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Motor unit discharge rate is correlated within individuals: A case for multilevel model statistical analysis

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ABSTRACT

Statistical analysis of motor unit discharge rate commonly uses the ordinary least squares based ANOVA and regression analyses or a repeated-measures ANOVA is used to account for within motor unit variance when the same motor unit is assessed multiple times. Both of these methods assume statistical independence of multiple motor units assessed within an individual. This investigation details two studies which quantify the statistical dependence of motor units within an individual. During a ramp contraction, motor unit initial discharge rate is mildly correlated within an individual (ICC: 0.11), though accounting for this effect significantly impacts regression analysis ($p = 0.01$). When a contraction is held at constant force and multiple observations are made on a motor unit, the motor unit discharges are more highly correlated (ICC: 0.41), even after accounting for the effects of multiple motor unit observations. A subject-level ICC of 0.01 can increase Type 1 error rate to 3.9–19.7%, depending on the number of motor units and study subjects. The increase in Type 1 error due to subject-level effects can be mitigated through the use of multilevel modeling techniques. This study details the use and benefit of multilevel models when statistically analyzing motor unit discharge data.

1. Introduction

The assessment of human motor unit discharges has been an ongoing area of research for over 30 years. Many early studies used 3–7 subjects, who were frequently the authors of the manuscript and no inferential statistical tests were performed (Bellemare et al., 1983; Grimby, 1984; Grimby et al., 1979, 1981). Given the limited availability of personal computing and lack of user-friendly statistical programs during that era, this practice was understandable. The approach of applying a purely descriptive analysis to motor units can also be found in more recent publications (Collins et al., 2001; Harris et al., 2005; Howell et al., 1995). However, most current researchers employ inferential statistics based on ordinary least-squares (OLS), such as *t*-tests and ANOVAs, or their non-parametric variants, to assess differences in the discharge rates of motor units.

When making multiple measurements from a single motor unit, a repeated-measures ANOVA (Carpentier et al., 2001; Laidlaw et al., 2000; Semmler and Nordstrom, 1998; Sohn et al., 2000) or paired-samples *t*-test (Garland et al., 1994; Macefield et al., 1993) is typically used to control for the presumed intra-correlation that is observed within the same motor unit. Notably, both of these OLS statistical approaches assume a compound symmetry covariance structure or homogeneous variance between two measurements regardless of the distance between the measurements (in time or contraction intensity). To an extent, this assumption can be tested with Mauchly's sphericity test and corrections can be applied to limit Type 1 error rate if the sphericity assumption is violated; however, the application of sphericity corrections may be unnecessary if an appropriate covariance structure is used at the onset. Using the maximum-likelihood (ML) method, available in most modern statistical programs (SAS, SPSS, Stata, R, etc.), allows researchers to choose from a library of covariance structures that may be more appropriate to their data.

Some studies have assumed that all motor units are statistically independent observations when recorded from subjects before and after a training protocol (Van Cutsem et al., 1998) or when cross-sectionally compared between young and old subjects (Kamen et al., 1995) because differing motor units have diverse biophysical properties impacting their discharge pattern. Contrary to the assumption that motor units within a subject are independent

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statistical observations, we recently demonstrated that significant subject-level variation was observed during a ramp task (Tenan et al., 2013). Indeed, Kamen et al. (1995) reported a subject-level intraclass correlation (ICC) of 0.90 for motor units discharging at maximal effort, indicating that 90% of the variability in motor unit discharge rate was explained by the particular subject, not the individual motor unit. An ICC of that magnitude is striking, and it is clear that the importance of this finding was overlooked at the time because an independent samples ANOVA was used to further assess the data. Depending on the number of motor units and subjects involved, a subject-level ICC as small as 0.01 can increase Type 1 error rate to more than 19% and an ICC of 0.30 can increase Type 1 error rate to more than 76% (Musca et al., 2011). Our approach to control for subject-level variation in motor unit discharge pattern was to use the ML-based multilevel statistical modeling procedure which allows for subject-level and motor unit-level variation as well as appropriate covariance structure specification. A multilevel model, also referred to as a hierarchical linear model, random effects generalized linear model or mixed effects generalized linear model, is able to account for the hierarchical structure of the data.

The goal of the current analysis is to demonstrate that subject-level variation in motor unit discharge is present in multiple types of muscle contractions and to explore the amount of subject-level variation during both a previously reported ramp contraction and a fatiguing task in a novel cohort. The effect of uncontrolled subject-level correlation is discussed and the use of multilevel models to control for subject-level variation is suggested.

2. Methods

2.1. Experiment 1

The details of this experiment have been reported previously (Tenan et al., 2013), the present study deviates from the previous analysis by exploring the amount of correlation that exists amongst motor units from individual subjects. Briefly, eleven males attended one visit and seven eumenorrheic women attended five visits. From this cohort, 510 motor units were recorded for analysis. The time of study visit was the same within each subject. The women attended visits during five different phases of their menstrual cycle. All subjects were free from neurologic, cardiovascular, endocrine or metabolic disorders and had no history of surgery, arthritis or immobilization to the examined leg. All subjects gave their informed consent and all experimental procedures were approved by the University of Texas at Austin Institutional Review Board.

2.1.1. Data collection apparatus and set-up

Subjects were secured in a chair with their dominant hip and knee fixed at 90°. Prior to insertion of an intramuscular fine-wire electrode (0.002 mm diameter recording area, California Fine Wire Company, Grover Beach, CA) into the vastus medialis (VM) and vastus medialis oblique (VMO), 12 knee extensions were performed without load. The signals from the inserted electrodes were pre-amplified and bandpass filtered at 8 Hz–3.12 kHz with a gain of 330 (B&L Engineering, Tustin, CA). The dominant ankle was affixed to a strain gauge to record knee extension force (Entran Sensors & Electronics, Fairfield, NJ). Data for electromyography (EMG) and force was A/D converted (Micro 1401 Cambridge Electronic Design, Cambridge, England) and collected through Spike2 (version 5.21, Cambridge Electronic Design, Cambridge, England). Force and intramuscular EMG were sampled at 1 kHz and 30 kHz, respectively.

2.1.2. Exercise protocol and motor unit data reduction

The subjects were trained on the performance of a ramp contraction up to 30% of maximal voluntary contraction (MVC) with a 7.5% MVC rate of rise. This ramp was practiced 3–6 times each study visit before data collection.

Motor units were assessed offline in Spike2. All data was 100 Hz high-pass filtered using a 4th order recursive Butterworth filter. Motor units were visually assessed and identified based upon shape, amplitude and discharge timing. The initial firing rate was the average of the first three interspike intervals converted to hertz.

2.1.3. Statistical analysis

In the first step, a null multilevel model, in which there are no independent variables, was constructed. The null model only includes the hierarchical structure where motor units observations are contained within a person. The model contained random subject-level intercept coefficients with an unstructured covariance structure. The composite multilevel structure can be written as:

$$\text{Frequency}_{ij} = \gamma_{00} + u_{0j} + r_{ij}$$

where Frequency_{ij} is the observed discharge rate for motor unit i within the jth subject, γ_{00} is the model-wide intercept, u_{0j} is the intercept variance term for the subject-level unit j, and r_{ij} is the model variance term.

The subject ICC is calculated as (Singer, 1998):

$$\text{ICC} = u_{0j}/(u_{0j} + r_{ij})$$

SAS PROC MIXED computes the statistical significance of the covariance parameter coefficient (u_{0j}) using a z-value based Wald test.

2.2. Experiment 2

Experiment 2 involves a novel cohort of eight subjects, four males and four females. From this cohort, 20 motor units were collected for analysis across an endurance task. Eleven motor units were observed under normal conditions and 9 were observed during ischemia. All subjects performed two study visits for data collection and were free from neurologic, cardiovascular, endocrine or metabolic disorders and had no history of surgery, arthritis or immobilization to the examined leg. All subjects gave their informed consent and experimental procedures were approved by the University of Texas at Austin Institutional Review Board.

2.2.1. Data collection apparatus and set-up

Subjects were seated with their right knee and ankle fixed at 90°. The right foot was affixed to a metal plate with a strain gauge to measure dorsiflexion force. The same fine wire techniques described in Experiment 1 were used in this Experiment; however, in Experiment 2 fine-wire electrodes were inserted into the proximal portion of the tibialis anterior. The force and EMG were sampled at 1 kHz and 20 kHz, respectively.

2.2.2. Exercise protocol and motor unit data reduction

Each subject attended two study visits. At each visit, a sustained isometric dorsiflexion contraction was held at 20% MVC to task failure (tremor exceeding ±5% MVC). One visit was performed under normal conditions and one was performed while a pressure cuff, placed above the knee, was inflated to 180 mmHg to induce ischemic conditions.

Motor units were assessed off-line using Spike2's waveform discrimination system. All classified motor units were also individually assessed by one investigator. The mean discharge rate for the single motor unit was measured in 5 s time bins assessed every

5% of the endurance task. All interspike intervals ⩽20 ms and ⩾200 ms were excluded from the analysis. The average of all interspike intervals within a given time bin is converted to hertz.

2.2.3. Statistical analysis

The goal of the analysis is first to assess overall subject-level intra-correlations after accounting for within motor unit correlations of spike train data. The secondary goal is to demonstrate the different variance–covariance techniques for repeated-measures data and process of model building.

The primary goal is to determine the subject-level variation while controlling for the within-motor unit variation. Thus, an unconditional linear growth model containing only an intercept and time slope was fit. The within-motor unit variation was modeled with a first-order autoregressive covariance structure which has heterogeneous variances and correlations that decline exponentially with distance (i.e., relative contraction time). This structures the within-motor unit variance so that observations close together in time are more highly correlated than observations further apart in time. The random subject-level effects were fit with an unstructured covariance structure. The multilevel structure can be written as:

$$\text{Frequency}_{ti} = \gamma_{00} + \gamma_{10}\text{TIME}_{ti} + u_{0j} + u_{1i}\text{TIME}_{ti} + r_{ti}$$

where Frequency_{ti} is the observed value for motor unit time point t within the ith subject, γ_{00} is the model-wide intercept, $\gamma_{10}\text{TIME}_{ti}$ is the coefficient for time point t within the ith subject, u_{0i} is the intercept variance term for the subject-level unit i, $u_{1i}\text{TIME}_{ti}$ is the variance term for time point t within the ith subject, and r_{ti} is the model variance term.

The subject ICC was calculated as (Singer, 1998):

$$\text{ICC} = u_{0i}/(u_{0i} + u_{1i}TIME_{ti} + r_{ti})$$

As the ICC equation indicates, the subject-level variance is separate from the variance explained within the multiple observations of each motor unit.

SAS PROC MIXED computes the statistical significance of the covariance parameter coefficients (u_{0i} and $u_{1i}\text{TIME}_{ti}$) using a z-value based Wald test.

A secondary goal of this investigation is to provide context for the use of multilevel models as a solution for subject-level correlations in motor units; an OLS repeated-measures ANOVA was used to assess the motor unit discharge rates at time intervals of 5% of time to tremor with a main effect of muscle ischemia and an interaction of the main effects. The OLS repeated-measures ANOVA assumes a compound symmetry within motor unit measurements; therefore, the first multi-level model replicates the repeated-measures ANOVA with the exception that the estimation technique is the maximum likelihood estimation technique. The second model retains the compound symmetry for repeated motor unit observations but allows for random subject-level variation with an unstructured covariance structure. The third model replicates the repeated-measures ANOVA, but uses a first-order autoregressive covariance structure instead of compound symmetry for repeated motor unit observations. The final model uses both the first-order autoregressive structure for repeated motor unit measurements and unstructured subject-level variation. The fit and relative quality of the models will be assessed with the −2 Log Likelihood and Akaike Information Criterion (AIC). The AIC is not a test of absolute or overall quality of the model; rather, the AIC is used to compare different candidate models to assess which model most accurately fits the data. When comparing models, a lower AIC indicates a better fitting model. Burnham and Anderson's (2002) conventions for AIC comparisons between competing models are: AIC differences <2 indicates the models are equivalent, AIC differences >4 and <7 indicates clearly distinguishable models and AIC differences >10 indicates models that are definitely different.

2.3. Type 1 error rate simulation

The simulation paradigm utilized by Musca et al. (2011) can be generalized to the study design and ICC of Kamen et al. (1995), Experiment 1/Tenan et al. (2013) and Experiment 2 to produce theoretically simulated Type 1 error rates for typical studies with single motor units. The simulation methodology has been explained in-depth previously (Musca et al., 2011). Briefly, the Type 1 error rate is examined as a function of the ICC and the number of level-2 and level-1 units. The data is simulated 5000 times for each study with the equal number of people (level-2 units) assigned to a "treatment" or "control" condition and an equal number of motor units (level-1 units) collected from each person. Furthermore, no systematic difference was assumed between the "treatment" or "control" conditions. The total number of subjects from each simulated study was used with the closest approximation of motor units that satisfied the perfectly symmetrical group distribution for effective simulation (Table 1).

All simulations were performed in R (R Core Team, 2014) using the arm package (Gelman and Hill, 2006). Different from the simulation by Musca et al. (2011), the parameter σ_α was varied to obtain the ICC for the previously reported motor unit studies: $\sigma_\alpha = 15.7$ for ICC = 0.90 (Kamen et al., 1995), $\sigma_\alpha = 1.8$ for ICC = 0.11 (Experiment 1) and $\sigma_\alpha = 4.6$ for ICC = 0.41 (Experiment 2).

3. Results

The subject ICC during the ramp task (Experiment 1) was 0.11 and the effect of the random coefficient on the model was significant ($p = 0.01$). A dot plot of the motor unit discharge rates during the ramp contraction are distributed by subject (Fig. 1).

The subject ICC during the fatiguing task (Experiment 2) was 0.41 and the variability in the random coefficient on the model was significant ($p = 0.04$). This ICC is separate from and accounts for the within motor unit variance observed across the fatiguing task. A dot plot of the motor units and their discharge rates across a fatiguing task are distributed by subject (Fig. 2).

The means and standard errors for the OLS-based repeated measures ANOVA (Fig. 3) can be visually contrasted with the final ML-based model (Fig. 4). The various ML-based models can be compared via the fit statistics where both lower −2 Log Likelihood and AIC represent increases in model fit (Table 2). The models utilizing a first-order autoregressive covariance structure are better fitting than the compound symmetry models. Furthermore, the first-order autoregressive model with random subject-level effects is clearly different from the model without subject-level effects.

The simulation Type 1 error rate results are in Table 1. The error rate is the percentage of times a significant difference ($p < 0.05$) was observed between the two simulated conditions when no difference exists.

4. Discussion

This study demonstrates that motor unit discharges are correlated within a subject. Furthermore, the amount of subject-level correlation may be dependent upon the task performed. Indeed, there is evidence of substantial subject-level correlation during ramp contractions up to 30% MVC and constant force contractions at 20% of MVC in addition to previous findings at 100% MVC (Kamen et al., 1995). These data, from three different cohorts of 39 participants (577 total motor units), allow for the reasonable expectation that motor units collected from the same subject

Table 1
Study specific simulation parameters and resulting Type 1 error rates.

Study/experiment	Subjects	Motor units per subject	ICC	Type 1 error rate (%)
Kamen et al. (1995)	14	8	0.90	52.7
Experiment 1/Tenan et al. (2013)	18	28	0.11	35.1
Experiment 2	8	5	0.41	31.2

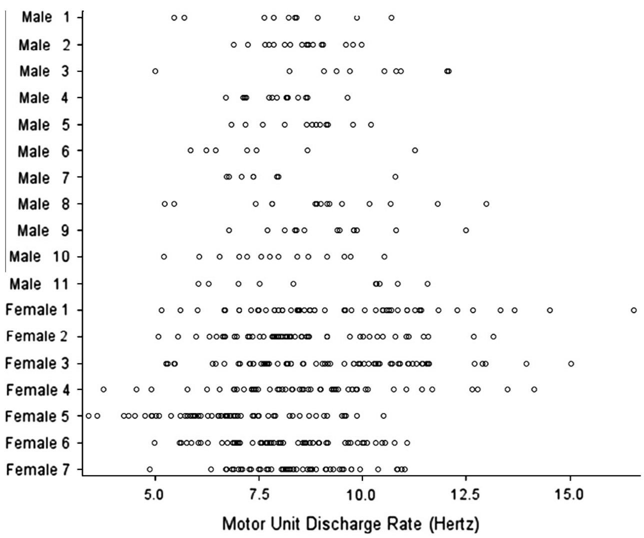

Fig. 1. Initial discharge rate of motor units during the ramp contraction task distributed by subject (Experiment 1).

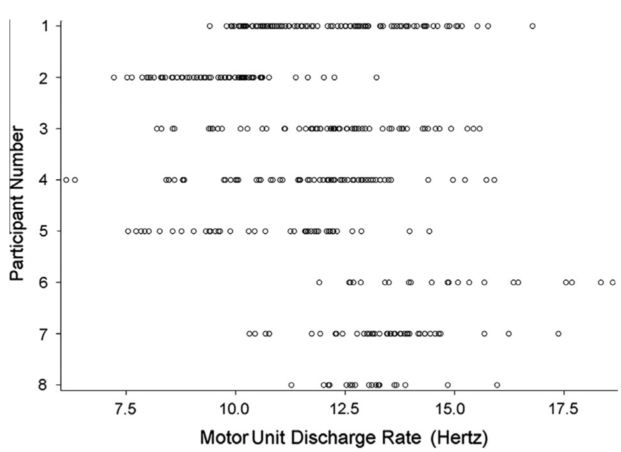

Fig. 2. The discharge rates of motor units during the isometric hold contraction task distributed by subject. The discharge rates for all motor units at all time points are pooled according to subject (Experiment 2).

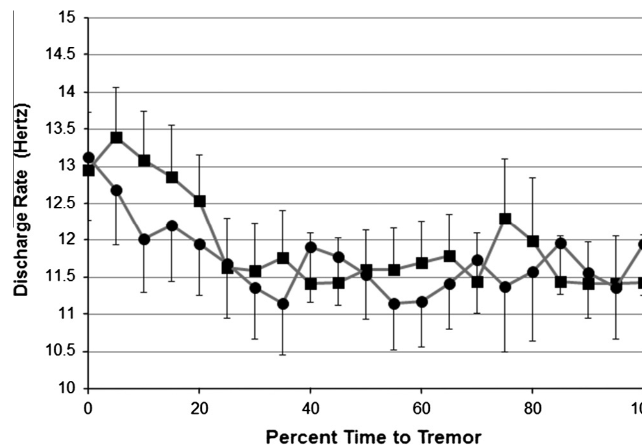

Fig. 3. Means (±standard error) computed across relative time using repeated measures ANOVA for motor units collected in Control (circles) and Ischemic (square) conditions (Experiment 2).

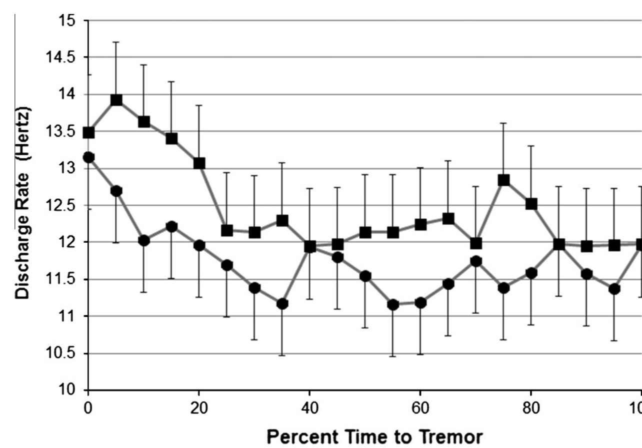

Fig. 4. Means (±standard error) computed across relative time using a maximum likelihood multi-level model with a first-order autoregressive covariance structure for repeated motor unit observations and unstructured subject-level effects for motor units collected in Control (circles) and Ischemic (square) conditions (Experiment 2).

across one or multiple visits are not statistically independent observations.

The statistical implications of this subject-level correlation have a clear and substantial impact on hypothesis testing. The convention of setting global $\alpha = 0.05$ indicates that the researcher is willing to accept the 5% chance of making a Type 1 error. Failing to account for correlated data can substantially inflate Type 1 error rate due to artificially low standard errors that do not account for subject-level variability. The paradigm utilized by Musca et al. (2011) can be applied to the present data as well as that of Kamen et al. (1995) to provide a real-world illustration of not controlling for subject-level variation. These simulated rates are not the actual error rate of the aforementioned studies because in no case is there perfectly balanced study design, zero systematic group differences cannot be assumed, and Tenan et al. (2013) accounted for subject-level variation. In the case of the present Experiment 2, the difference in estimate and standard error calculations between a conventional OLS ANOVA and ML-based multilevel analysis can be viewed in Figs. 3 and 4. There are noticeable differences in standard error estimates between the models. In this case, it may have affected what time point significant differences are observed between time points or between groups.

The use of ML-based calculation methods also allow for different covariance structures than the compound symmetry structure imposed in a repeated measures ANOVA. It is clear when comparing the ML-based models that the model using the first-order autoregressive covariance structure has superior fit to the model employing the more restrictive compound symmetry

Table 2
ML-based models and fit statistics. ICC's cannot be computed from models without subject-level intercept.

Covariance structure	Random subject-level intercept	−2 Log likelihood	AIC	Subject-level ICC
Compound symmetry	None	1387.6	1475.6	–
Compound symmetry	Unstructured	1386.6	1476.6	0.39
First-order autoregressive	None	1271.1	1359.1	–
First-order autoregressive	Unstructured	1262.4	1352.4	0.41

structure. In fact, the most strict AIC interpretation concludes that the compound symmetry models have "essentially no empirical support" when compared to the autoregressive models (Burnham and Anderson, 2002). The differential specification of the covariance structure for repeated-measures on the same motor unit can mathematically affect the calculation of the ICC in multilevel models (see Table 2). Indeed, it is this model specific ICC that allows the investigator to simulate the actual α-level for the specific model. In a properly parameterized statistical model where normality of errors, equal variance and model fit are correctly assessed, minor alterations to the motor-unit level co-variance structure should not impede similar interpretive results of the ICC.

The additional benefit of using ML-based models not restricted to the compound symmetry covariance structure is an increased flexibility in data analysis. For the sake of OLS ANOVA and ML multilevel model comparison, we needed to assume that the data was equally spaced in intensity by normalizing it to data points collected in 5 s time bins every 5% of time to tremor. These 5% intervals are differently spaced in absolute time due to differences in overall exercise time. However, a ML-based model can handle unequally spaced observations. For example, Fig. 5 depicts the model estimates (±standard error) for a multilevel model in absolute time. The last data point recorded for ischemia was at 340 s, which is why we do not report data after that time point. However, if the researcher is interested in forecasting beyond a recorded time point, appropriate standard errors and confidence intervals can be generated.

The ischemia intervention can be viewed as a confounding effect when determining the subject-level correlation of motor units; however, the substantial ICC recorded indicates that subject-level correlations of motor unit discharges are robust even during strong, acute interventions. Although the number of motor units recorded across three studies indicating subject-level correlations is large ($n = 577$), the number of motor units recorded in Experiment 2 alone is modest ($n = 20$). When only a modest number of motor units are sampled from a small number of

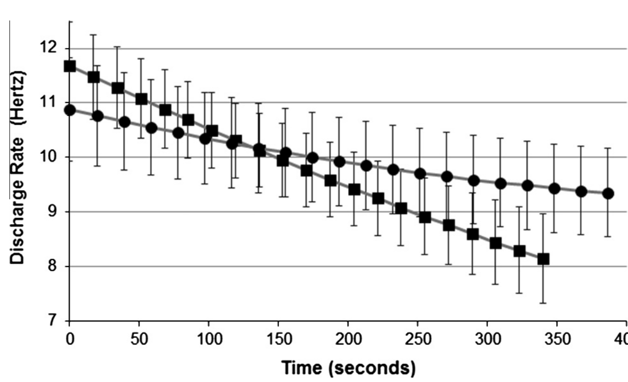

Fig. 5. Model estimates (±standard error) computed across absolute time using a maximum likelihood multi-level model with a first-order autoregressive covariance structure for repeated motor unit observations and unstructured subject-level effects for motor units collected in Control (circles) and Ischemic (square) conditions (Experiment 2).

participants, investigators must be careful to ensure that one "outlier" participant is not biasing the results. One approach may be to constrain the data such that each individual contributes the same number of motor units. A second option, favored by the authors, is to evaluate the influence of each participant using Cooks Distance statistic (Cook, 1979) and determine that no subject is exerting an outsized influence on the overall model.

Multilevel data structures, where lower-level units are a part of (or "nested within") a higher level unit, are common within the social sciences. Classically, this is illustrated with an example of a cluster of students within a classroom unit, which is a unit within a school, which is a unit within a school district. In the context of the present research, a motor unit is nested within an individual human. There may be justification to nest motor units within an anatomical muscle within a human, but that is an area for future research.

Simply because data can be visualized in a hierarchical structure does not mean that a multilevel analysis is necessary. For this reason, we advocate the use of the ICC which is the ratio of the between-group variance to the total data variance (Fidell and Tabachnick, 2006; Gelman and Hill, 2006; Singer, 1998). If the ICC and data structure are clearly reported, then the readership can determine if a statistical approach was reasonable. Conversely, a more conservative approach would be to always use a multilevel analysis for motor unit data. The latter convention should have no adverse effects, but it will add unnecessary complexity to the analysis if the ICC is very low and other data structure criteria are met (Musca et al., 2011). With added model complexity, researchers should be cautioned to check model specification for correlated error structures, normality of errors and equal variance of errors to ensure their analysis is unbiased (Gelman and Hill, 2006).

5. Conclusions

Motor unit discharge data is correlated within a subject even across testing days. This may result in inappropriate conclusions if statistically analyzed with ordinary least squares-based tests (ie. ANOVA, *t*-test, OLS regression, etc.). Subject level ICCs should be reported when analyzing multiple motor units from a subject. If the ICC is substantial enough to bias the statistical test, the use of multilevel models is suggested.

Conflict of interest

The authors have no conflicts of interest to declare.

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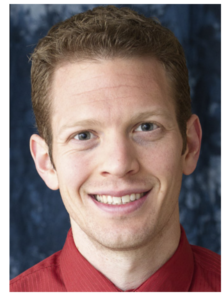

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