High-density surface electromyography allows for longitudinal assessment of the neural drive to muscle in individuals with acute stroke

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***Abstract*—Previous work on neuromuscular impairments following stroke has mainly focused on the chronic phase of recovery, and relatively little is known regarding the acute phase. Studies demonstrating impairments in muscle activation have typically used single bipolar surface electromyography (sEMG) recordings, which may lead to a mischaracterization of muscle excitation. In this study, we assessed neuromuscular function of patients undergoing rehabilitation therapy in the acute phase post-stroke, combining high-density sEMG (HDsEMG) decomposition with isometric force recording to quantify changes in force production and motor unit discharge rates in comparison with global amplitude of a single bipolar sEMG. Seven patients with acute hemiparetic stroke were tested, beginning when a detectable dorsi- and plantarflexion movement could be observed (T0) and then again 15 and 30 days later (T15 and T30). The isometric maximal voluntary contraction (MVC) in dorsi- and plantarflexion were measured at these time points. HDsEMG signals recorded from tibialis anterior, gastrocnemius lateralis and medialis, and soleus muscles during isometric contractions at 10% and 30% MVC were decomposed into motor unit discharge offline. Our main results revealed significant impairments in maximal force production at T0, which improved over the 30 days of inpatient rehabilitation therapy. There were also increases in mean motor unit discharge rate for TA and SOL muscles at 10% MVC. These neuromuscular changes could not be captured by using the classical, bipolar sEMG approach. Our results suggest that the combination of force recordings with HDsEMG analysis may provide useful information in the acute phase of stroke and, longitudinally, during inpatient rehabilitation therapy.**

Keywords— acute stroke, motor unit discharge behavior, motor recovery, high-density surface EMG

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I. Introduction

It is well established that hemiparetic stroke results in motor impairments that vary according to the area of the central nervous system affected by the ischemic or hemorrhagic event [1]. These impairments can significantly affect the ability of stroke survivors to perform daily activities and are compounded by subsequent inactivity and physical deconditioning [2]. Early rehabilitation therapy can improve a patient's functional mobility and independence in daily life [3-6]; however, for many patients, motor recovery is suboptimal and substantial motor deficits persist. The vast majority of our knowledge about neuromuscular alterations following a stroke has come from studies examining patients in the chronic phase (> 6-12 months post-stroke), but little is known about how they evolve in the acute phase [7]. A more complete understanding of early neuromuscular changes and their time course during acute rehabilitation therapy would help the development of novel, more effective rehabilitation interventions.

Compelling evidence suggests that post-stroke dysfunction in force production is likely caused by impaired excitability in descending motor pathways [8] and resulting changes in motor unit frequency coding and recruitment [9]. In both clinical and research settings, bipolar surface electromyography (sEMG) has typically been used to infer these neurological alterations and the following recovery. For instance, sEMG has been applied for detecting fibrillation potentials in the hemiparetic side [10], and as a biofeedback intervention, for targeting motor impairments in stroke patients [11]. However, there are several non-physiological factors affecting the acquisition and interpretation of bipolar sEMG, which may result in a mischaracterization of muscle excitation during specific motor tasks [12]. One possibility for minimizing these factors to apply several electrodes over the muscle of interest (i.e., high-density surface electromyography; HDsEMG), which provides a more accurate assessment of

muscle excitation than single bipolar EMG recordings [13]. Additionally, when combined with convolutive blind source separation techniques, HDsEMG allows for the non-invasive assessment of the discharge patterns of many individual motor units [14]. Considering the motor units are the final common pathway of the neuromuscular system, their assessment provides a direct window to the neural control of human movement. Indeed, HDsEMG decomposition combined with isometric strength measurements has provided insights into the motor unit neural drive from multiple muscles in chronic stroke patients [15, 16]. However, there is still a lack of this information in the acute phase of the disease and the initial stages of rehabilitation and recovery of function.

The primary objective of this study was to combine HDsEMG decomposition with isometric force recordings to quantify changes in force production and motor unit discharge rates in acute stroke patients during different time points of inpatient rehabilitation therapy. Additionally, we aimed to compare results from two different approaches, bipolar EMG and HDsEMG decomposition, for assessing neuromuscular alterations during the acute phase of stroke. By comparing the results obtained from each method, we sought to provide insights that can guide researchers in selecting the most appropriate approach for evaluating stroke patients in cross-sectional neuromechanistic studies as well as when assessing the efficacy of rehabilitation interventions.

II. METHODS

A. Participants

Seven patients in the acute phase of stroke (mean ± SD age 52 ± 19.98 years; time post-stroke < 12 weeks; 4 females) were recruited at Fondazione Teresa Camplani - Casa di Cura Domus Salutis (Brescia, Italy). All patients had severe lower limb impairment.

The inclusion criteria required that the participants have suffered from either ischemic or hemorrhagic stroke and be in the acute phase of the disease (< 12 weeks post-stroke). Being able to perform plantarflexion and dorsiflexion of the ankle and understanding and following the protocol were also inclusion criteria. This ensured that only patients who were cognitively and physically able to perform the tasks were included in the study. All participants signed a written informed consent before starting the experiments. This study was conducted in accordance with the latest version of the Declaration of Helsinki and approved by the local Ethics Committee (NP2490).

B. Experimental protocol

The study consisted of three experimental sessions during hospitalization and rehabilitation therapy of the patients: when they recovered detectable dorsi- and plantarflexion movements following stroke (T0), 15 days after T0 (T15), and 30 days after T0 (T30). An additional session was conducted to measure the ankle force production of the non-affected side. During these 30 days, they followed standard acute rehabilitation therapy. In each experimental session, patients had their legs comfortably positioned on a wooden design ergometer with their knee fully extended [17]. The foot of the tested leg was fixed with straps to an adjustable footplate that held the ankle at specific angles. This footplate was connected to a load cell

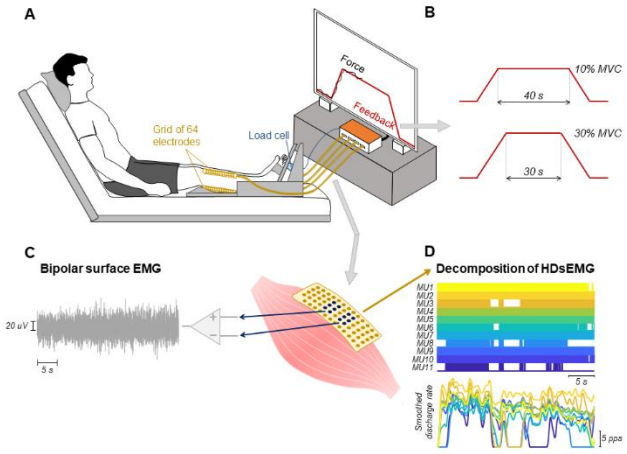

Fig. 1. (A) Schematic representation of the position of the participants, the electrodes position for TA and GL, and the screen used for visual feedback. (B) Trapezoidal profiles provided for the patients during the submaximal tasks, with the plateau region reaching 10% and 30% MVC. (C) and (D) shows the two approaches used to assess the neuromuscular alterations in acute stroke patients: simulated bipolar surface EMG from the grid (C) and decomposition of HDsEMG signals (D).

(SM-500 N, Interface, Arizona, USA) to record the dorsi- and plantarflexion isometric forces produced by the ankle (Fig. 1A).

Before starting the data collection, participants underwent a brief training session to familiarize themselves with the procedures. After that, they were asked to perform three isometric maximum voluntary contractions (MVC) in which they were instructed to achieve their MVC within three seconds and hold it for five seconds [16]. The highest force value achieved across the three trials was then set as the reference value for the submaximal tasks. At least 2 min after MVCs, participants were asked to follow trapezoidal profiles at two force levels, 10%, and 30% MVC. Specifically, they had to isometrically increase the force from 0% MVC to the target in 5 s, hold it for 40 s (10% MVC) or 30 s (30% MVC), and decrease it to 0% MVC in 5 s (Fig. 1B). This trapezoidal profile was repeated two times for each of two force levels, with a rest-in period of 2 min. During all submaximal trials, participants were provided with visual feedback of the ankle force on a screen (Fig. 1A). The protocol was repeated for both dorsi- and plantarflexion contractions in a randomized order. The ankle joint was positioned at 110° and 90° for the dorsiflexion and plantarflexion tasks, respectively [18].

C. Data collection

In addition to the acquisition of isometric ankle forces, HDsEMG were recorded using four 64-electrode matrices (Fig. 1A; 8 mm inter-electrode distance; OT Bioelettronica, Turin, Italy). The matrices were positioned longitudinally on the belly of the tibialis anterior (TA), gastrocnemius lateralis (GL), soleus (SOL), and gastrocnemius medialis (GM) muscles. Prior to electrode placement, the skin was cleaned with abrasive paste (EVERI, Spes Medica, Genova, Italy), and shaved when necessary. The reference electrode was positioned on the ankle.

HDsEMG signals were recorded in monopolar derivation and amplified by a variable factor across subjects (from 2,000 to 5,000) using a 12-bit A/D converter (20-500 Hz; EMG-USB2+, OT Bioelettronica, Turin, Italy). The

force signal provided by the load cell and HDsEMGs were sampled synchronously at a frequency of 2048 Hz.

D. Data Analysis

Force and HDsEMG were analyzed offline using Matlab (The MathWorks Inc., Natick, Massachusetts, USA). First, raw force signals acquired during MVCs were low-pass filtered at 15 Hz using a third-order Butterworth filter. To quantify changes in dorsi- and plantarflexion force production, the peak force was computed as the average force value over a 100 ms window centered at the peak. The values obtained at T0, T15, and T30 were then normalized with respect to the non-affected side.

Monopolar HDsEMG signals were filtered with a third-order Butterworth filter (20-500 Hz cut-off frequencies). After visual inspection, channels with low signal-to-noise ratio or artifacts were discarded. We then used two approaches to assess neuromuscular alterations in acute stroke patients during different time points of inpatient rehabilitation therapy: classical bipolar sEMG (Fig. 1C) and decomposition of HDsEMG signals (Fig. 1D).

1) Approach 1: classical bipolar sEMG

For this approach, we simulated one bipolar EMG detection site over the muscle belly. We specifically calculated the difference between the average monopolar signals from two groups of 6 channels within the electrode grid (dark blue electrodes in Fig. 1). Then the root-mean-square (RMS) amplitude was calculated to estimate the degree of TA, GL, SOL and GM activation. For the MVCs, a time window of 250 ms before the peak force was used. For the submaximal tasks, the RMS was calculated over the steady part of the contraction (30 or 40 s) using non-overlapping windows of 250 ms, and the average was considered for further analyses. The averaged RMS amplitude obtained was then normalized with respect to the RMS of MVC, separately for each time (T0, T15 and T30)

2) Approach 2: decomposition of HDsEMG

For this approach, HDsEMG signals were decomposed into motor unit spike trains using a convolutive blind-source separation algorithm [14]. Briefly, after extending and whitening HDsEMG signals, a fixed-point algorithm that seeks sources that maximize a measure of sparsity was applied to identify the motor unit pulse trains (see raster plot in Fig. 1D). The spikes were then separated from the noise and other potential sources using K-means and, while iteratively updating the motor unit separation vectors, the discharge times estimation was further refined by minimizing the coefficient of variation of the inter-spike intervals. This decomposition method has been previously validated in simulated and experimental signals [14]. After the automatic identification of motor units, all the motor unit spike trains were visually inspected. Missing pulses or incorrectly assigned pulses producing non-physiological discharge rates were manually and iteratively edited by an experienced operator [18]. Subsequently, the instantaneous discharge rate of individual motor units was calculated as the multiplicative inverse of the inter-spike interval (bottom part of Fig. 1D). The mean discharge rate was then obtained by averaging discharge rate values during the steady part of the submaximal tasks.

E. Statistical analysis

All statistical analyses were performed in R (version 4.2.2), using RStudio environment. Linear mixed-effect models (LMM) were applied for all statistical analysis, as they account for the non-independence of data points within each participant. For all variables (i.e., Peak MVC, RMS amplitude, and mean discharge rate), random intercept models with time (T0, T15 and T30) as fixed effect and participant as random effect were applied. LMMs were implemented using the package *lmerTest* [19] with the Kenward-Roger method to approximate the degrees of freedom and estimate the p-values. The *emmeans* package was used, when necessary, for multiple comparisons and to determine estimated marginal means with 95% confidence intervals.

III. RESULTS

Fig. 2A shows a representative example of the dorsiflexion isometric MVCs acquired for each condition. It is possible to see that there was a reduction of ~40% in the dorsiflexion MVC at T0 (orange line) compared to the non-affected side (dark blue line). Conversely, the dorsiflexion peak MVC at T15 (green line) and T30 (yellow line) was much closer to the non-affected side (reduction of less than 10%). Similarly, for the group data, there was a significant effect of time on the peak MVC values for both dorsiflexion (Fig. 2A; LMM; F = 14.82; P = 0.002) and plantarflexion (Fig. 2B; LMM; F = 7.54; P = 0.010). Specifically, for dorsiflexion (Fig. 2B), the median peak MVC significantly increased from 51.4 [22.2, 80.7] % at T0 to 94.8 [65.5, 124] % and 78.8 [49.5, 108] % at T15 and T30, respectively (P < 0.028 for both), with no significant differences between T15 and T30 (P = 0.246). For the plantarflexion (Fig. 2C), there was also a significant increase from 62.2 [26.3, 110] % at T0 to 95.7 [53.8, 137] % at T30 (P = 0.011), but without significant differences between T0 and T15 (P = 0.069), as well as between T15 and T30 (P = 0.895).

In order to assess the neuromuscular alterations in acute stroke patients as classically done in the literature, we estimated the degree of TA, GL, SOL and GM activation

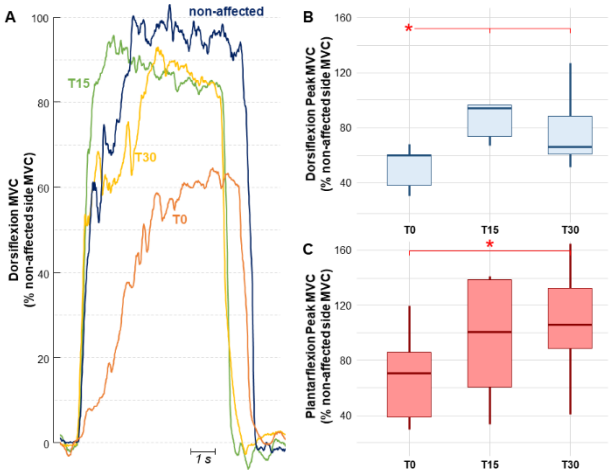

Fig. 2. (A) Example of dorsiflexion isometric MVCs acquired at T0 (orange), T15 (green), T30 (yellow), and for the non-affected side (dark blue) of a representative participant. (B) and (C) shows the group results of dorsi- and plantarflexion peak MVCs. Values are normalized with respect to non-affected side. Horizontal traces, boxes, and whiskers, respectively, denote median value, interquartile interval, and distribution range.

through the calculation of the RMS amplitude of a simulated bipolar from the grid of electrodes (normalized by RMS at MVC). Fig. 3 shows the RMS amplitude results for all muscles and for both force levels. At 10% MVC, there was no significant effect of time on RMS amplitude for any of the investigated muscles (LMM; $F < 2.55$ and $P > 0.128$ for all cases). The SOL and GM activation also did not differ over time at 30% MVC (LMM; $F < 2.35$ and $P > 0.146$ for both). Conversely, the TA and GL activation significantly changed over time at 30% MVC (LMM; $F > 4.98$ and $P < 0.032$ for both). For the TA, there was a significant reduction in RMS amplitude between T0 and T15 ($P = 0.032$), with no changes between T0 and T30 ($P = 0.285$) or between T15 and T30 ($P = 0.672$). For the GL, the RMS amplitude significantly decreased at T15 and T30 when compared to T0 ($P < 0.047$ for both), with no significant differences between T15 and T30 ($P = 1.000$).

Another approach we used to investigate the neuromuscular changes during the acute phase of stroke and subsequent days of rehabilitation was to assess the mean discharge rate of individual motor units using the decomposition of HDsEMG signals into motor unit spike trains. The number of identified motor units varied according to the participants, time of assessment, and muscle. Overall, a greater number of motor units was decomposed from the TA muscles than from the GL, GM, and SOL muscles. Within the triceps surae, the SOL muscle yielded the largest number of identified motor units. All details regarding the number of motor units identified for each participant are provided in Table 1 (10% MVC) and Table 2 (30% MVC).

Mean discharge rate results for all muscles and both force levels are shown in Fig. 4. For TA and SOL muscles, there was a significant effect of time on mean discharge at 10% MVC (LMM; $F > 6.72$ and $P < 0.002$ for both), but not at 30% MVC (LMM; $F < 2.46$ and $P > 0.090$ for both). Specifically, the TA mean discharge rate significantly increased from 8.47 [6.78, 10.2] pps at T0 and 8.47 [7.08, 10.5] pps at T15 to 9.98 [8.28, 11.7] pps at T30 (Fig. 4A; $P < 0.001$ for both). Moreover, as displayed in Fig. 4C, the SOL mean discharge rate significantly changed from 5.88 [5.28, 6.49] pps at T0 to 6.80 [6.12, 7.42] pps at T15 ($P = 0.027$) and 7.20 [6.48, 7.92] pps at T30 ($P = 0.004$).

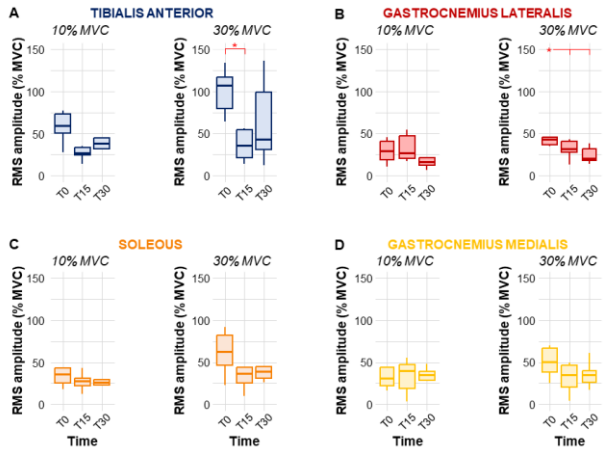

Fig. 3. Group results of RMS amplitude for tibialis anterior (A), gastrocnemius lateralis (B), soleous (C), and gastrocnemius medialis (D) muscles. Values are normalized with respect the RMS value obtained at MVC. Horizontal traces, boxes, and whiskers, respectively, denote median value, interquartile interval, and distribution range.

Conversely, for GL and GM muscles (Fig. 4B and 4D), the mean discharge rate did not significantly change over time neither at 10% MVC (LMM; $F < 1.45$ and $P > 0.246$ for both) or 30% MVC (LMM; $F < 2.95$ and $P > 0.059$ for both).

TABLE I. NUMBER OF MOTOR UNITS DECOMPOSED FOR 10% MVC

Muscle	Condition	Participants						
		S01	*S02*	*S03*	*S04*	*S05*	*S06*	*S07*
TA	T0	4	1	-	0	22	5	6
	T15	0	1	-	0	28	7	15
	T30	12	0	-	6	26	19	9
	Non-paretic	15	0	0	7	18	15	4
GL	T0	3	1	7	-	5	2	4
	T15	2	4	5	-	0	1	10
	T30	1	3	0	-	1	1	9
	Non-paretic	2	1	0	4	3	3	8
SOL	T0	7	6	13	-	6	1	15
	T15	2	1	11	-	2	4	15
	T30	3	3	0	-	5	4	8
	Non-paretic	7	1	0	10	11	5	7
GM	T0	1	1	2	-	5	1	5
	T15	6	0	1	-	3	1	3
	T30	3	0	0	-	5	4	7
	Non-paretic	0	0	0	2	5	4	9

*A dash indicates data was not collected for the given condition.

TABLE II. NUMBER OF MOTOR UNITS DECOMPOSED FOR 30% MVC

Muscle	Condition	Participants						
		S01	*S02*	*S03*	*S04*	*S05*	*S06*	*S07*
TA	T0	3	0	-	2	15	4	11
	T15	0	1	-	1	29	9	17
	T30	4	0	-	7	20	13	9
	Non-paretic	19	0	21	4	2	19	12
GL	T0	0	2	1	-	7	2	7
	T15	1	1	1	-	7	2	12
	T30	3	0	11	-	6	3	8
	Non-paretic	5	0	3	6	7	2	14
SOL	T0	0	5	11	-	6	1	19
	T15	12	2	10	-	4	2	7
	T30	6	6	14	-	9	3	8
	Non-paretic	8	1	7	9	18	5	7
GM	T0	0	0	2	-	7	0	9
	T15	5	0	1	-	9	3	7
	T30	6	0	3	-	12	6	7
	Non-paretic	6	0	4	1	3	4	6

*A dash indicates data was not collected for the given condition.

IV. DISCUSSION

In this paper, we combined isometric force recordings with HDsEMG decomposition to longitudinally assess changes in force production and motor unit discharge rates during the initial stages of rehabilitation of individuals with stroke. Moreover, we simulated a single bipolar sEMG from the grid of electrodes to investigate changes in global muscle activation, as typically done in the literature. As previsouly discussed, our main findings revealed that the HDsEMG decomposition approach combined with isometric force recordings, which has been previously applied in chronic stroke patients, may provide rich information in the acute phase of stroke and, longitudinally, during the first 30 days of inpatient rehabilitation therapy.

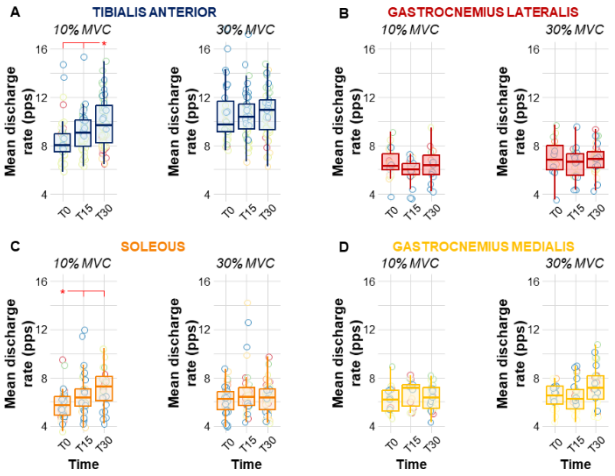

Fig. 4. Group results of mean discharge rate for tibialis anterior (A), gastrocnemius lateralis (B), soleous (C), and gastrocnemius medialis (D) muscles. Horizontal traces, boxes, and whiskers, respectively, denote median value, interquartile interval, and distribution range

Results from seven individuals with acute stroke showed significant decreases of ~50% and ~40% on average of dorsiflexion and plantarflexion MVCs, respectively, when compared to the non-paretic side at T0 (i.e., time point when they recovered detectable dorsi- and plantarflexion movements following stroke). These results are in line with the well-established loss in force production capability following stroke [20, 21]. Interestingly, our study found that dorsiflexion appears to be more affected than plantarflexion at T0, as indicated by the interquartile ranges of peak MVC in Fig. 2A and Fig. 2B. These findings are consistent with previous research on stroke-related impairments in dorsiflexor function in the chronic phase, which can lead to an inability to effectively lift the toes during the swing phase of walking, increasing the risk of falls and reducing mobility and independence [23]. Another interesting finding from our results is that these impairments identified at T0 progressively improved during 30 days of inpatient rehabilitation therapy. As further discussed below, we adopted two approaches to examine underlying mechanisms of changes in force control in acute stroke individuals: a classical bipolar EMG approach and HDsEMG decomposition.

When assessing changes in muscle excitation using the RMS amplitude of single bipolar sEMG, we did not find differences over time for any muscle investigated at 10% MVC. In contrast, at 30% MVC, there was a significant reduction in normalized RMS amplitude over time for TA and GL but not for SOL and GM. The decrease in amplitude in T15 and T30 relative to T0 is most notable in TA (10% and 30% MVC; Fig. 3). The comparability high level of activation at T0 may indicate that acute stroke patients require a larger neural drive input to perform submaximal tasks at the acute phase. It could also indicate that isometrically contractions for a duration of 40 s (10% MVC) or 30 s (30% MVC) induced fatigue, which is typically accompanied by marked increases in EMG amplitude [24]. However, there are several confounding factors affecting the acquisition and interpretation of bipolar sEMG that, when disregarded, may potentially lead to equivocal conclusions [25,26]. In addition, global sEMG limits the possibility to separate central and peripheral motor units changes. Therefore, the results provided by this approach need to be interpreted carefully.

Our data suggest that post-stroke individuals exhibit decreased discharge rates of motor units on the paretic side during the acute phase, as previously demonstrated in the literature during the chronic phase [16, 27]. In addition, we demonstrated that there were significant increases in the mean discharge rate during one month of inpatient rehabilitation therapy. These modifications were specific to the TA and SOL muscles, and for the 10% MVC task (see Fig. 4A and Fig. 4C). This may indicate that changes in force control in acute stroke patients are muscle- and force-level dependent, which is in line with muscle-specific adaptations in motor unit behavior in chronic stroke individuals [16]. Notably, these changes over time in TA and SOL muscles at 10% MVC were not revealed using the classical, bipolar sEMG approach, indicating that this approach may not completely capture the changes underlying the improvements in force control during the 30 days following the stroke event. Although some of these changes could be inferred from global sEMG, the confounding variables are fewer when interpreting results from individual motor units, enhancing the robustness of the obtained inferences.

In conclusion, gaining a deeper understanding of neural adaptations following stroke and determining how to optimizing physical rehabilitation interventions are crucial goals for preventing long-term motor impairment in people with hemiparetic stroke. As one step toward these goals, it can be helpful to analyze the activity of multiple muscles simultaneously [16] and objectively evaluate modified muscle control strategies in the early stages of rehabilitation [28]. Our results suggest that combining force recordings with HDsEMG analysis may be a useful approach for achieving these objectives. By providing a more comprehensive understanding of muscle activation patterns and force production capabilities, such approach could help clinicians tailor rehabilitation programs to individual patients' needs and improve their overall outcomes. Ultimately, future research in this area may lead to the development of more effective, personalized rehabilitation strategies that can help stroke patients recover their motor function and improve their quality of life.

REFERENCES

[1] F. N. Shelton, and M. J. Reding, "Effect of lesion location on upper limb motor recovery after stroke," *Stroke*, vol. 32, no. 1, pp. 107-12, Jan, 2001.

[2] S. A. Billinger, R. Arena, J. Bernhardt, J. J. Eng, B. A. Franklin, C. M. Johnson, M. MacKay-Lyons, R. F. Macko, G. E. Mead, E. J. Roth, M. Shaughnessy, and A. Tang, "Physical activity and exercise recommendations for stroke survivors: a statement for healthcare professionals from the American Heart Association/American Stroke Association," *Stroke*, vol. 45, no. 8, pp. 2532-53, Aug, 2014.

[3] G. Kwakkel, B. J. Kollen, and R. C. Wagenaar, "Long term effects of intensity of upper and lower limb training after stroke: a randomised trial," *J Neurol Neurosurg Psychiatry*, vol. 72, no. 4, pp. 473-9, Apr, 2002.

[4] E. S. Koroleva, S. D. Kazakov, I. V. Tolmachev, A. J. M. Loonen, S. A. Ivanova, and V. M. Alifirova, "Clinical Evaluation of Different Treatment Strategies for Motor Recovery in Poststroke Rehabilitation during the First 90 Days," *J Clin Med*, vol. 10, no. 16, Aug 21, 2021.

[5] E. R. Coleman, R. Moudgal, K. Lang, H. I. Hyacinth, O. O. Awosika, B. M. Kissela, and W. Feng, "Early Rehabilitation

After Stroke: a Narrative Review," *Curr Atheroscler Rep,* vol. 19, no. 12, pp. 59, Nov 7, 2017.

[6] G. N. Davidoff, O. Keren, H. Ring, and P. Solzi, "Acute stroke patients: long-term effects of rehabilitation and maintenance of gains," *Arch Phys Med Rehabil,* vol. 72, no. 11, pp. 869-73, Oct, 1991.

[7] S. Li, "Spasticity, Motor Recovery, and Neural Plasticity after Stroke," *Front Neurol,* vol. 8, pp. 120, Apr, 2017.

[8] S. A. Murphy, F. Negro, D. Farina, T. Onushko, M. Durand, S. K. Hunter, B. D. Schmit, and A. Hyngstrom, "Stroke increases ischemia-related decreases in motor unit discharge rates," *Journal of Neurophysiology,* vol. 120, no. 6, pp. 3246-3256, 2018.

[9] L. W. Chou, J. A. Palmer, S. Binder-Macleod, and C. A. Knight, "Motor unit rate coding is severely impaired during forceful and fast muscular contractions in individuals post stroke," *J Neurophysiol,* vol. 109, no. 12, pp. 2947-54, Jun, 2013.

[10] I. Kouzi, E. Trachani, E. Anagnostou, C. A. Rapidi, J. Ellul, G. C. Sakellaropoulos, and E. Chroni, "Motor unit number estimation and quantitative needle electromyography in stroke patients," *J Electromyogr Kinesiol,* vol. 24, no. 6, pp. 910-6, Dec, 2014.

[11] X. Zhang, D. Wang, Z. Yu, X. Chen, S. Li, and P. Zhou, "EMG-Torque Relation in Chronic Stroke: A Novel EMG Complexity Representation With a Linear Electrode Array," *IEEE J Biomed Health Inform,* vol. 21, no. 6, pp. 1562-1572, Nov, 2017.

[12] T. Lulic-Kuryllo, F. Negro, N. Jiang, and C. R. Dickerson, "Standard bipolar surface EMG estimations mischaracterize pectoralis major activity in commonly performed tasks," *J Electromyogr Kinesiol,* vol. 56, pp. 102509, Feb, 2021.

[13] T. M. Vieira, and A. Botter, "The Accurate Assessment of Muscle Excitation Requires the Detection of Multiple Surface Electromyograms," *Exercise and Sport Sciences Reviews,* vol. 49, no. 1, pp. 23-34, 2021.

[14] F. Negro, S. Muceli, A. M. Castronovo, A. Holobar, and D. Farina, "Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation," *Journal of Neural Engineering,* vol. 13, no. 2, pp. 026027, 2016/02/29, 2016.

[15] S. Murphy, M. Durand, F. Negro, D. Farina, S. Hunter, B. Smith, D. Gutterman, and A. Hyngstrom, "The Relationship Between Blood Flow and Motor Unit Firing Rates in Response to Fatiguing Exercise Post-stroke," *Front Physiol,* vol. 10, pp. 545, May, 2019.

[16] L. C. Miller, C. K. Thompson, F. Negro, C. J. Heckman, D. Farina, and J. P. Dewald, "High-density surface EMG decomposition allows for recording of motor unit discharge from proximal and distal flexion synergy muscles simultaneously in individuals with stroke," *Annu Int Conf IEEE Eng Med Biol Soc,* vol. 2014, pp. 5340-4, 2014.

[17] M. Cogliati, A. Cudicio, F. Toscani, P. Gaffurini, L. M. Bissolotti, C. Orizio, and F. Negro, "Normalized maximal rate of torque development during voluntary and stimulated static contraction in human tibialis anterior: Influence of age," *Exp Gerontol,* vol. 138, pp. 110999, Sep, 2020.

[18] A. Cudicio, E. Martinez-Valdes, M. Cogliati, C. Orizio, and F. Negro, "The force-generation capacity of the tibialis anterior muscle at different muscle-tendon lengths depends on its motor unit contractile properties," *Eur J Appl Physiol,* vol. 122, no. 2, pp. 317-330, Feb, 2022.

[19] A. Kuznetsova, P. B. Brockhoff, and R. H. B. Christensen, "lmerTest Package: Tests in Linear Mixed Effects Models," *Journal of Statistical Software,* vol. 82, no. 13, pp. 1 - 26, 12/06, 2017.

[20] V. Gray, C. L. Rice, and S. J. Garland, "Factors that influence muscle weakness following stroke and their clinical implications: a critical review," *Physiother Can,* vol. 64, no. 4, pp. 415-26, Fall, 2012.

[21] N. Arene, and J. Hidler, "Understanding Motor Impairment in the Paretic Lower Limb After a Stroke: A Review of the Literature," *Topics in Stroke Rehabilitation,* vol. 16, no. 5, pp. 346-356, 2009/09/01, 2009.

[22] B. Balaban, and F. Tok, "Gait disturbances in patients with stroke," *Pm r,* vol. 6, no. 7, pp. 635-42, Jul, 2014.

[23] Y. H. Wang, F. Meng, Y. Zhang, M. Y. Xu, and S. W. Yue, "Full-movement neuromuscular electrical stimulation improves plantar flexor spasticity and ankle active dorsiflexion in stroke patients: a randomized controlled study," *Clin Rehabil,* vol. 30, no. 6, pp. 577-86, Jun, 2016.

[24] R. Merletti, M. Knaflitz, and C. J. De Luca, "Myoelectric manifestations of fatigue in voluntary and electrically elicited contractions," *J Appl Physiol (1985),* vol. 69, no. 5, pp. 1810-20, Nov, 1990.

[25] L. Mesin, R. Merletti, and A. Rainoldi, "Surface EMG: the issue of electrode location," *J Electromyogr Kinesiol,* vol. 19, no. 5, pp. 719-26, Oct, 2009.

[26] A. Rainoldi, G. Melchiorri, and I. Caruso, "A method for positioning electrodes during surface EMG recordings in lower limb muscles," *J Neurosci Methods,* vol. 134, no. 1, pp. 37-43, Mar 15, 2004.

[27] J. J. Gemperline, S. Allen, D. Walk, and W. Z. Rymer, "Characteristics of motor unit discharge in subjects with hemiparesis," *Muscle Nerve,* vol. 18, no. 10, pp. 1101-14, Oct, 1995.

[28] F. Negro, M. Cogliati, A. Cudicio, L. Bissolotti, and C. Orizio, "Neural Biomarkers of Functional Recovery in Patients with Injured Motor System." pp. 907-910.