

# Sag During Unfused Tetanic Contractions in Rat Triceps Surae Motor Units

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**Carp, J. S., P. A. Herchenroder, X. Y. Chen, and J. R. Wolpaw.** Sag during unfused tetanic contractions in rat triceps surae motor units. *J. Neurophysiol.* 81: 2647–2661, 1999. Contractile properties and conduction velocity were studied in 202 single motor units of intact rat triceps surae muscles activated by intra-axonal (or intra-myelin) current injection in L<sub>5</sub> or L<sub>6</sub> ventral root to assess the factors that determine the expression of sag (i.e., decline in force after initial increase during unfused tetanic stimulation). Sag was consistently detected in motor units with unpotentiated twitch contraction times <20 ms. However, the range of frequencies at which sag was expressed varied among motor units such that there was no single interstimulus interval (ISI), with or without adjusting for twitch contraction time, at which sag could be detected reliably. Further analysis indicated that using the absence of sag as a criterion for identifying slow-twitch motor units requires testing with tetani at several different ISIs. In motor units with sag, the shape of the force profile varied with tetanic frequency and contractile properties. Simple sag force profiles (single maximum reached late in the tetanus followed by monotonic decay) tended to occur at shorter ISIs and were observed more frequently in fatigue-resistant motor units with long half-relaxation times and small twitch amplitudes. Complex sag profiles reached an initial maximum early in the tetanus, tended to occur at longer ISIs, and were more common in fatigue-sensitive motor units with long half-relaxation times and large twitch amplitudes. The differences in frequency dependence and force maximum location suggested that these phenomena represented discrete entities. Successive stimuli elicited near-linear increments in force during tetani in motor units that never exhibited sag. In motor units with at least one tetanus displaying sag, tetanic stimulation elicited large initial force increments followed by rapidly decreasing force increments. That the latter force envelope pattern occurred in these units even in tetani without sag suggested that the factors responsible for sag were expressed in the absence of overt sag. The time-to-peak force (TTP) of the individual contractions during a tetanus decreased in tetani with sag. Differences in the pattern of TTP change during a tetanus were consistent with the differences in force maximum location between tetani exhibiting simple and complex sag. Tetani from motor units that never exhibited sag did not display a net decrease in TTP during successive contractions. These data were consistent with the initial force decrement of sag resulting from a transient reduction in the duration of the contractile state.

## INTRODUCTION

Mammalian motor units exhibit considerable variation in force generation, endurance, and speed of contraction and relaxation (Burke 1981). A substantial portion of this diversity

can be attributed to the presence of distinct sets of myofibrillar constituents that define two general classes of muscle fibers, commonly referred to by their hallmark contractile feature as fast-twitch (F) and slow-twitch (S) fibers (Pette and Staron 1990; Stephenson et al. 1998). In attempting to identify motor units solely on the basis of contractile properties, Burke et al. (1973) proposed a classification scheme in which the presence or absence of “sag” (i.e., a decline in force after the initial increase during unfused tetanic stimulation) distinguished F and S motor units, respectively. Originally developed for medial gastrocnemius motor units of the cat, this scheme has been applied widely in other muscles and species (Burke 1981).

Studies of rat triceps surae (TS) motor unit contractile properties have produced qualitatively similar results, but there has not been general agreement on the specific criteria for type identification. The presence or absence of sag as a lone predictor of the speed of contraction has been confirmed in some studies (Chamberlain and Lewis 1989; Einsiedel and Luff 1993; Kanda and Hashizume 1989) but not in others (Bakels and Kernell 1993a,b; Grottel and Celichowski 1990). In these studies, sag was evaluated under widely varying conditions (e.g., at fixed stimulation frequencies or at frequencies based on the speed of contraction and with single trains or with batteries of trains at different stimulation frequencies). Sag has been demonstrated to be present at some stimulation frequencies in a given unit, but not all units display the same pattern of frequency dependence or even the same force envelope shapes (Celichowski 1992a; Einsiedel and Luff 1993; Gardiner 1993; Grottel and Celichowski 1990). Thus it is not surprising that a consistent classification scheme for F versus S units has not emerged for rat motor units.

We are currently developing a model for performing motor unit typing in the intact triceps surae (TS) of the anesthetized rat as part of this laboratory’s ongoing investigation of adaptive plasticity in the spinal cord (reviewed in Wolpaw 1997). The goal of the present study was to identify factors that contribute to variation in sag conformation to develop criteria for rapid and reproducible motor unit typing in the rat. Portions of this work has been reported previously in abstract form (Carp et al. 1997, 1998).

## METHODS

### *Animal preparation*

Experiments were performed in 24 male Sprague-Dawley rats weighing 461–554 g. All procedures conformed to National Institutes of Health Publication 85-23, Guide for the Care and Use of Labora-

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tory Animals and had been reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. Animals were anesthetized initially with 70 mg/kg ip of pentobarbital and supplemented with 10 mg/kg iv about every 30 min to suppress corneal and limb withdrawal reflexes and maintain heart rate between 300 and 350 beats per minute. The trachea and a jugular vein were cannulated. A mixture of dextran, sodium bicarbonate and glucose was infused continuously at 1.7–3.4 ml/h (Quintin et al. 1989). End tidal CO<sub>2</sub> was 32–42 mm of Hg during recording sessions. Rectal temperature was maintained at 37–38°C by feedback-controlled heat lamps.

Animals were mounted in the prone position in a rigid frame and secured by ear and jaw bars and a pair of hip pins applied 12–15 mm caudal to the front end of the ilium. The right hip and knee were extended and inserted into a latex surgical glove. The anterior surface of the hindlimb from the foot to the middle of the thigh was attached with cyanoacrylate glue to the glove, leaving the posterior side accessible for surgery. The hindlimb was immobilized by a 2-cm-wide clamp that gripped the anterior side of the tibia with its proximal edge 3 mm distal to the knee. The clamp was anchored in this position by inserting No. 62 drill bits through the tibia through two pairs of holes in the clamp located 2 mm from each end. This permitted the clamp to be tightened without slipping or impeding blood flow to the TS muscles.

The TS [i.e., medial and lateral gastrocnemius (MG and LG) and soleus (SOL)] muscles of the right hindlimb were dissected from surrounding tissue. The tendon of the plantaris muscle was dissected from the common tendon of the TS muscles and cut, but its position relative to the TS muscles was not altered. To avoid interruption of perfusion, the TS muscles were not separated from each other, and small vessels along the lateral borders of the muscles were left intact to the extent that permitted unimpeded passive stretch of the muscle. The calcaneus was cut leaving a small bone chip attached to the TS tendon. The bone chip was secured in a clamp, which was connected by a 50 × 0.6 mm steel wire to a force transducer (FT03, Grass Instruments).

Electromyographic activity (EMG) from each of the TS muscles (i.e., SOL, MG, and LG) was recorded with three intramuscular pairs of fine stainless steel wires that had 4 (SOL) or 6 (MG and LG) mm of Teflon insulation removed from each tip. Postmortem inspection confirmed electrode positions to be wholly within the targeted muscle in all but one experiment, where one of the wires intended for SOL was inserted into LG.

Motor unit recordings were performed by impalement of the motoneuron axon in the ventral root (modification of the method of Cope and Clark 1991). A dorsal laminectomy was performed from the L<sub>1</sub> to L<sub>5</sub> vertebrae and the dura mater was cut. The cut skin flaps were pulled up to hold a mineral oil pool. The L<sub>5</sub> or L<sub>6</sub> ventral root was manipulated with fine glass hooks onto a pair of stainless steel hook electrodes embedded in a longitudinally hemisected 10-mm-long piece of Silastic tubing (3 mm OD × 2 mm ID) 1–3 mm above the surface of the spinal cord. In addition, the tibial nerve was dissected from surrounding tissue, and its electroneurographic activity (ENG) was recorded using a cuff electrode formed from a longitudinally hemisected 4-mm-long piece of silastic tubing (0.4 mm OD × 0.26 mm ID) fitted with a semicircular loop of 50- $\mu$ m OD stainless steel wire at its midpoint. The cuff was positioned underneath the tibial nerve ~10 mm proximal to the TS muscles. A small incision was made with fine scissors in the epineurium opposite to the wire loop in the cuff. The tip of a 50- $\mu$ m OD stainless steel wire from which the final 0.5 mm of Teflon insulation had been removed was inserted gently just beneath the epineurium. Application of a rapid-cure silicone elastomer (Kwik-Cast, World Precision Instruments) to the cuff stabilized and electrically isolated the electrodes.

The TS muscles and tibial nerve then were submerged in a mineral oil pool formed by lifting the edges of the latex surgical glove that had been glued previously to the anterior skin of the hindlimb. Mineral oil

pools in the leg and back were maintained at 36–37 and 37–38°C, respectively, by feedback-controlled heat lamps.

### *Electrophysiological recording*

Ventral root axons were impaled by advancing glass microelectrodes filled with 3 M potassium acetate (12–25 M $\Omega$ ) in 1- to 3- $\mu$ m steps at a 45° angle to the long axis of the nerve with tip pointing caudally. As the electrode advanced, depolarizing current pulses (2–4 nA, 1 ms) were applied at 1 Hz until an action potential was elicited. Stimulation frequency was immediately reduced to 0.2 Hz, and motor units were identified by the presence of reproducible all-or-none EMG and twitch responses to single stimuli over at least a fourfold range of current intensities. Plantaris axon impalements sometimes elicited measurable EMG in the TS electrodes. These units were abandoned after observing movement of its cut tendon and transient force responses at the onset and offset of tetanic stimulation with little or no sustained force during the tetanus. Ventral root impalements produced negative resting potentials and action potential amplitudes of  $37 \pm 18$  mV (mean  $\pm$  SD, measured from spike threshold to peak).

A series of twitch and tetanic responses were elicited by current injection to characterize the properties of each motor unit. The following sequence of stimulation and recording protocols was initiated and continued until it was completed or until the unit was lost. First, unpotentiated twitch force, EMG, and axon voltage were recorded during 0.2-Hz stimulation and averaged (4–16 responses, with more averaged for low force units). Second, tetanic and twitch force were recorded during a 600-ms, 200-Hz train of stimuli followed 2 s later by a single stimulus. This sequence was repeated at 10-s intervals until the maximum potentiation of the single twitch was seen (usually 5–10 sequences). Third, tetanic force (and EMG in 26 motor units) was recorded during trains of 25 stimuli repeated at 10-s intervals to detect the presence of sag. In most units, the first sag train was elicited with an interstimulus interval (ISI) equal to the unpotentiated twitch contraction time (CT<sub>tw</sub>), estimated during the first step of this protocol as the time from twitch onset to maximum force. Longer initial intervals were used if this ISI elicited fused tetani. Stimulus trains were applied with at least three different progressively longer ISIs incremented in steps of 0.125–0.25 × CT<sub>tw</sub>. In all experiments, minimum and maximum values (in terms of CT<sub>tw</sub>) of the range of ISIs tested varied across units due to errors in estimating CT<sub>tw</sub>. An additional series of tetani were elicited in 12 motor units to evaluate the effect of repetition rate on the reproducibility of sag. In these units, two series of six tetani each were repeated at 10- and 0.5-s intervals for up to four different ISIs. Fourth, fatigue was assessed by recording tetanic force (and EMG in 36 motor units) during application of 70-Hz trains of 14 stimuli delivered at 1-s intervals for 2 min. Stimulation at 40 Hz is the de facto standard of motor unit typing in cats (Burke et al. 1973). However, CT<sub>tw</sub> of TS motor units is substantially shorter in rats (Bakels and Kernell 1993b; Chamberlain and Lewis 1989; Einsiedel and Luff 1993; Gardiner 1993; Grottel and Celichowski 1990) than that in cats (Burke 1967; Burke et al. 1973; Proske and Waite 1974; Wuerker et al. 1965). Therefore a higher stimulation frequency was chosen to maintain a comparable degree of tetanic fusion.

A fifth protocol was employed in which individual action potentials were elicited by injection of 2- to 3-ms depolarizing current pulses at 8–16 Hz, and axon voltage and tibial nerve ENG were recorded. Typically, 400–800 responses were averaged, but as many as 4,000 were averaged when the ENG had a low signal-to-noise ratio and recording stability permitted.

Typical recording sessions lasted 6–9 h. TS whole muscle twitch in response to supramaximal stimulation of the tibial nerve was monitored during all experiments. Experiments were terminated when twitch force fell to <75% of its initial value. Animals were killed at the end of the experiment by intravenous pentobarbital overdose. In experiments with ENG recordings, the conduction distance between

the impalement site and the subepineurial electrode was measured with the animal still mounted in the experimental apparatus.

### Data collection and analysis

EMG and ENG were band-pass filtered (0.03–3 and 0.003–10 kHz, respectively) and force and axon voltage were low-pass filtered (0.7 and 10 kHz, respectively). All signals were amplified to maximize resolution during A/D conversion by computer (sampling rate:  $\geq 2$  kHz for force,  $\geq 20$  kHz for EMG, ENG, and axon voltage).

Peak force ( $P_{tw}$ ),  $CT_{tw}$ , and half-relaxation time ( $HRT_{tw}$ ; i.e., time from maximum force to 50% of maximum force) were calculated for the averaged unpotentiated twitches. Maximum tetanic and potentiated twitch forces were calculated for each of the alternating pairs of tetani and twitches, and from these the responses with the largest amplitude tetanus ( $P_{tet}$ ) and potentiated twitch ( $P_{pot}$ ) were identified. Potentiated twitch contraction time ( $CT_{pot}$ ) and half-relaxation time ( $HRT_{pot}$ ) were also calculated for the largest potentiated twitch.

Preliminary experiments were performed to evaluate the effect of background passive force on twitch and tetanic amplitudes. Over a 50- to 550-mN range of forces produced by varying muscle length,  $P_{tw}$  did not vary with background passive force, but  $P_{tet}$  decreased by 2 mN per 100 mN increase in background passive force ( $P > 0.5$  and  $< 0.001$  for regression of  $P_{tw}$  and  $P_{tet}$ , respectively, on background passive force by analysis of covariance with motor unit as variate and background passive force as covariate). The mean background passive force  $\pm$  SE was  $133 \pm 14$  mN for the entire data pool (range = 101–203 mN, with only 1% of the units tested  $> 163$  mN). The error in measuring  $P_{tet}$  introduced by differences in background passive force were estimated to be  $< 2\%$  of  $P_{tet}$  in all units, which corresponded to projected errors of  $< 1$  mN in all cases. All tetani that were evaluated for sag were elicited in a background passive force range of 111–163 mN. Within this range, there was no significant correlation between background passive force and the likelihood of detecting sag ( $P > 0.2$  and  $r^2 = 0.01$  for linear regression of the percentage of trials tested in which sag was detected on background passive force).

The presence or absence of sag (see *Criteria for determination of sag status*) was noted for all tetani tested in a given motor unit. Failure to detect sag in any of at least four trains (median number of trains = 7) with ISIs falling in the range of  $1.0$ – $3.0 \times CT_{tw}$  (median minimum and maximum  $ISI/CT_{tw} = 1.20$  and  $2.69$ , respectively; mean  $\pm$  SD of ratio of maximum:minimum ISIs =  $2.1 \pm 0.3$ ) resulted in classification as a nonsagging unit. The presence of sag in one or more of those tetani resulted in classification as a motor unit with sag.

The pattern of force accumulation in unfused tetani in which sag was sought was quantified in two ways. First, a sequential force index (SFI) was calculated as the difference in amplitude between a given peak and its predecessor during the tetanus (with the SFI for the first peak being the difference between that peak and the pretetanic force level) divided by the maximum tetanic force. Second, the time from contraction onset to maximum force (TTP) was calculated for each peak in the tetanus.

Fatigue was quantified by calculating a fatigue index (FI) defined as the ratio of the maximum force during the 120th tetanus to that of the first tetanus.

Motor units were provisionally type-identified according to the criteria of Burke et al. (1973). Motor units with or without sag were classified as F or S, respectively. F motor units were subdivided further according to their fatigability such that units with a  $FI \geq 0.75$  were classified as fatigue-resistant (FR), those with  $0.25 < FI < 0.75$  as having intermediate fatigability [F(int)] and those with  $FI \leq 0.25$  as fatigable (FF).

Orthodromically conducted action potentials evoked by intra-axonal current injection usually were detected as triphasic ENG waveforms. Axonal conduction time was calculated as the latency from the onset of the current-evoked action potential to the onset of the large negative component (usually the second peak) of the ENG waveform.

The latter time point was considered to reflect the timing of the nodal action potential threshold (i.e., the time when net current flow at the subepineurial electrode was 0). Axonal conduction velocity (CV) was calculated as the ratio of the conduction distance to the conduction time.

The muscle containing the stimulated motor unit was identified nominally by the electrode pair with the largest evoked peak-to-peak amplitude EMG. No qualitative differences were observed in the force envelope shapes of motor units with or without sag among motor units from the different TS muscles. Thus the data were pooled across muscles.

Comparisons of motor unit properties by type were performed using ANOVA. Differences among treatment groups were assessed by Tukey's honestly significant difference test. Relationships between motor unit properties were assessed by linear regression. Differences with  $P < 0.01$  were considered to be statistically significant.

## RESULTS

A total of 202 motor units are included in this study. The number of motor units studied per experiment varied widely, with 15 experiments having 1–7 units and 9 experiments having 9–14 units. Of these motor units, 158 (78%) had at least one occurrence of sag at an ISI in the range of  $1.0$ – $3.0 \times CT_{tw}$ .

### Force profiles of unfused tetanic contractions

The force envelope formed by the peaks of unfused tetanic contractions exhibited several characteristic patterns, which are illustrated by data from four motor units in Fig. 1. *Unit 1* is representative of the 44 units in which sag was not detected at any ISI tested, even with ISIs of up to  $3.4 \times CT_{tw}$ . The peak force envelope rose steadily throughout the stimulus train without detection of local maxima. *Unit 2* is one of seven motor units (5–9 different ISIs tested) with peak force envelopes that increased to maxima (wide arrowheads) and decreased steadily thereafter. This uncomplicated force accumulation pattern is identified as "simple" sag ( $sag_S$ ). *Unit 3* is 1 of 63 motor units that exhibited force envelopes that rose to initial peaks (narrow arrowheads), decreased for one to three stimuli, and then increased again. This "complex" sag configuration ( $sag_C$ ) has been observed in both rats (Bakels and Kernell 1993a; Celichowski 1992a,b) and cats (Jami et al. 1983). The two sag patterns were both observed in each of the remaining 88 motor units (exemplified by *unit 4*), where  $sag_S$  was observed at some ISIs (ISI 1–2) and  $sag_C$  was observed at others (ISIs 4–7). Motor units in which at least one tetanus with sag was observed often had individual tetani in which sag was not detected (Fig. 1, ISIs 1–3 from *unit 3* and ISI 3 from *unit 4*). These are referred to as  $sag_N$  tetani.

The shape of the force envelope was also dependent on the time between successive tetanic contractions. Sets of six tetani at one to four different ISIs were repeated every 10 s and then every 2 s in 11 motor units. Repetition every 2 s resulted in qualitative changes in the shape of the force envelope of the second to sixth tetani, either through conversion among different sag patterns or by losing the appearance of sag entirely (16 or 30 of 140 tetani tested, respectively; Fig. 2, *top*). Stimulation at the same ISIs every 10 s produced little change in force envelope shape (Fig. 2, *bottom*), such that sag status (i.e., sag vs. no sag) did not vary during repetition of tetani in any motor unit and conversions among sag subtypes were detected in only 3 of 138 tetani tested.

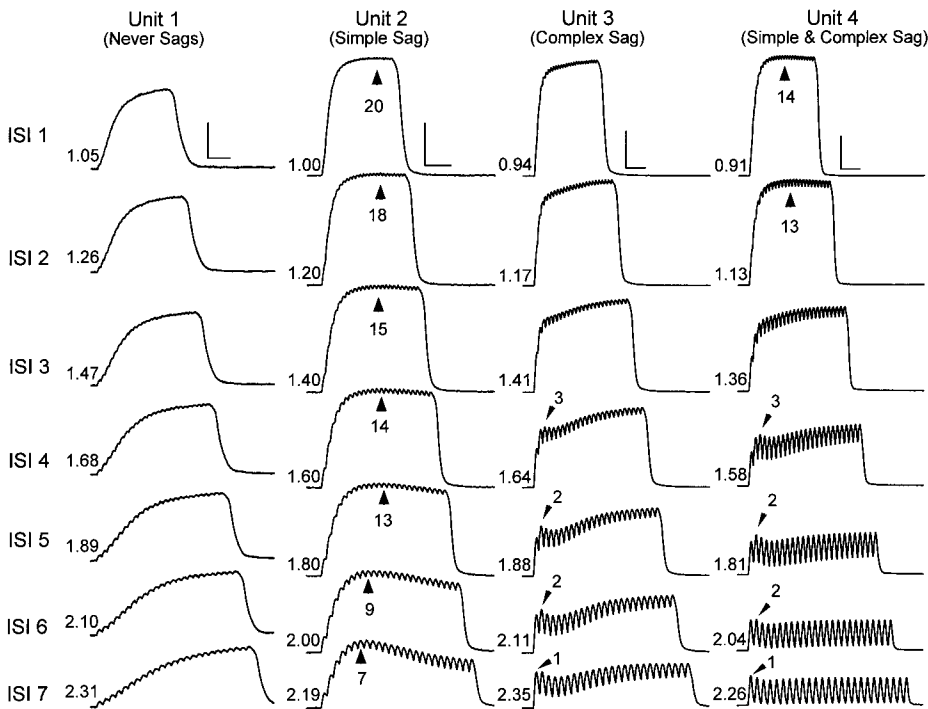


FIG. 1. Four different motor units (*units 1–4*) illustrate the most common patterns of force increment during tetanic contractions elicited by trains of 25 stimuli at different interstimulus intervals (ISIs) (*bottom left* of each trace as ISI 1–7) expressed as multiples of contraction times of unpotentiated twitch ( $CT_{tw}$ ; 24, 11, 14 and 15 ms for *units 1–4*, respectively). *Unit 1* did not display sag at any ISI multiple tested. *Unit 2* showed a consistent pattern of sag in which the force envelope formed by the peaks of each contraction reached a maximum (wide arrowheads) and then steadily decreased (i.e.,  $sag_s$ ). In this unit, the same pattern of sag was seen at ISIs of up to  $3.19 \times CT_{tw}$  (not shown). *Unit 3* showed a different pattern of sag in which the force envelope increased to an initial maximum (narrow arrowheads), decreased over the next few contractions, and then increased again [i.e., complex sag ( $sag_c$ )]. *Unit 4* exhibited both patterns of sag, with simple sag ( $sag_s$ ) at short ISIs (maxima indicated by wide arrows) and  $sag_c$  at longer ISIs (maxima indicated by narrow arrowheads). Calibrations: 330, 150, 200, and 200 ms and 25, 50, 115, and 130 mN for *units 1–4*, respectively.

EMG did not vary substantially during the course of the 207 tetani from 26 motor units in which it was recorded. For example, the peak-to-peak amplitude of the EMG response to the last tetanic stimulus of the train was not significantly different from that of the first EMG response (mean % difference  $\pm$  SE =  $0.7 \pm 1.0\%$ ;  $P = 0.53$  by  $t$ -test). In addition, the ratio of the last: first peak-to-peak EMG amplitudes did not

vary significantly with tetanic ISI (slope =  $0.03 \pm 0.02$ ,  $r^2 = 0.01$  and  $P = 0.12$  for regression of EMG amplitude ratio on  $ISI/CT_{tw}$ ). This relationship predicts only a 6% change in EMG for tetani elicited over the entire twofold range of  $ISI/CT_{tw}$  values used in this study. Thus any contribution of neuromuscular transmission failure to sag under the conditions described here was probably minimal.

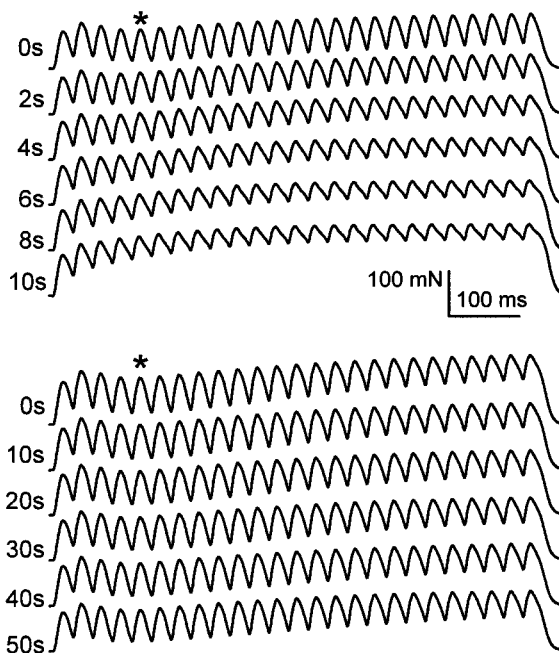


FIG. 2. Repetition of tetanic stimulus trains at 2-s intervals led to qualitative changes in the shape of the force envelope (*top*; decrement in peak amplitude at \* in 1st trace diminishes and then disappears in subsequent tetani). Tetanic trains applied at 10-s intervals in the same motor unit elicited consistently shaped unfused contractions (*bottom*; note that the decrement in peak amplitude at \* present in subsequent tetani). ISI within the tetanus was 27.9 ms, and the contraction time of the unpotentiated twitch was 13.4 ms.

#### Criteria for determination of sag status

On the basis of the different patterns of force accumulation described above, two sets of criteria were established to identify sag during an unfused tetanic contraction. The initial approach used to detect sag was to identify the earliest local maximum (and subsequent local minimum, if present) in the force envelope (1st-order method). As illustrated in Fig. 3, *bottom*, the second peak of the tetanus was identified as the first maximum of the force envelope because it was larger than at least two subsequent peaks ( $\Delta$  values under  $\dots$ ) by  $\geq 1\%$  of the maximum tetanic amplitude and by 1 SD of the baseline force level before stimulation. Subsequently, the third peak was defined as the first local minimum because it was smaller than at least two subsequent peaks by  $\geq 1\%$  of the maximum tetanic amplitude and by 1 SD of the baseline force level before stimulation. A tetanus with both a maximum and a minimum such as this is classified as having  $sag_{C1}$  (i.e., complex sag identified by the 1st-order method). Had no local minimum been located after detection of the initial maximum, the tetanus would have been classified as  $sag_s$ . Requiring interpeak differences to meet criteria based on both tetanus magnitude and background passive force variability provided a stringent approach to identifying maxima and minima. However, one limitation of these criteria was that tetani with substantial force decrements in only a single peak subsequent to the maximum were not detected as  $sag_{C1}$ . Thus in cases where sag was not detected using the first-order method, an alternate approach

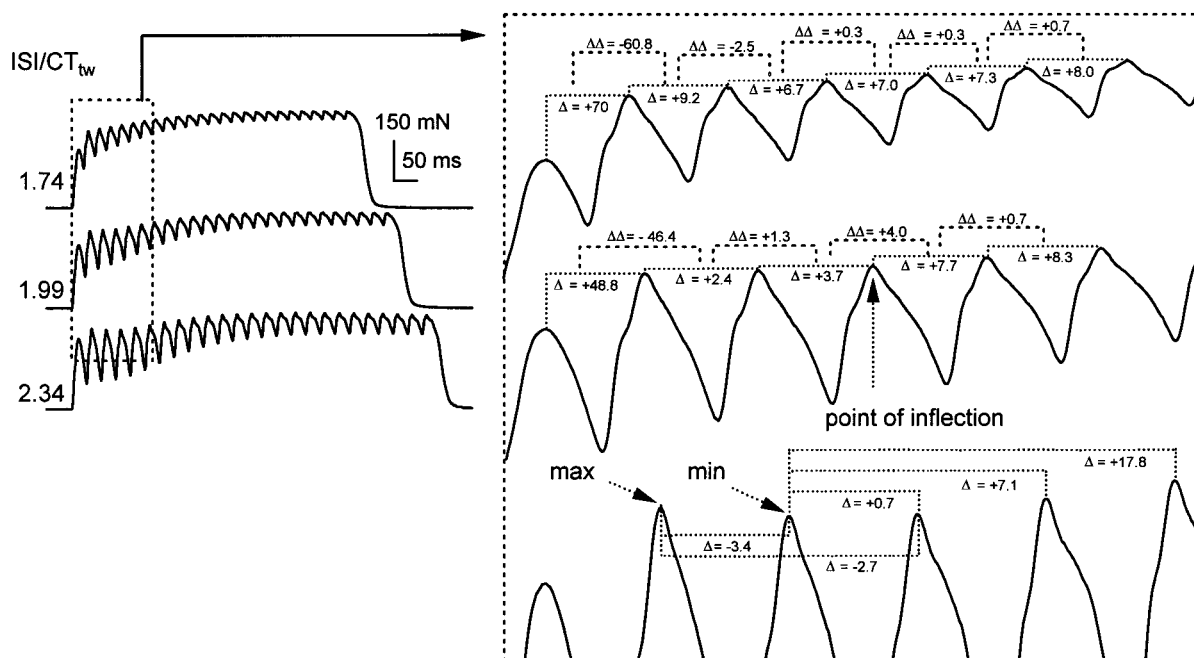


FIG. 3. Three unfused tetani elicited at different ISIs (expressed as multiples of  $CT_{tw}$  at the left end of each trace) from a single motor unit had different force envelopes. *Enlarged region*: 2 schemes used to detect sag (see text for full description). *Bottom*: (longest ISI) had a transient decrease in peak force after the 2nd peak (1st-order sag;  $\Delta$  values are differences between adjacent peaks in mN). *Middle*: (intermediate ISI) did not show any force decrement between sequential peaks but had a marked increase in the difference between the differences in adjacent peak amplitudes ( $\Delta\Delta$  values), which identified a point of inflection in the force envelope (2nd-order sag). *Top*: (shortest ISI) rose steadily (no sag).

based on the detection of an inflection point in the amplitudes of sequential force peaks was used (2nd-order method). In this method, second-order differences ( $\Delta\Delta$  values) were calculated between adjacent differences in peak force ( $\Delta$  value, as in the preceding text). Units with long  $CT_{tw}$  and monotonically rising peak force profiles (i.e., presumed S units) never exhibited  $\Delta\Delta$  values greater than +1.4 mN. Thus this  $\Delta\Delta$  value was used as the minimum criterion for identifying the presence of sag. This method readily identified tetani with substantial single-peak decrements (as described earlier) and an additional group of tetani with points of inflection that did not exhibit overt force decrements (Fig. 3, *middle*). All tetani identified with points of inflection more than +1.4 mN were defined as having a complex sag configuration as detected by the second-order method ( $sag_{C2}$ ). Tetani so identified were considered to have their first local minima at the middle peak of the three that produced the largest  $\Delta\Delta$  value. The peak before the one defined as minimum was defined as the first local ‘‘maximum,’’ even if it was not numerically higher than the middle peak. Tetani that failed to meet criteria for both first- and second-order methods were considered to not exhibit sag (Fig. 3, *top*).

#### Average force profiles in motor units with or without sag

Using the criteria described above, sag was identified in 933 (66%) of 1,406 tetani. The tetani with sag consisted of 310 (22%) with  $sag_S$ , 545 (39%) with  $sag_{C1}$ , and 78 (6%) with  $sag_{C2}$ . Of the units with  $sag_{C2}$ , 35 (2%) had overt force decrements. Of the remaining 473 tetani without sag, 173 (12%) were from motor units in which sag was elicited in at least one

other tetanus with a different ISI (i.e.,  $sag_N$  tetani), and the remaining 300 (21%) were from motor units that failed to display sag at any stimulation frequency.

Figure 4 shows the average increment in force (normalized to maximum tetanic force) produced by sequential stimuli in the train (i.e., SFI) for units with and without sag elicited at ISIs in the range of 1.1–1.3, 1.7–1.9, and 2.5–2.7  $\times CT_{tw}$  (Fig. 4, A–C, respectively). In motor units that failed to exhibit sag at any ISI tested (Fig. 4,  $\times$ ), the force of the first contraction represented a larger fraction of the maximum force as ISI increased. The subsequent 4–10 contractions added a near-constant fraction of the maximum tetanic force at any ISI, declining gradually thereafter toward an asymptote. Motor units that displayed sag during at least one sag train (Fig. 4,  $\bullet$ ,  $\circ$ ,  $\Delta$ ,  $\blacklozenge$ ) had larger force increments in response to the first stimulus than those that never sagged. The large initial increments diminished rapidly during the subsequent two to six stimuli. Significant differences were detected in the SFI between each group of tetani with sag and those from nonsagging motor units for short (peaks 1–3 and 5–25 for  $sag_S$ ; peaks 1–3 and 5–12 for  $sag_{C1}$ ; and peaks 1–2, 4–17, and 21 for  $sag_{C2}$ ), intermediate (peaks 1–3 and 6–25 for  $sag_S$ ; peaks 1–2, 4–16, 18, and 20 for  $sag_{C1}$ ; and peaks 1–2, 4–16, and 20 for  $sag_{C2}$ ), and long (peaks 1–2, 5–15, 17, 19, 22, and 24 for  $sag_S$ ; peaks 1, 4–15, 17, 19, 22, and 24 for  $sag_{C1}$ ; and peaks 1 and 12–14 for  $sag_{C2}$ ) ISIs in Fig. 4, A–C, respectively ( $P < 0.01$  for all by ANOVA).

The range of SFI values for motor units without sag overlapped those of motor units with sag for each stimulus in all ISI ranges. The absence of sag in a motor unit usually was pre-

dicted in a given tetanus by a stable (or gradually decreasing) pattern of SFI values in the second to final contractions of the tetanus (the SFI of the 1st contraction of tetani from motor units without sag varied substantially with ISI). For example, the sag status of a motor unit was correctly identified in 147 of

152, 164 of 166, and 113 of 115 tetani (97, 99, and 98% agreement, respectively) with nonsagging units defined by SFI of 0.007–0.18, 0.016–0.13, and 0.006–0.11 in the 2nd–12th contractions for ISI/CT<sub>tw</sub> values in the range of 1.1–1.3, 1.7–1.9, and 2.5–2.7, respectively. Slightly stronger relationships between sag and SFI pattern were observed for tetani limited to absolute ISI ranges. For example, the sag status of a motor unit was correctly identified in 266 of 268 tetani (99% agreement) when nonsagging units were defined by a SFI of 0.013–0.16 in the 2nd–12th contractions for ISIs in the range of 22.5–27.5 ms. The >97% covariation of SFI values and motor unit sag status could not be achieved with any single range of SFI values for all ISI or ISI/CT<sub>tw</sub> ranges nor for any other combination of sequential contractions within the tetanus. The striking difference in the patterns of force accumulation between sagging and nonsagging motor units is consistent with those reported for 40-Hz tetani in histochemically defined F and S motor units (Tötösy de Zepetnek et al. 1992).

Differences in force profiles among tetani from motor units with sag were most pronounced at intermediate duration ISIs (Fig. 4B). Differences among tetani elicited at shorter or longer ISIs were more subtle, but the overall patterns were qualitatively similar. Tetani displaying sag<sub>s</sub> decayed gradually, eventually exhibiting their characteristic force decrements toward the end of the stimulus train at intermediate ISIs ( $\Delta$  at stimuli 13 and 25) or earlier in the stimulus train at long ISIs ( $\Delta$  at stimuli 6 and 7). The first force increments of tetani with sag<sub>C</sub> ( $\bullet$  and  $\circ$ ) were larger than those with sag<sub>s</sub> ( $P < 0.001$ , ANOVA) and decreased more rapidly thereafter. The SFIs of motor units with sag<sub>C1</sub> ( $\bullet$ ) were nearly identical to those with sag<sub>C2</sub> ( $\circ$ ). No significant differences were detected between them at any stimulus number ( $P > 0.05$  for all except for stimulus 3 in A, where  $P > 0.01$  by ANOVA). The sag<sub>s</sub> groups were significantly different from the sag<sub>C1</sub> and sag<sub>C2</sub> groups at several stimuli (short ISIs: peaks 5–7, 12–23, and 25 and peaks 1 and 3–6, respectively; intermediate ISIs in B: peaks 1, 3–7, 10–22, and 24–25 and peaks 1, 3–5, 10–14, and 15–16, respectively; long ISIs in C: peaks 1, 3–4, 7–8, 10, and 12 and peaks 7–8, respectively;  $P < 0.01$  for all by ANOVA). Both sag<sub>C</sub> groups reached minima by the fourth stimulus, but the sag<sub>C1</sub> force increment became negative, while the sag<sub>C2</sub> force increment only reached zero. These patterns reflect the methods used to identify sag<sub>C</sub> (i.e., based on force decrement for sag<sub>C1</sub> and point of inflection for sag<sub>C2</sub>). The sag<sub>N</sub> force increment pattern ( $\blacklozenge$ ) was more similar to that of tetani with sag (lacking only the early and late negative components of sag<sub>C</sub> and sag<sub>s</sub> tetani, respectively) than to that of units that never sagged.

In addition to differences in the force envelope profiles

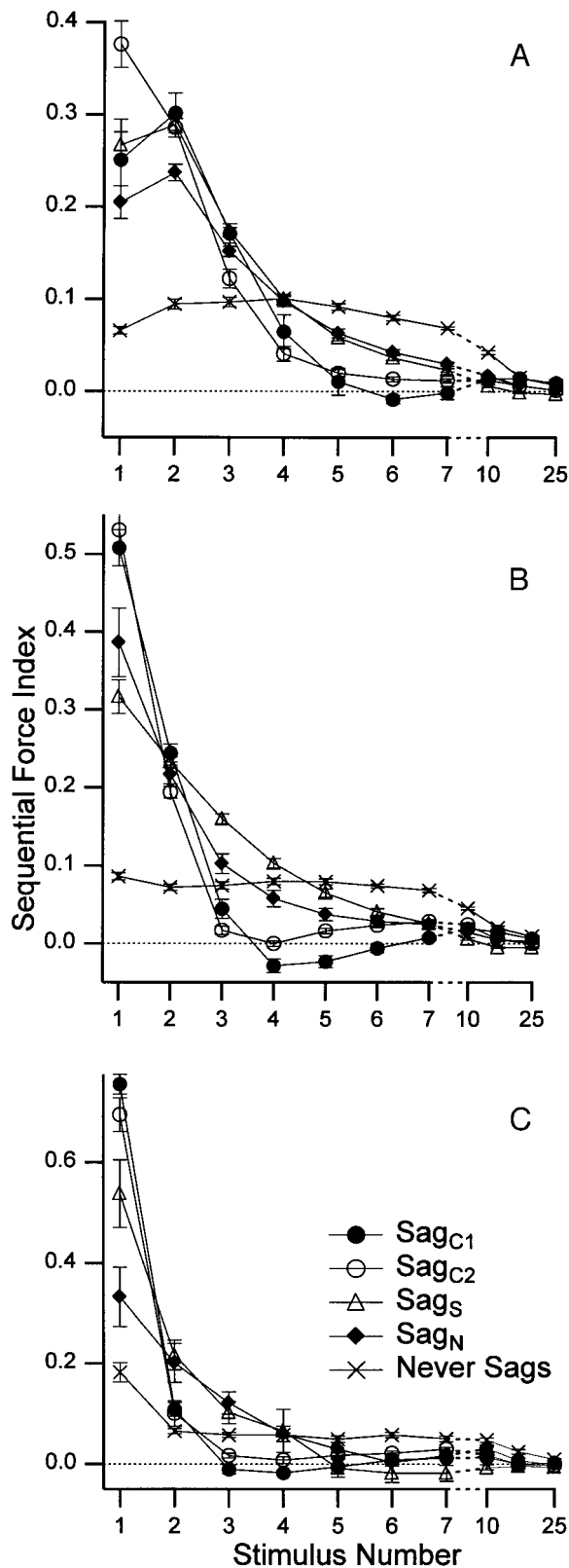


FIG. 4. Average change in force of adjacent peaks (normalized to maximum force)  $\pm$  SE is shown for individual trials with sag<sub>s</sub> ( $\Delta$ ), complex sag identified by the 1st-order method (sag<sub>C1</sub>;  $\bullet$ ), complex sag identified by 2nd-order method (sag<sub>C2</sub>;  $\circ$ ), and no sag ( $\blacklozenge$ ) from motor units with  $\geq 1$  tetanus with sag, and for trials from motor units which never displayed sag ( $\times$ ) in ISI/CT<sub>tw</sub> ranges of 1.1–1.3 (A), 1.7–1.9 (B), and 2.5–2.7 (C). On average, units with sag had large initial force increments that decreased over subsequent stimuli. Early force decrement was seen in both types of sag<sub>C</sub> but was negative only in sag<sub>C1</sub>. Motor units that never displayed sag had force increments that were constant during the 2nd to the 5th–7th stimuli, decreasing gradually thereafter. Force changes of tetani without sag recorded from motor units with sag were more similar to those of other tetani with sag than to those from units that never sagged.

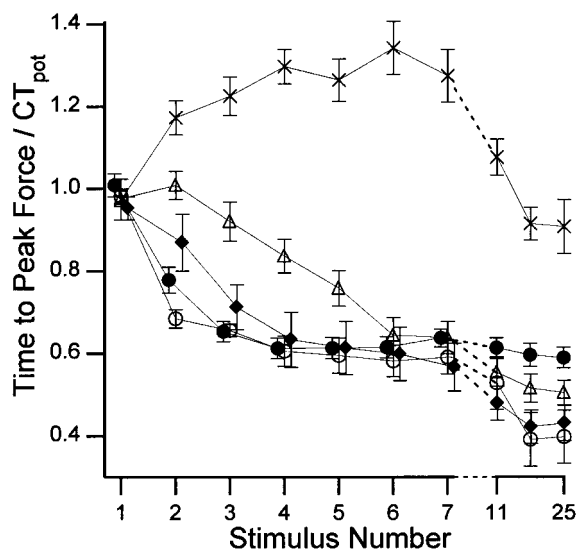


FIG. 5. Average time to peak force [expressed as a fraction of contraction time of potentiated twitch ( $CT_{pot}$ )]  $\pm$  SE for sequential peaks of tetani elicited at  $ISI/CT_{tw}$  of 1.7–1.9 is shown for tetani which elicited no sag ( $\blacklozenge$ ),  $sag_S$  ( $\triangle$ ),  $sag_{C1}$  ( $\bullet$ ), and  $sag_{C2}$  ( $\circ$ ) from motor units with sag in  $\geq 1$  tetanus and for trials from motor units which never displayed sag ( $\times$ ). All tetani with some form of sag began to exhibit a decrease in time to peak force within the 1st 3 stimuli of the train. Tetani from motor units that never sagged had an increase in time to peak force over the same time course.

among sagging and nonsagging motor units, there were also differences in TTP of the individual contractions comprising the tetani used to test for sag. For example, the TTP of tetani elicited in the  $ISI$  range of  $1.7\text{--}1.9 \times CT_{tw}$  decreased for all tetani from motor units with sag and increased for tetani from motor units that never sagged during peaks 2–6 (Fig. 5). The decrements in TTP in motor units with sag are associated with force decrements, while the increasing TTP of motor units that never sagged are associated with stable force increments (see SFI from peaks 2–6 of comparable tetani in Fig. 4B). TTP tended to decrease most rapidly in tetani with  $sag_C$  ( $\bullet$  and  $\circ$ ), less rapidly in  $sag_N$  tetani ( $\blacklozenge$ ), and least rapidly in  $sag_S$  tetani ( $\triangle$ ). The differences in the pattern of change in TTP among tetani from motor units with sag were subtle ( $sag_S$  tetani  $\neq$   $sag_{C1}$  tetani at peaks 2–4,  $sag_S$  tetani  $\neq$   $sag_{C2}$  tetani at peak 2, and  $sag_S$  tetani  $\neq$   $sag_N$  tetani at peak 4;  $P < 0.01$  by ANOVA) compared with differences in TTP between motor units with and without sag [ $sag_{C1}$ ,  $sag_{C2}$ ,  $sag_N$ , and  $sag_S \neq$  tetani from motor units that never sagged at peaks 2–6;  $P < 0.0001$  for all (except  $P < 0.01$  for  $sag_S$  at peak 2) by ANOVA]. After peak 6, the TTP subsequently decreased in tetani from motor units that never sagged and in tetani with  $sag_S$ ,  $sag_N$ , and  $sag_{C2}$  (but not  $sag_{C1}$ ).

Decreases in TTP are associated with force decrements to varying degrees in motor units displaying different types of sag. Figure 6 shows that the force increment provided by sequential stimuli during a tetanus varies directly with TTP for the first three to seven contractions in tetani with  $sag_{C1}$  (A) and  $sag_{C2}$  (B). Subsequent peaks displayed little dependence of force increment on TTP. Tetani with  $sag_S$  also displayed a similar phenomenon, but the relationship continued from the 2nd to 12th contractions before dissipating (C). These data suggest that the decrease in TTP during the initial contractions contributed to the development of sag in both subtypes, but

other factors determined the pattern of force accumulation during latter contractions of tetani. That tetani from motor units that never sagged also had force increments that varied with TTP during the majority of the tetanus (D) suggests that this relationship is not specific to sag but rather is a common outcome of unfused tetanic stimulation.

During tetanic stimulation, motor units displaying  $sag_S$  reached peak amplitude by a median of 13 stimulations within the train of 25 (middle 90% of units ranged from 5 to 20). Motor units displaying  $sag_C$  reached an initial force maximum by a median of two stimulations (middle 90% of values between 2 and 4). For both sag subtypes, the peak number of the first amplitude maximum tended to decrease at higher  $ISI$  multiples of  $CT_{tw}$  (e.g., compare ordinal position of peaks among traces from units 2 and 3 in Fig. 1). Regression analysis indicated that the location of the initial force maximum was related inversely to the  $ISI$  with ordinal peak location (Fig. 7). This difference in relative location was also evident in the absolute times of the force maxima for sag subtypes. For example, the mean time to the initial maximum  $\pm$  SD for all tetani elicited at  $ISIs = 1.7\text{--}1.9 \times CT_{tw}$  was  $303 \pm 132$  and  $58 \pm 28$  ms for  $sag_S$  and  $sag_{C1}$ , respectively ( $P < 0.0001$  by  $t$ -test). Differences in peak location and frequency dependence seen in the entire sample also are seen in the 88 units in which both sag subtypes were observed. The mean peak position  $\pm$  SD of pairs of trains with  $sag_S$  and pairs of trains with  $sag_C$  decreased by  $1.5 \pm 4.1$  and  $0.5 \pm 0.9$  stimulations per  $0.25 \times CT_{tw}$  change in  $ISI$ , respectively. The mean peak position  $\pm$  SD of  $sag_S$ -to- $sag_C$  decreased by  $9.8 \pm 8.7$  stimulations per

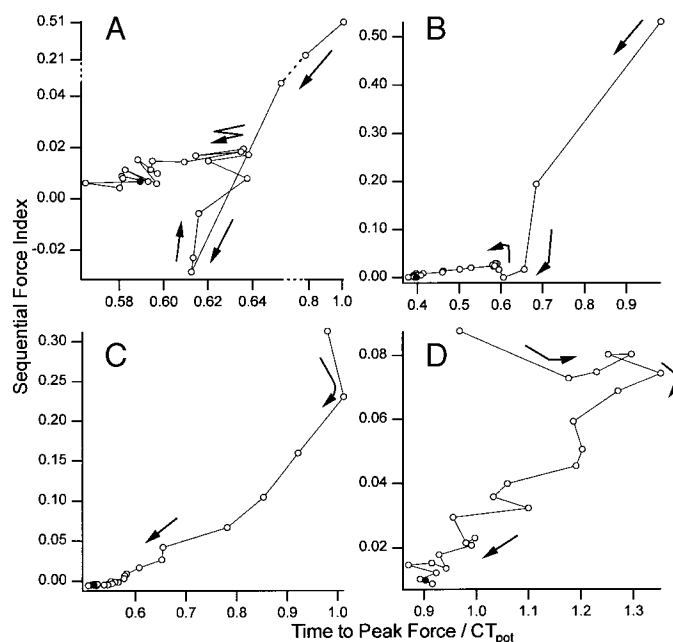


FIG. 6. Relationships between average sequential force index (i.e., difference in force between adjacent peaks divided by the maximum force during the tetanus) and average time to peak force (adjusted to the potentiated contraction time).  $\rightarrow$ , temporal sequence from the 1st to the last contraction, the latter being indicated by  $\bullet$ . Error bars have been left out for clarity. Sequential force index varied directly with time to peak force over the 1st few contractions of the tetanus with  $sag_{C1}$  (A) and  $sag_{C2}$  (B) and over a larger range of contractions for tetani with  $sag_S$  (C). Sequential force index was independent of time to peak force for the first several contractions in tetani from motor units that never displayed sag (D), but these variables did vary together during subsequent contractions.

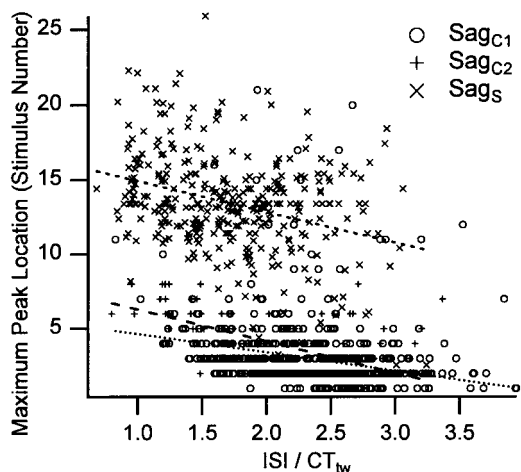


FIG. 7. Ordinal locations (based on the stimulus number in the tetanic train) of the 1st maximum in individual trains with  $sag_S$  ( $\times$ ),  $sag_{C1}$  ( $\circ$ ), and  $sag_{C2}$  ( $+$ ) varied inversely with  $ISI/CT_{pot}$  (slope  $\pm$  SD =  $-2.08 \pm 0.33$ ,  $-1.24 \pm 0.13$ , and  $-2.13 \pm 0.24$  and  $r^2 = 0.11$ ,  $0.14$ , and  $0.48$  for  $sag_S$ ,  $sag_{C1}$ , and  $sag_{C2}$ , as illustrated by regression lines with  $---$ ,  $\cdots$ , and  $---$ , respectively;  $P < 0.001$  for all by linear regression). All location values have had subtracted the difference between the mean location value of its unit and that of the entire population to account for between-unit variation without altering the within-unit relationship between ordinal location and  $ISI/CT_{tw}$ .

$0.25 \times CT_{tw}$  change in ISI. These data suggest that the transition from  $sag_S$  to  $sag_C$  does not represent a continuous process with the peak shifting smoothly from later to earlier peaks.

Tetani with  $sag_{C2}$  shared many features in common with those expressing  $sag_{C1}$  (e.g., force envelope shape, time course of TTP of sequential peaks, ordinal location of the initial maximum peaks), suggesting that they are manifestations of the same underlying phenomenon. The  $sag_{C2}$  tetani without force decrements, although not ordinarily defined as sag, had qualitatively similar force envelopes to  $sag_{C2}$  tetani with force decrements and to  $sag_{C1}$  tetani. Thus tetani with  $sag_C$  detected by either method are treated here as a single subtype of sag force profiles.

#### Frequency dependence of sag

Accurate type identification of motor units requires knowing the probability of detecting sag at a given frequency. For the 158 motor units with sag, Fig. 8, *top*, shows the total number of motor units tested (indicated by the total height of each bar) as a function of the train ISI. The number of motor units with or without sag in each ISI bin is indicated by the size of the hatched and open part of each bar, respectively. Stimulation at a given ISI can elicit tetani with very different degrees of fusion in F or S motor units. Therefore we also evaluated these distributions as a function of ISI expressed as multiples of  $CT_{tw}$ ,  $CT_{pot}$ ,  $HRT_{tw}$ , or  $HRT_{pot}$  (Fig. 8, *2nd–5th* from *top*, respectively). For all five distributions, there was a wide range of ISIs in which sag was not detected during individual trains, as indicated by the distribution of  $\square$  over several ISI bins. The probability of observing sag during any single train was highest for long ISIs (raw or normalized).  $ISI/CT_{tw}$ ,  $ISI/CT_{pot}$ , and ISI alone produced distributions with comparable frequency dependence and maximum probability of detecting sag (i.e., sag was correctly detected in  $\geq 95\%$  of the units in the uppermost

4–5 ISI bins).  $ISI/HRT_{tw}$  or  $ISI/HRT_{pot}$  produced distributions with lesser frequency dependence and maximum probability of detecting sag (i.e., sag was detected  $\geq 95\%$  of the units in only the upper 2 ISI bins). However, the advantage of using tetani elicited at long ISIs to detect sag was counterbalanced by the reduced number of tetani available for analysis. Tetani elicited at long ISIs were often of low amplitude with little fusion of consecutive contractions, making the determination of sag problematic. Tetani elicited at short ISIs had a lower probability of detecting sag correctly. This was due in large part to variability in the lowest ISI (raw or normalized) at which sag could be detected. For example, the lowest  $ISI/CT_{tw}$  value that elicited sag in a given motor unit ranged from 0.68 to 2.72 (median = 1.52, with the middle 90% of units in the range of 0.93–2.14). In addition, some motor units failed to sag at intermediate ISIs where trains at higher or lower multiples had already elicited sag (e.g., compare ISI 3 to ISI 2 and ISI 4 from *unit 4* in Fig. 1). Thus it is unlikely that stimulation at a single ISI value (or multiple of CT or HRT) could provide a reliable

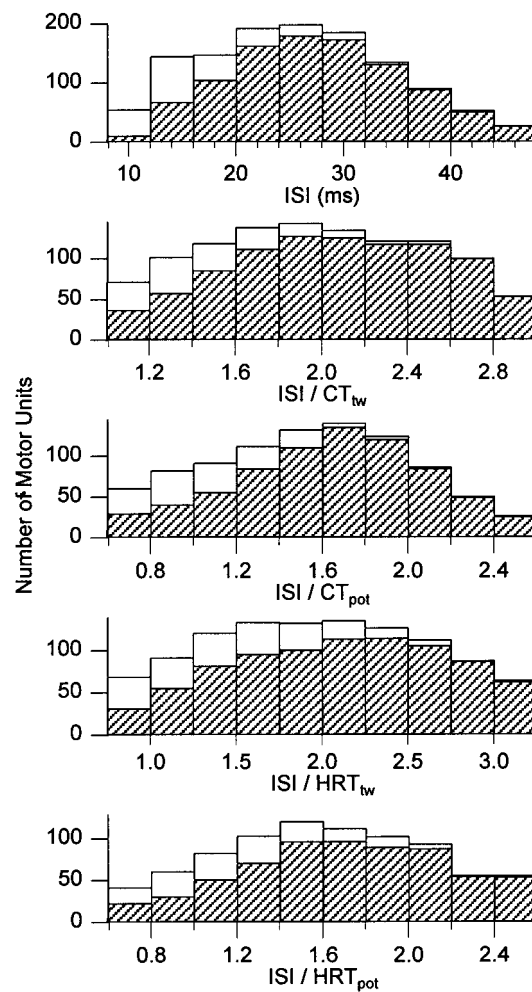


FIG. 8. In motor units where sag was detected during  $\geq 1$  tetanus, the number of motor units in which sag was or was not detected (hatched or open part of bar, respectively) are displayed according to the ISI,  $ISI/CT_{tw}$ ,  $ISI/CT_{pot}$ ,  $ISI/HRT_{tw}$ , or  $ISI/HRT_{pot}$ , as indicated on the abscissa. At least 4 different ISIs were assessed for each motor unit. Number of tetani with sag approached the total number of tetani tested (indicated by total bar height) at longer ISIs (raw or normalized), but the total number of motor units in which sag could be evaluated decreased at longer ISIs.



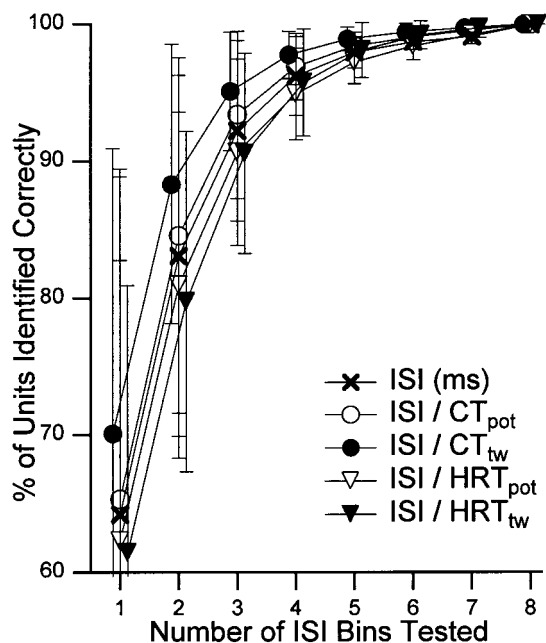


FIG. 9. In a post hoc analysis of the ability to detect sag in motor units with sag (4–13 tetani per unit), individual trials were sorted into 8 bins according to ISI ( $\times$ ; range = 13–45 ms, 158 units),  $ISI/CT_{pot}$  ( $\circ$ ; range = 0.8–2.4, 120 units),  $ISI/CT_{tw}$  ( $\bullet$ ; range = 1.0–3.0, 158 units),  $ISI/HRT_{pot}$  ( $\nabla$ ; range = 0.8–2.8, 120 units) or  $ISI/HRT_{tw}$  ( $\blacktriangledown$ ; range = 0.9–3.6, 156 units). The number of units in which sag was expressed in  $\geq 1$  of the trials tested (i.e., units that would have been identified correctly as exhibiting sag had only those tetani been applied) was determined for all combinations of ISI bins. For each number of ISI bin combinations tested, the number of units that were correctly identified as having sag were averaged and displayed on the ordinate as a percentage ( $\pm$ SD) of the total number of motor units tested. Percentage of motor units that were identified correctly as exhibiting sag increased as more tetani having different ISI ranges were used for the identification and was highest using ISI ranges defined by  $ISI/CT_{tw}$ .

method of determining the presence or absence of sag in a motor unit.

To evaluate the probability of correctly determining sag status using different numbers of tetani, a simulation was performed in which individual tetani with and without sag from all units with at least one tetanus with sag were sorted into eight bins according to their ISI (raw or normalized to CT or HRT). Although each motor unit in the simulation had some form of sag, not all units displayed sag in each of the ISI bins. The number of motor units that would have been identified successfully as having sag were noted for each ISI bin alone and for all possible combinations of those ISI bins to simulate the effect of testing for sag with different numbers of tetani. For example, tetani with  $ISI/CT_{tw} = 1.0$ –3.0 were divided among eight bins at  $0.25 \times CT_{tw}$  intervals. A single test tetanus in any one of the eight bins resulted in 61–137 of 158 units being correctly identified as having sag. Tests that sought the presence of sag in either one of two bins (28 combinations) resulted in 84–152 units being correctly identified. As more tetani were used, more units were correctly identified. The mean number of units correctly identified (expressed as a percentage of the total number of units tested) for up to eight tetani is shown in Fig. 9 ( $\bullet$  and  $\circ$ ). On average, motor units with sag in at least one tetanus were correctly identified 95% of the time with three or more tetani and 99% of the time with five or more tetani. Similar results were obtained using ISI alone or

normalized ISIs. Each method of normalizing ISI had slightly different abilities to distinguish sag correctly according to the following order:  $ISI/CT_{tw} > ISI/CT_{pot} > ISI > ISI/HRT_{tw} > ISI/HRT_{pot}$ .

Responses to individual tetani also were evaluated for the presence of sag with two different patterns of force decrement (i.e.,  $sag_S$  and  $sag_C$ ). For the 158 units tested, 108 exhibited  $sag_C$  and 77 exhibited  $sag_S$ . Figure 10 shows the probability of detecting each type of sag subtypes when distributed according to raw or normalized tetanic ISI. The frequency dependence of the different sag subtypes is clear, regardless of ISI treatment.  $sag_S$  ( $\square$ ) occurred at all ISIs tested, but was more prevalent at short ISIs, while  $sag_C$  ( $\boxtimes$ ) occurred preferentially at higher ISIs. For example, the median minimum  $CT_{tw}$  multiples at which  $sag_S$  and  $sag_C$  were observed were 1.37 (mid-90% of ISIs between  $0.91$  and  $2.34 \times CT_{tw}$ ) and  $1.96$  (mid-90% of ISIs between  $1.28$  and  $2.95 \times CT_{tw}$ ), respectively. The frequency dependence of sag configuration also was seen within units where both subtypes were elicited, i.e.,  $sag_S$  occurred in tetani with shorter ISIs than  $sag_C$  in 78 of 98 units.

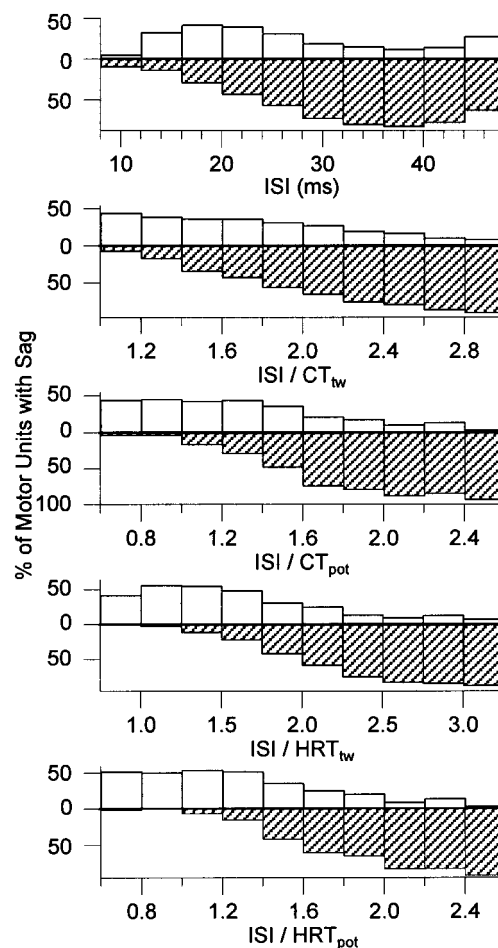


FIG. 10. Percentage of motor units with sag ( $100 \times$  number of tetani with sag/number of tetani tested) in units with sag in  $\geq 1$  tetanus binned according to the ISI,  $ISI/CT_{tw}$ ,  $ISI/CT_{pot}$ ,  $ISI/HRT_{tw}$ , or  $ISI/HRT_{pot}$  (as indicated on the abscissa) was highest for  $sag_S$  ( $\square$ ) at short ISIs, whereas  $sag_C$  ( $\boxtimes$ ) was detected more readily at long ISIs. Median number of tetani per bin (and ranges) for  $sag_S$  and  $sag_C$  were 141 (28–199) for ISI, 122 (54–144) for  $ISI/CT_{tw}$ , 90 (27–141) for  $ISI/CT_{pot}$ , 117 (65–136) for  $ISI/HRT_{tw}$ , and 52 (42–121) for  $ISI/HRT_{pot}$ .

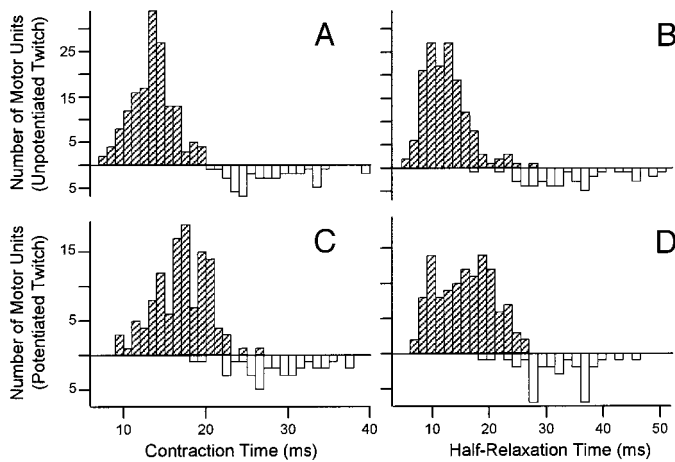


FIG. 11. Number of motor units with (▨) and without (□) sag are shown as a function of  $CT_{tw}$  (A),  $HRT_{tw}$  (B),  $CT_{pot}$  (C), and  $HRT_{pot}$  (D). Sag was preferentially distributed among the more rapidly contracting and relaxing motor units.

#### Relationship between sag and other motor unit properties

The presence of sag was distributed preferentially among motor units having short contraction and relaxation measurements (Fig. 11). This effect was shown most clearly for separation by  $CT_{tw}$  (Fig. 11A): all units with  $CT_{tw} < 20$  ms displayed sag in at least one tetanus (▨), whereas all those with  $CT_{tw} > 20$  did not exhibit sag in any tetanus (□). Similar results were obtained, but with a greater degree of overlap between sagging and nonsagging units, by basing the distributions of sag on  $HRT_{tw}$ ,  $CT_{pot}$ , and  $HRT_{pot}$  (Fig. 11, B–D, respectively).

Sag, force accumulation pattern, and measurements of con-

tractile speed each defined two populations of motor units that largely covaried, presumably reflecting the intrinsic differences between F and S motor units. We have applied the four-part scheme of Burke et al. (1973) to the 176 motor units in which both sag and fatigue tests were performed. Motor units with sag (presumed F units) displayed a wide range of fatigue sensitivity ( $FI = 0.01$ – $1.12$ ). Of the motor units that never sagged (presumed S units), most were fatigue resistant ( $FI = 0.93$ – $1.11$  in 39 of 42 units), but three motor units displayed a modest degree of fatigue sensitivity ( $FI = 0.85$ ,  $0.84$ , and  $0.72$ ). The latter FI value is below the commonly used criterion value of 0.75 for defining fatigue resistance. However, FI values from units with small tetanic force at the onset of the fatigue test (15, 33, and 26 mN, for these 3 units, respectively) may not be entirely reliable indicators of fatigue sensitivity due to random variation in maximum force during repeated tetanization. The long  $CT_{tw}$  values of these three motor units (28, 33, and 29 ms, respectively) led to their inclusion with other motor units that never sagged.

The properties of 49 FF, 43 F(int), 42 FR, and 42 S motor units are summarized in Table 1. Results are generally consistent with those of previous studies in rats. Potentiated and unpotentiated twitch and tetanic maximum amplitude followed the expected sequence of  $FF > F(int) > FR > S$  type units. S units contracted and relaxed more slowly than F motor units. No differences were found among F units in CT, but FR and F(int) motor units had longer  $HRT_{tw}$  values than FF units.  $P_{tw}:P_{tet}$  was higher in FF than in S, FR, or FI motor units. Previous studies found similar differences between FF and S motor units in twitch:tetanus ratio, but all F units had similar values (Celichowski and Grottel 1993; Einsiedel and Luff 1993). A greater degree of twitch potentiation (i.e., lower  $P_{tw}:P_{pot}$  ratio) was seen in F than in S motor units; this was

TABLE 1. Properties of rat triceps surae motor units by type

	Unit Type				Statistical Differences at $P < 0.01$
	FF	F(int)	FR	S	
$P_{tw}$ , mN	55 ± 41	24 ± 29	11 ± 7	3 ± 2	FF ≠ F(int), FR, S; F(int) ≠ S
$CT_{tw}$ , ms	14 ± 2	13 ± 3	14 ± 3	28 ± 5	S ≠ FF, F(int), FR
$HRT_{tw}$ , ms	10 ± 2	12 ± 3	16 ± 4	34 ± 8	S ≠ FF, F(int), FR; FR ≠ FF, F(int)
$P_{pot}$ , mN	98 ± 59	65 ± 61	24 ± 25	3 ± 2	FF ≠ FR, S; F(int) ≠ FR, S
$CT_{pot}$ , ms	17 ± 2	17 ± 3	17 ± 4	28 ± 5	S ≠ FF, F(int), FR
$HRT_{pot}$ , ms	16 ± 4	14 ± 5	18 ± 5	32 ± 6	S ≠ FF, F(int), FR
$P_{tet}$ , mN	240 ± 131	191 ± 160	101 ± 62	41 ± 19	S ≠ FF, F(int); FR ≠ F(int), FF
$P_{tw}:P_{pot}$	0.50 ± 0.17	0.31 ± 0.19	0.51 ± 0.24	0.78 ± 0.16	S ≠ FF, F(int), FR; F(int) ≠ FR, FF
$CT_{tw}:CT_{pot}$	0.80 ± 0.08	0.78 ± 0.12	0.82 ± 0.14	0.98 ± 0.13	S ≠ FF, F(int), FR
$HRT_{tw}:HRT_{pot}$	0.74 ± 0.23	0.93 ± 0.31	0.93 ± 0.17	1.09 ± 0.24	FF ≠ S, F(int), FR
$P_{tw}:P_{tet}$	0.21 ± 0.09	0.10 ± 0.07	0.09 ± 0.05	0.07 ± 0.04	FF ≠ S, F(int), FR
<i>n</i>	38–49	31–43	30–42	35–42	
%sag <sub>S</sub>	13 ± 24	20 ± 23	48 ± 26	—	FR ≠ FF, F(int)
%sag <sub>C</sub>	76 ± 24	62 ± 21	33 ± 20	—	FR ≠ FF, F(int)
<i>n</i>	49	43	42	—	
CV, m/s	75 ± 4	77 ± 7	75 ± 6	67 ± 4	S ≠ FF, F(int), FR
<i>n</i>	30	13	14	21	

Values are means ± SD. FF, fast-twitch units with sag and fatigue index ( $FI \leq 0.25$ ); F(int), fast-twitch units with sag and  $0.25 < FI < 0.75$ ; FR, fast-twitch units with sag and  $FI \geq 0.75$ ; S, slow-twitch units without sag;  $P_{tw}$ , maximum force of unpotentiated twitch;  $CT_{tw}$ , contraction time of unpotentiated twitch;  $HRT_{tw}$ , half-relaxation time of unpotentiated twitch;  $P_{pot}$ , maximum force of potentiated twitch;  $CT_{pot}$ , contraction time of potentiated twitch;  $HRT_{pot}$ , half-relaxation time of potentiated twitch;  $P_{tet}$ , maximum tetanic force;  $P_{tw}:P_{pot}$ , ratio of unpotentiated to potentiated twitch force maxima;  $CT_{tw}:CT_{pot}$ , ratio of unpotentiated to potentiated twitch contraction times;  $HRT_{tw}:HRT_{pot}$ , ratio of unpotentiated to potentiated half-relaxation times;  $P_{tw}:P_{tet}$ , ratio of unpotentiated twitch to tetanic force maxima; %sag<sub>S</sub>,  $100 \times$  number of motor units with sag<sub>S</sub>/number of motor units tested; %sag<sub>C</sub>,  $100 \times$  number of motor units with sag<sub>C</sub>/number of motor units tested; CV, conduction velocity.

TABLE 2. Correlation coefficients for linear regressions of motor unit properties on percent of tetani with sag

Motor Unit Property	Correlation Coefficient for Regression of MU Property on Percent of Trials with Sag		
	%Sag	%Sag <sub>S</sub>	%Sag <sub>C</sub>
P <sub>tw</sub>	+0.32*	-0.29†	+0.51*
CT <sub>tw</sub>	+0.21†	+0.11	+0.03
HRT <sub>tw</sub>	-0.04	+0.50*	-0.55*
P <sub>pot</sub>	+0.28†	-0.27†	+0.47*
CT <sub>pot</sub>	+0.21	+0.32†	-0.20
HRT <sub>pot</sub>	+0.11	+0.46*	-0.42*
P <sub>tet</sub>	+0.20	-0.29†	+0.44*
CV	-0.26	-0.17	-0.05
FI	-0.19	+0.50*	-0.64*

Values are correlation coefficients for linear regressions of each motor unit property on %sag (100 × number of tetani with sag/number of tetani tested in each motor unit), %sag<sub>S</sub>, and %sag<sub>C</sub>. \* $P < 0.0001$ ; † $P < 0.01$ .

consistent with previous results in cats (Stephens and Stuart 1975). CV was slower in S than in F motor units with no differences found among FF, F(int), and FR motor units.

Although sag was, by definition, present in all F motor units, the sag subtypes were not equally distributed among units of different fatigue sensitivity. The relative frequencies of sag subtypes were quantified by calculating the number of occurrences of sag<sub>S</sub> and sag<sub>C</sub> for each motor unit as a percentage of the total number of trains applied (%sag<sub>S</sub> or %sag<sub>C</sub>, respectively). %Sag<sub>S</sub> was significantly lower in FF and F(int) units than in FR units, whereas the reverse was true for %sag<sub>C</sub> ( $P < 0.01$  by ANOVA; see Table 1). The differences in sag subtype distribution by motor unit type could not be attributed to differences in background passive force at which tetani were elicited in that no significant correlations were found between background force and %sag<sub>S</sub> or %sag<sub>C</sub> ( $r^2 < 0.03$  and  $P > 0.05$  for both by linear regression). This suggested the possibility that motor unit contractile properties might be distributed differentially among motor units with different sag subtype prevalences. Several significant correlations were found between the probability of detecting sag subtypes and motor unit contractile properties related to size, speed, and fatigue (linear regression analyses summarized in Table 2). Most notably, the frequency of occurrence of sag<sub>C</sub> varied inversely with FI (Fig. 12A), HRT<sub>tw</sub> (Fig. 12C), and HRT<sub>pot</sub> and directly with P<sub>tw</sub> (Fig. 12B), P<sub>pot</sub>, and P<sub>tet</sub>.

The FI is linearly related to several of the other contractile properties that vary significantly with %sag<sub>S</sub> and %sag<sub>C</sub> ( $r = -0.55, 0.57, \text{ and } -0.56$ ;  $P < 0.0001$  for linear regressions of P<sub>tw</sub>, HRT<sub>tw</sub>, and P<sub>pot</sub> on FI). Thus the many relationships between contractile properties and sag subtype prevalence probably arise at least in part from covariation in contractile properties according to motor unit type. This was confirmed by further analysis of the relationship between %sag<sub>C</sub> and P<sub>tw</sub>, HRT<sub>tw</sub>, and FI, the three variables that alone accounted for the greatest amount of variation in %sag<sub>C</sub> ( $r^2 = 0.25, 0.31, \text{ and } 0.40$ , respectively, by linear regression). Multiple regression of %sag<sub>C</sub> on all three of these variables together only accounted for 47% of the total variation in %sag<sub>C</sub> ( $P < 0.0001$  overall). This indicated that much of the variation in %sag<sub>C</sub> accounted for by each variable alone was common to that accounted for

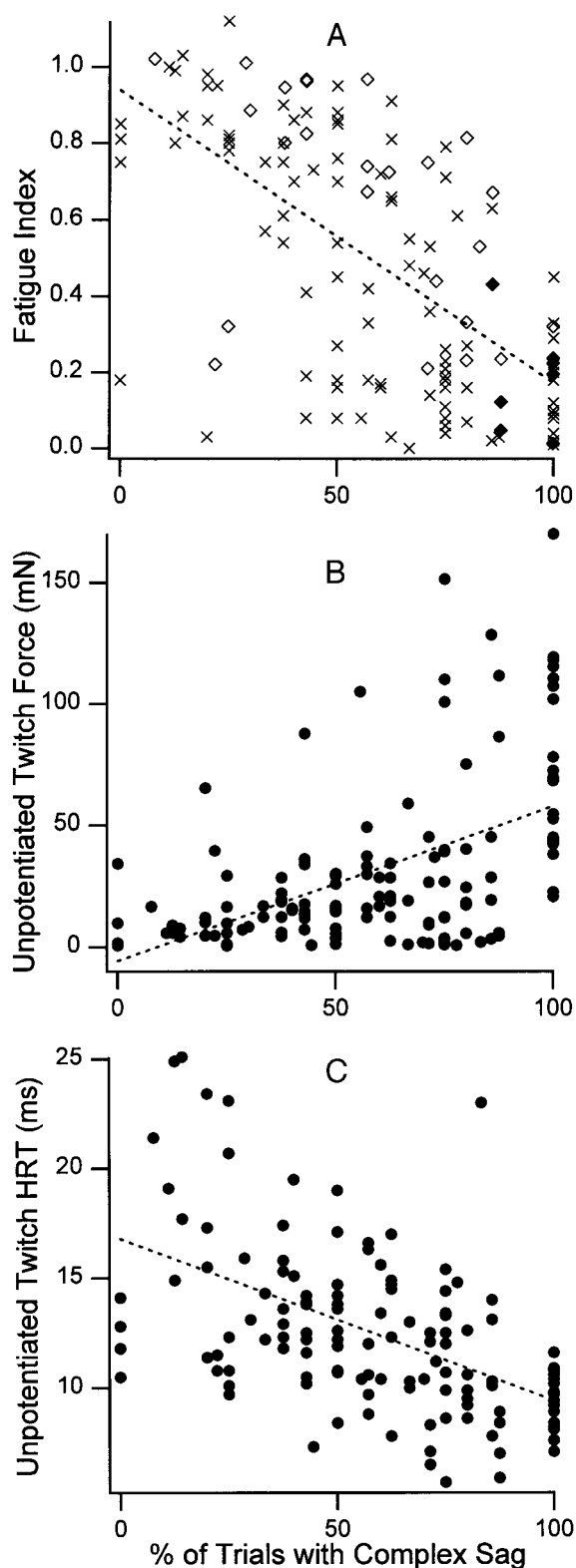


FIG. 12. Significant correlations exist between the percentage of trials with sag<sub>C</sub> and the fatigue index (A; maximum force during 120th tetanus divided by that during the first tetanus), unpotentiated twitch force (B), and half-relaxation time (C; see Table 2 for summary of statistical analysis). A: ♦ and ◇, motor units in which an electromyogram (EMG) was recorded during the fatigue test. EMG was not recorded from the other motor units (×). Substantial decreases in EMG amplitude were detected in some (♦) but not other (◇) motor units (see text for details).

by the other variables (i.e.,  $r^2$  unique to  $P_{tw}$ ,  $HRT_{tw}$ , and FI alone was 0.02, 0.04, and 0.09 at  $P < 0.02$ , 0.003, and 0.0001, respectively;  $r^2$  common to all three variables was 0.32).

A consequence of using a relatively high-frequency tetanus for fatigue testing (70 Hz vs. the more commonly used 40 Hz) is an increase in the contribution of neuromuscular transmission failure to reduced force production (Krnjevic and Miletić 1958; Kugelberg 1973). Of the 33 motor units with sag in which EMG was recorded during fatigue testing, 8 exhibited marked EMG decrements. In these units, the maximum EMG values during the 120th tetanus of the fatigue test was 11–64% of that during the 1st tetanus, and FI values were 0.01–0.43 (◆ in Fig. 12A). All of these motor units had %sag<sub>C</sub> values >0.86. However, motor units frequently expressing sag<sub>C</sub> were not uniquely sensitive to EMG failure during repeated tetanic stimulation. Several of the 25 motor units with little or no EMG decrement (maximum EMG in 120th tetanus >87% of that during the 1st tetanus; ◇ in Fig. 12A) were fatiguable and tended to express sag<sub>C</sub> (note the 3 motor units with FI values <0.25 and with %sag<sub>C</sub> = 0.71–0.88 in Fig. 12A). Thus there was no indication of selective susceptibility to neuromuscular fatigue among motor units with a high likelihood of expressing sag<sub>C</sub>.

#### *Relationship between body weight and sag*

The distributions of motor unit histochemical and contractile properties change with age in rats (Edström and Larsson 1987; Kugelberg 1976). In the present study, male animals of different weights (and, presumably, age) contributed different numbers of motor units to the data set. Differences in animal age (estimated from body weight to be 4–6 mo) could have confounded the present results. However, no linear relationships were found between body weight and %sag, %sag<sub>C</sub>, %sag<sub>S</sub>,  $P_{tw}$ ,  $P_{pot}$ ,  $P_{tet}$ ,  $CT_{tw}$ ,  $CT_{pot}$ ,  $HRT_{tw}$ ,  $HRT_{pot}$ , or FI ( $P > 0.15$  for all linear regressions). Thus the modest differences in animal ages are likely to have had little effect on the findings described in the preceding text.

#### DISCUSSION

The F and S classifications identify motor units with different ranges of properties related to contractile speed and tetanic force accumulation. The different distributions of properties are presumably the manifestation of their distinct histochemical profiles (Burke et al. 1973, 1974; Edström and Kugelberg 1968; Kugelberg 1973; Larsson et al. 1991). Histochemical or immunohistochemical classification is not feasible in studies requiring sampling from even modest numbers of motor units. Thus type-identification of motor units often has been performed according to criteria based on contractile properties alone.

#### *Sag and other correlates of contractile speed*

Three approaches have been employed to distinguish physiologically S and F motor units. The first and most direct approach is to base criteria on the distributions of CT or HRT. Histochemically defined F and S units tend to have short and long CT and HRT measurements, respectively, but the degree of overlap varies among muscles and species (Burke et al. 1973; Chamberlain and Lewis 1989; Kugelberg 1973; Kwa et

al. 1995; Sieck et al. 1996; Tötösy de Zepetnek et al. 1992). Thus use of these measurements as the sole means of classifying large samples of F and S motor units is straightforward when their distributions are clearly bimodal (Close 1967), but is problematic when their distributions are continuous (Kernell et al. 1983; Reinking et al. 1975). Placement of a contraction time criterion at the minimum between apparent clusters of samples may be questionable in cases of limited sample size. This issue has been addressed by using the longest contraction time found in motor units of intermediate-to-high fatigability as the criterion value for dividing fatigue-resistant F from S units (Bakels and Kernell 1993b; Grottel and Celichowski 1990; Reinking et al. 1975). In either case, the validity of this approach relies on the assumption of nonoverlapping distributions of  $CT_{tw}$  in F and S motor units.

The second approach relies on differences in the pattern of force accumulation during unfused tetanic contractions to distinguish F and S motor units (Gardiner 1993; Gillespie et al. 1986). Motor units with near-linear force increments in response to successive tetanic stimuli have physiological properties similar to those of histochemically defined S units, whereas those with large initial force increments followed by rapidly decreasing increments to subsequent stimuli have properties consistent with histochemically defined F units (Tötösy de Zepetnek et al. 1992).

The third approach to distinguish F and S motor units by physiological testing is based on the expression of sag during unfused tetanic contractions. In the first study to apply the sag phenomenon in this way (Burke et al. 1973), the presence or absence of sag divided their motor unit sample into F and S units with short and long  $CT_{pot}$ , respectively. The two-part separation by this contractile property was consistent with histochemical differences between these types. Several subsequent studies based on histochemical analyses of modest numbers of motor units have supported the separation of F and S units by sag (Chamberlain and Lewis 1989; Dum and Kennedy 1980; Edström and Larsson 1987; Kwa et al. 1995; Martin et al. 1988; Sieck et al. 1996). This approach is attractive because it provides a binary result once criteria for identifying sag have been defined. It has been observed in different mammalian skeletal muscles from different species, e.g., cat and rat gastrocnemius (Burke et al. 1973; Gardiner 1993; Gillespie et al. 1986; Proske and Waite 1974), cat extensor and flexor digitorum longus (Dum and Kennedy 1980; Dum et al. 1985), cat and rat tibialis anterior (Hamm et al. 1988; Larsson et al. 1991; Martin et al. 1988; Tötösy de Zepetnek et al. 1992), cat diaphragm (Sieck et al. 1996), cat peroneus longus (Kernell et al. 1983), rat soleus (Chamberlain and Lewis 1989; Kugelberg 1973), and rabbit masseter (Kwa et al. 1995) but not in cat extraocular muscles (Nelson et al. 1986). In addition, it is independent of differences in absolute contraction time among species and muscles. For example, sag distinguishes fast- and slow-contracting units in cat and rat MG motor units even though the  $CT_{tw}$  of cat F units overlaps that of rat S units (Burke et al. 1973; Einsiedel and Luff 1993).

The similarity between motor units with sag and with a rapidly decrementing pattern of force accumulation is not surprising because the methods are based on detecting different aspects of the same underlying deviations from linearity in the shape of the force envelope during unfused tetani. The absence of detectable sag in a given tetanus does not imply that the

phenomenon does not exist under those conditions but rather that whatever is responsible for the diminished force production during the tetanus is present but of insufficient magnitude to cause an overt reduction in force. This is supported by the observation that the SFI and TTP of tetani without sag from units with sag more closely matched the behavior of  $\text{sag}_S$  and  $\text{sag}_C$  tetani than those from motor units that never sagged.

Although most studies in which sag was assessed have supported the relationship between sag and motor unit type, Tötösy de Zepetnek et al. (1992) reported that only 31% of F motor units (as identified by CT) displayed sag. In a subset of 23 histochemically identified F motor units, 5 displayed discrepancies between histochemical and physiological classification: one represented a failure to detect sag, whereas the other four reflected discrepancies between oxidative status and fatigability. The present data suggest that the specific stimulation conditions used explained the failure to detect sag in many motor units with short CTs. First, sag was tested most thoroughly at two different ISIs. The limitations on correctly detecting sag with small numbers of trains indicated by the present study (Fig. 9) is consistent with the poor correlation between sag and time course of contraction and relaxation in studies using one- or two-train sag tests (Bakels and Kernell 1993b; Gardiner 1993; Grottel and Celichowski 1990; Reinking et al. 1975) and the good correlation observed in studies in which sag tests were performed at three or more different ISIs (Chamberlain and Lewis 1989; Einsiedel and Luff 1993; Kanda and Hashizume 1992). Second, one of the ISIs used to test for sag was at  $1.25 \times \text{CT}_{\text{tw}}$ , an ISI multiple at which there is a relatively low probability of detecting sag (Fig. 8). Third, tetani for assessing sag were repeated at short intervals; this would have reduced the likelihood of detecting sag in motor units capable of expressing it (Fig. 2) (also see Celichowski 1992b).

It is also possible that varying degrees of correlation between sag and histochemical and/or other contractile properties arose at least in part from differences in the criteria used to define sag. The contribution of this factor is difficult to evaluate because criteria for defining sag rarely have been discussed explicitly (but see Kwa et al. 1995). In the present study, some tetani exhibiting  $\text{sag}_C$  did not have overt local minima in their force envelopes (e.g., Fig. 3, *middle*). Although these were unlikely to have been identified as having sag in other studies, these tetani represented only 3% of the total number of tetani evaluated, and their inclusion did not alter the sag status of any motor unit (i.e., not considering these tetani as having  $\text{sag}_C$  did not cause any unit with sag to become a nonsagging unit nor did it substantially alter the values of  $\%\text{sag}_S$  and  $\%\text{sag}_C$  or their relationships with other contractile properties). Exclusion of these data from the post hoc analysis illustrated in Fig. 9 resulted in a decrease of  $<5\%$  in the probability of correctly detecting sag in any individual tetanus or combination of tetani. Thus after taking into account the factors that confound the ability to detect sag and the differences in methodology among studies, it seems likely that the presence or absence of sag is a reasonable physiological correlate of F and S motor unit type, respectively.

The present study has sought to identify factors that contribute to variability in sag conformation that could affect the classification of F and S motor units according to their sag status. The data demonstrate substantial variability in the te-

tan frequencies at which sag can be elicited, suggesting that a large part of the variability in sag testing among studies has arisen from use of inappropriate tetanic ISIs and/or an insufficient number of tetani. The present analysis indicates that several tetani at ISIs between  $1.5$  and  $2.5 \times \text{CT}_{\text{tw}}$  (or  $1.3$ – $2.0 \times \text{CT}_{\text{pot}}$ ) should be tested to ensure detection of sag in those units capable of expressing it. Under most circumstances, the improvement in reliability of detecting sag would be worth the modest cost in time for performing the additional tests.

### *Mechanism of sag*

The limited information available on the physiological expression of sag has provided some insight into its mechanism. Burke et al. (1976) observed that the TTP of individual contractions decreased during the course of an unfused tetanus. The present results confirmed their observation and also showed: that the decrease in TTP was delayed in tetani with  $\text{sag}_S$  relative to those with  $\text{sag}_C$  (Fig. 5); this is consistent with the difference in location of maximum tetanic force in tetani with these sag subtypes (Fig. 7); that TTP did not decrease from its value during the first contraction in motor units that never exhibited sag (Fig. 5); and that the increment in force added by a given contraction during the tetanus varied with its TTP (Fig. 6). These observations are consistent with the hypothesis of Burke et al. (1976) that the diminished capacity for force production that characterizes sag reflects a reduced duration of the contractile state. Their suggestion that such an effect could be due to differences in the sarcoplasmic reticulum between F and S muscle fibers is consistent with more recent information on the many type-dependent differences in the calcium release and sequestration properties of this structure (reviewed in Stephenson et al. 1998).

The present data also indicate that sag is not a single phenomenon. The initial maxima in the force envelopes of tetani with  $\text{sag}_S$  and  $\text{sag}_C$  have different ordinal and temporal locations, suggesting that these phenomena represent two discrete processes. The observations that the TTP decreases during tetani with either  $\text{sag}_S$  and  $\text{sag}_C$  and that the frequency dependence of the location of the initial maximum in the force envelope of the two sag subtypes is similar (Fig. 7) suggest that the mechanism underlying the initial decrement in force in  $\text{sag}_S$  and  $\text{sag}_C$  may be qualitatively similar. The subsequent increase in force seen only in tetani with  $\text{sag}_C$  appears to be the result of a different mechanism. The relationship between force increment and TTP is strong only until the initial tetanic maximum is reached, becoming substantially weaker at later peaks (Fig. 6). The lack of a sustained relationship between force increment and TTP beyond the tetanic maximum suggests that additional factors play a role in the later components of sag.

The secondary rise at the end of the force envelope during  $\text{sag}_C$  could result from transient changes in the phosphorylation state of the myosin light chain subunits responsible for twitch potentiation (Sweeney et al. 1993). The increase in twitch amplitude associated with repeated activation is more pronounced in motor units with sag than in those without it (see  $P_{\text{tw}}:P_{\text{pot}}$  of F and S units in Table 1). The most fatigable motor units with the shortest  $\text{HRT}_{\text{tw}}$  (i.e., those with the highest probability of exhibiting  $\text{sag}_C$ ; see Fig. 12, A and C) exhibit the greatest degree of increase in HRT during potentiation (compare  $\text{HRT}_{\text{tw}}$  and  $\text{HRT}_{\text{tw}}:\text{HRT}_{\text{pot}}$  of FF with those of other types

in Table 1). With a time constant for phosphorylation on the order of seconds (Moore and Stull 1984), potentiation would not have a substantial effect on force production until late in the tetanus (median and range of mid-95% of time to last peak for all tetani with  $\text{sag}_C = 729$  ms and 387–1,122 ms, respectively). The prolonged course of recovery from potentiation (Brown et al. 1998; Moore and Stull 1984) could contribute to the changes in force envelope that occur with repeated tetanic activation at 2-s intervals but not at 10-s intervals (Fig. 2).

The presence of sag and the magnitude of  $\text{CT}_{\text{tw}}$  are distributed similarly among motor units. However, this relationship is not invariant, in that sag and  $\text{CT}_{\text{tw}}$  can be dissociated after reinnervation after peripheral nerve injury to the extent that some motor units defy classification as described in the preceding text (Rafuse and Gordon 1998). The incomplete control by the reinnervating motoneurons over the expression of muscle fiber protein isoforms indicates that at least some of the determinants of sag and  $\text{CT}_{\text{tw}}$  are distinct but are co-expressed under normal conditions. Twitch contraction time is determined by several factors including calcium release and reuptake mechanisms of the sarcoplasmic reticulum and by myosin heavy and light chain isoform content (Pette and Staron 1990; Stephenson et al. 1998). In addition to the potential relationship between sag and sarcoplasmic reticulum function discussed in the preceding text, an intriguing relationship emerged between expression of sag and the distribution of contractile properties of fibers containing different myosin heavy chain (MHC) isoforms. MHC I and II are distributed differentially among S and F motor units, respectively. The distributions of CT among fibers expressing MHC IIA, IIX, or IIB overlap, but fatigability,  $P_{\text{tw}}$ , and  $P_{\text{tet}}$  increase with isoform expression in the following order: MHC IIA < MHC IIX < MHC IIB (Larsson et al. 1991). In the present study, these three contractile properties also varied with  $\% \text{sag}_C$ . This raised the possibility that the presence of any form of MHC II is related to the ability to express sag and that differential expression of MHC II isoforms is associated with the probability of exhibiting  $\text{sag}_C$ . However, the present data are not entirely consistent with this hypothesis in that the differential distribution of  $\text{HRT}_{\text{tw}}$  in motor units of different  $\% \text{sag}_C$  and fatigue sensitivities was not manifested among motor units containing different MHC II subtypes.

Differences in contractile properties among motor units could reflect in part differences in the arrangement of their individual muscle fibers such that F motor units tend to exert their force on adjacent fibers or connective tissue more than S motor units (Ounjian et al. 1991). However, cat caudofemoralis motor units display sag despite the minimal contribution of series elastic elements (Brown et al. 1998). Thus systematic variation among motor unit types in the series elastic component is not necessary for (but could contribute to) the differential expression of sag.

The deviations in linearity of force production that characterize sag appear to be related most directly to changes in the duration or efficacy of the contractile state. Such changes could result from transient alterations in the release of calcium from the sarcoplasmic reticulum and/or its subsequent sequestration, the sensitivity of the contractile machinery to calcium, or the efficacy of crossbridge formation. The covariation of sag expression with several different contractile properties indicates

that sag reflects the interaction of multiple processes within the muscle fibers.

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