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Age-related reductions in PICs

TITLE: Estimates of persistent inward currents are reduced in upper limb motor units of older adults

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ABSTRACT

Aging is a natural process that causes alterations in the neuromuscular system, which contribute to weakness and reduced quality of life. Reduced firing rates of individual motor units (MUs) likely contribute to weakness, but the mechanisms underlying reduced firing rates are not clear. Persistent inward currents (PICs) are crucial for the initiation, gain control, and maintenance of motoneuron firing, and are directly proportional to the level of monoaminergic input. Since the concentration of monoamines (i.e. serotonin and norepinephrine) are reduced with age, we sought to determine if estimates of PICs are reduced in older (>60 years old) compared to younger adults (<35 years old). We decomposed MU spike trains from high-density surface electromyography over the biceps brachii and triceps brachii during isometric ramp contractions to 20% of maximum. Estimates of PICs (i.e. ΔF) were computed using the paired MU analysis technique. Regardless of the muscle, peak firing rates of older adults were reduced by ~1.6 pulses per second (pps) ($P = 0.0292$), and ΔF was reduced by ~1.9 pps ($P < 0.0001$), compared to younger adults. We further found that age predicted ΔF in older adults ($P = 0.0261$), resulting in a reduction of ~1pps per decade, but there was no relationship in younger adults ($P = 0.9637$). These findings suggest that PICs are reduced in older adults, and, further, age is a significant predictor of estimates of PICs in older adults. Reduced PIC magnitude represents one plausible mechanism for reduced firing rates and weakness in older individuals.

Keywords (3-5): Aging, motor unit decomposition, persistent inward current, motoneuron, neuromodulation

51 KEY POINTS

- 52 • Persistent inward currents play an important role in the neural control of human
53 movement and are influenced by neuromodulation via monoamines originating in the
54 brainstem.
- 55 • During aging, motor unit firing rates are reduced, and there is deterioration of
56 brainstem nuclei, which may reduce persistent inward currents in alpha motoneurons.
- 57 • Here we show that estimates of persistent inward currents (ΔF) of both elbow flexor
58 and extensor motor units are reduced in older adults.
- 59 • Estimates of persistent inward currents have a negative relationship with age in the
60 older adults, but not young.
- 61 • This novel mechanism may play a role in alteration motor firing rates that occurs with
62 aging, which may have consequences for motor control.

INTRODUCTION

Aging is a natural process that causes alterations within the neuromuscular system, which can have severe consequences on health and quality of life in older adults (McNeil & Rice, 2018). Even in the absence of disease, there is age-related loss of muscle or lean mass (i.e. sarcopenia), and, perhaps more importantly, age-related loss of strength (i.e. dynapenia). Emerging evidence suggests that dynapenia is a significant contributor to quality of life in the elderly (Mitchell *et al.*, 2012). Indeed, biophysical properties of the muscle play a role in the reduced force generating capacity, but neural factors are likely to contribute as well.

There is a progressive loss in the number of motor units (MUs) with age (McNeil *et al.*, 2005), which comprise the muscle fibers and their parent motoneurons (Heckman & Enoka, 2012). As such, death of motoneurons is widely accepted as a precursor for many of the age-related adaptations in the nervous system (McNeil & Rice, 2018). Following death of motoneurons, the nervous system displays astounding plasticity, as evidenced by the reinnervation of orphaned muscle fibers by axonal sprouting (Gordon *et al.*, 2004), a process known as MU remodeling (Hepple & Rice, 2016). Since it is speculated that the loss of larger/faster motoneurons precedes the loss of smaller/slower type motoneurons (Kanda & Hashizume, 1989), reductions in MU firing rates are typically ascribed to this mechanism. Dalton *et al.* (Dalton *et al.*, 2010) previously showed that the firing rates of both biceps brachii (BIC) and triceps brachii (TRI) MUs are reduced across a wide range of contraction intensities in older, compared to young, adults. They suggested that the relatively higher proportional loss of higher threshold motoneurons may play a role in the age-related decline in firing rates, but alterations in the biophysical properties of the motoneurons are also likely to contribute.

Altered intrinsic motoneuron excitability may play a major role in age-related changes in motoneuron firing patterns. Although motoneurons were once believed to integrate their synaptic inputs passively, many studies have demonstrated that this integration is a highly

active process due to voltage-sensitive ion channels in their dendrites (Heckman *et al.*, 2008a; Heckman *et al.*, 2008b). Persistent inward currents (PICs) amplify and prolong excitatory synaptic input to the motoneuron (Lee & Heckman, 1998, 2000), which are the result voltage-gated slow activating L-type Ca^{2+} and fast activating persistent Na^{+} currents (Heckman *et al.*, 2008b). PICs are activated near threshold and can amplify synaptic currents by as much as 3-5 fold (Binder & Powers, 2001), and the level of PIC activation is highly dependent on the neuromodulatory drive from the monoaminergic system (i.e. serotonergic and noradrenergic drive) (Lee & Heckman, 1998, 2000). In addition, PICs are reduced with antagonist muscle afferent input (i.e. reciprocal inhibition), illustrating a role for inhibition in the control of PIC activity (Heckman *et al.*, 2008a; Powers *et al.*, 2012). Therefore, changes in levels of monoaminergic drive, intrinsic motoneuron excitability (i.e. monoamine receptor or ion channel function), and inhibition may alter MU firing patterns, as well as estimates of PICs, with age (Johnson *et al.*, 2017).

Furthermore, recent work has called for the investigation of PIC estimates in the aging neuromuscular system (Latella, 2021). The function of two primary monoaminergic nuclei in the brainstem, the raphe nuclei and locus coeruleus, have been shown to deteriorate with age (Shibata *et al.*, 2006; Pagano *et al.*, 2017). This deterioration likely results in reduced monoaminergic drive, and consequently reduced activation of PICs.

Fortuitously, recent advances in technology have enabled us to sample from large populations of concurrently active MUs, by using high-density surface electromyography (HD-sEMG) array electrodes and blind source separation algorithms (Holobar & Zazula, 2007; Negro *et al.*, 2016) with great success (Yavuz *et al.*, 2015; Del Vecchio *et al.*, 2018; Thompson *et al.*, 2018; Cogliati *et al.*, 2020; Del Vecchio *et al.*, 2020; Hassan *et al.*, 2020; Kim *et al.*, 2020; Martinez-Valdes *et al.*, 2020). This non-invasive technology has created an opportunity to further study the age-related alterations in the neuromuscular system by sampling from many concurrently active MUs. Using this technology allows us to gain better appreciation of the

population behaviour and provide more insights about the control of large portions of the motor pool, which was difficult to achieve with intramuscular EMG approaches.

In this study, we examined whether the MU firing characteristics in a large population of concurrently active MUs differed between a group of younger and older adults. More specifically, we compared MU firing patterns of the elbow flexor and extensors during triangular isometric contractions. We hypothesized that since previous work has shown MU firing rates are reduced in older adults, we would observe reductions in peak firing rates of both BIC and TRI MUs, as well as estimates of PIC magnitude (i.e. ΔF), in the older group. In addition, since healthy younger adults are unlikely to have an impairment in PIC function, we investigated the relationship between age and estimates of PICs in both groups separately. We hypothesized that there would be no relationship between age and estimates of PICs in the younger group, however there would be a negative relationship between age and estimates of PICs in the older group.

METHODS

Participants

In order to compare MU firing behaviour between healthy younger and older individuals, we recruited 10 younger (26 [2.87] years old, 3 female) and 10 older (67 [4.40] years old, 2 female) adults. At the time of testing, all participants were free of neurological, motor, and muscular impairments. All participants provided written informed consent in accordance with the Declaration of Helsinki, which was approved (STU00084502-CR0003) by the Institutional Review Board of Northwestern University.

Experimental Apparatus

The experimental apparatus, protocol, and data processing methods utilized is similar to those used in a previous experiment in our lab (Hassan *et al.*, 2020). Participants were secured in a Biodex chair (Biodex Medical Systems, Shirley, NY) with their dominant upper limb rigidly fixed to a six degree-of-freedom load cell with a fiberglass cast (JR3, Inc., Woodland, CA). The casted arm was positioned at a shoulder abduction angle of 75° and an elbow flexion angle of 90°. An illustration of the experimental setup is shown in Figure 1.

Figure 1: An illustration of the experimental setup. High-density surface electromyography (HD-sEMG) arrays were placed on the lateral head of the TRI and along the muscle belly of the BIC.

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161 High-density surface EMG (HD-sEMG) arrays (64 electrodes, 13x5, 8mm I.E.D.,
162 GR08MM1305, OT Bioelettronica, Inc., Turin, IT) were placed on the BIC and the lateral
163 head of the TRI on the casted limb. HD-sEMG data were sampled (2048 Hz), amplified
164 (x150), and band-pass filtered (10-500Hz) using a Quattrocentro signal amplifier (OT
165 Bioelettronica, Inc., Turin, IT). A reference electrode was placed on the acromion process of
166 the casted arm. Prior to collecting experimental data, real-time HD-sEMG recordings were
167 checked visually to ensure high signal-to-noise ratios.

168

169 Torque was sampled at 1024 Hz with a forearm-load cell interface. The limb segment lengths
170 and joint angles were converted into elbow flexion and extension torques using a Jacobian
171 based algorithm implemented by a custom MATLAB software (MathWorks). As EMG and
172 force/torque recordings were collected using separate computers, a 1 second TTL pulse was
173 transmitted to both computers for data alignment. Each trial was temporally synced offline
174 using cross-correlation of the TTL pulses.

175

176 *Experimental Protocol*

177 Participants were initially asked to produce their maximum voluntary torques (MVTs) in the
178 directions of elbow flexion or extension. A wall-mounted computer monitor placed directly
179 in front of participants provided real-time feedback of torque performance. MVT trials were
180 repeated until three trials in which the peak torque was within 10% of each other were
181 collected. If the last trial had the highest peak torque, an additional MVT trial was collected.
182 Participants were verbally encouraged during MVT trials to ensure peak torque performance
183 and were given adequate rest between trials to prevent muscle fatigue.

184

185 Each of the subsequent experimental trials consisted of three triangular isometric elbow
186 extension/flexion torque ramps, separated by 10 seconds of rest. Each ramp required

participants to increase torque (2% MVT/s) to 20% MVT over 10 seconds, and then decrease (-2% MVT/s) to 0% MVT over the next 10 seconds. During all trials, real-time torque feedback, as well as the desired experimental torque profile, were provided to the participants. In order to avoid fatigue, a minimum of 2 minutes of rest was given to participants between trials. Trials that did not exhibit a smooth increase of torque from 0% to 20% MVT and a smooth decrease of torque from 20% to 0% MVT over the desired timeframe were discarded, as were trials that display any sudden jerks in torque.

Data Analysis

MU Decomposition & Variables of Interest

After data acquisition, each EMG channel of the surface array was manually inspected and any channels with substantial artifacts, noise, or analog to digital saturation were removed. A convolutive blind-source separation algorithm (Negro *et al.*, 2016) with a silhouette threshold of 0.85 was used to decompose HD-sEMG into individual MU spike trains. All decomposed MU spike trains were visually inspected for each participant and trial through a custom-made graphical user interface in MATLAB. Any minor errors were corrected by local re-optimization of decomposition parameters in a manner similar to recent studies using the same blind source separation algorithm (Boccia *et al.*, 2019; Afsharipour *et al.*, 2020; Del Vecchio *et al.*, 2020; Hassan *et al.*, 2020; Martinez-Valdes *et al.*, 2020). Instantaneous MU firing rates were calculated as the inverse of the interspike intervals of each MU spike train and smoothed using a 2 second Hanning window using a custom-written MATLAB script.

Peak, total duration, and total range of smoothened decomposed MU firing rates were extracted through custom written MATLAB scripts for each muscle. MU range and duration during the ascending and descending phases of torque production were also extracted (see Figure 2).

214 *Estimating Persistent Inward Currents (PICs)*

215 The effects of persistent inward currents (PICs) on motoneuronal firing patterns can be
 216 appreciated through MU onset-offset hysteresis. The best approximation of this hysteresis,
 217 Delta F (ΔF), is calculated as the difference in the smoothed firing rate of a reporter (lower
 218 threshold) MU between the times of recruitment/derecruitment of a test (higher threshold)
 219 MU (Gorassini *et al.*, 2002). All ΔF values used throughout this study are “unitwise” mean
 220 values. That is, the average of ΔF values obtained for each test unit from all possible reporter
 221 units (see Figure 2). Criteria for inclusion of ΔF values from MU pairs were that the test MU
 222 was 1) recruited at least 1 second after the control unit to ensure full activation of PIC, and
 223 2) derecruited at least 1.5 seconds prior to the control MU to prevent ΔF overestimation (see
 224 Hassan et al. (Hassan *et al.*, 2020) for more details on utilizing ΔF for paired MU analysis).

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Figure 2: Top left shows the paired MU analysis method used to estimate persistent inward current magnitude, which quantifies the onset-offset hysteresis (i.e. ΔF) of a higher threshold (test unit; blue) MU with respect to a lower threshold (reporter unit; red). In the center, a typical extension torque trace (black line) from one younger participant is shown. Underneath the torque ramp, 3 out of the 12 decomposed MU firing patterns are shown. Each point indicates the instantaneous firing rate for each interstimulus interval, and the thick coloured lines indicate the smoothed firing rates of each MU. At the ends of the green and pink unit are vertical dotted lines that extend downward to the units below them (i.e. recruited at lower torque), which helps indicate the onset and offset of firing with respect to their reporter units. Individual ΔF values obtained from each reporter unit, and the mean of those values ($\Delta F_{\text{unitwise}}$) is shown for each test unit. The vertical black dotted line indicates peak torque in this ramp for ease of viewing the time point between ascending and descending duration.

227 Statistical Analysis

228 All data was imported into GraphPad (version 9.0.1 for Windows, GraphPad Software, San
229 Diego, California USA) where descriptive statistical analyses were performed. Hedge's g
230 effect sizes (ES) were calculated to provide a standardized effect for the mean differences
231 between the younger and older subjects for each variable. Mean and standard deviation

values for each variable reported are group means, which represent the average and error of the individual means computed for each participant.

We detail the effects of healthy aging on MU firing characteristics using linear mixed effects models. More specifically, we take into consideration all of our data points rather than averaging across them and basing our analysis on the mean within an individual trial or subject (Giboin *et al.*, 2020). All of these analysis were performed in R (R Core Team 2020, R Foundation for Statistical Computing, Vienna, AUT) using the lme4 package (Bates & Maechler, 2015) and significance was calculated using the lmerTest package (Kuznetsova *et al.*, 2017), which applies Satterthwaite's method to estimate degrees of freedom and generate p-values for mixed effects models by comparing the full model including the effect of interest against a null model excluding the effect of interest.

We used linear mixed effects models to determine if age group (categorical) and muscle were significant predictors for our MU variables. We employed age group (younger vs. older), muscle (BIC vs TRI), and their interaction as fixed effects. As random effects, we included a random intercept for each subject as well as a random slope accounting for the muscle within each subject. Effects estimated from the linear mixed effects models are presented as parameter estimates $\pm$ SE.

In order to avoid the bimodal distribution of ages created by our selective sampling of healthy younger and older adults, we used separate generalized linear mixed effects models (computed by the MuMIn R package (Barton, 2018) to identify significant relationships between ΔF and age in the younger and older groups. This was done to assess the degree to which the age of the subjects in each group could account for variance in ΔF . Specifically, we analyzed whether age was able to predict ΔF . For each age group, we included age (continuous variable), muscle (TRI vs. BIC), and their interaction as fixed effects. As random effects, we included a random intercept for each subject as well as a random slope accounting

260 for the muscle within each subject. Variance accounted for by the model is reported as
 261 conditional R^2 GLMM values, whereas variance accounted for by only the fixed effects is
 262 reported as marginal R^2 GLMM values (Nakagawa & Schielzeth, 2013; Johnson, 2014;
 263 Nakagawa & Schielzeth, 2017).

RESULTS

In the ten younger participants, decomposition yielded 1002 MU spike trains from the BIC, and 1211 MU spike trains from the TRI. In the ten older participants, decomposition yielded 533 MU spike trains from the BIC, and 827 from the TRI. All participants completed a minimum of 10 submaximal torque ramps in the directions of EF and EE. An average of 6.2 (3.81) and 9.0 (4.99) MUs per trial were decomposed from the BIC and TRI, respectively, of younger participants, and 4.4 (1.60) and 6.7 (3.39) MUs per trial were decomposed from the BIC and TRI, respectively, of older participants. Following the visual inspection and removal of erroneous spike times, the mean silhouette values of the decomposed motor units were 0.91 (0.05) from the BIC and 0.90 (0.05) from the TRI of the younger participants. The mean silhouette values from the older participants was 0.91 (0.04) from the BIC and 0.93 (0.05) from the TRI. An example of smoothed MU firing rate patterns of decomposed TRI MUs in a single trial from one younger and older individual are shown in figure 3, which shows many of the features that will be quantified below.

Figure 3: Single trial elbow extension torque (top; black traces) and smoothed firing rates of all decomposed triceps brachii MUs (bottom; coloured traces) for a younger (left; A) and an older (right; B) participant. Smoothed firing rates are darker at lower thresholds, and lighter for higher thresholds for both participants.

Comparison of peak MU firing rates

The peak firing rates from BIC and TRI MUs are shown in Figure 4. In this, and in the following figures, each data point represents the mean value from all MUs collected from one subject during the submaximal EF torque ramps (BIC data) or submaximal EE torque ramps (TRI data). In congruence with previous findings (Dalton *et al.*, 2010), older participants had reduced peak firing rates compared to younger participants. Group mean peak firing rates for the younger participants were higher than observed in the older participants in both the BIC (16.0 [1.74] pps vs 14.5 [2.22] pps, $ES = 0.71$) and the TRI (17.9 [1.52] pps vs 16.3 [1.90] pps, $ES = 0.84$). A linear mixed effects model revealed that both age group ($\chi^2 [1] = 4.7564$, $P = 0.0292$) and muscle ($\chi^2 [1] = 15.731$, $P < 0.0001$) were significant predictors of peak firing rate, but the interaction between the two variables was not significant ($P = 0.9980$). Peak MU firing rates were lowered by 1.6 (0.72) pps ($P = 0.0412$) in older participants, and were 1.8 (0.38) pps ($P = 0.0001$) higher in TRI, compared to BIC.

Figure 4: Individual participant means (younger = blue; older = red) and group mean and SD (black) for the peak firing rate of BIC (biceps brachii) MUs during a 20% elbow flexion ramp, and TRI (triceps brachii) MUs during a 20% elbow extension ramp.

Range of MU firing rates

Figure 5A shows the range of MU firing rates from BIC and TRI MUs. The group mean range of firing rates from the younger participants were higher than the MU firing rate ranges in the older participants in both BIC (12.0 [1.45] pps vs 9.4 [1.66] pps, $ES = 1.48$) and TRI (13.6 [1.22] pps vs 10.5 [1.97] pps, $ES = 1.70$). Age group ($\chi^2 [1] = 7.7509$, $P = 0.0053$) and

muscle ($\chi^2 [1] = 15.570, P < 0.0001$) were both significant predictors of firing rate range; the interaction of age group and muscle was not significant ($P = 0.8687$). The range of MU firing rates was 1.7 (0.60) pps ($P = 0.0091$) higher in younger participants than older participants, and 1.5 (0.32) pps ($P = 0.0001$) higher in MUs from the TRI than the BIC.

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Figure 5: Participant means (younger = blue; older = red), along with group mean and SD (black), for the range of MU firing rates during the full ramp (A), the ascending limb of the ramp (B), and the descending limb of the ramp (C). BIC (biceps brachii) data is from elbow flexion ramps, and TRI (triceps brachii) data is from elbow extension ramps.

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The observed range of MU firing rates for the ascending and descending limbs of the torque ramps are displayed in Figure 5B and 5C, respectively. During the ascending torque phase, the group mean firing rate range in the younger participants was 9.3 (0.96) pps in the BIC and 10.4 (0.79) pps in the TRI. While in the older participants, the group mean firing rate range was 8.7 (1.41) pps in the BIC and 9.7 (1.50) pps in the TRI. In the BIC, the effect size of the difference in firing rate range on the ascending limb between younger and older participants was 0.44, and in the TRI the effect size was 0.60. However, age group was not a significant predictor ($P = 0.1112$) nor was the interaction between muscle and age group ($P = 0.7546$). Muscle was the only significant predictor of MU firing rate range over the ascending limb of the torque ramp ($\chi^2 [1] = 10.9590, P = 0.0009$). The ascending limb firing rate range was 1.1 (0.29) pps higher in the TRI than in the BIC ($P = 0.0014$).

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During the descending torque phase, the group mean MU firing rate ranges were higher in the younger participants compared to the older participants for both muscles (BIC: 11.0 [1.49] pps vs 9.4 [1.66] pps, $ES = 0.91$; TRI: 12.7 [1.37] pps vs 10.5 [1.97] pps, $ES = 1.18$). Both age group ($\chi^2 [1] = 7.7199$, $P = 0.0055$) and muscle ($\chi^2 [1] = 10.856$, $P = 0.0010$) were significant predictors of descending limb firing rate range, in our model. The interaction between age group and muscle was not significant ($P = 0.4002$). During the descending torque ramps, the firing rate range was 1.9 (0.62) pps higher in younger participants ($P = 0.0083$), as compared to older participants, and 1.4 (0.37) pps higher in the TRI ($P = 0.0015$), as compared to the BIC.

Similar to the peak firing rates, older participants showed a reduced range of MU firing rates overall, as well as a reduction in firing rate range on the descending limb. However, the firing rate range during the ascending portion of the torque ramp was not significantly affected by aging. The difference in firing rate range between younger and older participants can be appreciated in an example of smoothed firing rates from the TRI of one younger and one older participant in figure R1.

Estimates of PIC amplitude using ΔF

Subject mean values for the ΔF calculation are shown in Figure 6. Group means for ΔF were substantially higher in the younger participants than in the older participants in the BIC (4.1 [1.35] pps vs 2.3 [0.84] pps, $ES = 1.47$) and in the TRI (5.2 [0.94] pps vs 3.2 [1.10] pps, $ES = 1.84$). Age group ($\chi^2 [1] = 18.326$, $P < 0.0001$) and muscle ($\chi^2 [1] = 17.796$, $P < 0.0001$) were both significant predictors for ΔF , in our model, however the interaction between those variables was not significant ($P = 0.2848$). ΔF was reduced by 1.9 (0.36) pps in the older participants ($P < 0.0001$), and 1.3 (0.24) pps lower in the BIC than in the TRI ($P < 0.0001$).

Figure 6: ΔF estimates from the BIC (biceps brachii) during elbow flexion and TRI (triceps brachii) during elbow extension. Participant means in color (younger = blue; older = red), with the black bars denoting group mean and SD.

We then determined whether any relationship existed between the reported age of participants and ΔF within each of these age groups. In Figure 7, ΔF is plotted as a function of participant age along with regression lines from our model. In the younger participants, the generalized linear mixed effects model accounted for 31.96% of the variance in ΔF , with the fixed effects of muscle and age accounting for 5.28% of the variance. Muscle was a significant predictor of ΔF ($\chi^2 [1] = 5.3981$, $P = 0.0202$), however, age was not a significant predictor of ΔF in the younger participants ($\chi^2 [1] = 0.0021$, $P = 0.9637$). The interaction between age and muscle was also not a significant predictor ($\chi^2 [1] = 0.3742$, $P = 0.5407$).

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Figure 7: The relationship between ΔF and age of the younger (blue; left) and older (red; right) group of participants. The lines indicate individual generalized linear mixed effects models. We show only the overall slope of the model, which includes fixed effects of age and muscle. For clarity of display, individual participants are not distinguished from one another, however the random effect in the model does account for the variability within each participant. Dark and light data points indicate ΔF values from TRI (triceps brachii) and BIC (biceps brachii) MUs, respectively. Some jitter was added to the data points x-value (age) for clarity of display. Equations derived from the model are displayed for the younger and older participants along with the marginal R^2 GLMM, which indicates the variance accounted for by our fixed effects of muscle and age.

369 In the older participants, the model accounted for 45.98% of the observed variance in ΔF ,
 370 with the fixed effects accounting for 29.32% of the variance. Both muscle ($\chi^2 [1] = 14.75$, P
 371 $= 0.0001$) and age ($\chi^2 [1] = 4.9504$, $P = 0.0261$) were significant predictors of ΔF , but the
 372 interaction between them was not ($\chi^2 [1] = 0.8031$, $P = 0.3702$). Greater reductions in ΔF
 373 were associated with increasing age, in the older participants ($-0.097 [0.041]$ pps/year, $P =$
 374 0.0473). In summary, ΔF was reduced in older participants compared to younger participants
 375 and a negative relationship existed between age and ΔF in the older participants, but not in
 376 the younger participants.

377

378 **MU firing duration**

Provoked by the reduction in firing rate hysteresis in older participants (i.e. reduced ΔF), we investigated whether the duration of the MU firing differed between age groups; the subject and group means for MU duration are shown in Figure 8A. The group means for total MU firing duration in the younger participants was 9.7 (1.95) s in the BIC and 8.9 (1.38) s in the TRI. In the older participants, the group mean for MU firing duration was 9.8 (1.83) s in the BIC and 9.6 (1.64) s in the TRI. The effect sizes between younger and older participants are 0.05 for the BIC and 0.43 in the TRI. However, the linear mixed effects model found that age group ($P = 0.3534$), muscle ($P = 0.3182$), and the interaction between age group and muscle ($P = 0.5084$) were not significant predictors of MU firing duration.

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Figure 8: Participant means (color) and group means (black), showing the firing duration of MUs for the full torque ramp (A), the ascending limb of the torque ramp (B), and the descending limb of the torque ramp (C).

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MU firing was further investigated by comparing the duration of firing during the ascending and descending limbs of the torque ramps, as shown in Figure 8B and C, respectively. On the ascending limb of the ramp, the group means for MU firing duration were shorter in the younger participants than in the older participants (see Figure 3 for example) in both the BIC (3.6 [0.99] s vs 4.6 [0.82] s, $ES = 0.97$) and in the TRI (4.1 [0.78] s vs 5.1 [0.83] s, $ES = 1.10$). Our model found age group ($\chi^2 [1] = 9.3990$, $P = 0.0022$) and muscle ($\chi^2 [1] = 4.5555$, $P = 0.0328$) were both significant predictors of firing duration on the ascending limb of the torque ramp, while the interaction between the two variables was not significant ($P =$

0.9224). Ascending limb firing duration was increased by 1.0 (0.30) s in the older participants ($P = 0.0039$), compared to younger participants, and 0.5 (0.22) s longer in TRI MUs than BIC MUs ($P = 0.0401$).

On the descending limb of the torque ramps, the group means for MU firing duration were longer for the younger participants than the older participants in both muscles (BIC: 6.1 [1.22] s vs 5.2 [1.24] s, $ES = 0.64$; TRI: 4.8 [0.70] s vs 4.6 [1.24] s, $ES = 0.24$). Muscle was revealed to be a significant predictor of firing duration on the decreasing torque ramp ($\chi^2 [1] = 7.4705$, $P = 0.0063$), but age group was not a significant predictor ($P = 0.1765$) and the interaction of age group and muscle was not significant ($P = 0.3589$). Descending limb firing duration was 1.0 (0.33) s longer in BIC MUs than in TRI MUs.

Age was only associated with an increased duration of firing on the ascending limb of the torque ramp, but did not significantly affect the overall firing duration or the firing duration on the descending limb of the torque ramp. As shown in figure 3, a longer duration of firing during the ascending phase of the ramp without a difference during the descending phase of the ramp or total duration of firing would indicate a leftward shift, and more symmetrical pattern of firing.

DISCUSSION

The present study aimed to investigate the effects of healthy aging on the MU firing patterns from the biceps brachii (BIC) and triceps brachii (TRI) by comparing younger and older healthy adults. In agreement with previous literature, we have found lower peak firing rates in both the BIC and TRI of older adults. Further, and perhaps most novel, we found substantial and significant reductions in estimates of persistent inward currents (PICs; ΔF) in older adults irrespective of muscle. In older adults, we also found that age was a significant predictor of ΔF (i.e. ΔF decreased with respect to age); however, there was no such relationship in the younger group. Additional characteristics of MU firing patterns support the notion of reduced onset-offset hysteresis, such as reduced firing rate range during the descending phase of the ramp and a leftward shift in MU firing in older compared to younger adults. These findings suggest that MU firing patterns in older people exhibit less PIC activity, which may have implications for motor control.

Reduced motor unit firing rates in older people

Reductions in MU firing rates in older adults here are similar to those reported in previous studies. For example, using intramuscular EMG, Dalton et al (Dalton *et al.*, 2010) found that mean firing rates are reduced in both the BIC and TRI across a variety of voluntary effort levels. The difference between younger and older adults was substantial (~45%) at high levels of effort for both muscles, but the differences at 25% MVC were modest and similar in magnitude to what we observed. Indeed, firing rates have been shown to be reduced in older adults in other studies as well, with larger differences at higher contraction intensities (Kamen *et al.*, 1995; Connelly *et al.*, 1999; Kamen & Knight, 2004; Barry *et al.*, 2007; Kirk *et al.*, 2018; Kirk *et al.*, 2019).

Organizational changes to the MUs following age-related loss of motoneurons (Doherty *et al.*, 1993) could play a role in the observed reductions in firing rate. With re-modelling there are changes in MU twitch contraction times such that twitch fusion occurs at a lower rate

(Bellemare *et al.*, 1983; Newton *et al.*, 1988; Connelly *et al.*, 1999; Roos *et al.*, 1999). However, investigations into motoneuron loss with age have shown a very moderate loss in individuals below the age of 60 (Tomlinson & Irving, 1977). As the mean age of the older participants in this group is only 67.8, some of our sample may not have substantial loss of motoneurons, and the subsequent reorganization of the motor pool would lead to only modest reductions of firing rates.

Changes in the pattern or level of excitatory synaptic input to the motoneurons are also likely to contribute to the observed reduction in firing rates with age. For instance, excitatory post-synaptic potential (i.e. EPSP) amplitudes, as estimated with peristimulus time histograms, in response to transcranial magnetic stimulation of the motor cortex, are reduced by ~50% in older people (> 55 years old). Hypoexcitability of the corticospinal pathway corresponds with weakness in older adults (Clark *et al.*, 2015), and ~33% of the variance in weakness in seniors can be accounted for by measures of corticospinal excitability (i.e. motor evoked potential amplitudes) and inhibition (i.e. corticospinal silent period). Stretch reflexes are also reduced with aging, suggesting that homonymous 1a input from muscle spindles is reduced (Bryndum & Marquardsen, 1964; Milne & Williamson, 1972), and slowed (i.e. longer half relaxation times) with advancing age (Carel *et al.*, 1979), which could be due to a combination of changes in muscle spindle sensitivity (Swash & Fox, 1972), Ia-motoneuronal transmission, and/or spinal motoneuron excitability (Geertsen *et al.*, 2017). Interestingly, the Hoffman (H-) reflex, which bypasses the muscle spindles by direct peripheral nerve stimulation, is also reduced with age, providing further support for Ia-motoneuronal transmission and/or spinal motoneuron excitability alterations with age (deVries *et al.*, 1985; Scaglioni *et al.*, 2003). Reductions in any combination of the above mentioned excitatory input would surely result in reduced MU firing rates in older adults.

Inhibitory spinal circuits can also affect descending input and/or Ia afferent input to spinal motoneurons via inhibitory interneurons. Older adults also have reduced indices of reciprocal

inhibition, both at the spinal (Kido *et al.*, 2004) and cortical levels (Hortobagyi *et al.*, 2006), which may alter the commands to agonist-antagonist muscle pairs. Although the effects of aging on Ia presynaptic inhibition at rest are unclear (Butchart *et al.*, 1993; Morita *et al.*, 1995; Earles *et al.*, 2001), there is a decrease in the amount of modulation of Ia presynaptic inhibition with increasing contraction intensity in older adults (Butchart *et al.*, 1993), which would lead to differences in the pattern of inhibition and have profound effects on the balance of excitation, inhibition, and neuromodulation (Johnson *et al.*, 2017) required to perform a task. The imbalance of inhibition and excitation could therefore play a role in the reduced firing rates we observed in our older adults.

MU firing rates are highly dependent upon the biophysical properties of the parent motoneurons, and age-related changes of such properties can lead to reductions in firing rates. Spike after-hyperpolarization (AHP) duration is increased in aged rodents (Cameron *et al.*, 1991; Kalmar *et al.*, 2009) and cats (Morales *et al.*, 1987). Similarly, AHP duration increases gradually with age (Piotrkiewicz *et al.*, 2007), and when compared directly, AHP is longer in older compared to younger adults (Christie & Kamen, 2010), which can contribute to reductions in MU firing rates. Other biophysical properties that are altered with age can affect recruitment and repetitive firing of MUs, such as increases in the input resistance (Chase *et al.*, 1985; Morales *et al.*, 1987; Kalmar *et al.*, 2009), and reduced rheobase current (Morales *et al.*, 1987; Kalmar *et al.*, 2009), suggesting that aged motoneurons are less excitable. Indeed motoneuron recruitment gain is reduced with age, as evidenced in a recent investigation by Nielsen and colleagues (Nielsen *et al.*, 2019). Therefore, changes to the biophysical properties of the motoneurons likely contribute to the reduced firing rates we observed in older adults.

Not only were peak firing rates reduced in the current investigation, but older adults also showed a compressed range of MU firing rates. This compressed range of firing can arise from similar mechanisms that underlie the reductions in peak firing rates, but it is interesting

to note that the firing rate range during the ascending phase of the contraction was similar for both younger and older adults. That is, the rate modulation from the onset of firing to peak firing was similar (see figure 5B). On the contrary, the reduction in overall firing rate range (~1.7 pps) seen in older participants, is primarily attributed to the reduction in firing rate range seen on the descending limb of the torque ramp (~1.9 pps). The reduced firing range seen on the descending limb (I.e. reduced hysteresis) of the torque ramps is most likely related to a decrease in PIC activity, which brings us to the next topic of our discussion (i.e. reduced estimates of PICs in older adults).

Reduced estimates of PICs in older people

We have shown that ΔF is substantially lower, regardless of muscle, in the upper limb of older adults, compared to younger adults. Further, in our older group, we found that increasing age was associated with reductions in ΔF (see figure 7). In addition, the relative firing duration of motor units is shifted to the left in older adults, such that the duration of firing is symmetrical during the ascending and descending phases of the ramp contraction, indicating less onset-offset hysteresis. Although ΔF and the leftward shift in firing patterns are an indirect estimate of PIC activity, they do support our hypothesis that PICs are reduced in older people. Such age-related changes in estimates of PICs are most likely due to either changes in 1) monoaminergic input to the motoneurons, 2) the amount or pattern of inhibition, and/or 3) Na^+ or Ca^{2+} channel function.

The overall leftward shift in the firing patterns of older individuals provides further insights into the effects of aging on MU firing. Based on the reduced ΔF and reduced firing rate range on the descending limb in the older adults, we expected to see a reduced duration of firing on the descending limb in those participants. Instead, we found an increased duration of firing on the ascending limb of the torque ramps in older participants, without significant changes to the overall duration and descending duration of firing, which may lead to the observed ΔF differences. This may suggest that motor units in older participants were recruited earlier

than those from younger participants. Indeed, this would be similar to other reports that have shown a lower average recruitment threshold for motor units recorded from older compared to younger adults (Erim *et al.*, 1999; Klass *et al.*, 2005, 2008; Fling *et al.*, 2009; Pascoe *et al.*, 2011). Most intriguing though is the fact that the firing patterns of older individuals were more symmetrical (~approximately equal time on the ascending and descending portion of the ramps – see figure 3), indicating less hysteresis, a behaviour that is a hallmark of PICs.

Schwindt and Crill (Schwindt & Crill, 1980) first discovered PICs in motoneurons by blocking K^+ currents in anesthetized preparations, but by using un-anesthetized decerebrate preparation, Hounsgaard and colleagues (Hounsgaard *et al.*, 1984; Hounsgaard & Kiehn, 1989) showed that PICs emerged in the presence of serotonin (5HT) or norepinephrine (NE), meaning that PICs are a natural consequence of endogenous neurotransmitters. Although important for initiation of repetitive firing, the component of the PIC mediated by Na^+ is not responsible for the long-lasting prolongation of firing due to the inactivation time constant of just a few seconds (Lee & Heckman, 1998; Binder & Powers, 2001). Instead, it is the component mediated by L-type Ca^{2+} channels that causes prolonged self-sustained firing and profound onset-offset hysteresis (Lee & Heckman, 2001; Harvey *et al.*, 2006; Kuo *et al.*, 2006), in particular, the $Ca^{2+}V1.3$ channels show little or no inactivation (Moritz *et al.*, 2007; Binder *et al.*, 2020). This “self-sustained” firing, or simply maintained motoneuron firing with decreased synaptic input, was emphasized as being quite important for postural behaviours because sustained force generation can be maintained without the need for sustained descending input (Hounsgaard *et al.*, 1984; Hounsgaard & Kiehn, 1989). Therefore, the functional role of PICs in normal human behaviours can easily be appreciated (Heckman *et al.*, 2008b). With a reduction in PIC-related prolongation of motoneuron firing, simple tasks would require greater synaptic input to maintain activities such as standing or carrying objects. Since the aging process is associated with reduced excitatory input to motoneurons, the role of PICs in maintaining forces required for everyday activities may be increased with advancing age.

Initial attempts to understand PIC effects of human MU firing behaviour focused on self-sustained firing or “bi-stability” (Kiehn & Eken, 1997). In such experiments, MUs are tracked during low-level voluntary efforts and an additional source of synaptic input (i.e. vibration) causes the recruitment of an additional MU (test unit) that maintains firing after the additional input is removed (Gorassini *et al.*, 1998). MUs can then be classified as either having PICs, or not, based on the occurrence of test units that maintain firing after the additional synaptic input is removed. Using this approach, Kamen and colleagues (Kamen *et al.*, 2006) showed that older individuals have a similar occurrence (23.1%) of MUs that exhibit self-sustained firing as younger adults (22.8%). As such, and contrary to the findings in our current investigation, they concluded that PIC-like behaviour does not seem to be affected by the aging process. They did, however, report that the mean drop-out torque of newly recruited MUs was slightly higher for older adults (3.26% maximal voluntary contraction (MVC) vs 2.43% MVC), although variability was high and therefore no statistical differences were reported. It is important to note that the occurrence of self-sustained firing may not be the best all-end-all method to quantify whether PICs are present during voluntary motor behaviour in humans. This is because PICs almost certainly contribute to motoneuron firing during all voluntary behaviours, because without the amplification effects of PICs, the small currents produced by descending and sensory inputs are too weak to have much of an effect on motoneuron firing (Binder & Powers, 2001). More important to the understanding of human motor output is the magnitude of PICs, rather than the presence.

Hysteresis of MU firing rates, on the other hand, has proven to be the most consistent hallmark for non-invasive estimation of the magnitude of PICs in humans, as was first realized by Gorassini, Bennett and colleagues (Bennett *et al.*, 2001a; Gorassini *et al.*, 2002). The now standard paired-MU analysis technique (ΔF) has been subject to rigorous investigations interested in the accuracy of these estimates (Bennett *et al.*, 2001a; Bennett *et al.*, 2001b; Powers *et al.*, 2008; Revill & Fuglevand, 2011; Powers & Heckman, 2015; Afsharipour *et al.*, 2020; Hassan *et al.*, 2020). Bennett and colleagues (Bennett *et al.*, 2001a; Bennett *et al.*, 2001b) used parallel MU and intracellular recordings in rat motoneurons to

clearly demonstrate that ΔF reflects features of PICs. With advances in technology, these estimates of PICs have been obtained across hundreds of MUs (Afsharipour *et al.*, 2020; Hassan *et al.*, 2020; Kim *et al.*, 2020; Trajano *et al.*, 2020), which likely provides a better overall estimation of PIC magnitude across the entire motor pool. Even though MUs of older adults in our experiment certainly displayed onset-offset hysteresis (i.e. positive ΔF values overall), the magnitude of this hysteresis was markedly reduced compared to the sample of younger adults that were recruited. In fact, estimates of PICs (ΔF) were reduced by $\sim 40\%$, with very large effect sizes (all ES > 1.45). In addition, the age of individuals was a significant predictor of ΔF , suggesting that PICs may deteriorate with age at a rate of ~ 1 pps/decade, but only in older adults.

The magnitude of PICs is directly proportional to the level of NE and 5HT (Lee & Heckman, 1998, 2000), which are monoamines released from the from the caudal raphe nucleus and locus coeruleus, respectively. These monoaminergic nuclei of the brainstem deteriorate with age (Shibata *et al.*, 2006; Pagano *et al.*, 2017), and in particular, the age-related reduction in locus coeruleus structural integrity is associated with impaired cognitive and behavioural function (Liu *et al.*, 2020), as well as reductions in central pain modulation (Grashorn *et al.*, 2013; Damien *et al.*, 2018). Deterioration of these nuclei could also lead to reductions in neuromodulatory drive to motoneurons, reducing PIC activity, which would ultimately explain some of the reductions observed in ΔF . NE-mediated effects are likely predominantly due to degradation of the locus coeruleus because older rodents maintain only $\sim 30\%$ NE nuclei compared to $\sim 90\%$ 5HT nuclei (Tatton *et al.*, 1991). Despite the evidence that a greater proportion of raphe nuclei are maintained with age, spinal 5HT is greatly reduced (Johnson *et al.*, 1993; Ko *et al.*, 1997). Therefore, the potential of 5HT-mediated effects with the aging process are more likely to occur peripherally. With aging, there is increased circulation of cytokines (so-called “inflamm-aging”) (Michaud *et al.*, 2013), which affect 5HT receptors and increase re-uptake of 5HT (Steinbusch *et al.*, 2021). In sum, less availability of monoamines would result in reduced PIC magnitude at the same relative effort, which is what we observed as a reduction in ΔF .

615

616 PICs are also highly sensitive to inhibitory inputs (Hultborn *et al.*, 2003; Kuo *et al.*, 2003;
617 Heckman *et al.*, 2008a; Hyngstrom *et al.*, 2008; Revoll & Fuglevand, 2017). Thus, changes
618 to the amount or pattern of inhibition may lead to reduced estimates of PICs as estimated by
619 ΔF . As mentioned above, there are age-related alterations in spinal and supraspinal inhibitory
620 circuits (Butchart *et al.*, 1993; Kido *et al.*, 2004; Hortobagyi *et al.*, 2006) that could modify
621 the synaptic input to motoneurons. While difficult to measure, the temporal pattern of
622 inhibitory commands can also affect the ΔF estimate (Powers *et al.*, 2012; Johnson *et al.*,
623 2017). Push-pull inhibition, where inhibition varies inversely with excitation, can lead to
624 reductions in MU hysteresis (Powers *et al.*, 2012). It, therefore, remains possible that the
625 pattern of the inhibitory commands is altered with age to compensate for the various
626 structural and functional changes in the neuromuscular system (Hepple & Rice, 2016;
627 McNeil & Rice, 2018) associated with the aging process and may contribute to our observed
628 reductions in ΔF .

629

630 Alterations in the integrity and function of 5HT/NE receptors and voltage sensitive ion
631 channels must also be considered in relation to age-related changes in the nervous system.
632 Basic (i.e. larger and longer AHP, lower rheobase, greater input resistance) and rhythmic (i.e.
633 slower minimum and maximum steady-state firing frequencies and lower f-I slopes)
634 motoneuron properties are consistent with reduced motoneuron excitability in very old (>30
635 months) rodents. However, Kalmar et al (Kalmar *et al.*, 2009) also showed an increased
636 incidence of PIC-like behaviour in very old rodent motoneurons, which they suggested to
637 have resulted from increased 5HT and NE receptor sensitivity to residual endogenous
638 monoamines (Harvey *et al.*, 2006) as a compensatory mechanism to counteract the reduced
639 motoneuron excitability. Although this increased incidence of PIC may seem to contradict
640 our findings, this type of analysis simply determines the relative number of motoneurons that
641 have hysteresis in response to current injection to the soma, whereas we quantified the
642 average magnitude of hysteresis during voluntary activation (i.e. axo-dendritic synaptic
643 input). L-type Ca^{2+} channels are concentrated in the dendritic tree, far away from the soma,

meaning that the levels of injected current may have underestimated PICs in a healthy younger motoneuron receptor hypersensitivity due to the inability to activate PICs from the soma (Bennett *et al.*, 1998; Lee & Heckman, 1998). Aging may also result in changes in the expression of receptor subtypes or the downstream signaling of various receptors. Indeed, there are age-related reductions in the duration of Ca²⁺-mediated plateau potentials in striatal neurons (Dunia *et al.*, 1996), and more generally, deregulated Ca²⁺ is an active component of healthy aging that can increase the risk of cell death and neurodegenerative disorders (Nikoletopoulou & Tavernarakis, 2012). As such, it is difficult to pinpoint the exact monoaminergic receptor or ion channel dysfunction that may contribute to the observed reductions in estimated PICs during voluntary contractions with aging.

Methodological considerations

Since this experiment was conducted in a non-invasive fashion, we were unable to directly determine the PIC magnitude. Instead, we relied on the best estimation of PICs available in humans (i.e. ΔF), which has undergone rigorous scrutiny to ensure accuracy of the estimates (Bennett *et al.*, 2001a; Bennett *et al.*, 2001b; Powers *et al.*, 2008; Revill & Fuglevand, 2011; Powers & Heckman, 2015; Afsharipour *et al.*, 2020; Hassan *et al.*, 2020). Nonetheless, it is difficult to determine whether the reduction in ΔF is the result of alterations in monoaminergic drive, the amount or pattern of inhibition, and/or changes to the monoaminergic receptor sensitivity or ion channel function. This delineation will require further work.

Overall, the MUs decomposed in the older participants had longer durations on the ascending phase of the ramp, suggesting a lower relative threshold of units decomposed for older adults. This relatively lower recruitment torque could lead to a ceiling effect in terms of how much hysteresis those units could exhibit. However, as the average duration of MU firing on the descending limb was 5.2 s and 4.6 s for the BIC and TRI respectively in the older participants

with the descending limb of the torque ramp being 10 seconds long, we do not believe the reduced ΔF observed was due to early derecruitment due to the time constraints of the task.

Practical considerations

In the words of Power and colleagues (Power *et al.*, 2016) – “If you don’t use it, you’ll likely lose it.” Whether this holds true for PICs is unclear at the moment, although Latella (Latella, 2021) recently made a compelling argument for studying the efficacy of strength training to mitigate the effects of aging on MU firing behaviour. Indeed, the work of Power and colleagues (Power *et al.*, 2010) suggests that estimates of MU numbers are greater in masters runners compared to their sedentary counterparts. Further, strength training-induced plasticity of motoneurons is not limited to younger adults. AHP duration is longer in older compared to younger adults, but that duration can be reduced with strength training in both age groups (Christie & Kamen, 2010). Thus, it remains possible that strength training, which necessitates high levels of effort (likely utilizing high levels of monoaminergic drive), could mitigate deterioration of monoaminergic function and/or PIC behaviour seen in older adults.

CONCLUSION

The present study compared the firing patterns of MUs from the elbow extensors and flexors of healthy younger and older adults during isometric ramp contractions. Irrespective of muscle, age was a significant predictor of peak firing rate and firing rate hysteresis, such that both were reduced in older adults. In addition to the differences observed between age groups, the age of individuals within the older group predicted a ~1pps per decade reduction in ΔF , a non-invasive estimate of PIC magnitude across the motor pool. This reduced estimate of PIC magnitude likely arises from reductions in monoaminergic input, alterations in the amount or pattern of inhibition, and/or alterations in monoamine receptor or ion channel function. It remains unclear whether alterations in firing rate hysteresis are a compensatory adjustment or impairment that occurs with aging, however, it remains possible that physical training may be able to mitigate such changes.

References

- Afsharipour B, Manzur N, Duchcherer J, Fenrich KF, Thompson CK, Negro F, Quinlan KA, Bennett DJ & Gorassini MA. (2020). Estimation of self-sustained activity produced by persistent inward currents using firing rate profiles of multiple motor units in humans. *J Neurophysiol* **124**, 63-85.
- Barry BK, Pascoe MA, Jesunathadas M & Enoka RM. (2007). Rate coding is compressed but variability is unaltered for motor units in a hand muscle of old adults. *J Neurophysiol* **97**, 3206-3218.
- Barton K. (2018). Multi-model inference. R package version 1.42. 1.
- Bates D & Maechler M. (2015). M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-7.
- Bellemare F, Woods JJ, Johansson R & Bigland-Ritchie B. (1983). Motor-unit discharge rates in maximal voluntary contractions of three human muscles. *J Neurophysiol* **50**, 1380-1392.
- Bennett DJ, Hultborn H, Fedirchuk B & Gorassini M. (1998). Synaptic activation of plateaus in hindlimb motoneurons of decerebrate cats. *J Neurophysiol* **80**, 2023-2037.
- Bennett DJ, Li Y, Harvey PJ & Gorassini M. (2001a). Evidence for plateau potentials in tail motoneurons of awake chronic spinal rats with spasticity. *J Neurophysiol* **86**, 1972-1982.
- Bennett DJ, Li Y & Siu M. (2001b). Plateau potentials in sacrocaudal motoneurons of chronic spinal rats, recorded in vitro. *J Neurophysiol* **86**, 1955-1971.
- Binder MD & Powers RK. (2001). Relationship between simulated common synaptic input and discharge synchrony in cat spinal motoneurons. *J Neurophysiol* **86**, 2266-2275.
- Binder MD, Powers RK & Heckman CJ. (2020). Nonlinear Input-Output Functions of Motoneurons. *Physiology (Bethesda)* **35**, 31-39.
- Boccia G, Martinez-Valdes E, Negro F, Rainoldi A & Falla D. (2019). Motor unit discharge rate and the estimated synaptic input to the vasti muscles is higher in open compared with closed kinetic chain exercise. *Journal of Applied Physiology* **127**, 950-958.

- Bryndum B & Marquardsen J. (1964). The Tendon Reflexes in Old Age. *Gerontol Clin (Basel)* **6**, 257-265.
- Butchart P, Farquhar R, Part NJ & Roberts RC. (1993). The effect of age and voluntary contraction on presynaptic inhibition of soleus muscle Ia afferent terminals in man. *Exp Physiol* **78**, 235-242.
- Cameron WE, Jodkowski JS, Fang H & Guthrie RD. (1991). Electrophysiological properties of developing phrenic motoneurons in the cat. *J Neurophysiol* **65**, 671-679.
- Carel RS, Korczyn AD & Hochberg Y. (1979). Age and sex dependency of the Achilles tendon reflex. *Am J Med Sci* **278**, 57-63.
- Chase MH, Morales FR, Boxer PA & Fung SJ. (1985). Aging of motoneurons and synaptic processes in the cat. *Exp Neurol* **90**, 471-478.
- Christie A & Kamen G. (2010). Short-term training adaptations in maximal motor unit firing rates and afterhyperpolarization duration. *Muscle Nerve* **41**, 651-660.
- Clark BC, Taylor JL, Hong SL, Law TD & Russ DW. (2015). Weaker Seniors Exhibit Motor Cortex Hypoexcitability and Impairments in Voluntary Activation. *J Gerontol A Biol Sci Med Sci* **70**, 1112-1119.
- Cogliati M, Cudicio A, Martinez-Valdes E, Tarperi C, Schena F, Orizio C & Negro F. (2020). Half marathon induces changes in central control and peripheral properties of individual motor units in master athletes. *J Electromyogr Kinesiol* **55**, 102472.
- Connelly DM, Rice CL, Roos MR & Vandervoort AA. (1999). Motor unit firing rates and contractile properties in tibialis anterior of young and old men. *J Appl Physiol (1985)* **87**, 843-852.
- Dalton BH, Jakobi JM, Allman BL & Rice CL. (2010). Differential age-related changes in motor unit properties between elbow flexors and extensors. *Acta Physiol (Oxf)* **200**, 45-55.
- Damien J, Colloca L, Bellei-Rodriguez CE & Marchand S. (2018). Pain Modulation: From Conditioned Pain Modulation to Placebo and Nocebo Effects in Experimental and Clinical Pain. *Int Rev Neurobiol* **139**, 255-296.

- Del Vecchio A, Holobar A, Falla D, Felici F, Enoka RM & Farina D. (2020). Tutorial: Analysis of motor unit discharge characteristics from high-density surface EMG signals. *J Electromyogr Kinesiol* **53**, 102426.
- Del Vecchio A, Negro F, Felici F & Farina D. (2018). Distribution of muscle fibre conduction velocity for representative samples of motor units in the full recruitment range of the tibialis anterior muscle. *Acta Physiol (Oxf)* **222**.
- deVries HA, Wiswell RA, Romero GT & Heckathorne E. (1985). Changes with age in monosynaptic reflexes elicited by mechanical and electrical stimulation. *Am J Phys Med* **64**, 71-81.
- Doherty TJ, Vandervoort AA, Taylor AW & Brown WF. (1993). Effects of motor unit losses on strength in older men and women. *J Appl Physiol (1985)* **74**, 868-874.
- Dunia R, Buckwalter G, Defazio T, Villar FD, McNeill TH & Walsh JP. (1996). Decreased duration of Ca(2+)-mediated plateau potentials in striatal neurons from aged rats. *J Neurophysiol* **76**, 2353-2363.
- Earles D, Vardaxis V & Koceja D. (2001). Regulation of motor output between young and elderly subjects. *Clin Neurophysiol* **112**, 1273-1279.
- Erim Z, Beg MF, Burke DT & de Luca CJ. (1999). Effects of aging on motor-unit control properties. *J Neurophysiol* **82**, 2081-2091.
- Fling BW, Knight CA & Kamen G. (2009). Relationships between motor unit size and recruitment threshold in older adults: implications for size principle. *Exp Brain Res* **197**, 125-133.
- Geertsen SS, Willerslev-Olsen M, Lorentzen J & Nielsen JB. (2017). Development and aging of human spinal cord circuitries. *J Neurophysiol* **118**, 1133-1140.
- Giboin LS, Tokuno C, Kramer A, Henry M & Gruber M. (2020). Motor learning induces time-dependent plasticity that is observable at the spinal cord level. *J Physiol* **598**, 1943-1963.

- Gorassini M, Yang JF, Siu M & Bennett DJ. (2002). Intrinsic activation of human motoneurons: possible contribution to motor unit excitation. *J Neurophysiol* **87**, 1850-1858.
- Gorassini MA, Bennett DJ & Yang JF. (1998). Self-sustained firing of human motor units. *Neurosci Lett* **247**, 13-16.
- Gordon T, Hegedus J & Tam SL. (2004). Adaptive and maladaptive motor axonal sprouting in aging and motoneuron disease. *Neurol Res* **26**, 174-185.
- Grashorn W, Sprenger C, Forkmann K, Wrobel N & Bingel U. (2013). Age-dependent decline of endogenous pain control: exploring the effect of expectation and depression. *PLoS One* **8**, e75629.
- Harvey PJ, Li X, Li Y & Bennett DJ. (2006). Endogenous monoamine receptor activation is essential for enabling persistent sodium currents and repetitive firing in rat spinal motoneurons. *J Neurophysiol* **96**, 1171-1186.
- Hassan A, Thompson CK, Negro F, Cummings M, Powers RK, Heckman CJ, Dewald JPA & McPherson LM. (2020). Impact of parameter selection on estimates of motoneuron excitability using paired motor unit analysis. *J Neural Eng* **17**, 016063.
- Heckman CJ & Enoka RM. (2012). Motor unit. *Comprehensive Physiology* **2**, 2629-2682.
- Heckman CJ, Hyngstrom AS & Johnson MD. (2008a). Active properties of motoneurone dendrites: diffuse descending neuromodulation, focused local inhibition. *J Physiol* **586**, 1225-1231.
- Heckman CJ, Johnson M, Mottram C & Schuster J. (2008b). Persistent inward currents in spinal motoneurons and their influence on human motoneuron firing patterns. *Neuroscientist* **14**, 264-275.
- Hepple RT & Rice CL. (2016). Innervation and neuromuscular control in ageing skeletal muscle. *J Physiol* **594**, 1965-1978.
- Holobar A & Zazula D. (2007). Multichannel blind source separation using convolution kernel compensation. *IEEE Transactions on Signal Processing* **55**, 4487-4496.

- 851 Hortobagyi T, del Olmo MF & Rothwell JC. (2006). Age reduces cortical reciprocal
852 inhibition in humans. *Exp Brain Res* **171**, 322-329.
- 853
- 854 Hounsgaard J, Hultborn H, Jespersen B & Kiehn O. (1984). Intrinsic membrane properties
855 causing a bistable behaviour of alpha-motoneurons. *Exp Brain Res* **55**, 391-394.
- 856
- 857 Hounsgaard J & Kiehn O. (1989). Serotonin-induced bistability of turtle motoneurons
858 caused by a nifedipine-sensitive calcium plateau potential. *J Physiol* **414**, 265-282.
- 859
- 860 Hultborn H, Denton ME, Wienecke J & Nielsen JB. (2003). Variable amplification of
861 synaptic input to cat spinal motoneurons by dendritic persistent inward current. *J*
862 *Physiol* **552**, 945-952.
- 863
- 864 Hyngstrom AS, Johnson MD & Heckman CJ. (2008). Summation of excitatory and
865 inhibitory synaptic inputs by motoneurons with highly active dendrites. *J*
866 *Neurophysiol* **99**, 1643-1652.
- 867
- 868 Johnson H, Ulfhake B, Dagerlind A, Bennett GW, Fone KC & Hokfelt T. (1993). The
869 serotonergic bulbospinal system and brainstem-spinal cord content of serotonin-,
870 TRH-, and substance P-like immunoreactivity in the aged rat with special reference
871 to the spinal cord motor nucleus. *Synapse* **15**, 63-89.
- 872
- 873 Johnson MD, Thompson CK, Tysseling VM, Powers RK & Heckman CJ. (2017). The
874 potential for understanding the synaptic organization of human motor commands via
875 the firing patterns of motoneurons. *J Neurophysiol* **118**, 520-531.
- 876
- 877 Johnson PC. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes
878 models. *Methods in ecology and evolution* **5**, 944-946.
- 879
- 880 Kalmar JM, Button DC, Gardiner K, Cahill F & Gardiner PF. (2009). Caloric restriction does
881 not offset age-associated changes in the biophysical properties of motoneurons. *J*
882 *Neurophysiol* **101**, 548-557.
- 883
- 884 Kamen G & Knight CA. (2004). Training-related adaptations in motor unit discharge rate in
885 young and older adults. *J Gerontol A Biol Sci Med Sci* **59**, 1334-1338.
- 886
- 887 Kamen G, Sison SV, Du CC & Patten C. (1995). Motor unit discharge behavior in older
888 adults during maximal-effort contractions. *J Appl Physiol (1985)* **79**, 1908-1913.

- Kamen G, Sullivan R, Rubinstein S & Christie A. (2006). Evidence of self-sustained motoneuron firing in young and older adults. *J Electromyogr Kinesiol* **16**, 25-31.
- Kanda K & Hashizume K. (1989). Changes in properties of the medial gastrocnemius motor units in aging rats. *J Neurophysiol* **61**, 737-746.
- Kido A, Tanaka N & Stein RB. (2004). Spinal excitation and inhibition decrease as humans age. *Can J Physiol Pharmacol* **82**, 238-248.
- Kiehn O & Eken T. (1997). Prolonged firing in motor units: evidence of plateau potentials in human motoneurons? *J Neurophysiol* **78**, 3061-3068.
- Kim EH, Wilson JM, Thompson CK & Heckman CJ. (2020). Differences in estimated persistent inward currents between ankle flexors and extensors in humans. *J Neurophysiol* **124**, 525-535.
- Kirk EA, Gilmore KJ & Rice CL. (2018). Neuromuscular changes of the aged human hamstrings. *J Neurophysiol* **120**, 480-488.
- Kirk EA, Gilmore KJ, Stashuk DW, Doherty TJ & Rice CL. (2019). Human motor unit characteristics of the superior trapezius muscle with age-related comparisons. *J Neurophysiol* **122**, 823-832.
- Klass M, Baudry S & Duchateau J. (2005). Aging does not affect voluntary activation of the ankle dorsiflexors during isometric, concentric, and eccentric contractions. *J Appl Physiol* (1985) **99**, 31-38.
- Klass M, Baudry S & Duchateau J. (2008). Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. *J Appl Physiol* (1985) **104**, 739-746.
- Ko ML, King MA, Gordon TL & Crisp T. (1997). The effects of aging on spinal neurochemistry in the rat. *Brain Res Bull* **42**, 95-98.
- Kuo JJ, Lee RH, Johnson MD, Heckman HM & Heckman CJ. (2003). Active dendritic integration of inhibitory synaptic inputs in vivo. *J Neurophysiol* **90**, 3617-3624.

- 927 Kuo JJ, Lee RH, Zhang L & Heckman CJ. (2006). Essential role of the persistent sodium
928 current in spike initiation during slowly rising inputs in mouse spinal neurones. *J*
929 *Physiol* **574**, 819-834.
- 930
- 931 Kuznetsova A, Brockhoff PB & Christensen RH. (2017). lmerTest package: tests in linear
932 mixed effects models. *Journal of statistical software* **82**, 1-26.
- 933
- 934 Latella C. (2021). Pick me, Pick me! Rationale for investigating persistent inward currents
935 (PICs) and associated exercise effects in the ageing neuromuscular system. *J Physiol*
936 **599**, 1957-1959.
- 937
- 938 Lee RH & Heckman CJ. (1998). Bistability in spinal motoneurons in vivo: systematic
939 variations in persistent inward currents. *J Neurophysiol* **80**, 583-593.
- 940
- 941 Lee RH & Heckman CJ. (2000). Adjustable amplification of synaptic input in the dendrites
942 of spinal motoneurons in vivo. *J Neurosci* **20**, 6734-6740.
- 943
- 944 Lee RH & Heckman CJ. (2001). Essential role of a fast persistent inward current in action
945 potential initiation and control of rhythmic firing. *J Neurophysiol* **85**, 472-475.
- 946
- 947 Liu KY, Kievit RA, Tsvetanov KA, Betts MJ, Duzel E, Rowe JB, Cam CAN, Howard R &
948 Hammerer D. (2020). Noradrenergic-dependent functions are associated with age-
949 related locus coeruleus signal intensity differences. *Nat Commun* **11**, 1712.
- 950
- 951 Martinez-Valdes E, Negro F, Falla D, Dideriksen JL, Heckman CJ & Farina D. (2020).
952 Inability to increase the neural drive to muscle is associated with task failure during
953 submaximal contractions. *J Neurophysiol* **124**, 1110-1121.
- 954
- 955 McNeil CJ, Doherty TJ, Stashuk DW & Rice CL. (2005). Motor unit number estimates in
956 the tibialis anterior muscle of young, old, and very old men. *Muscle Nerve* **31**, 461-
957 467.
- 958
- 959 McNeil CJ & Rice CL. (2018). Neuromuscular adaptations to healthy aging. *Appl Physiol*
960 *Nutr Metab* **43**, 1158-1165.
- 961
- 962 Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B, Cesari M & Nourhashemi
963 F. (2013). Proinflammatory cytokines, aging, and age-related diseases. *J Am Med Dir*
964 *Assoc* **14**, 877-882.

- 965
- 966 Milne JS & Williamson J. (1972). The ankle jerk in older people. *Gerontol Clin (Basel)* **14**,
- 967 86-88.
- 968
- 969 Mitchell WK, Williams J, Atherton P, Larvin M, Lund J & Narici M. (2012). Sarcopenia,
- 970 dynapenia, and the impact of advancing age on human skeletal muscle size and
- 971 strength; a quantitative review. *Front Physiol* **3**, 260.
- 972
- 973 Morales FR, Boxer PA, Fung SJ & Chase MH. (1987). Basic electrophysiological properties
- 974 of spinal cord motoneurons during old age in the cat. *J Neurophysiol* **58**, 180-194.
- 975
- 976 Morita H, Shindo M, Yanagawa S, Yoshida T, Momoi H & Yanagisawa N. (1995).
- 977 Progressive decrease in heteronymous monosynaptic Ia facilitation with human
- 978 ageing. *Exp Brain Res* **104**, 167-170.
- 979
- 980 Moritz CT, Lucas TH, Perlmuter SI & Fetz EE. (2007). Forelimb movements and muscle
- 981 responses evoked by microstimulation of cervical spinal cord in sedated monkeys. *J*
- 982 *Neurophysiol* **97**, 110-120.
- 983
- 984 Nakagawa S & Schielzeth H. (2013). A general and simple method for obtaining R2 from
- 985 generalized linear mixed-effects models. *Methods in ecology and evolution* **4**, 133-
- 986 142.
- 987
- 988 Nakagawa S & Schielzeth H. (2017). Extending R2 and intra-class correlation coefficient
- 989 from generalized linear mixed-effects models: capturing and characterizing
- 990 biological variation. *bioRxiv*, 095851.
- 991
- 992 Negro F, Muceli S, Castronovo AM, Holobar A & Farina D. (2016). Multi-channel
- 993 intramuscular and surface EMG decomposition by convolutive blind source
- 994 separation. *J Neural Eng* **13**, 026027.
- 995
- 996 Newton JP, Yemm R & McDonagh MJ. (1988). Study of age changes in the motor units of
- 997 the first dorsal interosseous muscle in man. *Gerontology* **34**, 115-119.
- 998
- 999 Nielsen JB, Morita H, Wenzelburger R, Deuschl G, Gossard JP & Hultborn H. (2019).
- 1000 Recruitment gain of spinal motor neuron pools in cat and human. *Exp Brain Res* **237**,
- 1001 2897-2909.
- 1002

1003 Nikolettou V & Tavernarakis N. (2012). Calcium homeostasis in aging neurons. *Front*
1004 *Genet* **3**, 200.

1005

1006 Pagano G, Niccolini F, Fusar-Poli P & Politis M. (2017). Serotonin transporter in Parkinson's
1007 disease: A meta-analysis of positron emission tomography studies. *Ann Neurol* **81**,
1008 171-180.

1009

1010 Pascoe MA, Holmes MR & Enoka RM. (2011). Discharge characteristics of biceps brachii
1011 motor units at recruitment when older adults sustained an isometric contraction. *J*
1012 *Neurophysiol* **105**, 571-581.

1013

1014 Piotrkiewicz M, Kudina L, Mierzejewska J, Jakubiec M & Hausmanowa-Petrusewicz I.
1015 (2007). Age-related change in duration of afterhyperpolarization of human
1016 motoneurons. *J Physiol* **585**, 483-490.

1017

1018 Power GA, Dalton BH, Behm DG, Vandervoort AA, Doherty TJ & Rice CL. (2010). Motor
1019 unit number estimates in masters runners: use it or lose it? *Med Sci Sports Exerc* **42**,
1020 1644-1650.

1021

1022 Power GA, Dalton BH, Doherty TJ & Rice CL. (2016). If you don't use it you'll likely lose
1023 it. *Clin Physiol Funct Imaging* **36**, 497-498.

1024

1025 Powers RK, Elbasiouny SM, Rymer WZ & Heckman CJ. (2012). Contribution of intrinsic
1026 properties and synaptic inputs to motoneuron discharge patterns: a simulation study.
1027 *J Neurophysiol* **107**, 808-823.

1028

1029 Powers RK & Heckman CJ. (2015). Contribution of intrinsic motoneuron properties to
1030 discharge hysteresis and its estimation based on paired motor unit recordings: a
1031 simulation study. *J Neurophysiol* **114**, 184-198.

1032

1033 Powers RK, Nardelli P & Cope TC. (2008). Estimation of the contribution of intrinsic
1034 currents to motoneuron firing based on paired motoneuron discharge records in the
1035 decerebrate cat. *J Neurophysiol* **100**, 292-303.

1036

1037 Revill AL & Fuglevand AJ. (2011). Effects of persistent inward currents, accommodation,
1038 and adaptation on motor unit behavior: a simulation study. *J Neurophysiol* **106**, 1467-
1039 1479.

1040

- 1041 Revill AL & Fuglevand AJ. (2017). Inhibition linearizes firing rate responses in human motor
1042 units: implications for the role of persistent inward currents. *J Physiol* **595**, 179-191.
- 1043
- 1044 Roos MR, Rice CL, Connelly DM & Vandervoort AA. (1999). Quadriceps muscle strength,
1045 contractile properties, and motor unit firing rates in young and old men. *Muscle Nerve*
1046 **22**, 1094-1103.
- 1047
- 1048 Scaglioni G, Narici MV, Maffiuletti NA, Pensini M & Martin A. (2003). Effect of ageing on
1049 the electrical and mechanical properties of human soleus motor units activated by the
1050 H reflex and M wave. *J Physiol* **548**, 649-661.
- 1051
- 1052 Schwindt PC & Crill WE. (1980). Properties of a persistent inward current in normal and
1053 TEA-injected motoneurons. *J Neurophysiol* **43**, 1700-1724.
- 1054
- 1055 Shibata E, Sasaki M, Tohyama K, Kanbara Y, Otsuka K, Ehara S & Sakai A. (2006). Age-
1056 related changes in locus ceruleus on neuromelanin magnetic resonance imaging at 3
1057 Tesla. *Magn Reson Med Sci* **5**, 197-200.
- 1058
- 1059 Steinbusch HWM, Dolatkhah MA & Hopkins DA. (2021). Anatomical and neurochemical
1060 organization of the serotonergic system in the mammalian brain and in particular the
1061 involvement of the dorsal raphe nucleus in relation to neurological diseases. *Prog*
1062 *Brain Res* **261**, 41-81.
- 1063
- 1064 Swash M & Fox KP. (1972). The effect of age on human skeletal muscle. Studies of the
1065 morphology and innervation of muscle spindles. *J Neurol Sci* **16**, 417-432.
- 1066
- 1067 Tatton WG, Greenwood CE, Verrier MC, Holland DP, Kwan MM & Biddle FE. (1991).
1068 Different rates of age-related loss for four murine monoaminergic neuronal
1069 populations. *Neurobiol Aging* **12**, 543-556.
- 1070
- 1071 Thompson CK, Negro F, Johnson MD, Holmes MR, McPherson LM, Powers RK, Farina D
1072 & Heckman CJ. (2018). Robust and accurate decoding of motoneuron behaviour and
1073 prediction of the resulting force output. *J Physiol* **596**, 2643-2659.
- 1074
- 1075 Tomlinson BE & Irving D. (1977). The numbers of limb motor neurons in the human
1076 lumbosacral cord throughout life. *J Neurol Sci* **34**, 213-219.
- 1077

1078 Trajano GS, Taylor JL, Orssatto LBR, McNulty CR & Blazeovich AJ. (2020). Passive muscle
1079 stretching reduces estimates of persistent inward current strength in soleus motor
1080 units. *J Exp Biol* **223**.

1081
1082 Yavuz US, Negro F, Sebik O, Holobar A, Frommel C, Turker KS & Farina D. (2015).
1083 Estimating reflex responses in large populations of motor units by decomposition of
1084 the high-density surface electromyogram. *J Physiol* **593**, 4305-4318.

1085

1086