Cortical re-organization after traumatic brain injury elicited using functional electrical stimulation therapy: A case report

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Abstract

Functional electrical stimulation therapy (FEST) can improve motor function after neurological injuries. However, little is known about cortical re-organization after FEST and weather it can improve upper-limb motor function after traumatic brain injury (TBI). Therefore, our study examined cortical and motor changes in a single male participant with chronic TBI suffering from mild motor impairment during 3-months of FEST and at 3-months follow-up. FEST was applied to enable upper-limb grasping and reaching movements during each session, which was performed for 45-60 min, 3 days per week, over 12-weeks. Short-term assessments were examined before and after each session, while long-term assessments were performed at baseline, after 6- and 12-weeks of FEST, and during follow-up 6- and 12-weeks after completing FEST. Short-term assessments carried out using transcranial magnetic stimulation (TMS) showed reduced cortical silent period (CSP), which is related to cortical and/or subcortical inhibition. At the same time, no changes in motor evoked potentials (MEP) were observed, suggesting corticospinal excitability was unaffected. Long-term assessments indicate increased MEP corticospinal excitability after 12-weeks of FEST, which remained 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, fMRI imaging data showed M1, as well as sensory, premotor, parietal area, and supplementary motor area activations increased after 12-weeks of FEST and during both follow-ups. While clinical scores did not change considerably, writing test performance indicates mild improvements after FEST. Our results suggest that FEST can effectively increase cortical activations, while writing tests confirmed functional improvements in fine motor function even after chronic TBI. These results demonstrated long-

term recovery mechanisms of FEST, which include cortical re-organization or neuroplasticity to improve motors function after neurological injury.

Key words: functional electrical stimulation; traumatic brain injury; neuroplasticity; rehabilitation; case report.

1. Introduction

Acquired brain injuries, such as stroke or traumatic brain injury (TBI) can often 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 limb (Nudo et al., 2013). Such injuries may have far-reaching consequences beyond physical impairment, possibly affecting emotional and economic status of injured individuals. Most TBI injuries were caused as a result of falls or motor vehicle accidents (Badhiwala et al. 2019), while demographics of both incidence and prevalence is predominantly the elderly populations (Peeters et al. 2015). Neurological injuries resulting from trauma, such as motor vehicle accidences, are typically diffuse and affect widespread changes in cortical activation patterns 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 after the injury, resulting in loss of fine motor control (Nudo et al., 2013). Specifically, it was shown in rodent models that downregulation of $GABA_A$ (inhibitory) receptors and upregulation of NMDA (excitatory) receptors occurs following focal brain injury in both ipsilesional and contralesional hemispheres (Redecker et al. 2000). Widespread effects in contralesional hemisphere are likely mediated via complex intra-cortical networks that facilitate communication between sensory and motor areas of the brain. Using magnetic stimulation in humans post-stroke, it was shown that excitability of the motor cortex was considerably reduced near the injury site, likely resulting in decreased cortical motor map representations of the affected muscles (Traversa et al 1997; Butefisch et al 2006). Therefore, both focal and diffuse brain injuries typically result in widespread cortical effects, having multifaceted consequences on motor control.

Considerable spontaneous (natural) recovery can occur even in absence of rehabilitative intervention after neurological injury (Nudo et al. 2013). Compensating behaviours are common after such injuries. For instance, individuals may use altered trunk activations during reaching (Cirstea and Levin 2000). Similarly, learned non-use can occur in the acute stage of injury if unsuccessful attempts to use affected limbs persist (Taub et al. 1998). In absence of behavioural conditioning or rehabilitation, plasticity in the motor cortex that occurs spontaneously may therefore be related to compensatory motor patterns, rather than recovery of original function (Nudo et al. 2013). By restraining use of the non-affected limb, constraint-induced movement therapy has been shown to improve use of the affected limb in animal models with deafferented muscles (Knapp et al. 1963). It was also shown as an effective clinical intervention in humans for improving motor control after a stroke (Wolf et al. 2006). Intact motor areas adjacent to the injury site and areas outside of the motor cortex such as the premotor cortex or ipsilateral cortical areas may contribute to cortical recovery via intracortical connectivity networks (Weiller et al. 1992; Seitz et al. 2005; Nudo et al. 2013). Therefore, understanding spontaneous recovery may help optimize novel neurorehabilitation interventions after TBI.

Functional electrical stimulation (FES) is a neurorehabilitation approach that can be used to apply short electric impulses on the muscles using transcutaneous electrodes applied to the skin surface, which can cause action potentials and generate muscle contractions in otherwise impaired muscles due to neurological injuries. Typically, an anode electrode is placed over the motor point on the muscle belly of the targeted muscle, while the cathode is placed at a convenient location to ensure that the current flow will reach the desired motor point for the targeted muscle. During stimulation biphasic constant-current stimulation is applied at frequencies ranging between 20-50 Hz and pulse widths ranging between 30-500 μs. The

amplitudes are varied in the range from 5-10 mA and up to 100 mA with the goal of assisting motor function through generating muscle contractions (Popovic, et al. 2012; Quandt and Hummel 2014; Carson and Buick 2019). When stimulation is sequenced spatiotemporally over the appropriate muscles, FES can generate functional movements, including grasping and/or reaching (e.g., Popovic et al. 2001; Popovic et al. 2012). Applications of electrical stimulation of muscles include recovering voluntary limb movements in individuals who have sustained neurological injuries such as stroke and spinal cord injury (SCI). Using this type of FES therapy or FEST (Popovic et al. 2002), our group has previously demonstrated recovery of upper-limb function in a randomized control trial with stroke patients (Thrasher et al., 2008; Marquez-Chin et al. 2017). Specifically, FEST was delivered along with conventional occupational and physical therapy in the intervention group, while the control group received 45 min of conventional therapy for 3 to 5 days per week for a total of 12 to 16 weeks (40 sessions in total). Compared to the control group, the acute stroke injury FEST group improved in terms of object manipulation, palmar grip torque, pinch grip force as well as on several other clinical measures, while chronic injury patients had smaller effects (Thrasher et al., 2008). Moreover, a randomized trial with cervical incomplete SCI (C4-C7 level) 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). Overall, FEST was shown as effective treatment to improve long-term voluntary upper-limb motor function in individuals with both acute and chronic neurological injuries (Popovic et al. 2012).

However, despite evidence for recovery of voluntary function after FEST, relatively little is known about the cortical re-organization after the interventions. Several recent review papers (Chipchase et al., 2011; Quandt and Hummel 2014; Carson and Buick 2019) synthesized proposed cortical re-organization mechanisms after FES in stroke patients. Specifically, it is known that FES applied at supra motor threshold intensities generates tetanic muscle contractions via the efferent pathway, which may also activate antidromically and affect ventral horn interneurons (Rushton 2003) to inhibit spinal reflex excitability (Hortobagyi et al. 2013; Kawashima et al. 2013; Milosevic et al. 2019). These electrical impulses activate the mixed nerve bundle and not only to recruit the efferent axons, but also afferent sensory nerve fibers directly and via reafference through muscle and joint movement-induced (e.g., muscle spindle) feedback (Bergquist et al. 2011), which may have direct effects on cortical activations in the sensorimotor areas (SMA) (Quandt and Hummel 2014; Carson and Buick 2019). Overall, the consensus is that neuroplasticity resulting from FES interventions can cause cortical activations changes. Various neuroimaging studies showed evidence demonstrating changes in the somatosensory cortex through cutaneous and muscle contraction-induced afference, which can be relayed to the primary motor cortex (M1) possibly via cortico-cortical connections (for a review, see Carson and Buick 2019). It was also suggested that FES interventions in more severely impaired stroke patients may evoke enhanced activations the contralesional somatosensory cortex, while those less impaired tend to show reduced and less diffuse ipsilesional activations (Quandt and Hummel 2014), suggesting patient-specific and injury-dependant modulation. These effects also seem to have dose-dependant characteristics, with above motor threshold intensity and longer durations of stimulation inducing more consistent and sustained cortical changes (Chipchase et al. 2011), while parameters such as frequency and pulse

width as well as location of stimulation (i.e., nerve or muscles) may change how spinal and supra-spinal circuits are recruited (Bergquist et al. 2011; Carson and Buick 2019). Given little or no consensus between studies about methodological considerations of FEST delivery (i.e., number and duration of sessions as well as intervention durations) and parameters of stimulation (e.g., frequency and intensity of stimulation), cortical changes can vary widely between studies.

During FEST, task-specific and repeated training is delivered with the assistance of a therapist. Participants are first asked to attempt to perform a motor task, while the therapist provides reinforcement by triggering appropriate muscles to assist completion of attempted tasks (Popovic et al. 2012). Similarly, repetition, temporal coincidence, and context-specific reinforcement during motor task performance were suggested as mechanism for inducing experience-dependant cortical plasticity after TBI (Nudo et al. 2013). 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 recent randomized trial (de Sousa et al. 2016). Therefore, the objective of the current study was to investigate possible 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 on cortical re-organization in a detailed clinical case study with an individual suffering from upper-limb motor impairment after chronic TBI. Specifically, the objective of the study was to understand temporal characteristics of recovery using neuroimaging and neurophysiological evaluations as well as to examine motor function 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 will be correlate to specific cortical re-organization outcomes.

2. Methods

2.1. Clinical presentation

A participant was a 39-year old male with a TBI resulting from a motor vehicle accident. The accident occurred 7 years prior to start of the study. In the initial assessment, which was administered after the accident, the participant was diagnosed as having suffered a diffuse brain injury, multiple trauma, skull fracture, pulmonary contusion, and hemorrhagic shock. At the time of injury, the participant was diagnosed as a serious condition by the Glasgow Coma Scale (GCS) (Teasdale et al. 1974): no eye opening, no verbal response, and no motor response. Physiological testing concluded that there was no injury to the spinal cord. The participant received skull reconstructive surgery and remained in intensive care unit for 3-weeks, which was followed by one-month of monitoring, before 5-months of inpatient rehabilitation, where he received standard rehabilitation for 3-hours per day. After discharge, he was still unable to walk independently and required assistance during daily living. Over the ensuing six years, he continued various rehabilitation and training programs, including Pilates and brain gymnastics. Ultimately his lower-limb function improved, and he was able to walk independently, while his upper-limb impairment persisted. 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.

At the study onset, symptoms related to movement function included: (1) ataxia, specifically characterized by tremor in the right upper- and lower-limbs (i.e., contralateral to the trauma) during movement initiation, as well as trunk, whole body movement, and balance disorders; (2) involuntary movements in the right thumb, and tremor during performance of fine

motor tasks such as writing and using chopsticks; (3) mild hemiplegia mainly affected the right foot; and (4) eye movement disorder, characterized by poor eye movement control. Symptoms related to higher brain dysfunction, included: (1) memory loss, related to pre-accident and recall of new events after the accident; (2) attention disorder, characterized by decline in arousal, decline in attention (sleeping or drowsiness), specifically during multi-tasking activities; (3) performance impairment, including impulsive behaviour; and (4) social behavior disorders, characterized by decline in recognition of anger and emotional control.

As a result of the upper-limb motor impairment, the participant enrolled in the study aiming to improve upper-limb function using FEST. Prior to the study, the participant was informed about the study objectives and signed a written informed consent in accordance with the principles of the Declaration of Helsinki, which was approved by the local institutional research ethics committee at the University of Tokyo.

2.2. Functional electrical stimulation therapy (FEST)

Functional electrical stimulation (FES) was delivered using the Complex Motion (Compex, Switzerland) 4-channel constant current electrical stimulation system. Electrical stimulation was used to activate the muscles by applying a rectangular, biphasic, asymmetric charge balanced stimulation pulses at a 40 Hz stimulation frequency and 300 μsec pulse width (Popovic et al. 2001; Popovic et al. 2002). Electrical stimulation was applied on the muscles transcutaneously via surface electrodes (5×5 cm square electrodes on larger muscles and 2 cm diameter circular electrodes on the smaller muscles). During each training session, the therapist determined the stimulation amplitude for each muscle by gradually increasing the stimulation amplitude with 1 mA increments until they identified palpable contractions. The stimulation

amplitude was then set to 1.5x the amplitude that evoked palpable contractions, and adjusted if necessary, to produce smooth muscle contractions of each muscle (Popovic et al. 2001).

The FEST training protocol is summarized in Figure 1. Training was delivered over the course of 12 weeks (three months), with 3 sessions per week, each lasting between 45 and 60 min and with at least one day between sessions (Figure 1A). Each training session consisted of three functional training protocols (for more details, see Thrasher et al. 2008 and Kapadia et al. 2011), as illustrated in Figure 1B: (1) palmar grasp - to generate hand opening, a cathode was placed on the wrist extensors (extensor carpi radialis: 19.6±4.2 mA) and the anode on the extensor tendons (dorsal side of the wrist); to generate a palmar grasp, the cathodes were placed on the thumb (abductor pollicis brevis: 9.6±2.4 mA) and wrist flexors (flexor carpus radialis: 9.6±2.9 mA and flexor carpus ulnaris: 10.5±3.1 mA) and the anodes on the flexor tendons (palmar side of the wrist); (2) hand-mouth - to generate elbow and shoulder flexion, the cathodes were placed on the biceps (biceps brachii: 17.8±6.1 mA) and shoulder (anterior deltoid: 15.7±4.5 mA) with the anode also placed on the muscle belly away from the cathodes; to generate elbow and shoulder extension, the cathodes were placed on the triceps (triceps brachii: 18.8±4.5 mA) and the shoulder (posterior deltoid: 21.3±4.7 mA) and the anodes on the muscle belly away from the cathodes; and (3) point forward - to generate hand pointing forward, the cathodes were placed on the triceps (triceps brachii: 18.2±2.7 mA) and shoulder (anterior deltoid: 17.1±3.9 mA) with the anodes on the muscle belly away from the cathodes; to generate hand retraction, the cathodes were placed on the biceps (biceps brachii: 16.9±5.2 mA) and shoulder (posterior deltoid: 21.2±4.6 mA) and the anodes on the muscle belly away from the cathodes (Figure 1B). Each movement was delivered independently during the sessions. In each protocol, participant performed a specific functional task, including grasping a water bottle (palmar grasp), bringing

an object to their mouth (hand-mouth), and pointing towards a target (pointing forward). For each trial, the participant was first asked to attempt to perform the task himself without the help if FES, while the therapist triggered a pre-programmed FES sequence after allowing the participant to initiate the movements to assist his voluntary effort.

2.3. Assessments protocol

Timeline of assessments is summarized in Figure 1A. Assessments were carried out to examine cortical and corticospinal circuits associated with upper-limbs, as well as functional performance and clinical scores related to hand function. Long-term assessments were carried out twice over the course of the 12-weeks of FEST and twice during the 12-weeks follow-up period after the intervention was completed (Figure 1A): 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 functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS), while functional performance was assessed using an instrumented drawing test and clinical scores. Short-term cortical changes were assessed immediately before and after each FEST session over the course of 12-weeks of training, once per week, using TMS. A detailed description of assessment protocols follows.

2.3.1. Transcranial magnetic stimulation (TMS)

TMS sessions were carried out during both long-term assessments (i.e., every 6 weeks) and short-term assessments (i.e., before and after each FEST session). During the assessments,

the participant wore a tight-fitting cap and remained seated comfortably on the chair with his right hand and forearm relaxed and supported on the table. Electromyographic (EMG) activities were recorded from the intrinsic hand muscles unilaterally. Bipolar Ag/AgCl surface electrodes (Vitrode F-150S, Nihon Koden, Tokyo, Japan) were placed on the right (i.e., intervention) hand with 1 cm separation on the: (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 approximately on the same locations of the muscle between assessment days. Prior to application of EMG electrodes, skin was prepared using an abrasive and alcohol to reduce skin impedance. EMG signals were band-pass filtered (15-1,000 Hz), amplified (1000×; MEG-6108, Nihon Koden, 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, Magxtim Co., Whitland, UK) through a figure-of-eight coil, single-pulse TMS was delivered over the area of the left primary motor cortex (M1) that was optimal for inducing MEP in the right FDI. The “hot spot” location was determined and defined with respect to cranial landmark as references during the baseline assessment (Pre). This “hot spot” location was used as a starting point for all subsequent assessments (During, Post0, Post1, and Post2), while the exact location was confirmed on each assessment day. The MEPs were always evoked with the participant keeping voluntary contraction at 10% MVC 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 MVC level was determined prior to each assessment by performing and averaging three MVC trials.

The motor threshold (MT) for evoking MEPs was as the minimum TMS intensity at which five MEPs had peak-to-peak amplitudes of at least 50 μV and were evoked from the FDI 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 the long-term assessments and short-term assessments, the input-output relationship between the TMS stimulation intensity and the MEP responses amplitude was obtained by applying TMS stimulations at 60, 70, 80, 90 and 100% of the TMS stimulator intensity. Three trials were performed at each TMS intensity and the responses averaged for each muscle (FDI and APB) at each intensity (Ridding et al. 2001). Since MEPs were recorded during active contractions (i.e., 10% MVC), it was also possible to record the cortical silent period (CSP) of the MEPs from the same trials. CSP was calculated from the responses evoked at 70% of the stimulator output (Farzan 2014).

Moreover, 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 1.2x 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 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 sec and averaged to obtain response peak-to-peak amplitude for each location (Mortifee et al. 1994; Ridding et al. 2001).

2.3.2. Functional magnetic resonance imaging (fMRI)

During fMRI sessions, which were carried out during long-term assessments (i.e., every 6 weeks), the participant remained in the supine position in an MRI scanner 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 target force level for the grip and pinch task was shown on a visual display and it was set at 20% of maximal voluntary contraction (MVC) effort (Ward et al. 2003). The MVC levels were determined prior to the experiment by performing and averaging three MVC trials, for the hand grip and finger pinch tasks, after a warm-up and task practice. During fMRI assessments, the participant was asked to follow the target force trajectories as precisely as possible. The target force trajectories consisted of four phases: rest (10 sec), ascending (10 sec), keep at 20% MVC (10 sec), and descending (10 sec) (Kuhtz-Buschbeck et al. 2001). fMRI scan sessions were repeated four times for each task and averaged for the four hand grip and four finger pinch tasks. A rest period of at least 20 sec was given between each trial. Force data was recorded using a custom program written in LabVIEW (National Instruments, Austin, TX, USA) and digitized at 1,000 Hz sampling frequency using an analog-to-digital converter (USB-6259 BNC, National Instruments, Austin, TX, USA). Force data during fMRI sessions was used to ensure that the participant was following the target force trajectories during fMRI scans.

All MRI images were acquired using a 3T MRI scanner with a 64-channel head coil (MAGNETOM Plisma, Siemens, Germany). Functional T2*-weighted echo-planar images to reflect blood oxygenation level-dependent (BOLD) responses (Ogawa et al. 1990) were collected using the following parameters: TR=2,000 ms, TE=25 ms, flip angle=90°, FOV=192 mm, 39

contiguous axial slices acquired in interleaved order, thickness=3.0 mm, in-plane resolution = 3.0×3.0 mm, bandwidth =1,776 Hz/pixel, as in previous studies using similar force match tasks (Noble et al. 2013; Naito and Hirose 2014). Auto-align was run at the start of each session. High-resolution T1-weighted structural images were also acquired, using the 3D MPRAGE (T1-weighted anatomical images) pulse sequence: TR=2,000 ms, TE=2.9 ms, flip angle=9.0°, FOV=256 mm, 176 contiguous axial slices, thickness = 1.0 mm, in-plane resolution: 1.0×1.0 mm (Noble et al. 2013; Naito and Hirose 2014).

2.3.3. Drawing tests

To evaluate upper-limb fine motor function, which was carried out during long-term assessments (i.e., every 6 weeks), the participant was asked to perform: (i) tracking and (ii) sine wave tracing tasks (wavelength: 50 mm, amplitude: 25 mm, 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^{o}. For the tracking task, the participant was instructed to follow the moving target on the tablet screen which moved on a sine wave at 12 mm/sec, while during the sine wave tracing task, the participant was instructed to follow the outline of a sine wave at his preferred speed, without a moving target. For both tasks, the participant was asked to draw as precisely as possible. Two trials were recorded for each of the tacking and sine wave tracing tasks and averaged. Before each assessment, a brief practice period was given to familiarize the participant.

2.3.4. Clinical assessments

Clinical scores, which were evaluated during long-term assessments (i.e., every 6 weeks), 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 physical therapist.

2.4. Data analysis

2.4.1. MEPs

All MEP analysis was performed using a custom program written in Matlab (2017a, The MathWorks Inc., Massachusetts, USA). 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, which were averaged and each stimulation intensity (i.e., 60, 70, 80, 90, and 100% of the TMS stimulator output), were first calculated. The average MEP amplitudes were plotted relative to the TMS stimulation intensities and a linear fit was obtained using simple linear regression. The slope of the linear regression line was used to define the gain parameter of the input-output relationship curve (Figure 2A and Figure 2E) (Farzan 2014).

The cortical silent period (CSP) duration was defined as the absolute CSP for each muscle as the time between the end of the MEP (i.e., the first point at which the rectified EMG after the stimulus was below 3SD of the mean pre-stimulus EMG activity) and the time at which the post-stimulus EMG returned to the pre-stimulus EMG activity (i.e., the time at which the EMG exceed 3SD of the mean pre-stimulus EMG activity) (Figure 2E and Figure 2F) (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 10% of maximum MEP ($aMEP_{10\%}$) relative to all partitions (N_{total} = 2,500): $area = \frac{N\,(aMEP_{10\%})}{N_{total}} \times area_{map}$, where $area_{map}$ is 100 cm^2 (Figure 2C) (van den Ruit et al. 2015).

2.4.2. fMRI

All fMRI data analysis was performed using Statistical Parametric Mapping (SPM12, Wellcome Trust Center for Neuroimaging, London, UK) software implemented in Matlab (2017b, The MathWorks Inc., Massachusetts, USA). Prior to data analysis, DICOM image files were converted to NIFTI format. First, preprocessing was performed in the following order: (1) Realignment - excessive head movement was corrected using the realignment procedure by applying a threshold of 2 mm for translation and 2° for rotation (NOTE: since no excessive movements were identified in any of the images, no scans were excluded); (2) Coregistration - the T1-weighted structural scan and the average EPI-scan in each of the four experimental conditions were aligned to superimpose the head position information; (3) Normalization - segmentation of the structural scan was performed, providing normalization parameters, which were used to normalize the EPI-scans to the Montreal Neurological Institute (MNI) space (resized voxels 3×3×3 mm) (Kuhtz-Buschbeck et al. 2001); (4) Smoothing - EPI-scans were smoothed with a Gaussian kernel of 8 mm (Naito and Hirose 2014); and (5) Scaling - the value in each voxel was normalized by converting it into a percent signal change (PSC), which was the

percentage increase from the mean of the whole brain in each session and an indicator of the intensity of the BOLD signal (Noble et al. 2013). The PSC value was calculated on a voxel-wise basis, for each condition (NOTE: during the preprocessing stage, the first 30 scans were discarded for the finger pinch task for During assessment because these contained excessive pulse noises in whole brain areas, which was above 3SD of the mean). 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; Friston et al. 1995). Whole brain analysis was then 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 (Figure 3A and Figure 3D) (Naito and Hirose 2014). The threshold was set at voxel level $p<.001$ (uncorrected) and cluster level $p<.050$ (Familywise error correction: FWE) (Naito and Hirose 2014; Woo et al. 2014).

Next, we set the region of interest (ROI) in six anatomical areas defined bilaterally: hand primary motor cortex (M1; $x=\pm37$, $y=-21$, $z=58$) (Mayka et al. 2006), sensory cortex (S1; $x=\pm 40$, $y=-24$, $z=50$) (Mayka et al. 2006), secondary somatosensory cortex (S2; $x=\pm58$; $y=-27$; $z=30$) (Iftime-Nielsen et al. 2012), parietal rostroventral area (PR; $x=\pm54$; $y=-13$; $z=19$) (Hinkley et al. 2007), supplementary motor area (SMA; $x=\pm20$; $y=-8$; $z=64$) (Ciccarelli et al. 2006), premotor cortex (PM; $x=\pm8$; $y=-6$; $z=64$) (Ciccarelli et al. 2006). These ROI regions were chosen based on the previous studies that investigated cortical effects of FES (Blickenstorfer et al. 2009; Joa et al. 2012; Gandolla et al. 2016). In addition, the most activated voxel in the contralateral M1 region (peak voxel) was calculated to define the most active ROI location (Verstynen et al. 2005). For these regions, PSC was calculated with the MarsBar toolbox (MRC Cognition and Brain

Sciences Unit, Cambridge, UK) for the SPM12 software (Brett et al. 2002). Finally, a control region was defined as the hippocampus gyrus (HC; left: x=−22; y=−34; z=−8 and right: x=32; y=−30; z=−8) (Hayes et al. 2011), which was not associated with hand movements.

2.4.3. Drawing tests

Tracking and sine wave tracing tasks were evaluated using the following parameters to assess performance: (i) error - for the tracking task, error was the distance between the target point and the position of the participant's pen, while for the sine wave tracing task, error was the shortest distance between the coordinates of the sine wave and the position of the participant's pen; (ii) mean velocity - mean velocity during the tasks; (iii) coefficient of variation (CV) of velocity - the ratio between the standard deviation and the mean velocity during the tasks; and (iv) mean acceleration - mean acceleration during the tasks (Figure 4). Two repeated trials were averaged for each task (i.e., tracking and sine wave tracing) and each assessment. All parameters were calculated using the instrumented tablet software (TraceCoder®, Version 1.0.8, SystemNetwork, Osaka, Japan) (Itotani et al. 2016).

2.4.4. Clinical assessments

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

2.5. Statistics

Short-term assessments were analyzed using the paired samples t-test to compare the input-output curve slope and CSP before vs. after each FEST session for a single subject

obtained over the course of 12-weeks. Shapiro-Wilk test was used to confirm that data were normally distributed. Statistical comparisons were performed using SPSS Statistics (IBM Corp., Armonk, NY, USA). Significance level was set to $p<.050$.

3. Results

3.1. Short-term effects

Short-term assessment TMS results are summarized in Figure 2A and B. Input-output curve showed no statistically significant differences between slopes of FDI (t-test, p=.056) and APB (p=.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 (p=.002) and APB (p=.029) muscles after each FEST session, compared to before the session (Figure 2B).

3.2. Long-term effects

3.2.1. TMS

Long-term assessment TMS results are summarized in Figure 2C, D, and E. Input-output curve showed that slope of 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 intervention was completed (Post1 and Post2), compared to baseline (Pre) (Figure 2C). CSP showed that there were no changes in both FDI and APB muscles after 6-weeks (During) and after 12-weeks (Post0) of FEST as well as in the 12-week follow-up period (Post1 and Post2), compared to baseline (Pre) (Figure 2D). Finally, MEP maps showed that area in the motor cortex in both FDI and APB muscles increased immediately after 12-weeks of FES training (Post0) and that it remained for at

least another 12-weeks after the FEST intervention was completed (Post1 and Post2), compared to baseline (Pre) (Figure 2E).

3.2.2. fMRI

Long-term assessment fMRI results are summarized in Figure 3, with activations of the whole brain during the grip task shown in Figure 3A and the finger pinch task in Figure 3D. Peak activated voxel in the primary motor cortex (M1) showed that activations in the M1 area for both the grip (Figure 3B) and finger pinch (Figure 3E) tasks increased after 12-weeks of FEST (Post0) and remained for at least another 12-weeks after the FEST intervention was completed (Post1 and Post2), compared to baseline (Pre). The location of the peak activated voxel for both the grip (Figure 3B) and finger pinch (Figure 3E) tasks did seem to shift. Moreover, ROI analysis showed that contralateral M1 region activations for the grip (Figure 3C: Cont M1) and finger pinch (Figure 3F: Cont M1) tasks increased after 12-weeks of FEST (Post0) and remained for at least another 12-weeks after the FEST intervention was completed (Post1 and Post2), compared to at baseline (Pre). Similarly, activations in other defined cortical areas, including the sensory cortex (S1), secondary somatosensory cortex (S2), parietal area (PR), supplementary motor area (SMA), and the premotor area (PM) showed similar patterns during both the grip (Figure 3C) and finger pinch (Figure 3F) tasks in the contralateral (top) as well as the ipsilateral hemisphere (bottom), although ipsilateral activations seemed to be affected to a smaller extent. Finally, the control area (HC) activations did not seem to change over the course of the FEST intervention in the contralateral and the ipsilateral hemisphere (Cont HC and Ipsi HC).

3.2.3. Drawing tests

Long-term assessment drawing test results are summarized in Figure 4. Mean error during the tracking task (Figure 4C - top) increased after 6-weeks (During) and after 12-weeks (Post0) of FEST, while it seemed to decrease during follow-up assessments at 6-weeks (Post1) and 12-weeks (Post2) after the FEST intervention was completed, compared to the baseline (Pre); however, during the sine wave tracing task (Figure 4C - bottom), the mean error seemed to decrease after 6-weeks (During) and after 12-weeks (Post0) of FEST as well as during follow-up assessments at 6-weeks (Post1) and 12-weeks (Post2) after the FEST intervention was completed, compared to the baseline (Pre). Mean velocity, CV of velocity, and mean acceleration during both the tracking and sine wave tracing tasks seemed to decrease after 12-weeks of FEST (Post0) and remain for at least another 12-weeks after the FEST intervention was completed (Post1 and Post2), compared to baseline (Pre) (Figure 4C).

3.2.4. 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).

4. Discussion

The current study investigated short- and long-term cortical re-organization and motor improvements resulting from an upper-limb FEST intervention (Thrasher et al. 2008; Kapadia et

al. 2011) in a detailed clinical case study with an individual suffering from mild motor impairment resulting from chronic TBI (> 7 years). Specifically, our results 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 induce long-term cortical re-organization that lasted for at least another 12-weeks after the intervention was over, similar to clinical carry-over effects (Kapadia et al. 2011). Assessments during the intervention suggest that cortical changes were not apparent after 6-weeks of FEST, rather they required 12-weeks of training. Therefore, like in stroke and incomplete SCI (Thrasher et al. 2008), it seems that FEST can be successfully applied in the chronic TBI patients to induce cortical re-organization, offering the prospect of increased therapeutic effectiveness. Although clinical and motor improvements were relatively minor in our current case study, it should be noted that the participant presented with relatively mild upper-limb motor impairment at the beginning of the intervention (Table 1). A discussion about cortical re-organization mechanisms and functional changes after FEST follows.

4.1. Evidence of cortical re-organization after FEST

Our results showed the time course of short- and long-term cortical re-organization during and after a FEST intervention aiming to improve upper-limb motor function in an individual with chronic TBI. Short-term assessment results indicate reduced cortical silent period (Figure 2B - CSP), while corticospinal excitability which was evaluated by MEP input-output curve (Figure 2A) after each FEST session, was not affected. Cortical silent period refers to an interruption of voluntary muscle activity by TMS applied over the contralateral motor cortex (Wilson et al. 1993; 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). Specifically, cortical silent period following TMS of the motor cortex may be related to changes in spinal motoneuron excitability resulting from activation of muscle spindle receptors and/or activation of inhibitory Renshaw cells (Wilson et al. 1993; Wolters et al. 2008), as well as have cortical origins based on intracortical inhibition (Wilson et al. 1993; Knash et al. 2003). Contrary to our findings, some previous studies have reported increased corticospinal excitability after extended application of electrical stimulation (Ridding et al. 2000; Luft et al., 2002; Kaelin-Lang et al. 2002), which suggests that changes in excitability reflect, at least in part, modifications in cortical re-organization (Chipchase et al. 2011). Perhaps, 45-60 min during our FEST session was insufficient to facilitate cortical excitability, while 2-hours of stimulation may be required (Luft et al. 2002; Kaelin-Lang et al. 2002; Ridding et al. 2000). However, it must also be acknowledged that most of these previous studies were done in able-bodied participants, while our current study participant was an individual with TBI. Facilitation of corticospinal excitability after repetitive nerve stimulation was also shown to increase the cortical silent period, but only after changes in MEP amplitude during the stimulation (Knash et al. 2003). Consistent to our results, electrical stimulation of cutaneous nerves in the upper-limbs was shown to shorten the cortical silent period (Hess et al. 1999; Classen et al. 2000), which suggests its involvement in sensorimotor integration (Wolters et al. 2008). Similarly, cutaneous and afferent feedback from FEST may activate the somatosensory cortex, which may over the long-term affect cortico-cortical connections (Carson and Buick 2019). Short-term electrical stimulation may also antidromically activate the Renshaw cells interneurons (Rushton 2003) to inhibit spinal reflex excitability (Hortobagyi et al. 2003; Kawashima et al. 2013; Milosevic et al. 2019). Therefore, short-term effects of FEST could possibly be related to intracortical inhibition, while our results

suggest that changes in cortical silent period, without any changes in corticospinal excitability, are more likely related to 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 considerable facilitation after 12-weeks, which remained even after completion of FEST during follow-up for at least 12-weeks (Figure 2C). On the other hand, cortical silent period remained unaffected (Figure 2D). Previous studies showed increased MEP amplitudes after 2-hours of electrical stimulation in animal models (Luft et al. 2002) and after ulnar nerve stimulation in humans (Ridding et al. 2000; Ridding et al. 2001). Using the MEP input-output curve, increased corticospinal excitability was also shown after 2-hours of sensorimotor electrical nerve stimulation (Kaelin-Lang et al. 2002). Moreover, no changes were observed in excitability of M-responses and cervicomedullary junction (subcortical) stimulation evoked responses, suggesting lack of modulation of excitability at muscle or spinal cord level (Kaelin-Lang et al. 2002). The slope (and plateau) of the MEP input-output curve reflect the strength of corticospinal projections to the target muscles (Farzan 2014). It was shown that slope of the MEP input-output curve becomes less steep with $GABA_A$ (inhibitory) receptor agonist (e.g., lorazepam), while administration of an indirect dopaminergic-adrenergic (excitatory) agonist (e.g., D-amphetamine) increased the slope (Boroojerdi et al. 2001). Taken together, long-term assessments after FEST indicate increased cortical excitability, possibly via upregulation of dopaminergic excitatory receptors and/or downregulation of GABAergic inhibitory receptors. While consistent to our current findings of corticospinal excitability, previous studied also showed that aftereffects lasted less than 24-hours (Ridding et al. 2001) or as little as 8-20 min (Kaelin-Lang et al. 2002) after a 2-hour intervention. Our results showed

considerable long-term facilitation of corticospinal excitability not immediately after 45-60 min, but after 12-weeks of FEST and for at least another 12-weeks, even in absence of FEST.

Increased corticospinal excitability can probably be explained by larger area over which MEPs can be obtained in the hand (FDI and APB) muscles using MEP maps, which indicate enlarged hand muscle representations within M1 after 12-weeks of FEST and during follow-up (Figure 2E). Motor maps obtained using TMS-evoked MEPs were shown as reliable for extracting useful somatotopic information from the primary motor cortex (Wilson et al. 1993b; Wassermann et al. 1992). Specifically, it was shown that 2-hours of electrical nerve stimulation can produce larger areas over which MEPs can be evoked (Ridding et al. 2001). Moreover, a shift in the cortical representation zones after electrical stimulation was shown to be larger compared to the control group (Ridding et al. 2001). Although our study findings could not suggest a trend in the shift of motor maps, which were previously shown in healthy individuals, likely due to their non-uniform expansion in their motor cortex representation (Ridding et al. 2001; Byrnes et al. 1999), we confirmed considerable expansion of the motor areas which are consistent with the time-course of changes of MEP amplitude facilitation evoked over a single "hot spot" location in an individual with chronic stage TBI. While motor evoked responses could reflect cortical and/or spinal level excitability, changes in motor map representations confirmed that effects of FEST most likely occurred at the cortical level. Moreover, it was previously shown that shift of the motor map representations after stroke are not stable (Byrnes et al. 1999), possibly due to the location of the lesion of the surrounding cortical areas or other spontaneous recovery effects (Ridding et al. 2001; Nudo et al. 2013). Nonetheless, increased motor map area and subsequent MEP amplitude facilitation (Ridding and Rothwell 1997) confirm cortical-level re-organization after FEST.

Cortical re-organization was further corroborated by our fMRI data, which showed larger BOLD responses after 12-weeks of FEST and during follow-up, but not after 6 weeks, compared to baseline assessments (Figure 3). The time course of cortical changes obtained using fMRI in the M1 is consistent to the MEP maps obtained using TMS. Specifically, peak activated area in the M1 was considerably increased, while the location did not change consistently during both hand grip and finger pinch tasks (Figure 3 - peak activated voxel in M1). Our results also showed that not only was M1 activation increased, but also the primary somatosensory cortex (S1), secondary somatosensory cortex (S2), parietal rostroventral area (PR), supplementary motor area (SMA), and premotor cortex (PM) all showed larger BOLD signal in both the contralateral as well as smaller ipsilateral hemisphere activations during both hand grip and finger pinch tasks (Figure 3). On the other hand, the control region, did not exhibit any changes (Figure 3 - HC). Strong evidence using various neuroimaging techniques suggested that somatosensory cortices, including both S1 and S2 areas, are activated during electrical stimulation of muscles and nerves (Korvenoja et al. 1999; Boakye et al. 2000; Nihashi et al. 2005; Carson and Buick 2019). Electrical stimulation at intensities above the motor threshold give rise to cutaneous afferents as well as muscle contraction-induced reafference activity in the S1 (Wiesendanger and Miles, 1982; Carson and Buick 2019). Moreover, contralateral S1 activation increases with the increased intensity of stimulation (Krause et al. 2001), while S2 activation appeared at lower intensities compared to S1 area (Backes et al. 2000), suggesting afferent recruitment has intensity-dependant effects in the somatosensory cortex. Moreover, state of cortical circuits is not only altered in the somatosensory areas, but also the motor cortical networks via multi-stage hierarchical processing in which various parts of the motor system are engaged (Avanzini et al. 2018). Somatosensory cortex changes can be relayed to the motor cortical areas via cortico-

cortical connections and/or directly via cerebello-thalamo-cortical connections (Carson and Buick 2019). Specifically, electrical stimulation was shown to cause activations in both contralateral S1 and M1 when median nerve stimulation was applied at the motor threshold intensity (Spiegel et al. 1999), as well in the SMA using similar stimulation protocols (Manganotti et al. 2012). Intensity-dependant effects were shown in motor cortical networks as well, with progressively larger M1 activations at maximal motor response intensity, compared to sensory-level stimulation intensity (Smith et al. 2003). Importantly, consistent to our current study FEST protocols, functional level of stimulation, which generated flexion and extension of the wrist resulted in fMRI-registered simultaneous cortical activations in the contralateral M1, S1 and PM areas, bilateral S2 and SMA, as well as ipsilateral cerebellum (Blickenstorfer et al. 2009). Although our study could not quantify cerebellum activations, which is thought to be a part of the motor control network and affected by electrical stimulation of the periphery (Iftime-Nielsen et al. 2012; Carson and Buick 2019), we showed that the PR area, a site of potential sensorimotor integration (Hinkley et al. 2007), was considerably affected by FEST. It has also been suggested that stimulation patterns that mimic voluntary-like activations (i.e., FEST) are required to induce reliable cortical changes (Carson and Buick 2019). However, magnitude of cortical activation change relative to rest are larger during voluntary movement compared to FES-induced movements in the M1, S1 and SMA areas, while S2 activations were larger during FES condition (Joa et al. 2012). On the other hand, activations in the ipsilateral cerebellum and contralateral M1 and S1 were larger during combined voluntary and FES-induced contractions compared to FES condition alone (Joa et al. 2012). Adjuvant techniques combining the central drive at the level of the cortex using voluntary movement intention or motor imagery tasks and consequential muscle contractions using FES, may be crucial in associative forms of neural plasticity (Carson and

Buick 2019). Similarly, brain-machine interface-controlled FEST, which can be viewed as a form of associative intervention, have been shown as extremely effective to restore motor function after various neurological injuries (Daly et al. 2009; Biasiucci et al. 2018; Marquez-Chin et al. 2016). In our study, the participant was asked to actively attempt each movement and contraction before the therapist applied appropriate sequence of FES to activate the appropriate muscles. Taken together, these findings emphasize the importance of associative interventions that combine central activations at the cortical level and peripheral electrical stimulation to induce cortical re-organization.

While most abovementioned studies demonstrated how electrical stimulation can engage cortical networks during the stimulation, evidence also exists that sustained cortical changes can outlast the stimulation intervention. For instance, 2-hours of median nerves stimulation at intensities above the motor threshold was shown to cause increased cortical activations in the M1, S1 and dorsal premotor cortex, which persisted for up to 60 min after the stimulation (Wu et al. 2005). Similarly, using mesh glove stimulation at intensities below the sensory threshold for a period of 30 min was shown to induce cortical activations in the contralateral M1 and S1 regions for a period of 2-hours following cessation of stimulation (Golaszewski et al. 2004). On the other hand, therapeutic application of electrical stimulation delivered over longer periods of time, which used similar intervention protocols to our current study, showed evidence of sustained cortical re-organization (Shin et al. 2008; Sasaki et al. 2012; Gandolla et al. 2016). Specifically, 30 min of finer flexion / extension induced using an upper-limb FES orthosis once per day for a total of 12-weeks was shown to improve motor function of chronic hemiplegia patients, which was accompanied by fMRI-registered cortical changes in the somatosensory cortex either distributed bilaterally in some patients or localized unilaterally within the somatosensory area in

others after the intervention (Sasaki et al. 2012). Moreover, 1-hour of muscle activation-triggered FES wrist extension applied 5 days per week for a total of 10-weeks significantly improved motor function in chronic stroke patients, which was accompanied by shifting in the somatosensory area activations from ipsilateral to contralateral hemisphere after the cessation of stimulation (Shin et al. 2008). In the lower-limbs, 30 min of FES per day for applied for foot-drop correction over the peroneal nerve for 5 days per week for a total of 4-weeks showed that SMA and angular gyrus were the key regions involved in mediating therapeutic carryover effects in stroke patients who improved the functional outcomes (Gandolla et al. 2016). Taken together, our results therefore suggest that at least 40-hours of FEST are required to induce cortical re-organization in the upper-limbs (Shin et al. 2008; Sasaki et al. 2012), while there were no changes with less training (i.e., after 6-weeks of FEST). Lower-limb interventions may require shorter interventions (Gandolla et al. 2016). Importantly, our current study also demonstrated long-term cortical re-organization not just immediately after the intervention, but also several months (i.e., at least 12-weeks) after cessation of FEST, which is consistent with clinical recovery profiles (Thrasher et al. 2008; Kapadia et al. 2011; Marquez-Chin et al. 2017). Considering that the individual in our current study was in the chronic stage (> 7 years) after the injury, spontaneous recovery mechanisms can be ruled out. Evidence points that long-term repeated sensory (afferent) and motor recruitment using FES during task-specific upper-limb training, can induce experience-dependant cortical plasticity after brain injuries (Nudo et al., 2013). While, somatosensory cortices (S1 and S2) may be activated via cutaneous and contraction-induced reafference from FEST (Wiesendanger and Miles, 1982; Carson and Buick 2019), intact motor areas topologically adjacent to the damaged site within the primary motor cortex (M1) and areas outside of M1 such as the premotor cortex and supplementary motor areas

(PM and SMA) in contralateral and ipsilateral hemispheres may assume control over the affected muscles via intracortical connectivity networks (Weiller et al. 1992; Seitz et al. 2005; Nudo et al. 2013). Specifically, dopamine rewards system (Boroojerdi et al. 2001; Kaelin-Lang et al. 2002) and Hebbian associative learning (Hebb 1949), exposed through long-term task-specific repeated training with cortical engagement during voluntary intention and FES-induced functional consequence, are the likely mechanisms of FEST cortical re-organization, i.e., neuroplasticity.

4.2. Functional changes in hand motor function after FEST

Clinical scores and drawing test results suggest that the individual who participated in our study had a relatively high level of motor function at the onset of FEST intervention, suggesting a relative plateau in motor function, while the intervention resulted in minor improvements. Specifically, the FIM score evaluates activities of daily living, including motor scores, communication, and social cognition (Granger and Hamilton 1992) with excellent reliability in TBI patients (Donaghy and Wass 1998). The FMA evaluates the motor function, sensation, joint movement, and pain components, also with excellent test-retest reliability in TBI patients (Platz et al. 2005). At the start of the intervention (Pre), the FIM score was 42 out of 42, indicating complete independence, while the upper-limb portion of the FMA score was 63 out of 66, indicating high level of upper-limb function. As expected, neither FIM nor FMA scores changes as a result of the intervention (Table 1) due to ceiling effect on these clinical scores. On the other hand, the MAL score increased from 78 to 79 out of 92 after 6-weeks of FEST and lasted for at least another 18-weeks after FEST (Table 1). The MAL score is a structured semi-interview that can assess upper-limb function, which consists of 30 functional daily tasks, and evaluation of the amount-of-use scale as well as quality-of-movement scale (Lee et al. 2004). Minimal clinically

important difference of MAL is 1.0-1.1 (Simpson and Eng 2013). Previous studies have shown improvements in functional impairments using clinical scores after the FEST intervention in people with stroke (Thrasher et al. 2008) as well as incomplete cervical SCI (Kapadia et al. 2011), which lasted well after the intervention period (Kapadia et al. 2011). Our results suggest possible mild improvements using MAL score after FEST in an individual with chronic TBI.

Drawing test results, which can assess fine motor function, also showed minor changes in motor function immediately after 6-weeks of FEST, which seemed to progress further after the intervention and during follow-up (Figure 4B and C). 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 some effects on the drawing tests after FEST, which are indicative of improved performance and may be related to the cortical changes. Specifically tracking task (Figure 4C - top), which required following a moving target on the tablet screen, initially showed deteriorated performance (increased mean error), while there was improvement during follow-up. These were accompanied by a decrease in mean velocity and acceleration, which may suggest less abrupt movements. On the other hand, tracking task (Figure 4C - bottom), which required following the outline of a sine wave a self-selected speed, showed progressive improvements in performance (decreased mean error) immediately after 6-weeks and 12-weeks of FEST, which were accompanied by decreased mean velocity and acceleration. Similarly, improved square 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). Using similar, but more intense FEST protocols, improved performance during circle-drawing test was suggested to be associated with reduced spasticity (Kawashima et

al. 2013). Considerable improvements in drawing accuracy on a tracking task was reported in individuals with chronic stroke after 10-weeks of FES upper-limb therapy, consistent to increased cortical activations, while the control group that did not exhibit altered cortical activations also did not improve on the drawing test (Shin et al. 2008). Electrical stimulation is known to affect the same brain networks that ultimately serve as a basis for improved functional capacity (Traversa et al. 1997; Fraser et al. 2002; Carson and Buick 2019). Specifically, if changes can be made to persist indefinitely, they can cause motor improvements (Ridding et al. 2001). Considering that stimulation parameters and modes of delivery of electrical stimulation can vary in their effectiveness to evoke changes in the central nervous system (Chipchase et al. 2011; Bergquist et al. 2011; Carson and Buick 2019), the current study utilized the FEST protocols developed by our group, which were shown in randomized clinical trials to improve motor function after neurological injuries (Thrasher et al. 2008; Kapadia et al. 2011; Marquez-Chin et al. 2017). Using these FEST protocols, we demonstrated considerable cortical re-origination beyond the intervention period. Therefore, although clinical scores and functional motor performance improvements in our study were relatively mild, the results of cortical re-organization after FEST suggest that functional motor improvements can be induced in individuals suffering from motor impairment after TBI.

4.3. Limitations and future work

A limitation of our current study is the small sample size (n=1) and no control group to examine the benefits of equal conventional upper-limb therapy, compared to FEST. 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 (Thrasher et al. 2008; Kapadia et al. 2011). Therefore, superiority of FEST has previously been shown in larger innervational studies, while cortical mechanism of the FEST intervention, remained unclear and variable between studies (Carson and Buick 2019), especially in individuals with TBI. Our study utilized a detailed assessment over the course of 3-month of FEST intervention as well as during 3-months follow-up period with an individual suffering mild upper-limb motor impairment after chronic stage TBI to understand mechanisms of recovery and time course of cortical re-organization after FEST. As recently pointed out case study observations utilizing detailed aspects of interventions can serve as a basis for future studies targeting larger populations (Bloem et al. 2020). Specifically, such investigations have led to many important clinical and neurophysiological discoveries (Bloem et al. 2020). Therefore, our current study results should be used to test specific hypothesis related to cortical mechanisms of motor function improvement using FEST in the TBI population. Moreover, another limitation of our study is that we did not investigate short- or long-term spinal reflex excitability effects resulting from FEST. It is generally known that even short-term application of FES can inhibit the spinal reflex excitability (Hortobagyi et al. 2003; Milosevic et al. 2019), which may help to reduce spasticity. Similarly, long-term application of FEST was shown to inhibit spinal reflex excitability (Kawashima et al., 2013). Considering that simulation parameters and models of delivery of electrical stimulation can alter its physiological effectiveness (Chipchase et al., 2011; Bergquist et al. 2011), future studies are warranted to examine subcortical excitability in parallel with cortical re-organization during and after FEST.

5. Conclusions

Using detailed assessments, our clinical case study results showed that FEST intervention can be effective for facilitating cortical re-organization that can improve voluntary upper-limb motor function after brain injuries. Although motor improvements were relatively small, our study showed motor changes, correlated to cortical re-organization in an individual with mild motor impairment. Specifically, our results showed long-term effects of FEST on corticospinal excitability, likely due to larger motor map representations in and around the primary motor cortex area. These findings were corroborated by neuroimaging results, which showed enlarged activations in the somatosensory areas, as well as the primary motor area, other areas related to voluntary motor control and sensorimotor integration. These findings should serve as evidence to develop and test specific hypotheses in larger cohorts related to effectiveness of FEST for recovery of upper-limb motor function after TBI.

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Conflicts of interest

M.R.P. is a shareholder in company MyndTec Inc. The remaining authors have no conflicts of interest.

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Tables

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

Figure Captions

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-weeks and 12-weeks of FEST (During and Post0), as well as during follow-up 6-weeks 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.

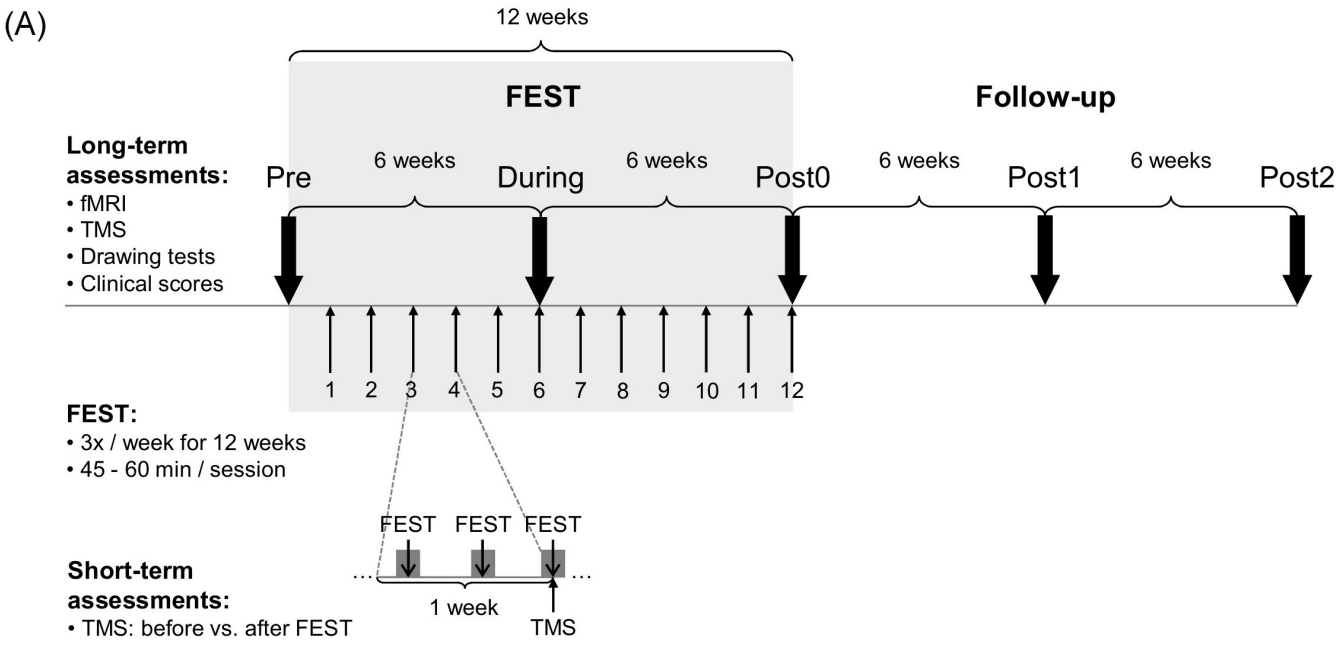
Figure 2: *Motor evoked potential (MEP) results for the short-term assessments* - **(A)** Input-output relationship curve for the first dorsal interosseous (FDI) and abductor pollicis brevis (APB) muscles. Dotted lines indicate simple linear regression lines of the curves before and after one functional electrical stimulation therapy (FEST) session. Each point is indicated as the mean amplitudes and standard error (SE). Bar graphs indicate values of regression line slope; **(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-weeks and 12-weeks of FEST (During and

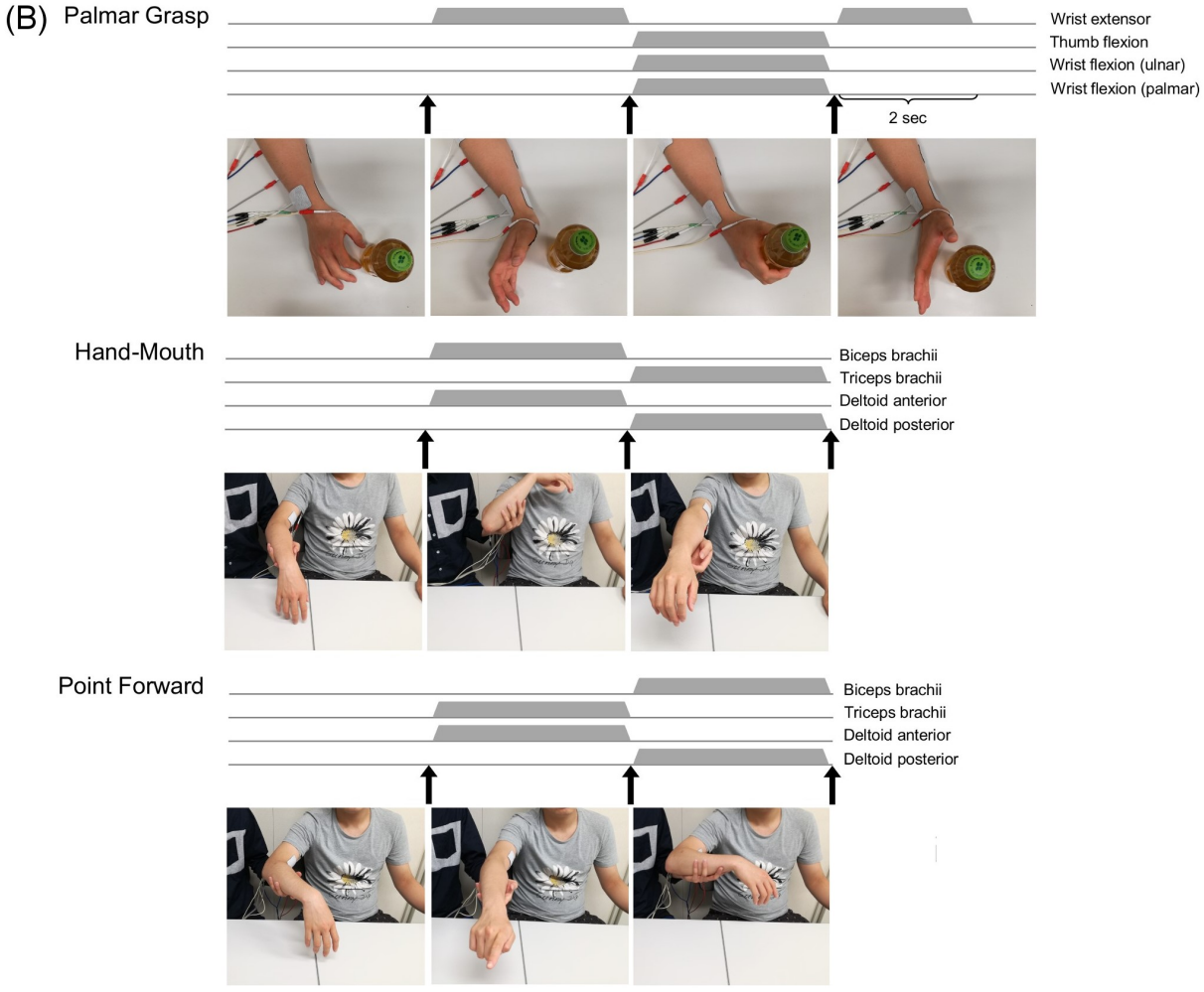
Post0) as we as during follow-up assessments 6-weeks and 12-weeks after FEST (Post1 and Post2). Each point is presented as the mean amplitudes and standard error (SE). Bar graphs indicate values of regression line slope. **(D)** CSP for the FDI and APB muscles during Pre, During, Post0, Post1 and Post2 assessments; **(F)** 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. Legend: n.s., not significant; *p<.05.

Figure 3: *Functional magnetic resonance imaging (fMRI) during the hand grip task* - **(A)** Activated regions during right (intervention) hand grip force matching 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<.001 (uncorrected) and cluster level p<.05 (FWE). Assessments were carried out at baseline (Pre), after 6-weeks and 12-weeks of FEST (During and Post0), as we as during follow-up assessments 6-weeks and 12-weeks after FEST (Post1 and Post2); **(B)** ROI analysis and the coordinates of the most activated voxel in the primary motor cortex (M1) for each assessment; **(C)** 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 the activity of the contralateral hemisphere (Contra) and the lower bar graphs shows the activity of the ipsilateral hemisphere (Ipsi). *fMRI during the finger pinch task* - **(D)** Activated regions during right (intervention) finger pinch force matching 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<.001 (uncorrected) and cluster level p<.05

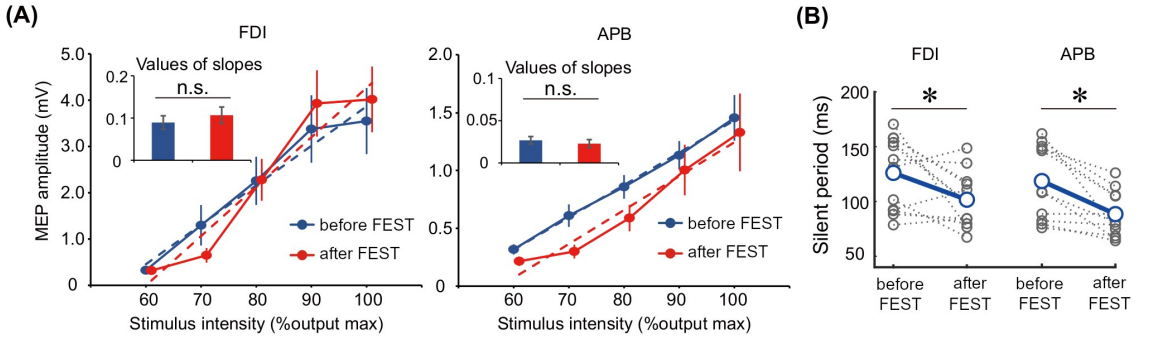
(FWE). Assessments were carried out at Pre, During, Post0, as we as Post1 and Post2; **(E)** ROI analysis and the coordinates of the most activated voxel in the primary motor cortex (M1) for each assessment; **(F)** ROI results based on anatomical regions in the M1 as well as S1, S2, PR, SMA, PM, and HC. The upper bar graphs show the activity of the contralateral hemisphere (Contra) and the lower bar graphs shows the activity of the ipsilateral hemisphere (Ipsi).

Figure 4: *Drawing test results* - **(A)** Experimental setup showing the instrumented tabled with the participant, who was instructed to track a sine wave displayed on the screen; **(B)** Representations of the participant's performances on the drawing tests at baseline (Pre), after 6-weeks and 12-weeks of FEST (During and Post0), as we as during follow-up assessments 6-weeks and 12-weeks after FEST (Post1 and Post2). Tracking performance is shown in the upper row, with the round target, which moved over the sine wave at 12mm/sec and while the participant was instructed to follow it. Sine wave tracing performance is shown in the lower row where the participant had to follow the outlined at self-selected speed; **(C)** The error, velocity, coefficient of variation (CV) of velocity and acceleration performance, with tracking shown in the upper row and sine wave tracing in the lower row.

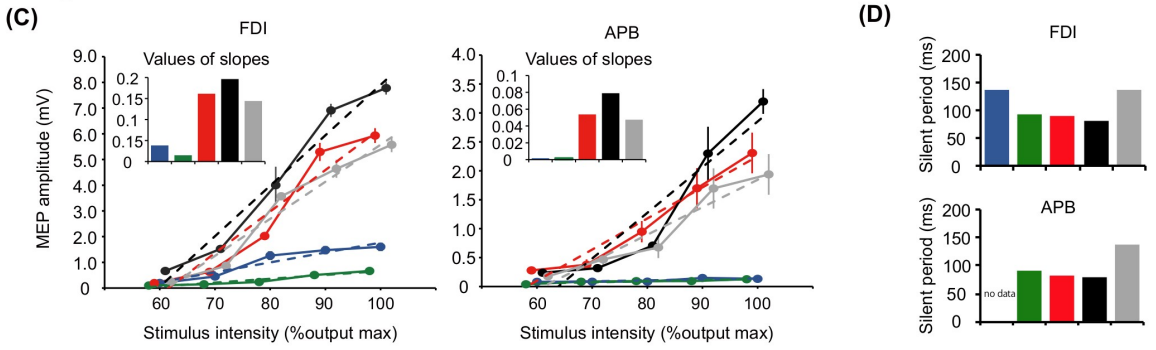


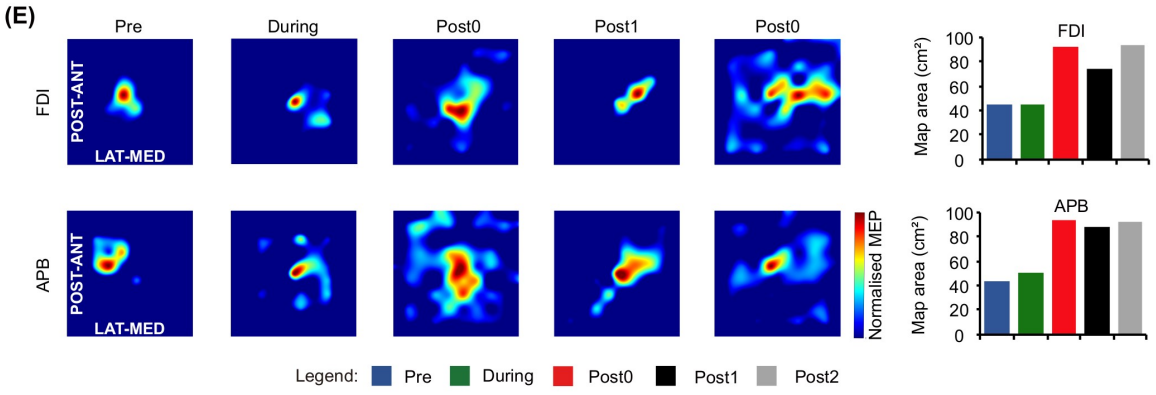


Short-term assessments

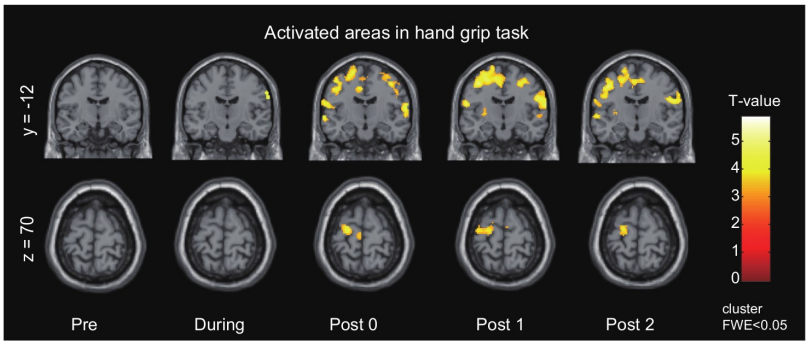


Long-term assessments

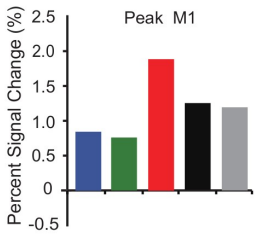




(A) Hand grip task



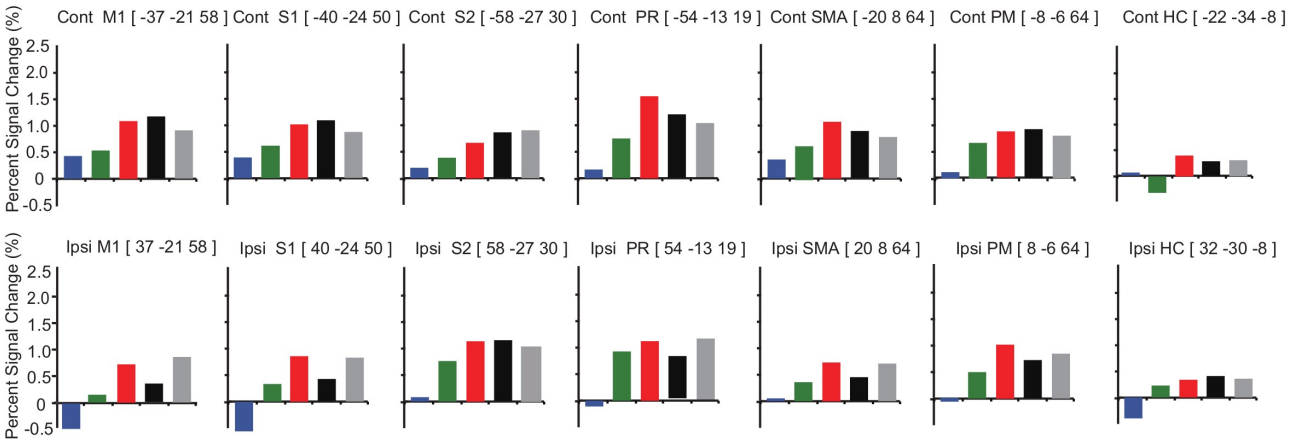
(B)



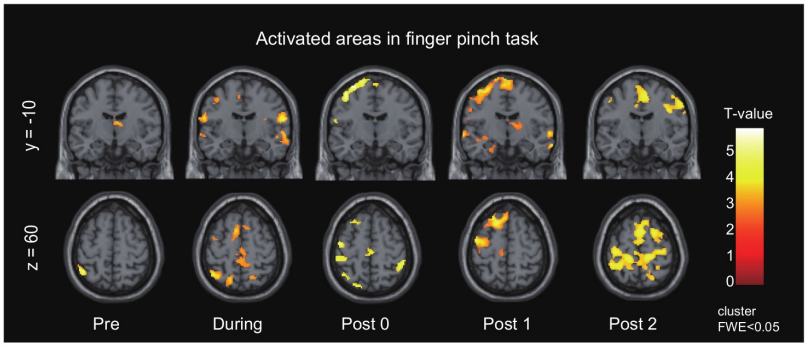
Locations of peak activated voxel in M1

	MNI coodinates			
Assesments	x	y	z	Z
Pre	-54	10	44	4.61
During	-38	-16	54	2.94
Post 0	-48	-2	38	7.1
Post 1	-40	-10	50	5
Post 2	-44	-8	46	5.41

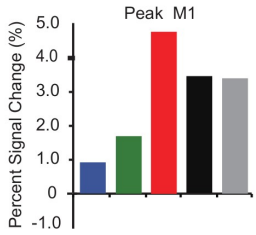
(C)



(D) Finger pinch task



(E)



Locations of peak activated voxel in M1

	MNI coodinates			
Assesments	x	y	z	Z
Pre	-56	2	48	4.16
During	-40	0	54	5.92
Post 0	-38	-8	68	6.87
Post 1	-46	-2	58	5.84
Post 2	-34	-20	68	4.72

(F)