Stability of Motor-Unit Force Thresholds in the Decerebrate Cat

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Cope, Timothy C., Alan J. Sokoloff, Stan M. Dacko, Rebecca Huot, and Eleanor Feingold. Stability of motor-unit force thresholds in the decerebrate cat. J. Neurophysiol. 78: 3077–3082, 1997. To further test the hypothesis that some fixed property of motoneurons determines their recruitment order, we quantified the variation in force threshold (FT) for motoneurons recruited in muscle stretch reflexes in the decerebrate cat. Motor axons supplying the medial gastrocnemius (MG) muscle were penetrated with micropipettes and physiological properties of the motoneuron and its muscle fibers, i.e., the motor unit, were measured. FT, defined as the amount of MG force produced when the isolated motor unit was recruited, was measured from 20 to 93 consecutive stretch trials for 29 motor units. Trials were selected for limited variation in base force and rate of rise of force, which have been shown to covary with FT, and in peak stretch force, which gives some index of motor-pool excitability. Under these restricted conditions, large variation in FT would have been inconsistent with the hypothesis. Analysis of the variation in FT employed the coefficient of variation (CV), because of the tendency for FT variance and mean to increase together. We found that CV was distributed with a median value of 10% and with only 2 of 29 units exceeding 36%. Some of this variation was associated with measurement error and with intertrial fluctuations in base, peak, and the rate of change of muscle force. CV was not significantly correlated with motor-unit axonal conduction velocity, contraction time, or force. In three cases FT was measured simultaneously from two motor units in the same stretch trials. Changes in recruitment order were rarely observed (5 of 121 stretch trials), even when FT ranges for units in a pair overlapped. We suggest that the large variation in recruitment threshold observed in some earlier studies resulted not from wide variation in the recruitment ranking of motoneurons within one muscle, but rather from variation in the relative activity of different pools of motoneurons. Our findings are consistent with the hypothesis that recruitment order is determined by some fixed property of α-motoneurons and/or by some unvarying combination of pre-synaptic inputs that fluctuate in parallel.

INTRODUCTION

It has been hypothesized that the orderly sequence in which motoneurons are typically recruited into activity is determined by a fixed property intrinsic to the motoneurons themselves (Henneman et al. 1974). The determining property or properties cannot be fixed, however, if recruitment order can be shown to vary in repeated trials (see Burke 1981). One result that appears to demonstrate substantial intertrial variation in recruitment was obtained from humans performing replicate voluntary contractions. Under these conditions, Romaiguere and colleagues (Romaiguere et al. 1989, 1993) reported large random fluctuations in force threshold (FT), which is defined as the force of muscular contraction measured at the moment when a motor unit reaches firing threshold. By this measure the threshold of one unit is judged in relation to the cumulative force produced by all previously recruited units. Thus FT provides an index of the variation in recruitment order among active motor units and variation in the FT might reflect instability in the process of recruitment order. In computer simulations, for example, Heckman and Binder (1993) demonstrate variation in recruitment sequence caused by the addition of random variance in motoneuron excitability.

There are, however, other potential sources of variation in FT that are introduced by the way in which this parameter is commonly measured. The majority of these studies have been performed on human subjects in whom motor unit threshold is actually measured in relation to joint torque rather than muscle force. Therefore the “torque threshold” of a motor unit may vary because of shifts in the relative contributions of different muscles to the joint torque and not because of variation in the recruitment of motor units within a single muscle. Additionally, intertrial fluctuations in either background force (Cordo and Rymer 1982) or in the rate of rise of force (Budingen and Freund 1976; Tanji and Kato 1973) may yield FT variation.

The purpose of this study was to limit the sources of variation in FT as much as possible to the recruitment process itself. To accomplish this, we measured the onset of firing for one motoneuron in relation to force generated by the medial gastrocnemius (MG) muscle in the decerebrate cat. Force records were taken directly and exclusively from the MG muscle. The MG muscle was selected because its recruitment behavior in the cat has been well documented (Bawa et al. 1984; Cope and Clark 1991; Tansey and Botterman 1996) and gives little evidence of functionally independent groups or pools of motoneurons (Clark et al. 1993; Cope and Clark 1991; Dacko et al. 1996; but see Kanda et al. 1977). In addition, examination of motor-unit FT was restricted to sequential reflex contractions exhibiting similar profiles of force in response to identical ramp stretches. Reflex contractions meeting these requirements were assumed to indicate the involvement of a similar set of active motor units from one recruitment trial to the next. We reasoned that substantial fluctuations in the FT of motor units under these conditions would indicate that the relative excitability of motoneurons cannot be determined by a fixed motoneuron property.

Some of the findings reported here appear in abstract form (Cope and Sokoloff 1996).

METHODS

Eight cats of either sex, ranging in weight from 2.5 to 3.5 Kg, were used in terminal experiments similar to those described in
were artificially respired to hold end-tidal CO2 between 3 and 4% for 20-30 minutes to induce changes in FT (cf. Denier van der Gon et al. 1985).

Dose of pentobarbital sodium (Nembutal, 150 mg/Kg) was given in some cases, but ramp stretches were only able to recruit units when the stimulus was strong enough to elicit a reflexive response. In other cases, the stimulus had to be lowered to levels that did not cause continuous firing of the unit. Across the majority of units (21 of 29), average base force, mainly attributable to passive stretch (as indicated by low or absent levels of resting EMG), ranged from 0.4 to 1.7 N, but was as high as 6.8 N. All 29 motor units, average values ranged from 2 to 26 N in peak force and from 17 to 153 N/s in rate of rise of force.

Records were collected simultaneously through separate A/D channels and stored in files on computer for later analysis. The records of motor-unit force and whole-muscle force were digitized at a sampling rate of 1 kHz and the recording of axonal action potentials were digitized at 20 kHz. In a few cases, files were constructed from records collected on VCR tape, as described in an earlier report (Dacko et al. 1996).

Data analysis

The parameters measured from whole-muscle force and motor-unit firing in response to each stretch trial are illustrated in Fig. 1B. Base and peak forces were determined, respectively, at single points measured immediately before and at the peak of the rising force trajectory. The rate of rise of force was measured from the force developed over time from base to peak force. FT was taken as the value of whole-muscle force measured when the isolated motor unit began firing. FTs were not normalized to base force, because differences in base force reflected differences in threshold for one unit to the next.

Sequential stretch responses were accepted for analysis when the SD of base and peak forces and of the rate of rise of force were not more than 20% of the respective mean values, i.e., when CVs were < 20%. This criterion was met by 29 different motor units for ≥ 20 stretch trials (range, 20–93 trials). We restricted our analysis to units with ≥ 20 trials to have large enough sample sizes to perform the multiple regression analyses described below.

Analysis was also restricted to those stretch trials in which the motor unit under study was recruited during the ramp portion of stretch; recruitment thresholds were not compared over trials in which the unit was recruited either during the hold or release phase of stretch. Recruitment thresholds reported here were obtained, therefore, under conditions of the same stimulus across different recruitment trials.

The contributions of two other sources of FT variation were considered. First, the discrete sampling of muscle force (1 kHz) introduced variation in our measurement of FT. FT was not precisely determined when the motor-unit action potential occurred in between force samples (taken every 1 ms). Instead, threshold was assigned the force designated by the closest sampling point (i.e., within 0.5 ms of action-potential occurrence). On the basis of the maximum differences in force observed between adjacent samples (60–200 mN), we estimate that this source of variation amounted to no more than 1.5% of the mean FT values. Second, the forces measured during stretch were produced not only by reflex contraction but also by the muscle’s passive resistance to stretch. To estimate variation introduced by this passive component, we measured variation in digitized records of the force generated by areflexive (denervated) muscle in response to stretch. Base and peak forces were measured from one denervated MG muscle.
FORCE THRESHOLD STABILITY

FIG. 1. Reflex recruitment of motor axons in sequential trials of muscle stretch. A, top: record of ramp-hold-release length perturbations of fixed time course (100, 300, and 100 ms, respectively) and amplitude (4 mm) applied every 4 s by servomotor to distal tendon of medial gastrocnemius (MG) muscle. A, middle: continuous record of MG muscle force showing responses to stretch. A, bottom: event markers denoting times of occurrence of action potentials from 1-MG motor axon recruited in stretch reflexes. B: expanded records of MG muscle force (top) and action potential times (bottom) identify parameters measured from each stretch trial.

Stability

fixed at different base-force levels ranging from 1.3 to 3.5 N and stretched over many trials of either 4, 6, or 8 mm. The CVs measured under these conditions ranged from 3 to 11%, with the largest variation associated with the 8-mm stretches.

Statistical methods

To characterize relationships between pairs of variables, we used the Spearman rank correlation, because most distributions were highly skewed and/or multimodal. To measure the variation in FT for each unit, we used the CV because the relationship between the means and SDs for all 29 units was approximately linear.

Multiple linear regression (Sokal and Rohlf 1995) was applied to fit a linear regression model of FT on the base, peak, and rate of rise of whole-muscle force for records collected from each motor unit. We used these regression models to calculate adjusted CVs for each unit, using the formula adjusted $\text{CV} = \text{CV}(1 - R^2)$, where $R^2$ is the unadjusted $R^2$ from the multiple regression. The adjusted CV should be interpreted as a measure of variation in FT that is not explained by variation in base, peak, and rate of rise in muscle force. Residual analysis showed that a few of these linear regressions were not good fits because of outliers, but we included these anyway because they provide a conservative bound on the possible amount of reduction in the CV. That is, a better model could only make the adjusted CV smaller. In addition, we performed a multiple linear regression analysis to examine the relationship between the CV and various unit characteristics. All regression models were extensively checked by residual analysis.

RESULTS

The findings described below were taken from 29 MG motor units. In comparison with previously reported values (e.g., Cope and Clark 1991; Tansey and Botterman 1996; see McDonagh et al. 1980), ranges in conduction velocity (59–115 m/s) and in twitch contraction time (21–95 ms) approximated those for the whole population of MG motor units. The range in tetanic force (20–314 mN) was biased however toward lower values, probably reflecting the insufficiency of muscle stretch alone to recruit high-threshold motor units in decerebrate cats (see Cope and Clark 1991).

Variation in motor-unit FT

The purpose of this study was to quantify the intertrial variation in FT for single motor units. Fig. 2 illustrates this variation for one motor unit. Unadjusted values of CV were $\leq 10\%$ for 15 of 29 units and $< 36\%$ for all but two units.

Fig. 2 shows how shifts in the muscle’s response to stretch, in this case small fluctuations in base force, was associated with variation in FT. In this case, a shift to a lower base force seen in the bottom of the three force traces corresponds with a lower FT. Evaluation of the whole sample revealed statistically significant ($P < 0.05$) Spearman rank correlations between FT and base force for 13 units, between FT and peak force for 11 units, and between FT and rate of rise in force for 7 units. Thus despite selection of recruitment trials in which parameters of whole-muscle force were broadly the same, slight variations in these parameters appeared to contribute variation in FT. To remove this contribution and to generate CV values that express the amount of variation in FT not explained by variation in base, peak, and rate of rise in force, we performed multiple regression as described in METHODS. The resultant adjustment reduced the CV of FT by as much as 20%. In most cases, however, the reduction was small ($< 4\%$ for 26 of 29 units). The distribution of the adjusted values for CV in FT is shown in Fig. 3.

To investigate possible explanations for the remaining variation in FT, we performed a linear regression of the

FIG. 2. Intertrial variation in force threshold (FT) for 1 MG motor unit. Superimposed lines show beginning portions of muscle force responses to 3 stretch trials, all positioned relative to stretch onset and zero force. FTs indicated by ● for the three traces, and by entries in the histogram for all trials ($n = 39$). →, location of FTs for the 3 trials within histogram; $X$, FT mean.
natural log of the adjusted CV on the three motor-unit properties (axonal conduction velocity, tetanic force, and twitch contraction time). This analysis showed no systematic relationships. Graphic analysis gave some indication of possible cat-to-cat differences, with some cats seeming to have all low-variation motor units and with other cats having some low-variation and some high-variation units. The small sample sizes of at most five to seven motor units per cat made this impossible to test statistically, however.

**FTs of motor-unit pairs**

To determine whether the residual variation in FT might reflect variation in recruitment order, we examined variation in FT for three pairs of motor units. Fig. 4 presents the data collected from two pairs of MG units. Lines connect the FTs measured in single stretch trials. Unit 1 designates the unit commonly exhibiting the lowest FT and lines with positive slopes identify stretch trials in which unit 2 was recruited at a higher level of muscle force than unit 1. In Fig. 4A, FT for unit 2 was higher than for unit 1 in all 49 trials. Regularity in the recruitment ranking for this unit pair is not surprising given that there was very little overlap in the ranges of FT. By contrast, the FTs of units 1 and 2 overlapped substantially in Fig. 4B and even more so in the third pair (data not shown). Even so, the rank order of FTs changed infrequently from one stretch to the next; unit 2 had the higher FT in 33 of 35 trials for the pair in Fig. 4B and 34 of 37 trials in the remaining pair. These findings indicate that fluctuations in FT were correlated among units, as verified for units in all three pairs by statistically significant ($P < 0.005$) Spearman rank correlation coefficients. These findings demonstrate that the FT of single units can vary widely without causing changes in the rank order in threshold of one motor unit relative to another.

**DISCUSSION**

The force of muscular contraction increases as the number of motor units recruited into activity increases. This relationship makes it possible to estimate the order in which motor units are recruited by noting the force generated at the time individual motor units are first recruited into activity (see Calancie and Bawa 1990; Henneman and Mendell 1981).

The present study focused on variation in FT over replicate recruitment trials. We argued, as have others (e.g., Burke 1981), that large variation in FT, especially under the rigidly controlled conditions used in this study, would indicate that recruitment order is not fixed and, therefore, could not be determined by any fixed element of neuronal integration, either pre- or postsynaptic. In our experiments we limited potential sources of variation by studying FT against force generated by a single muscle and by selecting recruitment trials for similarity in whole-muscle contraction. Under these conditions, the CV fell below 10% for one-half of the sample of motor units and below 36% for all but two motor units. Part of this variation was attributable to measurement error and sources of variation other than the recruitment process itself.

The magnitude of variation observed by us was much lower than that reported by Romaiguere et al. (1989). The latter study identified only 3 of 50 motor units with CVs <10% and values reached as high as 117%. Among several differences between that study and our own was that thresholds for human motor units were measured by Romaiguere et al. in relation to wrist-extension torque generated by multiple muscles. It is possible under this condition that what varied was not the relative recruitment threshold of one motor unit among other active units in the parent muscle, but rather the relative contribution to force made by the parent muscle among all participating muscles. Differential activation of multiple muscles was not a factor either in our study or in the extensive investigation of FT variation performed by Tanji and Kato (1973) on motor units in the human hand muscle, abductor digiti minimi. This muscle is the only one involved in producing abduction of the fifth digit, and in 5–10 trials of slow (5 s) ramp isometric contractions, the CVs in FTs averaged about 8% and did not exceed 20%. Similarly, the first dorsal interosseous muscle acts alone in abducting the first digit, and its motor units show little intertrial variation in FT (Freund et al. 1975).

Low variation (usually <10%) was reported by Denier van der Gon et al. (1985) for the FTs of motor units isolated from one of the multiple muscles that produce flexion of the human elbow. Thus the contribution of force from multiple muscles need not result in as large variation in FT as reported by Romaiguere et al. (1989, 1993). Perhaps the greater variation found by Romaiguere et al. signals less stability in the
relative force contributions made by muscles producing wrist extension than by muscles generating elbow flexion. Another possibility is suggested by our finding that unit FT co-varies with even small irregularities in force production, irregularities that were not quantitatively analyzed in human studies. Whatever the reason for high FT variation found in one study, the relatively low variation found here is comparable with that reported in all other studies that we are aware of, even though obtained from different species (cat vs. human), different muscles (muscles in lower vs. upper extremities), and different tasks (lengthening vs. isometric contractions initiated reflexively vs. voluntarily). This similarity suggests that constraints on the thresholds of motoneurons are tight and widely applied.

Although relatively small, the unexplained variation in FT measured here was consistently observed. Of central importance to our objective was evidence that the magnitude of variation observed here did not reflect switches in motor-unit recruitment order. Switches in the recruitment order of units studied in pairs were rare, even in cases of substantial overlap in the ranges of unit FTs. Switches in recruitment order were also scarce in the face of intertrial threshold variation for motor units in the first dorsal interosseous (Tanji and Kato 1973) and tibialis anterior (Kato et al. 1985) in human subjects. These findings must mean that threshold fluctuations are correlated among motor units, as has been discussed most recently by Gossard et al. (1994). Another factor that may have limited switches in order in our data set was the relatively low numbers of motor units recruited during the 100-ms period of muscle stretch, as suggested by the relatively low levels of reflex force and EMG. With few motor units active, there would be greater tolerance for threshold variation without causing recruitment order variation than with greater numbers of active units.

Motor-unit recruitment is only one of several factors that determine muscle force and that might result, therefore, in FT variation. Force generated by stretching the passive elements of muscle contributed to the records obtained in this study and this component of force exhibited some degree of variation. In addition, the active force generated by muscle contraction was partially attributable to motor-unit firing rate that was variable enough (rate variation up to 10 pps) to produce intertrial variation in contraction force (cf. Kermell et al. 1983). It is likely that both of these sources of variation in muscle force produced some of the observed variation in motor-unit FT, independently of any variation in motor-unit recruitment.

Perspectives on the mechanism of recruitment order

Variation in the recruitment of motoneurons provides a valuable test of the hypothesis that some fixed property of neuronal integration establishes recruitment order; low but not high variation is compatible with this hypothesis. Two kinds of variation have been examined. One is a systematic variation in which recruitment order or measures of recruitment threshold are shown to vary depending on, for example, the source of excitatory drive to the motoneurons (e.g., Garnett and Stephen 1981; see also Burke 1991; Thomas et al. 1986). In consideration of this kind of variation, we suggested previously that there is little evidence for functionally relevant variation in the order in which active motoneurons are recruited (Cope and Clark 1995). The other kind of variation is random, or seemingly uncontrolled variation in recruitment order or threshold, and was the focus of the present study. Some other studies have demonstrated substantial random variation (Gossard et al. 1994; Rall and Hunt 1956; Romaiguere et al. 1989, 1993), thereby giving evidence of a degree of variation in recruitment that is not compatible with a fixed determinant of order. In these studies, however, there was uncertainty about the identity of the motoneurons and/or muscle under study. In the present study, where this uncertainty was eliminated, variation in FT was limited and variation in recruitment order was rarely observed. Our findings are compatible, therefore, with the abundant evidence for stability in recruitment order (description and citations in Calancie and Bawa 1990; Cope and Clark 1995; Desmedt 1981; Henneman and Mendell 1981).

All considered, we believe that the weight of evidence supports the view that some fixed factor(s) dominates in establishing the orderly recruitment of active motoneurons. This factor might derive from determinants of motoneuron excitability (Binder et al. 1996; Cope and Pinter 1995; Gustafsson and Pinter 1985) and/or synaptic input to motoneurons from interneurons or neurons such as Ia afferents (Heckman and Binder 1993).

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REFERENCES


