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REVIEW ARTICLE

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# A novel paired associative stimulation protocol with a high-frequency peripheral component: A review on results in spinal cord injury rehabilitation

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## Abstract

In recent decades, a multitude of therapeutic approaches has been developed for spinal cord injury (SCI), but few have progressed to regular clinical practice. Novel non-invasive, cost-effective, and feasible approaches to treat this challenging condition are needed. A novel variant of paired associative stimulation (PAS), high-PAS, consists of non-invasive high-intensity transcranial magnetic stimulation (TMS) and non-invasive high-frequency electrical peripheral nerve stimulation (PNS). We observed a therapeutic effect of high-PAS in 20 patients with incomplete SCI with wide range of injury severity, age, and time since injury. Tetraplegic and paraplegic, traumatic, and neurological SCI patients benefited from upper- or lower-limb high-PAS. We observed increases in manual motor scores (MMT) of upper and lower limbs, functional hand tests, walking tests, and measures of functional independence. We also optimized PAS settings in several studies in healthy subjects and began elucidating the mechanisms of therapeutic action. The scope of this review is to describe the clinical experience gained with this novel PAS approach. This review is focused on the summary of our results and observations and the methodological considerations for researchers and clinicians interested in adopting and further developing this new method.

**KEYWORDS**

paired associative stimulation, peripheral electrical stimulation, spinal cord injury, transcranial magnetic stimulation

**Abbreviations:** GRASSP, Graded and Redefined Assessment of Strength Sensibility and Prehension; MI, motor imagery; MMT, manual muscle test; PAS, paired associative stimulation; PNS, peripheral nerve stimulation; RMT, resting motor threshold; (r)TMS, (repetitive) transcranial magnetic stimulation; SCI, spinal cord injury; tDCS, transcranial direct current stimulation.

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## 1 | INTRODUCTION

Spinal cord injury (SCI) has a devastating effect on the quality of life of an individual and constitutes a substantial economic burden for society (Chen & DeVivo, 2019). The estimated number of patients with SCI is over 2.5 million worldwide (Thuret et al., 2006). Although SCI accounts for only a small proportion of all injuries, the associated consequences make SCI one of the most life-changing injuries (Chen & DeVivo, 2019). Although several therapeutic approaches have been recently developed, only a few have progressed from laboratories to actual clinical practice (Jack et al., 2020; Ramer et al., 2014; Tohda & Kuboyama, 2011). There is a great demand for safe, non-invasive, and feasible treatments for both the acute and chronic stages of SCI.

The majority of SCIs are incomplete (Ackery et al., 2004; Chen & DeVivo, 2019; Fawcett et al., 2007). Moreover, non-functional connectivity can also be preserved in clinically complete injuries (Kirshblum & Solinsky, 2019; Squair et al., 2016). Over the last 40 years, pre-hospital management of SCI has dramatically improved, leading to an increased amount of spared residual connectivity and consequent improvement in prognosis after SCI (Whetstone, 2019). Strengthening these residual pathways through a wide range of non-invasive methods has gained considerable attention in human SCI research (Field-Fote, 2015; Field-Fote et al., 2016). Activity-based rehabilitation has been identified as number one in a top-10 research priority list for SCI (van Middendorp et al., 2014). Long-term potentiation (LTP) (Nicoll, 2017), the result of the cooperativity and associativity of neuronal activation, is one of the central targets in counteracting the weakness of the damaged connections after neuronal trauma and disease. Indeed, evidence from animal studies indicates that stimulation protocols inducing spike-time-dependent (STDP)-like plasticity between upper and lower motor neurons are promising approaches for strengthening the residual connections and promoting motor recovery (Ahmed, 2013; McPherson et al., 2015; Nishimura et al., 2013).

To this end, non-invasive stimulation techniques, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), are gaining increased attention as potential tools to improve rehabilitation outcomes through activity-dependent mechanisms (Rossini et al., 2015). TMS is a safe, non-invasive technique that enables the specific activation of part of the cortex (Rossi et al., 2009; Rossi et al., 2018). Cortical activation by TMS depends on the coil type (Deng et al., 2013), its positioning and orientation (Laakso et al., 2014), on the stimulation intensity, and on the type of navigation (Hannula & Ilmoniemi, 2017). When a figure-of-eight-coil (half value depth 0.9–3.4 cm and tangential spread 5 $cm^2$) (Deng et al., 2013) is combined with E-field navigation (Hannula & Ilmoniemi, 2017), motor cortex identification precision with no more than 2-mm error comparable to direct electrical stimulation can be achieved (Schmidt et al., 2015). Some clinical trials have been conducted on the efficacy of repetitive TMS (rTMS) in SCI patients. Multisession rTMS can induce some functional improvement in SCI individuals. Overall, the available data are inconsistent and likely depend on the parameters of the rTMS protocol and on the severity and level of SCI (Ellaway et al., 2014). tDCS delivers a continuous subthreshold current over the scalp. Anodal tDCS may promote neuroplasticity by depolarizing intracortical axons and pyramidal neurons and may lead to increased cortical excitability that alters the neuronal firing rate (Nitsche et al., 2004). A meta-analysis of randomized sham-controlled blinded clinical trials in SCI patients indicated functional recovery with a small effect size by anodal tDCS. However, no significant difference in muscle strength was observed between active and sham tDCS (de Araujo et al., 2020).

Paired associative stimulation (PAS) is a combination of TMS with electrical peripheral nerve stimulation (PNS). PAS for motor rehabilitation combines TMS of the primary motor cortex (M1) with PNS of the contralateral limbs (Chen & Udupa, 2009). The idea of PAS is to synchronously activate pre- and postsynaptic neurons to induce plastic changes by mechanisms utilizing LTP (Stefan et al., 2000). PAS-induced changes in neuronal connectivity represent a form of STDP (Stefan et al., 2000). STDP can result in either synaptic LTP or inhibition (LTD), depending on the timing of the pre- and postsynaptic stimuli (Dan & Poo, 2004). In healthy individuals, PAS can induce either LTP-like or LTD-like plasticity in the corticospinal tract (CST), as indicated by the facilitation or inhibition of motor-evoked potentials (MEPs; responses to TMS recorded from muscles). The interstimulus interval (ISI) between TMS and PNS is the strongest factor determining the polarity of the response (Suppa et al., 2017). The first PAS protocols targeted the cortical level (Stefan et al., 2000; Suppa et al., 2017). However, PAS stimuli can also be timed to converge on the spinal cord level (Taylor & Martin, 2009). PAS can be applied to upper and lower motor neurons; it thus appears to be an attractive possibility to strengthen weakened connectivity between these neuronal populations (Jack et al., 2020; Suppa et al., 2017).

Data on conventional PAS (single TMS and single PNS pulses) on functionally meaningful outcomes in neurological patients are scarce. A single session of conventional PAS resulted in a transient increase of MEP amplitudes (Carson & Kennedy, 2013) and transiently improved motor function in SCI patients (Bunday & Perez, 2012). Most PAS experiments have studied the efficacy of a single PAS session. A 4-week PAS improved leg muscle function in some stroke patients (Uy et al., 2003). Ten sessions of PAS, with parameters timed to occur at the spinal cord level and combined with exercise, modified motor performance in chronic SCI patients. Maximal voluntary contraction in targeted muscles increased after PAS with or without exercise but not after sham-PAS with exercise; this was preserved at 6 months in

the group receiving PAS with exercise. Graded and Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP) and the 10-m walk test outcomes did not differ significantly between the groups (Jo & Perez, 2020).

In addition to dependence on the exact determination of ISI, numerous other conditions can affect the presence, the polarity, and the magnitude of MEP amplitude changes after conventional PAS (Carson & Kennedy, 2013). The outcome can depend on genetic factors, neuroanatomical differences, age, alertness, and focus of attention at the time of the experiment, time of the day, and medication, among others (Carson & Kennedy, 2013; Suppa et al., 2017). Conventional PAS protocols are excellent for studying and inducing cortical plasticity in humans. However, we believed that the demand for high timing precision and dependence on the aforementioned factors might not be suitable for clinical work. Measurements of SCI patients can be hampered by muscle spasticity and small or absent MEPs, medications affecting the central nervous system that cannot be avoided, and the fact that stimulation cannot always be performed at the same time of the day, among other considerations. Moreover, when timing is critical for strengthening plasticity, erroneous timing might lead to weaker connectivity (Jack et al., 2020). If PAS was applied in multiple successive sessions to enhance corticospinal connections as a tool for long-term rehabilitation, the initially calculated ISI might need to be adjusted constantly, as neuronal conductivity may change over time as a result of stimulation.

To this end, we developed a modified version of PAS that utilizes high-intensity TMS and high-frequency PNS ("high-PAS") and depends less on the detailed precision of the stimulation parameters. The scope of this review is to present the clinical experience gained with this novel PAS approach, which has been developed by our group since 2016 (Shulga, Lioumis, et al., 2016). We refer to other recent, excellent reviews on neuromodulation approaches in SCI, including conventional PAS, for a broad overview of the field (Carson & Kennedy, 2013; Christiansen & Perez, 2018; Field-Fote, 2015; Jack et al., 2020; Suppa et al., 2017). Here, we provide a summary of our clinical results in SCI subjects as well as our work in healthy subjects aimed at optimizing the PAS protocol. We also share observations and considerations for those interested in adopting and further developing this new method.

## 2 | NOVEL MODIFIED PAS PROTOCOL (HIGH-PAS): STIMULATION SETUP AND RATIONALE FOR PARAMETER SELECTION

The current recommendation for conventional PAS includes a single TMS pulse at the optimum cortical stimulation site (hotspot) of the target muscle at an intensity producing a MEP of 1 mV in a small hand muscle (Suppa et al., 2017). PNS is delivered either as single pulses (Stefan et al., 2000) or in 10-Hz trains (McKay et al., 2002). As described above, conventional PAS may employ fixed ISIs between TMS and PNS (Carson & Kennedy, 2013), or if individually adjusted, exact determination of ISIs is required to ensure correct timing of the stimuli (Bunday & Perez, 2012).

The STDP model in which synaptic input to dendrites is active just before a somatic input is now regarded as highly simplified (Suppa et al., 2017). Spike timing is not the only requirement to induce plasticity; it depends also on the firing rate, postsynaptic voltage, and synaptic cooperativity (Feldman, 2012). For example, in experiments using brain tissue slices, connections exhibited Hebbian STDP only when presynaptic and postsynaptic spikes occurred at moderate firing rates (10–20 Hz); higher firing rates (>30 Hz) induced LTP independent of spike timing (Feldman, 2012).

High-PAS utilizes high-frequency PNS trains and TMS pulses given at 100% of stimulator output (Figure 1). The idea is to generate multiple orthodromic volleys in the corticospinal tract by high-intensity TMS pulse (see below) and multiple antidromic activations by a peripheral stimulus train. Importantly, in cellular-level studies, multiple interactions induce LTP-like effects that overcome their long-term depression (LTD)-like counterparts (Sjostrom et al., 2001). Therefore, it is plausible that the net result of multiple collisions at the level of the spinal cord would be an LTP-like effect. Indeed, we have shown that this protocol effectively potentiates MEPs, indicating LTP-like plasticity, even when errors of ±10 ms are introduced into the calculation of ISI (Shulga et al., 2016). We have shown that in the lower limbs, high-PAS with 100-Hz PNS upregulates MEPs in healthy subjects by 100%–150% (Figure 2) with a responder rate of 100% (Mezes et al., 2020; Tolmacheva et al., 2019). The corresponding values for the upper limbs in healthy subjects are yet to be determined.

## 3 | CLINICAL IMPROVEMENTS IN CHRONIC SCI PATIENTS

Regardless of the level of completeness, most of the recovery after SCI occurs within 6 months post-injury and the rate of change plateaus at 9 months post-injury (Oleson & Flanders, 2019). Although some motor recovery may continue for 2 years or more, the degree is generally small and unlikely to improve function significantly (Oleson & Flanders, 2019). We have recruited patients at the chronic phase (1–15 years post injury) to investigate the impact of 4–12 weeks of high-PAS (up to over a year in one patient (Rodionov et al., 2019)) on motor performance and other outcomes. As extensive spontaneous recovery at

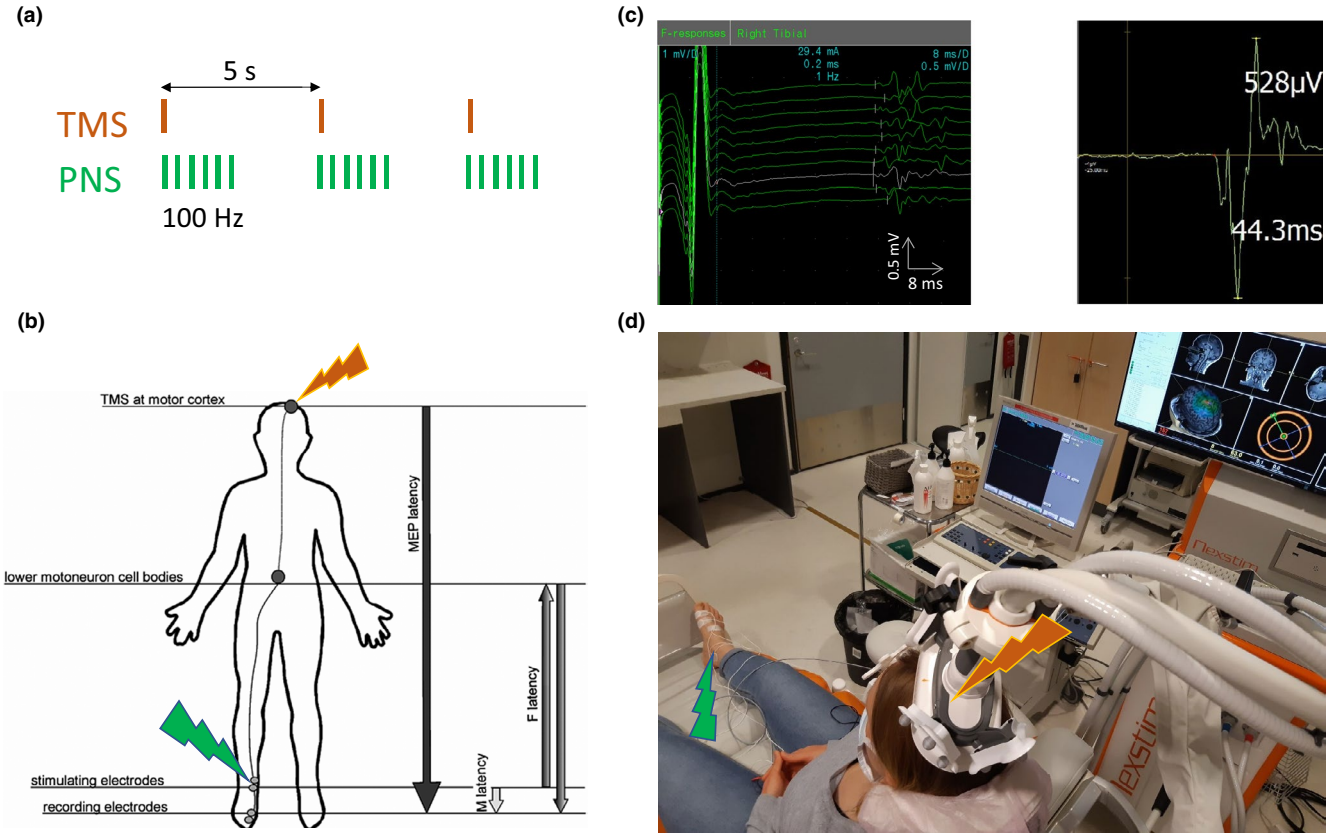


**FIGURE 1** PAS protocol. (A) PAS stimulation pattern. TMS – transcranial magnetic stimulation, PNS – peripheral electrical nerve stimulation. (B) Schematic representation of PAS setup. F-latency and MEP latency are measured before the PAS session to calculate the interval between TMS and PNS with the formula [$F_{latency} - MEP_{latency}$]. See Shulga et al., (2015) for the calculation yielding the formula. (B, D) stimulation of the tibial nerve (green lightning) and corresponding M1 hotspot (orange lightning). (C) Representative F-responses (left) and motor-evoked potential (MEP) (right) recorded from right tibial nerve. (D) Photo of the setup

this stage is highly improbable, the patients served as their own control. We have by now reported altogether 20 patients, all of whom benefited from PAS, as described below (Rodionov et al., 2019, 2020; Shulga, Lioumis, et al., 2016; Shulga et al., 2020; Tolmacheva, Savolainen, et al., 2019; Tolmacheva et al., 2017; Vaalto et al., submitted). The clinical characteristics of these patients are summarized in Table S1. PAS can be applied to upper (Rodionov et al., 2019; Shulga, Lioumis, et al., 2016; Tolmacheva, Savolainen, et al., 2019; Tolmacheva et al., 2017) and lower (Rodionov et al., 2020; Shulga, Lioumis, et al., 2016; Shulga et al., 2020) limbs. It increases manual muscle test (MMT) scores in both traumatic (Rodionov et al., 2019, 2020; Shulga, Lioumis, et al., 2016; Shulga et al., 2020; Tolmacheva et al., 2017) and neurological (Rodionov et al., 2020; Tolmacheva, Savolainen, et al., 2019) SCI and in both paraplegic (Shulga, Lioumis, et al., 2016; Shulga et al., 2020) and tetraplegic (Rodionov et al., 2019, 2020) patients. In all studies, PAS was conducted in parallel with continuous conventional and individually tailored rehabilitation (Table S1), which remained the same as before PAS and was not influenced by the researchers.

### 3.1 | Manual motor test

Patients were evaluated by Manual Muscle Testing (MMT) (Hislop et al., 2014) on 0–5 scale and with functional tests. The interpretation of MMT values is listed in Table 1. MMT scores ≥3 are considered as functional (Oleson & Flanders, 2019). In each study, we calculated the average of the change in MMT score for each muscle. Only the muscles with abnormal values in the initial evaluation were considered.

After documenting the increase in MMT in two pilot patients, one para- and one tetraplegic (Shulga, Lioumis, et al., 2016), we conducted three case series (5 patients each) where MMT increased in each individual patient of each series (Rodionov et al., 2020; Tolmacheva, Savolainen, et al., 2019; Tolmacheva et al., 2017). We observed that upper limb PAS for 4 weeks increased MMT on average by 1 point, measured at follow up across all hand muscles in patients with traumatic injury (Tolmacheva et al., 2017). Thereafter, we further developed the PNS settings (Tolmacheva, Makela, et al., 2019) (see "PNS settings" below). With this new protocol applied for 6 weeks, we achieved a 1.7-point average increase in upper

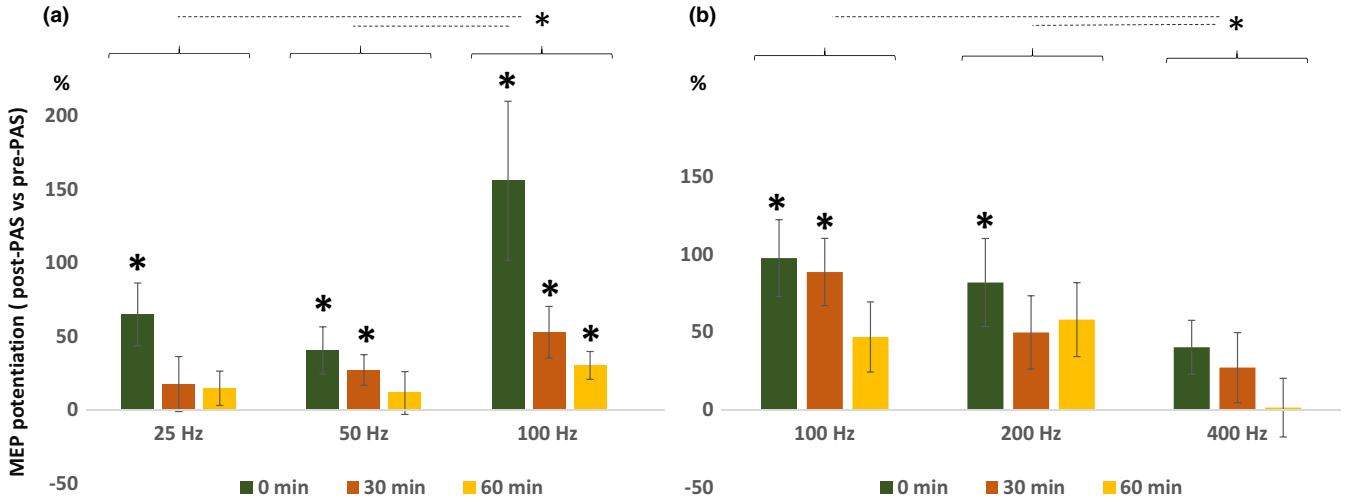


**FIGURE 2** Results from (Tolmacheva, Makela, et al., 2019) (A) and (Mezes et al., 2020) (B) showing that the strongest and most durable MEP potentiation is obtained with the 100-Hz PNS. (A) 100 Hz was significantly more efficient than 50 Hz and 25 Hz (star above the brackets) and was the only protocol that significantly potentiated MEPs at 0 min, 30 min, and 60 min after PAS (stars above the columns). See Figure 2b in (Tolmacheva, Makela, et al., 2019) for pre-and post-PAS raw data. (B) 100 Hz was the only protocol that significantly potentiated MEPs at 0 min and 30 min after PAS (stars above the columns). The 400-Hz protocol was significantly weaker than 100 Hz and 200 Hz (star above the brackets). See Supplementary Data in Mezes et al., (2020) for raw data

**TABLE 1** Manual Muscle Test (MMT) evaluation scale. ROM indicates range of movement.

| Value | Meaning |
|---|---|
| 0 | No visible or palpable contraction. |
| 1 | Visible or palpable contraction. |
| 2 | Full ROM gravity eliminated. |
| 3 | Full ROM against gravity. |
| 4 | Full ROM against gravity, moderate resistance. |
| 5 | Full ROM against gravity, maximum resistance. |

limb muscles in patients with non-traumatic SCI, measured in the follow-up (Tolmacheva, Savolainen, et al., 2019). We have also applied 8 weeks of stimulation to lower limbs of tetraplegic patients (both traumatic and non-traumatic) and observed a 1.2-point average MMT increase across all muscles (Rodionov et al., 2020). It is noteworthy that as MMT measures function against gravity, larger strength changes in the leg muscles are required to reach similar MMT results as in the hand muscles. In a case study where PAS was given as long as improvement was ongoing, we achieved an MMT increase of 3.2/1.8 in the right/left hand, which led to full MMT scores at the end of the experiment (Rodionov et al., 2019) (see also Table 2).

## 3.2 | Functional gains

Apart from MMT, we have also observed significant improvements in functional hand tests. In a study with non-traumatic tetraplegic patients, at 6-month evaluation we documented a 35% increase in Box and Block test results, a 60% increase in palm pinch test, and a 110% increase in grip dynamometry after upper limb PAS (Tolmacheva, Savolainen, et al., 2019). We also observed an average 22% improvement in walking speed in ambulatory participants after lower limb PAS (Rodionov et al., 2020). Subjective functional improvements were also reported by many patients who underwent 4–12 weeks of PAS. For example, patients reported improved use of the stimulated hand for hair washing, food slicing, dressing, handling a steering wheel (Tolmacheva, Savolainen, et al., 2019), opening doors, or opening bottles (Tolmacheva et al., 2017). Patients also reported generally more versatile use of the hands (Tolmacheva, Savolainen, et al., 2019; Tolmacheva et al., 2017). Eight weeks of PAS for lower limbs improved Spinal Cord Independence Measure (SCIM) in two patients of five by 15 and 9 points, mostly in Mobility subscales (Rodionov et al., 2020). In one patient, 12 weeks of PAS 1 year after injury enabled the participation in walking rehabilitation deemed futile before PAS, and improved walking distance by more than 100% when repeated for another 12 weeks 2 years after the injury. The patient was originally non-ambulatory; after the whole intervention he ambulated 50% of the time at home (Shulga et al., 2020), i.e., in this patient PAS was the major factor that restored the ability to walk. A remarkable increase in SCIM (especially in self-care, see Table 2) was achieved in a case study (Rodionov et al., 2019) where PAS was applied to the hands for as long as they improved (for over a year). Before PAS, the subject needed total or partial assistance in eating, bathing,

**TABLE 2** Spinal Cord Independence Measure (SCIM) self-care subscore before and after high-PAS was applied for as long as improvement continued (for over 1 year) (Rodionov *et al*., 2019)

| BEFORE, score and meaning | AFTER, score and meaning |
|---|---|
| 1. Needs partial assistance for eating and/or drinking, or for wearing adaptive devices | 3. Eats and drinks independently; does not require assistance or adaptive devices |
| 1. Requires partial assistance for bathing upper body (soaping, washing, drying body and head, manipulating water tap) | 3. Washes independently; does not require adaptive devices or specific setting |
| 0. Requires total assistance with dressing upper body (clothes, shoes, permanent orthoses: dressing, wearing, undressing) | 3. Independent with clothes without buttons, zippers or laces; does not require adaptive devices or specific settings |
| 0. Requires total assistance with transfer from bed to wheelchair | 2. Independent with transfer from bed to wheelchair |
| 1. Requires partial assistance with grooming (washing hands and face, brushing teeth, combing hair, shaving) | 3. Grooms independently without adaptive devices |

dressing, and grooming. During the follow up, he could perform these tasks independently and without adaptive devices. In all patients who were able to utilize the improved muscle activity in daily life, the achieved scores either persisted or often even improved during the 1–4 month follow up. See Supplementary Links for example patient videos.

### 3.3 | Other effects

In all of our patients, the results of sensory testing performed according to American Spinal Injury Association impairment scale (AIS) classification did not change significantly. Spasticity measured by the modified Ashworth scale was also not affected.

Eight of 20 patients had neuropathic pain, which was mild to moderate in 7 patients (Rodionov et al., 2019, 2020; Shulga, Lioumis, et al., 2016; Shulga et al., 2020; Tolmacheva et al., 2017) and severe in 1 (Vaalto et al., submitted). Neuropathic pain decreased or unpleasant sensations disappeared in seven patients. A more detailed summary on neuropathic pain results is presented in Vaalto et al. (submitted).

## 4 | IMPLEMENTATION OF PAS

### 4.1 | Pre-PAS measurements and calculations

Although the implementation of PAS is individualized for each patient, it follows the same procedures for everyone. Below, we describe the setup and the underlying rationale. TMS is always delivered at 100% SO, whereas PNS intensity and the ISI between PNS and TMS are individually adjusted. Depending on the stimulation setup and need of the patient, nerves to be stimulated are selectively chosen based on the distribution of the weakness. One or both limbs can be chosen for stimulation. Preparation for PAS includes measurements of F-responses and mapping the motor cortex and measurement of MEP latencies (Figure 1).

#### 4.1.1 | F-responses and determination of PNS intensity

F-waves are late orthodromic responses produced by a pool of motoneurons that is antidromically activated by peripheral motor or mixed-nerve stimulation. F-waves enable assessment of transmission between the stimulation site in a limb and the corresponding motor neuron in the spinal cord. The afferent and efferent pathway for the F-wave is the alpha motor neuron without an intervening synapse. Thus, F-waves reflect conduction to and from the spinal cord. The conduction velocity is identical in afferent and efferent fibers (Mesrati & Vecchierini, 2004). As we sought to estimate conduction along the entire motor axis in the upper and lower motor neurons, we used F-responses to define individual ISI between TMS and PNS (Shulga et al., 2015). For comparison, H reflex is a proprioceptive reflex elicited by posterior pathway activation. It is a muscle reaction to electrical stimulation of Ia sensory fibers activating the motoneuron pool and the efferent motor fibers. The conduction velocity is more rapid on afferent than on efferent fibers (Mesrati & Vecchierini, 2004). H reflex is a probe to study sensorimotor integration (Knikou, 2008) and thus is not an optimal tool for study of purely motor conduction.

First, 10 responses to 0.2-ms pulses at supramaximal intensity are recorded from the muscles innervated by the nerves to be stimulated (Table 3) and minimum F-latency is determined for ISI calculation (see "Calculation of ISI"). Thereafter, F-responses to single 1-ms pulses are recorded and the minimum PNS intensity required to produce persistent F-responses is determined; this intensity defines the

**TABLE 3** The positions of the stimulating electrodes and the corresponding muscles for MEP/F-recordings for each stimulated nerve. If MEPs cannot be found from the designated muscles, alternative muscles innervated by the same nerve can be used. In case of two different movements, the patient is instructed to do each movement 50% of the time. However, clearly weaker movements can be done 100% of the time. Other movements that particularly need improvement and are innervated by the selected nerve can also be chosen (Rodionov *et al*., 2019)

| Nerve | Stimulating electrode location | Movement for preactivation or motor imagery | Muscle for F-response and MEP measurements |
|---|---|---|---|
| Median | palmar wrist (carpal tunnel) | opposition of I-II-III fingers | Abductor pollicis brevis (APB) |
| Ulnar | at the wrist proximally to Guyon canal | spreading fingers, flexion of IV-V fingers | Abductor digiti minimi (ADM) |
| Radial | proximally to lateral epicondyle; electrodes are pressed against the skin to ensure the contact between electrodes and nerve | wrist and finger extension | Extensor digitorum or brachioradialis |
| Femoral | crossing of the inguinal crease and femoral artery; the electrodes are slightly pressed manually to ensure that the stimulation reached the nerve. The contraction of the quadriceps muscle during femoral nerve stimulation is monitored and the optimal site of stimulation is adjusted to achieve maximal contraction. | hip flexion, knee extension | Vastus medialis |
| Tibial | behind the medial malleolus | plantarflexion, knee flexion | Abductor hallucis (AH) |
| Peroneal | the frontal midline of the ankle | dorsiflexion | Extensor digitorum brevis (EDB) |
| Gluteal | the electrode placement is determined by an anatomical landmark centred at the ischial tuberosity (59); a tape roll (45×25 mm) is attached on top of the electrodes and the patient sits on it, thus pressing the electrodes towards the nerve | gluteal muscle contraction, hip abduction | Gluteus maximus |

individual PNS intensity for each nerve. This procedure ensures that the motoneurons of the spinal cord are stimulated (Mesrati & Vecchierini, 2004).

### 4.1.2 | Motor cortex mapping and MEP latency

The selection of muscles for motor cortex mapping depends on the peripheral nerves to be stimulated. The list of nerves that we have stimulated and the corresponding muscles are presented in Table 3. For example, for median nerve PAS, we defined the TMS hotspot in the primary motor cortex (M1) for abductor pollicis brevis (APB). Mapping starts at the presumed anatomical location of the representation of the corresponding muscle at the intensity slightly above the resting motor threshold (RMT) of this area. The location and direction of the coil are thereafter varied to define the sites (hotpots) where TMS elicits the largest and most consistent MEPs recorded with the surface electrodes placed on the corresponding muscle belly. If the RMT is over 100% of the maximum stimulator output (MSO) of the TMS device, mapping is performed with motor pre-activation. Motor imagery (MI) can also be utilized. Mapping requires some experience, as in some patients the need to use pre-activation, the abnormal appearance of MEPs, and contamination of the electromyogram (EMG) by spasticity make mapping more challenging than in healthy subjects or in some other patient groups.

Once the hotspot is identified, we obtain 15 MEPs at 100% SO (the same intensity that will be used for the stimulation) and calculate their average latency; this value is used for the calculation of ISI (see "Calculation of ISI"). If a follow up of MEP amplitudes is planned before and after the PAS period, MEP amplitudes should also be measured at 120% RMT,

according to conventional standards. However, in our experience, MEP evaluation before and after PAS in SCI patients is strongly compromised by the variability in spasticity, which affects MEP amplitudes. RMT can also be measured at this stage for follow up; in some SCI patients RMT is over 100% SO (see also "Motor imagery and pre-activation").

#### 4.1.3 | Calculation of ISI

During high-PAS, each TMS pulse is synchronized with the first pulse of PNS train at a pre-calculated ISI. The ISI is calculated by the formula [$F_{latency} - MEP_{latency}$] to make the stimuli coincide at the level of the spinal cord. This formula utilizes minimum F-latency (see "F-responses and determination of PNS intensity") and mean latency of 15 MEPs (see "Motor cortex mapping and MEP latency"), thus emphasizing the fastest conducting peripheral fibers and all central fibers (Shulga et al., 2015). The calculations that yielded the formula are presented in Shulga et al., (2015) (see also Figure 1). Although ISI is individually determined for each nerve, we have shown that small inaccuracies in defining ISI (e.g., due to challenges in recordings of F-response or MEP) do not abolish the PAS effect on MEP potentiation (Shulga, Zubareva, et al., 2016) (see also "Potential mechanisms of action").

### 4.2 | PAS session

TMS of each muscle representation area is paired with PNS of the nerve innervating that muscle to activate all major muscle groups of the upper or lower limb. The nerves and corresponding muscles for hotspot determination are listed in Table 3. If MEPs are not elicited from the muscles indicated in Table 3, other muscles that are innervated by the nerve of interest can also be used.

#### 4.2.1 | PNS settings

The stimulating electrode locations are listed in Table 3. PNS consists of trains of six 1-ms pulses delivered at 100 Hz. In our first protocols, we used PNS of 50-Hz frequency with clinically beneficial outcome (Shulga, Lioumis, et al., 2016; Shulga, Zubareva, et al., 2016; Tolmacheva et al., 2017). We subsequently demonstrated in healthy subjects that 100-Hz PNS potentiates MEPs more than 50-Hz PNS (Tolmacheva, Makela, et al., 2019). Thereafter, we successfully used 100-Hz PNS in our clinical studies (Rodionov et al., 2019, 2020; Shulga et al., 2020; Tolmacheva, Savolainen, et al., 2019). Tests of 200-Hz or 400-Hz PNS did not potentiate MEPs more than 100-Hz PNS (Mezes et al., 2020) (Figure 2).

The determination of the individual PNS intensity based on F-response measurement is described in "F-responses and determination of PNS intensity". Some patients can perceive PNS as unpleasant, particularly at the beginning of the session. Anesthetizing the skin with EMLA lidocaine–prilocaine ointment reduces these unpleasant sensations (Gajraj et al., 1994). EMLA penetrates 3–5 mm into the skin (Gajraj et al., 1994) and thus does not affect the conductivity of the stimulated nerve. Moreover, gradual adaptation to PNS takes place. The first stimulation session is usually started with the stimulation intensity below the targeted intensity. The intensity is gradually increased during the session, and the tolerability of the stimulation after each step is carefully confirmed. The target intensity is usually achieved during the first session, but sometimes two to three sessions are needed for full adaptation. Ensuring the patient that the stimulation intensity can be reduced immediately at their request is essential in preventing possible anxiety associated with the stimulation. In some SCI patients, skin sensation is weakened or absent, providing a natural anesthesia. This makes adaptation to PNS easier and is not a contraindication for PNS (see also "Safety"). Only a minority of our patients chose to use EMLA continuously.

All proximal muscles of the upper limb cannot be covered by the stimulation of the corresponding nerves, as the nerves innervating these muscles are deep and not accessible to PNS. However, in both upper and lower limbs, some improvements were also observed in muscles not directly stimulated (Rodionov et al., 2020; Shulga, Lioumis, et al., 2016; Tolmacheva, Savolainen, et al., 2019; Tolmacheva et al., 2017). Spreading of the activation to the neighboring motor cortex and peripheral nerves adjacent to stimulated ones is possible; there are also alternative explanations for this phenomenon (see "Potential mechanisms of action").

#### 4.2.2 | TMS intensity

TMS is delivered at 100% during PAS. As described above, this plausibly generates multiple orthodromic volleys, increasing PAS efficacy. All our patients have tolerated this intensity without adverse events. During hand representation area stimulation, slight face sensations or twitches may occur in some patients; leg area stimulation is targeted more medially and does not induce such sensations.

#### 4.2.3 | PAS frequency and duration of stimulation

Paired stimulations are delivered at 0.2 Hz. The initial selection of the frequency was based on conventional PAS protocols (Carson & Kennedy, 2013). We have later shown that

higher frequency of 0.4 Hz with an identical total number of pulses is not as effective in potentiating MEPs in healthy subjects (Mezes et al., 2020). We have not investigated the effect of lower frequencies, as prolonging the PAS session would not be feasible in the clinical setting.

The duration of stimulation for each peripheral nerve-M1 hotspot pair is 20 min (240 PAS pulses). The time required for preparations is usually approximately 30 min. A session for the upper limb, including medial, ulnar, and radial nerves, thus takes about 1.5 hr. The corresponding time for the lower limb session (4 nerves) is 1 hr 50 min. We have limited the total duration of a therapy session to a maximum of 3 hr; to strengthen both lower limbs, 6 nerves innervating the weakest muscles were stimulated (Rodionov et al., 2020).

We have stimulated patients 5 days per week during the first 2 weeks and 3 days per week thereafter. In therapy of a patient who was stimulated for over 1 year, we had occasional breaks of 1–2 weeks as long as improvement was observed (Rodionov et al., 2019).

### 4.2.4 | Motor imagery and pre-activation

Attention is crucial for the success of conventional PAS protocols; stimulation failed to induce plasticity when the subject's attention was directed away from the hand targeted by PAS (Stefan et al., 2004). Therefore, in our first clinical protocols, we combined PAS to motor imagery (MI) (Shulga, Lioumis, et al., 2016; Tolmacheva, Savolainen, et al., 2019; Tolmacheva et al., 2017). In addition, decreasing the motor thresholds by MI may enhance generation of multiple anti- and orthodromic volleys, which is the goal of the stimulation (see "Potential mechanisms of action"). Furthermore, MI also plausibly activates secondary motor cortical areas, which enhances the effectiveness of the stimulation (Carrillo-de-la-Pena et al., 2008).

We have shown that in a high-PAS protocol, MI is not required for MEP amplitude potentiation in healthy individuals (Shulga, Zubareva, et al., 2016). However, this observation should be interpreted with caution when designing protocols for neurological patients (due to their higher RMTs). We have not yet conducted studies without MI or pre-activation in SCI patients. Although we have shown that MI is not required in healthy subjects, we conduct all healthy subject experiments with MI to closely mimic the situation with a SCI patient.

Slight motor pre-activation can presumably serve the same purpose as MI. The neural networks activated in the microelectrode recordings from the primary motor cortex of SCI patients during mental imagery and attempted movements appear to be overlapping, albeit with slight differences (Vargas-Irwin et al., 2018). In one study, we used pre-activation in the muscles with RMT exceeding 100% of MSO (to ensure that TMS reaches the spinal cord level) and MI in the remaining muscles (Rodionov et al., 2019). A slight motor pre-activation appeared to be easier for patients to accomplish than MI and was also easier to instruct, control, and correct. Consequently, we have used slight pre-activation regardless of RMT in later studies (Rodionov et al., 2020; Shulga et al., 2020). Moreover, pre-activation can potentiate the effects of conventional PAS in SCI patients and healthy subjects (Bunday et al., 2018).

### 4.2.5 | Other considerations

Based on the considerations presented in "Motor imagery and pre-activation", the patients were not allowed to watch TV or use their smartphone, to have long discussions with the therapist, or to engage in other distracting activities during the session; silent concentration on the movement was a condition of choice. One patient who found the long sessions particularly boring listened to music of his own choice during the PAS with motor pre-activation (Shulga et al., 2020). This did not prevent the therapeutic effect. It would be interesting to study if listening to music has an additional facilitatory or inhibitory therapeutic effect (Sarkamo & Soto, 2012; Thaut, 2005).

Some patients adapt to the stimulation so completely that they fall asleep during the stimulation; overall tiredness unrelated to high-PAS can predispose to this tendency. This should be avoided, as sleep may profoundly change functional connectivity and motor thresholds, making the therapeutic response unpredictable. MI or pre-activation is also not performed while the patient is sleeping. Listening to music might help maintain vigilance (Davenport, 1972).

A proper comfortable position of the patient is particularly important during long sessions to avoid neck tension and related headache. In our studies, the patients were seated in a comfortable armchair provided by the manufacturer of the TMS device in a semi-seated position. If transfers to and from the wheelchair are not feasible, PAS delivered to the patient sitting in a wheelchair can also be considered.

Our patients continued their normal medication regimen during high-PAS and follow-up periods. Medication for pain, spasticity, or other indications used by our patients represented the usual repertoire for this patient group and included baclofen, tizanidine, pregabalin, clonazepam, tramadol, zopiclone, mirtazapine, and amitriptyline, among others (Table S1). These drugs did not prevent the therapeutic effect of high-PAS. It is not possible to say in each single case if the drugs diminished, enhanced, or did not influence the outcome. This important topic requires further investigation.

# 5 | POTENTIAL MECHANISMS OF ACTION

## 5.1 | Spinal level

The level of reorganization that can occur both supraspinally and spinally within the sensorimotor system is far greater than previously assumed. A high level of motor control seems possible with relatively few descending axons extending below the level of the lesion (Edgerton et al., 2019). As outlined above, multiple antidromic activations triggered by high-frequency PNS trains and multiple orthodromic volleys triggered by 100% SO TMS pulses lead to multiple interactions. As interactions leading to LTP-like effects overcome their long-term depression (LTD)-like counterparts (Sjostrom et al., 2001), the net result of multiple collisions at the level of the spinal cord is an LTP-like effect. Due to the use of the [$F_{latency} - MEP_{latency}$] formula, the arrival timing between the pre- and postsynaptic volleys at the spinal cord level is considerably closer than possible interactions occurring at the cortical level; it is thus plausible that the observed MEP potentiation originates mainly from induction of plasticity at corticomotoneuronal synapses of the spinal cord. Corticospinal fibers terminate both on interneurons and directly on alpha and gamma motoneurons of the spinal cord (Goshgarian, 2019). Thus, high-PAS might both reroute interneurons (Ling et al., 2020) and directly strengthen synaptic connections between upper and lower and motor neurons, which leads to the net effect of improved corticospinal conduction.

High-intensity TMS can induce one D-wave and four I-waves with an interval of approximately 1.5 ms in between within a time window of no longer than 10 ms (Rothwell et al., 1991). A TMS pulse administered at 100% SO activates several neural populations of variable conductivity (Edgley et al., 1997). Activation also occurs in the wider vicinity of the stimulated cortical site, resulting in inducing neuronal firing of the neighboring cortex in a slightly different time frame. In neurological patients, this temporal dispersion of responses of different neural populations can be even broader. Increasing the frequency of PNS increases the probability for coincidence of ascending and descending volleys within the effective time window for inducing an LTP-like effect. Moreover, it is plausible that the remaining neural pathways in patients with neurological diseases have a wide range of conductivities due to partial injuries and partial recovery, leading to dispersion of activations.

We have demonstrated in healthy subjects that high-PAS induces robust potentiation of MEPs at a wide range of intervals between TMS and PNS and tolerates at least ±10 ms errors in ISI determination (whereas conventional PAS protocols require a very narrow, precisely defined interval) (Shulga, Zubareva, et al., 2016). We have also shown that the protocol is resilient to small errors in mapping, which may be a reality in clinical practice (Tolmacheva, Makela, et al., 2019). This insensitivity to errors may be one of the reasons why this protocol has led to favorable clinical outcomes. Although some functional and anatomical reorganization after SCI occurs spontaneously, the efficacy of the neural pathways to rewire themselves is use dependent (Edgerton et al., 2019). Temporally and spatially dispersed anti- and orthodromic volleys produced by high-PAS plausibly affect a wide net of connections that are strengthened upon multiple repetitions. Therefore, although our protocol is resilient to initial errors in the exact determination of ISI, exact repetition of the same stimulation setup (ISI, electrode placement, TMS navigation, stimulation intensities) is probably important for the therapeutic effect.

We have demonstrated that although 100-Hz PNS is more efficient than 50- and 25-Hz PNS (Tolmacheva, Makela, et al., 2019), further increase in frequency of PNS up to 200 and 400 Hz does not provide additional efficacy (400 Hz was less efficient than 100 Hz) (Mezes et al., 2020). Thus, bringing the PNS frequency closer to the frequency of I-waves (Rothwell et al., 1991) does not produce stronger potentiation (Mezes et al., 2020); the exact coincidence of each PNS pulse with each TMS-induced volley does not appear to be the strongest determining factor for MEP potentiation. Rather, the specific frequency of PNS appears to be important, although PNS by itself does not produce MEP potentiation (Tolmacheva, Makela, et al., 2019). LTP is thought to have early and late phases; plasticity-promoting events of the early phase lead to the stable late-phase LTP (L-LTP) if sufficiently repeated (Panja & Bramham, 2014). L-LTP is dependent on protein synthesis and is associated with enlargement and remodeling of postsynaptic density (PSD), enlargement of pre-existing dendritic spines, and de novo synapse formation (Panja & Bramham, 2014). Brain-derived neurotrophic factor (BDNF) is thought to play a critical role in stimulating the formation of L-LTP at glutamatergic synapses (Panja & Bramham, 2014). Importantly, the 50–100 Hz stimulation induces activity-dependent release of BDNF in vitro (Balkowiec & Katz, 2000; Gartner & Staiger, 2002; Lever et al., 2001).

We have shown that increasing the frequency of PAS from 0.2 Hz to 0.4 Hz and increasing the frequency of PNS to >100 Hz diminishes the efficacy of the high-PAS protocol (Mezes et al., 2020). Consistently, when vagus nerve stimulation (VNS) was paired with an auditory stimulus to induce recovery-promoting plasticity, shortening the interval between VNS-tone pairs also reduced plasticity and abolished therapeutic effect in the auditory cortex (Borland et al., 2018). The authors concluded that longer intervals between the events generate more plasticity and better recovery because the structural changes underlying these improvements may require several seconds to minutes to develop

(Borland et al., 2018). The possible role of activity-dependent plasticity-inducing molecules (such as neurotrophins) in the PAS effect might explain why sufficiently low frequencies are required. A frequency that is too high might deplete relevant components of the neurotrophin release machinery, such as vesicles and calcium stores. This would not allow sufficient time for plastic response to occur and therefore would render particularly the long-term plasticity less effective.

When only the weaker hand was stimulated, some (albeit smaller) improvements were also observed in the contralateral hand (Tolmacheva, Savolainen, et al., 2019). Peripheral nerve lesions affect spinal cord structures on the side of the peripheral nerve, but also modify contralateral, non-lesioned structures. These contralateral modifications are qualitatively similar but smaller and less persistent (for a review, see Koltzenburg et al., 1999). It is possible that unilateral PAS improves both the ipsilateral and contralateral modified spinal cord networks by normalizing the excitation-inhibition balance at the intraspinal level (Koltzenburg et al., 1999). Epidural electric stimulation (EES) has recently been shown to be useful in rehabilitation of patients with SCI; EES has been suggested to mediate its effect on stimulation of dorsal roots that mediate proprioceptive information from the limbs and activate spinal networks regulating automated motor programs (Formento et al., 2018). PNS applied to the nerve trunks containing both motor and sensory activation can naturally activate proprioceptive input to the spinal cord and contribute to such reorganization.

Although we stimulate M1 in our protocols, it is highly plausible that corresponding S1 areas are also activated by TMS delivered at 100% SO. Peripheral sensory nerves are also activated by PNS. However, we have not observed any improvement in the sensory outcomes. It has recently been proposed that the lack of sensory improvement in our PAS work and those of others were due to anatomical reasons. Stimulation might strengthen the connections between neighboring neurons and reroute interneurons leading to new connections between cortical and motor neurons, but it cannot induce regeneration of the sensory axons that have disconnected from their cell bodies in the dorsal root ganglion and have degenerated (Ling et al., 2020). The ISIs are not optimized for sensory tracts, and S1 stimulation intensity is naturally weaker than M1 stimulation intensity. The effect of PAS on the sensory system merits further research, and probably requires more sensitive measurement than the 0–2 scale of the AIS examination sheet. It is also not clear why we did not observe an effect on spasticity. The modified Ashworth scale measures spasticity only in the large joints and therefore might lack sensitivity to detect smaller changes in the fingers. Spasticity is multifactorial and is also strongly affected by spasticity medication. As we did not change the medication of our patients, this might be another confounding factor in spasticity evaluation.

## 5.2 | Cortical level

A severe impairment in one limb might worsen the impairment of the contralateral limb through unfavorable interhemispheric cortico-cortical or intraspinal neuronal interactions. For example, patients with chronic post-stroke motor impairment recruit larger portions of secondary motor areas than patients with no residual impairment (Ward & Cohen, 2004). Larger activation of supplementary motor areas was also observed in patients with incomplete SCI (Sharp et al., 2017; Zdunczyk et al., 2018); this increased activation might impair the function of the less-affected limb via interhemispheric inhibition (Boddington & Reynolds, 2017). When the weakest nerves were selected for stimulation, achieving a more normal state in the sensorimotor network governing the more severely affected parts might also alleviate other impairments through normalization of the excitation-inhibition balance at the cortical level (Boddington & Reynolds, 2017).

Recruitment of proprioceptive pathways by EES in SCI patients has been shown to modify cortical excitability (Wagner et al., 2018). SCI patients have reduced event-related synchronization (ERS) of beta band spontaneous cortical activity related to motor execution, and the amplitude of ERS decreases in proportion to severity of SCI (Gourab & Schmit, 2010). The ERS is enhanced during EES and correlates with improved motor performance of SCI patients (Wagner et al., 2018). We have observed a similar enhancement of ERS after PAS therapy in a subset of 5 SCI patients (Vanhanen et al.,, submitted), suggesting that PAS modifies cortical excitability.

## 5.3 | Contribution of individual PAS components

It is important to stress that the therapeutic effect originates from dual stimulation and not from TMS or PNS alone. When TMS is used alone, lasting inhibitory aftereffects can be achieved with 1-Hz repetitive TMS and facilitatory aftereffects with high-frequency (>1 Hz) repetitive TMS (Rossi et al., 2009). We have shown that the 0.2-Hz TMS that we use does not lead to MEP potentiation (Shulga, Zubareva, et al., 2016). Thus, it is highly improbable that TMS alone would have accounted for the obtained results.

We have additionally shown that 50-Hz PNS (Shulga, Zubareva, et al., 2016) or 100-Hz PNS (Tolmacheva, Makela, et al., 2019) alone has no effect on MEPs. In a double-blind sham-controlled setup where one hand of the patient was randomly selected for PAS and the contralateral hand received only PNS (combined to sham TMS), we showed that PAS is more efficient than PNS in SCI patients (Tolmacheva et al., 2017). In addition, we recently applied PAS without

TMS (PNS and MI only) to five tetraplegic patients, otherwise replicating the previous PAS study (Tolmacheva, Savolainen, et al., 2019) in terms of patient group and stimulation parameters. Consistent with our previous result (Tolmacheva et al., 2017), we observed that without TMS, the MMT score increase was 59% smaller than that obtained with PAS. In addition, omitting TMS abolished the therapeutic effect on functional hand tests (Pohjonen et al., 2021). The effect on PNS combined to MI on MMT is plausibly explained by the fact that MI activates M1. When this weak activation is combined with high-frequency PNS, which does not require the exact adjustment of ISI, a weaker but similar effect to a high-PAS protocol is achieved through activation of upper and lower motor neurons. However, upper motor neuron activation by MI is neither as precisely timed nor as specific as the combination of TMS and PNS, where motor cortex stimulation sites and ISIs are defined precisely and individually, are easy to monitor, and do not require any effort from the patient.

As mentioned above, we observed that PAS can also positively affect the unstimulated contralateral hand (Tolmacheva, Savolainen, et al., 2019). At the behavioral level, the improved use of the more severely affected hand might encourage the patient to engage in bilateral tasks in a more versatile way. In the lower limbs, improvements in weak muscles also plausibly affect the whole limb by making walking easier and thus promoting increased use of all muscles. When only part of the lower limb nerves was stimulated to restrict the duration of the stimulation, the muscles innervated by the unstimulated nerves also improved (Rodionov et al., 2020).

## 6 | SAFETY

No serious adverse effects were observed in any of our studies (Rodionov et al., 2019, 2020; Shulga, Lioumis, et al., 2016; Shulga et al., 2020; Tolmacheva, Savolainen, et al., 2019; Tolmacheva et al., 2017).

In one patient with over 12 years since injury, lower back pain, which was a problem already before high-PAS, was temporarily and reversibly exacerbated during lower limb PAS (Rodionov et al., 2020). However, the patient did not discontinue the stimulations. This might have occurred due to factors unrelated to PAS, or alternatively may have been caused by an altered balance between leg and trunk muscles after high-PAS enabled better function of the lower limbs. Most patients who received lower limb high-PAS had < 3 years since SCI, and one patient had SCI 8 years ago (Rodionov et al., 2020; Shulga et al., 2020).

In one patient receiving upper limb PAS as a long-term treatment, during PAS of the radial nerve around week 12, the subject reported a sensation resembling electrical stimulation in both legs, spasticity, and spasticity-related leg pain. A simultaneous urinary tract infection unrelated to stimulation was detected and treated. Psychological stress unrelated to stimulation also occurred simultaneously. PAS was interrupted for 2 weeks and the patient increased on-demand pain medication. The patient subsequently reported a decrease in spasticity and pain. The subject's position in the chair was adjusted to increase comfort, and PNS intensity was slightly decreased. These symptoms gradually disappeared over weeks 16 to 19 of stimulation (Rodionov et al., 2019).

SCI patients have general susceptibility to skin problems. Consequently, the skin under the PNS electrodes must be monitored after each session; slight redness of the skin after stimulation is attributed to increased blood flow and does not require further attention if reversible within 2 to 3 hr. We have not observed any permanent skin damage due to stimulation. We have been using abrasive paper and ethanol for skin preparation for the recording and stimulating electrodes. If skin irritation occurs due to skin preparation, abrasion can be omitted at least temporarily.

## 7 | SUMMARY AND FUTURE DIRECTIONS

The specificity of neuromodulation techniques applied to the whole spinal cord is generally limited (Jack et al., 2020). High-PAS is an option that can selectively activate weakened connections. We have observed that high-PAS can benefit a wide range of patients with incomplete SCI. Patients with milder and more recent injuries benefit more and improve more quickly than those with more severe and chronic lesions, and will require shorter interventions than those with more severe or older injuries (Rodionov et al., 2020; Tolmacheva, Savolainen, et al., 2019; Tolmacheva et al., 2017). However, sufficiently long treatment in people with more severe injuries can also lead to significant increases in independence (Rodionov et al., 2019). We have included patients of a wide age range; this is important as the incidence of SCI is increasing in older populations (Chen & DeVivo, 2019). As residual connectivity is a prerequisite for high-PAS, this technique would not be beneficial for patients with complete injuries. It remains to be determined where high-PAS can be an adjunct treatment potentiating other approaches that would restore connectivity after complete SCI (such as cell transplantation).

We have performed studies in healthy subjects and optimized PNS and PAS frequencies (Mezes et al., 2020; Tolmacheva, Makela, et al., 2019). Further research is necessary to optimize the intensities of stimulation to find settings that achieve maximum efficacy with minimum discomfort. Further studies to optimize the TMS settings are also needed. As comorbid traumatic brain injury occurs in 16% to 74% of SCI cases (Chen & DeVivo, 2019), the safety of TMS in these patients should be considered carefully.

Although we have not documented any significant changes in sensory function, we did observe diminishing neuropathic pain in most of our patients. The involvement of the somatosensory system and its role in the therapeutic effect of high-PAS requires further research.

Considering the anatomy of small finger muscles and larger muscles of the lower limbs and the fact that MMT and AIS scores measure performance against gravity, it is clear that the therapeutic effects can be detected faster in the upper than in the lower limbs. Whereas even small improvements in the upper limbs almost immediately benefit everyday life functions (e.g., by enabling more effective grasping), larger changes in the lower limbs are required for improvements in walking. Therefore, the upper limbs of patients with milder tetraplegia are the easiest group to study and further develop high-PAS; stronger innervation directly translates into more independent activities of daily living. However, we have also obtained promising results on lower limb function. It is worth noting that even walking part-time or being in an upright position is highly beneficial for overall health (Bauman & Nash, 2019) as this improves blood-pressure regulation, bone density, weight control, bowel function, and psychological well-being and prevents pressure sores and heterotopic ossification and enables smoother transfers to and from the wheelchair.

Currently, the major limitation of the high-PAS approach is the relatively small number of published clinical studies. In some of our experiments, the rehabilitation personnel not involved in the research were aware of the fact that the patient was participating in a research project. Although these personnel were not aware of the details of the studies, this can nevertheless introduce an additional bias (both positive expectations and fear of unknown adverse effects). Randomized sham-controlled trials are essential for the transfer of high-PAS to clinical practice. It is also important that its benefits are confirmed by other independent laboratories and clinical teams.

The equipment for high-PAS is already available and has a well-characterized safety profile. Experience in clinical neurophysiology is required for defining the stimulation parameters at the beginning of a stimulation. A team of a physiatrist or a neurologist together with a physiotherapist is useful for selection of the optimal PAS targets for the stimulation and for evaluation of safety aspects. Thereafter, daily stimulation sessions can be performed by personnel with a diverse educational background, for example, by nurses or physiotherapists. The reduced need of external assistance and increase in quality of life in SCI patients justifies the therapy costs. These considerations, together with the results and observations described above, encourage further research of this promising new approach.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

AS produced the first draft of the manuscript. AS and PL produced figures. All authors contributed to writing, ideas, and editing of the manuscript and approved the final version.

## SUPPLEMENTARY LINKS

**Upper limb function**

**Video from** (Rodionov et al., 2019) demonstrating functional improvement in the upper limb of a patient with severe traumatic tetraplegia who received high-PAS treatment for as long as improvement was observed.

https://static-content.springer.com/esm/art%3A10.1038%2Fs41394-019-0225-5/MediaObjects/41394_2019_225_MOESM1_ESM.mp4S

**Video from** (Tolmacheva, Savolainen et al., 2019) demonstrating a rapid improvement after 10 days of high-PAS in a patient with milder non-traumatic tetraplegia:

https://ars.els-cdn.com/content/image/1-s2.0-S2467981X19300290-mmc2.mp4

**Lower limb function**

A series of videos in (Shulga et al., 2020) demonstrating the progress of the patient with traumatic paraplegia from non-ambulatory to ambulating independently with a walker:

https://www.nature.com/articles/s41394-020-0320-7#Sec4

## ETHICS STATEMENT

This work was approved by Ethics Committee of Medicine of Helsinki University Hospital (written approval).

## PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ejn.15191.

## DATA AVAILABILITY STATEMENT

Not applicable (this article reports no primary data).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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