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RESEARCH ARTICLE

Does strict validation criteria for individual motor units alter population-based regression models of the motor unit pool?

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Abstract

The purpose of this study was to determine if the implementation of a strict validation procedure, designed to limit the inclusion of inaccuracies from the decomposition of surface electromyographic (sEMG) signals, affects population-based motor unit (MU) analyses. Four sEMG signals were obtained from the vastus lateralis of 59 participants during isometric contractions at different relative intensities [30%, 70%, and 100% of maximal voluntary contraction (MVC)], and its individual motor unit potential trains (MUPTs) were extracted. The MUPTs were then excluded (ISIval) based on the coefficient of variation and histogram of the interspike intervals (ISI), the absence of additional clusters that reveals missed or additional firings, and more. MU population-based regression models (i.e., modeling the entire motor unit pool) were performed between motor unit potential size (MUP_{SIZE}), mean firing rate (MFR), and recruitment threshold (RT%) separately for $DSDC_{Only}$ (includes all MUPTs without the additional validation performed) and ISIval data at each contraction intensity. The only significant difference in regression coefficients between $DSDC_{Only}$ and ISIval was for the intercepts of the MUP_{SIZE}/MFR at 100% MVC. The validation had no other significant effect on any of the other regression coefficients for each of the contraction intensities. Our findings suggest that even though the decomposition of surface signals leads to some inaccuracies, these errors have limited effects on the regression models used to estimate the behavior of the whole pool. Therefore, we propose that motor unit population-based regression models may be robust enough to overcome decomposition-induced errors at the individual MU level.

Keywords Decomposition of surface electromyography · Recruitment threshold · Mean firing rate · Action potential size

Introduction

The electrical activity of human muscles during contraction can be assessed using electromyographic (EMG) electrodes placed either inside (intramuscular EMG) or on the skin over (surface EMG) the muscle of interest (De Luca et al. 2006; Latash 2008). Regardless of the method being used to record the electrical activity, the obtained interference signal will reflect the composite of motor unit potentials (MUPs) from muscle fibers pertaining to multiple motor units (MUs) (De Luca et al. 2006; Latash 2008). Since these MUPs can cancel each other out when summated (Keenan et al. 2005), the amplitudes of interference signals provide a poor estimate of the neural drive that the central nervous system uses to control motor units (Dideriksen and Farina 2019; Martinez-Valdes et al. 2018). Therefore, techniques have been developed to decompose the interference EMG signals into the constituent MUPTs

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to further our understanding of motor control (e.g., Chen and Zhou 2015; De Luca et al. 2006; Holobar and Zazula 2007; Negro et al. 2016). Initially, the EMG decomposition method was developed for signals obtained with intramuscular EMG (iEMG). However, the small yield of MUs sampled and the invasiveness of iEMG led to the natural progression to decomposition methods for surface EMG (sEMG) signals (Chen and Zhou 2015; De Luca et al. 2006; Holobar and Zazula 2007; Negro et al. 2016).

Generally speaking, decomposition of interference EMG signals consists of identifying individual MUP shapes that repeat on a regular basis and assigning them to individual MUPTs (De Luca et al. 2006; Farina and Holobar 2016; Holobar and Zazula 2004; LeFever and De Luca 1982; Mambrito and De Luca 1984). Nevertheless, as more MUs are detected (e.g., at higher contraction intensities), the superposition (i.e., overlap) of multiple MUP shapes makes it increasingly difficult to distinguish the activity of separate motor units (especially those associated with small MUPs) (Hu et al. 2014a). Several methods to decompose the sEMG signal have been proposed, such as the "Precision Decomposition III" (PDIII) algorithm, developed by De Luca et al. (2006) and later improved by Nawab et al. (2010), and the "convolutive blind source separation" method, developed by Holobar and Zazula (2007).

A major advantage the sEMG decomposition has over iEMG is the increased pickup area, which leads to a higher yield of MUs sampled with a larger range of contraction intensities (Colquhoun et al. 2018; De Luca and Hostage 2010; Farina et al. 2010; Hu et al. 2014b). Therefore, sEMG decomposition is uniquely positioned for population-based regression models, which allow extrapolation to the entire MU pool. However, the decomposition algorithms introduce an additional source of error, as the superposition of MUPs mentioned previously can lead to missed or additional firings for each MU sampled. Therefore, during the last decade, concerns regarding the validity of the PDIII method have been raised publicly (e.g., De Luca and Nawab 2011; Farina and Enoka 2011) which has led to multiple studies to explore the validity by an independent research group (Hu et al. 2012, 2013, 2014a, b). One validation technique that has recently been applied is to spike-trigger average the interference EMG signals from the decomposed firings, and then examine the variation of the waveforms (e.g., McManus et al. 2016). Another technique recently recommended is to validate motor units based on their firing variability. For example, under the assumption that motor unit interspike intervals (ISIs) show a Gaussian distribution, as shown by Clamann (1969), Hu et al. (2014b) examined the ISI distributions of common MUs obtained from iEMG and sEMG. They found that MUs with a high number of correctly identified firings showed a Gaussian ISI distribution.

As such, it is possible that a strict validation of firings, such as the one performed by Hu et al. (2014b) using ISI distributions, may be necessary to accurately model the MU pool. Therefore, the purpose of this study was to determine if a strict validation procedure to limit the inclusion of decomposition-based inaccuracies affects population-based MU analyses. We hypothesized that these types of regression models are robust, and would be minimally affected by the occasional errors in firing times of individual MUPTs.

Methods

Experimental design

This study consists of aggregated data from two separate experiments, each including motor unit recordings of the vastus lateralis (VL) muscle during isometric knee extensions. Experiment 1 consisted of a ramp and hold contraction at 100% of maximal voluntary contraction (MVC). Experiment 2 consisted of two submaximal ramp and hold contractions: one at 30% MVC, and the other at 70% MVC performed by each subject in different days. Mean firing rate (MFR), recruitment threshold (RT%), and peak-to-peak action potential amplitude (MUP_{SIZE}) were calculated from individual MUPs from each of the three contraction intensities (30%, 70%, and 100% MVC) after undergoing two different analysis conditions. The first condition ($DSDC_{Only}$) used only the pairs (to compare against the second condition) of the commonly utilized "Decompose-Synthesize-Decompose-Compare" accuracy test prior to calculating the MU variables. The second condition (*ISIval*) included the same accuracy test followed by an additional, strict validation process using ISI distribution and variability as the primary criteria.

Participants

After approval by the university's Institutional Review Board, 32 subjects for experiment 1, and 27 subjects for experiment 2, between the ages of 19 and 74 agreed to participate in our study. All participants completed an informed consent and a health questionnaire where they reported not having any neuromuscular or musculoskeletal problem in their dominant leg.

Isometric contractions

The participants sat in an upright position in an isokinetic dynamometer (Biodex System 4; Biodex Medical Systems, Shirley, NY, USA) with a knee angle of 120° and hip angle of ~ 100°. In Experiment 1 (100% MVC), the torque (Nm) signal from the dynamometer was recorded and utilized for later calculations, while in Experiment 2 (30–70% MVC), force (N) was recorded from an S-beam load cell (Model SSM-AJ-500; Interface, Scottsdale, AZ, USA). Participants warmed up and familiarized with the protocol by performing submaximal ramp and hold contractions. For all ramp contractions, participants had to follow a force trajectory that was displayed on the screen overlaid with real-time torque/force feedback. Before participants successfully traced each ramp and hold contraction, they performed three MVCs lasting 3–4 s (2–3 min rest between trials); the highest 100 ms epoch was considered peak MVC and was used to normalize each participant's ramp and hold contractions. Each participant, depending on the experiment and visit, then performed a ramp and hold contraction at either 100%, 70% or 30% MVC. The duration and rate of each ramp and hold contraction varied: 100% MVC lasted 15 s with a 5 s plateau and the ramps increasing/decreasing at a rate of ± 20% MVC/s; the 70% MVC tracing lasted 18 s with a 10 s plateau at the target force and the ramps increasing/decreasing at a rate of ± 17.5% MVC/s; and the 30% MVC tracing lasted 16 s with a 10 s plateau and the ramps increasing/decreasing at a rate of ± 10% MVC/s.

Motor unit potential recordings and processing

Four separate sEMG signals were recorded from each contraction using a 5-pin array sensor (Delsys, Inc., Natick, MA) placed over the VL muscle at either 2/3 (Experiment 1) or 1/2 the distance (Experiment 2) between the center of the muscle and the lateral condyle of the femur's dominant leg (Zaheer et al. 2012). The reference electrode was placed over the spinous process of the C7 vertebrae during both experiments. Before electrode placement, the skin surface was prepared by removing hair, abrading, and cleansing it with alcohol (Isopropyl 70%).

Torque/Force and EMG signals were sampled simultaneously at a rate of 20 kHz using a 16-channel acquisition system (Bagnoli system, Delsys Inc., Natick, MA, USA). Motor unit trains were then decomposed using the "Precision Decomposition III" algorithm. Once decomposed, only those MUPTs with an accuracy > 90% as determined by the "Decompose-Synthesize-Decompose-Compare" test with dEMG Analysis Software 1.1.3 (Delsys, Inc., Natick, MA) were kept for signal processing and validation. After decomposition, motor unit action potential trains were exported and analyzed with a custom-written LabVIEW program (LabVIEW 18.0; National Instruments, Austin, TX, USA), which calculated the following variables for each MUPT: RT% (the % of MVC at the onset of firing), MUP_{SIZE} (the peak-to-peak amplitude of the averaged waveform template, as measured by Pope et al. (2016)), MFR (the mean during the plateau of a smoothed MFR curve, smoothed with a 1-s Hanning window), and ISI (the time, in ms, between each firing). In addition, the mean, coefficient of variance (CoV; standard deviation normalized by the mean), minimum and maximal values, and range were calculated from the ISI's for subsequent data analysis.

For the *ISIval* condition, ISI histograms were displayed, along with the RT%, CoV, ISI range (ms) and mean for each MU.

Manual decisions were then made (by J.H.S.) to keep or discard each individual MUPT based on the following criteria: the ISI histogram must have had a normal or positive kurtotic distribution, a CoV < 30%, a range of ≤ 100 ms; absence of any firings prior to force onset (i.e., RT% > 0%); an absence of excess (> 2) counts (i.e. distinct, separate clusters) in regions which could indicate missed or additional firings. MUPTs with bimodal ISI histograms were immediately discarded as they likely reflect either poor decomposition accuracy or contributions originating from two distinct MUs erroneously being identified as one (Hu et al. 2014b). Examples of these ISI histograms along with their accompanying decisions (keep or discard) are provided in Fig. 1. Additionally, an entire contraction was discarded if it did not have at least eight MUPTs and there was not a sufficient spread/distribution in the RT% values of the MUs sampled. Sufficient spread was defined as a RT% range of at least 25% for the 100% and 70% MVC contractions, and at least a 10% range for the 30% MVC contractions.

Statistical analyses

Simple linear regression coefficients (slopes and y-intercepts) and exponential regression coefficients (decay rates and intercepts) were calculated for $DSDC_{Only}$ and *ISIval*. Specifically, linear regression coefficients were calculated for the RT% vs MFR relationship at each contraction intensity (30%, 70%, and 100% MVC), and for the MUP_{SIZE} vs MFR and RT% vs MUP_{SIZE} at 30% MVC, respectively. Exponential regression was performed for the MUP_{SIZE} vs MFR and RT% vs MUP_{SIZE} at 70% and 100% MVC, as used in Contessa et al. (2016); Herda et al. (2019); Miller et al. (2018); Sterczala et al. (2018). Using SPSS Statistics 24 (International Business Machines Corp., Armonk, NY, USA), a one-way ANOVA was used to examine the CoV of $DSDC_{Only}$ among contraction intensities. Follow-up analysis included a Bonferroni post hoc test to determine any statistical difference between contraction intensities. A dependent samples *t* test was used to examine the mean CoV of MUPT ISIs for each subject for all conditions [*ISIval* MUPTs vs. Discarded MUPTs (*Discard*)] and to examine the regression coefficients between $MUP_{SIZE}/MFR_{100\%}$, RT% /$MUP_{SIZE100\%}$, and RT% /$MFR_{100\%}$. Several Wilcoxon Signed-Rank tests, due to a small sample size, were used to examine the regression coefficients between $MUP/MFR_{30\%}$, $MUP/MFR_{70\%}$, RT%/$MUP_{SIZE30\%}$, RT%/$MUP_{SIZE70\%}$, RT%/

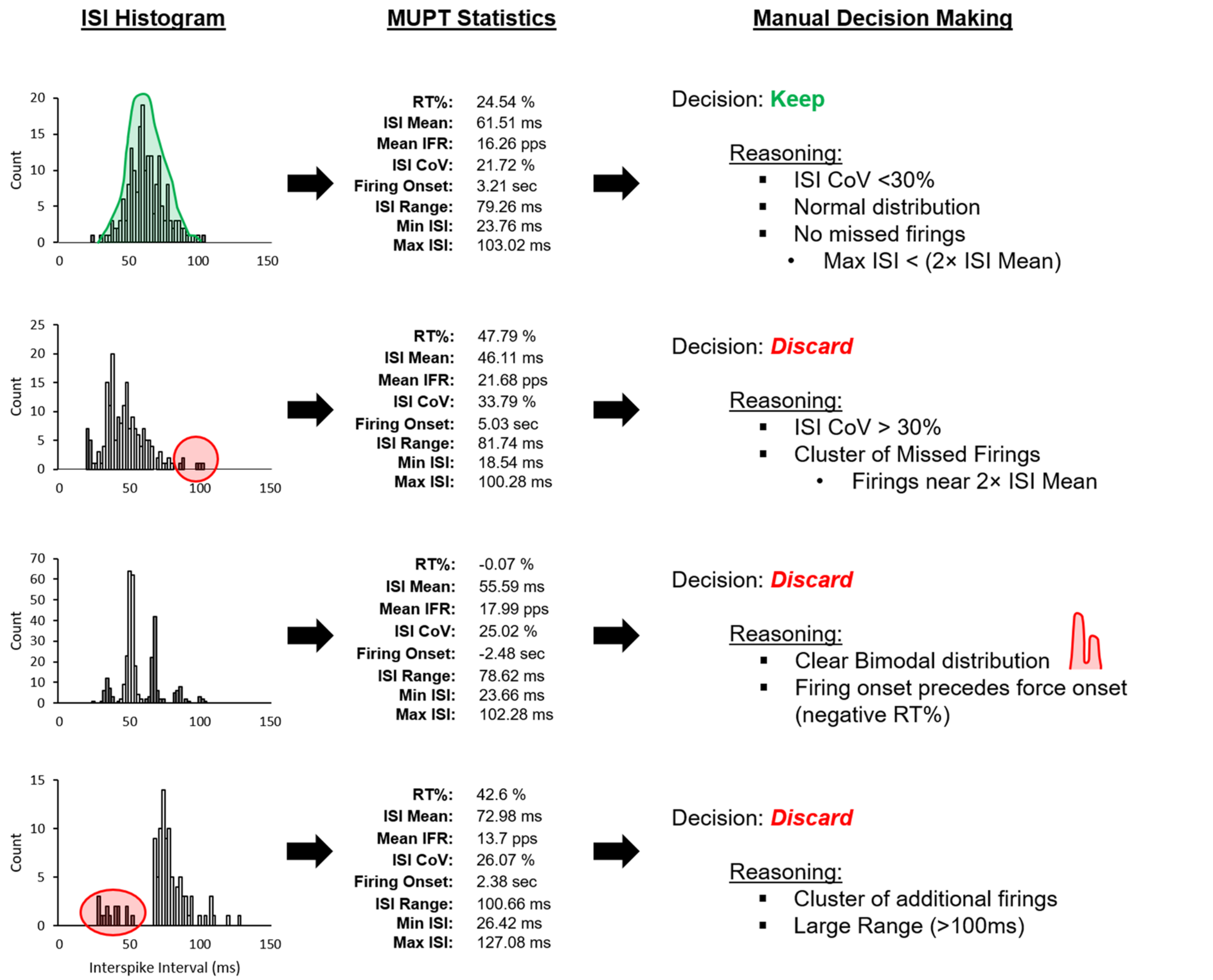

Fig. 1 Four examples of decisions to keep or discard motor unit potential trains based on the interspike interval (ISI) validation criteria. RT%: relative recruitment threshold, *IFR* instantaneous firing rate, *CoV* coefficient of variation

$MFR_{30\%}$, and RT%/$MFR_{70\%}$. Two-way [condition ($DSDC_{Only}$ and *ISIval*) × contraction (30%, 70%, and 100%)] repeated measures ANOVAs were not utilized because there was no interest in examining relationships between contraction levels (e.g., it is already known that MFR increases as intensity increases). To account for familywise error rates, our a priori alpha level was Bonferroni corrected (0.05/3 = 0.016) for testing regression coefficients within each contraction intensity (Vincent and Weir 2012). Therefore, alpha < 0.016 was used to identify potential meaningful differences.

Results

The decomposed sEMG signal from 85 contractions, belonging to 59 subjects, passed the "Decompose-Synthesize-Decompose-Compare test" set to an accuracy ≥ 90%. From these decomposed sEMG signals, a total of 27 contractions—(100% MVC = 16, 70% MVC = 5, 30% MVC = 6) passed the strict criteria to be part of the *ISIval* condition, with none of them, from experiment 2, being from the same subject. $DSDC_{Only}$ consisted of 493 MUPTs ($DSDC_{Only100\%} = 283$, $DSDC_{Only70\%} = 104$, and $DSDC_{Only30\%} = 106$), from these, 334 (67.7%) passed the strict criteria to be part of the *ISIval* condition ($ISIval_{100\%} = 63.25\%$, $ISIval_{70\%} = 70.19\%$, and $ISIval_{30\%} = 77.36\%$), and 159 did not pass ($Discard_{100\%} = 36.75\%$, $Discard_{70\%} = 29.81\%$, and $Discard_{30\%} = 22.64\%$), as shown in Fig. 2.

Coefficient of variance

A visual depiction of *Discard* (red crosses) and *ISIval* at 30% MVC (a), 70% MVC (b), 100%MVC (c) and the linear regressions of CoV vs RT% for the *ISIval* (d) are shown in Fig. 3. A dependent samples t test showed a significant difference in CoV for *ISIval* (21.83 ± 2.2%) and *Discard* (25.85 ± 2.89%); t (26) = 8.88, p < 0.001. $DSDC_{Only100\%}$ (24.14 ± 4.31) had a significantly higher CoV ($p < 0.01$) than

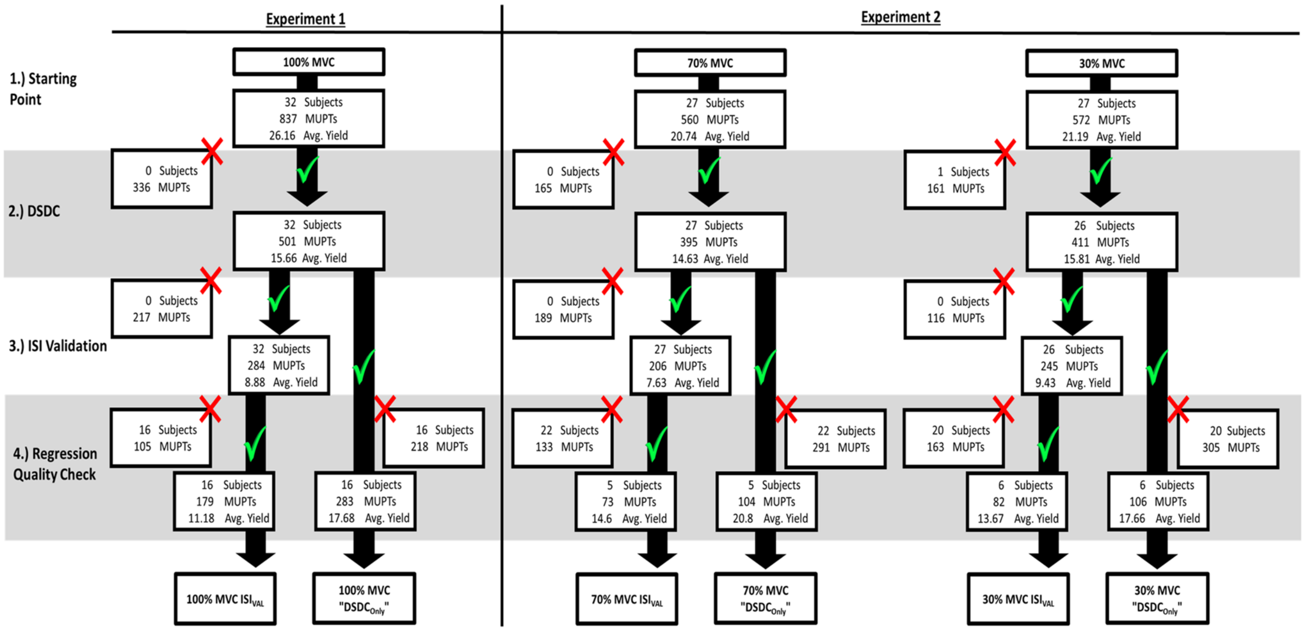

Fig. 2 Visual depiction of the steps taken to obtain the ISI_{val} and $DSDC_{Only}$ datasets from experiment 1 and 2. *MUPT* motor unit potential train, *DSDC* decompose-synthesize-decompose-compare test, *MVC* maximal voluntary contraction, *ISI* interspike interval

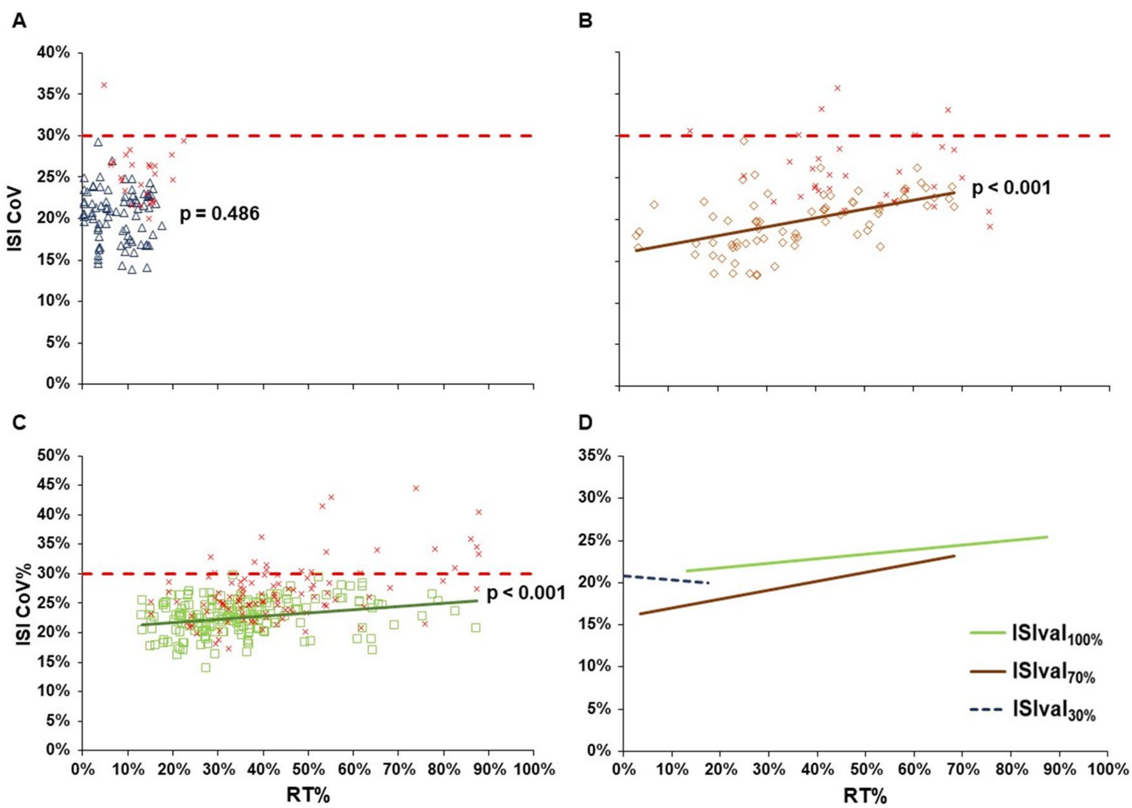

Fig. 3 The interspike interval (ISI) coefficient of variation (CoV) for each motor unit potential train plotted as a function of its recruitment threshold (RT%). Plots A, B, and C show the motor units detected at 30%, 70%, and 100% MVC, respectively. The red X's are the discarded motor units, which are not included in the regressions. Plot D shows the same 3 regression lines from **a**–**c** for easy comparison. Note that the dashed regression line in plot **d** was not significant, as shown in Plot (**a**)

$DSDC_{Only70\%}$ (21.61 ± 4.52) and $DSDC_{Only30\%}$ (21.54 ± 3.78), as revealed by one-way ANOVA post hoc test. Moreover, Fig. 4 depicts the amount of MUPTs included and excluded as a function of RT% for each contraction intensity.

Motor unit potential size and mean firing rate

As shown in Fig. 5, a dependent samples t test revealed a no significant change in decay rate ($p = 0.154$) but a significant difference in intercepts ($p = 0.014$) for the 100% MVC condition. Wilcoxon Signed-Rank Test revealed no significant change in decay rate ($p = 0.144$) and y-intercepts ($p = 0.144$), and in slope ($p = 0.116$) and y-intercept ($p = 0.028$), for the 70% MVC and 30% MVC conditions, respectively.

Recruitment threshold and motor unit potential size

Comparing the $DSDC_{Only}$ and *ISIval*, dependent samples t test revealed no significant change in decay rate ($p = 0.077$) and intercepts ($p = 0.028$), for the 100% MVC condition. Wilcoxon Signed-Rank Test revealed a non-significant change in decay rate ($p = 0.715$) and y-intercepts ($p = 0.465$), and in slope ($p = 0.112$) and y-intercept ($p = 0.028$), for the 70% MVC and 30% MVC conditions, respectively (see Fig. 6).

Recruitment threshold and mean firing rate

A dependent samples t test revealed no significant difference, between $DSDC_{Only}$ and *ISIval*, in slope ($p = 0.112$) and y-intercepts ($p = 0.072$), for the 100% MVC condition. Wilcoxon Signed-Rank Test revealed a non-significant change, between $DSDC_{Only}$ and *ISIval*, in the 70% MVC slope ($p = 0.5$) and y-intercept ($p = 1$), and in the 30% MVC slope ($p = 0.345$) and y-intercept ($p = 0.075$), as seen in Fig. 7.

Discussion

The purpose of this study was to determine if a strict validation procedure to limit the inclusion of decomposition-based inaccuracies affects population-based motor unit analyses. Therefore, we thoroughly examined the ISIs of individual MUPTs, as proposed by Hu et al. (2014b), and compared regression coefficients for both conditions ($DSDC_{Only}$ and *ISIval*). The primary findings were that (1) the slopes or decay rates of the $DSDC_{Only}$ and *ISIval* conditions were

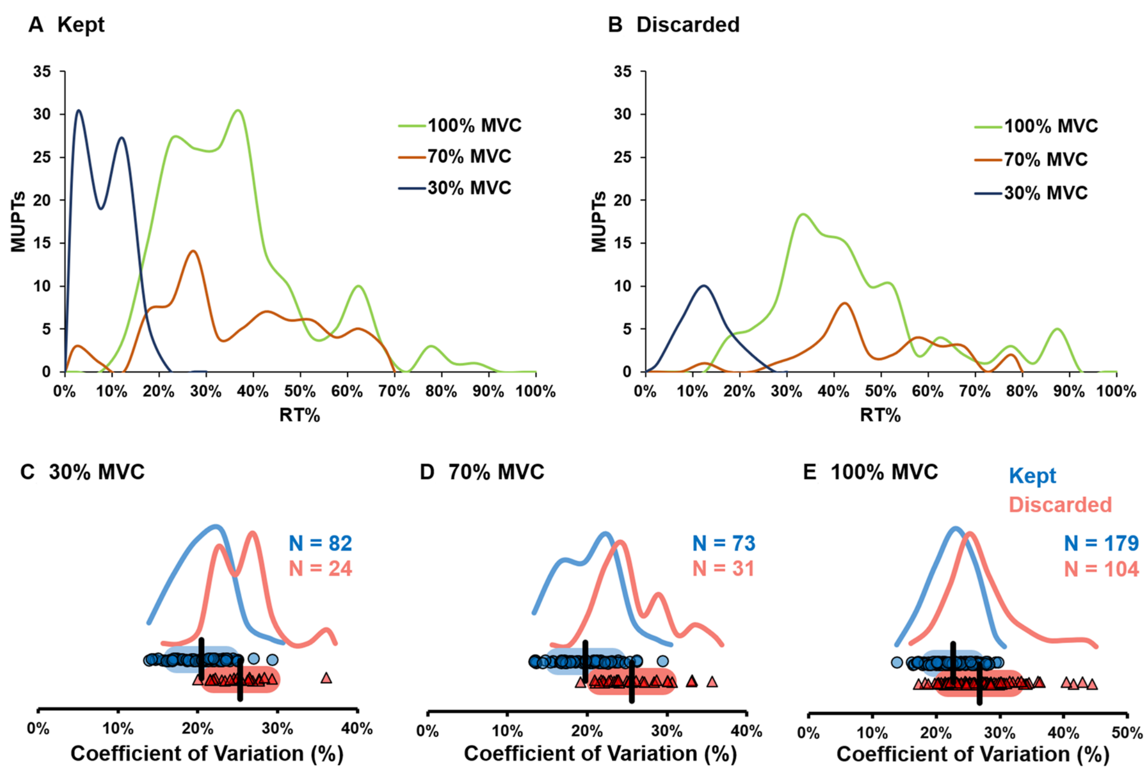

Fig. 4 **a** The count and distribution of motor unit potential trains (MUPTs) that passed the ISI validation criteria based on their recruitment threshold (RT%). **b** The count and distribution of MUPTs that were discarded (i.e., did not pass the validation). **c**–**e** The relative count and distribution of MUPTs based on their coefficient of variation (CoV) for each contraction intensity (30%, 70%, and 100% MVC). Blue = MUPTs that passed validation and were kept, Red = MUPTs that were discarded

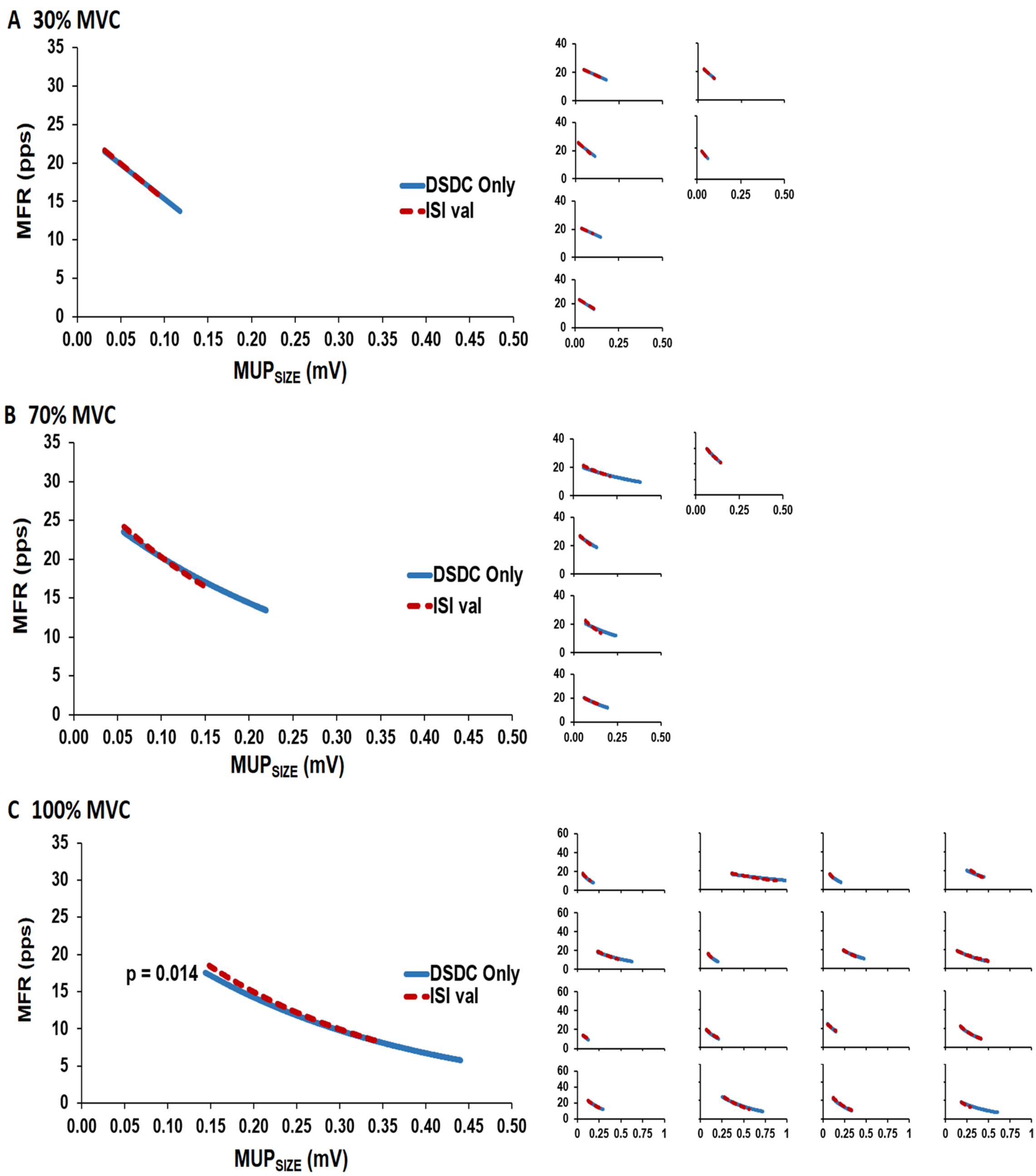

Fig. 5 Regression models for mean firing rates (MFR) as a function of their motor unit potential amplitude (MUP_{SIZE}) at 30% (plot **a**), 70% (plot **b**), and 100% (plot **c**) of maximal voluntary contraction (MVC). The regression lines in the large plots represent the mean decay rate and intercept from the individual-subject regressions (smaller plots on the right). The blue solid lines represent all of the motor unit potential trains (i.e., without the validation), and the red lines represent the motor unit potential trains that passed the inter-spike interval validation criteria (ISIval)

not statistically different for any relationship, but (2) the intercept for the MUP_{SIZE}/MFR relationship at 100%MVC ($MUP_{SIZE}/MFR_{100\%}$) did differ ($p = 0.014$). Secondary findings show that (3) there was a significant difference ($p < 0.001$) in ISI variance between the MUPTs that passed the validation (*ISIval)* and those that were discarded. Finally, (4) there was a significantly higher CoV in the MUPTs ISI's during the 100% MVC contraction compared to the submaximal contractions ($p < 0.01$).

As mentioned above, slopes and/or decay rates were not statistically different for any of the relationships at any contraction intensity. This would suggest that regression models to estimate and extrapolate the behavior of the entire motor unit pool from a small number of MUPTs (~ 13 MUPTs/

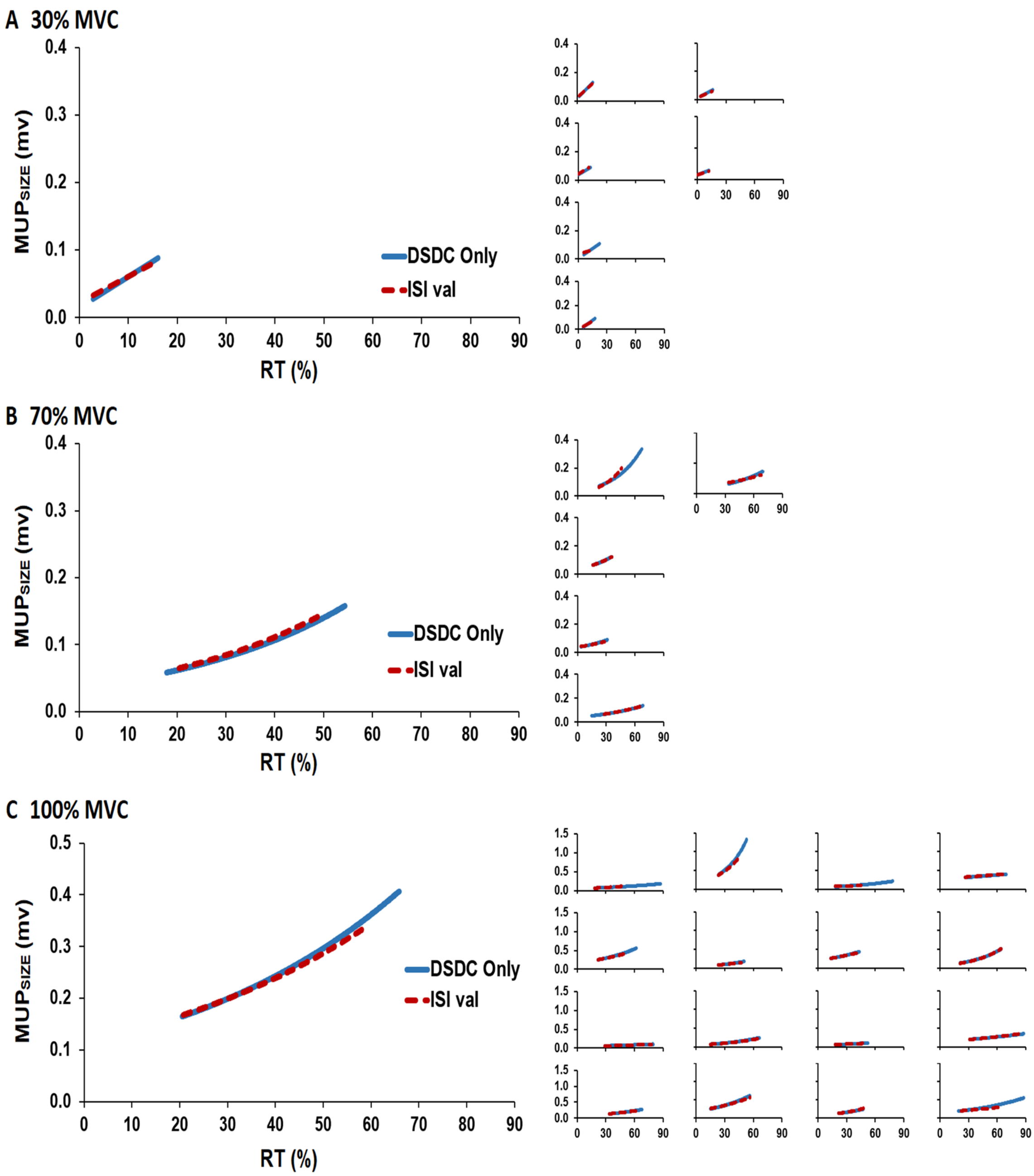

Fig. 6 Regression models for motor unit potential size (MUP_{SIZE}) as a function of their recruitment threshold (RT%) at 30% (plot **a**), 70% (plot **b**), and 100% (plot **c**) of maximal voluntary contraction (MVC). The regression lines in the large plots represent the mean decay rate and intercept from the individual-subject regressions (smaller plots on the right). The blue solid lines represent all of the motor unit potential trains (i.e., without the validation), and the red lines represent the motor unit potential trains that passed the interspike interval validation criteria (ISIval)

contraction in the present study) may be robust enough to overcome high ISI variability or decomposition-based inaccuracies in a few of the MUPTs. As such, decomposition inaccuracies in a few of the individual firings may not affect that MUPT's utility and ability to contribute towards estimating behavior of the pool. However, some caution is warranted as the intercept coefficient of the $MUP_{SIZE}/MFR_{100\%}$ model was significantly altered by the inclusion of MUPTs that did not meet the strict validation criteria. Therefore, while robust, population-based analyses of MUs are certainly not unsusceptible to inaccuracies.

It is also worth mentioning that there is a tradeoff to requiring that each MUPT meets a strict criteria, as this could substantially decrease the MU yield from a given

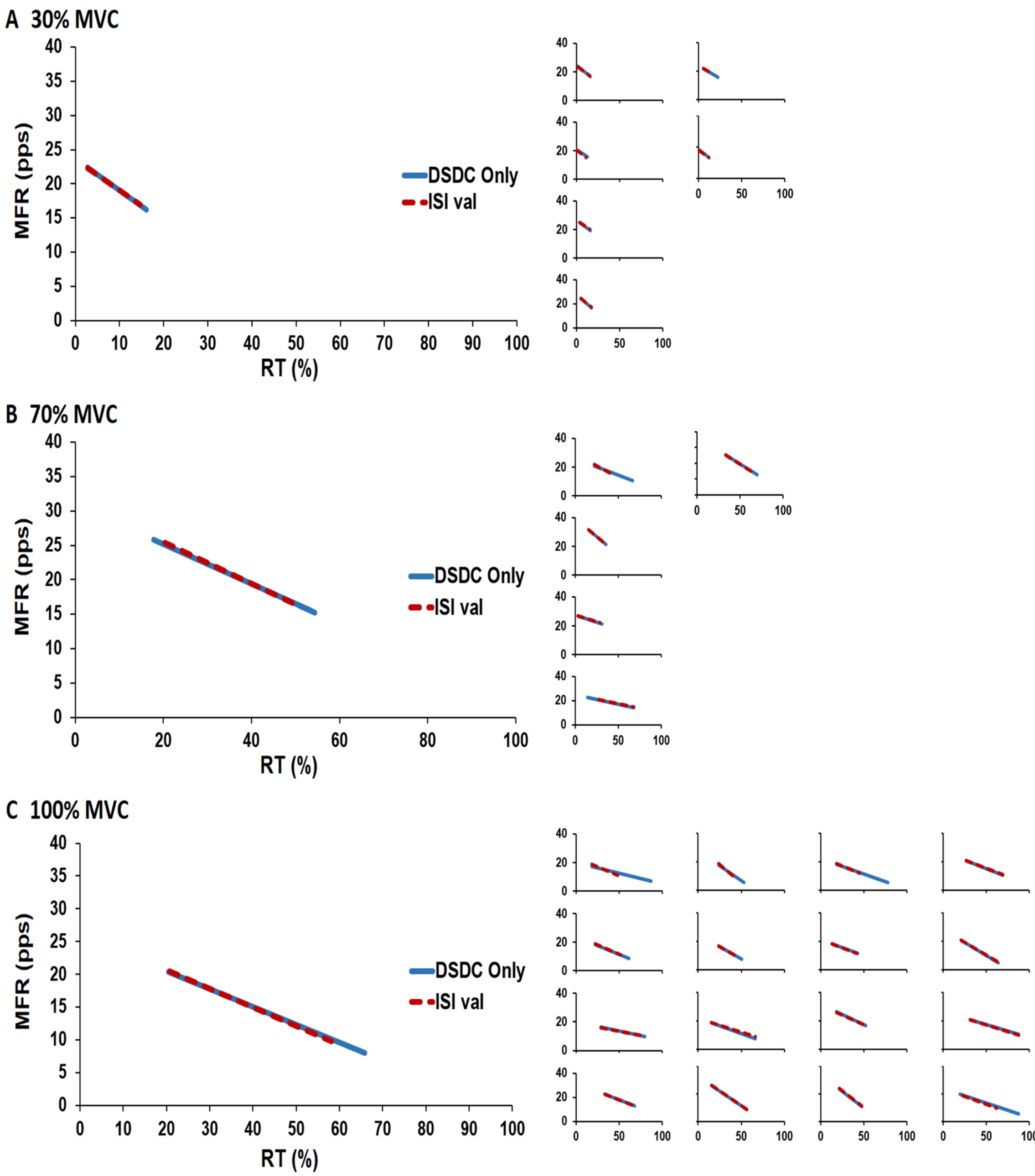

Fig. 7 Regression models for mean firing rate (MFR) as a function of their recruitment threshold (RT%) at 30% (plot **a**), 70% (plot **b**), and 100% (plot **c**) of maximal voluntary contraction (MVC). The regression lines in the large plots represent the mean slope and intercept from the individual-subject regressions (smaller plots on the right). The blue solid lines represent all of the motor unit potential trains (i.e., without the validation), and the red lines represent the motor unit potential trains that passed the interspike interval validation criteria (ISIval)

contraction (> 30% of the MUPTs were eliminated in the present study). Given that the predictive powers of regression models are heavily reliant on sample size (n), the elimination of too many MUPTs (e.g., decreased n) may also lead to the elimination of many contractions. In the present study, we discarded ~ 68% of our initial contractions (58 eliminated of 85 total) because they no longer met our minimum criteria to fit with a population-based regression model (at least 8 MUs with a sufficient spread or range of RT%). CoV was significantly different ($p < 0.001$) between *ISIval* (21.83 ± 2.2%) and *Discard* (25.85 ± 2.89%). Mean CoV of *Discard* is < 30% due to other, non-CoV-related exclusion criteria, as shown in Fig. 1. CoV has been used as an indicator of variance in different studies examining the decomposed signal of surface EMG (e.g. Hu et al. 2013, 2014a, b). Based on the statistical distribution findings of Clamann (1969) regarding motor unit firing patterns in skeletal muscle, Hu et al. (2014b) performed a two-source validation by examining the ISI histograms of common MUs, sampled using both, iEMG and sEMG. For each common MU, they measured the accuracy percentage of individual MU firings by counting the number of correctly identified firings (same firings detected by iEMG and sEMG) divided by the total number of firings (correctly identified firings plus false positives and negatives, as explained later). Hu et al. (2014b) found that, after visual inspection, most of the ISI histograms showed a Gaussian distribution, as expected based on findings of Clamann (1969). Specifically, those with high accuracy had little or absence of secondary peaks at the short ISI distribution (false positives) and/or at the long ISI distribution (false negatives). In addition, from 67 common MUs, Hu et al. (2014b) found a significant negative correlation ($r=-0.65$, $p<0.001$) between accuracy percentage and CoV, in other words, the less variation of ISIs, the more accurate the identified firings. As shown in Fig. 3 of Hu et al. (2014b), most of the accurate MUs (~90%) showed a coefficient of variation ≤ 35%. Holobar et al. (2010) found that at CoV < 30%, the rate of agreement of firings between iEMG and HD-sEMG signals was high (~84). Also, they found that CoV was significantly correlated to decomposition accuracy. Based on the mentioned studies, it seems likely that a CoV < 30%, like the one used in this study, can accurately identify valid and spurious firings of individual MUs, therefore eliminating potential errors produced during the "Decompose-Synthesize-Decompose-Compare test".

Differences in the mean intercept of the MUP_{SIZE} vs. MFR relationship at 100% MVC, after Bonferroni correction, might be explained by the lack of statistical significance of some of the relationships within individual subjects, and the superposition of APs at higher forces. One subject's MUP_{SIZE} vs. MFR relationship in $DSDC_{Only100\%}$ and one subject's in $ISI_{val100\%}$ were not significant (R^2 0.292–0.305, $p>0.05$) (shown in Supplementary Data – Table 1). Furthermore, as shown in Fig. 5 and 6, even though the intercept of MUP_{SIZE} vs. MFR (at 30% MVC) and the intercept of RT% vs. MUP_{SIZE} (at 30% and 100%) were not statistically significant (p > 0.016), they showed a p-value < 0.05. Coincidentally, the same relationships, excluding the 30% MVC MUP_{SIZE}, vs. MFR, showed a non-statistically significant relationship (R^2 0.184–0.388, $p>0.05$) for some subjects (shown in Supplementary Data – Table 1). Regarding superposition of APs at high forces, it remains to be known to what extent the lack of detection of smaller, very low threshold MUs during the high-force contractions (see Fig. 3c and Fig. 4a, b) might have influenced the results of the regression models (both $DSDC_{Only}$ and *ISIval*). Therefore, caution should be taken to make inferences.

Limitations

First, one of the limitations of this study is that, to our knowledge, all the studies examining the ISI histograms and its CoV are based on findings using iEMG. As mentioned in the introduction section, iEMG is often limited to low force contractions, and just a few motor units are obtained per contraction (e.g., a small yield), Therefore, ISI characteristics of the motor unit pool and MUs at high-force contractions remain unknown, especially as recorded from multi-channel surface EMG. Second, as shown in Fig. 3 by Hu et al. (2014b), some MUs with an ISI CoV > 0.3 (30%) might show an accuracy ≥ 90%. As a result, some of the discarded MUs may have still been accurate and able to contribute to the population-based models. Third, the short duration of the ramp-up (5 s) of the 100% MVC contraction provides an additional challenge to the decomposition algorithm since a short ramp-up makes the location of recruitment times difficult to identify (as mentioned by Nawab et al. (2010)). However, while a longer ramp with slower, more subtle increases in force may improve the accuracy of detecting RT% (and minimize the effects of neuromechanical delay), it may also lead to fatiguing effects.

Conclusion

Our primary finding was that, after strict examination of ISI histograms of individual MUs obtained from sEMG decomposition, the additional validation had little to no effect on the population-based models of motor unit action potential amplitude (AP_{SIZE}) and firing properties (MFR and RT%). However, there was one exception in that the inclusion of motor units that did not meet our strict validation criteria did affect the mean intercept coefficient of the AP_{SIZE} vs. MFR model during maximal contractions. We also found that implementation of strict validation criteria comes with the consequence in that the motor unit yield or number of usable contractions may be substantially reduced. We propose that, even though the decomposition of surface signals leads to some inaccuracies in motor unit firing times, overall the motor unit population-based models may be robust enough to overcome many of the errors at the individual motor unit level.

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