

1 **TITLE: A computational approach for generating smooth estimates of motor unit**
2 **discharge rates and visualizing population discharge characteristics**

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19 **ABSTRACT**

20 *Objective:* Successive improvements in high density surface electromyography and decomposition
21 techniques have facilitated an increasing yield in decomposed motor unit (MU) spike times. Though these
22 advancements enhance the generalizability of findings and promote the application of MU discharge
23 characteristics to inform the neural control of motor output, limitations remain. Specifically, 1) common
24 approaches for generating smooth estimates of MU discharge rates introduce artifacts in quantification,
25 which may bias findings, and 2) discharge characteristics of large MU populations are often difficult to
26 visualize. *Approach:* In the present study, we propose support vector regression (SVR) as an improved
27 approach for generating continuous estimates of discharge rate and compare the fit characteristics of SVR
28 to traditionally used methods, including Hanning window filtering and polynomial regression. Furthermore,
29 we introduce ensembles as a method to visualize the discharge characteristics of large MU populations. We
30 define ensembles as the average discharge profile of a subpopulation of MUs, composed of a time
31 normalized ensemble average of all units within this subpopulation. Analysis was conducted with MUs
32 decomposed from the tibialis anterior (N = 2128), medial gastrocnemius (N = 2673), and soleus (N = 1190)
33 during isometric plantarflexion and dorsiflexion contractions. *Main Result:* Compared to traditional
34 approaches, we found SVR to alleviate commonly observed inaccuracies and produce significantly less
35 absolute fit error in the initial phase of MU discharge and throughout the entire duration of discharge.
36 Additionally, we found the visualization of MU populations as ensembles to intuitively represent population
37 discharge characteristics with appropriate accuracy for visualization. *Significance:* The results and methods
38 outlined here provide an improved method for generating smooth estimates of MU discharge rate with SVR
39 and present a unique approach to visualizing MU populations with ensembles. In combination, the use of
40 SVR and generation of ensembles represent an efficient method for rendering population discharge
41 characteristics.

42 INTRODUCTION

43 Following its wide adoption into research, the electromyographic signal has been increasingly realized as
44 a rich source of information regarding supraspinal and spinal mediated mechanisms for motor control.
45 Specifically, given the tight coupling of action potentials (1:1 discharge) between spinal motor neurons and
46 the muscle fibers that they innervate, collectively termed the motor unit, electric potentials recorded by both
47 intramuscular and surface electromyography (EMG) function as a unique window into the central nervous
48 system (Heckman & Enoka, 2012; Johnson, Thompson, Tysseling, Powers, & Heckman, 2017). Indeed,
49 EMG signals are comprised of the superimposed action potentials of many motor units (MUs), which allows
50 for individual MU discharge instances to be estimated with decomposition techniques. (De Luca, Adam,
51 Wotiz, Gilmore, & Nawab, 2006; Farina, Holobar, Merletti, & Enoka, 2010; Holobar, Minetto, & Farina,
52 2014; Nawab, Chang, & De Luca, 2010; Rau & Disselhorst-Klug, 1997). These estimated MU discharge
53 instances can then be used to characterize central nervous system function and garner insights into both
54 healthy and pathological motor control, an approach bolstered through advancements in high density
55 surface EMG (HD-sEMG) approaches (Kallenberg & Hermens, 2009; Li et al., 2015; Murphy et al., 2018).

56 Though the use of HD-sEMG and recent improvements in decomposition approaches have facilitated the
57 application of estimated MU discharge characteristics to inform physiological understanding, its adoption
58 into common practice and large scale application have introduced potential pit-falls in data estimation and
59 visualization. Specifically, successive improvements in decomposition algorithms, HD-sEMG electrode
60 arrays, and amplifier technology have continued to provide a greater yield in discriminated motor units.
61 Though this increased MU yield has facilitated a greater confidence in the generalizability of findings and
62 insight into population MU behavior, large increases in sample sizes often persuade researchers to reduce
63 the dimensionality of their dataset through averaging, which fails to adequately account for variance, or be
64 met with difficulties in accurately portraying the qualitative aspects of their data. This limitation will only
65 magnify as an increasing amount of parameters are found to affect MU discharge patterns and is evident in
66 recent papers from the field, where adequate qualitative representations of entire datasets are difficult to
67 achieve and single choice trials are often displayed. A concise and informative methodology for portraying
68 large sets of MU discharge profiles, or neuronal discharge in the general sense, would vastly improve the
69 capability of researchers to relay their findings in a consistent and intuitive way.

70 More importantly, in addition to the difficulties of efficiently visualizing these increasingly large datasets,
71 the process of extracting physiologically relevant metrics from decomposed MU discharge profiles is often
72 variable amongst research groups and has considerable potential for biasing findings. Specifically, the
73 process used to generate smooth estimates of MU discharge rates lacks an agreed upon computational
74 method and has the potential to substantially influence frequently characterized outcome metrics.

75 Commonly employed methods for obtaining smooth estimates of MU discharge rates include filtering of
76 binary spike trains with a window function, such as the Hanning (Hann) window, or fitting instantaneous
77 discharge rates with various degrees of lower order polynomial functions (Afsharipour et al., 2020; De
78 Luca, LeFever, McCue, & Xenakis, 1982a; Gorassini, Yang, Siu, & Bennett, 2002; F. Negro & Farina,
79 2012). Though commonly used, filtering with the Hanning window introduces undesirable characteristics
80 at the onset and offset of discharge, which biases the estimated recruitment and derecruitment discharge
81 rates, and lower order polynomial functions can potentially remove relevant characteristics of MU
82 discharge (i.e. over-smoothing). Indeed, a recent study demonstrated how fit method (Hanning, Gaussian,
83 5th-order polynomial) can affect estimates of persistent inward currents (PICs) generated by the paired MU
84 analysis technique (i.e. ΔF), showing the edge effects of the Hanning window to bias motor unit recruitment
85 and derecruitment estimates (Hassan et al., 2020). To minimize the introduction of biases in the data
86 analysis pipeline, a method of generating smooth estimates of MU discharge rates that accurately represent
87 the end conditions and more effectively balances the tradeoff between noise mitigation and retaining
88 relevant discharge characteristics is necessary.

89 To address these problems, we 1) investigated support vector regression (SVR) as a more effective means
90 of producing smooth continuous estimates of MU discharge rates and 2) propose that large populations of
91 MUs be quantified and visualized in ensembles, or average traces of MU discharge rates for subpopulations
92 of MUs separated by a metric of interest (e.g. torque at MU recruitment). Each ensemble represents the
93 average behavior of motor units within a subpopulation and is composed of a time normalized estimate of
94 discharge rate for each individual MU, generated through SVR. Support vector regression, with its ability
95 to independently weight observations (e.g. MU recruitment and derecruitment) and tune hyperparameters
96 to optimize fit, offers a level of control far superior to traditional fitting schemes (Alex J. Smola &
97 Schölkopf, 2004; Vapnik, 1995). In specific, weighting the end conditions alleviates the biasing effects at
98 recruitment and derecruitment introduced by the edge effects of the Hanning window while hyperparameter
99 tuning tempers the unnecessary smoothing introduced by fitting with lower-order polynomial functions.
100 We hypothesized that: 1) compared to the Hanning window and a 5th and 6th order polynomial, the
101 capabilities inherent to SVR would facilitate a more accurate representation of estimated MU discharge
102 rates, and 2) visualizing groups of MU discharge rates as ensembles would provide an intuitive method to
103 convey findings in a compelling manner, where one figure can visually display the potential findings of an
104 entire dataset.

105 **METHODS**

106 Dataset

107 *Participants:* Motor unit spike trains were obtained from multiple ongoing human subject studies. This
108 included twenty-one young participants (F: 5, M: 16; Age: 26.4 ± 1.7) with no known neuromuscular,
109 musculoskeletal, or cardiovascular impairments. All participants provided written and informed consent
110 (Northwestern University Institutional Review Board STU00202964) in accordance with the Declaration
111 of Helsinki.

112 *Overview:* Given that a primary goal of this effort was to provide data quantification and visualization
113 methodologies for studies that employ estimates of smooth MU discharge rates, the contraction profile and
114 muscles were chosen accordingly. Specifically, to assist in generalizability to future studies, a ramp
115 contraction, consisting of a linear increase and subsequent decrease in effort, was chosen because it provides
116 desirable MU recruitment spacing and is commonly used in the field (De Luca, LeFever, McCue, &
117 Xenakis, 1982b; Farina et al., 2009; Kim, Wilson, Thompson, & Heckman, 2020; Orssatto et al., 2021;
118 Oya, Riek, & Cresswell, 2009). Similarly, given frequent use of the lower limb in HD-sEMG studies, ramp
119 contractions were generated through either ankle dorsiflexion or plantarflexion with grid electrodes fixed
120 atop the skin overlying the tibialis anterior (TA), medial gastrocnemius (MG), and soleus (SOL) muscle
121 bellies.

122 *Experimental Setup:* For each experimental session, participants were seated in a Biodex chair, with their
123 left foot securely attached to a footplate fixed onto a Systems 4 Dynamometer (Biodex Medical Systems,
124 Shirley, NY) such that the axis of rotation aligned with the center of rotation of the ankle joint. Throughout
125 the session a participants' hips were maintained at approximately 80 degrees of flexion, left knee at 20
126 degrees flexion, and left ankle at 10 degrees of plantarflexion with thigh and shoulder straps used to
127 minimize movement. Target torque ramps and visual feedback (i.e. dorsiflexion or plantarflexion torque)
128 were provided on a television screen via a custom Matlab interface (MATLAB (R2020b), The Mathworks
129 Inc., Natick, MA). Torque about the ankle was filtered with a 125 ms moving average window before being
130 provided as visual feedback to the participant. For subsequent analysis, raw torque signals were amplified
131 ($150\times$) and digitized (2048 Hz) using a 16-bit analog-to-digital converter (Quattrocento, OT Bioelettronica,
132 Turin, IT) and lowpass filtered (50 Hz) with a fifth order Butterworth filter.

133 *Experimental Protocol:* Prior to commencement of ramp contractions, participants were asked to generate
134 maximal voluntary isometric contractions of the plantarflexors and dorsiflexors, with 2 minutes of rest
135 separating contractions. At least two contractions were performed, and repeated until the peak torque within
136 the last contraction was no larger than 5% of the previous contraction. We then used the maximum voluntary
137 torque (MVT) achieved during these contractions to normalize all subsequent ramp contractions. Ramp

138 contractions started from rest and consisted of a 10 second linear increase to 30% MVT and a 10 second
139 decrease back to rest (i.e. 3% MVT/s rise and decay speeds). To mitigate learning effects and ensure smooth
140 contractions, participants completed a minimum of 6 dorsiflexion and plantarflexion practice ramps.
141 Following practice trials, each experimental session consisted of 4-12 ramp contractions for each
142 dorsiflexion and plantarflexion, conducted in random order.

143 *High Density Surface EMG (HD-sEMG):* HD-sEMG was collected via 64 channel electrode grids
144 (GR08MM1305, OT Bioelettronica, Turin, IT) placed atop the skin with adhesive foam (KITAD064, OT
145 Bioelettronica, Turin, IT) overlying the TA, MG, and SOL muscle bellies. The location of the muscles were
146 identified via palpation by a clinical exercise physiologist. Prior to electrode placement, the left leg was
147 shaved and the skin overlying the muscles was abraded with abrasive paste and cleaned with isopropyl
148 alcohol. Two Ag/AgCl ground electrodes were placed bilaterally on the right and left patella and a moist
149 band electrode was placed around the right ankle. HD-sEMG signals were acquired with differential
150 amplification (150 x), digitized (2048 Hz), and bandpass filtered (10-900 Hz) using a 16-bit analog-to-
151 digital converter (Quattrocento, OT Bioelettronica, Turin, IT).

152 *Motor Unit Decomposition:* In preparation for decomposition, all surface EMG channels were bandpass
153 filtered at 20–500 Hz (second-order, Butterworth) and visually inspected to remove channels with
154 substantial artifacts, noise, or saturation of the A/D board (typically 2-3 channels). The remaining EMG
155 channels were decomposed into individual MU spike trains using convolutive blind source separation and
156 successive sparse deflation improvements (Martinez-Valdes et al., 2017; Francesco Negro, Muceli,
157 Castronovo, Holobar, & Farina, 2016). The silhouette threshold for decomposition was set to 0.87. To
158 improve decomposition accuracy and correct spikes that indicated non-physiological MU discharge,
159 experienced investigators conducted manual editing of the spike trains. Specifically, automatic
160 decomposition results were improved through iteratively re-estimating the spike train and correcting for
161 missed spikes or substantial deviations in the discharge profile (Boccia, Martinez-Valdes, Negro, Rainoldi,
162 & Falla, 2019; Del Vecchio et al., 2020; Hug et al., 2021).

163 Computational Fitting Methods

164 To compare support vector regression (SVR) with commonly employed methods, smooth MU discharge
165 rates were generated with the following computational approaches. For Hanning (Hann) window filtering,
166 analysis began with decomposed binary spike trains, whereas SVR and polynomial regression was initiated
167 with discrete estimates of instantaneous discharge rate. To obtain these discrete values, estimated MU
168 discharge times were obtained from decomposed MU spike trains and used to quantify the inter-spike
169 interval (ISI), or the time between each consecutive spike. A discrete estimate of instantaneous discharge
170 rate was then calculated as the reciprocal of the time series ISI for each MU. For all trials, any MU which

171 failed to sustain a minimum of 10 consecutive discharges was removed from analysis. This resulted in a
172 total MU Yield of 2128 for TA, 2673 for MG, and 1190 for SOL.

173 *Hanning (Hann) Window Filtering*

174 The use of the Hanning window to generate smooth discharge rate estimates from decomposed motor unit
175 spike trains has been a popular computational approach within the field (De Luca et al., 1982a). To obtain
176 these estimates, a binarized motor unit spike train is filtered with a Hanning window of pre-specified length.
177 We have chosen to employ a Hanning window with length equivalent to 1 s in duration, though windows
178 of various durations have previously been employed (De Luca et al., 1982a; Hassan et al., 2020; F. Negro
179 & Farina, 2012).

180 *Polynomial Regression*

181 Fitting instantaneous discharge rates with a polynomial function is becoming a common practice amongst
182 the field to provide smooth discharge rate estimates, with a 5th order polynomial commonly used
183 (Afsharipour et al., 2020; Gorassini et al., 2002). To compare SVR and polynomial regression, we have
184 chosen to use a 5th and 6th order polynomial function to fit instantaneous discharge rates. Given that a 5th
185 order polynomial necessitates opposite end conditions, an even degree polynomial should theoretically
186 better represent the discharge profiles observed during isometric ramp contractions. Furthermore, an even
187 degree polynomial greater than a 5th order function is likely more desirable (i.e. 6th not 4th order), given the
188 known smoothing properties. Polynomial regression of the instantaneous discharge rates was accomplished
189 through employing least squares with the time vector centered at zero and scaled to one standard deviation.
190 The polynomial coefficients produced by this operation were then used to generate smooth estimates of
191 discharge rate along a prediction time vector from MU recruitment to derecruitment sampled at 2048 Hz.

192 *Support Vector Regression*

193 Support vector regression was first introduced by Vapnik and colleagues, who outlined the application of
194 traditional support vector machine classification to a regression problem (Drucker, Burges, Kaufman,
195 Smola, & Vapnik, 1996; Vapnik, 1995). Like classification with support vector machines, support vector
196 regression (SVR) employs much of the same principles, including use of kernels to represent data in a
197 higher dimensional space, a hyperplane separating data points in this higher dimensional space, and a
198 margin about this hyperplane. In depth discussions on SVR and its algorithmic implementation can be found
199 elsewhere and will not be included here (Cristianini & Shawe-Taylor, 2000; Alex J Smola & Schölkopf,
200 1998; Alex J. Smola & Schölkopf, 2004). For ease of translation across research groups, we implemented
201 SVR with Matlab's inbuilt function *fitrsvm* to train an SVR model with L1 soft-margin minimization
202 (MATLAB (R2020b), The Mathworks Inc., Natick, MA). For each MU, training data included the
203 instantaneous discharge rate estimates and corresponding time instances. Smooth estimates of discharge

204 rate were then generated using Matlab's inbuilt *predict* function to generate an estimated discharge rate
205 along a prediction time vector from MU recruitment to derecruitment sampled at 2048 Hz (MATLAB
206 (R2020b), The Mathworks Inc., Natick, MA).

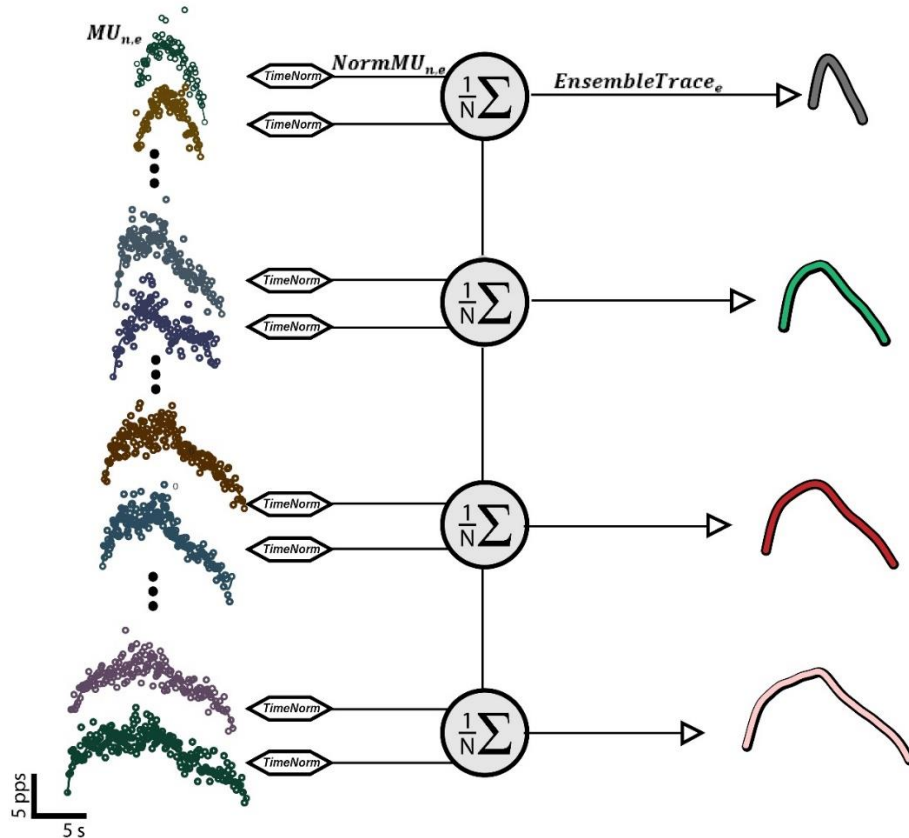
207 Support vector regression contains various parameters that can be tuned to optimize fitting characteristics.
208 For our purposes, this included the kernel that is employed, the kernel scale factor, epsilon, and the
209 regularization parameter (Alex J. Smola & Schölkopf, 2004). The kernel and kernel scale factor define the
210 function employed in expanding dimensionality and was chosen as a radial basis function. Epsilon defines
211 one-half of the margin, or width about the hyperplane in which no penalty is assigned to the cost function.
212 The regularization parameter indicates the penalty that is assigned to points outside this margin.

213 To account for the inherent differences in variability of discharge between muscles, we chose an epsilon
214 value that was MU specific and scaled based on the discharge variability for that unit. Specifically, we
215 chose an epsilon value equal to one-eleventh of the interquartile range of the discharge rate, which generates
216 an approximate epsilon insensitive region (margin) of one quarter of a standard deviation. To ensure
217 desirable end characteristics, the initial and final five discharge instances were weighted five times greater
218 than the remainder of discharge instances for a given unit. To optimize the kernel scale and regularization
219 parameters, we performed a grid search across a range of 0-1000 for both terms and used the values that
220 most closely replicated the average sum of squared error achieved with the 1 s Hanning window throughout
221 the middle 80% of discharge. That is, the error seen at the first and last 10% of discharge was not considered
222 given the known edge effects introduced by filtering with the Hanning window. The 1s Hanning window
223 was chosen as a comparator, given that this fit is generally believed to retain and accurately portray the
224 relevant characteristics of MU discharge throughout the *middle* portion of discharge. This produced a
225 regularization parameter of 370 and a kernel scale factor of 1.6.

226 Ensembles

227 An overview of the construction of ensembles can be seen in Figure 1. For each muscle, all discriminated
228 MUs across participants (TA: 2128, MG: 2673, SOL: 1190) were separated into ten equally spaced bins
229 based upon the percent of Maximum Voluntary Torque (MVT) that a MU was recruited at (i.e. 3% MVT
230 increments for 30% MVT ramps). The MUs within each of these 3% MVT bins were then fit with the three
231 computational fitting methods. For each fit method, we utilized a time normalization procedure to generate
232 MUs of a pre-specified length within each ensemble. For SVR and polynomial regression, we adjusted the
233 sampling rate of the prediction vector such that smooth discharge rate estimates within an ensemble were
234 vectors of equal length, equivalent to the length of the time vector from average MU recruitment to
235 derecruitment sampled at 2048 Hz. Similarly, for Hanning window filtering, the smooth discharge rate
236 estimates generated by the Hanning window were resampled using linear interpolation to generate vectors

237 of identical length within a given ensemble. Following this normalization, we then quantified the ensemble
 238 average of all MU fits within each 3% MVT cohort and mapped these traces from the average recruitment
 239 to derecruitment instance of each group to generate the quantized ensemble traces. This was done such that
 240 each ensemble trace represented a true “average”, with the ensemble discharge rate traces representing the
 241 average discharge profile from MU recruitment to MU derecruitment for all units within that cohort.



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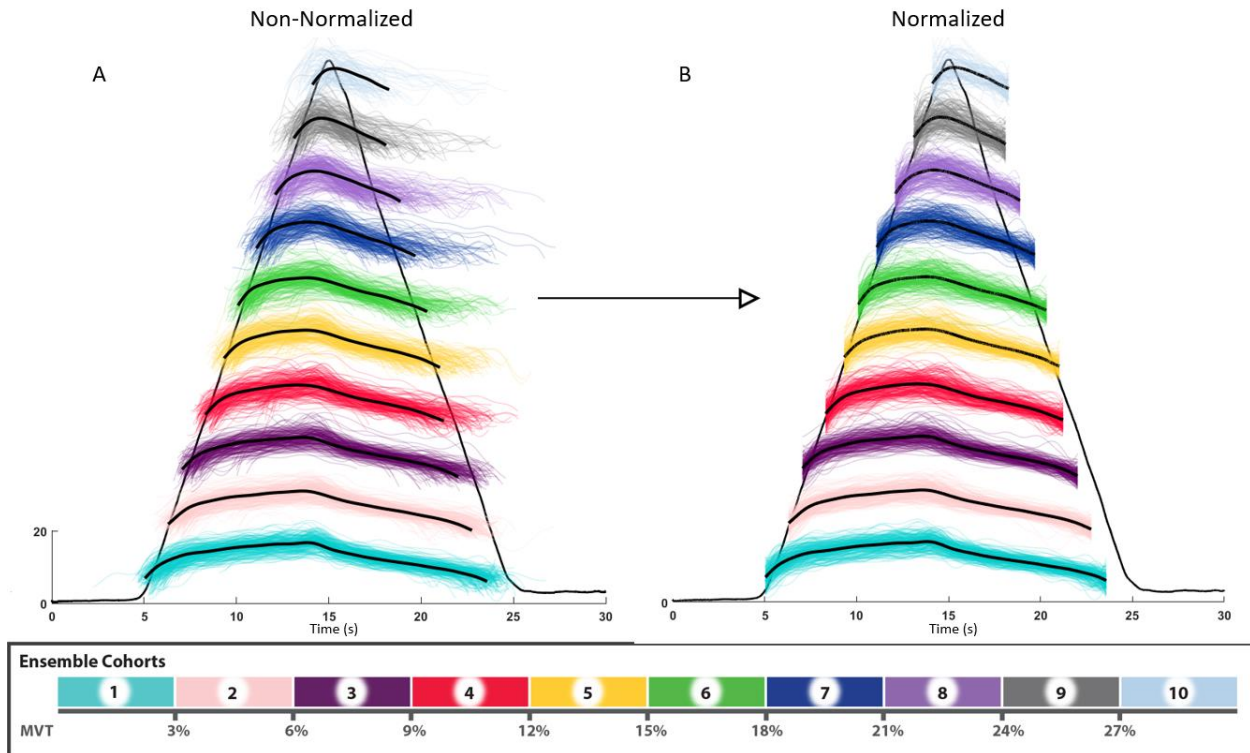
243 Figure 1: Overview of ensemble construction. Populations of estimated motor unit discharge profiles were
 244 subdivided into cohorts based upon a metric of interest (four groups on left), filtered or fit with an estimating
 245 function with the time axis (x-axis) normalized such that motor units within a subdivision align from onset
 246 to offset, and ensemble averaged (four traces on right). (pps: pulses per second; s: second)

247 To further illuminate the time normalization process, Figure 2 shows the SVR estimates for all TA MUs (N
 248 = 2128) separated into ten ensemble cohorts both before (Figure 2A; non-normalized) and after (Figure 2B;
 249 normalized) normalization. In this figure, the quantized SVR ensemble traces (Figure 2A: black traces) can
 250 be seen to unsurprisingly represent the average onset and offset of discharge across MUs, given that this is
 251 how they were defined. Furthermore, in the time normalized traces, representation of MUs on an identical
 252 timescale from recruitment to derecruitment can be observed. This allows for the ensemble trace to
 253 represent the true average shape of MUs within each ensemble, with distinct modes of discharge mapped

254 from onset to offset. The ability of these ensemble traces to represent the average discharge profile of each
255 MU cohort can be seen with the black traces overlying the non-normalized fits.

256

257



258

259 Figure 2: Normalization in ensemble construction. Shown above is the ensemble construction process for
260 all decomposed TA motor units ($N = 2128$). Units are first separated into ten cohorts based upon their
261 recruitment threshold, with the color bar indicating the recruitment range of this group normalized to
262 individual participants' MVT and the black triangular trace representing the average torque across all trials
263 and participants. Smoothed estimates of MU discharge rate are then created with support vector regression
264 (A), and projected onto time vectors of identical length (B). These normalized estimates are then ensemble
265 averaged to generate the overlying black traces. These ensemble traces in black are shown overlying the
266 normalized and non-normalized estimates for comparison. (pps: pulses per second; s: second)

267 Fit Comparison & Ensemble Accuracy

268 To compare the accuracy of the three computational fitting methods we quantified the residual error, or
269 absolute deviation between the continuous and discrete discharge rate estimates, for each individual MU.
270 This was conducted to provide insight into the type of bias introduced by each fit, spanning from MU
271 recruitment to derecruitment. To observe the qualitative effects of the various fitting schemes, ensemble
272 figures were constructed for each fit method.

273 *Simulations*

274 To characterize the accuracy of representing populations of MUs as ensembles, we utilized a Monte Carlo
275 type simulation. Specifically, we treated the process of creating ensemble traces as a transfer function,
276 decomposed MU spike trains as inputs, and used outcome metrics for each individual MU and ensemble
277 trace of discharge rate at recruitment, discharge rate at derecruitment, peak discharge rate, time to peak
278 discharge rate, and ΔF .

279 For each iteration, we separated the total MU dataset for the TA into ensemble groups based upon their
280 torque at recruitment, as before, and iteratively resampled two-thirds of this population. This was conducted
281 for 100 iterations, with the average population outcome metrics for all units within each ensemble and the
282 outcome metrics for the ensemble trace used to generate estimated distributions for each metric of interest.
283 The difference between the estimated distribution of the sampled MU populations and ensemble traces was
284 then used to garner insight into the ability of the ensemble traces to capture the characteristics of the
285 populations of MUs within them.

286 Discharge rate at recruitment and derecruitment were calculated as the discharge rate of the first and last
287 instance of the smooth SVR fits, respectively. Peak discharge was calculated as the maximum discharge
288 rate of each MU SVR fit or ensemble trace, with time to peak calculated as the time between MU
289 recruitment and this peak value.

290 ΔF is a commonly employed metric used estimate the magnitude of PICs and represents the discharge
291 hysteresis of a higher threshold MU with respect to a lower threshold unit. To quantify ΔF , we employed a
292 paired MU analysis technique such that ΔF for a given MU (test unit) represented the change in discharge
293 rate of a lower threshold unit (reporter unit) between the recruitment and derecruitment of this test unit.
294 This was conducted for every possible combination of MU pairs within a trial where the reporter unit
295 exhibited sustained discharge throughout the test units recruitment and derecruitment. To account for the
296 pairing of a test unit with multiple reporter units, ΔF for a test unit was calculated as the average change in
297 discharge rate across all possible reporter unit pairs. To allow for full activation of the PIC in the reporter
298 unit, we excluded any pairs with recruitment time differences < 1 s (Bennett, Li, Harvey, & Gorassini, 2001;
299 Hassan et al., 2020; Powers, Nardelli, & Cope, 2008). Additionally, to avoid saturated reporter units, we
300 excluded test unit-reporter unit pairs in which the reporter unit discharge range was < 0.5 pps while the test
301 unit was active (Stephenson & Maluf, 2011). Furthermore, we only included test unit-reporter unit pairs
302 with rate-rate correlations of $r^2 > 0.7$ to ensure that MU pairs likely received common synaptic drive
303 (Gorassini et al., 2002; Udina, D'Amico, Bergquist, & Gorassini, 2010; Wilson, Thompson, Miller, &
304 Heckman, 2015).

305 To quantify ΔF for a given ensemble, we conducted a similar process treating each ensemble as either a test
306 or reporter ensemble. Specifically, all ensemble traces of a lower recruitment torque than a given ensemble
307 were used as reporter ensembles. ΔF for a test ensemble was then calculated as the average change in
308 discharge rate across all possible reporter ensembles from test ensemble recruitment to derecruitment.

309 Statistical Approach

310 To determine significant differences in fit error between the fitting methods, we employed linear mixed
311 effects models with either average absolute fit error across the first five discharges of a MU or the entire
312 duration of MU discharge as a dependent variable, fixed effects of torque recruitment cohort (ensemble),
313 muscle, and fit method, and random effects of participant and trial nested within participant. P-values were
314 obtained by likelihood ratio tests of the full model with the effect in question against the model without the
315 effect in question. For main effects, this included their subsequent interaction terms.

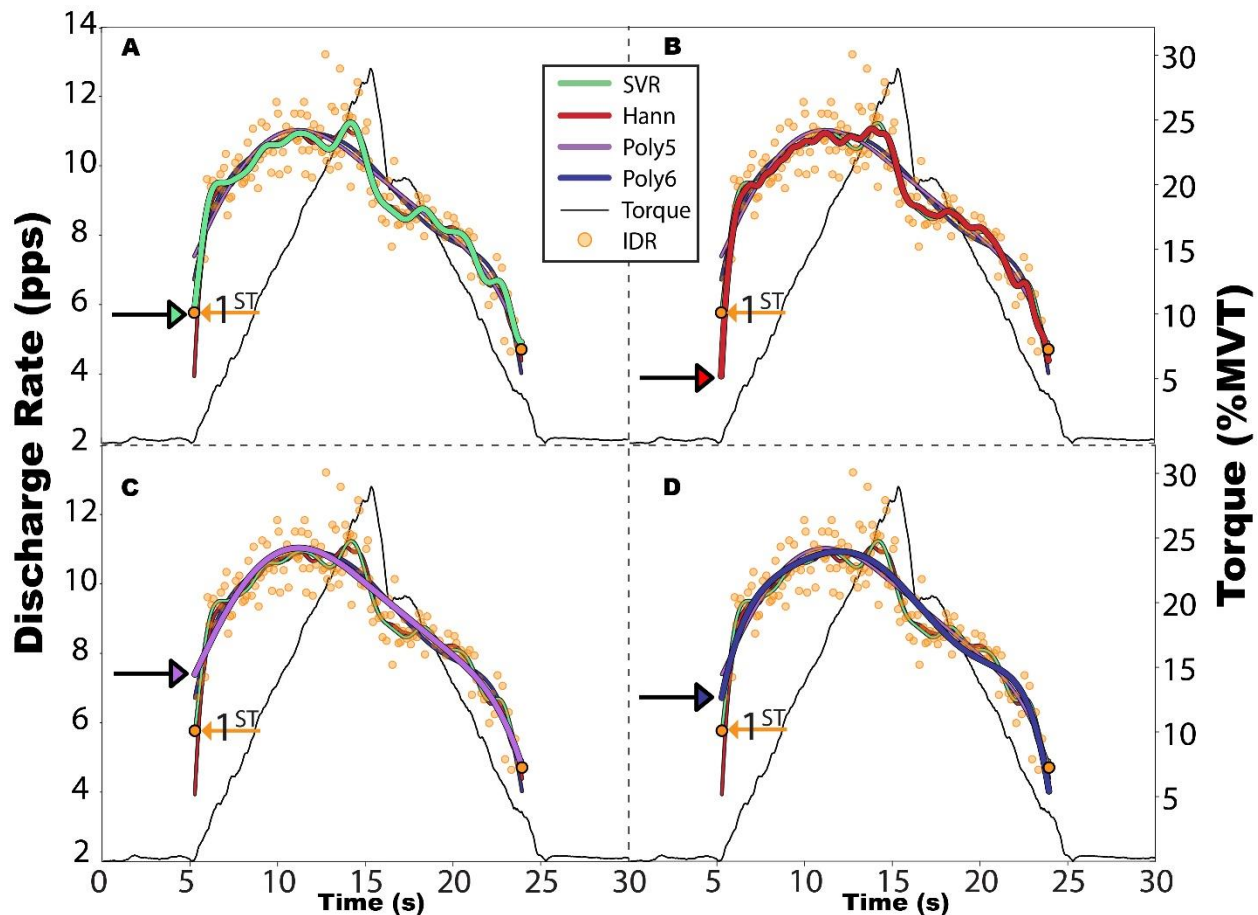
316 To test for statistically significant differences in discharge characteristics of interest between SVR
317 ensembles and the MU population characteristics, we used data generated with the Monte Carlo simulation.
318 Specifically, we employed linear models with dependent variables of the ensemble and sample estimates
319 for each outcome metrics of interest. For simplicity we only analyzed the TA data set and included a total
320 of 1000 observations for each method (ensemble or sample estimate) and outcome metric, or 100 iterations
321 for each of the ten ensemble cohorts. As fixed effects we included torque recruitment cohort (ensemble),
322 whether the estimate was from the ensemble trace or sample population average, and the interaction
323 between these factors.

324 All statistical analysis was performed with R (R Core Team, 2021). Mixed model analysis was achieved
325 via the *lme4* (Bates, Maechler, Bolker, & Walker, 2015) package. To ensure the validity of model fitting,
326 the assumptions of linearity and normal, homoscedastic residual distributions were confirmed. Estimated
327 marginal means were employed in pairwise post-hoc testing and achieved with the *emmeans* package
328 (Lenth, 2021). Significance was set at $\alpha = 0.05$ and pairwise and multiple comparisons were corrected using
329 Tukey's corrections for multiple comparison.

330 RESULTS

331 *Fit Method Comparison*

332 A randomly chosen MU from the MG muscle during a single plantarflexion trial can be seen in Figure 3
333 with each fit method applied. Qualitatively, polynomial regression has produced estimates that follow the
334 general trend of the MU discharge profile but fail to portray key attributes of discharge associated with the
335 activation PICs. This includes an initial high gain phase (PIC activation or acceleration of discharge)
336 followed by a gain attenuated phase (PIC saturation or post-acceleration discharge rate saturation)
337 (Heckman & Enoka, 2012). Additionally, on the descending portion of the ramp (~16-18 s) an abrupt
338 decrease in torque occurs with a corresponding decrease in discharge rate that is not captured by either
339 polynomial fit. Support vector regression and Hanning window filtering, with the employed parameters, do
340 appear to follow the discharge profile during this decrease in torque as well as highlight the expected
341 features introduced by PICs. That said, while these attributes are captured by the Hanning window filtering
342 approach, this fit appears to underestimate the initial discharge rate of 5.77 pps by a sizable margin (Hann:
343 3.94 pps, Poly-5: 7.41 pps, Poly-6: 6.72 pps, SVR: 5.73 pps).

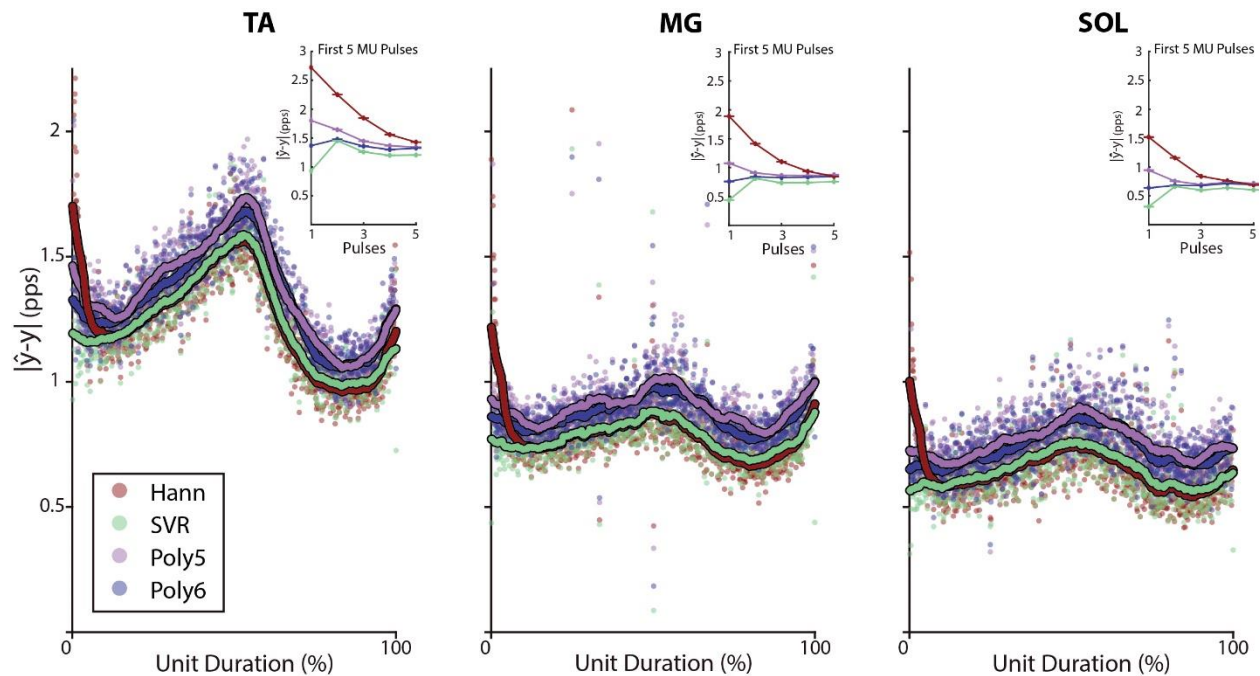


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345 Figure 3: Effects of fit method on smoothed estimates of discharge for a single motor unit. A single

346 decomposed medial gastrocnemius (MG) motor unit from a plantarflexion trial is shown with its estimated
347 instantaneous discharge rate (IDR) in pulses per second (pps) and orange. The discharge rate at recruitment
348 and derecruitment are shown outlined in black. The smooth estimates of discharge rate generated through
349 each investigated approach are shown in solid lines. This includes A) support vector regression (SVR), B)
350 Hanning (Hann) window filtering, and C-D) polynomial regression with either a 5th or 6th degree
351 polynomial. Plantarflexion torque about the ankle is shown in black, normalized to maximum voluntary
352 torque (MVT).

353 To highlight the fitting characteristic of each computational approach, Figure 4 shows the absolute fit error
354 for each method as a function of unit duration. As is observed, the fit error is unequally distributed across
355 the discharge profile for each fit method, with the various fitting schemes diverging at the onset and offset
356 of discharge and the polynomial regression fits systemically higher across the unit duration. The inset within
357 Figure 4 accentuates this divergence at onset, showing the absolute fit error averaged across all units for
358 the first five instances of all MUs discharge.



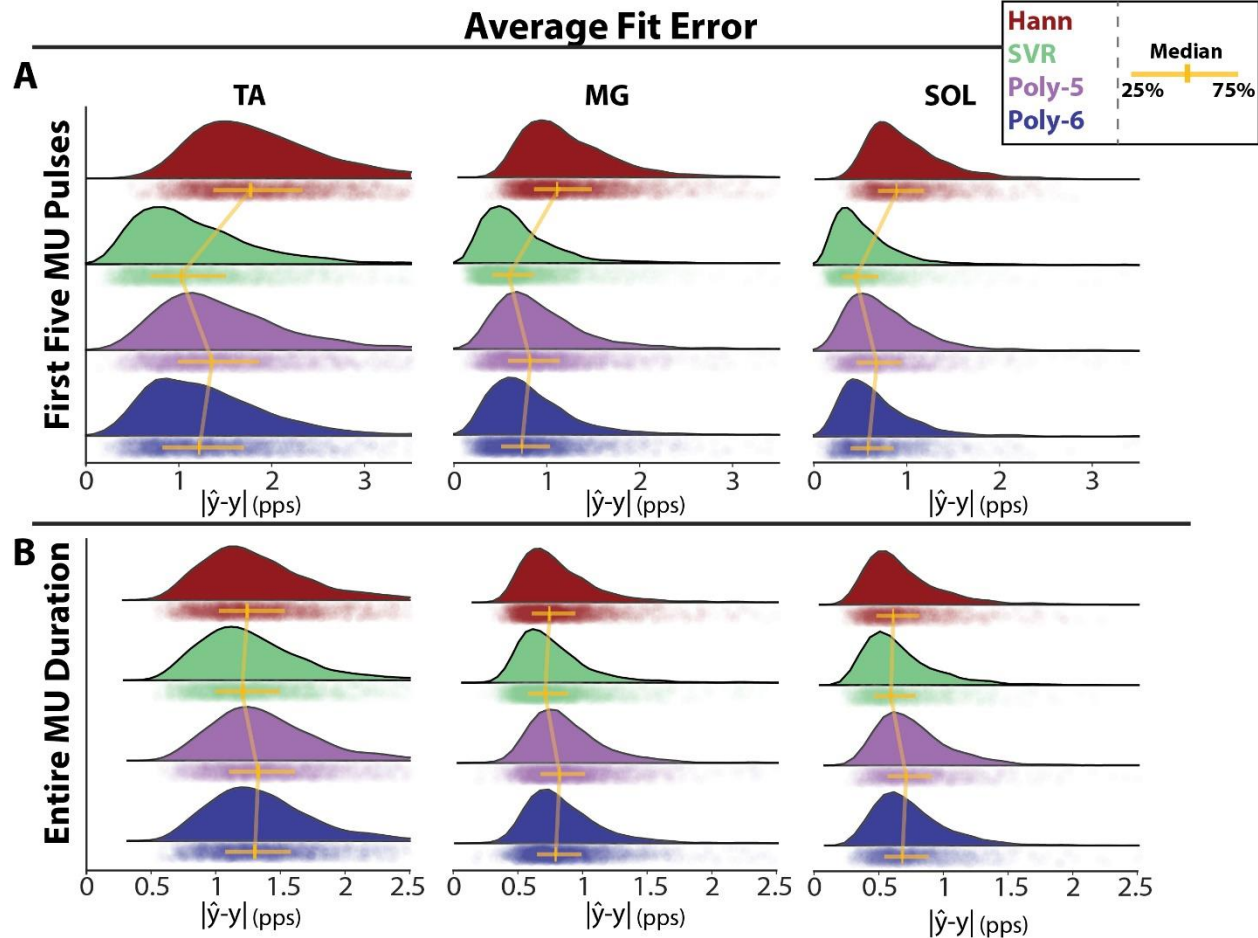
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360 Figure 4: Absolute fit error for each fit method across motor unit duration. The absolute difference between
361 the estimated discharge rate (\hat{y}) and instantaneous discharge rate (y) is shown as a function of motor unit
362 duration from recruitment (0%) to derecruitment (100%) for each fit method in pulses per second (pps). Fit
363 methods included support vector regression (SVR), Hanning (Hann) window filtering, and polynomial
364 regression with either a 5th or 6th degree polynomial. Colored dots indicate individual discharge instances
365 and the solid lines represent a moving average with width corresponding to a 6% unit duration. The inset
366 displays the absolute error plot as a function of the number of discharges from recruitment, for the first five

367 discharges for all motor units (avg. \pm SE). This is shown for the tibialis anterior (TA, N = 2128), medial
368 gastrocnemius (MG, N = 2673), and soleus (SOL, N = 1190).

369 To further investigate this divergence of fitting schemes, the average fit error for the first five discharges
370 for each MU can be seen in the top row of Figure 5A with a corresponding probability density function.
371 We used a linear mixed model and maximum likelihood estimation to predict this fit error within the first
372 five discharges of a MU (Absolute Error \sim FitMethod*Muscle*Ensemble + (1|PID:Trial)), showing fit
373 method ($\chi^2(90) = 4082.4$, $p < 0.001$), ensemble cohort ($\chi^2(108) = 1959.5$, $p < 0.001$), and muscle ($\chi^2(80) =$
374 1140.7 , $p < 0.001$) to be significant predictors of this fit error. Additionally, interactions between fit method
375 and ensemble ($\chi^2(81) = 394.2$, $p < 0.001$), fit method and muscle ($\chi^2(60) = 194.6$, $p < 0.001$), and muscle
376 and ensemble were observed ($\chi^2(72) = 548.3$, $p < 0.001$). Across all muscles and ensemble cohorts, a main
377 effect comparison of marginal means yields an estimated decrease in absolute error with SVR when
378 compared to Hanning (0.584 pps; 95%CI: [0.554, 0.614]), 5th degree polynomial (0.225 pps; 95%CI:
379 [0.195, 0.256]), and 6th degree polynomial (0.114 pps; 95%CI: [0.083, 0.144]). Significant decreases in
380 absolute error with SVR were also observed when separated by muscle for the difference between SVR and
381 Hanning (TA: 0.758 pps [0.714, 0.802]; MG: 0.564 pps [0.521, 0.608]; SOL: 0.430 pps [0.363, 0.496]),
382 SVR and 5th degree polynomial (TA: 0.297 pps [0.252, 0.341]; MG: 0.197 pps [0.154, 0.241]; SOL: 0.182
383 pps [0.116, 0.249]), and SVR and 6th degree polynomial (TA: 0.142 pps [0.098, 0.187]; MG: 0.098 pps
384 [0.055, 0.142]; SOL: 0.100 pps [0.034, 0.167]).

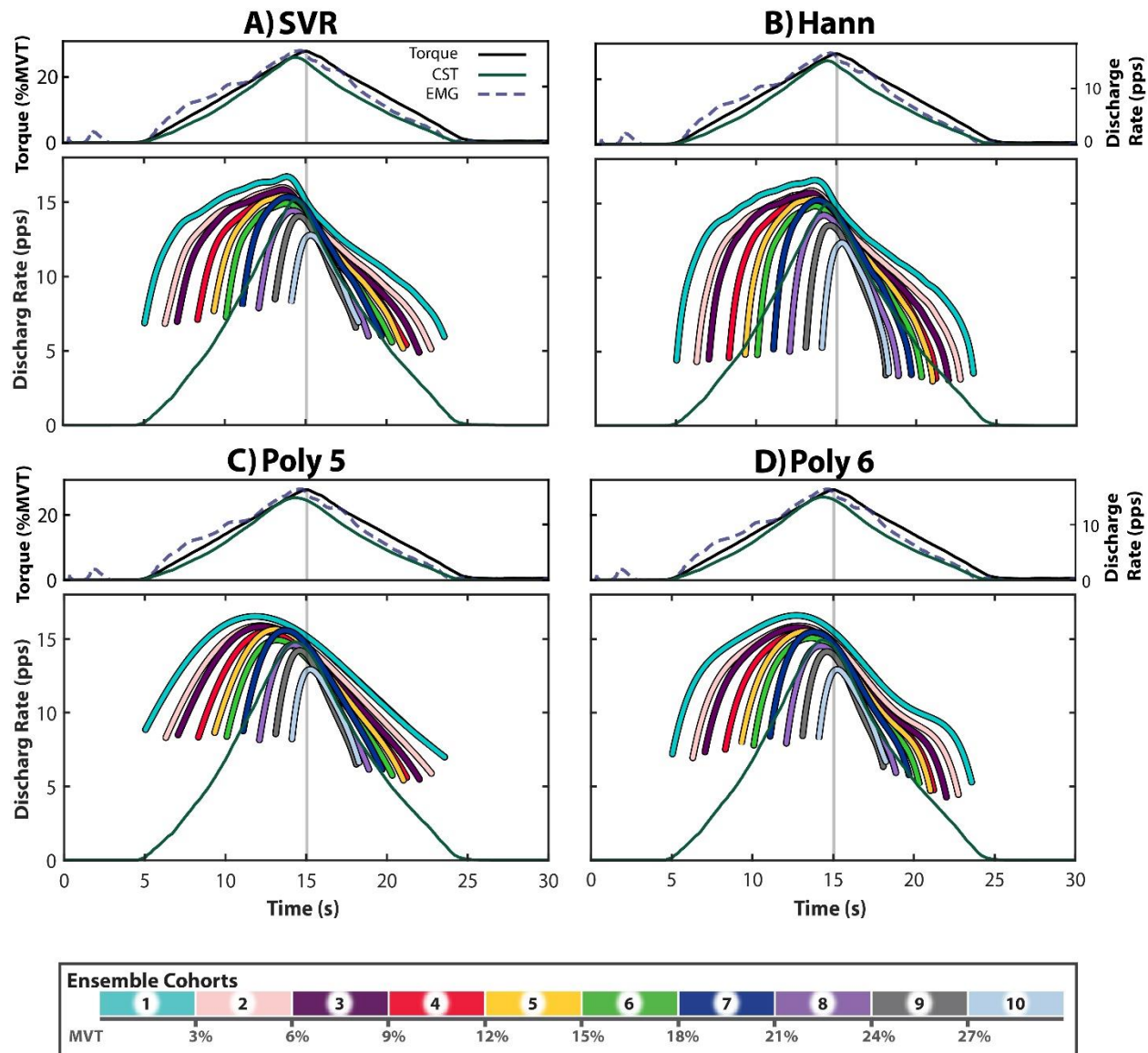
385 Using average fit error across the entire MU discharge duration (Figure 5B) as the dependent variable in a
386 linear mixed effects model (Absolute Error \sim FitMethod*Muscle*Ensemble + (1|PID:Trial)), we found fit
387 method ($\chi^2(90) = 637.1$, $p < 0.001$), muscle ($\chi^2(80) = 929.9$, $p < 0.001$), and ensemble ($\chi^2(108) = 2605.7$,
388 $p < 0.001$) to be significant predictors of fit error. Furthermore, we observed an interaction between fit
389 method and ensemble ($\chi^2(81) = 205.0$, $p < 0.001$) as well as muscle and ensemble ($\chi^2(72) = 397.0$, $p <$
390 0.001) with a non-significant fit method and muscle interaction ($\chi^2(60) = 15.0$, $p \sim 1$). Across all muscles
391 and ensemble cohorts, marginal means for the absolute error are estimated as 0.949 pps (95%CI: [0.915,
392 0.982]) for SVR, 1.052 pps (95%CI: [1.019, 1.086]) for the 5th degree polynomials, 1.021 pps (95%CI:
393 [0.987, 1.054]) for the 6th degree polynomials, and 0.990 pps (95%CI: [0.957, 1.024]) for the Hanning
394 window. When separated by muscle, we observed significant decreases in marginal means for absolute
395 error with SVR when comparing the difference between SVR and 5th degree polynomial (TA: 0.106 pps
396 [0.080, 0.132]; MG: 0.103 pps [0.077, 0.128]; SOL: 0.102 pps [0.063, 0.141]), SVR and 6th degree
397 polynomial (TA: 0.074 pps [0.048, 0.101]; MG: 0.068 pps [0.042, 0.094]; SOL: 0.075 pps [0.035, 0.114]),
398 and SVR and Hanning (TA: 0.049 pps [0.023, 0.075]; MG: 0.056 pps [0.030, 0.081]; SOL: 0.021 pps
399 [0.019, 0.060]).



400

401 Figure 5: Average absolute error across the fit methods. The average absolute difference between the
402 estimated discharge rate (\hat{y}) and instantaneous discharge rate (y) is shown for each fit method in pulses per
403 second (pps) for the first five motor unit discharges (A) and entire duration of discharge (B). Each data
404 point represents an individual motor unit with the corresponding probability density generated by a gaussian
405 kernel. Individual colors represent the various fit methods and include support vector regression (SVR),
406 Hanning (Hann) window filtering, and polynomial regression with either a 5th or 6th degree polynomial.
407 This is shown for the tibialis anterior (TA, $N = 2128$, left), medial gastrocnemius (MG, $N = 2673$, middle),
408 and soleus (SOL, $N = 1190$, right).

409 To illustrate the impact of each fit method on ensemble construction, we display the ensemble traces for
410 each of the four fit methods applied to all of the TA MUs in Figure 6. The over-smoothing created through
411 polynomial regression and the biased end conditions of Hanning window filtering are highly evident and
412 correspond to the fit errors that were observed for each method (Figures 4 & 5).

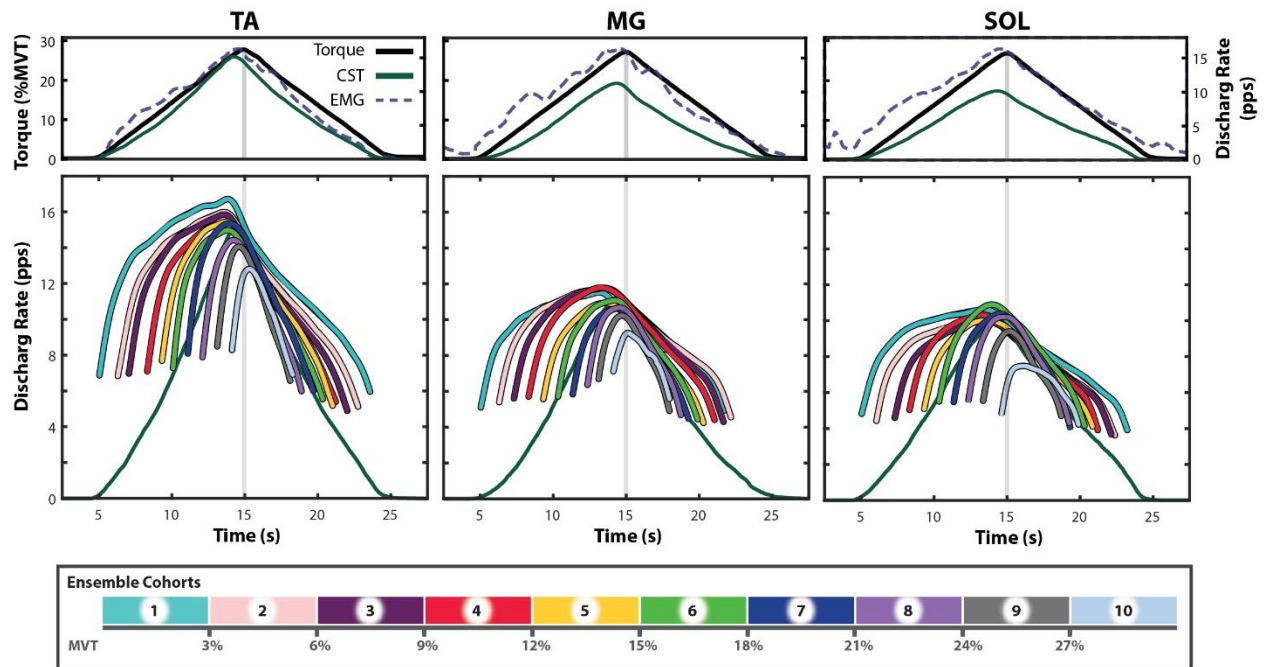


413

414 Figure 6: Ensembles for each fit method. Each quadrant represents one of the investigated fitting schemes
 415 and corresponds to A) support vector regression (SVR), B) Hanning (Hann) window filtering, and C-D)
 416 polynomial regression with either a 5th or 6th degree polynomial. Within each quadrant, the top plot depicts
 417 the average torque in black, the cumulative spike train (CST) across all units in green, and the average
 418 moving root mean squared EMG for all trials in dashed purple. Torque is shown as percent of maximum
 419 voluntary torque (MVT), the CSTs are shown in pulses per second (pps), and peak EMG is shown
 420 equivalent to maximum torque. The bottom plot within each quadrant houses the ensembles, color
 421 coordinated in accordance with the color bar, and the CST for reference. The light gray line across plots
 422 indicates the time of peak torque. (Ensemble cohorts: N = 240, 215, 202, 232, 232, 229, 210, 259, 202,
 423 107).

424 *SVR Ensemble Representative Capacity*

425 To highlight the distinct discharge behavior within and across muscles, we display SVR ensemble plots for
426 the TA, MG, and SOL in Figure 7. The visualization capacity and information density inherent to ensembles
427 are easily conveyed here, with distinct differences in discharge characteristics immediately perceptible
428 between muscles.



429

430 Figure 7: SVR ensembles across muscles. Shown are the ensemble traces for each muscle, generated from
431 motor unit discharge rates estimated with support vector regression (SVR). For each muscle, the top plot
432 depicts the average torque in black, the cumulative spike train (CST) across all units in green, and the
433 average moving root mean squared EMG for all trials in dashed purple. Torque is shown as percent of
434 maximum voluntary torque (MVT), the CSTs are shown in pulses per second (pps), and peak EMG is
435 shown equivalent to maximum torque. The bottom plot for each muscle houses the ensembles, color
436 coordinated in accordance with the color bar, and the CST for reference. The light gray line across plots
437 indicates the time of peak torque. (Ensemble cohorts: [TA: N = 240, 215, 202, 232, 232, 229, 210, 259,
438 202, 107]; [MG: N = 221, 261, 299, 310, 373, 383, 304, 287, 160, 75]; [SOL: N = 154, 146, 142, 171, 136,
439 150, 110, 97, 54, 30])

440 To investigate the ability of the ensembles to portray underlying MU population statistics, we ran a Monte
441 Carlo simulation and show the results in Figure 8. For each iteration of this simulation, we quantified the
442 discharge rate at recruitment, derecruitment, and peak, as well as time to peak discharge rate and ΔF for
443 each ensemble and corresponding population of MUs within that ensemble. To estimate the deviation
444 between ensemble traces and population averages, we fit a linear model to each outcome metric, with fixed
445 effects of ensemble cohort (1-10), whether an estimate was from the random sample or an ensemble trace

446 (method), and the interaction between these two. Results of these linear models are separated by outcome
447 metric as follows.

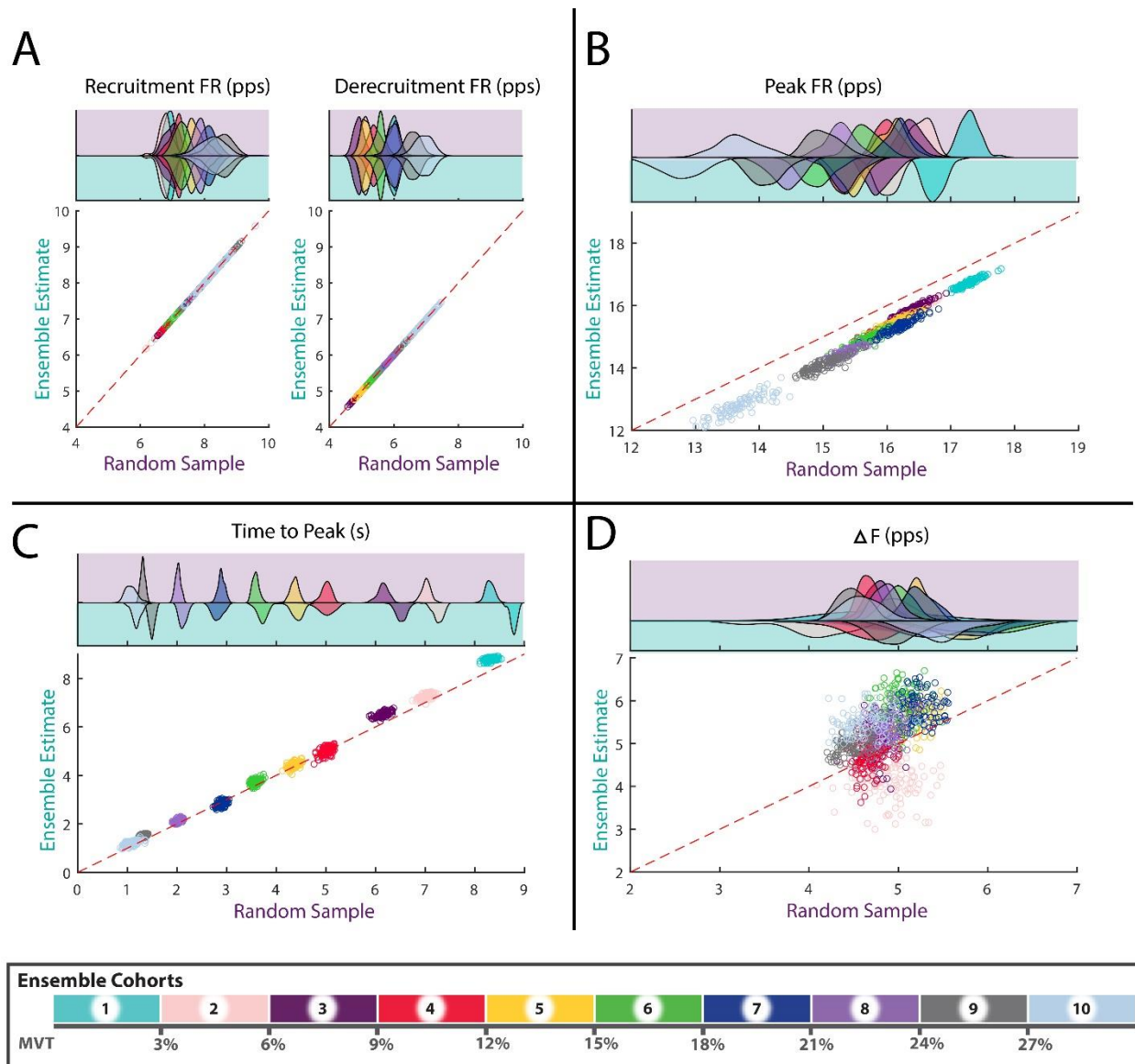
448 Peak Discharge Rate: We fit a linear model to peak discharge rate using ordinary least squares (Peak DR ~
449 Ensemble * Method). The model exhibits substantial explanatory power ($R^2 = 0.96$, $F(19, 1980) = 2614.2$,
450 $p < 0.001$, adj. $R^2 = 0.96$) with significant effects of ensemble ($F(9, 1980) = 4851.9$, $p < 0.001$), method
451 ($F(1, 1980) = 5762.7$, $p < 0.001$), and their interactions ($F(9, 1980) = 26.68$, $p < 0.001$). Averaging across
452 all ensemble cohorts, a main effect comparison of marginal means for method yields an estimated decrease
453 of 0.725 pps (95%CI: [0.706, 0.744]) in the ensemble estimates when compared to the MU population
454 averages. Separating by ensemble cohort, estimates range from a minimum decrease in the ensemble
455 estimates of 0.511 pps (95%CI: [0.452, 0.570]) in ensemble three to a decrease of 0.908 pps (95%CI: [0.849,
456 0.968]) in ensemble nine.

457 Recruitment & Derecruitment Discharge Rate: We fit a linear model to discharge rate at recruitment and
458 derecruitment, separately, using ordinary least squares (Estimate ~ Ensemble * Method). The models
459 exhibit substantial explanatory power for discharge rate at recruitment ($R^2 = 0.84$, $F(19, 1980) = 566.2$, p
460 < 0.001 , adj. $R^2 = 0.84$) and derecruitment ($R^2 = 0.92$, $F(19, 1980) = 1261.6$, $p < 0.001$, adj. $R^2 = 0.92$). For
461 both models, the fixed effect of ensemble cohort was significant (recruitment: $F(9, 1980) = 1195.4$, $p <$
462 0.001 ; derecruitment: $F(9, 1980) = 2663.5$, $p < 0.001$), but the method and interaction terms were not.

463 Time to Peak: We fit a linear model to the time from recruitment to peak discharge rate using ordinary least
464 squares (Time to Peak ~ Ensemble * Method). The model exhibits substantial explanatory power ($R^2 =$
465 0.998 , $F(19, 1980) = 72029.00$, $p < 0.001$, adj. $R^2 = 0.998$) with significant effects of ensemble ($F(9, 1980)$
466 $= 151721.00$, $p < 0.001$), method ($F(1, 1980) = 1547.77$, $p < 0.001$), and their interactions ($F(9, 1980) =$
467 168.25 , $p < 0.001$). Averaging across all ensemble cohorts, a main effect comparison of marginal means
468 for method yields an estimated increase of 0.160 s (95%CI: [0.152, 0.168]) in the ensemble estimates.
469 Separating by ensemble cohort, estimates range from a non-significant effect in ensemble four to a
470 maximum increase of 0.480 pps (95%CI: [0.451, 0.502]) in ensemble one.

471 ΔF : We fit a linear model to the estimates of ΔF for both the ensemble traces and average values within
472 each ensemble ($\Delta F \sim$ Ensemble * Method) for ensemble cohorts two through ten, due to a lack of test units
473 for the first ensemble trace. The model exhibits high explanatory power ($R^2 = 0.75$, $F(17, 1782) = 314.04$,
474 $p < 0.001$, adj. $R^2 = 0.75$) with significant effects of ensemble ($F(8, 1980) = 382.21$, $p < 0.001$), method
475 ($F(1, 1980) = 798.58$, $p < 0.001$), and their interactions ($F(8, 1980) = 185.29$, $p < 0.001$). Averaging across
476 all ensemble cohorts, a main effect comparison of marginal means for method yields an estimated increase
477 of 0.361 pps (95%CI: [0.336, 0.386]) in the ensemble estimates. Separating by ensemble cohort, estimates

478 range from a decrease of 0.833 pps (95% CI: [-0.908, -0.758]) in ensemble two, to an increase of 0.890 pps
 479 (95% CI: [0.815, 0.965]) in ensemble six.



480

481 Figure 8: Ensemble accuracy. Shown above are the results of the Monte Carlo simulation, treating the
 482 construction of ensembles as a process. This is shown for five parameters of interest, including discharge
 483 rate at recruitment (A, left), derecruitment (A, right), and peak (B) as well as time from recruitment to peak
 484 discharge rate (C) and ΔF (D). Within each quadrant, the bottom plot depicts the ensemble estimate of a
 485 given parameter against the average of that parameter estimate for all individual motor units. This is
 486 separated into ensemble cohorts according to the color bar with a data point for each iteration ($N = 1000$).
 487 The dashed red line depicts a theoretical 100% agreement between the ensemble estimates and the
 488 population average for a random sample. The probability density for each ensemble and parameter are
 489 displayed in the top row of each quadrant and follow an identical color scheme. Distributions outlined in
 490 purple on the top row correspond to population averages of the sample with those on the bottom row

491 outlined in blue corresponding to the ensemble estimates. Motor units were sampled from the decomposed
492 population of tibialis anterior units ($N = 2128$).

493 **DISCUSSION**

494 The increasingly prevalent implementation of HD-sEMG as a research tool has the potential to bolster
495 insights into not only basic human and non-human neurophysiology but both healthy motor control and
496 pathological motor dysfunction. In this paper, we addressed two potential limitations that researchers using
497 HD-sEMG often encounter. That is, 1) the computational approach used to generate smooth discharge rate
498 estimates and 2) the process of visualizing the discharge patterns of large populations of decomposed MUs.
499 To address these potential issues, we suggest support vector regression (SVR) as an improved
500 computational approach over traditionally used methods and put forth the visualization of large populations
501 of MUs as ensembles.

502 *Estimating Motor Unit Discharge Rate*

503 In its current state, motor neuron and MU research lacks an agreed upon computational method for creating
504 smooth continuous estimates of discharge rates. A recent comprehensive tutorial regarding HD-sEMG
505 expertly detailed the process of extracting neural information from HD-sEMG, including data acquisition,
506 decomposition, an overview of quantifiable MU properties, and motor unit tracking, but did not extend
507 commentary on the optimal fit methods for MU analysis techniques (Del Vecchio et al., 2020). Various
508 computational approaches are routinely employed to generate smooth estimates of discharge rate and have
509 the potential to introduce artifacts in MU quantification and characterization protocols (Hassan et al., 2020).
510 Commonly employed approaches include filtering of decomposed MU spike trains with a window function
511 (i.e. Hanning window) or fitting instantaneous discharge rates with a polynomial function of an arbitrary
512 degree. Though these methods are historically in wide use, investigation of their biasing effects on MU
513 discharge estimates is lacking. In this paper we have highlighted these potential effects and proposed an
514 alternative method, support vector regression, for creating smooth estimates of MU discharge rate.

515 Support vector regression (SVR), as a computational fitting approach, provides far greater flexibility than
516 Hanning (Hann) window filtering or polynomial regression and allows for greater optimization of fitting
517 characteristics. Specifically, much like the principles of support vector machines (Cortes & Vapnik, 1995;
518 Vapnik, 1995), SVR fits a hyperplane and margin to a collection of training data points, with use of a kernel
519 function to effectively fit the data points in a higher dimensional space. These characteristics, in addition
520 to the ability to independently weight observations, facilitates detailed SVR hyperplane optimization and
521 allows for a collection of data points to be estimated in a highly tunable manner. This is particularly useful
522 in creating smooth estimates of MU discharge rate when compared to traditional approaches, where edge
523 effects from filtering with a window or over smoothing through polynomial regression can perceptibly
524 affect data interpretation. Indeed, as can be seen in Figure 3 and Figure 4, SVR appears to offer a suitable

525 middle ground of mitigating noise (i.e. smoothing) while also retaining relevant characteristics of MU
526 discharge (e.g. discharge rate at recruitment, initial amplification, and rate modulation phases).

527 The ability of SVR to balance the tradeoff between bias and variance was achieved through the tuning of
528 parameters for the kernel and the cost functions and allows SVR to generate more accurate estimates. As
529 shown in Figures 4 and 5, this approach provides more accurate estimates when compared to both filtering
530 with the Hanning window and regression with a polynomial function. When comparing SVR and Hanning
531 window filtering, this is most evident in the fit error produced for the first five MU pulses. In these initial
532 MU pulses, the Hanning fits produced significantly higher deviations across all muscles with an estimated
533 increase in error over SVR fits of 0.758 pps (95%CI: [0.714, 0.802]) for the TA, 0.564 pps (95%CI: 0.521,
534 0.608]) for the MG, and 0.430 pps (95%CI: [0.363, 0.496]) for the SOL. When Comparing SVR and
535 polynomial regression, the over-smoothing effect of the polynomial fits can be seen in Figure 4 and Figure
536 5, with the polynomials absolute error higher than the SVR estimates for the first five MU pulses in Figure
537 5A and entire MU duration in Figure 5B. This increase in absolute error was most severe with the 5th degree
538 polynomial, which produced an average absolute error for the first five instances of discharge that was
539 0.225 pps (95%CI: [0.195, 0.256]) greater than the error observed with the SVR estimates. This increased
540 error was also apparent across the entire MU duration with an average absolute error at any given instance
541 of 1.052 (95%CI: [1.019, 1.086]) for the 5th degree polynomial, 1.021 pps (95%CI: [0.987, 1.054]) for the
542 6th degree polynomial, and 0.949 pps (95%CI: [0.915, 0.982]) for the SVR fits. Though the differences
543 between fits is small in magnitude, these values represent the average error at any given instance and will
544 accumulate across the MU duration. The potential effects of these absolute fit errors on choice outcome
545 metrics could be substantial and are apparent in the selected example MU in Figure 3. In this example, the
546 Hanning fit underestimates the initial discharge rate by almost 2 pps and the polynomial functions omit the
547 abrupt decrease in discharge rate on the descending portion of the ramp. The ramifications of these artifacts
548 are blatant in Figure 6, where visualization of the ensembles broadcast the fitting characteristics of each fit
549 method, a key strength of visualizing MU populations as ensembles.

550 *Population Visualization: Ensembles*

551 The increase in MU yield within studies employing modern HD-sEMG technology necessitates an intuitive
552 approach to displaying the findings of a dataset in an efficient manner. We suggest that large MU datasets
553 be visualized as ensembles. Ensembles are traces of MU discharge rate that represent the average discharge
554 profiles of a subpopulation of motor units and can be used to quickly convey the discharge characteristics
555 of a population of motor units. Here, we have subdivided our MU dataset into ten cohorts based upon torque
556 at recruitment, to observe changes in discharge profile as a function of recruitment threshold, though an
557 alternative parameter could have easily been chosen (e.g. discharge rate at recruitment). Depiction of these

558 ensembles can efficiently show characteristics of the MU populations discharge rate, including changes
559 across the subdividing parameter as well as changes following an intervention should ensembles be
560 constructed for both scenarios.

561 The capability of ensembles to convey the underlying discharge characteristics of the MU population can
562 be observed in both Figure 2 and Figure 7. In Figure 2A, the ensemble traces appear to overlay the non-
563 normalized SVR fits and represent the average discharge profile of the underlying TA MUs. This is apparent
564 across ensemble cohorts, with the lower cohorts easily represented and the higher ensemble cohorts
565 exhibiting a greater variability in derecruitment that the ensembles capture with the average instance of
566 derecruitment. In Figure 7, distinct modes of MU discharge rate can be observed for each of the ten
567 recruitment threshold cohorts across the TA, MG, and SOL muscles. This occurs in accordance with
568 expected findings and includes an initial acceleration phase, where activation of PICs take place, a gain
569 attenuation phase (post-acceleration rate saturation) where the PICs are likely saturated, a decay in
570 discharge rate with decreases in torque, and subsequent hysteresis where the derecruitment discharge rate
571 is decreased and occurring at lower torque values (Heckman & Enoka, 2012). Interestingly, a semblance of
572 the “onion-skin” phenomena may also be conveyed with these ensemble plots when subdivided by
573 recruitment threshold, of which could be used to further investigate this interesting phenomenon (De Luca
574 & Contessa, 2015; Inglis & Gabriel, 2021; Piotrkiewicz & Türker, 2017). Additionally, the differences
575 between muscles (Figure 7) is stark and highlights the ability of these ensemble traces to efficiently convey
576 differences in discharge rate between groups of MUs. When looking across muscles, the ensembles easily
577 represent muscle specific changes in discharge rate and display comparable trends observed in literature
578 (Kim et al., 2020).

579 To characterize the relationship between the ensembles and underlying population of MUs, we conducted
580 a simulation where we iteratively resampled our TA MU population, taking two-thirds of the population
581 each time to generate ensembles. Results of this simulation illuminate the rich density of information
582 garnered through the ensemble traces. Of note, the ensemble traces appear to represent the recruitment and
583 derecruitment discharge rate nearly identically (Figure 8A), a key feature woven into their construction,
584 while systemically under-estimating the peak discharge rate (Figure 8B). This under-estimation can likely
585 be attributed to the aligning process, where instances of recruitment and derecruitment are aligned rather
586 than the instance of peak discharge. Though the instances of peak discharge rate across MUs within a given
587 ensemble are likely similar, there is a slight smoothing effect as peaks are misaligned. Across all ensembles
588 this is estimated as an approximate attenuation of 0.725 pps (95%CI: [0.706, 0.744]). Furthermore, though
589 the time to peak discharge rate of the ensemble traces appear to closely track that of the MU population
590 (Figure 8C), they are not identical. Across all ensembles, a delay of 0.160 s (95%CI: [0.152, 0.168]) is

591 estimated and should be considered when employing ensemble traces for quantification. Similarly,
592 considering the quantification of ΔF across the population or through the ensemble traces, the ensembles
593 produce estimates approximately 0.361 pps (95%CI: [0.336, 0.386]) higher across all ensembles (Figure
594 8D).

595 Though the ensemble estimates of peak discharge rate, time to this peak, and ΔF are significantly different
596 than the underlying MU population tested here, their magnitudes and intended purpose must be considered.
597 Explicitly, these deviations must be interpreted with the understanding that the ensembles are designed as
598 a visualization tool to supplement the proper analysis and reporting of population statistics. With the proper
599 analysis and reporting of population statistics, ensemble traces can be employed to quickly convey changes
600 in discharge profile across the population with a level of accuracy sufficient for visualization purposes.
601 Indeed, depending on scale, qualitative considerations such as line thickness can easily obscure the
602 visualization of time to peak discharge by more than 160 ms. With this in mind, visualization of ensembles
603 allow for researchers to supplement their quantitative findings and portray MU discharge characteristics of
604 an entire dataset in an intuitive manner where the various modes of MU discharge are observed.

605 *Further Considerations*

606 On a fundamental level, the visualization of ensemble traces are meant to portray average discharge
607 characteristics of MU populations as an input-output function. Here we have chosen an input of torque at
608 recruitment and isolated ten cohorts of MUs, though this can conceivably be expanded to a variety of
609 neuronal discharge recordings and types of input variables. For example, the discharge patterns of cortical
610 neurons within the auditory cortex could be separated by the frequency of auditory stimulation and
611 visualized in a similar manner to what was conducted here (Bitterman, Mukamel, Malach, Fried, & Nelken,
612 2008; Montgomery & Wehr, 2010). Additionally, the modulation of biceps brachii MUs during isometric
613 elbow flexion ramps, subdivided by various degrees of deltoid activation, in individuals with chronic
614 hemiparetic stroke could be used in a similar fashion to construct ensemble traces. This would supply
615 unique insight into the prevalent coupling of shoulder abduction with elbow, wrist, and finger flexion post
616 stroke (Dewald & Beer, 2001; Dewald, Pope, Given, Buchanan, & Rymer, 1995). More closely related to
617 the paper at hand, we could have separated MUs into groups of equal sample sizes based on their torque at
618 recruitment and gauged insight into recruitment spacing at the cost of insight into distinct differences across
619 recruitment threshold.

620 *Potential Limitations*

621 Though the methods outlined here represent an incremental step in the analysis and visualization of MU
622 discharge profiles, a few limitations must be considered. In specific, the biases introduced by each fit
623 method highlighted here are only relevant to quantitative outcome metrics that employ smooth estimates of

624 MU discharge. The benefits of any computational approach are dependent on their application. Metrics that
625 analyze adjacent aspects of MU discharge (e.g. inter-spike interval) would remain unaffected by the various
626 fitting schemes. Furthermore, the improvement in estimates achieved through SVR are only improvements
627 upon the two most commonly employed methods used in fitting spinal MU discharge rates. We are not
628 proposing that SVR is the ultimate fitting method, that alternative approaches are inferior, or that
629 improvements to the current approaches (e.g. bias correction for the windowing) are unavailable. Instead,
630 we are emphasizing SVR as a modern approach that outperforms commonly employed methods. Of
631 particular note, though SVR outperforms a 6th degree polynomial, implementation of a 6th degree
632 polynomial appears to outperform the commonly employed 5th degree polynomial, and thus could be
633 considered in various circumstances. Lastly, more extensive hyperparameter optimization methods, such
634 as a Bayesian optimization scheme, may provide superior results.

635 **CONCLUSIONS**

636 To address potential limitations in the analysis and visualization of large MU populations in modern HD-
637 sEMG studies, smooth estimates of MU discharge rates can be generated with SVR and MU populations
638 may be visualized as ensembles. In this study, we have shown SVR to be an effective computational
639 procedure for generating smooth estimates of MU discharge rate. In addition to possessing superior
640 adaptability, when compared to Hanning window filtering and polynomial regression, SVR more accurately
641 estimates the recruitment region of MU discharge while maintaining adequate accuracy throughout the
642 duration of discharge. These desirable characteristics of SVR are highly evident when used to generate
643 ensembles. The generation of ensembles, as defined in this study, represents a novel method to visualize
644 the average discharge profiles of many MUs within a dataset. This allows for the efficient rendering of
645 discharge characteristics that are representative of the entire dataset and not comprised of single choice
646 example trials or a barrage of scatter plots. In combination, the use of SVR and generation of ensembles
647 represents an efficient approach for portraying population discharge characteristics with appropriate
648 accuracy for effective visualization.

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