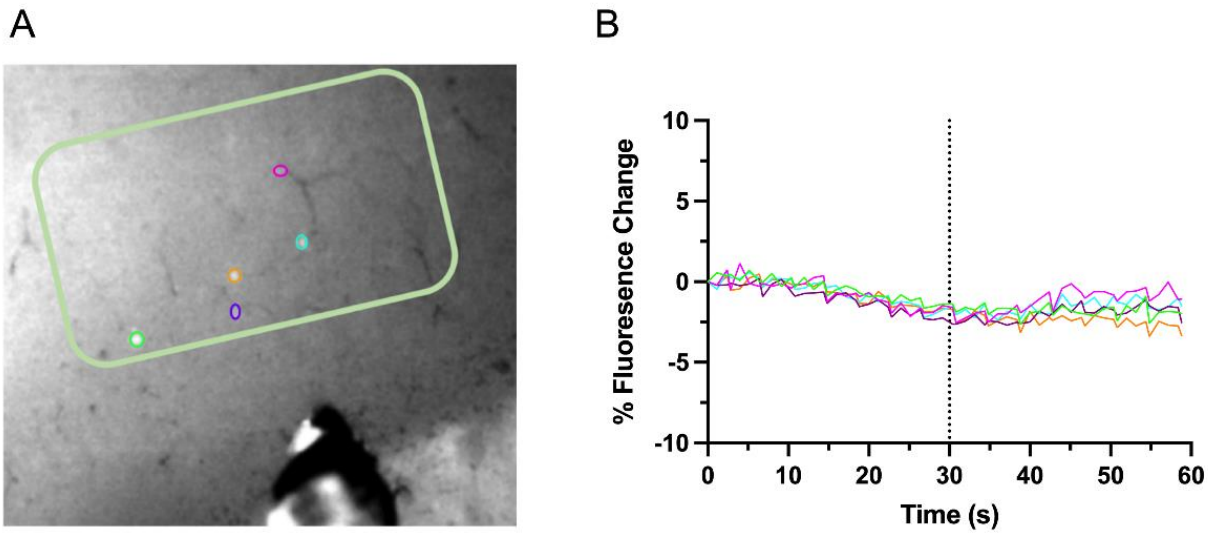

Figure 4. Interhemispheric connectivity in L5. (**A**), Representative image depicting the identified GAD1 neurons (ROIs shown as color coded circle). (**B**), Percent fluorescence change over time, with stimulation via bipolar tungsten electrode in the brain slice (pictured in (**A**)) occurring at 30 s.

Stimulation of CC induced an average fluorescence change of −0.67 ± 1.67% in denervated rats compared to 0.56 ± 2.06% in control rats (Figure 5A; Mann–Whitney Wilcoxon test, $p < 0.0001$). In addition, we found that CC stimulation evoked fewer neural responses in L5 neurons in denervated rats than in control rats. Only 3 out of 70 (4.29%) GAD1 neurons in denervated rats were deemed responsive to stimulation compared to 35 of 144 (24.31%) GAD1 neurons in control rats (Figure 5B; Chi–squared = 12.927, $p < 0.0003$). The average amplitude change of fluorescence between L5 responsive GAD1 neurons in the denervated and control rats was not statistically different (Figure 5C; 1.87 ± 0.83% in denervated rats, 3.02 ± 2.84%; Mann–Whitney Wilcoxon test, $p = 0.8385$).

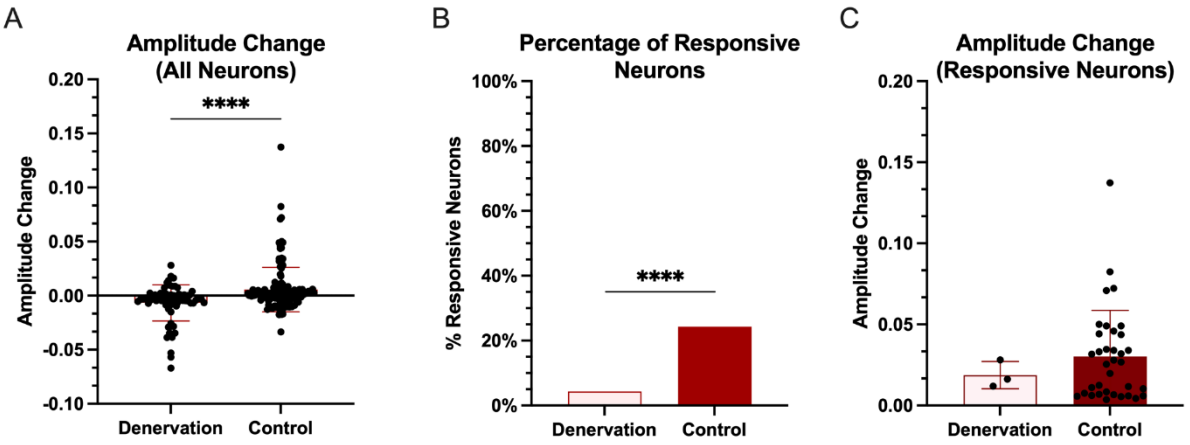

Figure 5. Interhemispheric downregulation of GAD1 neuron activity in L5 in the deprived S1 after injury. (**A**), Fluorescence change (mean + SD) of all identified GAD1 neurons after stimulation, (**B**), number of GAD1 neurons responsive to stimulation, and (**C**), the average fluorescence change of the responsive GAD1 neurons. ($p < 0.0001$, ****).

These results demonstrate that denervation led to an increase in the activity of L2/3 and L5 GAD1 neurons in response to local network activity, while denervation led to a decrease in the activity of L5 GAD1 neurons in response to interhemispheric stimulation of the CC.

4. Discussion

Ample research has found that cortical remapping occurs in the S1 following peripheral denervation. This remapping involves both inhibitory interneurons and excitatory neurons. Interneurons receive both excitatory and inhibitory inputs and project locally within the cortical layers [31]. fMRI of the intact and the deprived S1 of denervated rats have shown bilateral increases in both fMRI and single–unit responses following stimulation of the intact limb. The single unit increases were identified as inhibitory interneurons [9]. Li et al. [15] provided additional evidence demonstrating an upregulation in inhibitory interneurons and identified a potential pathway to restore levels of interneuron activity by inhibiting transcallosal communication. Recently, Cywiak et al. [18] demonstrated that excitation of the deprived S1 with non-invasive brain stimulation [32] and magnetogenetics technologies [33] could alleviate pain and improve performance in rats that previously underwent PNI. Moreover, studies show increases in excitatory neurons in the deprived S1 following stimulation, suggesting a shift in the balance of inhibition and excitation [11,13–15,17,34,35].

The development and use of transgenic animals for research has been limited to mice due to numerous biological limitations. Transgenic rats are a relatively newer model organism that serves as a better replicate for human disease. The development of genomic modifications in rats has transcended from using cre technology [36] to zinc finger nucleases [37], transcription activator-like effector nuclease (TALEN) [38], and the more revolutionary CRISPR technique [39]. Our research study necessitated the reliable imaging of GAD1 interneuron activity in brain slices in the deprived S1. To do so, we created a strain of transgenic rats that express genetically encoded calcium sensor GCaMP6s in GAD1 neurons. These novel transgenic rats were used to successfully image calcium dynamics of GAD1 neurons in all layers of the somatosensory cortex, with a specific interest in the activity in L2/3 and L5. Through confocal imaging, we identified two pathways, one intrahemispheric and one interhemispheric, that affected the activity of inhibitory interneurons.

Despite the novelty of our imaging technique, a meticulous approach is required to interpret GCaMP–associated changes. While increases in GCaMP responses are well established to be correlated to increases in neural activity, the cellular basis of decreases in

fluorescence may be less clear. A trend seen in our recordings is mild negative deviations of fluorescent changes from the baseline, specifically in denervated rats after transcallosal stimulation. This can be due to: (1) hyperpolarization responses in neurons that have been shown to decrease GCaMP responses [40], (2) small deflections that are a measure of constant calcium flux, (3) negative changes in the fluorescent signal due to a photo–bleaching effect, and/or (4) temporal resolution of GCaMP probe translates to deflections in the baseline. However, it is critical to use the right approach to extract meaningful information from datasets to remove this bias towards negative deflections in imaging [41].

Most of the interneuron projections are local [42,43]. Thereby, these locally connected inhibitory interneurons communicate within layers and are responsible for the mechanisms of intrahemispheric plasticity. In the current study, intrahemispheric stimulation of L2/3 and L5 in the deprived S1 of denervated rats led to increased inhibitory interneuron activity. Several mechanisms could lead to this phenomenon, including long–term depression of excitatory intracortical synapses [44] and potentiation of inhibitory synapses [45].

The CC transmits bilateral sensory signals to the contralateral hemisphere [34,46]. Disruption in interhemispheric connections can cause maladaptive changes, among them the development of phantom limb pain [47]. After unilateral whisker denervation, stimulation of the intact whisker has been shown to strengthen the synaptic connection between the CC and the remote deprived L5 neurons [14,15]. Changes in the functioning of GABAergic receptors in inhibitory interneurons have also been demonstrated post–injury [20,48] as the reduced presence of GABA in the presynaptic terminal post–injury lowers the action potential threshold of the neurons in the targeted region of the deprived S1 [13,14]. In the current study, a decrease in the activity of the inhibitory interneurons was seen in the deprived S1 of denervated rats compared to that of the controls. The strength of excitation–inhibition from the intact to the deprived cortex through the CC is primarily determined by the activity balance and communication between excitatory neurons and inhibitory interneurons across the cortical hemispheres. Injury leads to decreased activity of inhibitory neurons in the deprived S1 and allows for spontaneous activation of excitatory neurons in the remote S1 interhemispheric target [48].

The differences in network activity seen within local connections are opposite of that observed due to remote, interhemispheric differences. A possible mechanism behind this difference could be due to the nature of inhibitory interneurons having a non–homogenous mechanism of plasticity. For example, studies have shown that many populations of GABAergic interneurons fail to undergo the classical NMDA–mediated mechanisms of synaptic plasticity [49]. Also, the vast diversity in interneuron subtypes with 5 different subclasses accounts for innate differences in plasticity mechanisms [50]. Altogether, these studies suggest that both excitatory neurons and inhibitory interneurons are involved in post–injury plasticity. These changes in intercortical and cortical–cortical communication interfere with normal sensory processing and may be the foundation of sensory dysfunctions [19,51–53].

Clinical Translation

Interneuron dysfunction is involved in a variety of neuropathologies, such as schizophrenia [39], epilepsy [54], Alzheimer's disease [55,56], autism [57], and phantom limb pain [47,58]. Therefore, transgenic rats, such as the novel ones generated for the current study, would be a valuable tool for investigating such pathologies. This is the first time that the activity of inhibitory interneurons was directly visualized via acute brain slice imaging. Through the generation of our transgenic rats, we were able to identify two separate pathways leading to cortical remapping in the deprived S1. Pharmacological approaches [59] and guided neuroplasticity approaches [60] can be further used to specifically target mechanisms driving the changes in the activity of GAD1 neurons.

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Right median nerve electrical stimulation to hasten awakening from coma

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SUMMARY

Electrical stimulation of the right median nerve may hasten the awakening of closed head injured, comatose patients. A series of 25 comatose patients have been treated. These patients made better recoveries than similar individuals reported in the literature. In a double-blind pilot project patients in the treated group scored better on interval Glasgow Coma Scale scores, spent fewer days in the intensive care unit, and showed better Glasgow Outcome Scores at 1 month post-injury. Peripheral electrical stimulation of the right median nerve, through activation of the ascending reticular activating system, may be sufficient to arouse the moderate to severely comatose patient.

Remote changes in cortical excitability after experimental traumatic brain injury and functional reorganisation

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SUMMARY

Although cognitive and behavioral deficits are well known to occur following traumatic brain injury (TBI), motor deficits that occur even after mild trauma are far less known, yet are equally persistent. This study was aimed at making progress toward determining how the brain reorganizes in response to TBI. We used the adult rat controlled cortical impact injury model to study the ipsilesional forelimb map evoked by electrical stimulation of the affected limb, as well as the contralesional forelimb map evoked by stimulation of the unaffected limb, both before injury and at 1, 2, 3, and 4 weeks after using functional magnetic resonance imaging (fMRI). End-point c-FOS immunohistochemistry data following 1 h of constant stimulation of the unaffected limb were acquired in the same rats to avoid any potential confounds due to altered cerebrovascular coupling. Single and paired-pulse sensory evoked potential (SEP) data were recorded from skull electrodes over the contralesional cortex in a parallel series of rats before injury, at 3 days, and at 1, 2, 3, and 4 weeks after injury in order to determine whether alterations in cortical excitability accompanied reorganization of the cortical map. The results show a transient trans-hemispheric shift in the ipsilesional cortical map as indicated by fMRI, remote contralesional increases in cortical excitability that occur in spatially similar regions to altered fMRI activity and greater c-FOS activation, and reduced or absent ipsilesional cortical activity chronically. The contralesional changes also were indicated by reduced SEP latency within 3 days after injury, but not by blood oxygenation level–dependent fMRI until much later. Detailed interrogation of cortical excitability using paired-pulse electrophysiology showed that the contralesional cortex undergoes both an early and a late post-injury period of hyper-excitability in response to injury, interspersed by a period of relatively normal activity. From these data, we postulate a cross-hemispheric mechanism by which remote cortex excitability inhibits ipsilesional activation by rebalanced cortical excitation-inhibition.

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