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Pilot study: Neuromuscular function in healthy participants and subacute stroke patients following an associative BCI intervention assessed by HD-EMG

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Title page

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Abstract

Aim: To investigate changes in neuromuscular function measured by high density electromyography (HD-EMG) before and after an associative brain computer interface (BCI) intervention within healthy participants and subacute stroke patients.

Method: Eleven healthy participants and four subacute stroke patients (three in the intervention group and one in the sham group) took part. For the healthy participants, the protocol consisted of two sessions: one intervention session and one sham session. For the stroke patients the BCI protocol consisted of 12 sessions, where HD-EMG was assessed in sessions 2 and 11.

During one session, a 64 channel HD-EMG matrix electrode placed on the extensor carpi radialis of the dominant/paretic arm was used to assess changes in neuromuscular function before and after the BCI session. In the initial HD-EMG measurements the maximal voluntary contraction (MVC) was found and used to calculate submaximal force levels. Three ramps of force contractions were performed at each submaximal force level: 20% and 50% MVC, respectively. The BCI session consisted of 30 ballistic extensions of the wrist in response to a visual cue combined with an associative peripheral electrical stimulation. After the BCI session the post HD-EMG measurements were performed.

Results: For the healthy participants no significant interaction or main effects were observed pre and post one BCI session for MVC, force CV, total number of motor units (MU), recruitment threshold (RT), or discharge rate (DR).

For the stroke patients they all had force improvements. The data for the DR varied. For the RT two from the intervention group and the one from the sham group overall seemed to increase between sessions. However, the data from the last stroke patient in the intervention group varied.

Conclusion: For healthy participants no significant changes were found suggesting that if neuromuscular changes contribute to the positive effects of an associative BCI intervention, they do not change significantly after only one session. All stroke patients got stronger and better at maintaining a steady force. The DR data for all stroke patients varied suggesting that the force improvements were not primarily due to improvements in DR. Independent of treatment three stroke patients had an increase in RT and improvements in MU firing behavior was observed. This pilot study is the first to assess changes in neuromuscular function in subacute stroke patients following four weeks of associative BCI intervention. However, more patients are needed to make more general conclusions.

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Introduction

Stroke is currently one of the leading causes of disability and death among adults worldwide (Zhu, 2022). Following stroke, it is important to initiate rehabilitation to regain as much function as possible. It is in the first few months after a stroke that the plasticity in the brain is at its highest (Mrachacz-Kersting et al., 2019). This critical phase can be exploited by including brain computer interface (BCI) in the rehabilitation program (Mrachacz-Kersting et al., 2016).
Multiple studies have shown both on lower and upper extremities that by using an electroencephalography (EEG) based non-invasive BCI method combined with an associative peripheral electrical stimulation (PES), recovery after a stroke is improved, including increases in corticospinal excitability (CSE) and functional improvements (Kim et al., 2022; Mrachacz-Kersting et al., 2016, 2019; Mrachacz-Kersting & Aliakbaryhosseinabadi, 2018; Niazi et al., 2012)
However, at what level these improvements occur is yet not fully understood. One theory is that the improvements are due to changes in the neuromuscular function. The mechanisms of paresis following stroke are generally unclear and might include loss of motor units (MU), muscle atrophy, impairment in MU pool activation, and uncoordinated muscle activation (Li et al., 2015). Normally, maximal level of force in muscles is generated by an optimal combination of MU recruitment and rate modulation (Li et al., 2015). Evidence suggests that weakness following stroke may be a result of disorganized MU firing behavior, but the evidence is still incomplete and primarily comes from indirect measurements with surface electromyography (EMG) (Li et al., 2013, 2014, 2015; Zhou et al., 2007, 2013). Surface EMG is insufficient when investigating MU firing behavior (Li et al., 2015; Zhang & Zhou, 2012). However, high-density EMG (HD-EMG) has shown promising insights (Del Vecchio et al., 2019, 2020; Kallenberg & Hermens, 2009; Li et al., 2015; Zhang & Zhou, 2012).
HD-EMG is a novel non-invasive technique used to record MU action potentials (MUAP). It is based on multiple small surface electrodes, which enables a larger interpretation of muscle electrophysiological activity (Rojas-Martínez et al., 2020). Understanding MU firing behavior in stroke patients will help to analyze the disrupted mechanisms, which is crucial for the development of possible therapeutic interventions (Li et al., 2015).

The aim of this pilot study was to investigate changes in neuromuscular function measured by HD-EMG before and after an associative BCI intervention within healthy participants and subacute stroke patients. It was hypothesized that BCI intervention would increase discharge rate (DR) and decrease recruitment threshold (RT) within one session for healthy participants and after four-weeks of intervention for subacute stroke patients. Furthermore, force improvements were only expected for the stroke patients.

Method

Population

Eleven healthy participants and four stroke patients (Table 1) recruited from the neurorehabilitation center at Neuroenhed Nord in Brønderslev, Denmark, were included in the study.
All participants gave their written consent before the experiment, were above the age of 18 years, cognizant, and able to make informed decisions. Only stroke patients who had suffered from a clinical stroke within the last month (subacute stroke patients) and those for whom the cerebrovascular injury was verified by MRI scan were included. The stroke patients were excluded from the study if they were not able to complete the experiment because of an earlier stroke in the same cerebral region, a cognitive or verbal disturbance, a general weakness, or other severe comorbidities. Furthermore, if the patient was not able to generate a force in the paretic wrist, they were also excluded.
Approval for the pilot study was given by the Scientific Ethics Committee for Nordjylland, Denmark (reference no. N-20200004).

Table 1: Healthy participant and stroke patient characteristics. Median values and standard deviation (SD) in parentheses.

	Healthy Participants	**Stroke patient 1**		**Stroke patient 2**		**Stroke patient 3**		**Stroke patient 4**	
Sex	8 Men, 3 Female	M		M		F		M	
Age (years)	28.6 (± 4.9)	75		40		74		60	
Weight (kg)	78.3 (± 10.1)	108		91		63		63	
Height (cm)	182.7 (± 5.2)	189		175		162		175	
Affected/Dominant side	9 Right, 2 Left	Left		Left		Right		Right	
Treatment group	Intervention/Sham	Intervention		Sham		Intervention		Intervention	
MEP status	Non-relevant	+		+		-		-	
Pre session 2 (Pre2) Post session 11 (Post11)	Non-relevant	Pre2	Post11	Pre2	Post11	Pre2	Post11	Pre2	Post11
UE-FM (/66)	Non-relevant	46	53	63	66	8	22	42	41
ARAT (/57)	Non-relevant	31	52	54	57	3	7	23	32

Study design

The current study took place at the neurorehabilitation center at Neuroenhed Nord in Brønderslev, Denmark, and data were collected from the 24th of October to the 8th of December 2022. For healthy participants, the associative BCI protocol and the HD-EMG assessment consisted of two sessions with at least one week in between: one intervention session and one sham session. The order of the sessions was randomized by using the website rando.la. For stroke patients, the BCI protocol consisted of 12 sessions over a period of four weeks, where HD-EMG was assessed in sessions 2 and 11. The stroke patients had a stratified randomization based on their score on the upper extremity Fugl-Meyer scale (UE-FM) together with age and randomized by rando.la into either an intervention or a sham group. All participants were blinded. The experimenters of the BCI protocol were not blinded. CSE was measured by a transcranial magnetic stimulation (TMS) in sessions 1, 6, and 12. Before and after the 12 sessions, the stroke patients were clinically evaluated by physiotherapists

blinded to the intervention using the UE-FM and the action research arm test (ARAT). TMS and the clinical tests will not be further discussed in the current study.
A schematic overview of the experimental protocol for a single session is shown in Figure 1.

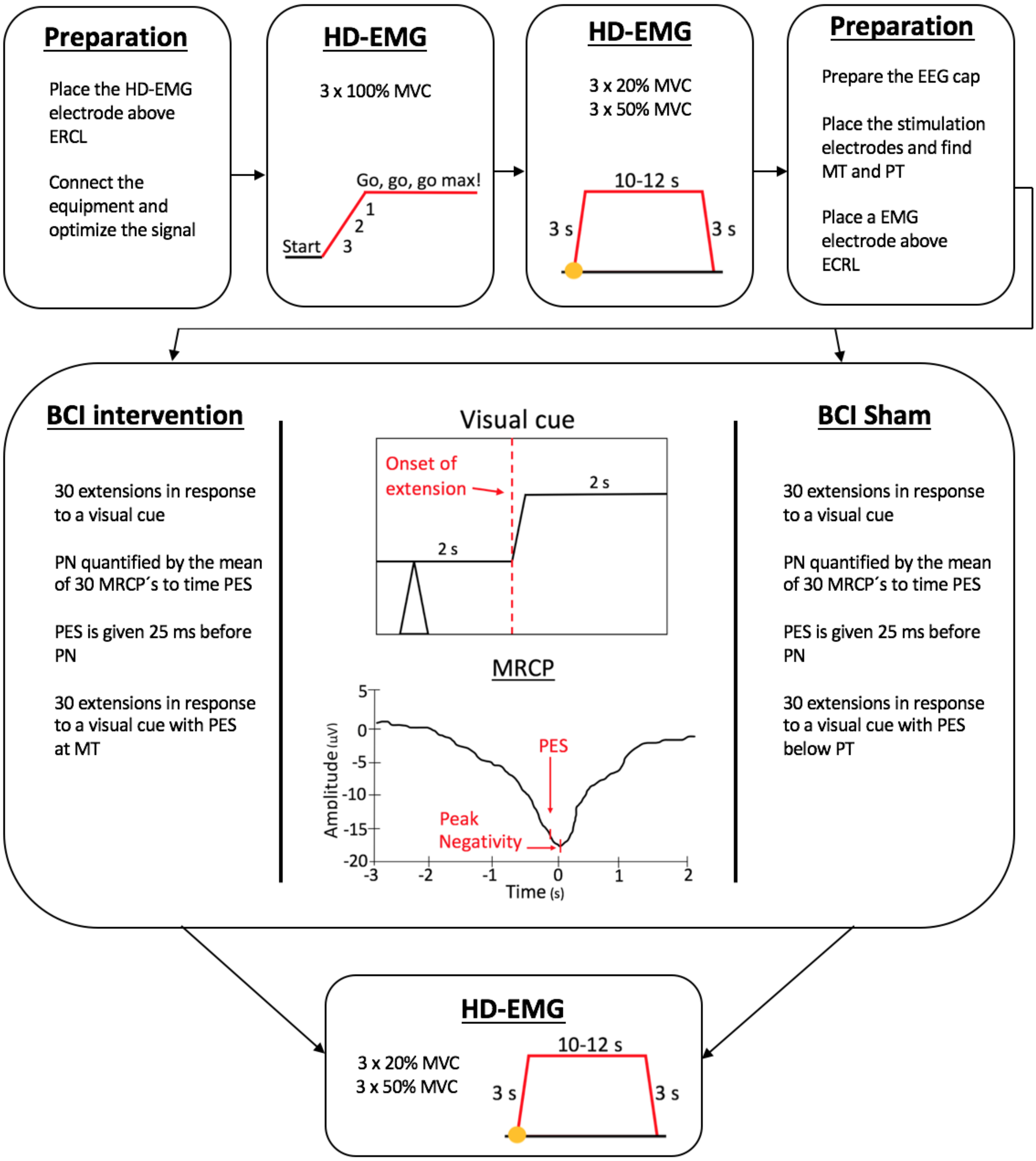

Figure 1: Schematic overview of the experimental protocol.
HD-EMG = high density electromyography; ECRL = extensor carpi radialis; MVC = maximal voluntary contraction; EEG = electroencephalography; MT = motor threshold; PT = perception threshold; EMG = electromyography; MT = motor threshold; PT = perception threshold; EMG = electromyography; PN = peak negativity; MRCP = movement-related cortical potential; PES = peripheral electrical stimulation.

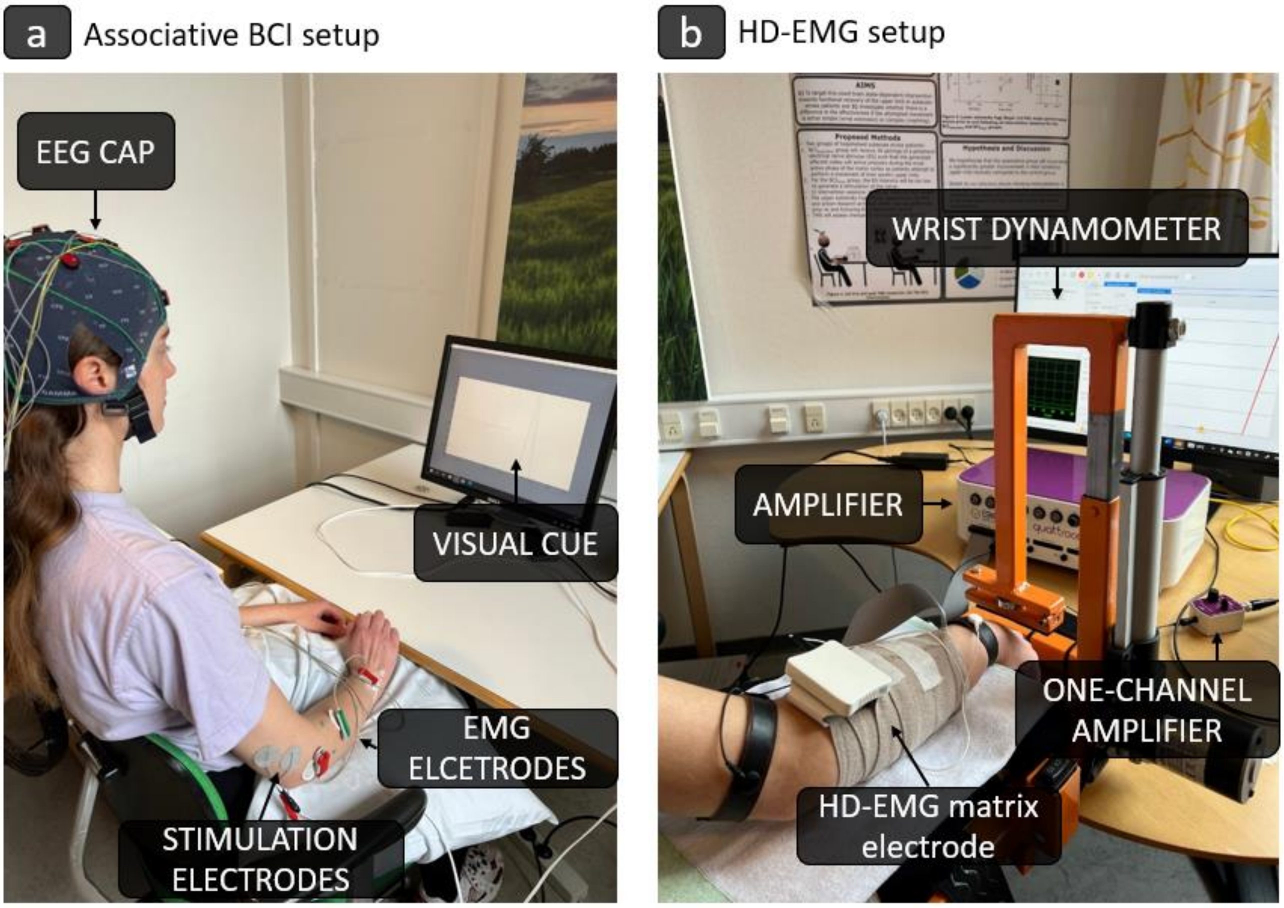

Figure 2: (a) Associative brain computer interface experimental setup. (b) High density electromyography experimental setup. EEG = electroencephalography; EMG= electromyography; HD-EMG = high density electromyography.

Associative brain computer interface

Associative BCI setup

The associative BCI setup is shown in Figure 2a and consisted of recording both EEG and EMG signals sampled at a frequency of 256 Hz and hardware filtered from 0 to 100 Hz. The EEG signals were recorded using an EEG cap (g.GAMMAcap2; G.Tec Medical Engineering, Schiedlberg, Austria) connected to a g.USBamp amplifier (G.Tec Medical Engineering). Electrodes were placed based on the international 10-20 system in the following positions: FZ, FC1, FC2, C5, CZ, C4, CP1, CP2, PZ, FP2 and a reference electrode on the earlobe. The EMG signals were recorded from a single channel on the extensor carpi radialis muscle (ECRL) with two reference electrodes placed over the lateral epicondyle and over the ulnar styloid process on the dominant arm for healthy participants and on the paretic arm for stroke patients. On the same arm, two simulation electrodes placed above the lateral bicipital sulcus to stimulate the radial nerve were connected to an electrical stimulator (NoxiTest IES 230). All participants were placed on a chair approximately 1 m from a computer screen. The dominant/paretic arm was placed on a pillow in their lap.

Timing and intensity of the peripheral electrical stimulation

PES was applied to the radial nerve through the stimulation electrodes. The PES in the BCI intervention sessions was set at motor threshold (MT), defined as the lowest stimulation at which a contraction in the wrist extensors could be palpated. The PES in the BCI sham sessions was set below the perception threshold (PT), which was the lowest stimulation registered by the participant. This was done to ensure no sensory neurons were recruited (Mrachacz-Kersting et al., 2019).
The participants were asked to perform 30 ballistic extensions of the wrist with the dominant/paretic arm in response to a visual cue on a computer screen. If the patients were not able to extend their wrist, they were asked to imagine the movement. The visual cue consisted of 2 s preparation time, 2 s hold-phase of the contraction, and 6 s resting time. The cue was timed by a custom-made MATLAB script (R2015a; MathWorks, Natick, MA). Based on the EEG recordings, the peak negativity (PN) was quantified by the mean of the 30 MRCP's to calculate the delivery of the PES later used in the associative BCI protocol. The following equation was used: time of delivery = 2 s - 0.025 s ± PN (Mrachacz-Kersting et al., 2019). The 2 s refers to the preparation time and the 0.025 s refers to the signal latency from the upper limb to the cortex (Mrachacz-Kersting et al., 2019).

Experimental session

The participants performed 30 ballistic extensions of the wrist in response to the same visual cue. The actual or imagined extension of the wrist was performed with the PES either at MT or below PT depending on whether it was a BCI intervention or sham session, respectively.
The healthy participants attended the sessions at similar times of the day. Most stroke patients attended the sessions in the afternoon after their regular training at the rehabilitation center.

High density electromyography measurements

HD-EMG setup

The HD-EMG measurements were performed before (pre) and after (post) a BCI session, the set-up is shown in Figure 2b. The participants were placed on a chair approximately 1 m away from a computer screen. The dominant/paretic arm was placed comfortably in a wrist dynamometer consisting of a customized load cell which was connected to an amplifier (Quattrocento; OT Bioelettronica). An HD-EMG matrix electrode (Matrix 64 electrodes; OT Bioelettronica) with 64 channels was placed on the skin above the belly of ECRL and connected to the amplifier. Two wet band reference electrodes were placed on the dominant/paretic arm; one around the wrist and one around the elbow and connected to the amplifier. The wrist dynamometer was connected to a one channel pre-amplifier (Forza-B; OT Bioelettronica) which was also connected to the amplifier. Both HD-EMG and force data were sampled at 2048 Hz.

Experimental session

The participants performed three maximal voluntary contractions (MVC) as an isometric extension of the wrist in response to a vigorous verbal encouragement with a 25 s resting time in between.

The force was measured by the customized load cell. The total duration of the contraction was 5 s, consisting of 3 s of acceleration and 2 s of maximal force contraction. The highest MVC value was used to calculate the submaximal force levels: 20% and 50% MVC, respectively. A new MVC was measured and used in each session. Each submaximal force level was performed three times as ramp contractions. The ramp for 20% MVC consisted of 3 s incline, 12 s hold-phase, and 3 s decline, whereas the hold-phase for 50% MVC consisted of 10 s. This was displayed on a computer screen with real time visual feedback. The resting time between each submaximal ramp contraction and force level was 30 s to avoid muscular and mental fatigue. Before each submaximal force level, the participants performed one to two trials as a familiarization.

Data Analysis

All data were processed offline using a customized MATLAB script (Negro et al., 2016). A schematic overview of the different steps in the data analysis is shown in Figure 3.
First, a visual inspection of the HD-EMG signals from all 64 channels was conducted to exclude bad signals. Figure 3b shows 8 out of 64 channels with a similar signal-to-noise ratio. The higher the signal-to-noise ratio the better the signal quality. A bad signal was detected by visualizing signal outliers from the baseline across the channels (Del Vecchio et al., 2020).
Second, the ramp of force contraction and the HD-EMG signals were cut to only include the HD-EMG signals during the ramp shown in Figure 3c.
Third, for each submaximal force level pre and post, the three ramps of force contractions were combined into one file as shown in Figure 3d before decomposition. This was done to ensure long enough HD-EMG signals for an optimal pattern recognition (Del Vecchio et al., 2020). The HD-EMG signals were decomposed with a blind-source separation method with a bandwidth of 20-405 Hz and MU pulse trains were extracted and manually improved and adjusted (Del Vecchio et al., 2019, 2020). An example is shown in Figure 3e. The heights of the MUAP's corresponds to the weight of how strong a MU firing pattern is formed. The stronger the pattern, the more certainty that the action potentials were fired by the same MU and the closer the silhouette calculation (SIL) is at 100% (Martinez-Valdes et al., 2017). SIL ≥ 0.87 were included. The blind source separation automatically identified MU discharges and the manual improvement consisted of adding or removing firings below a certain threshold to improve the SIL.
An example of an improved decomposition is shown in Figure 3f. In both plots the RT and the DR for the different MU's are represented. The order of the RT's in the clustered bar plot depends on the MU's extracted from the first out of the three trials.
The final data that were extracted for each time (pre and post), submaximal force level (20% and 50% MVC), and each condition (intervention and sham) consisted of: MVC (N), force coefficient of variation (CV) (%MVC, hold-phase), total number of MU's, mean RT (%MVC, the whole ramp), and mean DR (pps, hold-phase). However, MVC was only measured pre each condition.

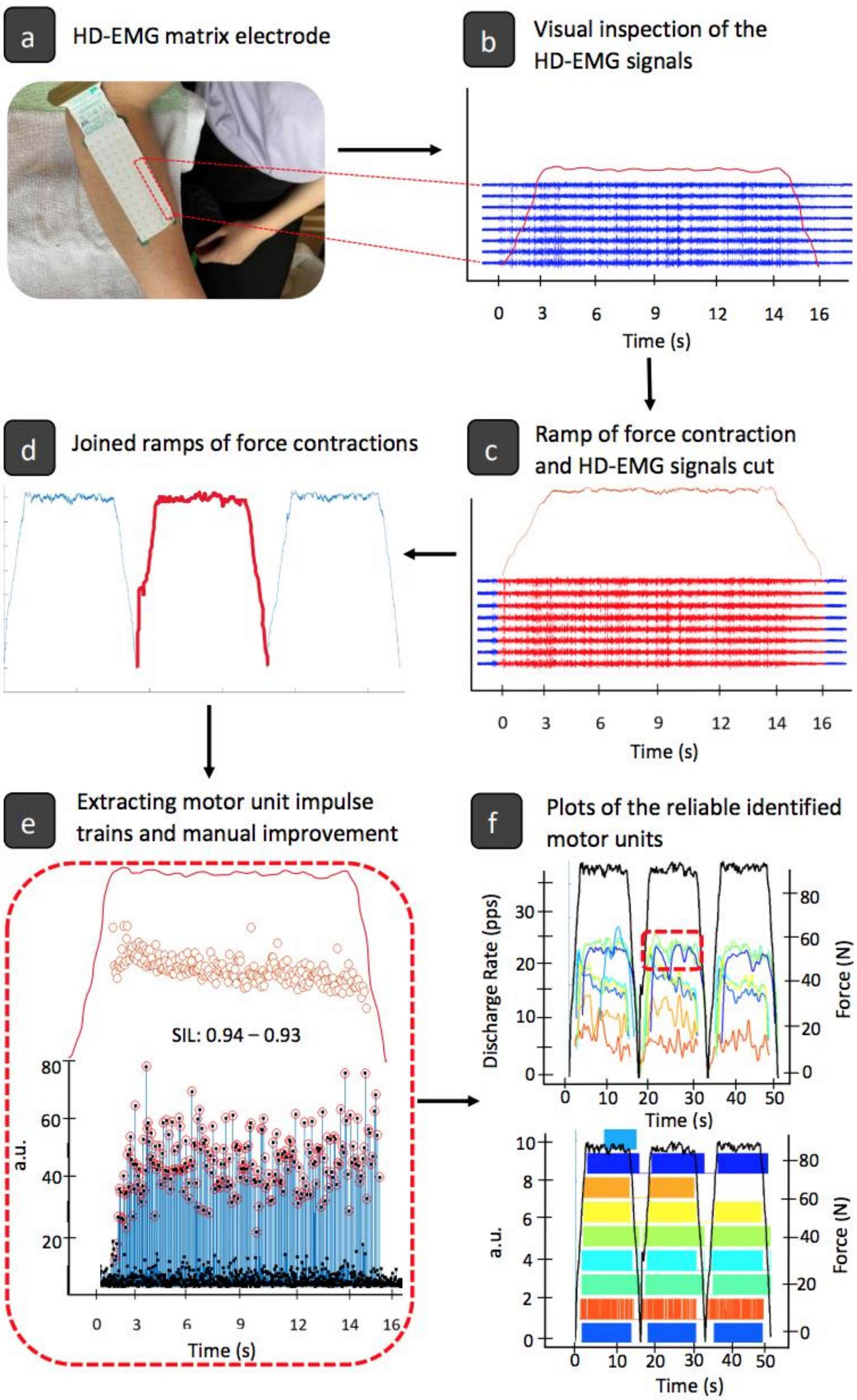

Figure 3: Schematic overview of the different steps of data analysis. The data are from healthy participant 2, sham session, pre 20% MVC.

(a) The HD-EMG matrix electrode with 64 channels placed on the skin above the belly of ECRL. (b) Visual inspection of the HD-EMG signals from 8 out of 64 channels (blue) and the ramp of force contraction (red) to exclude bad signals. (c) The ramp of force contraction (upper) and HD-EMG signals (lower) were cut to only include the HD-EMG signals during the ramp marked by red. (d) For each submaximal force level the three ramps of force contractions (blue, red) were combined into one file before decomposition. The HD-EMG signals were decomposed with a blind-source separation method and MU pulse trains were extracted. (e) Manual adjustment and improvement of the decomposition. Lower: the impulses from a single MU. Each blue spike illustrates a MUAP with heights corresponding to the weight of how strong a pattern in the upper part is formed. Upper: pattern formed by included spikes. SIL = 94 - 93%. The stronger the pattern, the closer the SIL calculation is at 100%. (f) The outcome of the improved decomposition. Upper: the plot shows the firing patterns for different MU's (the colored lines) that occurred during the three ramps of force contractions (black). The x-axis represents time, the y-axis on the left represents the DR, and the y-axis on the right represents force in %MVC. Lower: the plot shows the RT and DR for different MU's. Each colored bar represents a MU and matches the MU with the same color in the other plot. The bar in the bottom represents the MU with the lowest RT and the bar in the top represents the MU with the highest RT. The density of the bars corresponds to the number of discharges. The order of the RT's depends on the MU's extracted from the first out of the three trials.
HD-EMG = high-density electromyography, MU = motor unit, MUAP = motor unit action potential, SIL = silhouette index, RT = recruitment threshold, DR = discharge rate, a.u. = arbitrary unit, s = seconds, pps = pulse per second, N = newton, MVC = maximal voluntary contraction.

Statistical Analyses

Due to the limited number of stroke patients, only statistical analyses for the healthy participants were performed. Descriptive statistics are provided for stroke patients.
The assumption of normality was assessed through the Shapiro-Wilks test and visualized by QQ-plots for MVC, force CV, total number of MU's, mean RT, and mean DR. All data were approximately normally distributed and thereby met the assumptions needed for performing analyses of variance (ANOVA) or paired t-tests. A paired t-test for MVC was performed comparing the intervention and sham sessions. For each of the other dependent variables, a two-way repeated measures ANOVA compared two within-subjects factors, treatment (intervention, sham), and time (pre, post), respectively; one for each submaximal force level at 20% and 50% MVC. Statistically significant interactions and main effects were assessed through post hoc analyses with Bonferroni corrections. Statistical significance was determined as $p < 0.05$. Data are reported as mean ± SD within the text and in the figures unless otherwise stated.

Results

Healthy participants

Eleven healthy participants were included. However, due to lack of data from some MU's, some participants were excluded by the ANOVA. For pre and post 20% MVC for total number of MU's, mean RT, and mean DR, nine participants were included. For pre and post 50% MVC for total number of MU's, mean RT, and mean DR seven participants were included.

Figure 4 represents an example of the outcome from the decomposition including force, RT, and DR for one healthy participant's pre intervention session at 50% MVC. Figure 4a shows the firing patterns of six MU's and the order of their RT's are represented in Figure 4b. The order of the RT's depends on the MU's extracted from the first out of the three trials. The RT's at 50% MVC occurred at higher forces compared to the stroke patient in Figure 6.

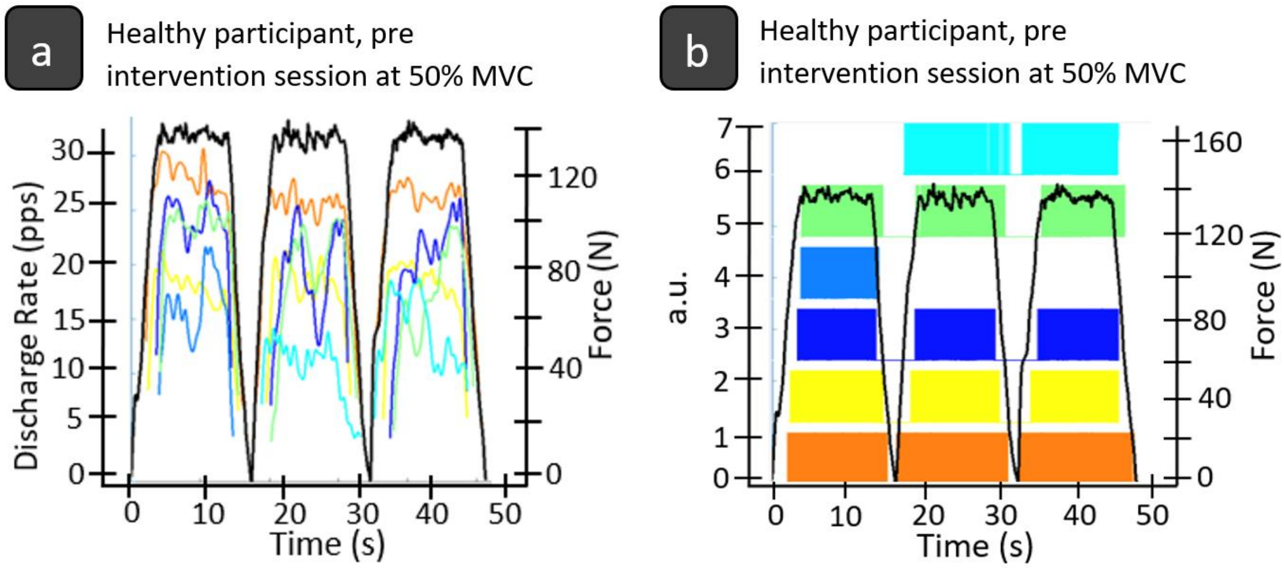

Figure 4: Clustered line and bar plots of the RT and DR for one healthy participants pre intervention session at 50 % MVC. (a) the plot shows the firing patterns for different MU's (the colored lines) that occurred during the three ramps of force contractions (black). The x-axis represents time, the y-axis on the left represents DR, and the y-axis on the right represents force in %MVC. (b) the plot shows the RT and DR for different MU's. Each colored bar represents a MU and matches the MU with the same color in plot a. The bar in the bottom represents the MU with the lowest RT and the bar in the top represents the MU with the highest RT. The density of the bars corresponds to the number of discharges. The order of the RT's depends on the MU's extracted from the first out of the three trials. RT= recruitment threshold; DR = discharge rate; MVC = maximal voluntary contraction; MU = motor unit; s = seconds; pps = pulse per second; N = newton; a.u. = arbitrary unit.

Maximal voluntary contraction, force coefficient of variation, recruitment threshold and mean discharge rate

Mean MVC was 482.8 ± 126.1 N for the intervention session and 456.8 ± 163.4 N for the sham session. No statistically significant difference between the intervention and sham sessions was observed (p = 0.50). Mean ± SD for force CV, total number of MU's, RT, and DR based on the measured MVC at the given session are shown in Table 2 and visualized in Figure 5. No statistically significant interactions or main effects of treatment and time were observed for force CV (all *p's* ≥ 0.28) or DR (all *p*'s ≥ 0.19) at 20% or 50% MVC.

For the total number of MU's, no statistically significant interaction or main effects of treatment and time were observed at 20% MVC (all *p*'s ≥ 0.10). A significant main effect of time ($F_{1,6}$ = 7.181, *p* = 0.037) revealed higher pre than post values at 50% MVC. There was no main effect of treatment or treatment by time interaction (both *p*'s ≥ 0.59) at 50% MVC.

For RT, no statistically significant interaction or main effects of treatment and time were observed for mean RT at 20% MVC (all *p*'s ≥ 0.21). A significant main effect of treatment ($F_{1,6}$ = 6.446, *p* = 0.044) revealed higher post than pre values at 50% MVC. There was no main effect of time or treatment by time interaction (both *p*'s ≥ 0.39) at 50% MVC.

Table 2: Mean ± SD for force CV, total number of MU's, RT, and DR for the healthy participants.
MU = motor unit; RT= recruitment threshold; DR = discharge rate; MVC = maximal voluntary contraction; CV = coefficient of variation; pps = pulse per second.

	Intervention				**Sham**			
Submaximal force level	20% MVC		50% MVC		20% MVC		50% MVC	
Time	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Force CV (%MVC)	1.5 ± 0.4	1.5 ± 0.4	2.0 ± 0.6	1.9 ± 0.3	1.5 ± 0.3	1.5 ± 0.4	2.0 ± 0.6	2.1 ± 0.7
Total number of MU´s	3 ± 2.2	3 ± 2.9	2.7 ± 1.7	2.5 ± 1.4	4.2 ± 3.3	4 ± 1.8	3.8 ± 2.6	2.8 ± 1.4
Recruitment threshold (%MVC)	12.8 ± 4.2	15.5 ± 5.	41.0 ± 3.9	37.9 ± 9.0	13.8 ± 4.0	15.4 ± 2.1	42.3 ± 5.5	42.5 ± 4.5
Discharge rate (pps)	16.8 ± 3.2	15.7 ± 3.0	15.9 ± 6.1	15.8 ± 4.8	16.0 ± 3.2	16.4 ± 2.4	14.7 ± 3.4	16.2 ± 6.3

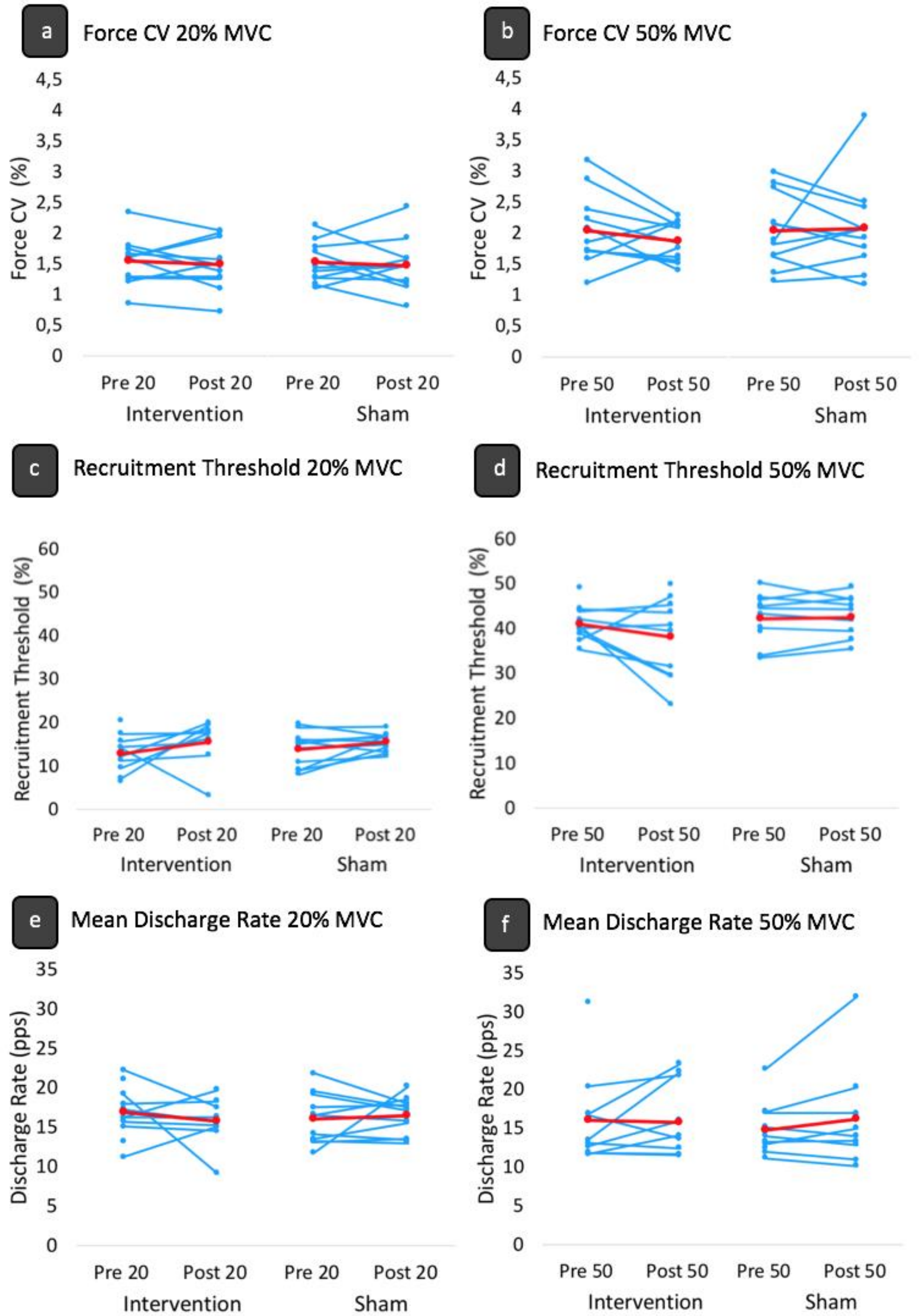

Figure 5: Force CV, RT, and DR for healthy participants. (a-b) Force CV as percentage of each healthy participants' 100% MVC. (c-d) RT as percentage of each healthy participants' 100% MVC. (e-f) Mean DR in pps. Each blue line represents a single participant, and the red lines represent the mean value. (a) Force CV at 20% MVC pre and post for both the intervention and sham session. (b) Force CV at 50% MVC pre and post for both the intervention and sham session. (c) RT at 20% MVC pre and post for both the intervention and sham session. (d) RT at 50% MVC pre and post for both the intervention and sham session. (e) DR at 20% MVC pre and post for both the intervention and sham session. (f) DR at 50% MVC pre and post for both the intervention and sham session.
CV = coefficient of variation; RT = recruitment threshold; DR = discharge rate; MVC = maximal voluntary contraction; pps = pulse per second.

Stroke patients

Figure 6 represents an example of the outcome from the decomposition including force, RT, and DR for stroke patient 3 (intervention group) for all sessions at 50% MVC. Stroke patient 3 was not able to hold a steady force, explaining the fluctuations in the ramps of force contractions (black) shown in Figure 6a-h.

Regarding the RT in Figure 6a-h, all the recruitments occurred at low force compared to the healthy participant in Figure 4. Regarding the DR it seems to be around the same level when comparing session 2 (Figure 6a-d) with 11 (Figure 6e-h).

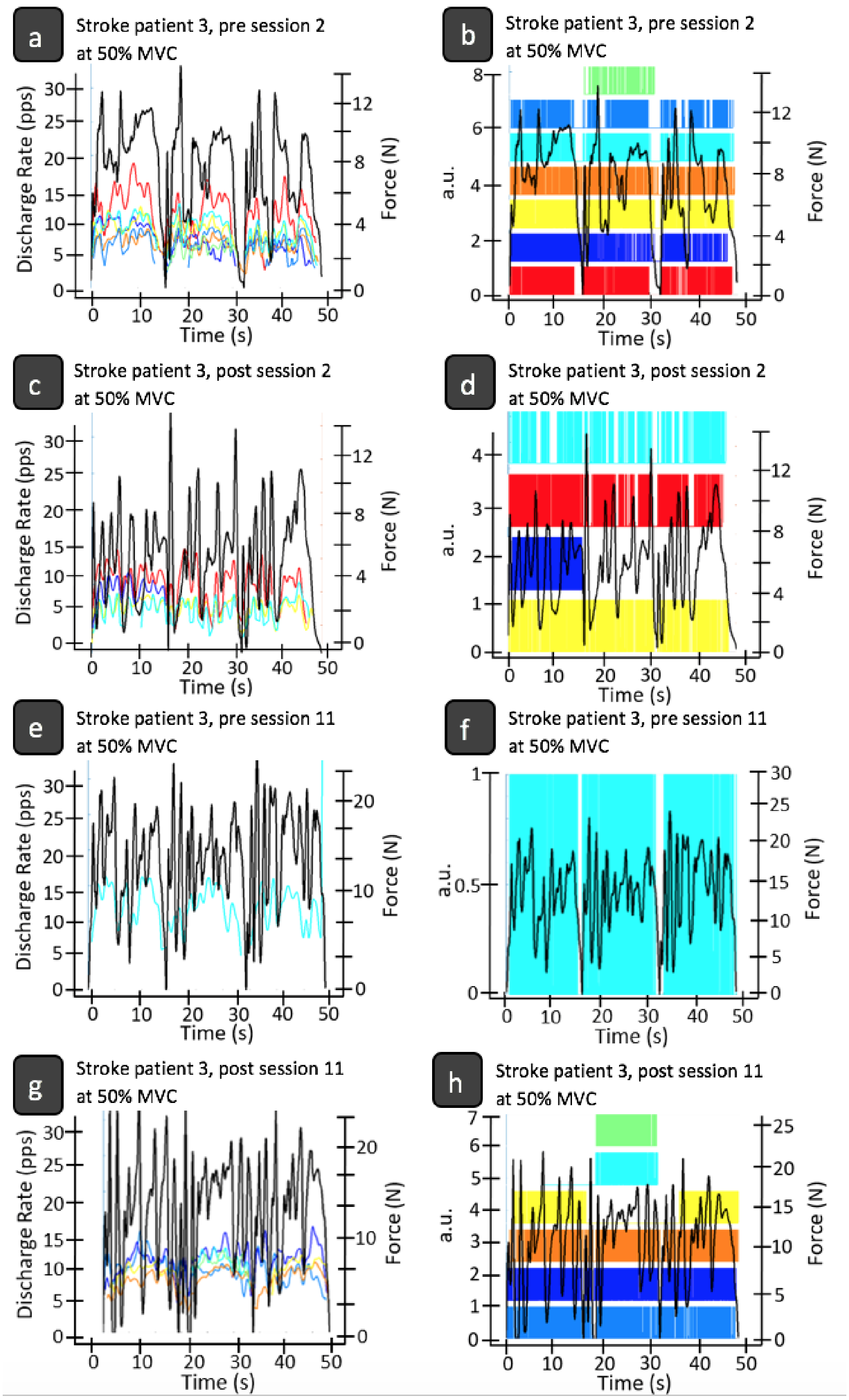

Figure 6: Clustered line and bar plots of the RT and DR for stroke patient 3 in the intervention group. (a-b) Represents pre intervention session 2 at 50% MVC. (c-d) Represents post intervention session 2 at 50% MVC. (e-f) Represents pre intervention session 11 at 50% MVC. (g-h) Represents post intervention session 11 at 50% MVC. (a, c, e, g) the clustered line plots show the firing patterns for different MU's (the colored lines) that occurred during the three ramps of force contractions (black). The x-axis represents time, the y-axis on the left represents DR, and the y-axis on the right represents force in %MVC. (b, d, f, h) the clustered bar plots show the RT and DR for different MU's. Each colored bar represents a MU and matches the MU with the same color in the other plot. The bar in the bottom

represents the MU with the lowest RT and the bar in the top represents the MU with the highest RT. The density of the bars corresponds to the number of discharges. The order of the RT's depends on the MU's extracted from the first out of the three trials.
RT = recruitment threshold; DR = discharge rate; MVC = maximal voluntary contraction; MU = motor unit; s = seconds; pps = pulse per second; N = newton; a.u. = arbitrary unit

Maximal voluntary contraction, force coefficient of variation, recruitment threshold and mean discharge rate

In the intervention group, stroke patient 1 had a MVC of 273 N in session 2 and 348 N in session 11, corresponding to an increase of 28%. Stroke patient 3 had a MVC of 31 N in session 2 and 43 N in session 11, corresponding to an increase of 38%. Stroke patient 4 had a MVC of 41 N in session 2 and 155 N in session 11, corresponding to an increase of 277%. In the sham group, stroke patient 2 had a MVC at 234 N in session 2 and 279 N in session 11, corresponding to an increase of 19%.
Results for force CV, total number of MU's, RT, and DR based on the measured MVC for the given session for both the intervention and sham groups are shown in Table 3. A visualization of the results together with the reference values ± SD error bars from the healthy participants are shown in Figure 7.
Figure 7a-b shows force CV at 20% and 50% MVC, respectively. Both the intervention and sham group had a decrease in force CV from session 2 pre 20% to session 11 post 20% MVC, whereas all except stroke patient 3 from the intervention group decreased from session 2 pre 50% to session 11 post 50% MVC.
Figure 7c-d shows RT´s at 20% and 50% MVC, respectively. Two in the intervention group (patients 1 and 4) and the patient in the sham group had an increase in RT from session 2 pre 20% to session 11 post 20% MVC and session 2 pre 50% and session 11 post 50% MVC. One in the intervention group (patient 3) had a decrease in RT from session 2 pre 20% to session 11 post 20% MVC and session 2 pre 50% and session 11 post 50 % MVC.
Figure 7e-d shows DR's at 20% and 50% MVC, respectively. For the intervention group, stroke patient 1 had an increase in DR from session 2 pre 20% to session 11 post 20% MVC and session 2 pre 50% to session 11 post 50% MVC. The DR for stroke patient 3 from the intervention group was approximately at the same level from session 2 pre 20% to session 11 post 20% MVC but increased from session 2 pre 50% to session 11 post 50% MVC. The DR's for stroke patient 4 in the intervention group and stroke patient 2 in the sham group were approximately at the same level from session 2 pre 20% to session 11 post 20% MVC but decreased from session 2 pre 50% to session 11 post 50% MVC.

Table 3: Force CV, total number of MU's, RT, and DR at 20% and 50% MVC for stroke patients.
CV = coefficient of variation; MU = motor unit; RT= recruitment threshold; DR = discharge rate; MVC = maximal voluntary contraction; pps = pulse per second.

	Intervention 20% MVC												**Sham 20% MVC**			
	Stroke patient 1				Stroke patient 3				Stroke patient 4				Stroke patient 2			
Session	Session 2		Session 11		Session 2		Session 11		Session 2		Session 11		Session 2		Session 11	
Time	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Force CV (%MVC)	7.2	2.6	3.3	2.2	70.4	43.1	48.9	56.5	7.7	6.1	4.9	4.8	4.9	4.9	4.9	3.0
Total number of MU´s	5	8	4	11	2	7	4	2	9	6	10	12	2	3	1	4
Recruitment threshold (%MVC)	12.7	17.3	11.5	13.7	10.8	8.0	7.3	4.3	9.6	10.7	9.0	13.2	11.1	5.9	9.2	12.9
Discharge rate (pps)	16.2	17.6	20.4	18.4	8.7	10.2	11.2	8.8	9.0	8.7	9.8	8.8	11.1	11.5	11.9	11.8
	Intervention 50% MVC												**Sham 50% MVC**			
	Stroke patient 1				Stroke patient 3				Stroke patient 4				Stroke patient 2			
Session	Session 2		Session 11		Session 2		Session 11		Session 2		Session 11		Session 2		Session 11	
Time	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Force CV (%MVC)	6.1	9.2	3.7	5.9	24.3	36.0	26.7	25.9	10.2	6.4	3.6	3.5	13.2	9.3	13.2	9.3
Total number of MU´s	2	6	7	3	7	4	1	6	5	10	14	11	2	1	3	1
Recruitment threshold (%MVC)	45.6	47.3	44.5	48.9	15.7	21.0	23.7	14.8	19.3	22.7	20.8	29.3	23.9	27.4	26.1	31.0
Discharge rate (pps)	21.5	11.9	19.9	21.9	9.4	9.5	13.5	10.4	17.7	10.1	9.5	10.7	14.4	14.4	11.9	13.2

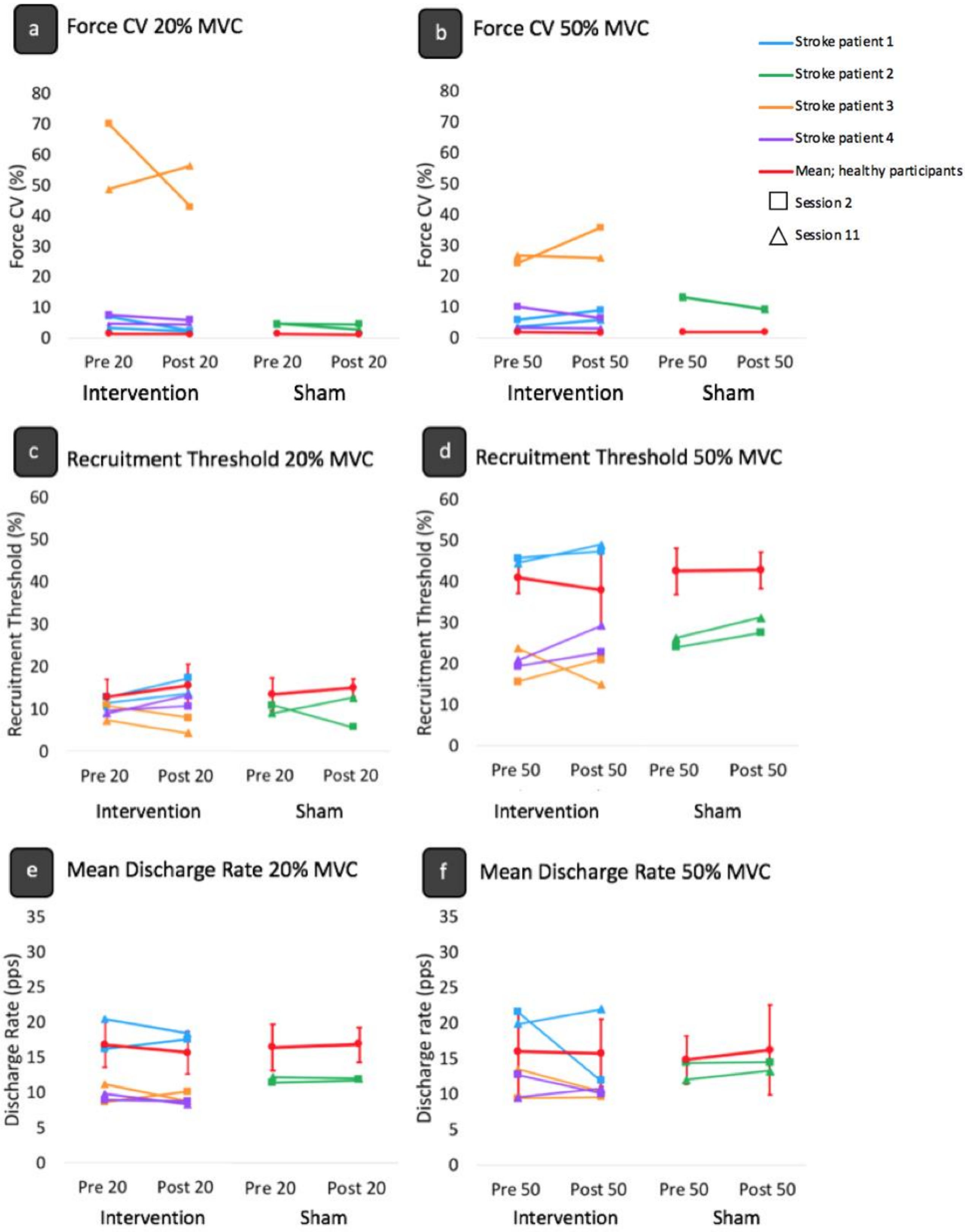

Figure 7: Force CV, RT, and DR for stroke patients with reference values (red lines ± SD error bars) from healthy participants. (a-b) Force CV as a percentage of each stroke patient´s 100% MVC. (c-d) RT as a percentage of each stroke patient´s 100% MVC. (e-f) Mean

DR in pps. The blue lines represent stroke patient 1. The green lines represent stroke patient 2. The orange lines represent stroke patient 3. The purple lines represent stroke patient 4. The square represents session 2 and the triangle represents session 11.
(a*) Force CV at 20% MVC pre and post for both the intervention and sham group. (b) Force CV at 50% MVC pre and post for both the intervention and sham group. (c) RT at 20% MVC pre and post for both the intervention and sham group. (d) RT at 50% MVC pre and post for both the intervention and sham group. (e) DR at 20% MVC pre and post for both the intervention and sham group. (f) DR at 50% MVC pre and post for both the intervention and sham group.*
CV = coefficient of variation; RT = recruitment threshold; DR = discharge rate; SD = standard deviation; MVC = maximal voluntary contraction; pps = pulse per second.

Discussion

The aim of this pilot study was to investigate whether the improvements in CSE together with the functional improvements observed following an associative BCI intervention (Kim et al., 2022; Mrachacz-Kersting et al., 2016, 2019; Mrachacz-Kersting & Aliakbaryhosseinabadi, 2018; Niazi et al., 2012) could be explained by possible changes in neuromuscular function measured by HD-EMG.
For the healthy participants, no significant changes were observed pre and post one intervention or sham BCI session regarding MVC, force CV, total number of MU's, RT, or DR.
The stroke patients all had force improvements consisting of a lower force CV and a higher MVC. The data for DR varied. For the RT, two patients from the intervention group and the one patient from the sham group overall seemed to increase between sessions. However, the data from the last stroke patient in the intervention group varied and all the recruitments occurred at low forces.

The findings in healthy participants suggest that if neuromuscular changes contribute to the positive effects of an associative BCI intervention, they do not change significantly in healthy participants after only one session. Other studies have suggested that the positive effects of only one associative BCI intervention are due to cortical/supraspinal changes (Jochumsen, Navid, Rashid, et al., 2019; Kim et al., 2022; Niazi et al., 2012). A study by Kim et al. (2022) used measures of M-max and the latency of M and F waves in both healthy participants and chronic stroke patients to investigate alterations at spinal and peripheral levels, while Niazi et al. (2012) used short latency stretch reflex (SLR) recordings in healthy participants instead. Both studies showed a significant increase in MEP amplitude in the intervention groups suggesting an increase in CSE. However, no changes in M-max or latencies of M and F waves or SLR amplitudes after only one BCI session were found, indicating that the changes do not happen at these levels (Kim et al., 2022; Niazi et al., 2012).
Even though the study by Kim et al. (2022) investigated chronic stroke patients as well as healthy participants, one cannot exclude whether the results would have been different for subacute stroke patients instead. This could be due to plasticity being at its highest in the first few months following the stroke (Mrachacz-Kersting et al., 2019).

To our knowledge, no other studies have investigated the possible effects of four weeks of an associative BCI intervention on the neuromuscular function in subacute stroke patients. However, a study by Del Vecchio et al. (2019) investigated how four weeks of strength training in 28 young healthy men would affect neuromuscular function using HD-EMG. MVC measurements were performed at the beginning of each session. The study showed a decrease in RT and an increase in DR in the same MU's. This demonstrates that the output from the spinal cord can be augmented after one month of strength training alone (Del Vecchio et al., 2019).

In the current study, four subacute stroke patients attended a four-week associative BCI protocol: three in the intervention and one in the sham group. In all four patients, a force improvement in MVC from session 2 to 11 was observed. Stroke patients in the intervention group (patient 1, 3, and 4) had percentage increases of 28%, 38%, and 277%, respectively. Whereas the stroke patient (patient 2) in the sham group had a percentage increase of 19%. Furthermore, both groups improved in force CV with a decrease in force variability. The overall force improvements suggest that all stroke patients got stronger and better at maintaining a steady force. However, none of them matched the mean MVC or force CV of healthy participants. These findings are consistent with other studies investigating MU behavior in chronic stroke patients proving a reduced MVC of the affected side (Kallenberg & Hermens, 2009; Liu et al., 2022).

When trying to understand force improvements, it is important to know that both recruitment and DR play a crucial role when generating muscle force. The improvements of these mechanisms can occur at different levels. However, the use of HD-EMG cannot assess at which levels possible improvements occur (Enoka, 2008; Kao et al., 2019).

For all stroke patients, their DR data varied both between and within patients. These findings might suggest that the observed force improvements were not primarily due to improvements in DR. Nevertheless, their different starting points represent another important factor. However, more patients are needed to make any conclusions. Other studies (Li et al., 2015; Negro et al., 2020) investigating MU firing behavior in chronic stroke patients found a general decrease in DR when comparing the affected and unaffected sides. Nevertheless, in the current study, both stroke patients 1 and 2 improved to have mean DR's comparable with the healthy participants at session 11 at 50% MVC.

For RT, two stroke patients in the intervention group (patients 1 and 4) and the stroke patient in the sham group (patient 2) seemed to increase from session 2 to 11. However, the data from the last stroke patient in the intervention group (patient 3) varied. As seen in Figure 6a-h and Table 3, all the recruitments at 50% MVC occurred at lower forces compared to the recruitments in healthy participants (Figure 4 and Table 2). These findings suggest that the recruitment of MU's was not force-related and did not follow the order of recruitment based on Henneman's size principle (Heckman & Enoka, 2012). Other studies investigating MU behavior in chronic stroke patients using HD-EMG found similar disturbances in recruitment order (Kallenberg & Hermens, 2009; Liu et al., 2022). Possible explanations could be due to no differentiation between low or high threshold MU's, premature recruitment of larger MU's, reinnervation of the bigger muscle fibers by low threshold MU's, or an increased contribution of low threshold MU's, possibly due to degeneration of high threshold MU's (Kallenberg & Hermens, 2009; Liu et al., 2022). Besides the increase in RT for all stroke patients except one in the intervention group (patient 3), the recruitments in session 11 at 50% MVC occurred at higher forces, suggesting improvements in the order of recruitment leaning towards a force-related recruitment (Heckman & Enoka, 2012). However, only one of the stroke patients (patient 1) matched the mean RT of the healthy participants. Common for the stroke patients with increased RT´s is that they either had a greater starting point or a greater functional improvement measured by FM and ARAT. However, more patients are needed to make any conclusions regarding whether four weeks of an associative BCI intervention affect the neuromuscular function in subacute stroke patients. This can be assessed in future studies.

Methodological considerations

To assess changes in neuromuscular function, the healthy participants and stroke patients had to perform an isometric ramp contraction at 20% and 50% MVC. A slow linear ramp contraction was chosen to ensure reliable recruitment and derecruitment thresholds compared to a ballistic contraction (Del Vecchio et al., 2020; Heckman & Enoka, 2012). The advantages of the ballistic contraction are that it is the movement of choice in most studies using a BCI-protocol and it is easier both to perform and imagine (Mrachacz-Kersting et al., 2016, 2019; Niazi et al., 2012). When identifying MU firing behavior, a ballistic contraction provides information about speed recruitment instead (Del Vecchio et al., 2019, 2020; Heckman & Enoka, 2012).

Most muscles have a maximum recruitment between 40-90% MVC (Del Vecchio et al., 2020; Liu et al., 2022) depending on the size of the muscle and the type of force contraction (Del Vecchio et al., 2020; Enoka, 2008; Heckman & Enoka, 2012; Liu et al., 2022). However, in most muscles the main recruitment of MU's happens long before reaching the maximum force level (Heckman & Enoka, 2012).

The choice of the submaximal force levels was furthermore limited due to the HD-EMG decomposition analysis, which states that MU firing patterns are easier to retrieve if a range of target forces between 30% to 70-90% MVC is used (Del Vecchio et al., 2020). However, a study by Martinez-Valdes et al. (2017) found that within one session, the least amount of MU's was observed at 70% compared to 10%, 30%, and 50% MVC. The same applied in the current study where more MU's were observed at 20% compared to 50% MVC, indicating that higher submaximal force levels makes the pattern recognition in the decomposition more difficult (Del Vecchio et al., 2020). A challenge with relatively high submaximal force levels is that the stroke patients have more difficulties maintaining a steady force and are more likely to experience fatigue (Zhang & Zhou, 2012). In the current study it resulted in fluctuations in the ramp of force contractions, especially at 50% MVC. In a study by Liu et al. (2022) it resulted in changing the hold phase in the ramp to 5 seconds instead of 10 s at 50% MVC. Both the fluctuations in force and the decrease of time in the hold-phase makes it more difficult for the decomposition analysis to ensure an optimal pattern recognition (Del Vecchio et al., 2020). To take both physical and mental fatigue into account, only two submaximal force levels with a resting time at 30 s were chosen in the current study (Jochumsen, Navid, Nedergaard, et al., 2019; Li et al., 2015; Liu et al., 2022; Martinez-Valdes et al., 2017).

In general, most studies (Del Vecchio et al., 2019; Liu et al., 2022; Martinez-Valdes et al., 2017) choose a range of submaximal force levels between 10-70% MVC, making the choice of 20% and 50% MVC in the current study comparable. Even though submaximal force levels below 30% MVC pose a problem for decomposition, they are very interesting to investigate from a clinical point of view because they reflect the percentage of MVC mostly used in activities of daily life (Bjarkam, 2015). This is important when talking about stroke rehabilitation. These methodological considerations should be considered in future studies.

Conclusion

For the healthy participants, no significant changes were observed pre and post one intervention or sham BCI session for MVC, force CV, total number of MU’s, RT, or DR. These findings suggest that if neuromuscular changes contribute to the positive effects of an associative BCI intervention, they do not change significantly in healthy participants after only one session.
After four weeks attending the associative BCI protocol, force improvements were observed in all subacute stroke patients both in the intervention and sham group, suggesting the patients got stronger and better at maintaining a steady force. The DR data for all stroke patients varied, suggesting that the force improvements were not primarily due to improvements in DR.
Independent of treatment, three stroke patients had an increase in RT and the recruitments at 50% MVC occurred at higher forces, suggesting improvements in MU firing behavior leaning towards a force-related recruitment. For the last stroke patient, the recruitment data varied and the recruitments at 50% MVC occurred at lower forces, suggesting that the recruitment of MU’s did not follow the order of recruitment based on Henneman’s size principle.
This pilot study is the first to assess changes in neuromuscular function in subacute stroke patients following four weeks of an associative BCI intervention. However, more patients are needed to make more general conclusions.

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