Stroke Increases Ischemia-related Decreases in Motor Unit Discharge Rates

Spencer A. Murphy[1], Francesco Negro[2], Dario Farina[3], Tanya Onushko[1], Matthew Durand[3], Sandra K. Hunter[4], Brian D. Schmit[1], and Allison Hyngstrom[4]

1 Department of Biomedical Engineering, Marquette University and the Medical College of Wisconsin, Milwaukee WI USA

[2] Department of Clinical and Experimental Sciences, University of Brescia, Brescia Italy

[3] Department of Bioengineering, Imperial College of London, London, England

[4] Department of Physical Medicine and Rehabilitation, Medical College of Wisconsin, Milwaukee WI, USA

[5] Department of Physical Therapy, Marquette University Milwaukee WI USA

Running Head: Ischemia and Paretic Motor Unit Firing Behavior

Correspondence to:

Allison Hyngstrom, Department of Physical Therapy, Marquette University Milwaukee WI 53201

Allison.hyngstrom@marquette.edu

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 sub-maximal isometric contractions. Ten chronic stroke survivors (>1 year post stroke) and 10 controls participated. During conditions of whole leg occlusion, the discharge timings of motor units were identified from decomposition of high-density surface EMG signals during repeated sub-maximal 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%; $p<0.001$). 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 %MVC 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 Nm vs 25.75 ± 6.78 Nm; control: 38.80±13.23 Nm vs 30.14±9.64 Nm). Controls had a greater exponential decline (lower time constant) in oxygen saturation blood flow as compared to 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 post stroke.

NEW AND NOTEWORTHY

Hyperexcitable inhibitory sensory pathways sensitive to ischemia may play a role in deficient motor unit activation post stroke. Using high-density surface EMG 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 to controls. These findings are impactful to neurophysiologists and clinicians because they implicate a novel mechanism of force generating impairment post stroke which likely exacerbates baseline weakness.

Key Words: motor unit firing behavior, stroke, ischemia, EMG

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 and resulting muscle activation and force generation is a combination of.excitatory and inhibitory synaptic inputs as well as baseline intrinsic excitability(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 (Gemperline, Allen et al. 1995, Chang, Francisco et al. 2013, Chou, Palmer et al. 2013, Li, Holobar et al. 2014, McNulty, Lin et al. 2014, Mottram, Heckman et al. 2014, Pollock, Ivanova et al. 2014) that 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, Palmer et al. 2013). In addition, recent studies have shown decreased ability to increase the magnitude of paretic global surface EMG during sub-maximal fatiguing contractions (Rybar, Walker 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 post stroke (Foltys, Krings et al. 2003, Schwerin, Dewald et al. 2008, Jang, Kim et al. 2017, Peters, Dunning et al. 2017). 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 post stroke.

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, Amann et al. 2016). In other neurological conditions, such as spinal cord injury, this pathway is hyperexcitable, (Schmit, Benz et al. 2002, Schmit, Hornby et al. 2003, Wu, Hornby et al. 2006, Theiss, Hornby et al. 2011) presumably due to decreased regulation by supraspinal centers. Understanding how activation of this pathway impacts motor output post stroke is important as 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 post stroke due to impaired blood flow to the paretic muscle. Several studies showed that individuals with stroke have impaired peripheral large and small conduit regulation of blood flow. First, resting blood flow in the femoral artery is lower in the paretic leg (Billinger, Gajewski et al. 2009, Sherk, Sherk et al. 2015) and there is an impaired flow mediated dilation response compared with the non-paretic leg and with individuals without stroke even when normalized to muscle mass and strength (Ivey, Gardner et al. 2004, Durand, Murphy et al. 2015). Moreover, during volitional contractions, the hyperemic response to contractions at various load levels is blunted compared to those without stroke (Durand, Murphy et al. 2015). Important to function, the paretic leg hyperemic response to volitional contractions has been shown to be positively correlated with clinical measures of function (Durand, Murphy et al. 2015). In summary, these studies indicate that there is impaired blood flow to resting and exercising muscles in the paretic leg that leads 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 post stroke, as seen with the group Ia afferents and resultant spasticity(Li 2017). Hidler and Schmit 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 (Hidler and Schmit 2004). Others have shown increased stroke-related reflex responses through cold and pain stimuli and increased central hypersensitivity in pain pathways (Soo Hoo, Paul 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, Venturelli et al. 2014) and motor performance is improved in healthy individuals when mechanosensitive group III/IV pathways are pharmacologically blocked (Amann, Blain 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. Here we quantified the effects of whole leg ischemia on paretic vastus lateralis motor unit firing rates during sub-maximal 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 consent before participation in the study, and procedures were approved by the Medical College of Wisconsin Institutional Review Board (PRO190103). Ten subjects with chronic hemiparetic stroke (8 male, 2 female, 58.9 ± 9.4 years) and ten age matched neurologically intact subjects (8 male, 2 female, 60.2 ± 9.5 years) participated in the study. Stroke subject inclusion criteria were: single, unilateral stroke (obtained through verbal communication from the physician and consistent with neurological physical examination); able to ambulate at least 30 feet (with or without an assistive device); ≥ 6 months post stroke. Stroke subject exclusion criteria included: brainstem stroke; any uncontrolled medical condition; contractures of any lower extremity joints; inability to follow 2-3 step commands. Table 1 reports the subject characteristics.

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 2048 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 electromyograms (EMGs) were obtained using a 64 channel 2-D 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 sterilizing the subject's skin with an alcohol swab and rubbing to remove superficial dead skin. The signals for each channel were differentially amplified between 1000 and 5000 V/V (subject dependent) and band-pass filtered between 10 and 500 Hz using the EMG-USB2+ amplifier. The signals were sampled at 2048 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 Inc., 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 subject sitting still and upright in the Biodex chair for 5 min prior to onset of any experimental procedures. To control for inter-subject variability,the NIRS measurements were normalized to the mean of the baseline period and collected throughout the experimental protocol.

Experimental Protocol

All control subjects performed the protocol with their right leg (dominant per subject report), and all stroke subjects 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. Subjects were given verbal and visual feedback. After at least a 5-min rest period following the final MVC, subjects performed a sub-maximal isometric ramp and hold torque tracking protocol. For the ramp and hold protocol, the subjects 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 subject indicating the torque produced by the knee extensor muscles. The trapezoid was 18 s in duration consisting of a 4s rising phase from rest, a 10s hold phase at 20% MVC, and a 4s 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 occluding blood flow. 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 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, Wales) 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 mA to 500 mA) was set at 20% above the level required to produce a maximal resting twitch amplitude that caused a supramaximal stimulation.

Data Processing and Statistical Analysis

Torque

Torque signals were zero phased low-pass filtered at 15 Hz using a 2nd order Butterworth filter prior to analysis and processed using Matlab (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 (Negro, Muceli 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 greater than 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 to the baseline noise. 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 lowpass filtered with a unit area Hanning window of 1-s duration. Motor units were identified applying the decomposition algorithm on six individual ramp and hold trials. The trials were concatenated from the original recordings with a resting segment of four seconds 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, Negro et al. 2017) .

The instantaneous firing rates of individual motor units were calculated as the inverse of the inter-spike interval. The firing rate at recruitment and derecruitment were defined as the mean of the first and last three inter-spike intervals. 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 a motor unit continuously fired with the final contraction that a motor unit continuously fired.

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 O_2 saturation were determined using Matlab's Curve Fitting Toolbox. The time constant ($\tau = \frac{1}{\lambda}$) of the exponential decay model $\left(N = N_0 e^{-\lambda t}\right)$ 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 O2 during the occlusion protocol. This was accomplished using firing rate during the last contraction a motor unit fired as a percent of the first contraction that a motor unit fired versus the change in O_2 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 non-occlusion conditions: firing rate during the hold phase, recruitment firing rate, derecruitment firing rate, recruitment threshold, and derecruitment threshold. The between group variables were 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 subjects for the exponential decay time constant and the mean slope value for the linear regression for change in firing rate per change in muscle O_2 saturation. All statistical tests were performed using an alpha level of 0.05 for significance. Data are reported as the mean ± standard deviation.

RESULTS

Mean Firing Rates

During the occlusion condition, there was a significant main effect of CONTRACTION as firing rates were lower for the final CONTRACTION (7.89 ± 1.93 pps) compared to 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 (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 as compared to controls ($p < 0.001$, See Fig. 2 single subject example, 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 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 sub-maximal 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 to the controls with occlusion. Finally, with stroke and control data combined, the percent decline in firing rates was positively correlated with net torque generated ($r^2= 0.262$, $p=0.30$). Whereby, the smaller the toque value generated (which reflects a smaller MVC value), the larger the relative decline in mean firing rates. There was a trend towards a positive correlation between the change in firing rate and the change in MVC, in the individuals with stroke, but it was not significant ($r^2=0.325$, $p = 0.85$).

In the condition without occlusion, there was 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 no occlusion condition.

On an individual level (Table 2), 7/10 individuals with stroke had lower mean firing rates (<8 pps) as compared to 2/8 controls. In addition, 8/10 individuals with stroke had absolute torque recruitment thresholds less than 20 vs 4/8 controls.

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.

In the no occlusion condition, 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 11.41 ± 4.37 %MVC) cycles. 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 versus control for the first contraction (stroke = 39.99%MVC;control=57.11%MVC) and the last contraction (stroke=43.19;control = 50.97).

Recruitment Firing Rates.

During occlusion, there was a significant main effect of CONTRACTION for recruitment firing rates as firing rates decreased in the final cycle (6.96 ± 1.85 pps) compared to 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 as control recruitment firing rates (7.95 ± 2.09 pps) were higher than stroke (6.73 ± 1.79 pps, p = 0.009),

but there was no main effect of CONTRACTION (p = 0.62) on recruitment firing rates as 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 to 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 (6.98 ± 1.66 pps) derecruitment firing rates higher than stroke (6.56 ± 2.60 pps) derecruitment firing rates. 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=6.99 ± 1.90 pps) cycle. 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 also observed (p = 0.57) because baseline (13.48 ± 5.42 %MVC) was similar to the final (13.34 ± 5.04 %MVC) cycle. 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 no occlusion 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 (13.27 ± 4.45 %MVC) cycles. There was also no interaction effect of CONTRACTION and GROUP ($p = 0.10$).

Local Muscle Oxygen Saturation

Figure 4 shows the fitted exponential decays for each individual subject's local oxygen saturation during occlusion. Individuals with stroke had a greater time constant for exponential decay of local muscle oxygen consumption compared to control subjects (22.90 ± 10.26 min vs. 5.46 ± 4.09 min, $p < 0.001$) during ischemia (Fig. 5). Stroke time constants ranged from 9.2 min to 35.6 min, and control time constants ranged from 0.4 min 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. 6); further, the average slope value of the linear regression line was significantly more negative for stroke than for control ($p < 0.001$, Fig. 7). Table 3 includes the mean relative change in oxygen saturation for each person. At an individual level, the change in firing rate was correlated with the relative change in oxygen saturation ($r^2 = 0.290$, $p = 0.021$).

Maximum Voluntary Contractions (MVCs)

Baseline MVCs were significantly lower for stroke compared to controls (123.21 ± 62.32 Nm 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 Nm to 25.65 ± 6.40 Nm) and control (38.80 ± 12.43 Nm 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) as both stroke (125.97 ± 35.61 ms to 165.42 ± 50.54 ms) and control (104.92 ± 43.11 ms 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

The magnitude of sEMG for the first cycles of the occlusion and no occlusion protocols were similar for both the stroke survivors (95.19 ± 34.00 μV vs 99.08 ± 28.76 μV, p = 0.80) and controls (123.06 ± 65.08 μV vs 121.08 ± 59.62 μV, p = 0.95).

DISCUSSION

In this study, we demonstrate that paretic motor unit firing behavior is more sensitive to inhibitory effects of transient occlusion 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 sub-maximal 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 MU 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 to 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 no occlusion 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 no occlusion condition seen here, are in contrast to other studies which have shown differences between controls and individuals with stroke in firing rates during sub-maximal contractions(Gemperline, Allen et al. 1995, Mottram, Heckman et al. 2014). On an individual level, the majority of the individuals with stroke did have lower firing rates than the controls (7/10) and where there were similar torques being generated the paretic motor units were firing less than controls (Table 2). Our results may differ from others due to key differences in the task: (1) relative effort vs matched target torque, (2) muscle groups (upper extremity vs lower extremity musces) and (3) contraction type (triangle ramp vs ramp and hold). Finally, we could not fully evaluate motor unit firing rate saturation as we did not perform multiple target torque conditions, but this will be addressed in future studies.

Because the occlusion condition was performed after the no occlusion and the occlusion + MVC protocol, it is possible that the decline in firing rates was due to increased fatigability in the stroke survivors (Knorr, Ivanova et al. 2011, Hyngstrom, Onushko et al. 2012). However, individuals were given at least 5 minutes rest between protocols and the respective magnitudes of the global surface EMG from the first cycle of the two protocols in people with stroke was similar indicating similar neural drive for a given target torque. If there was substantial muscle fatigue, the magnitude of

EMG would be expected to be greater in order to meet the force demands of the task (Enoka and Stuart 1992, Garland, Enoka et al. 1994).

A larger decrease in paretic motor unit firing rates could be attributed a larger relative decrease in oxygen saturation due to occlusion as compared to controls. However, in our study, individuals with stroke had a decreased rate and relative magnitude of change in oxygen saturation in response to occlusion as compared to 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.(Landin, Hagenfeldt et al. 1977, De Deyne, Hafer-Macko et al. 2004, Billinger, Coughenour et al. 2012) Because current methodology limits the absolute measurement of oxygen saturation at baseline, future studies will examine absolute measures 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 (Schmit, Benz et al. 2002, Schmit, Hornby et al. 2003, Hornby, Tysseling-Mattiace et al. 2004). In our study, we show that even though the mechanics of the stimulus was 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, Venturelli 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. Here, with respect to controls, we did not find a large inhibitory effect on control motor unit firing rates (Fig. 3) performing brief knee extension contractions. 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, Blain et al. 2011, Taylor, Amann 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 from this data set. Effects on recruitment threshold will be more fully explored in future studies that involve multiple load levels during contractions. We did see 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 conditions to strongly manipulate the group III/IV afferents. Although transient, the inhibitory response provided insight into what individuals may be experiencing during exercise or strenuous physical activity. Future studies will examine the effects of levels of ischemia and duration on motor performance.

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, Carey et al. 2008, Lodha, Naik et al. 2010, Chang, Francisco et al. 2013, Kuhnen, Rybar et al. 2015) and decreased task endurance. Our results corroborate previous studies demonstrating impaired motor unit firing and recruitment after stroke (Gemperline, Allen et al. 1995, Chou, Palmer et al. 2013) 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 were weaker at baseline. Future studies will examine the effects of ischemia on other aspects of force regulation such as force steadiness.

Here we show the effect of transient total occlusion on paretic motor unit firing behavior during a sub-maximal task. In daily life, many activities of daily living are performed under sub-maximal force conditions in which there is reciprocal activation of muscle groups- this may lessen the ischemia related inhibition. Indeed, during conditions 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 post stroke 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. This may reflect “muscle wisdom” in which there is prolonged contraction of the muscle in response to decreasing firing rates to maintain force levels. Future studies will address how the absolute magnitude of blow 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.

FIGURE LEGENDS

Table 1. Characteristics of all Stroke subjects.

Figure 1. Experimental protocol. A) The subject first performed 3-5 baseline maximum voluntary contractions (MVC) with the knee extensors with a one-minute break between contractions. B) The subject then performed 6 “ramp and hold” isometric knee extensor contractions, holding at 20% MVC, with a one-minute break between cycles. C) The subject performed 6 MVCs with a one-minute break between MVCs while the test leg was occluded. After each MVC when the muscle was fully relaxed, an electrical stimulation was applied to the quadriceps to elicit a resting twitch response. D) The subject then repeated the “ramp and hold” protocol during whole leg occlusion. A five-minute break is provided between each separate part of the protocol. NIRS and sEMG of the quadriceps muscle were recorded throughout the experiment.

Figure 2. Single subject examples of individual motor unit firing rates during the occlusion protocol for a control and stroke subject. The gray trace shows the filtered firing rates using a 1000 ms Hanning window. The bold squares represent the average firing rate of the motor unit for the ramp and hold cycle. A linear fit of the average firing rates for each cycle is represented using the dashed gray line. The black trace represents the torque. Consistent with the group effect, note the greater decline in motor unit firing rates (grey trace) with the stroke subject as compared to the control example.

Figure 3. Mean firing rates during the 20% ramp and hold occlusion protocol for the stroke and control subjects. Individuals with stroke had a larger decline in firing rates as compared to controls (CONTRACTION*GROUP effect, $p<0.001$).

Figure 4. Local oxygen saturation of the rectus femoris before the beginning of each ramp and hold cycle at 20% MVC for stroke and control. Minute zero represents the muscle oxygen saturation before the first ramp and hold cycle, and minute five represents the muscle oxygen saturation before the final cycle of the ramp and hold cycle at 20% MVC. A model of exponential decay was fitted to the data.

Figure 5. Mean time constant for the exponential decay of the local muscle oxygen saturation of the rectus femoris during occlusion at 20% MVC ramp and hold cycle for the stroke and control populations.

Figure 6. Linear regressions of each motor unit firing rate as a percentage of the First Cycle versus the local muscle oxygen saturation for all stroke and control recorded motor units during the 20% MVC occlusion protocol. Squares are the group mean for the firing rate and local muscle oxygen saturation.

Figure 7. The average slope value of the linear regression lines for the motor unit firing rate versus the oxygen saturation between stroke and control during the 20% MVC occlusion protocol.

GRANTS: Work from this paper was funded by the National Institute for Neurological Disorders and Stroke for the Nation Institutes for Health, award number: R21 NS088818. Spencer Murphy was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, award number: TL1TR001437**.** The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Francesco Negro has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 702491 (NeuralCon).

AUTHOR CONTRIBUTIONS: SM, BS, and AH conceived and designed research; SM performed experiments; SM analyzed data; SM, FN, SKH, MD, BS, and AH. interpreted results of experiments; SM prepared figures; SM, TO, and AH drafted manuscript; SM, FN, SKH, MD, BS, TO and AH edited and revised manuscript; SM, FN, SKH, MD, BS, TO and AH approved final version of manuscript.

ACKNOWLEDGMENTS

None.

REFERENCES

Amann, M., G. M. Blain, L. T. Proctor, J. J. Sebranek, D. F. Pegelow and J. A. Dempsey (2011). "Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans." J Physiol **589**(Pt 21): 5299-5309.

Amann, M., M. Venturelli, S. J. Ives, D. E. Morgan, B. Gmelch, M. A. Witman, H. Jonathan Groot, D. Walter Wray, J. Stehlik and R. S. Richardson (2014). "Group III/IV muscle afferents impair limb blood in patients with chronic heart failure." Int J Cardiol **174**(2): 368-375.

Billinger, S. A., E. Coughenour, M. J. Mackay-Lyons and F. M. Ivey (2012). "Reduced cardiorespiratory fitness after stroke: biological consequences and exercise-induced adaptations." Stroke Res Treat **2012**: 959120.

Billinger, S. A., B. J. Gajewski, L. X. Guo and P. M. Kluding (2009). "Single limb exercise induces femoral artery remodeling and improves blood flow in the hemiparetic leg poststroke." Stroke **40**(9): 3086-3090.

Blennerhassett, J. M., L. M. Carey and T. A. Matyas (2008). "Clinical measures of handgrip limitation relate to impaired pinch grip force control after stroke." J Hand Ther **21**(3): 245-252; quiz 253.

Chang, S. H., G. E. Francisco, P. Zhou, W. Z. Rymer and S. Li (2013). "Spasticity, weakness, force variability, and sustained spontaneous motor unit discharges of resting spastic-paretic biceps brachii muscles in chronic stroke." Muscle Nerve **48**(1): 85-92.

Chou, L. W., J. A. Palmer, S. Binder-Macleod and C. A. Knight (2013). "Motor unit rate coding is severely impaired during forceful and fast muscular contractions in individuals post stroke." J Neurophysiol **109**(12): 2947-2954.

De Deyne, P. G., C. E. Hafer-Macko, F. M. Ivey, A. S. Ryan and R. F. Macko (2004). "Muscle molecular phenotype after stroke is associated with gait speed." Muscle Nerve **30**(2): 209-215.
Durand, M. J., S. A. Murphy, K. K. Schaefer, S. K. Hunter, B. D. Schmit, D. D. Gutterman and A. S. Hyngstrom (2015). "Impaired Hyperemic Response to Exercise Post Stroke." PLoS One **10**(12): e0144023.
Enoka, R. M. and D. G. Stuart (1992). "Neurobiology of muscle fatigue." J Appl Physiol **72**(5): 1631-1648.
Foltys, H., T. Krings, I. G. Meister, R. Sparing, B. Boroojerdi, A. Thron and R. Topper (2003). "Motor representation in patients rapidly recovering after stroke: a functional magnetic resonance imaging and transcranial magnetic stimulation study." Clin Neurophysiol **114**(12): 2404-2415.
Garland, S. J., R. M. Enoka, L. P. Serrano and G. A. Robinson (1994). "Behavior of motor units in human biceps brachii during a submaximal fatiguing contraction." J Appl Physiol (1985) **76**(6): 2411-2419.
Gemperline, J. J., S. Allen, D. Walk and W. Z. Rymer (1995). "Characteristics of motor unit discharge in subjects with hemiparesis." Muscle Nerve **18**(10): 1101-1114.
Heckman, C. J. and R. M. Enoka (2012). "Motor unit." Compr Physiol **2**(4): 2629-2682.
Hidler, J. M. and B. D. Schmit (2004). "Evidence for force-feedback inhibition in chronic stroke." IEEE Trans Neural Syst Rehabil Eng **12**(2): 166-176.
Hornby, T. G., V. M. Tysseling-Mattiace, E. N. Benz and B. D. Schmit (2004). "Contribution of muscle afferents to prolonged flexion withdrawal reflexes in human spinal cord injury." J Neurophysiol **92**(6): 3375-3384.
Hyngstrom, A. S., T. Onushko, R. P. Heitz, A. Rutkowski, S. K. Hunter and B. D. Schmit (2012). "Stroke-related changes in neuromuscular fatigue of the hip flexors and functional implications." Am J Phys Med Rehabil **91**(1): 33-42.
Ivey, F. M., A. W. Gardner, C. L. Dobrovolny and R. F. Macko (2004). "Unilateral impairment of leg blood flow in chronic stroke patients." Cerebrovasc Dis **18**(4): 283-289.
Jang, S. H., D. H. Kim, S. H. Kim and J. P. Seo (2017). "The relation between the motor evoked potential and diffusion tensor tractography for the corticospinal tract in chronic hemiparetic patients with cerebral infarct." Somatosens Mot Res **34**(2): 134-138.
Knorr, S., T. D. Ivanova, T. J. Doherty, J. A. Campbell and S. J. Garland (2011). "The origins of neuromuscular fatigue post-stroke." Exp Brain Res **214**(2): 303-315.
Kuhnen, H. R., M. M. Rybar, T. Onushko, R. E. Doyel, S. K. Hunter, B. D. Schmit and A. S. Hyngstrom (2015). "Stroke-related effects on maximal dynamic hip flexor fatigability and functional implications." Muscle Nerve **51**(3): 446-448.
Landin, S., L. Hagenfeldt, B. Saltin and J. Wahren (1977). "Muscle metabolism during exercise in hemiparetic patients." Clin Sci Mol Med **53**(3): 257-269.
Li, S. (2017). "Spasticity, Motor Recovery, and Neural Plasticity after Stroke." Front Neurol **8**: 120.
Li, X., A. Holobar, M. Gazzoni, R. Merletti, W. Z. Rymer and P. Zhou (2014). "Examination of Post-stroke Alteration in Motor Unit Firing Behavior Using High Density Surface EMG Decomposition." IEEE Trans Biomed Eng.
Lodha, N., S. K. Naik, S. A. Coombes and J. H. Cauraugh (2010). "Force control and degree of motor impairments in chronic stroke." Clin Neurophysiol **121**(11): 1952-1961.
Martinez-Valdes, E., F. Negro, C. M. Laine, D. Falla, F. Mayer and D. Farina (2017). "Tracking motor units longitudinally across experimental sessions with high-density surface electromyography." J Physiol **595**(5): 1479-1496.
Matthews, P. B. C. (1972). Mammalian Muscle Receptors and Their Central Actions. London, Arnold.
McNulty, P. A., G. Lin and C. G. Doust (2014). "Single motor unit firing rate after stroke is higher on the less-affected side during stable low-level voluntary contractions." Front Hum Neurosci **8**: 518.
Mottram, C. J., C. J. Heckman, R. K. Powers, W. Z. Rymer and N. L. Suresh (2014). "Disturbances of motor unit rate modulation are prevalent in muscles of spastic-paretic stroke survivors." J Neurophysiol **111**(10): 2017-2028.
Negro, F., S. Muceli, A. M. Castronovo, A. Holobar and D. Farina (2016). "Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation." J Neural Eng **13**(2): 026027.

Peters, H. T., K. Dunning, S. Belagaje, B. M. Kissela, J. Ying, J. Laine and S. J. Page (2017). "Navigated Transcranial Magnetic Stimulation: A Biologically Based Assay of Lower Extremity Impairment and Gait Velocity." Neural Plast **2017**: 6971206.
Pollock, C. L., T. D. Ivanova, M. A. Hunt and S. J. Garland (2014). "Motor unit recruitment and firing rate in medial gastrocnemius muscles during external perturbations in standing in humans." J Neurophysiol **112**(7): 1678-1684.
Rybar, M., E. Walker, H. Kuhnen, D. Ouellette, R. Berrios, S. K. Hunter and A. S. Hyngstrom (2014). " The stroke-related effects of hip flexion fatigue on over ground walking." Gait and Posture **39**(4): 1103-1108.
Schmit, B. D., E. N. Benz and W. Z. Rymer (2002). "Reflex mechanisms for motor impairment in spinal cord injury." Adv Exp Med Biol **508**: 315-323.
Schmit, B. D., T. G. Hornby, V. M. Tysseling-Mattiace and E. N. Benz (2003). "Absence of local sign withdrawal in chronic human spinal cord injury." J Neurophysiol **90**(5): 3232-3241.
Schwerin, S., J. P. Dewald, M. Haztl, S. Jovanovich, M. Nickeas and C. MacKinnon (2008). "Ipsilateral versus contralateral cortical motor projections to a shoulder adductor in chronic hemiparetic stroke: implications for the expression of arm synergies." Exp Brain Res **185**(3): 509-519.
Sherk, K. A., V. D. Sherk, M. A. Anderson, D. A. Bemben and M. G. Bemben (2015). "Lower limb neuromuscular function and blood flow characteristics in AFO-using survivors of stroke." J Geriatr Phys Ther **38**(2): 56-61.
Soo Hoo, J., T. Paul, J. Chae and R. D. Wilson (2013). "Central hypersensitivity in chronic hemiplegic shoulder pain." Am J Phys Med Rehabil **92**(1): 1-9; quiz 10-13.
Taylor, J. L., M. Amann, J. Duchateau, R. Meeusen and C. L. Rice (2016). "Neural Contributions to Muscle Fatigue: From the Brain to the Muscle and Back Again." Med Sci Sports Exerc **48**(11): 2294-2306.
Theiss, R. D., T. G. Hornby, W. Z. Rymer and B. D. Schmit (2011). "Riluzole decreases flexion withdrawal reflex but not voluntary ankle torque in human chronic spinal cord injury." J Neurophysiol **105**(6): 2781-2790.
Wu, M., T. G. Hornby, J. H. Kahn and B. D. Schmit (2006). "Flexor reflex responses triggered by imposed knee extension in chronic human spinal cord injury." Exp Brain Res **168**(4): 566-576.

Table 1

Table 2

Subject	Firing Rate (PPS)		Recruitment Threshold (Nm)		Recruitment Threshold (%MVC)		MVC (Nm)	Torque (Nm)	
	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

Table 3

Subject	%Δ Motor Unit Firing Rate	%Δ O^2 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

Figure 1

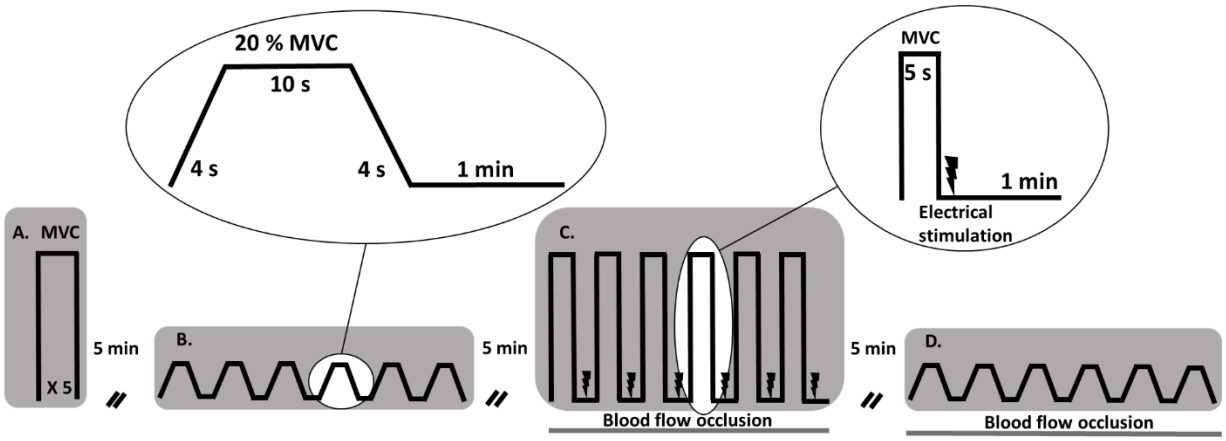
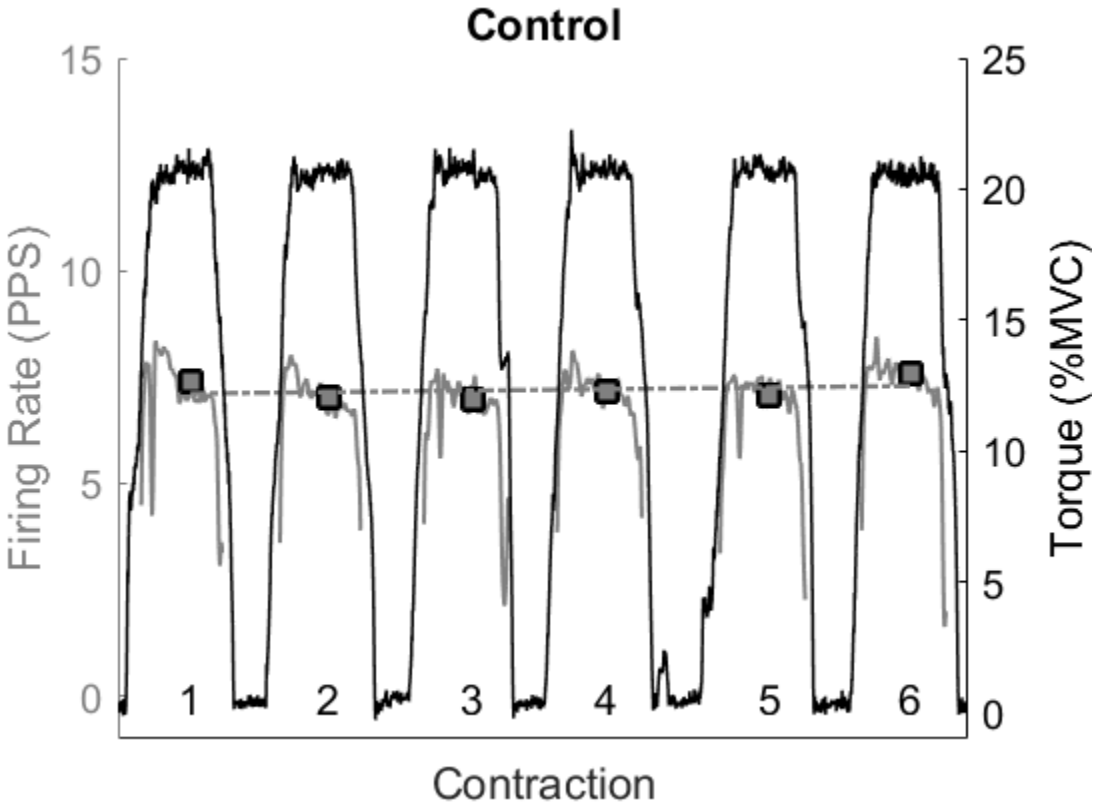

20 % MVC
10 s
4 s
4 s
1 min
MVC
5 s
1 min
Electrical stimulation
A. MVC
X 5
5 min
B.
5 min
C.
5 min
D.
Blood flow occlusion
Blood flow occlusion

Figure 2



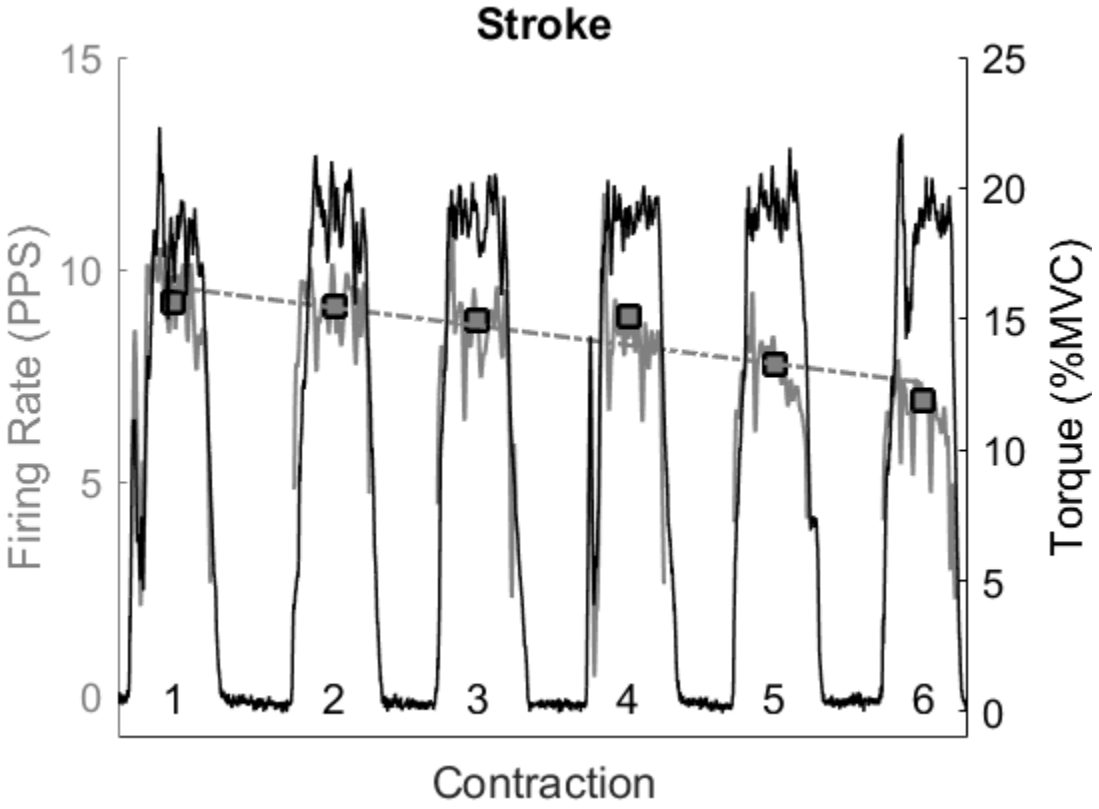


Figure 3

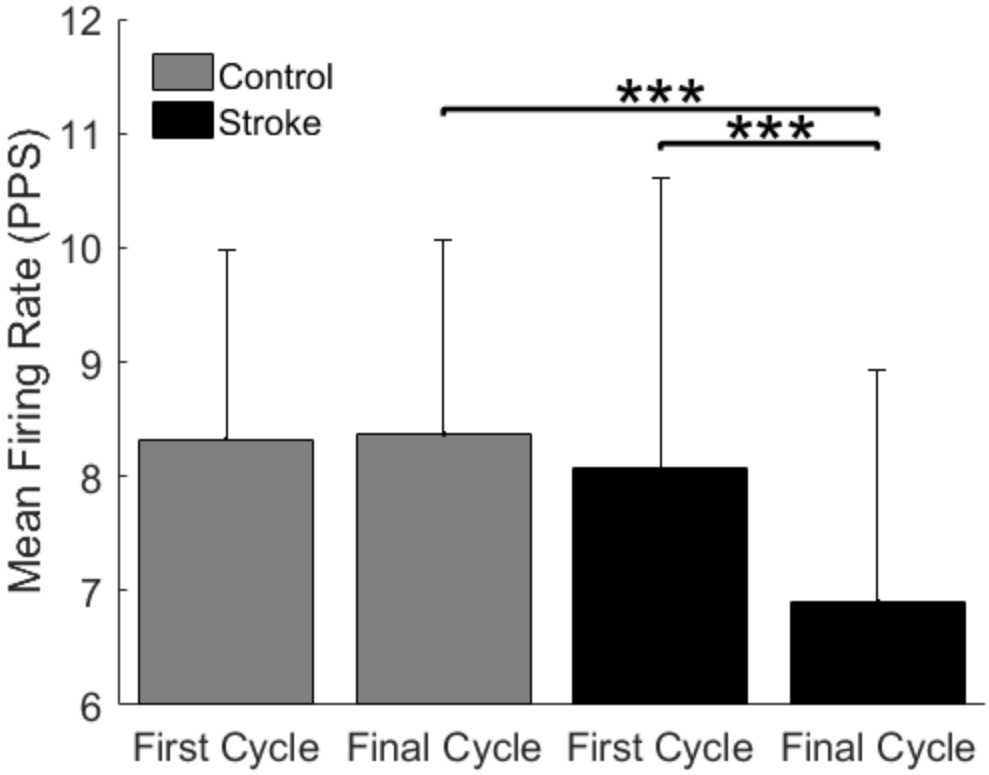

12
11
10
9
8
7
6
Mean Firing Rate (PPS)
Control
Stroke
***
***
First Cycle
Final Cycle
First Cycle
Final Cycle

Figure 4

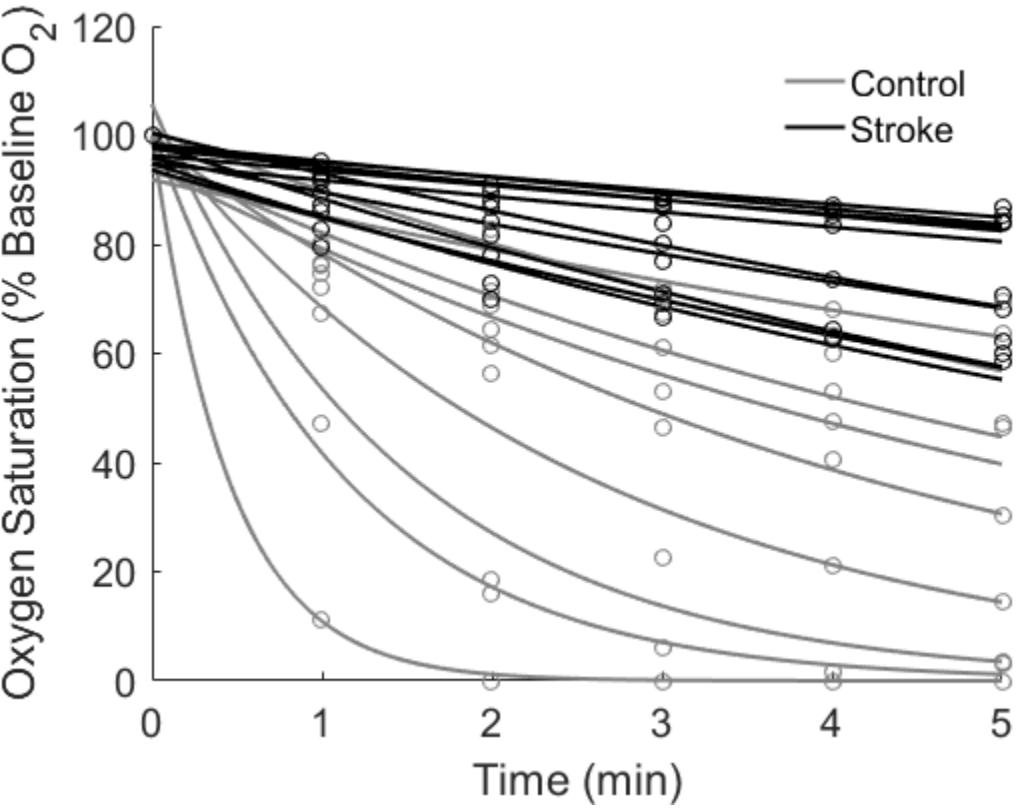

Oxygen Saturation (% Baseline O_2)
120
100
80
60
40
20
0
0
1
2
3
4
5
Time (min)
Control
Stroke

Figure 5

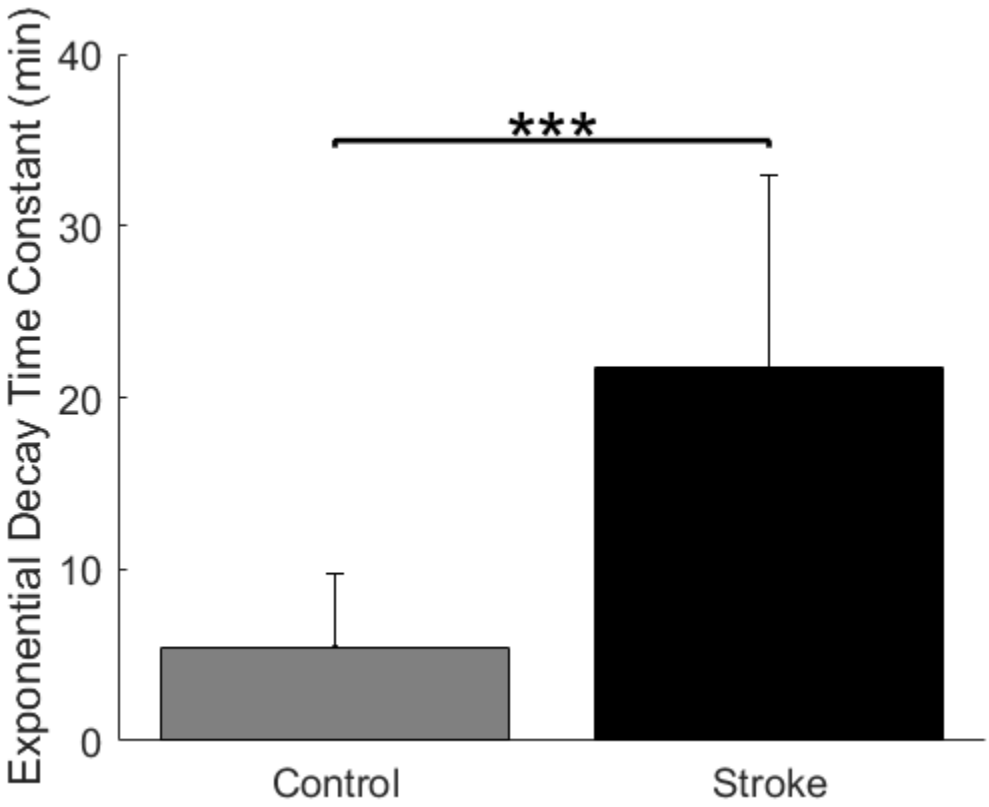

Exponential Decay Time Constant (min)
40
30
20
10
0
***
Control
Stroke

Figure 6

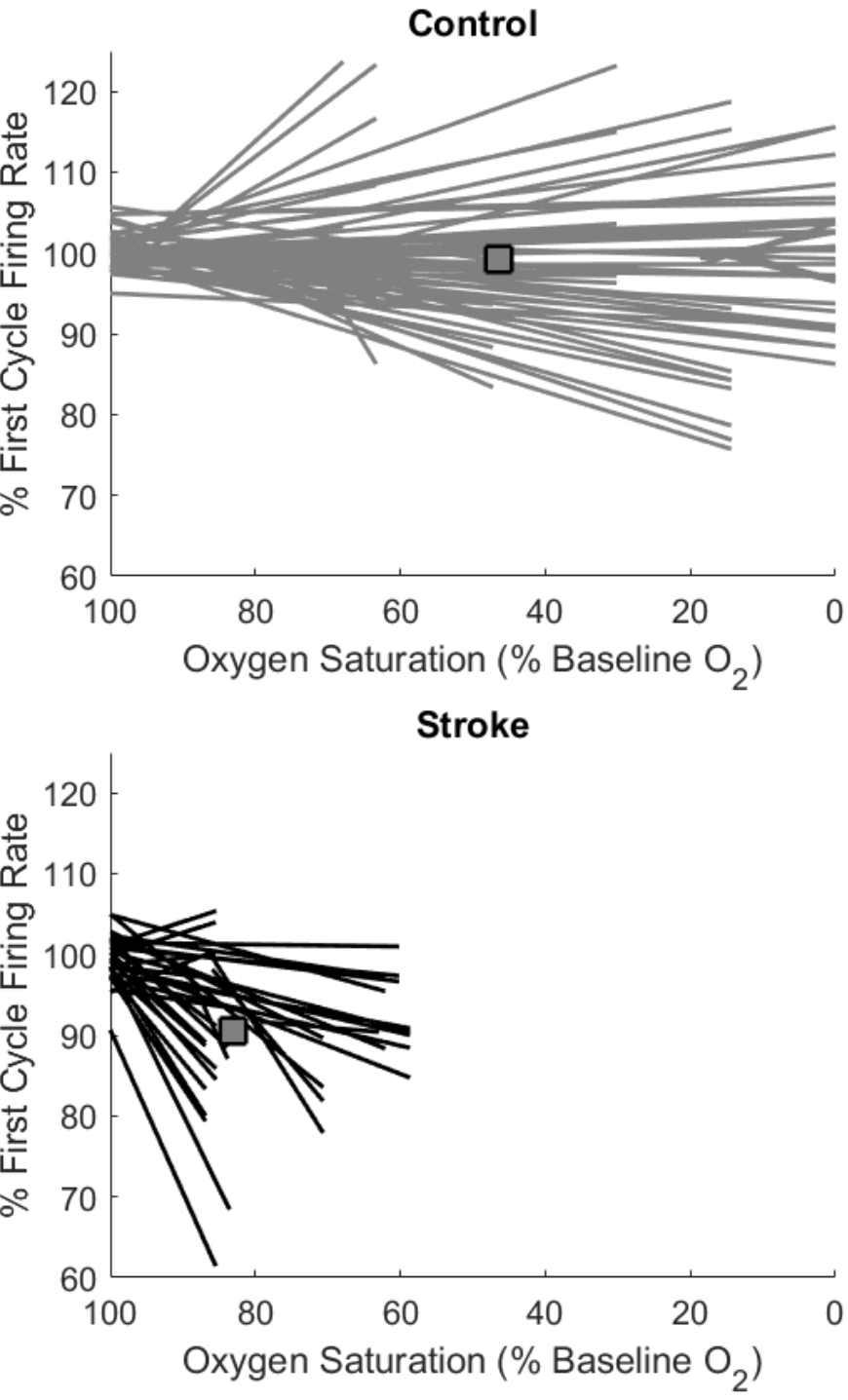

Control
% First Cycle Firing Rate
120
110
100
90
80
70
60
100
80
60
40
20
0
Oxygen Saturation (% Baseline O_2)
Stroke
% First Cycle Firing Rate
120
110
100
90
80
70
60
100
80
60
40
20
0
Oxygen Saturation (% Baseline O_2)

Figure 7