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Examination of Afterhyperpolarization Duration Changes in Motoneurons Innervating Paretic Muscles in Stroke Survivors

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Abstract— **The after hyperpolarization (AHP) of a motoneuron is a primary determinant of motoneuron firing rate. Any increase in its duration or amplitude could alter normal motor unit (MU) firing rate properties in stroke, and potentially impact muscle force generation. The objective of this preliminary study was to examine potential differences in afterhyperpolarization (AHP) duration of motoneurons innervating paretic and contralateral limb muscles of hemiparetic stroke survivors. A novel surface EMG (sEMG) electrode was used to record from the first dorsal interosseous muscle (FDI) of three hemiparetic stroke survivors. sEMG data was decomposed to derive single motor unit (SMU) events, which were subsequently utilized to produce interval (ISI) histograms of the motor unit discharge. Interval Death Rate (IDR) analysis was then used to transform ISI histograms into death rate plots. [1] The prescribed IDR analysis method [1] involves a final transformation of death rate plots into an estimated AHP time course. The present study uses a modified method of interpreting death rate plots in order to determine AHP duration. AHP durations from this analysis are similar to durations obtained from ISI variability analysis. [2] Results from three subjects indicate that on average, motor units on the paretic side have a longer AHP duration than the contralateral side, potentially contributing to lower firing rates, and to less efficient force production in paretic muscles.**

I. Introduction

Cerebrovascular disease (stroke) in the US alone affects an estimated 7,000,000 people over the age of twenty. Consequently, stroke is the leading cause of long-term disability in the US. [3] One of the most common motor impairments post-stroke is paresis, or muscle weakness. While progressive changes in mechanical properties of paretic muscles, such as muscle atrophy, metabolic changes, or connective tissue infiltration are prevalent and could contribute to paresis, the possibility that changes at the spinal neuronal level occur also needs to be examined. Understanding potential changes in the properties and control of motoneurons could offer further insight towards mechanisms that may contribute to muscle weakness post-stroke.

Earlier electrophysiological studies have reported reductions of motor unit number, fibrillation and sharp waves, reduced M wave, and lower firing rates in motoneurons of paretic muscles [4-8]. Gemperline et al. suggest that lower firing rates are attributed to one of the following mechanisms: changes in the biophysical properties of the neuron, changes in synaptic input, or a decrease in neuromodulator input. [6] The goal of the present study is to determine whether a biophysical mechanism -manifested within the afterhyperpolarization (AHP) period, is responsible for lower firing rates.

The AHP is a primary determinant of motoneuron firing rate, and any increase in its duration or amplitude could alter normal motor unit (MU) firing rate properties in stroke. The relative variability of discharge in motoneurons is dependent upon both the AHP size and time course, and the amplitude and frequency content of concurrent synaptic noise. While statistical methods have been used to estimate the AHP duration in neurologically intact human subjects [1, 9-11], little data exists in stroke survivors [12].

Currently, two distinct methods of estimating AHP duration in humans exist: interval death rate (IDR) analysis [1], and variability analysis of inter-spike intervals (ISIs) [13]. Both methods have been validated through animal models [14]. Variability analysis comparing average AHP duration of motor units in brachial biceps between post-stroke patients and healthy control subjects suggests a lengthened AHP duration after cerebral stroke [12]. However, as the authors state, comparison of ISI variability between groups may have been confounded by a difference in spike generating modes: rhythmic firing mode (short interval range) vs. occasional spike mode (long interval range). [15]

In order to assess AHP duration systematically during similar types of firing modes, as well as to determine whether paresis in hand muscle (FDI) can be partially attributed to changes in AHP time course, the present study utilizes a novel combination of techniques from both the IDR and ISI variability analysis methods. Using this combined method, along with data collected from a non-invasive novel sEMG sensor array (Delsys, Inc.), the objective of this preliminary study is to determine whether the AHP time course could contribute to lower firing rates in paretic muscles.

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II. METHODS

A. Subjects

Three chronic hemiparetic stroke subjects: one with moderate weakness, one with severe weakness, and one with mild weakness (see Table 1) of the extremities contralateral to the cerebral lesion were tested. The subjects were recruited from the outpatient clinics of the Rehabilitation Institute of Chicago. All participants gave informed consent via protocols approved by the Institutional Review Board at Northwestern University.

Table 1. Demographic information of stroke subjects.

ID	Sex	Age	A	FT	C	Duration (years)	Paretic side
1	M	66	3	16/66	4	9	L
2	M	65	2	17/66	2	17	R
3	F	53	1	63/66	7	3	R

B. Experimental Setup

The participants were seated upright in a Biodex chair with their upper arm comfortably resting on a plastic support. To standardize hand position and to minimize activity of unrecorded muscles, the forearm was placed in a brace that was linked to the table. The index finger was placed in line with the long axis of the forearm (Fig. 1A). The proximal phalanx of the index finger was fixed to a ring-mount interface attached to a six degrees-of-freedom load cell (ATI, Inc.). The recorded forces from the x (abduction/adduction) direction was low passed (cutoff =200 Hz) and digitized at a sampling frequency of 1 kHz.

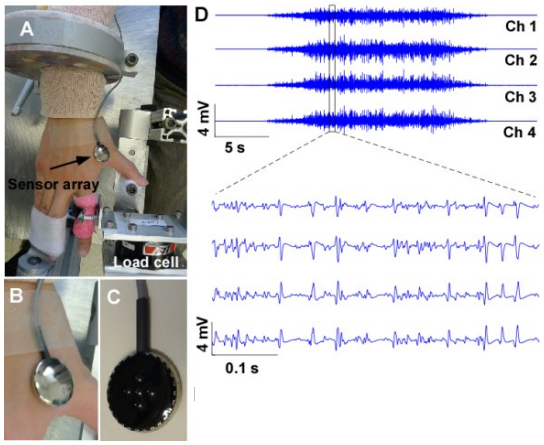

Fig. 1: Experimental setup, surface sensor array, sEMG signals, and force display. 1A: Brace and cast used to ensure index finger was aligned with the long axis of the forearm. 1B: Placement of Delsys Sensor Array on FDI muscle. 1C: 5 cylindrical probes of the sensor array, each located at the corners and center of 5x5 mm square. 1D: Pairwise differentiation of the 5 electrodes allows for 4 channels of sEMG signals.

sEMG were recorded from the first dorsal interosseous (FDI) muscle using a surface sensor array (Delsys, Inc.) as shown in Fig. 1B. This consists of 5 slender cylindrical probes, each located at the corners and at the center of a 5 × 5 mm square (Fig. 1C). Pairwise differentiation of the 5 electrodes yields 4 channels of sEMG signals (Fig. 1D). The sEMG sensor and a reference electrode were connected to 4 channels of a Delsys Bagnoli sEMG system. The signals were amplified and filtered with a bandwidth of 20 Hz to 2000 Hz. The signals were sampled at 20 kHz.

C. Procedures

Each subject was tested on both sides using the same protocol during one session. The paretic muscle was tested first. Participants were required to maintain a relatively stable firing rate of a single motor unit at its lowest firing rate. The subject was given both visual and audio feedback of motor unit activity. Once a distinct unit was audibly and visually distinct, the subject was requested to maintain that unit firing for as long as possible. All four EMG channels as well as the force signal were monitored online via Spike2 (Cambridge Electronic Design, UK) and subsequently stored for processing. An average of four motor units was collected from the paretic side, and five from the contralateral side.

D. Data Analysis

Spike2 software was used to classify motor units using a template matching algorithm based on amplitude and shape characteristics. The sEMG trials were selected for further analysis based on the following criteria: (1) More than 2,000 spikes were generated for a single motor unit; (2) The force variability during the steady state force was low.

Trains of single motor unit action potentials were analyzed using the IDR method [1] in Matlab. Each spike train was spliced into "subpopulations" based on a running mean firing rate determined by averaging 5 spikes before, and 5 spikes after each instantaneous spike. The resulting interval histograms of the subpopulation of spikes were then generated based on instantaneous intervals, not running mean intervals. Interval histograms (5ms bins) therefore each signified a distinct mean firing rate for a motor unit. All ISI histograms containing at least 1,000 intervals (mean: 1734 intervals) were used for further analysis. ISI interval death rates were subsequently calculated, representing the probability (*P)* of a motor unit discharge terminating the ISI. The calculation of *P* is described by the following equation:

$$P = \frac{\left[\ln\left(\frac{N_0}{N_1}\right)\right]}{bin\ width}$$

N_0 is the sum of following bins in the interval histogram, including the bin whose *P* is being measured, and N_1 is the sum of all following bins excluding the bin whose *P* is being measured. The final IDR plot eliminates the last few probabilities since the equation diverges quickly when remaining bin numbers are low.

In order to assess AHP duration, the interval at which the IDR plot "plateaus" was determined. The beginning

of the IDR plot plateau indicates the time at which the motoneuron starts being randomly excited by synaptic noise, after the AHP has concluded. [1] Since IDR plots are constrained by finite interval bins, the "plateau" period is often characterized by erratic behavior, where values substantially deviate from the initial smooth trajectory of the IDR.

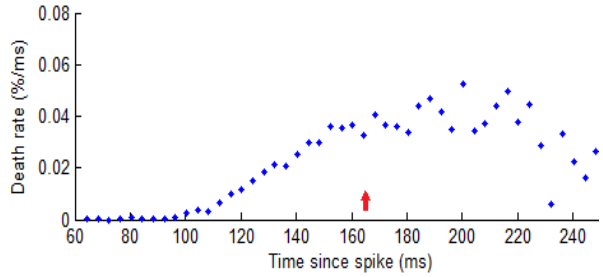

Fig. 2: Sample interval death rate plot from subject #1 (contralateral side), red arrow indicates beginning of plateau phase.

As Fig. 2 indicates, this point can easily be determined by the point at which the slope of the plot (qualitatively) reaches a mean of zero. To confirm, a quantitative approach was applied, where the transition interval was determined by fitting a line to the upwards trajectory of the plot. ISI variability analysis uses a similar quantitative approach to calculate the AHP estimate. The estimate, or "transition interval", is marked by the least-squares sum of two fitted lines (indicated by the operator). [12] Due to the erratic behavior of the plateau period, a secondary line was not fit. Beginning at the smooth upwards trajectory of the IDR plot, a linear regression was applied to the first 4, 5, … 4+x points of this portion of the IDR plot, until the time point where the r^2 value was below 0.95. In some cases, the calculated plateau interval was clearly a few milliseconds off the visually apparent plateau interval. Consequently the cutoff threshold ($r^2 < .95$) was slightly modified, but remained within the following range: [$.92 < r^2 < .95$]. Preliminary results from this modified analysis projected similar values to the published, animal model validated ISI variability analysis. [2, 14]

III. Results

Using the modified analysis method, we analyzed sEMG data of the FDI muscles of three hemiparetic stroke subjects. A total of 8 MUs from the paretic side and 7 MUs from the contralateral side were extracted from subject 1 (Chedoke score = 4). A total of 8 MUs from the paretic side and 4 MUs from the contralateral side were extracted from subject 2 (Chedoke score = 2). A total of 6 MUs from the paretic side and 4 MUs from the contralateral side were extracted from subject 3 (Chedoke score = 7). However, due to data analysis requirements, 3 MUs were analyzed for each subject on the contralateral side, 7 MUs were analyzed on the paretic side for subject 1, 6 MUs on the paretic side for subject 2, and 5 MUs on the paretic side for subject 3.

Fig. 3 illustrates a plot of the interval histogram and corresponding IDR plot derived from a paretic MU (subject 1). For subject 1, the average estimated AHP duration on the paretic side was 166.8 ms (SD: 13.4, 7 MUs), and the average firing rate was 6.8 Hz (SD: 0.34). For subject 2, the average estimated AHP duration on the paretic side was 177.8 ms (SD: 24.4, 6 MUs), and the average firing rate was 5.9 Hz (SD: 0.37). For subject 3, the average estimated AHP duration on the paretic side was 137.8 ms (SD: 16.9, 5 MUs), and the average firing rate was 7.7 Hz (SD: 0.69).

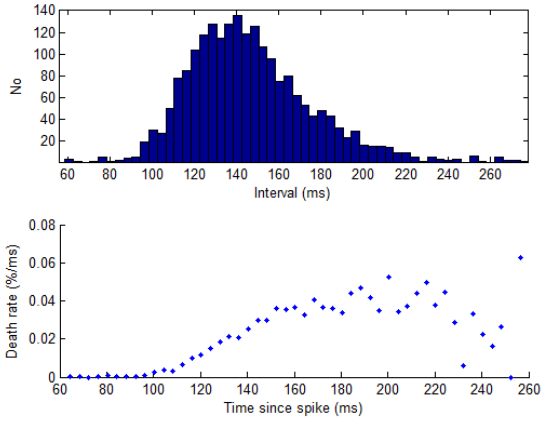

Fig. 3: Subject 1 - Interval Histogram of spike train from a paretic single motor unit (top). Corresponding Interval Death Rate plot (bottom). Mean firing rate: 7.01 Hz, estimated AHP duration: 164 ms.

Fig. 4 illustrates a plot of the interval histogram and corresponding IDR plot derived from a contralateral MU (subject 2). For subject 1, the average estimated AHP duration on the paretic side was 123.4 ms (SD: 7.5, 3 MUs), and the average firing rate was 8.6 Hz (SD: 0.07). For subject 2, the average estimated AHP duration on the paretic side was 155.2 ms (SD: 15.2, 3 MUs), and the average firing rate was 7.6 Hz (SD: 0.62). For subject 3, the average estimated AHP duration on the paretic side was 100.2 ms (SD: 13.3, 3 MUs), and the average firing rate was 11.1 Hz (SD: 0.74).

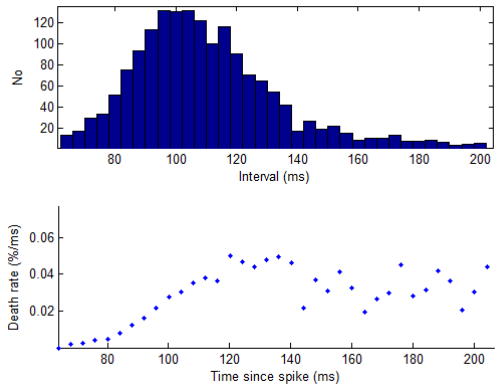

Fig. 4: Subject 2 - Interval Histogram of spike train from a contralateral single motor unit (top). Corresponding Interval Death Rate plot (bottom). Mean firing rate: 9.0 Hz, estimated AHP duration: 120.5 ms.

The Komogorov-Smirnov goodness-of-fit test was applied on the AHP estimates of both sides. Results indicated that the AHP estimate data on both the paretic and contralateral side followed a normal distribution. AHP estimates for each subject were averaged on the paretic and contralateral side. Subsequently, a paired t-test was used to statistically validate differences between the paretic and contralateral results. (See Table 2)

Table 2. Average AHP Duration Estimates

ID	Sex	Mean AHP (Contra)	Mean AHP (Paretic)
1	M	100.15	137.75
2	M	155.20	177.84
3	F	123.37	166.75

Results indicate that there is a significant increase ($p=.03$) of AHP durations on the paretic side compared to the contralateral side.

IV. Discussion

This preliminary study uses a modified IDR analysis to estimate AHP duration. A novel, non-invasive, sEMG recording electrode was used to record sEMG signals. Preliminary results reveal differences in motor unit firing rates between the paretic and contralateral side, corresponding with previous findings illustrating lower overall firing rates in paretic limbs [6]. These results also support findings from an earlier study which indicated an increased AHP duration in motor units of paretic biceps brachii, compared to healthy biceps. The modified IDR analysis may eliminate the confounding element of rhythmic firing vs. occasional firing [15], because the analysis does not rely on variability of the spike train. Instead, IDR analysis is governed by the "termination" probability of inter-stimulus intervals.

The modified IDR analysis may need to be refined in order to strengthen the method of qualitative assessment. Statistical validation, or perhaps animal model validation of the modified death rate analysis along with further subject recruitment may offer robust results supporting the hypothesis that a lengthened AHP contributes to lower MU discharge rates in cerebral stroke.

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