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Stroke Increases Ischemia-related Decreases in Motor Unit Discharge Rates

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Abstract

Following stroke, hyperexcitable sensory pathways, such as the group III/IV afferents that are sensitive to ischemia, may inhibit paretic motor neurons during exercise. We quantified the effects of whole leg ischemia on paretic vastus lateralis motor unit firing rates during submaximal isometric contractions. Ten chronic stroke survivors (>1 yr poststroke) and 10 controls participated. During conditions of whole leg occlusion, the discharge timings of motor units were identified from decomposition of high-density surface electromyography signals during repeated submaximal knee extensor contractions. Quadriceps resting twitch responses and near-infrared spectroscopy measurements of oxygen saturation as an indirect measure of blood flow were made. There was a greater decrease in paretic motor unit discharge rates during the occlusion compared with the controls (average decrease for stroke and controls, 12.3 ± 10.0% and 0.1 ± 12.4%, respectively; $P < 0.001$). The motor unit recruitment thresholds did not change with the occlusion (stroke: without occlusion, 11.68 ± 5.83%MVC vs. with occlusion, 11.11 ± 5.26%MVC; control: 11.87 ± 5.63 vs. 11.28 ± 5.29%MVC). Resting twitch amplitudes declined similarly for both groups in response to whole leg occlusion (stroke: 29.16 ± 6.88 vs. 25.75 ± 6.78 Nm; control: 38.80 ± 13.23 vs 30.14 ± 9.64 Nm). Controls had a greater exponential decline (lower time constant) in oxygen saturation compared with the stroke group (stroke time constant, 22.90 ± 10.26 min vs. control time constant, 5.46 ± 4.09 min; $P < 0.001$). Ischemia of the muscle resulted in greater neural inhibition of paretic motor units compared with controls and may contribute to deficient muscle activation poststroke.

NEW & NOTEWORTHY Hyperexcitable inhibitory sensory pathways sensitive to ischemia may play a role in deficient motor unit activation post stroke. Using high-density surface electromyography recordings to detect motor unit firing instances, we show that ischemia of the exercising muscle results in greater inhibition of paretic motor unit firing rates compared with controls. These findings are impactful to neurophysiologists and clinicians because they implicate a novel mechanism of force-generating impairment poststroke that likely exacerbates baseline weakness.

INTRODUCTION

The purpose of this study was to quantify the inhibitory effects of transient ischemia via whole limb blood flow occlusion on paretic motor units to further understand potential neural mechanisms of force-generating impairments during exercise in chronic stroke survivors. The firing behavior of motoneurons resulting in muscle activation is due to a combination of excitatory and inhibitory synaptic inputs and baseline intrinsic excitability of the motoneuron pool (Heckman and Enoka 2012). Following stroke, baseline impairments in the nervous system's ability to activate paretic motoneuron pools and muscles limits force generation. Several studies have documented stroke-related changes in paretic motor unit rate coding and recruitment (Chang et al. 2013; Chou et al. 2013; Gemperline et al. 1995; Li et al. 2015; McNulty et al. 2014; Mottram et al. 2014; Pollock et al. 2014), which are the two key neural strategies to grade force. During brief submaximal contractions, paretic motor unit firing rates have a compressed range compared with individuals without stroke, especially at higher force (Chou et al. 2013). In addition, recent studies have shown decreased ability to increase the magnitude of paretic global

surface electromyography (EMG) during submaximal fatiguing contractions (Rybar et al. 2014). Not surprisingly, given the primary pathology in the motor cortex, electrophysiological studies using transcranial magnetic stimulation have demonstrated decreased excitability in descending motor pathways poststroke (Foltys et al. 2003; Jang et al. 2017; Peters et al. 2017; Schwerin et al. 2008). However, motoneurons receive other synaptic inputs that can shape motor output (Heckman and Enoka 2012). For example, little is known on how inhibitory spinal pathways, such as the group III/IV pathways, may contribute to impaired motor unit firing behavior and muscle activation during exercise poststroke.

Group III/IV receptors are sensitive to muscle ischemia and compression associated with muscle contractions (Matthews 1972). In individuals without stroke and evidence from animal models, the group III/IV pathways are known to have an inhibitory effect on motor output by limiting muscle activation, force generation, and, during both single-limb and whole body fatiguing exercise, potentially decreasing voluntary drive (central fatigue) (Taylor et al. 2016). In other neurological conditions, such as spinal cord injury, this pathway is hyperexcitable (Schmit et al. 2002, 2003; Theiss et al. 2011; Wu et al. 2006), presumably due to decreased regulation by supraspinal centers. Understanding how activation of this pathway impacts motor output\post stroke is important because it may exacerbate baseline impairments in motor unit firing behavior and muscle activation, thus further limiting force-generating capabilities.

The inhibitory effects of ischemia on motor output may be enhanced poststroke due to impaired blood flow to the paretic muscle. Several studies have shown that individuals with stroke have impaired peripheral large and small arterial regulation of blood flow. First, resting blood flow in the femoral artery is lower in the paretic leg (Billinger et al. 2009; Sherk et al. 2015), and both resting and reactive hyperemic blood flow are reduced in the paretic leg compared with the nonparetic leg (Ivey et al. 2004). Moreover, our group has shown that during volitional contractions, at various load levels, the hyperemic response is blunted compared with those without stroke (Durand et al. 2015). Importantly, we also showed that the paretic leg hyperemic response to volitional contractions was positively correlated with clinical measures of function (Durand et al. 2015). In summary, these studies indicate that there is impaired blood flow to resting and exercising muscles in the paretic leg that may lead to an increased accumulation of metabolic byproducts and activation of group III/IV pathways compared with individuals without stroke.

In addition to impaired blood flow to exercising paretic muscle, the excitability of the group III/IV pathways may be increased poststroke, as seen with the group Ia afferents and resultant spasticity (Li 2017). Hidler and Schmit (2004) demonstrated experimentally and with computational modeling that group III/IV pathways contributed more than group Ib or Ia pathways to reflex force inhibition of the paretic elbow flexors. Others have shown increased stroke-related reflex responses through cold and pain stimuli and increased central hypersensitivity in pain pathways (Soo Hoo et al. 2013). The inhibitory impact of group III/IV activity on motor output is seen in other patient populations, such as congestive heart failure (Amann et al. 2014), and motor performance is improved in healthy individuals when mechanosensitive group III/IV pathways are pharmacologically blocked (Amann et al. 2011).

Thus it is plausible that the stroke-related changes in the regulation of peripheral blood flow to exercising muscle and a change in the excitability of the group III/IV spinal pathways may enhance the inhibitory response to ischemia, limiting motor unit firing behavior. In this study we quantified the effects of whole leg ischemia on paretic vastus lateralis motor unit firing rates during submaximal isometric contractions. We hypothesized that in response to ischemia, knee extensor paretic motor unit firing rates would decrease to a larger extent than those without stroke. Secondary measures of maximal voluntary contraction and resting twitch responses were made to provide mechanistic insights.

MATERIALS AND METHODS

Subjects

All participants gave informed written consent before participation in the study, and procedures were approved by the Medical College of Wisconsin Institutional Review Board (PRO190103). Ten individuals with chronic hemiparetic stroke (8 men, 2 women, 58.9 ± 9.4 yr) and 10 age-matched neurologically intact controls (8 men, 2 women, 60.2 ± 9.5 yr) participated in the study. Stroke inclusion criteria were single, unilateral stroke (confirmation obtained through verbal communication from the physician and consistent with neurological physical examination), ability to ambulate at least 30 ft. (with or without an assistive device), and ≥6 mo poststroke. Stroke exclusion criteria included brain stem stroke, any uncontrolled medical condition, contractures of any lower extremity joints, and inability to follow two- to three-step commands. Table 1 reports the subject characteristics.

Table 1. Characteristics of all stroke participants

Subject	Age, yr	Time Poststroke, yr	Fugl-Meyer
1	64	12	18
2	65	11	24
3	68	13	21
4	60	15	25
5	65	17	28
6	58	3	31
7	61	24	34
8	37	12	27
9	52	11	17
10	68	34	22

Fugl-Meyer score indicates Fugl-Meyer assessment of motor recovery after stroke.

Torque Measurements

Knee extension torque measurements were made using a Biodex dynamometer (Biodex Medical System, Shirley, NY). Participants sat on a Biodex chair with their knee flexed to 90° and the leg securely attached to the Biodex attachment 2 cm above the lateral malleolus. The torque was measured using the Biodex load cell (force-torque transducer), sampled at 2,048 Hz, acquired by an EMG-USB2+ amplifier (256-channel regular plus 16-auxiliary channels; OT Bioelettronica, Turin, Italy), and recorded using the OT Biolab software.

Surface EMG Recordings

Surface EMG recordings were obtained using a 64-channel 2-dimensional electrode array (13 rows, 5 columns). A double-sided adhesive sticker designed for and compatible with the array was placed over the array. The holes within the adhesive sticker were filled with a conductive electrode paste (Ten20; Weaver and Company, Aurora, CO). The array was placed over the belly of the vastus lateralis, midway between the patella and the greater trochanter, after the subject's skin was sterilized with an alcohol swab and rubbed to remove superficial dead skin. The signals for each channel were differentially amplified between 1,000 and 5,000 V/V (subject dependent) and bandpass filtered between 10 and 500 Hz using the EMG-USB2+ amplifier. The signals were sampled at 2,048 Hz and acquired with the OT Biolab software throughout the duration of the experimental protocol.

Near-Infrared Spectroscopy Measurements

Near-infrared spectroscopy (NIRS) measurements were obtained using a SenSmart Universal Oximetry System (Nonin Medical, Plymouth, MN). Near-infrared light was used to measure the regional skeletal muscle tissue oxygen saturation (rSO_2). Receiving optodes were placed over the rectus femoris of the test leg. The signals were sampled at 2 Hz and collected by the embedded software of the device. Continuous baseline measurements were taken with the participant sitting still and upright in the Biodex chair for 5 min before onset of any experimental procedures. To control for intersubject variability, the NIRS measurements were normalized to the mean of the baseline period and collected throughout the experimental protocol.

Experimental Protocol

All control participants performed the protocol with their right leg (dominant per participant report), and all stroke participants performed the protocol with the paretic leg. Figure 1 illustrates the timeline of the protocol. First, each participant performed at least five baseline maximum voluntary contractions (MVC) of the knee extensor muscles with 1 min of rest between trials. The peak force of all the trials was used as the MVC. Study participants were given verbal and visual feedback. After at least a 5-min rest period following the final MVC, individuals performed a submaximal isometric ramp-and-hold torque tracking protocol. For the ramp-and-hold protocol, the participants were instructed to trace a trapezoid trajectory displayed on a computer screen by contracting their knee extensor muscles to generate torque. Real-time visual feedback was provided to the participant indicating the torque produced by the knee extensor muscles. The trapezoid was 18 s in duration consisting of a 4-s rising phase from rest, a 10-s hold phase at 20% MVC, and a 4-s decline phase back to rest. For each ramp-and-hold trial, one trapezoid contraction was repeated six times with a 1-min resting period between trials. After at least a 5-min break, a second protocol of both MVCs and ramp and holds was performed while blood flow was occluded. A blood pressure cuff designed for the leg was inflated to 225 mmHg over the upper thigh of the test leg to transiently occlude blood flow to the test leg. The cuff remained inflated throughout the six isometric knee extension contractions (Fig. 1). We chose to fully occlude blood flow for a brief period of time to elicit a maximal response without injury. During the protocol with repeated MVCs only, after each MVC, a constant current generator (DS7A; Digitimer, Welwyn, UK) delivered a rectangular pulse of 100-μs duration with maximum amplitude of 400 V, which was used to percutaneously stimulate the quadriceps muscle. The stimulation intensity (usually 200–500 mA) was set at 20% above the level required to produce a maximal resting twitch amplitude that caused a supramaximal stimulation.

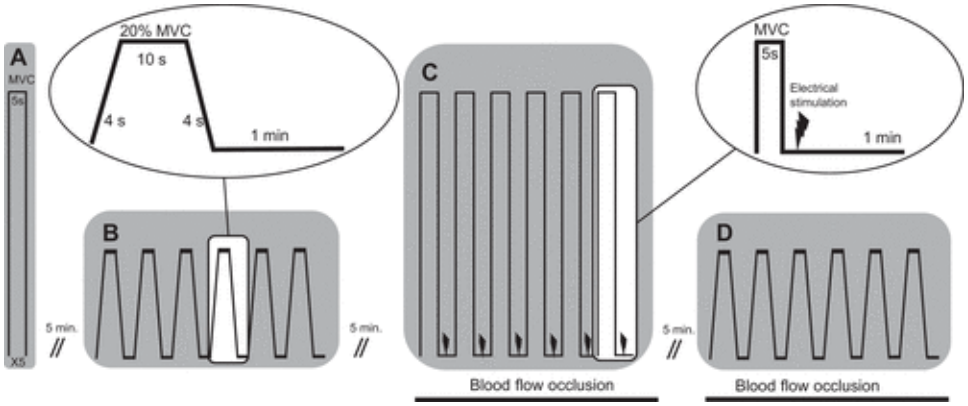

Fig. 1. Experimental protocol. *A*: participants first performed 3–5 baseline maximum voluntary contractions (MVC) with the knee extensors with a 1-min rest between contractions. *B*: participants then performed 6 "ramp-and-hold" isometric knee extensor contractions, at 20% MVC, with a 1-min rest between contractions. *C*: the subject performed 6 MVCs with a 1-min rest between contractions while the test leg was occluded. After each MVC when the muscle was fully relaxed, electrical stimulation was applied to the quadriceps to elicit a resting twitch response. *D*: subject then repeated the ramp-and-hold protocol during whole leg occlusion. Near-infrared spectroscopy and surface electromyography of the quadriceps muscle were recorded throughout the experiment.

Data Processing and Statistical Analysis

Torque.

Torque signals were zero-phased low-pass filtered at 15 Hz using a second-order Butterworth filter before analysis and processed using MATLAB (The MathWorks, Natick, MA). Peak torque was calculated for each MVC and elicited resting twitch. In addition, time from the peak amplitude of the twitch to a drop in 75% amplitude was determined to quantify relaxation rates for the resting twitches.

Motor unit decomposition.

The 64 (63 after differential amplification) individual EMG channels were visually examined to remove noisy channels. The remaining channels were decomposed to attain information of single motor unit action potential trains using a multichannel convolutive blind source separation algorithm previously described and validated by Negro et al. (2016). To provide a normalized index of reliability similar to the pulse-to-noise ratio, a silhouette measure (Sil) was computed on each estimated source, and the source was considered of acceptable quality if Sil was >0.90. Sil provides a measure of the quality of the extracted motor unit spike trains based on the relative amplitude of the deconvolved spikes compared with the baseline noise. Of the units with Sil > 0.90, we then calculated the two-dimensional correlation between motor unit action potential shapes of tracked units in different contractions and found correlation values >0.85. Because we wanted to examine motor units that were likely continuously contributing to force generation, an identified motor unit was excluded for further analysis if its firing rate (pulses per second, pps; Hz) was less than 5 Hz. A total of 75 units were accepted for the control group and 37 for the stroke group. The motor unit action potential timings were time-locked with the torque trace. The firing instances for each motor unit were low-pass filtered with a unit area Hanning window of 1-s duration. Motor units were identified by applying the decomposition algorithm on six individual ramp-and-hold trials. The trials were concatenated from the original recordings with a resting segment of 4 s between each ramp-and-hold trial. Under the assumption of stable motor unit action potential properties, this configuration and similar approaches provided the possibility to identify reliably the same motor units in different trials (Martinez-Valdes et al. 2017). However, in this study units were only tracked within a given protocol and not between the occlusion and nonocclusion protocols.

The instantaneous firing rates of individual motor units were calculated as the inverse of the interspike interval. The firing rates at recruitment and derecruitment were defined as the mean of the first and last three interspike intervals, respectively. Motor unit recruitment and derecruitment thresholds were determined as the torque (normalized to MVC) for the first and last discharge time for each motor unit, respectively. The mean firing rates during the hold phase of the ramp and hold contractions, de/recruitment firing rates, and de/recruitment thresholds, were compared for the first contraction that a motor unit continuously fired with the final contraction that a motor unit continuously fired.

Global surface EMG RMS measurements.

The EMG root mean square (RMS) for each channel of the HDsEMG array was calculated during the 10-s hold phase for each contraction

$$(RMS = \sqrt{\frac{1}{N}\sum_{begin\ hold}^{end\ hold} x_n^2}, n = data\ point, N = total\ data\ points)$$

The global surface EMG RMS was calculated as the mean RMS of all channels for each contraction.

Regional muscle oxygen saturation.

To determine relative levels of oxygen saturation within the quadriceps muscle, NIRS measurements of local oxygen saturation within the rectus femoris were determined before the beginning of each contraction by averaging the NIRS values during the 2-s interval before the ramp-and-hold contraction. During the occlusion protocol, the coefficients (with 95% confidence bounds) to model the exponential decay of oxygen saturation were determined using MATLAB's Curve Fitting Toolbox. The time constant ($\tau = 1/\lambda$) of the exponential decay model ($N = N_0 e^{-\lambda t}$) was determined, and the population means for both stroke (N = 10) and control (N = 10) subjects were compared.

A linear regression modeled the relation between the change in firing rate and the change in oxygen during the occlusion protocol. This was accomplished by using firing rate during the last contraction that a motor unit fired as a percentage of the first contraction that a motor unit fired vs. the change in oxygen saturation over the entire 20% MVC ramp-and-hold occlusion protocol. The slope of the regressions was calculated, and the mean of the linear regression slopes was compared between stroke and controls.

Statistical analysis.

We performed separate repeated-measures, mixed-model ANOVAs on the following dependent variables for the occlusion and nonocclusion conditions: firing rate during the hold phase, recruitment firing rate, derecruitment firing rate, recruitment threshold, derecruitment threshold, and mean global surface EMG. The between-group variable was group (paretic and control). Contraction (first contraction and last contraction) was the within-group comparison. A Bonferroni correction was used in all post hoc testing and only performed when an interaction effect between contraction and group was present. Separate two-sample *t*-tests were used to detect the difference between stroke and control participants for the exponential decay time constant and the mean slope value for the linear regression for change in firing rate per change in muscle oxygen saturation. All statistical tests were performed using an alpha level of 0.05 for significance. Data are means ± SD.

RESULTS

Mean Firing Rates

During the occlusion condition, there was a significant main effect of contraction, because firing rates were lower for the final contraction (7.89 ± 1.93 pps) compared with baseline (8.24 ± 1.99 pps; $P < 0.001$). There was also a main effect of group on motor unit firing rate ($P = 0.02$). Stroke participants (7.49 ± 2.36 pps) had lower mean firing rates compared with control participants (8.35 ± 1.67 pps; $P = 0.02$). There was a significant interaction effect between contraction and group in which the paretic leg had a larger decrease in firing rates compared with controls ($P < 0.001$; see Fig. 2, single subject example, and Fig. 3, group). Post hoc pairwise comparisons revealed a significant decrease ($P < 0.001$) in stroke motor unit firing rates between the baseline contraction and the final contraction during occlusion, but there was no significant difference ($P = 0.72$) for control motor unit firing rates between baseline and final contractions (Fig. 3). This equates to an average 12.32 ± 9.99% decrease in firing rates for stroke (final contraction vs. first contraction) and a 0.12 ± 12.42% increase in firing rate for control subjects. There was also a significant difference in firing rates between the paretic and control legs in the final contraction ($P < 0.001$), but not for the first contraction ($P = 0.52$) (Fig. 3). Thus, for submaximal contractions with similar relative efforts, both groups had similar firing rates at baseline, but paretic motor unit firing rates decreased to a larger degree compared with the controls with occlusion. Finally, with stroke and control data combined, the percent decline in firing rates was positively correlated with net torque generated during the submaximal contractions ($r^2 = 0.262$, $P = 0.030$), whereby the smaller the torque (which reflects a smaller MVC value), the larger the relative decline in mean firing rates. There was a

trend toward a positive correlation between the change in firing rate and the change in MVC in the individuals with stroke, but it was not significant (r^2 = 0.325, P = 0.085).

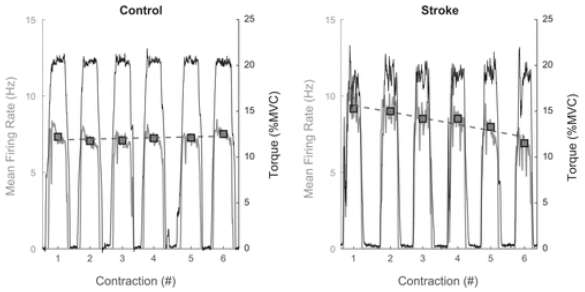

Fig. 2. Single-subject examples of individual motor unit firing rates during the occlusion protocol for a control and stroke survivor. The gray trace shows the filtered firing rates using a 1,000-ms Hanning window. Squares represent the average firing rate of the motor unit for the ramp-and-hold contraction. A linear fit of the average firing rates for each contraction is represented by the dashed gray line. The black trace represents the torque. Consistent with the group effect, note the greater decline in motor unit firing rates (gray trace) with the stroke survivor compared with the control example. MVC, maximum voluntary contraction.

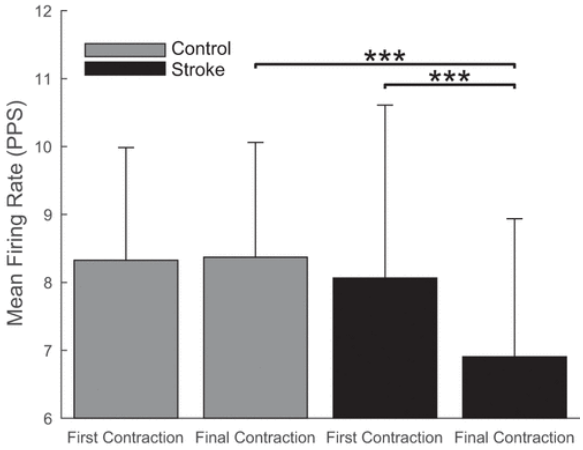

Fig. 3. Mean firing rates during the 20% ramp-and-hold occlusion protocol for the stroke and control participants. Individuals with stroke had a larger decline in firing rates compared with controls. ***P < 0.001, contraction × group effect. PPS, pulses/s.

In the condition without occlusion, there were no statistical differences for group (stroke: 8.24 ± 1.64 pps; control: 8.64 ± 2.09 pps; P = 0.40), contraction (baseline: 8.44 ± 2.05 pps; final: 8.55 ± 1.89 pps; P = 0.24), and contraction × group interaction (P = 0.98). In summary, stroke and control motor unit firing behavior was not statistically different during the nonocclusion condition. On an individual level (Table 2), 7/10 individuals with stroke had lower mean firing rates (<8 pps) compared with 2/8 controls. In addition, 8/10 individuals with stroke had absolute torque recruitment thresholds less than 20 compared with 4/8 controls.

Table 2. Mean values of variables describing motor unit firing behavior for all participants

Firing Rate, pps		Recruitment Threshold, Nm		Recruitment Threshold, %MVC		Baseline MVC, Nm	Torque, Nm		
Subject	*Contraction 1*	*Contraction 6*	*Contraction 1*	*Contraction 6*	*Contraction 1*	*Contraction 6*	*Protocol*	*Contraction 1*	*Contraction 6*
Control									
1							93.37	18.48	17.77
2	8.32	7.74	13.60	15.28	7.68	6.83	104.44	23.13	22.89

3	9.23	7.98	14.24	17.63	14.50	11.71	206.47	43.19	42.70
4	8.03	8.51	28.82	33.79	8.19	6.99	236.17	47.97	48.20
5							111.40	23.45	24.00
6	7.80	7.51	17.24	16.11	9.64	10.32	166.30	33.24	33.01
7	7.90	7.86	32.80	32.18	7.53	7.67	246.88	49.70	49.38
8	9.71	9.81	39.90	35.03	6.84	7.79	272.81	55.74	55.76
9	8.89	9.00	27.89	19.57	8.61	12.27	240.08	47.80	47.44
10	10.08	10.50	11.04	11.01	15.36	15.41	169.56	33.06	33.30
Stroke									
1	8.78	6.37	5.74	8.56	12.52	8.39	71.87	12.53	12.52
2	7.07	6.49	15.85	11.81	8.65	11.61	137.14	29.02	28.87
3	7.38	5.64	11.21	12.93	13.83	12.00	155.07	28.16	28.36
4	6.30	5.43	17.54	17.50	6.46	6.47	113.29	22.67	21.75
5	7.24	5.77	26.85	34.06	9.14	7.20	245.37	50.35	50.11
6	13.63	11.37	26.83	27.74	7.60	7.35	203.99	40.00	39.49
7	6.86	6.47	12.67	10.62	6.62	7.91	83.94	15.35	15.39
8	7.38	7.16	14.12	14.16	11.22	11.18	158.39	29.24	29.11
9	6.92	6.30	6.49	6.92	10.21	9.57	66.22	12.32	11.38
10	9.09	5.86	3.42	2.85	13.76	16.52	47.05	8.94	9.56

MVC, maximum voluntary contraction; pps, pulses/s.

Recruitment Threshold

During the occlusion condition, there was no significant contraction effect (baseline: 11.76 ± 5.79%MVC vs. final cycle: 11.04 ± 5.28%MVC; $P = 0.09$). There was not a significant main effect of group ($P = 0.87$) for motor unit recruitment thresholds (control: 11.44 ± 5.49%MVC vs. paretic: 11.31 ± 5.69%MVC). There was also no interaction effect of contraction and group ($P = 0.97$). These results suggest the same pool of units were recruited. In 23/37 units from the stroke group, the recruitment threshold decreased from the first to last contraction, and in 13/37 units, the recruitment threshold increased. Within the occlusion trials, there appears to have been a trend toward an increase in recruitment threshold when the first and the last contractions were compared (Fig. 4).

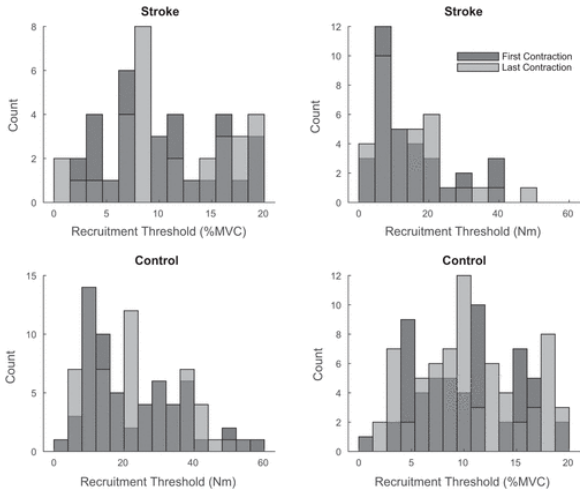

Fig. 4. Histogram of torque recruitment thresholds in stroke (*top*) and control (*bottom*) between the first and last contractions during the occlusion condition. MVC, maximum voluntary contraction.

In the condition without occlusion, control motor unit recruitment thresholds (11.42 ± 4.35%MVC) were not statistically different from the paretic (10.83 ± 5.63%MVC) motor unit recruitment thresholds (main effect of group: $P = 0.87$). There was no main effect of contraction ($P = 0.19$) (baseline: 10.98 ± 5.33%MVC vs. final cycle: 11.41 ± 4.37%MVC). There was also no significant interaction effect of contraction and group ($P = 0.17$). The range of recruitment thresholds was compressed in individuals with stroke vs. control for the first contraction (stroke: 39.99%MVC; control: 57.11%MVC) and the last contraction (stroke: 43.19%MVC; control: 50.97%MVC).

Recruitment Firing Rates

During occlusion, there was a significant main effect of contraction for recruitment firing rates, because firing rates decreased in the final cycle (6.96 ± 1.85 pps) compared with the baseline cycle (7.45 ± 2.05 pps; $P = 0.001$). There was no main effect of group (stroke: 6.79 ± 2.16 pps vs. control: 7.40 ± 1.83 pps; $P = 0.08$) and no significant interaction effect between contraction and group ($P = 0.16$).

During the condition without occlusion, there was a significant main effect of group, because control recruitment firing rates (7.95 ± 2.09 pps) were higher than for stroke (6.73 ± 1.79 pps; $P = 0.009$), but there was no main effect of contraction ($P = 0.62$) on recruitment firing rates, because baseline (7.53 ± 2.20 pps) and final (7.45 ± 1.93 pps) were similar. There was also no significant interaction effect between contraction and group ($P = 0.82$). These results show that when the protocol was performed without occlusion, stroke recruitment firing rates were lower than control motor units, but baseline and final cycles did not affect the recruitment firing for stroke or control motor units.

Derecruitment Firing Rates

During the occlusion condition, there was a significant main effect of group ($P = 0.004$), where individuals with stroke had lower rates at derecruitment (6.39 ± 2.42 pps) compared with controls (7.10 ± 2.02 pps). There was no significant main effect of contraction ($P = 0.53$) for motor unit derecruitment firing rates, where baseline (7.00 ± 2.19 pps) was similar to the final (6.73 ± 2.17 pps) cycle. There was no significant interaction effect of contraction and group ($P = 0.18$).

Likewise, in conditions without occlusion, there was a significant main effect of group ($P = 0.006$) with control derecruitment firing rates (6.98 ± 1.66 pps) higher than stroke derecruitment firing rates (6.56 ± 2.60 pps). There was not a significant main effect of contraction ($P = 0.07$) for motor unit derecruitment firing rates (baseline: 6.65 ± 2.21 pps vs. final cycle: 6.99 ± 1.90 pps). There was no significant interaction effect of contraction and group ($P = 0.34$).

Derecruitment Threshold

There was no main effect of group between control (13.39 ± 5.06%MVC) and stroke (13.44 ± 5.56%MVC; $P = 0.66$) for motor unit derecruitment threshold during occlusion. No main effect of contraction was observed ($P = 0.57$), because baseline derecruitment threshold (13.48 ± 5.42%MVC) was similar to that of the final cycles (13.34 ± 5.04%MVC). There was also no interaction effect of contraction and group ($P = 0.25$).

There was no main effect of group ($P = 0.62$) or contraction ($P = 0.83$) for motor unit derecruitment threshold during the nonocclusion condition. Control (13.25 ± 4.09%MVC) was similar to stroke (13.85 ± 5.05%MVC) for derecruitment threshold, and derecruitment threshold was similar for baseline (13.69 ± 4.51%MVC) and final cycles (13.27 ± 4.45%MVC). There was also no interaction effect of contraction and group ($P = 0.10$).

Local Muscle Oxygen Saturation

Figure 5 shows the fitted exponential decays for each individual's local oxygen saturation during occlusion. Individuals with stroke had a greater time constant for exponential decay of local muscle oxygen consumption compared with control subjects (22.90 ± 10.26 vs. 5.46 ± 4.09 min; $P < 0.001$) during ischemia (Fig. 6). Stroke

time constants ranged from 9.2 to 35.6 min, and control time constants ranged from 0.4 to 13.3 min. Only two of the control time constants overlapped into the lower range of the stroke time constants. Linear regressions of each motor unit firing rate with the local muscle oxygen saturation yielded an average r^2 value of 0.49 for stroke and 0.41 for control (Fig. 7); furthermore, the average slope value of the linear regression line was significantly more negative for stroke than for control ($P < 0.001$; Fig. 8). Table 3 includes the mean relative change in oxygen saturation for each participant. At an individual level, the change in firing rate was correlated with the relative change in oxygen saturation ($r^2 = 0.290$, $P = 0.021$).

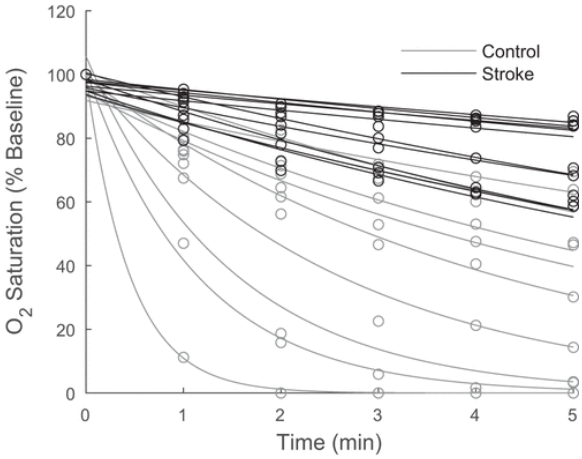

Fig. 5. Local O_2 saturation of the rectus femoris before the beginning of each ramp-and-hold contraction at 20% maximum voluntary contraction (MVC) for stroke and control. *Minute 0* represents the muscle O_2 saturation before the first ramp-and-hold cycle, and *minute 5* represents the muscle O_2 saturation before the final ramp-and-hold cycle at 20% MVC. A model of exponential decay was fitted to the data.

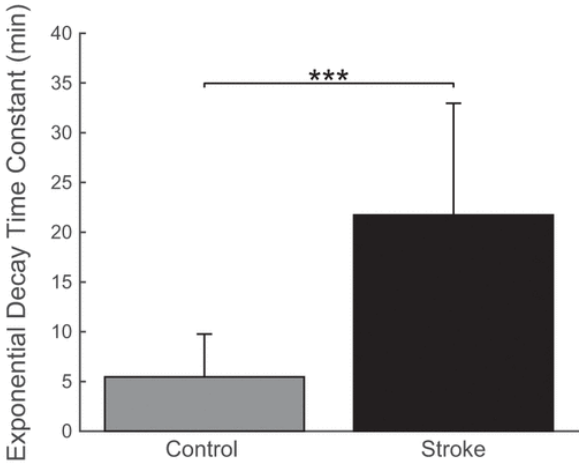

Fig. 6. Mean time constant for the exponential decay of the local muscle O_2 saturation of the rectus femoris during occlusion at 20% maximum voluntary contraction ramp-and-hold cycle for the stroke and control populations. ***$P < 0.001$.

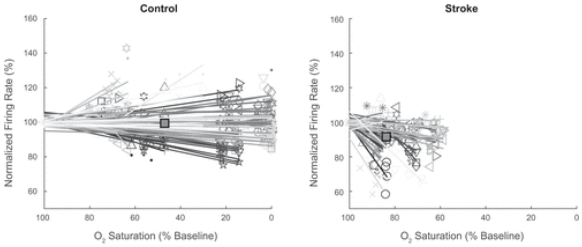

Fig. 7. Linear regressions of each motor unit firing rate as a percentage of the first contraction vs. the local muscle O_2 saturation for all stroke and control recorded motor units during the 20% maximum voluntary contraction occlusion

protocol. Each shape and color combination represents the firing rate at that local O_2 saturation. Bold squares are the group means for the firing rate and local muscle O_2 saturation.

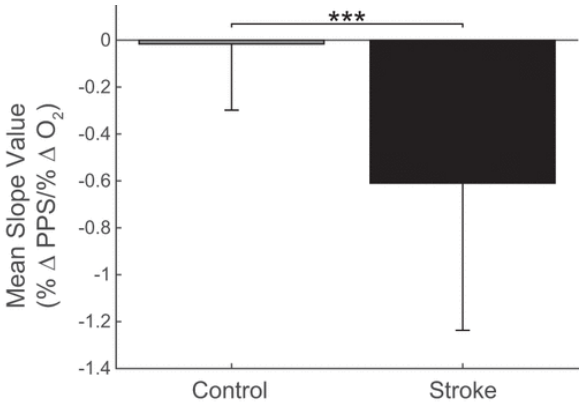

Fig. 8. Mean slope value of the linear regression lines for the motor unit firing rate vs. the O_2 saturation between stroke and control groups during the 20% maximum voluntary contraction occlusion protocol. ***P < 0.001. PPS, pulses/s.

Table 3. Mean values of variables describing O_2 saturation for each participant

Subject	%ΔMotor Unit Firing Rate	%ΔO2 Saturation	Time Constant, min	Slope	R^2
Control					
1		−52.77	5.79		
2	−6.92	−85.67	2.57	−0.17	0.36
3	−13.52	−100.00	1.47	−0.10	0.42
4	5.97	−96.76	1.13	0.08	0.49
5		−36.16	10.39		
6	−3.68	−30.50	13.28	−0.06	0.46
7	−0.52	−96.46	0.45	−0.05	0.53
8	0.95	−69.83	4.25	0.11	0.46
9	1.13	−53.49	6.59	−0.03	0.54
10	4.19	−41.57	8.73	0.17	0.55
Stroke					
1	−27.48	−15.81	30.54	−0.98	0.38
2	−8.17	−14.61	33.83	−0.94	0.56
3	−23.57	−29.37	14.77	−0.92	0.76
4	−13.87	−37.92	10.21	−0.21	0.23
5	−20.19	−16.19	29.88	−0.84	0.61
6	−16.54	−13.14	35.59	−1.10	0.61
7	−5.74	−41.32	9.33	−0.23	0.35
8	−3.02	−39.84	9.25	−0.07	0.21
9	−8.86	−14.55	30.93	−0.28	0.21
10	−35.53	−31.75	13.11	−0.92	0.70

Maximum Voluntary Contractions

Baseline MVCs were significantly lower for stroke compared with controls (123.21 ± 62.32 vs. 179.00 ± 61.20 Nm; P < 0.049; see Table 2). During the MVC occlusion, there was a significant main effect of contraction (first

contraction: 139.60 ± 64.92 Nm; last contraction: 107.19 ± Nm; $P < 0.001$). There was a significant interaction of contraction and group ($P = 0.015$) whereby the controls had a larger decline in MVC between cycles compared with the subjects with stroke (control: first contraction, 164.92 ± 66.75 Nm; last contraction, 116.75 ± 44.35 Nm; stroke: first contraction, 114.21 ± 54.93 Nm; last contraction, 97.62 ± 51.47 Nm). There was no main effect of group (control: 139 ± 64.92 Nm; stroke: 107.19 ± 47.78 Nm; $P = 0.161$).

Resting Twitch

During occlusion, there was a significant main effect of contraction (first contraction: 33.70 ± 10.87 Nm; last contraction: 27.81 ± 8.04 Nm; $P = 0.001$) for the resting twitch amplitude because amplitude decreased for both stroke (28.91 ± 6.49 to 25.65 ± 6.40 Nm) and control (38.80 ± 12.43 to 30.14 ± 9.02 Nm). There was not a significant main effect of group ($P = 0.126$) and no significant interaction effect of contraction and group ($P = 0.085$). Time to 75% relaxation also had a significant main effect of contraction (first contraction: 116.07 ± 40.70 ms; last contraction: 143.16 ± 50.49 ms; $P = 0.010$) because both stroke (125.97 ± 35.61 to 165.42 ± 50.54 ms) and control (104.92 ± 43.11 to 118.11 ± 36.85 ms) increased in relaxation time. There was also no significant main effect of group ($P = 0.105$) and no interaction effect of contraction and group ($P = 0.163$).

Global Surface EMG

Repeated-measures ANOVA during the occlusion protocol showed no main effect of contraction ($P = 0.303$) or group ($P = 0.825$) and no interaction effect ($P = 0.116$) for the mean global surface EMG. The nonocclusion protocol also showed no main effect of contraction ($P = 0.063$) or group ($P = 0.231$) and no interaction effect ($P = 0.268$) for the mean global surface EMG. Paired *t*-test showed that the stroke group was not significantly different during the nonocclusion and occlusion protocols at the first contraction (95.19 ± 34.00 vs. 99.08 ± 28.76 μV; $P = 0.80$) or the last contraction (99.03 ± 36.52 vs. 97.85 ± 46.07 μV; $P = 0.96$). This was also similar for the control group, because the mean RMS was not significantly different between the nonocclusion and occlusion protocols at the first (123.06 ± 65.08 vs. 121.08 ± 59.62 μV; $P = 0.95$) or the last contraction (133.25 ± 42.83 vs. 126.41 ± 79.13 μV; $P = 0.78$). These data show that the mean RMS was not impacted by the occlusion protocol between groups.

DISCUSSION

In this study, we demonstrate that paretic motor unit firing behavior is more sensitive to inhibitory effects of transient ischemia compared with responses in individuals without stroke. Occlusion of the paretic leg caused a larger decrease in the average motor unit firing rates during a submaximal contraction without substantial changes in muscle contractile properties. Remarkably, the decline in motor unit firing rates occurred despite individuals with stroke having a greater time constant for the rate of change of oxygen saturation and a lower relative change in oxygen saturation compared with the controls. Because group III/IV muscle afferents are sensitive to muscle ischemia and have an inhibitory effect on motor unit firing behavior, our results suggest that group III/IV afferents contribute to altered motor unit firing and may contribute to impaired force generation in chronic stroke during exercise.

Transient Group III/IV Muscle Afferent Feedback Inhibits Paretic Motor Unit Discharge

The major finding of this study was that motor unit firing rates decreased when blood flow was transiently occluded to the contracting paretic muscle (Fig. 3). Potential mechanisms for the larger decrease in paretic motor unit firing during occlusion compared with the controls include *1*) impaired descending drive to the paretic motoneuron pools with repeated contractions, *2*) greater decreases in paretic oxygen saturation during occlusion, and *3*) increased excitability in group III/IV spinal pathways. Because on average across the group, there was no detected decrease in firing rates for the stroke or control groups in the nonocclusion condition, the decrease in firing rates during occlusion was not likely due to a baseline inability of descending pathways to

activate the motor units or rate-coding impairments at the level of the motoneuron over multiple contractions across the 5-min condition.

The group findings during the nonocclusion condition in this study contrast with other studies that have shown differences between controls and individuals with stroke in firing rates during submaximal contractions (Gemperline et al. 1995; Mottram et al. 2014). On an individual level, most of the participants with stroke did have lower firing rates than the controls (7/10), and in instances where the torques were similar between a control and participant with stroke, the paretic motor units had lower firing rates than those of controls (Table 2). Our results may differ from those of others because of key differences in the task: *1*) relative effort vs. matched target torque, *2*) muscle groups (upper extremity vs. lower extremity muscles), and *3*) contraction type (triangle ramp vs. ramp and hold). Finally, we could not fully evaluate motor unit firing rate saturation because we did not perform multiple target torque conditions, but this is intended to be addressed in future studies.

Because the occlusion condition was performed after the nonocclusion and the occlusion + MVC protocols, it is possible that the decline in firing rates was due to increased fatigability in the stroke survivors (Hyngstrom et al. 2012; Knorr et al. 2011). However, individuals were given at least 5 min of rest between protocols, and the respective magnitudes of the global surface EMG from the first cycle of the two protocols in people with stroke were similar, indicating similar neural drive for a given target torque. In addition, there was no significant change in EMG amplitude within a protocol for both the stroke and control groups. If there was substantial muscle fatigue, the magnitude of EMG would be expected to be greater to meet the force demands of the task (Enoka and Stuart 1992; Garland et al. 1994). Because during the occlusion trial there was a decrease in firing rates but no change in EMG amplitude or force, this suggests that a recruitment vs. rate-coding strategy was used to maintain force across contractions.

A larger decrease in paretic motor unit firing rates could be attributed to a larger relative decrease in oxygen saturation due to occlusion compared with controls. However, in our study, individuals with stroke had a decreased rate and relative magnitude of change in oxygen saturation in response to occlusion compared with controls. Group differences in the oxygen saturation response due to occlusion are likely due to paretic muscle becoming less oxidative and/or having less mitochondrial content. Both of these changes are known secondary consequences of stroke, primarily due to limb disuse (Billinger et al. 2012; De Deyne et al. 2004; Landin et al. 1977). Because current methodology limits the absolute measurement of oxygen saturation at baseline, future studies need to examine absolute levels of oxygen saturation during transient and chronic baseline conditions and the relationship to motor unit firing behavior.

Previous studies in other neurological conditions, such as spinal cord injury, have demonstrated that group III/IV pathways are hyperexcitable. Although these studies focused on the flexor withdrawal and activation of flexor muscles, they demonstrate an exaggerated motor response for a given sensory input (Hornby et al. 2004; Schmit et al. 2002, 2003). In our study, we show that even though the mechanics of the stimulus were the same (same compression pressure), the inhibitory response was larger (greater decline in mean firing) in people with stroke compared with controls. Likewise, in congestive heart failure patients, the same input of muscle contraction and stimulation of group III/IV results in an overexaggerated vascular response (Amann et al. 2014). It is possible, therefore, that altered group III/IV muscle afferent activity in our stroke participants resulted in inhibition of motoneuron output. In the present study, with respect to controls, we did not find a large inhibitory effect on control motor unit firing rates (Fig. 3) during performance of brief knee extension contractions, although there was a trend for a shift in recruitment thresholds within the occlusion trial (Fig. 4). Certainly, under different exercise conditions, such as whole body and limb exercise in healthy controls, group III/IV pathways have inhibitory effects on motor output (Amann et al. 2011; Taylor et al. 2016). This difference may be because our protocol involved transient ischemia and brief, submaximal contractions in a single muscle group.

Although ischemia can also alter the exercising muscle contractile properties, our data support the idea of a change in central mechanisms. From our data, we demonstrate that twitch amplitude did not differ between stroke and control, suggesting that the observed changes in motor unit firing were not due to changes in muscle contractile properties. It may have been predicted that the inhibition would have affected other motoneuron properties such as a shift in recruitment threshold. However, no systematic shift in recruitment thresholds was detected in this data set. We did, however, observe a decrease in firing rates at recruitment for both groups during the occlusion condition, which supports an inhibitory effect on motoneuron firing properties. Because the transient occlusion was performed on the test leg and not remotely, we cannot say whether the effect was a spinal response or more centralized effect. Finally, our study used transient occlusion of blood flow to manipulate the activation of group III/IV afferents. Although transient, the inhibitory response provided insight into what individuals could experience during intense exercise or activity.

Implications for Motor Performance

In our study, we demonstrate that transient group III/IV feedback mechanisms also play a role in impaired motor unit firing in the paretic muscle. After stroke, there is an impaired ability to generate appropriate forces, resulting in functional consequences such as diminished capacity to make accurate movements, increased variability in movement output (Blennerhassett et al. 2008; Chang et al. 2013; Kuhnen et al. 2015; Lodha et al. 2010), and decreased task endurance. Our results corroborate previous studies demonstrating impaired motor unit firing and recruitment after stroke (Chou et al. 2013; Gemperline et al. 1995) and extend previous findings by demonstrating how transient sensory inputs can potentially contribute to force generating deficits during contractions. We show that individuals with the largest reductions in motor unit firing rates were also generating smaller target torques; in other words, they were weaker at baseline. Future studies are intended to examine the effects of ischemia on other aspects of force regulation such as force steadiness.

In this study we show the effect of transient total occlusion on paretic motor unit firing behavior during a submaximal task. In daily life, many activities of daily living are performed during submaximal force conditions in which there is reciprocal agonist-antagonist activation of muscle groups; this may lessen the ischemia-related inhibition. Indeed, during the condition with no occlusion, we found no decrease in firing rates during a sustained 20% contraction for either group. However, ischemia-related inhibition of paretic motor units might be relevant during sustained high-intensity contractions, such as carrying a heavy grocery bag or exercising against a heavy load. Because of the limited total range in the change oxygen saturation in response to full occlusion poststroke and the likelihood of increased excitability in the group III/IV polysynaptic spinal pathways, the relationship between ischemia and motor unit firing rates may not be linear. Related to this, we found that resting twitch relaxation time increased similarly for both the stroke and control groups in response to ischemia, possibly reflecting support of the “muscle wisdom” hypothesis (Bigland-Ritchie et al. 1983; Marsden et al. 1983), which proposes the prolonged force relaxation of the muscle is a response to decreases in motor unit firing rates so as to maintain force levels. Future studies are intended to address how the absolute magnitude of blood flow to an exercising muscle tracks with motor unit firing behavior in chronic stroke and if therapies that increase blood flow during exercise improve motor performance.

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DISCLAIMERS

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.A.M. performed experiments; S.A.M. and A.S.H. analyzed data; S.A.M., F.N., T.O., S.K.H., B.D.S., and A.S.H. interpreted results of experiments; S.A.M. prepared figures; S.A.M., T.O., and A.S.H. drafted manuscript; S.A.M., F.N., D.F., T.O., M.J.D., S.K.H., B.D.S., and A.S.H. edited and revised manuscript; S.A.M., F.N., D.F., T.O., M.J.D., S.K.H., B.D.S., and A.S.H. approved final version of manuscript.

AUTHOR NOTES

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