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ADAPTATION OF CAT MOTONEURONS TO SUSTAINED AND INTERMITTENT EXTRACELLULAR ACTIVATION

BY J. M. SPIELMANN*, Y. LAOURIS†, M. A. NORDSTROM‡, G. A. ROBINSON§, R. M. REINKING AND D. G. STUART

From the Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724, USA

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SUMMARY

1. The main purpose of this study was to quantify the adaptation of spinal motoneurons to sustained and intermittent activation, using an extracellular route of stimulating current application to single test cells, in contrast to an intracellular route, as has been used previously. In addition, associations were tested between firing rate properties of the tested cells and other type (size)-related properties of these cells and their motor units.

2. Motoneurons supplying the medial gastrocnemius muscle of the deeply anaesthetized cat were stimulated for 240 s with microelectrodes which passed sustained extracellular current at 1·25 times the threshold for repetitive firing. Many cells were also tested following a rest period with intermittent 1 s current pulses (duration 600 ms) at the same relative stimulus strength. Cell discharge was assessed from the EMG of the motor unit innervated by the test neuron. The motoneurons and their motor units were assigned to four categories (i.e. types FF, FR, S and F; where F = FF+FR) based on conventional criteria. In all, twenty F (16 FF, 4 FR) and fourteen S cells were studied with sustained stimulation. Thirty of these cells (17 F, 13 S) and an additional two cells (1 F, 1 S) were studied with intermittent stimulation.

3. The mean threshold current required for sustained firing for a period of $\geqslant 2$ s was not significantly different for F and S cells. However, most of the other measured parameters of motoneuron firing differed significantly for these two cell groups. For example, at 1·25 times the threshold current for repetitive firing, the mean firing duration in response to 240 s of sustained activation was 123 ± 88 s ($\pm$S.D.) for F cells *vs.* 233 ± 19 s for S cells. These values were significantly longer than those from a comparable, previously reported study that employed intracellular stimulation.

* Present address: Department of Behavioural Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260, USA.

† Present address: Department of Neural Control, Cyprus Neuroscience and Technology Institute, Nicosia, Cyprus.

‡ Present address: Department of Physiology, University of Adelaide, Adelaide 5001, Australia.

§ Present address: Department of Physiology, University of North Carolina, Chapel Hill, NC 27514, USA.

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With intermittent stimulation, the firing durations of F and S cells were not significantly different from each other.

4. All cells exhibited a delay from the onset of current to the first spike, followed by a brief accelerating discharge that was followed by a slower drop in firing rate. Some cells (21 of 34 with sustained activation; 20 of 32 with intermittent) exhibited doublet discharges (interspike intervals $\leqslant 10$ ms) that were intermingled with the more predominant singlet discharges. Doublets were more common in the S cell type.

5. With sustained activation, the mean delay from the onset of current to the first spike was $2{\cdot}6 \pm 1{\cdot}1$ s for F cells, and $3{\cdot}2 \pm 1{\cdot}9$ s for S cells. The time required to reach peak frequency of singlet discharge following repetitive firing onset was significantly shorter for F than S cells ($7{\cdot}0 \pm 5{\cdot}0$ *vs.* $14{\cdot}3 \pm 13{\cdot}6$ s) and the peak singlet frequencies also differed significantly (F, $28{\cdot}0 \pm 7{\cdot}7$ Hz *vs.* S, $15{\cdot}6 \pm 2{\cdot}5$ Hz). Subsequently, the mean magnitude of firing rate reduction from the peak to 24 s later was significantly greater for F cells than that for S cells ($16{\cdot}2 \pm 6$ Hz *vs.* $5{\cdot}8 \pm 3$ Hz). These gradual reductions in firing frequency for both F and S cells during the course of their sustained stimulation were qualitatively similar to the late adaptation observed in previous studies that had employed intracellular stimulation.

6. The time course of firing frequency for each unit with sustained activation was fitted with a double-exponential equation: the first time constant (τ_1) for the initial increase in frequency was relatively short (F, $2{\cdot}5 \pm 2{\cdot}1$ s *vs.* S, $3{\cdot}7 \pm 4{\cdot}1$ s). The second time constant (τ_2) was significantly shorter for F than S cells ($130{\cdot}7 \pm 98{\cdot}4$ s *vs.* $750{\cdot}0 \pm 402{\cdot}4$ s). It is argued that the τ_2 values provided a quantitative description of the type of adaptation termed 'late' in previous studies.

7. The responses to intermittent stimulation were qualitatively similar to those seen with sustained activation. Cells responded to intermittent stimulation with trains of impulses, which began after several cycles of stimulation, with a mean frequency per train that first increased and then declined slowly over time (termed between-train adaptation). The thirty cells which received both stimulation protocols were compared for differences in their discharge under the two conditions of activation. For these comparisons, doublet intervals were included in the frequency calculations in each case. The time required to reach peak firing frequency was significantly different for intermittent *vs.* sustained stimulation (F cells, $19{\cdot}9 \pm 18{\cdot}1$ s *vs.* $7{\cdot}9 \pm 9{\cdot}9$ s; S cells, $78{\cdot}3 \pm 64{\cdot}7$ s *vs.* $39{\cdot}0 \pm 43{\cdot}6$ s). Peak firing frequencies were not significantly different in the two protocols (F, $39{\cdot}4 \pm 14$ Hz *vs.* $35{\cdot}9 \pm 15{\cdot}8$ Hz; S cells, $26{\cdot}4 \pm 10{\cdot}1$ Hz *vs.* $25{\cdot}4 \pm 7{\cdot}8$ Hz). The magnitude of firing frequency reduction from the peak to 24 s later was not significantly different for the F and S cells between protocols (F cells, $12{\cdot}0 \pm 12{\cdot}4$ Hz *vs.* $17{\cdot}0 \pm 15{\cdot}7$ Hz; S cells, $4{\cdot}3 \pm 3{\cdot}1$ Hz *vs.* $6{\cdot}3 \pm 5{\cdot}7$ Hz).

8. Associations were tested between firing rate properties of the test cells brought out by their extracellular activation, and other well-known, type (size)-related properties of motoneurons and motor units. For sustained activation, peak singlet firing frequency and τ_2 were each significantly associated with axonal conduction velocity, motor unit twitch contraction time, and peak tetanic force. The extent of between-train adaptation (intermittent activation), as quantified by the drop in mean frequency per train from its peak to 24 or 58 s later, was significantly correlated to axonal conduction velocity and peak tetanic force. The peak firing frequency at

the onset of the adaptive process was correlated with the extent of adaptation for both sustained and intermittent activation.

9. The overall activity of the F and S cell populations were compared using an ensemble average of firing frequency. The population mean is influenced by both adaptation, and discharge duration, of individual cells. Over 240 s of sustained stimulation there was a 94% reduction in the ensemble mean frequency for the F cell population, compared to a 24% reduction for S cells. For intermittent stimulation, the population of type F cells showed a smaller reduction of ensemble mean firing frequency (64%), while the reduction in ensemble mean firing frequency for S cells (20%) was similar to that seen with sustained activation. These findings emphasize the advantages of the S cell type for prolonged discharge with sustained (continuous) activation.

10. These results show that the extracellular activation technique is a reliable means with which to study motoneuron adaptation in the mammalian spinal cord. The associations demonstrated between peak firing frequency and measures of adaptation with other type (size)-related properties of the motor units extend the consideration of Henneman's size principle to include the *active* repetitive firing properties of mammalian motoneurons.

INTRODUCTION

Motoneuron adaptation refers to a slowing in the discharge rate of this cell type during the application of constant-intensity sustained or intermittent stimulation. Adaptation is a fundamental neuronal process that has been demonstrated for single motoneurons (via their motor unit discharges) during *sustained* maximum voluntary contractions of whole muscles in conscious humans (e.g. Bigland-Ritchie, Johansson, Lippold & Woods, 1983), and during *sustained* activation of single motoneurons in anaesthetized reduced-animal preparations via intracellular recording and stimulation (e.g. Kernell & Monster, 1982*a*, *b*).

Adaptation has also been demonstrated, but in passing, for single motoneurons during *intermittent* muscle contractions in the high decerebrate cat (Zajac & Young, 1980) and the conscious rhesus macaque (Palmer & Fetz, 1985). For other cell types, there is a recent detailed analysis of adaptation to intracellularly applied *intermittent* current pulses by Llinás & Lopez-Barneo (1988), who studied tectal neurons in a guinea-pig slice preparation. Despite the fundamental importance of motoneuron adaptation for development of muscle force in posture and movement, the literature on this intrinsic cell property is unusually sparse, particularly for that associated with rhythmic, intermittent muscle contractions that are an essential feature of animal existence (e.g. locomotion, mastication and respiration).

In the human muscle fatigue literature, there has been substantial recent interest in the observation that as muscle fatigues during a maximal voluntary contraction, there is a decline in both the rate of relaxation in force for whole muscle and the mean activation rate of motor unit populations; an association that apparently serves to optimize muscle force by matching discharge rate to fusion frequency (e.g. Bigland-Ritchie *et al.* 1983). This fatigue-induced association between relaxation rate and discharge rate has also led to the hypothesis that 'during fatigue, motoneuron firing

rates may be regulated by a peripheral reflex originating in response to fatigue-induced changes within the muscle' (Bigland-Ritchie, Dawson, Johansson & Lippold, 1986). Already, this hypothesis has stimulated new work on both experimental animals and conscious humans (for a review see Enoka & Stuart, 1992). However, in one of the original promulgations of this hypothesis, the authors emphasized that 'the decline in motoneuron firing rates seen during fatigue of a sustained maximum voluntary contraction may result primarily from changes in central motoneuron excitability; the time course of frequency changes are quite similar to that reported by Kernell and colleagues for changes in the discharge rates of cat single motoneurons in response to constant current injection (Kernell, 1965*a*, *b*; Kernell & Monster, 1982*a*, *b*)' (Bigland-Ritchie *et al.* 1986).

The cited work from Kernell's laboratory is the most substantial yet undertaken on motoneuron adaptation, particularly as it relates to muscle fatigue. The main purpose of the present work was to quantify the motoneuron adaptation that is an intrinsic property of the cell, under conditions of both sustained and intermittent stimulation. A second goal was to test for associations between intrinsic firing-rate properties of motoneurons and other type (size)-related properties of motoneurons and motor units.

The main technical difference between the present study and Kernell's work is a unique attempt to study adaptive properties using extracellular instead of intracellular stimulation of the test cell. Motoneurons supplying the medial gastrocnemius muscle of the cat hindlimb were stimulated extracellularly with current set at 1·25 times the threshold for repetitive firing and the discharge rate of functionally isolated motoneurons was monitored via their EMG patterns. The slowing in discharge rate of this cell type during sustained and intermittent activation was confirmed (cf. Kernell & Monster, 1982*a*, *b*) and characteristics of the adaptive behaviour were quantified.

It was shown that the extracellular stimulation technique increased the proportion of cells that responded with prolonged firing periods, possibly by reducing cell damage. Furthermore, several significant associations were demonstrated between the firing rate properties of the tested cells and other type (size)-related properties of the test cells and their motor units. The present use of the extracellular stimulation technique has also brought out a number of new and provocative associations between the adaptation displayed by motoneurons during their sustained *vs.* intermittent activation.

METHODS

Muscle model

The test muscle was the cat medial gastrocnemius, a bulky unipennate, hindlimb ankle extensor composed of about 300 motor units: 50% FF + FI, 25% FR, and 25% S, accounting for about 80, 15, and 4%, respectively, of the cumulative force output of the muscle (for review see McDonagh, Binder, Reinking & Stuart, 1980). Medial gastrocnemius was also the test muscle in the Kernell & Monster (1982*a*, *b*) studies, which allowed for direct comparison between the two sets of data.

Surgical procedures

Experiments were performed on adult cats of either sex weighing between 2·5 and 3·5 kg. Anaesthesia was induced by sodium pentobarbitone (40–60 mg/kg, I.P.), followed by intravenous maintenance doses as needed throughout the experiment to maintain a deeply anaesthetized state (constricted pupils, lack of corneal and pinnal reflexes, etc.). Dexamethasone (2 mg, I.M.) was

routinely administered to reduce oedema in the spinal cord. A tracheal cannula, and intravenous, intra-arterial, and bladder catheters were inserted. Blood pressure (via carotid cannula), rectal and muscle temperature, and percentage expired CO_2 were monitored and maintained at approximately $\geqslant$ 80 mmHg, 37 °C, and $\leqslant$ 3·5%, respectively. Artificial ventilation was used, if required. A lumbosacral laminectomy was performed to expose the spinal cord from L4 to L7. The left hindlimb was denervated except for medial gastrocnemius, while ensuring that the blood supply to the test muscle was not compromised.

After completion of the initial surgery, the preparation was mounted in a rigid frame. The left leg was fixed at the knee and ankle in a flexed position (approximately 105 deg at the knee) and a paraffin oil bath formed from the leg skin. Medial gastrocnemius was attached to a force transducer via a low compliance Dacron line and passive force was maintained at approximately 1 N (cf. Burke, Levine, Tsairis & Zajac, 1973), with all subsequent tests conducted at a length corresponding to that passive force. A bipolar electrode was placed on the nerve to medial gastrocnemius approximately 10 mm from the entry point into the muscle. Bipolar electrodes were also placed on tibial, hamstring, lateral gastrocnemius–soleus, and lateral peroneal nerves for antidromic stimulation to facilitate the localization of the test cells (i.e. supplying medial gastrocnemius) with the microelectrode. Dorsal roots L6 and L7 were sectioned on the left side. The experiment was terminated if the blood pressure fell below 80 mmHg or if whole-muscle twitch force fell below 70% of initial. Animals were killed at the conclusion of the experiment by I.V. injection of a 20% solution of magnesium sulphate. Conduction distance was measured along the sciatic nerve from the L6–L7 dorsal root junction to the medial gastrocnemius muscle–nerve electrode for determination of axonal conduction velocity.

Stimulation and data recording arrangement

Figure 1 shows the stimulation and data recording arrangement. Broken-tipped microelectrodes (o.d., 10–14 μm; resistance, 0·8–1·2 MΩ), filled with 2 M potassium citrate were used. Initial experiments with NaCl electrodes were unsuccessful in producing sustained repetitive discharge for $\geqslant$ 20 s. A Ag–AgCl reference electrode (surface area, *ca* 160 mm^2) was placed in adjacent spinal musculature. Test cells were antidromically activated with 0·1 ms pulses at one pulse per second and the intraspinal field potentials were monitored with the microelectrode. Every effort was made to limit the number of stimuli delivered to the medial gastrocnemius muscle nerve. Initially, the tip of the microelectrode was positioned in a region of the motoneuron pool supplying medial gastrocnemius which corresponded to the maximum amplitude of the extracellularly recorded antidromic response to stimulation of the nerve to medial gastrocnemius. The final tip position was obtained by passing 2–5 ms depolarizing (cathodal) current pulses (at 1 Hz) and placing the tip in the position corresponding to the site of lowest threshold for initiation of a single action potential. At this point, the amplitude of the extracellular field potential was typically 1 mV or larger. Motor unit activity was monitored with a fine-wire electrode embedded in the test muscle and referenced to adjacent denervated leg musculature. Motoneuron activation was monitored via the motor unit EMG. The low stimulus currents ($\leqslant$ 1 μA) required to stimulate motoneurons were considered to indicate that the tip position was as close to the initial segment/soma region as possible (cf. Gustafsson & Jankowska, 1976).

Proof of functional isolation of a single cell was based on all-or-none EMG and force profiles in response to a graded stimulus, and the appearance of a single spike in the averaged muscle–nerve neurogram (32 sweeps) following stimulation with short (2–5 ms) extracellular current pulses delivered through the microelectrode. Although extracellular current may have activated other motoneurons of different muscle species, this was not considered to be of consequence to the present results.

Experimental procedures

Following the functional isolation of a single motoneuron, its muscle unit was characterized using the following criteria: (1) twitch contraction time (CT) averaged from eight or sixteen single stimuli (pulse duration here and for criteria (2) and (3) 2–5 ms) delivered by the extracellular (EC) microelectrode at 0·1 Hz, (2) a 500 ms unfused tetanus delivered at a stimulation rate of 1·25 × CT to assess the presence (F cells) or absence (S cells) of 'sag' (i.e. the sag test of Burke *et al.* 1973), and (3) peak force in response to tetanic stimulation at either 200 Hz (train duration, 250 ms) for F cells or 100 Hz (train duration, 500 ms) for S cells. Subsequently, the threshold current for repetitive firing in response to sustained activation (current pulse duration 240 s) was determined

by gradually increasing the current strength until the cell responded with a repetitive discharge of $\geqslant 2$ s duration. The incremental increase in current strength was continued over 30–60 s to avoid problems with threshold determination due to the delay in the onset of spiking associated with the extracellular technique (see below). With the citrate electrode, few cells with acceptably low single-spike thresholds failed to fire repetitively for 2 s with sustained stimulation.

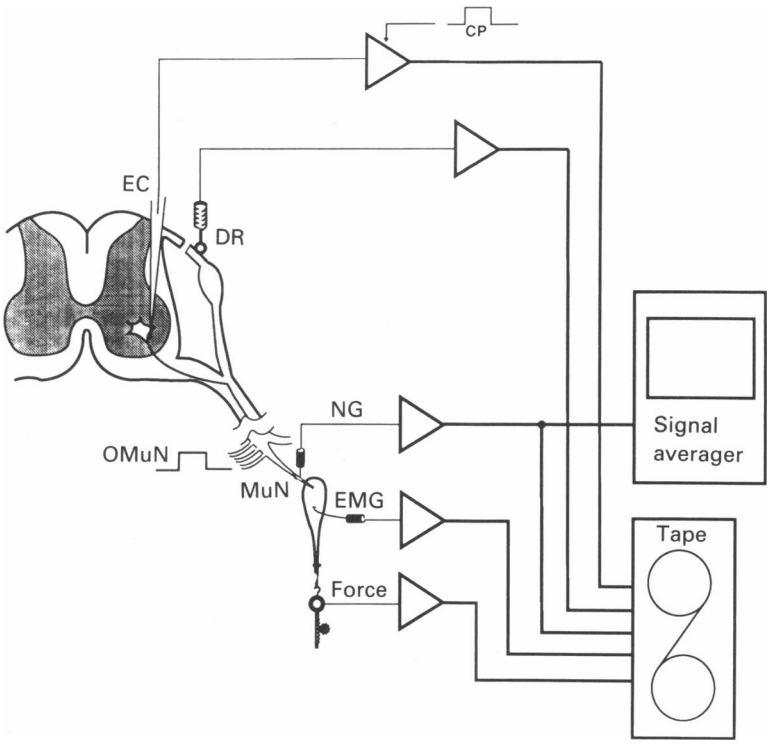

Fig. 1. Schematic of the experimental arrangement. An extracellular (EC) microelectrode was placed near the soma of a medial gastrocnemius motoneuron. A dorsal root volley (DR) was used to verify that stimulation of peripheral nerves was suprathreshold for group I axons. EC positioning was guided by antidromic stimulation of either the medial gastrocnemius muscle nerve (MuN) or the proximal stumps of other muscle nerves (OMuN). Functional isolation of single medial gastrocnemius motoneurons was assured by averaging (32 sweeps) the neurogram (NG) recording following a brief (2–5 ms) command pulse (CP) delivered by the EC electrode via a microelectrode amplifier. A sustained or intermittent CP was used to induce repetitive discharge for up to 240 s in the test cells. Relevant signals including EMG, NG, Force, and CP (actual injected current) were recorded on analog tape for later analysis.

Cells with acceptably low threshold current for repetitive firing ($\leqslant 1$ μA) were subjected to four sequences (protocols) of 240 s stimulation, including (1) sustained extracellular stimulation at 1·25 × the threshold for sustained firing, (2) a 2 min rest followed by sustained stimulation with a superimposed subthreshold pink noise, (3) a 2 min rest followed by intermittent stimulation with 600 ms current pulses delivered at 1 Hz (at 1·25 × the threshold for repetitive firing), and (4) following another 2 min rest, sequence (3) with superimposed subthreshold pink noise. All data were stored on a 7 channel FM tape-recorder for subsequent analysis. Two cells (1 F, 1 S), discharged for less than 10 s in response to the first stimulation protocol, and were eliminated from the present study.

The present report deals only with data obtained using the first and third stimulation protocols. For the intermittent stimulation trial, the microelectrode remained in the same position established during the motoneuron isolation procedure, unless the short-pulse threshold (assessed at the onset of each new protocol, see above) changed by *ca* 10% from the initial value.

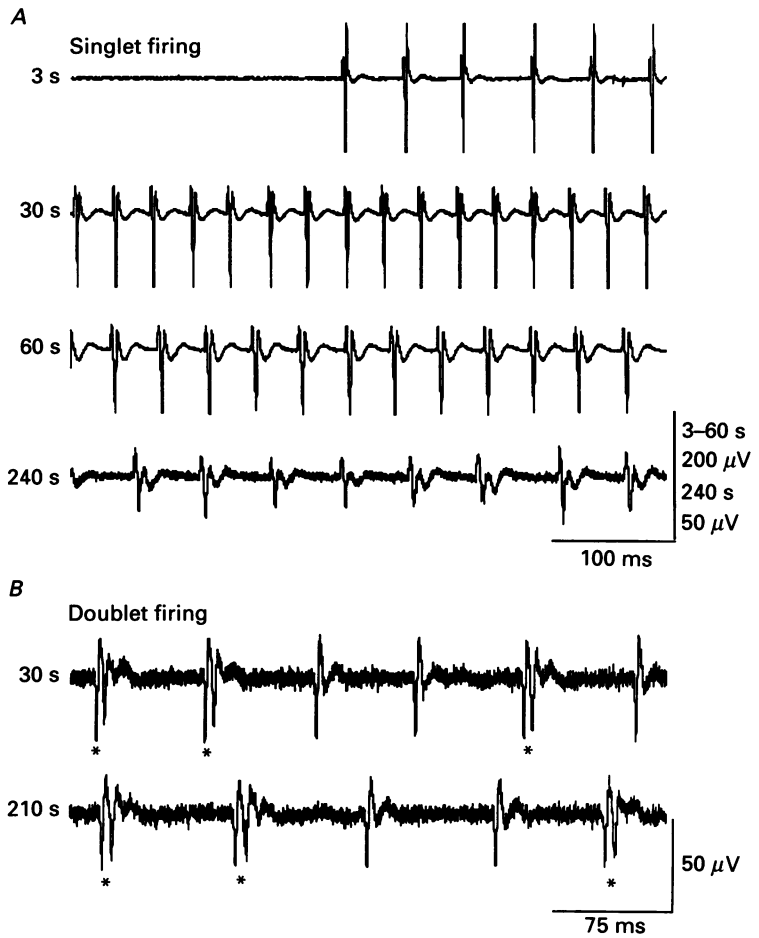

Fig. 2. Examples of motor unit EMG changes during the course of the 240 s period of sustained stimulation of a spinal motoneuron. Shown in *A* are epochs of the EMG record beginning at different times following the onset of the stimulating current (3, 30, 60 and 240 s) for a cell that exhibited only singlet discharge (i.e. all interspike intervals > 10 ms). For this illustration, the EMG signal was sampled at 61 μs per point. The motor unit EMG declined markedly in amplitude (note different calibration values for 3–60 s, and 240 s) and changed in shape. Such changes in the EMG waveform occurred slowly and progressively. *B* shows epochs of the EMG record at different times following the onset of stimulation (30 and 210 s) for a cell (different from cell shown in *A*) that exhibited doublet discharge (indicated by asterisks; interspike intervals ⩽ 10 ms) in addition to singlet discharge throughout most of the stimulation period.

Subsequently in this report, the motoneuron responses to the intermittent stimulus pulses are referred to as spike *trains*. Each train and its subsequent 400 ms rest period (i.e. at 1 train/s) is referred to as a *cycle*. The threshold current strength required for repetitive discharge was

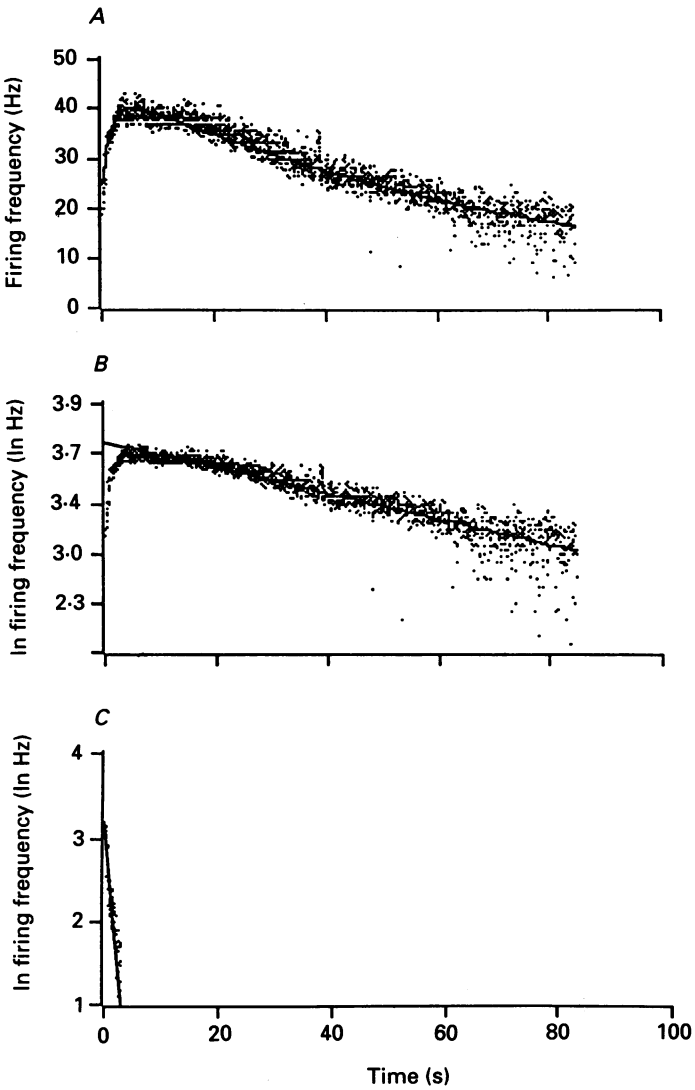

Fig. 3. Method used to fit a double exponential function to the instantaneous firing frequency profile of a motoneuron responding to sustained activation. The firing pattern of a type F cell with a firing duration of 84 s is shown. *A*, instantaneous frequency (dots) plotted *vs.* time and the corresponding double exponential fit: $Y = -K_1 \exp(-t/\tau_1) + K_2 \exp(-t/\tau_2)$ (continuous line). *B*, same data as in *A* with firing rate expressed in units of natural logarithm (ln Hz), to aid in determining the separation of τ_1 from τ_2 (see below). The optimal time point (t_{opt}) for separation of these data was 3 s. The linear regression line shown in *B* was fitted to the frequency values at $t > 3$ s. It was used to calculate a late time constant (τ_2) of 81 s and an ordinate intercept (antilog), K_2, of 45 Hz. *C*, after subtraction of the remaining values (i.e. at $t < 3$ s) from this fit, the residual data points were fitted with another (steep) regression line, which provided an initial time constant (τ_1) of 1·88 s, and an ordinate intercept (antilog), K_1, of 24 Hz. The equation of the fitted line calculated in this manner and shown in *A* was: $Y = -24\exp(-t/1{\cdot}88) + 45\exp(-t/81)$.

determined by gradually increasing the current to the point where the functionally isolated cell responded with several spikes per train for two trains. The intermittent stimulation trial continued for 240 s, or until repetitive discharge ceased. Units that did not respond with $\geqslant 2$ spikes per train for $\geqslant 10$ s were not included in the present analysis.

Following the final period of 240 s stimulation, and a subsequent 2 min rest period, the test cells were stimulated with a train of thirteen brief pulses at 40 Hz for 330 ms, repeated at 1 Hz for 2 min (Burke *et al.* 1973). A standard fatigue index was calculated based on the ratio of the peak force developed in the first train divided by that developed in the 120th train. This test was only useful for classification when the units remained capable of generating force following the prior tests (cf. Botterman & Cope, 1988). for some units (Table 1), fatigability was judged from the initial sustained stimulation trial. Motor units were classified as fast (type F) if their twitch contraction times were < 38 ms and slow (type S) if > 38 ms. All units were characterized as either FF, for fast-contracting, highly fatigable motor units; FR, for fast-contracting, fatigue-resistant units; or S, for slowly contracting, fatigue-resistant units.

The fatigue-based classification (i.e. FF *vs.* FR *vs.* S) is considered conventional (Burke *et al.* 1973). However, the fatigue indices derived from the standard fatigue test in the present study are not considered reliable indicators of absolute fatigue (cf. Enoka & Stuart, 1992), because the test was applied after four, 240 s prior periods of stimulation (i.e. two sustained, two intermittent; see above). Although the standard fatigue test was useful for unit classification, it was not considered appropriate to test for an association between this index and other type (size)-related properties of motoneurons and motor units.

Data analysis

The discharge pattern of single cells was determined off-line from the EMG signal using a computer-based spike-discrimination system employing a template-matching algorithm (SPS 8701; Signal Processing Systems, 23 Airlie Avenue, Prospect 5082, Australia) which measured the firing time with ± 125 μs resolution. The EMG waveforms of F cells tended to decline in amplitude and change in shape during the course of stimulation, which meant that discrimination was not always straightforward. Figure 2*A* shows such an example. The motoneuron began firing 3 s after the onset of current application (Fig. 2*A*, first trace). After 30 s of stimulation (Fig. 2*A*, second trace), the cell was firing at a higher frequency, and the EMG was already reduced in amplitude. At 60 s (Fig. 2*A*, third trace), the firing frequency had declined from its earlier peak, the amplitude was reduced further and a slower, late component in each EMG wavelet became more prominent. By 240 s (Fig. 2*A*, fourth trace), the firing frequency had declined further and the EMG amplitude was markedly diminished. Discrimination accuracy in such cases was facilitated by the use of the SPS 8701.

Figure 2*B* shows the EMG from a unit that discharged with doublets (interspike intervals $\leqslant 10$ ms *vs.* > 10 ms for singlet discharge, Zajac & Young, 1980) throughout most of the 240 s stimulation period. These doublet discharges were easily recognized, so they presented no special problem in assessing the interspike intervals of the test cell discharge.

The EMG interspike intervals were used to analyse the entire, instantaneous discharge profile on an Apple Macintosh computer (Apple Computer, Inc., 20525 Mariani Avenue, Cupertino, CA 95014, USA) using laboratory-developed softward written in LabVIEW, an icon-based programming environment (National Instruments Corporation, 6504 Bridge Point Parkway, Austin, TX 78730–5039, USA). The analysis involved several phases, as summarized below.

Sustained activation: fitting of time constants to the instantaneous discharge frequency profile. The usual response of a motoneuron to extracellular stimulation was an initial increase in firing frequency of the singlet discharge followed by a slower decline (e.g. Fig. 3*A*). The response of cells to sustained activation was quantified by modelling the change in firing rate for each motoneuron as a second-order system and fitting a double exponential equation:

$$Y = -K_1 e^{-t/\tau_1} + K_2 e^{-t/\tau_2}. \quad (1)$$

The first step in the fitting process was to plot the natural logarithm of the instantaneous frequency *vs.* time (Fig. 3*B*). For each cell, time was referenced from the onset of firing. The optimal time point (t_{opt}) for separating the period of increasing and decreasing rate was found by iteration to minimize the mean square error of the total fit. Linear regression performed on the data associated with the period of decreasing discharge rate (Fig. 3*B*) provided the time constant (τ_2,

slope of the regression line) and the intercept (K_2, antilogarithm of the ordinate intercept of the semilogarithmic plot) for the second term of eqn (1). Once the decreasing rate values were fitted, the deviation of the residual data points from this regression line was used to extract the parameters of the first term (increasing rate) of eqn (1). To determine these parameters, a 'peeling' procedure was employed. The residual data points (i.e. points at time $\leqslant t_{opt}$) and the ordinate values of the regression line at corresponding times were expressed in linear terms (antilogarithm). Their corresponding *differences* were determined and plotted in semilogarithmic scale (Fig. 3*C*). A regression line was fitted to these points to obtain the time constant (τ_1, slope of the regression line) and coefficient for the first term of eqn (1) (Fig. 3*C*). In order to determine K_1, the intercept was corrected for the shift imposed by the method by calculating its difference from the intercept of the second term. Note that in eqn (1) the term derived from the data shown in Fig. 3*C* is *subtracted* from the second (late adaptation term) to produce a fitted line which followed the increasing firing rate profile of the data in the first few seconds. Use is made subsequently of the term *peak singlet firing frequency*. This parameter is defined as the peak value of the double exponential fit for singlet firing.

Many cells responded with varying degrees of doublet firing (e.g. Fig. 5*C* and *D*). In these instances the doublet intervals were excluded from the calculation, and a double exponential fit was applied to the singlet firing profile alone. Time constants are reported for singlet discharge profiles (interspike intervals > 10 ms) only. The rationale for separating the doublet and singlet firing profiles for the fitting of time constants involved four points. (1) The doublet and singlet interspike intervals were grouped into clearly separate frequency ranges. Doublet intervals did not merge into the singlet discharge range with adaptation. Discharge in the instantaneous frequency range between that of singlet and doublet firing (60–100 Hz) was very infrequent. (2) When fitted separately, the adaptation of doublet and singlet discharge yielded different time constants of adaptation. (3) The mean time constants of adaptation of singlet discharge were not significantly different in cells which exhibited doublet discharge and those that did not. (4) Doublet discharge was often sporadic. For these four reasons, adaptation of doublet discharge was considered to represent a different process to adaptation of singlet discharge, and doublets were excluded from the calculation of the time constants of late adaptation for singlet discharge.

Sustained activation: interspike interval distribution and changes in the mean interval. Interval histograms (bin width, 5 ms) were plotted for all cells. These were used to identify doublet discharge (intervals $\leqslant 10$ ms). The mean interval (including doublets, if present) was calculated over 1 s epochs and expressed as mean firing frequency *vs.* time. These values were used in the population ensemble means used to describe adaptation in the different cell types (Fig. 10). Here it was considered appropriate to use a measure of firing frequency that included all interspike intervals, since the emphasis of this comparison was a functional one; i.e. the amount each cell type contributes to the total muscle output at intervals following the onset of a constant excitatory drive. This measure of firing frequency was used in all comparisons of motoneuron responses with sustained *vs.* intermittent stimulation.

Intermittent activation: quantification of discharge rate and between-train adaptation. In the analysis of motoneuron responses to intermittent activation, firing rate was quantified by determining the mean interspike interval per train (including doublets) expressed in terms of mean firing frequency per train. In the present study, motoneurons responded to intermittent stimulation with a relatively fast increase in firing frequency per train that peaked several seconds after stimulation commenced and then declined more slowly over time. Between-train adaptation was quantified for each cell by calculating the drop in firing frequency from the mean peak firing frequency per train to points 24, and 58 s later, and to the end of stimulation. The peak-to-24 s later analysis was analogous to the method used by Kernell & Monster (1982*a*, *b*) to quantify late adaptation during sustained activation by the drop in frequency from 2 to 26 s of discharge.

Individual time constant values were not fitted to the instantaneous firing frequency profiles of each unit as was done for the sustained discharge profiles. In the comparison of discharge with intermittent *vs.* sustained stimulation, the frequency values from sustained stimulation were calculated by including doublets, so as to provide a meaningful comparison. For within-cell comparisons of adaptation with respect to the number of spikes produced (cf. Fig. 12) in the two activation protocols the mean frequency data for individual cells were fitted with a high-order polynomial to provide interpolated values for comparison. With doublets included in the frequency calculation, this method provided a tighter temporal fit of individual cell data than the double exponential method.

Intermittent activation: quantification of within-train adaptation. Within-train adaptation was quantified by plotting the first interval (i) in the train *vs.* the second interval in the train ($i+1$) for all trains comprising the stimulation period. Similar joint-interval density plots were constructed for the second *vs.* third interval and so on (ith *vs.* ($i+1$)th) up to the seventh and eighth interval.

Relation between neuromechanical properties and discharge characteristics. Correlations were sought between the neuromechanical properties of the motor units (axonal conduction velocity, twitch contraction time, and peak tetanic force) and the active discharge properties of the motoneurons revealed with sustained (peak singlet firing frequency, time constant of late adaptation) and intermittent (peak firing frequency, extent of between-train adaptation) stimulation. With sustained stimulation, firing duration was defined as the period for which the motor unit fired continuously with a mean frequency > 5 Hz. If the mean frequency fell below 5 Hz in any 1 s epoch once firing had commenced, repetitive discharge was considered to have ceased (even if it recommenced later; cf. Kernell & Monster, 1982*a*). With intermittent stimulation, repetitive discharge was considered to have ceased if the motoneuron responded with < two action potentials per train.

RESULTS

Sustained stimulation

Sampling issue

Results with sustained stimulation were obtained from thirty-four medial gastrocnemius motor units (20 F, 14 S) in ten experiments. Distribution of the neuromechanical properties of the units are summarized in Table 1. The axonal conduction velocities reported in Table 1 are low compared to previously published data (McDonagh *et al.* 1980) obtained with intracellular stimulation. This difference may be due to increased activation time associated with extracellular, intraspinal stimulation of motoneurons (cf. Gustafsson & Jankowska, 1976). Note that the relative mean difference in conduction velocity for the F and S cells was similar to previously reported values (McDonagh *et al.* 1980).

Threshold for sustained firing: F vs. S cells

The mean threshold current for sustained firing for a period $\geqslant 2$ s was not significantly different for the F and S cells (Table 2). Corresponding values for intracellular stimulation have been reported by Kernell & Monster (1981) for motoneurons of type-identified motor units. These investigators reported significant differences between the threshold current for repetitive firing for each of the reported cell types (nA: FF, 21, FR, 11; S, 6·5). On this basis, it is obvious that to elicit repetitive discharge, extracellular stimulation required at least 10× the current required during intracellular stimulation.

Firing duration: F vs. S cells and extracellular vs. intracellular activation

Figure 4*A* and *B* compares the firing durations with sustained activation for the motor unit sample obtained here (*A*) to the motor unit sample of a previously published intracellular study (*B*, Kernell & Monster, 1982*a*). Figure 4*A* shows the distribution of firing duration for the presently studied motoneurons, with stimulus strength at 1·25× the threshold for repetitive firing. The mean firing duration was 168 ± 87 s ($\pm$S.D.; $n = 34$). The corresponding values (Fig. 4*B*) for motoneurons activated by intracellular stimulation (Kernell & Monster, 1982*a*) were 128 ± 82 s (calculated from Kernell & Monster, 1982*a*; their Fig. 1*A*, by assuming that cells in

each bin fired for the entire time for that particular bin). The two frequency distributions in Fig. 4*A* and *B* were significantly different. This suggests that extracellular stimulation significantly increased the proportion of cells capable of 240 s of continuous firing.

The differences in motoneuron response to extracellular and intracellular stimulation are revealed in more detail in Fig. 4*C* and *D*. It was clear that S cells

TABLE 1. Neuromechanical properties of the motor unit sample studied with sustained activation

Motor unit type	FF	FR	F*	S
Sample size (n)	16**	4	20	14
Sag	+	+	+	−
Twitch contraction time (ms)	24·9 ± 5·0 (17·4–37·3)	22·0 ± 7·2 (14·6–31·7)	24·4 ± 5·4 (14·6–37·3)	53·4 ± 12·4 (38·6–75·5)
Twitch force (mN)	27·1 ± 29·9 (2·1–111·8)	2·2 ± 1·6 (1·2–4·6)	22·1 ± 28·5 (1·2–111·8)	1·4 ± 1·2 (0·2–4·5)
Tetanic force (mN)	320·5 ± 183·3 (65·2–719·6)	88·2 ± 66·1 (32·8–169·7)	274·1 ± 190·6 (32·8–719·6)	18·8 ± 18·9 (1·1–71·6)
Conduction velocity (m/s)	87·6 ± 9·0 (73·6–109·7)	81·3 ± 9·0 (69·3–91·0)	86·4 ± 9·2 (69·3–109·7)	74·2 ± 7·4 (62·5–90·5)
Fatigue index	0·24 ± 0·11 (0·12–0·35) $n = 4$	0·88 ± 0·26 (0·51–1·10) $n = 4$	0·56 ± 0·39 (0·12–1·10) $n = 8$	1·04 ± 0·13 (0·73–1·17) $n = 12$

Values are means ± S.D. (range). Twenty units (4 FF, 4 FR, 12 S) were classified on the basis of the standard fatigue index (FF < 0·25; FR + S > 0·75). For the remaining fourteen units (12 FF, 2 S), the test neuron was either lost prior to the standard fatigue test (2 FF), or no measurable force was produced by these units during the application of the fatigue test (10 FF, 2 S). For these fourteen cells, their fatigue classification was assessed qualitatively during the first 240 s (sustained) stimulation period: the twelve FF units exhibited a pronounced reduction of force during the course of stimulation whereas the two S units maintained *ca* > 70% of their initial force.

* F = FF + FR motor units.

** One F cell classified as FF had a negative sag test.

comprised the majority of those firing for 240 s of extracellular stimulation (Fig. 4*C*). Nevertheless, it is worth noting that 25% of the F cells were capable of sustained firing for longer than 180 s when stimulated extracellularly. Mean firing duration for the present F cell sample was 123 ± 88 s *vs.* 233 ± 19 s for the S cells. The data from Kernell & Monster (1982*b*; their Fig. 3 of which three of their data points were indistinguishable to us due to superimposition) have been redrawn in Fig. 4*D* in a similar manner to Fig. 4*C*. With intracellular stimulation (Fig. 4*D*) there was a fairly even distribution of firing duration for both F and S cells. Type S cells were clearly more likely to cease firing before 240 s with intracellular *vs.* extracellular stimulation, and the distribution of firing duration for S cells using the two routes of stimulation was significantly different. This was not the case for F cells.

Motoneuron adaptation: singlet firing

Four general patterns were seen in the spectrum of motoneuron responses to sustained extracellular stimulation, and a representative example of each of these is shown in Fig. 5*A–D*. Two of these firing profiles show singlet-discharging cells and

two show patterns in which doublet firing occurred. These records show the instantaneous (unsmoothed) firing frequency plotted *vs.* time for two F cells (Fig. 5*A* and *C*) and two S cells (Fig. 5*B* and *D*). The double exponential line of best fit is also plotted. The initial increase in firing frequency shown in Fig. 5*A* was a universal

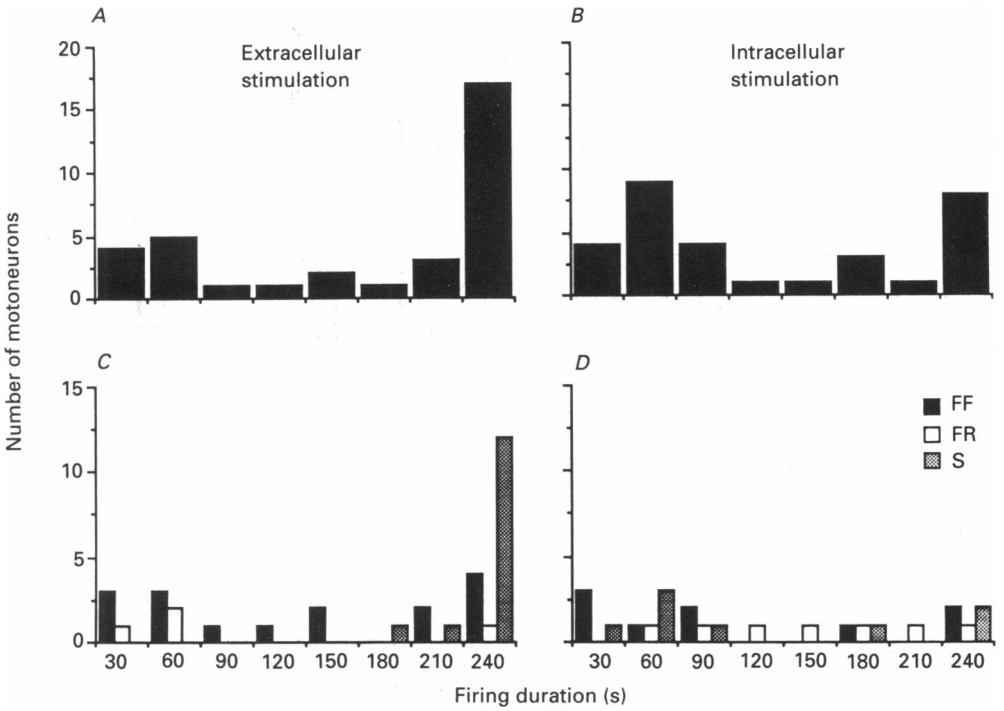

Fig. 4. Distributions of total firing duration with sustained activation: extracellular *vs.* intracellular stimulation. The upper two panels compare the corresponding firing duration data for the motoneurons activated with extracellular (*A*, present study; $n = 34$) and intracellular (*B*, Kernell & Monster, 1982*a*: $n = 31$; their Fig. 1*A*) sustained stimulation. The two frequency distributions were significantly different (Kolmogorov-Smirnov test; $P < 0{\cdot}01$). *C*, distribution of firing duration for the present sample of type-identified motoneurons activated with extracellular stimulation. Type S cells (Stippled bars; $n = 14$) comprised the majority of the cells that exhibited sustained firing for 240 s (12/14 cells fired throughout the entire test period; all fired for > 150 s). The distribution for type F cells was more uniform (FF, filled bars; $n = 16$. FR, open bars; $n = 4$). *D*, analogous data replotted from Kernell & Monster (1982*b*; their Fig. 3). The distribution of firing duration for both F and S cells was fairly uniform. The distributions for S cells were significantly different in the two studies (Kolmogorov–Smirnov test; $P < 0{\cdot}001$), but there was no significant difference between the two distributions of F cells ($P > 0{\cdot}05$).

finding for the singlet discharge profiles. The initial increase was followed in most cases (32 of 34) by a decline in firing frequency (late adaptation) which developed over the course of stimulation. However, the remaining two cells (both type S; one of which is shown in Fig. 5*B*) demonstrated a very slow increase in firing frequency that followed the initial rapid rise in discharge. Adaptation in these two cells may

have been better described with an equation other than the one used in this study. However, even in these two cases, the double exponential fit was better than that provided by either a linear or a single exponential fit. These two cells were omitted from any analyses that incorporated τ_2 since these would be of opposite sign to the

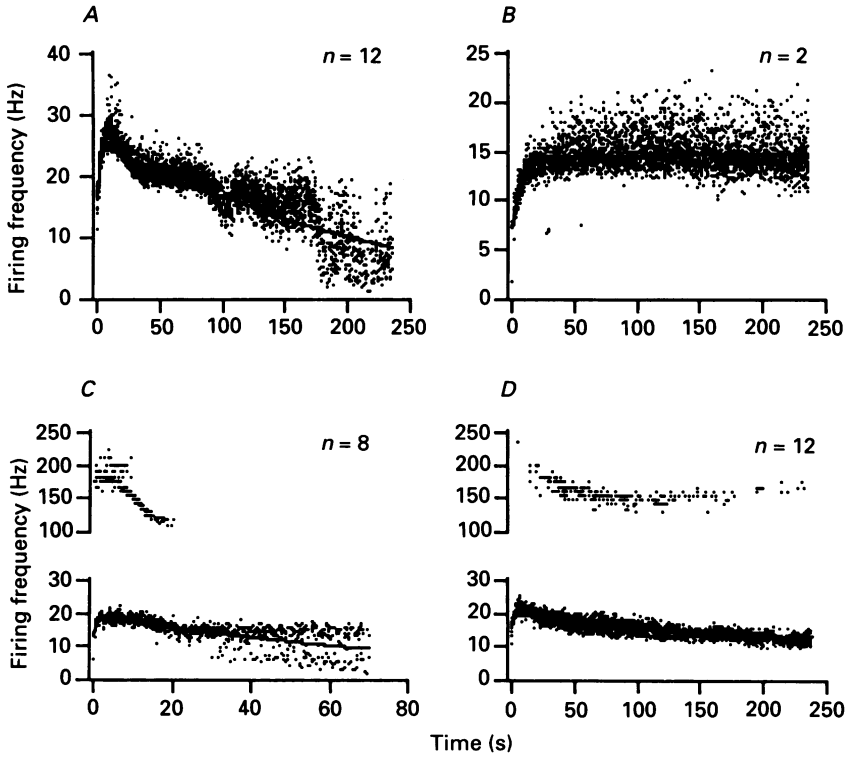

Fig. 5. Typical adaptation patterns of single motoneurons firing in response to sustained extracellular stimulation. The profile of the instantaneous firing frequency *vs.* time has been fitted with a double exponential equation. The number (n) of cells exhibiting qualitatively similar behaviour (A–D) is indicated. A, type F motoneuron ($\tau_1 = 2{\cdot}2$ s, $\tau_2 = 197$ s. B, type S motoneuron ($\tau_1 = 6{\cdot}0$ s, $\tau_2 = -16656$ s; unit showed a slight increase rather than decrease in firing frequency hence the change in sign for τ_2). C, type F motoneuron that exhibited doublet firing during the first 20 s of stimulation. A double exponential fit was applied to singlet firing alone ($\tau_1 = 0{\cdot}5$ s, $\tau_2 = 96$ s). Note the break in the Y-axis. D, type S cell whose response also featured doublet firing. A double exponential fit was applied to singlet firing alone ($\tau_1 = 0{\cdot}7$ s, $\tau_2 = 453$ s). Type F cells generally exhibited more adaptation than type S cells.

other reported τ_2s. Of the thirty-four motoneurons studied, fourteen exhibited singlet firing patterns consistent with those shown in Fig. 5A and B.

Motoneuron adaptation: doublet firing

The firing patterns of the remaining twenty F and S cells were similar to those already described, but complicated by the occurrence of doublet, or even triplet discharges characterized by short ($\leqslant 10$ ms) interspike intervals (Fig. 5C and D).

For many cells, the doublet discharge was intermittent. In general, it was more likely to be seen in the initial period of sustained stimulation as shown for an F cell in Fig. 5*C*. Instantaneous frequencies in excess of 200 Hz were reached in this cell, but the doublet intervals were intermittent, and their rate was clearly different from the far more numerous singlet-firing spikes generated at frequencies near 20 Hz. In general, the doublet firing was clearly separated from the lower frequency, singlet firing with only an occasional spike occurring in the intermediate frequency range outside of the clusters of points formed by the doublet and singlet spikes. Two FF cells showed doublet firing similar to that shown in Fig. 5*C*. The other seven F cells (3 FF, 4 FR) displayed non-adaptive or intermittent doublet firing.

Figure 5*D* shows the usual pattern in the S cells (12 of 14) when doublets were present. S cells demonstrated a greater incidence of doublet firing which usually displayed little adaptation. Peak instantaneous frequencies achieved during doublet firing for S cells were similar to those in F cells, as shown in Fig. 5*C* and *D*. Doublet firing, whether intermittent or nearly continuous, was more likely to continue throughout the 240 s course of stimulation for the S cells.

Singlet vs. doublet firing issues

The interspike interval histograms for the cells were grouped on the basis of the presence or absence of doublet discharge. Several analyses were performed to determine whether the presence of doublet discharge affected the singlet discharge component of the cells' firing patterns. Individual histograms from each of the doublet-discharging cells were summed to produce a population interspike interval histogram for the F and S cells. Similar histograms were produced for the singlet discharging cells in both the F and S cell populations. These population histograms are shown in Fig. 6 in order to illustrate the relative incidence of doublet spikes. The histogram for the population of singlet-firing type F cells is shown in Fig. 6*A*. Figure 6*B* shows the histogram for the population of singlet-firing type S cells. Population histograms are shown for the F cells that fired with doublet discharges (Fig. 6*C*) and the S cells which exhibited doublet discharges (Fig. 6*D*). Note that for cells in which doublet discharge was observed, the doublet peaks (Fig. 6*C* and *D*) comprised 6·2 and 31·8% of the total intervals for F and S cell populations, respectively. Also note that the mean intervals for the singlet discharge of F and S cells were similar in the presence or absence of doublet firing (i.e. Fig. 6: *A vs. C*, 50 *vs.* 54 ms; *B vs. D*, 66 *vs.* 54 ms). The histogram shown in Fig. 6*E* included intervals from all the F cells (i.e. the $A+C$ histograms of Fig. 6). Note the relatively small contribution (3·7%) that the doublets made to the entire population of intervals for F cells. Figure 6*F* shows the corresponding interval histogram for all the S cells (i.e. $B+D$ histograms in Fig. 6). Here, the relative contribution of doublets to the entire population of intervals was considerably more than in the F cell case, being 26·4% of the total number of intervals.

To investigate whether late adaptation of singlet discharge was complicated by doublet discharges, a comparison was made of the τ_2 values for F and S cells displaying singlet discharge alone *vs.* those displaying both singlet and doublet discharges. The mean τ_2 value for singlet-discharging type F cells was not significantly different from that for the type F cells that showed singlet and doublet

firing (94±85 s, $n = 11$ *vs.* 175±99 s, $n = 9$). The only S cell that showed a singlet discharge pattern and late adaptation had a τ_2 value of 650 s compared to a mean of 759±421 s for the eleven S cells that displayed both singlet and doublet firing. On the basis of these findings, it was concluded that the presence of doublets did not

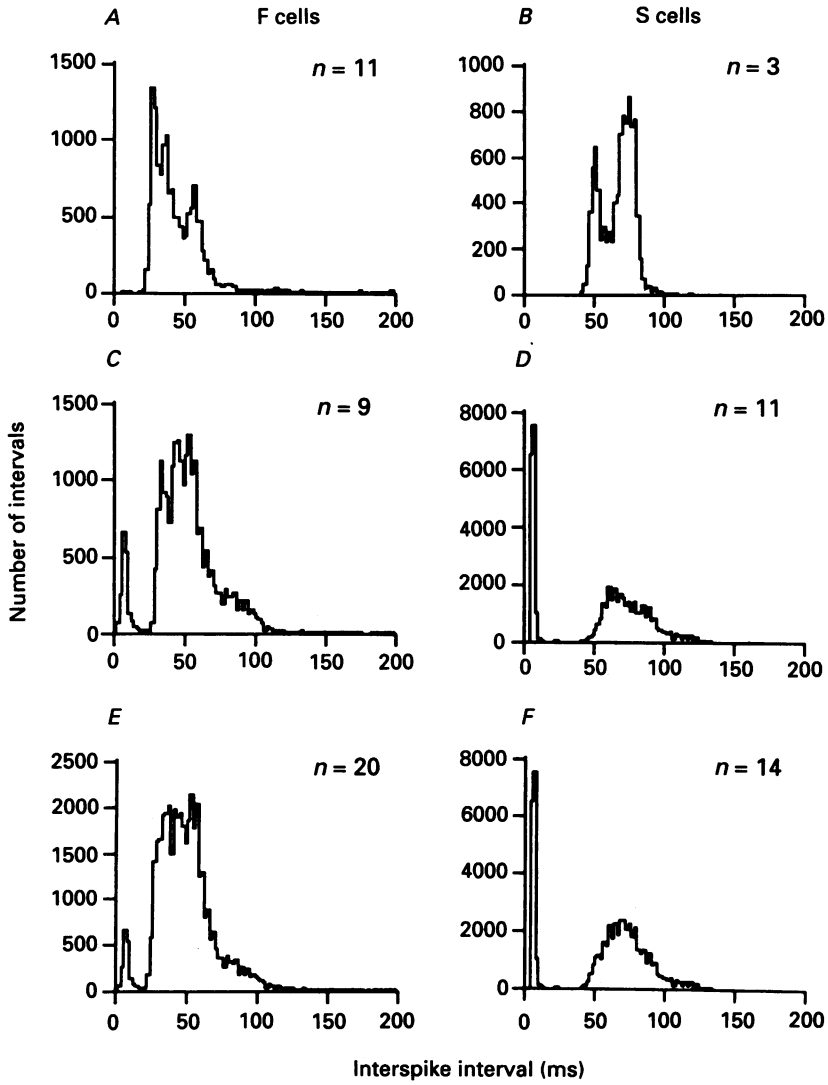

Fig. 6. Interspike interval histograms of singlet- and singlet plus doublet-discharging cells during sustained stimulation; F *vs.* S cells. *A* and *B*, interval histograms for the singlet-discharging F (*A*) and S (*B*) cells. *C* and *D*, interval histograms for F (*C*) and S (*D*) cells that displayed doublet discharge. The doublet peak ($\leqslant$ 10 ms) for the type F cells was a small component (6·2%) of the total number of spikes in this population. For the type S cells, the doublet peak was considerably larger, 31·8% of the total spike count. *E* and *F*, entire population of F (*E*) and S (*F*) cells (i.e. combinations of the singlet and doublet firing cells). The number of cells (n) comprising each population histogram is indicated on each plot.

significantly influence the late adaptation of singlet discharge. It was therefore considered acceptable to pool the τ_2 values for singlet discharge of the cells that displayed both singlet and singlet plus doublet discharge.

Firing rate properties: F vs. S cells

Several firing properties of cells activated with sustained extracellular stimulation are given in Table 2. Each measurement in Table 2 is briefly discussed below in

TABLE 2. Firing rate properties of the motoneuron sample in response to sustained activation

Motoneuron type	FF	FR	F	S
Sample size (n)	16	4	20	12
Threshold current for repetitive firing (nA)	384±179 (130–830)	553±285 (150–760)	418±207 (130–830)	462±234 (120–1000)
Time to the onset of firing (s)	2·6±1·2 (1·0–4·5)	2·5±0·6 (1·7–3·1)	2·6±1·1 (1·0–4·5)	3·2±1·9 (0·9–6·5)
Initial firing frequency (Hz)	17·1±7·0[a] (4·0–28·0)	16·0±6·3[b] (10·0–24·0)	16·9±6·7[c] (4·0–28·0)	9·6±2·4[abc] (7·0–16·0)
Time to peak singlet firing frequency (s)	7·6±5·3 (1·9–17·9)	4·1±1·4 (2·7–6·0)	7·0±5·0[f] (1·9–17·9)	14·3±13·6[f] (5·0–52·4)
Peak singlet firing frequency (Hz)	28·3±7·3[a] (14·1–40·7)	27·2±10·5[b] (18·2–41·8)	28·0±7·7[c] (14·1–41·8)	15·6±2·5[abc] (11·0–19·6)
Time constant of first exponential fit τ_1 (s)	2·8±2·3 (0·5–8·0)	1·4±0·4 (1·0–2·0)	2·5±2·1 (0·5–8·0)	3·7±4·4 (0·7–16·7)
Time constant of second exponential fit τ_2 (s)	137·4±92·6[d] (17·4–302·8)	103·9±131·3[e] (27·6–299·6)	130·7±98·4[f] (17·4–302·8)	750·0±402·4[def] (160·5–1530·6)

Values are means±S.D. (range). The sample includes all thirty-two motoneurons that fired repetitively for > 10 s in response to sustained extracellular current and displayed late adaptation (i.e. had a positive τ_2 value). Two S units did not display any late adaptation and were not included in the values reported in this table. The τ_1 values were obtained from double exponential fits of the total. Superscript letters indicate significant differences between the various cell groups: [a]FF > S, [b]FR > S, [c]F > S, [d]FF < S, [e]FR < S, [f]F < S. Values for K_1 and K_2 are not presented, even though they were both necessary to determine the above-mentioned parameters. The biophysical and physiological meaning of these values is too obscure at this time to warrant discussion.

relation to previous literature on allied measurements. Five of the properties presented in this table are of biophysical rather than physiological interest, i.e. the threshold current for sustained firing, the time to the onset of firing, the initial firing frequency, the time to reach peak firing frequency, and its time constant (τ_1). They reflect events associated with the extracellular activation of nerve cells in a non-homogeneous volume conductor (i.e. the mammalian spinal cord; cf. Ranck, 1979).

The threshold current for sustained firing. As stated above, the mean magnitude of the current required to provoke sustained firing in the cells for > 2 s was not significantly different for F and S cells. At first glance, this finding was surprising given that previous work has shown that the surface area of the soma and dendrites of these two cell types differ significantly, S cells being smaller, and with a

significantly lower rheobase (reviewed in Stuart & Enoka, 1983). The similarities in threshold are presumably related to the way in which the neurons are activated by extracellular current (see Discussion), and to the varied distances between the extracellular electrode tip and the cell.

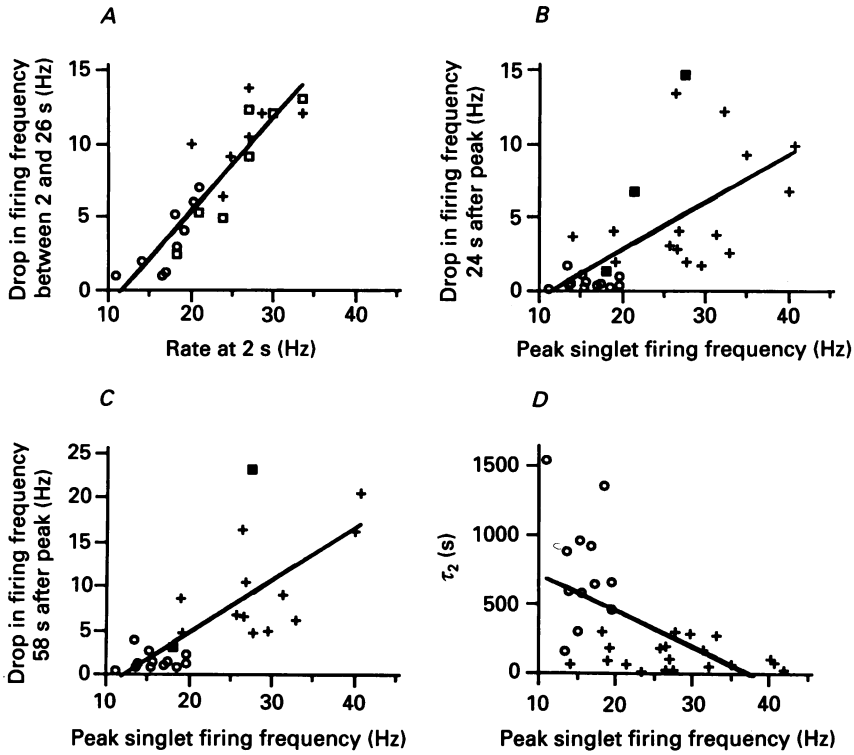

Fig. 7. Effect of peak firing frequency on late adaptation with sustained activation. *A*, data from Kernell & Monster (1982*b*) replotted to show mean firing frequency in the 2nd second of intracellular current injection *vs.* the change in mean frequency at 26 s. There was a significant correlation between the firing rate at 2 s and the extent of adaptation ($r = 0{\cdot}92$, $n = 23$, $P < 0{\cdot}001$). Significant correlations were also found for the S cells alone ($r = 0{\cdot}79$, $n = 9$, $P < 0{\cdot}05$) and for the F cells alone ($r = 0{\cdot}78$, $n = 14$, $P < 0{\cdot}05$). *B* and *D*, significant correlations among the present data. *B*, peak singlet firing frequency (PSFF) *vs.* the change in frequency 24 s after peak (as measured from the fitted double exponential curve; $r = 0{\cdot}63$, $n = 30$, $P < 0{\cdot}001$). *C*, PSFF *vs.* the change in rate 58 s after peak ($r = 0{\cdot}75$, $n = 26$, $P < 0{\cdot}001$). In both *B* and *C*, significant correlations were obtained when the population of cells was considered as a whole and not within individual cell types. *D*, PSFF *vs.* τ_2 ($r = -0{\cdot}57$, $n = 32$, $P < 0{\cdot}001$). Regardless of the method used to quantify late adaptation (*B–D*), there were significant correlations between the PSFF and the magnitude of the adaptation; a finding similar in many respects to the results obtained with intracellular current injection (*A*). Symbols for *A–D*: +, FF; ■, FR; ○, S.

Time to the onset of firing. Cells did not begin firing immediately following the onset of the sustained extracellular current pulse. The mean delay from current onset to the first spike was not significantly different for F and S cells.

The initial firing frequency. Recall that this value was obtained from the *Y*-intercept of the exponential fit. The mean value differed significantly for the F and S cells. Stimulating currents of relatively similar strength produced initial firing frequencies in F cells that were nearly *double* those in S cells when set at 1·25 × the threshold for sustained firing.

The time to peak singlet firing frequency. The mean time required to reach peak singlet firing frequency following the onset of spiking was significantly shorter for the F as compared to the S cells. This may simply reflect the shorter τ_2 values of the F cell population, since the time to peak will be influenced by both τ_1 and τ_2.

The time constant for accelerated firing (τ_1). The mean of τ_1 was not significantly different for the F and S cells, as if to suggest that the time to peak firing difference, while significant, was not a striking one.

The two remaining properties in Table 2, the peak singlet firing frequency, and the time constant of late adaptation (τ_2), have both biophysical and physiological implications, the latter particularly in relation to Henneman's size principle (see, for example, several chapters in Binder & Mendell, 1990, in particular, those of Kernell and Rall).

The peak singlet firing frequency. This value was obtained from the peak of the double exponential fit of singlet discharge. Its mean value also differed significantly for the F and S cells. As with the initial firing frequency, the F cells reached peak singlet firing frequencies that were, on average, almost twice those of S cells responding to extracellular stimulation of comparable strength. Interestingly, the ranges of the peak singlet firing frequency values in Table 2 for F and S cells (14–42 Hz and 11–20 Hz, respectively) span the range in the Kernell and Monster (1982*a*, *b*) study, using the intracellular route of stimulation. In their study, stimulus strength was set at 5 or 10 nA above the threshold for rhythmic firing. For stimulation at 5 nA above threshold, they obtained ranges of 18–33 Hz and 11–22 Hz for F and S cells, respectively (Kernell & Monster, 1982*b*; their Fig. 4). These data suggest that the present use of an extracellular stimulus strength set at 1·25 × the threshold for sustained firing provided a strength of activation quite similar to an intracellular setting of 5 nA above the same threshold.

The time constant of late adaptation. The τ_2 values of Table 2 are thought to quantify late adaptation because the profiles of the firing frequency reduction are so similar to those reported previously by Kernell & Monster (1982*a*, *b*) when using intracellular stimulation to activate the cells. For the present sample, the mean τ_2 value was significantly different for F and S cells.

Firing rate effects on late adaptation

The adaptive properties of motoneurons has been previously quantified by measuring the drop in firing frequency from the 2nd to the 26th seconds of firing (1 s averages; Kernell & Monster, 1982*a*, *b*), and we performed a similar analysis on our data (Fig. 7). In the present study, peak singlet firing frequency was considered analogous to the Kernell & Monster (1982*b*) measure of firing frequency at 2 s (see above). It could be argued that the present data could have been analysed in a manner entirely identical to that of Kernell & Monster (1982*a*, *b*; i.e. mean firing frequencies averaged over 1 s epochs). However, the present strategy was to restrict

this component of the analysis to singlet discharge, because there was no mention of doublet discharges in the Kernell & Monster (1982*a*, *b*) work. In the comparison of sustained *vs.* intermittent activation, we present an analysis of the data which includes doublets (Fig. 11), which also supports the present conclusions.

Figure 7*A* is data replotted from the intracellular work of Kernell & Monster (1982*b*). It shows a correlation between the firing frequency at 2 s and the drop in firing frequency between the 2nd and 26th seconds of discharge. Similar results obtained in the present extracellular study are shown in Fig. 7*B*. Here, as in Fig. 7*A*, there was a significant relationship between the peak singlet firing frequency and the drop in firing frequency measured 24 s after the peak.

Figure 7*C* shows the significant association between peak singlet firing frequency and the drop in firing frequency 58 s later (present study). The correlation was tighter in Fig. 7*C* compared to Fig. 7*B* ($r = 0{\cdot}60$ *vs.* 0·75), probably due to the exclusion of four neurons in the latter correlation because they failed to fire for 60 s. The significant correlations reported in Fig. 7*B* and *C* were not present when the F and S cells were considered separately.

A significant negative correlation was found in Fig. 7*D* between the peak singlet firing frequency and the time constant for late adaptation (τ_2). When the individual populations of F and S cells were considered separately, no significant associations were found.

In summary, the present work confirms the previous finding of Kernell & Monster (1982*a*) that the magnitude of late adaptation is strongly correlated to the firing frequency at the onset of the late adaptation process.

Size principle issues

The relationship between various motor unit type (size)-related measurements and firing rate parameters are shown in Fig. 8. Significant correlations were found for the full population of cells (i.e. F+S) between axonal conduction velocity and τ_2 (Fig. 8*A*, upper panel), and between conduction velocity and peak singlet firing frequency (Fig. 8*A*, lower panel, thick line). There was also a significant correlation between conduction velocity and peak singlet firing frequency for the S cells alone (Fig. 8*A*, lower panel, thin line).

Significant correlations were found between motor unit twitch contraction time and τ_2 (Fig. 8*B*, upper panel), and between contraction time and peak singlet firing frequency (Fig. 8*B*, lower panel). Contraction time was also significantly correlated with τ_2 for the S cells alone. This regression line ($n = 12$; $r = 0{\cdot}75$) was nearly identical to that reported for the full population, and for this reason, was omitted from Fig. 8*B* (upper panel).

Peak tetanic force was inversely related to τ_2 (Fig. 8*C*, upper panel) and positively correlated with peak singlet firing frequency (Fig. 8*C*, lower panel) when the entire population was considered. However, these correlations were not present when the individual cell types were considered separately.

Of the three motor unit parameters significantly associated with τ_2 contraction time appeared to be the best predictor (Fig. 8*B*, upper panel). Peak singlet firing frequency showed similar correlations regardless of the motor unit parameter with which it was compared.

Population adaptation responses with sustained activation: F vs. S cells

Based on the late adaptation (τ_2) measurements reported in this study, it is clear that the F cells exhibited more adaptation during sustained activation than did the S cells. There was a corresponding difference in the duration of firing in response to

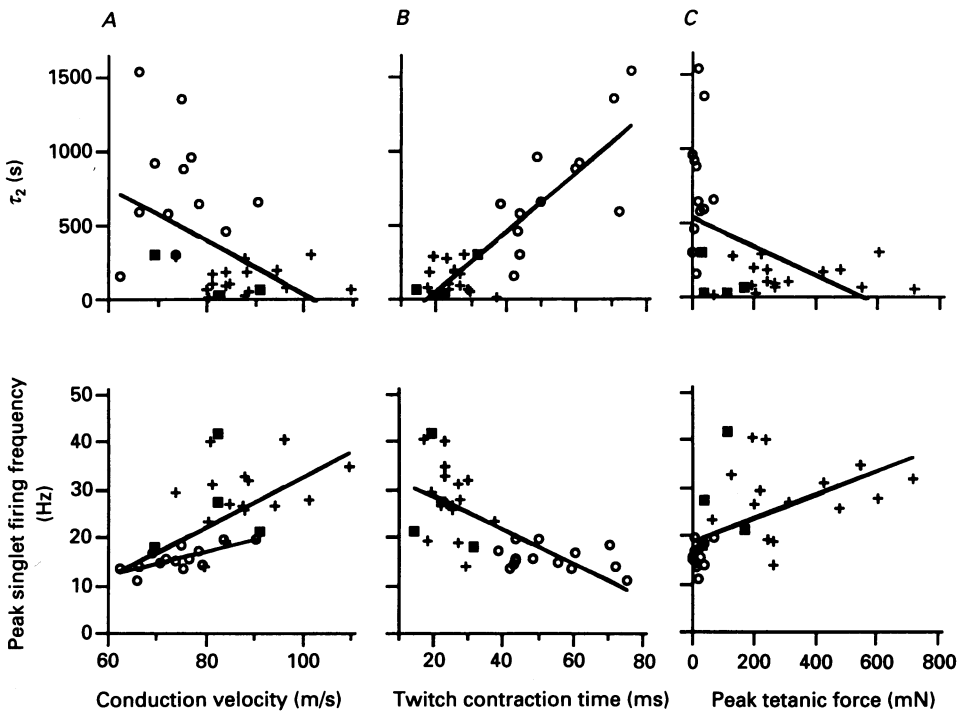

Fig. 8. Significant associations between size-related properties and motoneuron firing characteristics during sustained extracellular stimulation. *A*, upper panel, axonal conduction velocity (CV) *vs.* τ_2, the time constant for late adaptation ($n = 32$, $r = -0{\cdot}48$, $P < 0{\cdot}01$). *A*, lower panel, CV *vs.* peak singlet firing frequency, PSFF ($n = 34$, $r = 0{\cdot}62$, $P < 0{\cdot}001$). The lower regression line is for S cells when considered separately ($n = 14$, $r = 0{\cdot}73$, $P < 0{\cdot}005$). *B*, upper panel, twitch contraction time (CT) *vs.* time constant for late adaptation (τ_2; $n = 32$, $r = 0{\cdot}87$, $P < 0{\cdot}001$). The regression line was quite similar to that for the S cells when they were considered separately ($n = 12$, $r = 0{\cdot}75$, $P < 0{\cdot}05$). *B*, lower panel, CT *vs.* PSFF ($n = 34$, $r = 0{\cdot}69$, $P < 0{\cdot}001$). *C*, upper panel, peak tetanic force *vs.* τ_2 ($n = 32$, $r = -0{\cdot}48$, $P < 0{\cdot}005$). *C*, lower panel, peak tetanic force *vs.* PSFF ($n = 34$, $r = 0{\cdot}58$, $P < 0{\cdot}001$). Note that both PSFF and τ_2 showed significant relationships to the size-related properties: conduction velocity, twitch contraction time, and peak tetanic force. Symbols: FF, +; FR, ■; S, ○.

sustained activation, with S cells more likely to discharge repetitively for the entire 240 s of stimulation. These two interrelated features of cell discharge in response to a constant-intensity stimulus have important consequences for the contribution of F and S cell populations to muscle activity under conditions of sustained excitation. In order to provide a graphic illustration of the difference, a population procedure was

adopted. The mean firing frequency (i.e. inverse of the mean interval, including doublets) for each motoneuron was calculated over 1 s epochs for its entire firing duration. The motoneurons were grouped according to type (F or S), and the mean firing frequencies of all motoneurons of a particular type were summed for each 1 s epoch. This value was divided by the number of motoneurons in each sample (20 F, 14 S). This procedure provided the ensemble mean firing frequency of the F and S cell populations for each second of the 240 s period of stimulation. Some cells ceased firing before the end of the test, of course. In this case, they contributed no spikes to the summed frequency for each subsequent epoch, and the ensemble mean was still normalized to the original number of units. This approach was adopted because the intention was to illustrate a functional point, that is, the relative contribution of the different cell populations to muscle activation when the neurons receive a constant excitatory drive. The result of this procedure is shown in Fig. 9*A*. The mean response of the F cells (thick line) increased from 22 to 31 Hz in the first 5 s of firing. From this point, the mean showed a much more rapid decay than the S cell population mean, reducing to 2 Hz at 234 s following the onset of firing. In contrast, the mean for the S cells (thin line) increased from 12 Hz at the onset of firing to a peak of 22·5 Hz at 10 s, followed by a slow decay, still being 17 Hz at the end of the stimulation period.

For the type S population, the curve in Fig. 9*A* is predominantly influenced by changes in firing frequencies of the active cells; only two S cells ceased firing before the end of stimulation (at 175 and 210 s, respectively). In contrast, the F cell mean was influenced by the reduction in the number of active cells, in addition to firing frequency changes in the individual cells. To illustrate this point, the normalized F cell ensemble mean firing frequency is plotted *vs.* time in Fig. 9*B*, with the proportion of active F cells in each 1 s epoch indicated by the dashed line. In the absence of adaptation, the normalized ensemble mean frequency would overlie the plot of the proportion of active cells. The actual ensemble mean frequency curve was below the curve showing the proportion of active cells at all time points following the peak, and the difference between the two plots illustrates the added importance of adaptation for the population response. In the first 60 s, the normalized ensemble firing frequency declined more steeply than the proportion of active cells, indicating that adaptation effects strongly influenced the ensemble mean in the initial stages. Beyond the 60 s time point, the ensemble mean and the proportion of active cells declined roughly in parallel.

The increased susceptibility of the F cell population to adaptation and cessation of firing under conditions of constant excitation is evident from the Fig. 9 analysis. Over the 240 s of stimulation, the normalized contribution of the F cells declined from a peak of 31 Hz per motoneuron to 2 Hz per motoneuron, a 94% reduction in the ensemble firing frequency (Fig. 9*A*). In other words, the F motor unit population could only make approximately 6% of its peak contribution to total muscle force after 240 s of continuous activation with a constant excitatory command (disregarding frequency–force non-linearities and the effects of contractile fatigue which would reduce this contribution even further). In contrast, the S motoneuron population was much less affected, the reduction in ensemble frequency from the peak was just 24% during the course of the 240 s of stimulation.

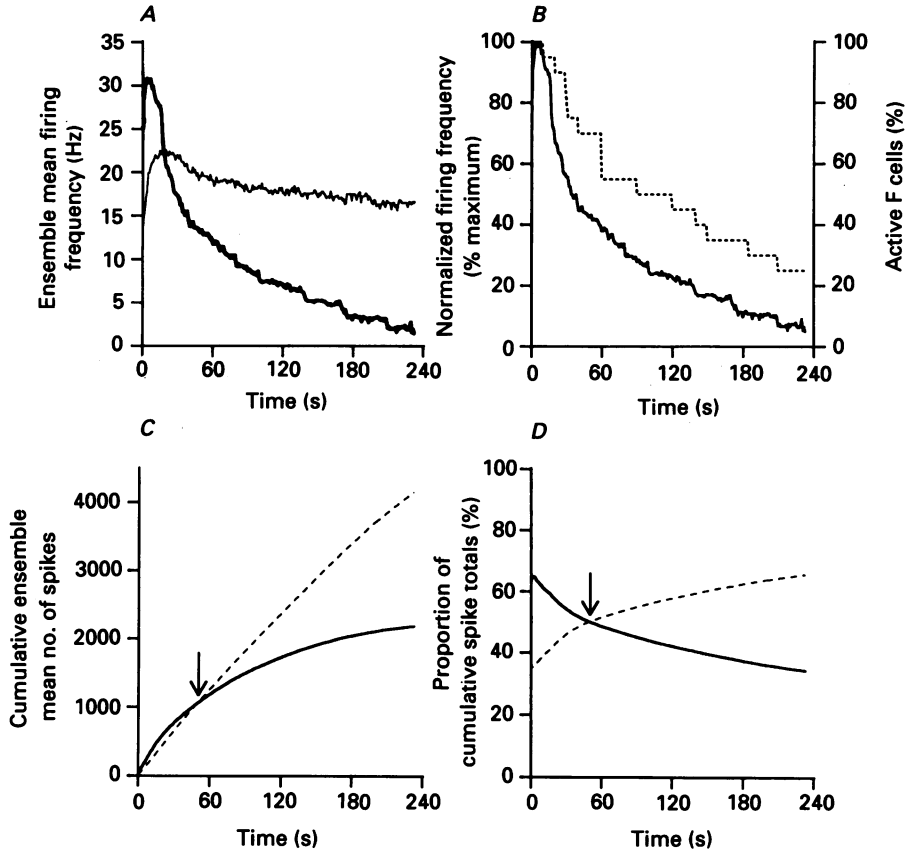

Fig. 9. Population mean responses to sustained extracellular stimulation; F *vs.* S cells. *A*, the ensemble mean firing frequency (calculated over 1 s epochs) plotted against time for F (thick line; $n = 20$) and S (thin line; $n = 14$) cell populations. For each unit contributing to the mean, time is referenced from the onset of spiking in that unit. The record is truncated at 234 s, which corresponds to the minimum period of stimulation received by all motoneurons following the onset of their firing. The mean 1 s values for the two sets of data were significantly different from 2 to 10 s ($P < 0{\cdot}05$; unpaired t test) and from 59 s to the end of the stimulation period (unpaired t test; $P < 0{\cdot}05$). Note that the F cell population mean declined considerably more than the S cell mean. *B*, normalized ensemble mean firing frequency for the F cell population (continuous line) and the proportion of active F cells (dashed line) are plotted *vs.* time. The ensemble mean declines more steeply than the proportion of active cells in the first 60 s, indicating the importance of adaptation for the population response in this period. Beyond this time point, the two curves decline in parallel. *C*, cumulative ensemble mean number of spikes *vs.* time. F cell population, continuous line; S cell population, dashed line. *D*, proportion of cumulative total number of spikes produced by the two cell types at times following the onset of spiking. F cell population, continuous line; S cell population, dashed line. In *C* and *D*, note that the relative contribution of S cells to the total number of spikes increased with longer durations of stimulation.

Another way to view the contribution of each cell type is to consider the total number of spikes produced by each type at times following the onset of spiking. In Fig. 9*C*, the cumulative ensemble mean number of spikes produced by the two cell

types is plotted *vs.* time (i.e. the integrals of curves in Fig. 9*A*). At time points less than 51 s (arrow), the F cell population (continuous line) had produced the greater number of spikes. From this point until the end of stimulation, the S cell population (dashed line) was superior to the F cells in terms of the total number of spikes produced in response to the constant-intensity stimulus. The proportion contributed by each cell type to the cumulative number of spikes at times following the onset of spiking is shown in Fig. 9*D*. The relative S cell contribution (dashed line) increased as the duration of stimulation increased, from 37% of total spikes after 1 s, to 50% at 51 s, and finally 66% at 234 s.

Intermittent stimulation

The results are based on analysis of thirty-two motoneurons (14 FF, 4 FR, and 14 S) supplying the medial gastrocnemius muscle. Thirty of these motoneurons (17 F, 13 S) were from the sample studied with sustained activation. Two additional cells (1 F, 1 S), studied only with intermittent activation, are included in the present sample.

Motoneuron response during the 240 s course of intermittent stimulation

Typical results from two cells that discharged for 240 s are shown in the raster plots of Fig. 10. Each of these cells discharged quite consistently and showed remarkably little change over time in either mean firing frequency per train or the number of spikes per train. Note the consistency of the firing time of the first spike following stimulus onset in both cells. Also note that the cell discharge was confined to the 600 ms period in which the stimulating current was applied each second.

Threshold current for repetitive firing and delay in firing onset: F vs. S cells and intermittent vs. sustained stimulation

The mean ($\pm$S.D.) threshold current to elicit $\geqslant$ two action potentials per train for at least two cycles (s) of stimulation was not significantly different for the F and S cells (578 ± 302 nA *vs.* 588 ± 228 nA). Both sets of values were not significantly different than their counterparts during sustained stimulation (cf. Table 2). As was the case with sustained extracellular stimulation, cells did not begin firing immediately following the onset of stimulation. Several cycles of stimulation were frequently necessary before spiking commenced. The mean delay from current onset to the first spike was $2{\cdot}5 \pm 2{\cdot}2$ s for type F cells, and $3{\cdot}1 \pm 2{\cdot}2$ s for type S cells, an insignificant difference (Student's unpaired t test, $P > 0{\cdot}05$). Both sets of values were not significantly different from their counterparts during sustained stimulation (cf. Table 2). It appears that the delays observed in the onset of spiking were due to a similar mechanism associated with each stimulation protocol, and was not something unique to either one.

Duration of repetitive firing: F vs. S cells and intermittent vs. sustained stimulation

For intermittent stimulation, the mean duration of F and S cell firing was not significantly different (194 ± 69 s *vs.* 231 ± 32 s). This result was in contrast to the mean durations of firing during sustained stimulation, which featured a significantly shorter time for F cell firing. For F cells, the mean firing duration was significantly

longer for intermittent *vs.* sustained firing (194 ± 69 s *vs.* 123 ± 88 s). In contrast, the duration of S cell firing was virtually identical for intermittent *vs.* sustained firing (231 ± 32 s *vs.* 233 ± 19 s).

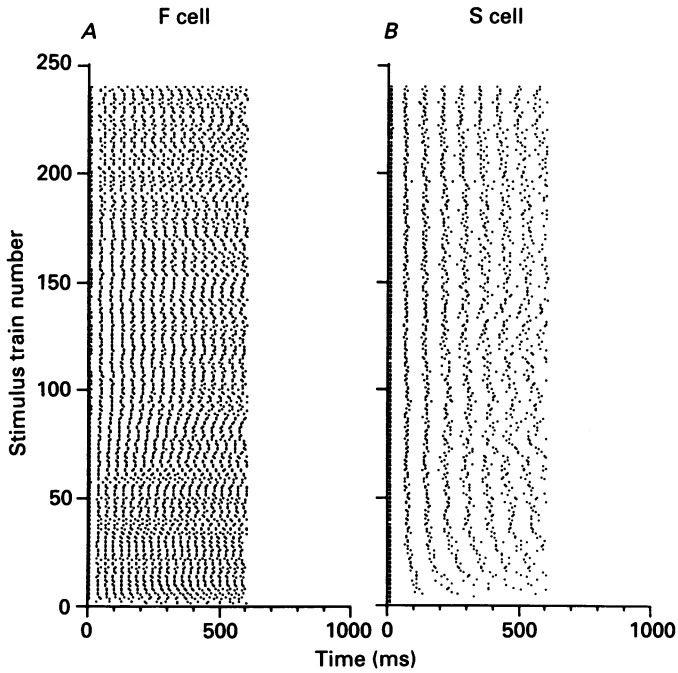

Fig. 10. Raster plots of the discharge for two representative motoneurons during 240 s of intermittent stimulation. Consecutive spike trains are shown (ordinate), with train number increasing from bottom to top. Each dot represents a single EMG compound action potential. The first spike in response to each stimulus cycle was tightly coupled to the onset of the current (delay, *ca* 8 ms). *A*, response of a typical F cell, stimulated with 862 nA. Note a slight decrease in the mean frequency per train during the 240 s course of stimulation. *B*, response of a typical S cell, stimulated with 525 nA. In general, F cells fired faster within each train and tended to show more adaptation between trains than did S cells.

Singlet vs. doublet discharge

Interval histograms were constructed for cells displaying singlet only, and singlet plus doublet discharge, in the same manner as for sustained stimulation (cf. Fig. 6). With intermittent stimulation, doublet intervals comprised 6·8% of total intervals for F cells, and 20·0% for S cells. The relative incidence of doublet discharge in the two cell types was similar to that seen with sustained stimulation. As was the case for sustained activation, no significant difference was found in the mean intervals between the doublet and non-doublet discharging cells for either the F or S cells responding to intermittent activation.

Within-train adaptation

Joint-interval density plots were used to identify within-train adaptation. For each cell, selected intervals (i) in each train were plotted *vs.* the next adjacent interval ($i+1$) for each train of a 240 s stimulation period. Eight such plots were examined for each neuron up to and including the 8th *vs.* the 9th interval of each train. A tendency for the ($i+1$)th interval to be larger than the ith interval was indicative of within-train adaptation, and was evident in the joint-interval density plot as a tendency for points to cluster above the line of identity.

Within-train adaptation, identified with the above method, was observed in ten (5 F, 5 S) of the thirty-two cells. In each case, the phenomenon faded when either the 2nd *vs.* 3rd, or the subsequent joint-interval density plot was examined.

Quantification of between-train adaptation

The term *between-train* adaptation is used here in the context of the term *late* adaptation used in the studies of adaptation during sustained stimulation (Kernell & Monster, 1982*a*, *b*) to allow qualitative description of the entire response profile on a per train basis during the intermittently applied activation. Similarly, Llinás & Lopez-Barneo (1988) used an analogous descriptor, *long-term* adaptation, to describe the decrease in the number of spikes per train during intermittent stimulation. Motoneurons responded to intermittent stimulation with a mean firing frequency per train which increased over the initial stimulation cycles, and then slowly declined from this peak value over the 240 s of stimulation.

Figure 11 shows that a procedure introduced by Kernell & Monster (1982*a*) for quantifying the magnitude of late adaptation to *sustained* stimulation was applicable to the between-train adaptation resulting from *intermittent* stimulation. Figure 11*A* shows the original Kernell & Monster (1982*a*) result: a correlation between the drop in firing frequency between the 2nd and 26th second of sustained discharge, and the firing frequency at 2 s. Similar results from the sustained extracellular stimulation protocol are shown in Fig. 11*B*. In this analysis of our data, doublet intervals have been included in the frequency calculation (cf. Fig. 7*B*) to allow comparison with the data obtained with intermittent activation. Here, as in Fig. 11*A*, there was a significant correlation between the peak firing frequency and the drop in frequency measured 24 s later.

For the between-train adaptation to intermittent stimulation, Fig. 11*C* shows a significant association between the peak firing frequency per train and the drop in frequency from that peak to a lesser value 24 s later. The correlation was also significant for the F and S cells when each group was considered separately. Similarly, there was a significant correlation between the peak firing frequency per train for intermittent stimulation and the drop in firing frequency from that peak to the firing frequency 58 s later (Fig. 11*D*). Again, the association was significant for the F and S cells when considered separately or together. The reduction in mean frequency per train was more pronounced for F *vs.* S cells.

Discharge characteristics of cells that received both sustained and intermittent stimulation

The discharge characteristics of the thirty cells (17 F, 13 S) that received both sustained and intermittent stimulation are of particular interest. Firing frequency was averaged over 1 s epochs for sustained stimulation, and for each 600 ms train for intermittent stimulation. Doublet discharge was included in each case. The results

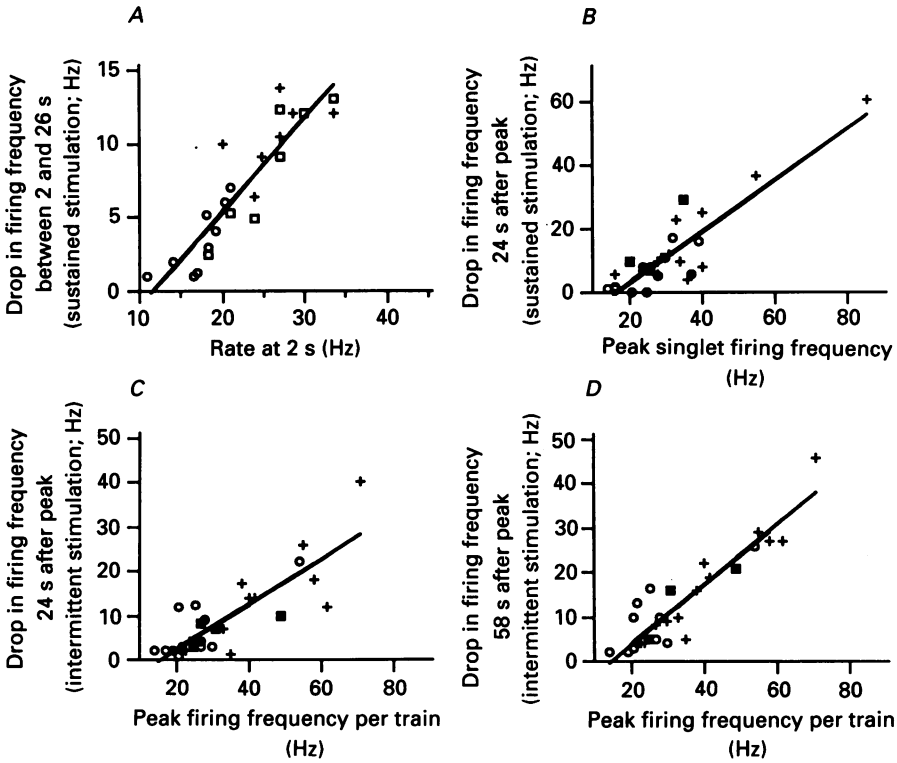

Fig. 11. Effect of peak firing frequency on the extent of motoneuron adaptation: sustained and intermittent stimulation. *A*, data from Kernell & Monster (1982*b*) replotted to show mean firing frequency in the 2nd second of *sustained* intracellular current injection *vs.* the change (drop) in mean frequency at 26 s. There was a significant correlation between the rate at 2 s and the extent of adaptation ($r = 0{\cdot}92$, $n = 23$, $P < 0{\cdot}001$). *B–D*, significant correlations among the present data. *B*, results obtained from the sustained-stimulation protocol (doublets included in frequency calculation), peak singlet firing frequency (PSFF) *vs.* the change in rate 24 s after peak ($r = 0{\cdot}87$, $n = 30$, $P < 0{\cdot}001$). Significant correlations were also found when F ($r = 0{\cdot}87$) and S cell populations ($r = 0{\cdot}76$) were considered separately. *C* and *D*, extent of between train-adaptation for intermittent extracellular stimulation. *C*, peak firing frequency (PFF) *vs.* the change in rate 24 s after peak ($r = 0{\cdot}83$, $n = 30$, $P < 0{\cdot}05$). *D*, PFF *vs.* the change in rate 58 s after peak ($r = 0{\cdot}92$, $n = 28$, $P < 0{\cdot}05$). Both *C* and *D* had significant correlations when the F and S cell populations were considered separately. Regardless of the value (24 or 58 s) used to quantify the extent of adaptation during intermittent stimulation (*C* and *D*), there were significant correlations between the PFF and the magnitude of the adaptation, similar in many respects to the results obtained with either sustained intracellular stimulation (*A*) or sustained extracellular stimulation (*B*). Symbols for *A–D*: +, FF; ■, FR; ○, S.

are summarized for five discharge properties in Table 3. With both sustained and intermittent stimulation, significant differences were generally found in all the measured discharge parameters for F *vs.* S cell comparisons; the only exception was the initial firing frequency with intermittent stimulation. In comparisons of the response of a cell type under the two different stimulation protocols, significant differences were found only for the time-to-peak firing frequency in both F and S cells.

TABLE 3. Discharge characteristics of motoneurons that received both sustained and intermittent stimulation protocols

Cell type	Sustained activation	Intermittent activation	Significant difference (sustained *vs.* intermittent)***
	Initial firing frequency (Hz)		
F	22·0 ± 12·7	17·9 ± 12·6	n.s.
S	12·8 ± 8·4*	11·1 ± 8·9	n.s.
	Time to peak firing frequency (s)		
F	7·9 ± 9·9	19·9 ± 18·1	$P < 0·03$
S	39·0 ± 43·6**	78·3 ± 64·7*	$P < 0·02$
	Peak firing frequency (Hz)		
F	35·9 ± 15·8	39·4 ± 14·2	n.s.
S	25·5 ± 7·8*	26·4 ± 10·1*	n.s.
	Peak to 24 s later drop in firing frequency (Hz)		
F	17·0 ± 15·7	12·0 ± 12·4	n.s.
S	6·3 ± 5·7*	4·3 ± 3·1*	n.s.
	Drop in firing frequency from the peak to end of firing (Hz)		
F	30·8 ± 16·8	25·7 ± 17·3	n.s.
S	11·1 ± 8·4**	7·8 ± 9·7**	n.s.

* Denotes significant difference F *vs.* S (unpaired *t* test, $P < 0·05$).
** Denotes significant difference F *vs.* S (unpaired *t* test, $P < 0·01$).
*** Paired *t* test; n.s. denotes not significant ($P > 0.05$).

Intermittent activation: relationship between motoneuron and motor unit properties

Associations were sought between two *active* motoneuron properties revealed with intermittent activation, peak firing frequency per train and the magnitude of between-train adaptation, and three type (size)-related properties of the motor units: axonal conduction velocity, motor unit twitch contraction time and peak tetanic force.

Peak firing frequency per train. As with the peak singlet firing frequency with sustained activation, peak firing frequency per train was significantly correlated with three type (size)-related motor unit properties. When the full (F + S) population was considered, a significant positive correlation was found between peak firing frequency per train and axonal conduction velocity ($n = 32$, $r = 0·47$, $P < 0·05$). A significant

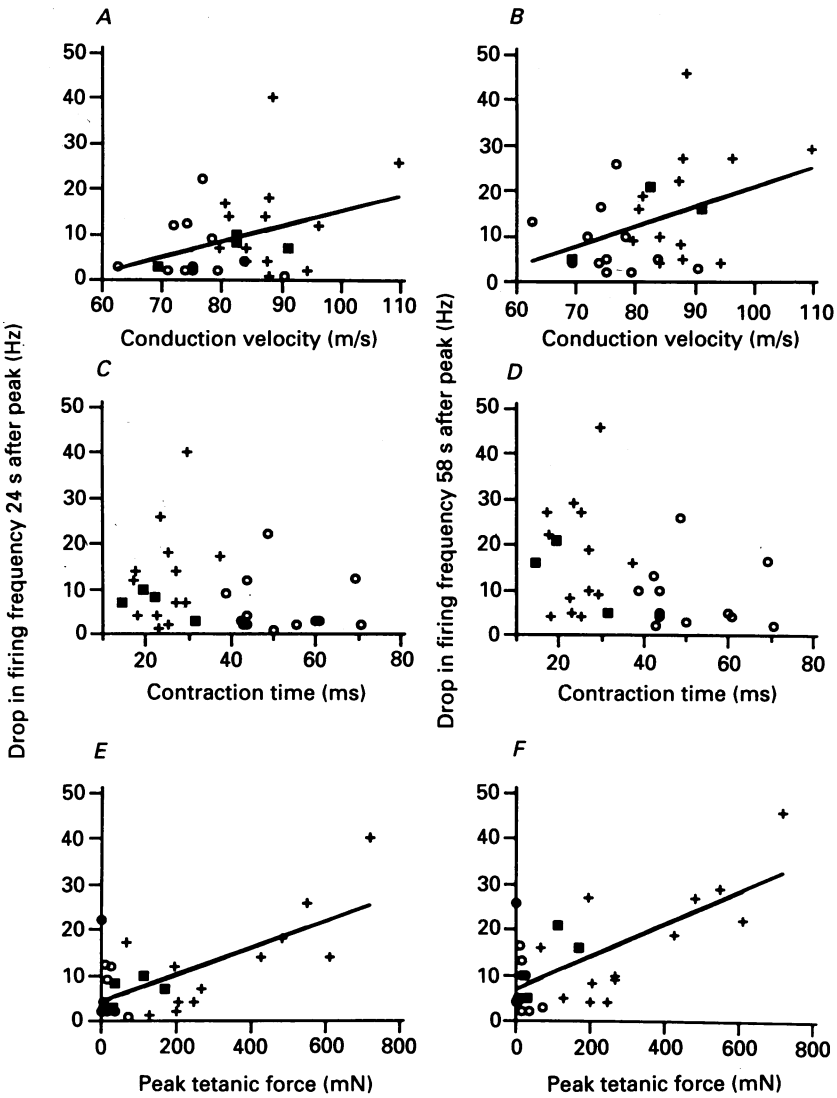

Fig. 12. Associations between the extent of between-train adaptation to intermittent stimulation and other type (size)-related motor unit properties. *A*, axonal conduction velocity (CV) *vs.* drop in firing frequency from its peak value to 24 s later (24 s drop; $n = 30$, $r = 0{\cdot}37$, $P < 0{\cdot}05$). *B*, CV *vs.* drop in firing frequency from its peak value to 58 s later (58 s drop; $n = 28$, $r = 0{\cdot}40$, $P < 0{\cdot}05$). *C*, contraction time (CT) *vs.* 24 s drop ($n = 30$, $r = 0{\cdot}22$, $P > 0{\cdot}05$). *D*, CT *vs.* 58 s drop ($n = 28$, $r = 0{\cdot}35$, $P > 0{\cdot}05$). Note that neither *C* nor *D* showed significant correlations between CT and the extent of adaptation. *E*, peak tetanic force *vs.* 24 s drop ($n = 30$, $r = 0{\cdot}67$, $P < 0{\cdot}05$). The regression line (not shown) was quite similar to that for the F cells when they were considered separately ($n = 17$, $r = 0{\cdot}75$, $P < 0{\cdot}05$). *F*, peak tetanic force *vs.* 58 s drop ($n = 28$, $r = 0{\cdot}69$, $P < 0{\cdot}05$). A similar regression line was observed for the F cells ($n = 16$, $r = 0{\cdot}73$, $P < 0{\cdot}05$). Symbols: +, FF; ■, FR; ○, S.

negative correlation was found between peak firing frequency per train and twitch contraction time ($n = 32$, $r = -0{\cdot}41$, $P < 0{\cdot}05$), and a significant positive correlation between peak firing frequency per train and peak tetanic force ($n = 32$, $r = 0{\cdot}66$,

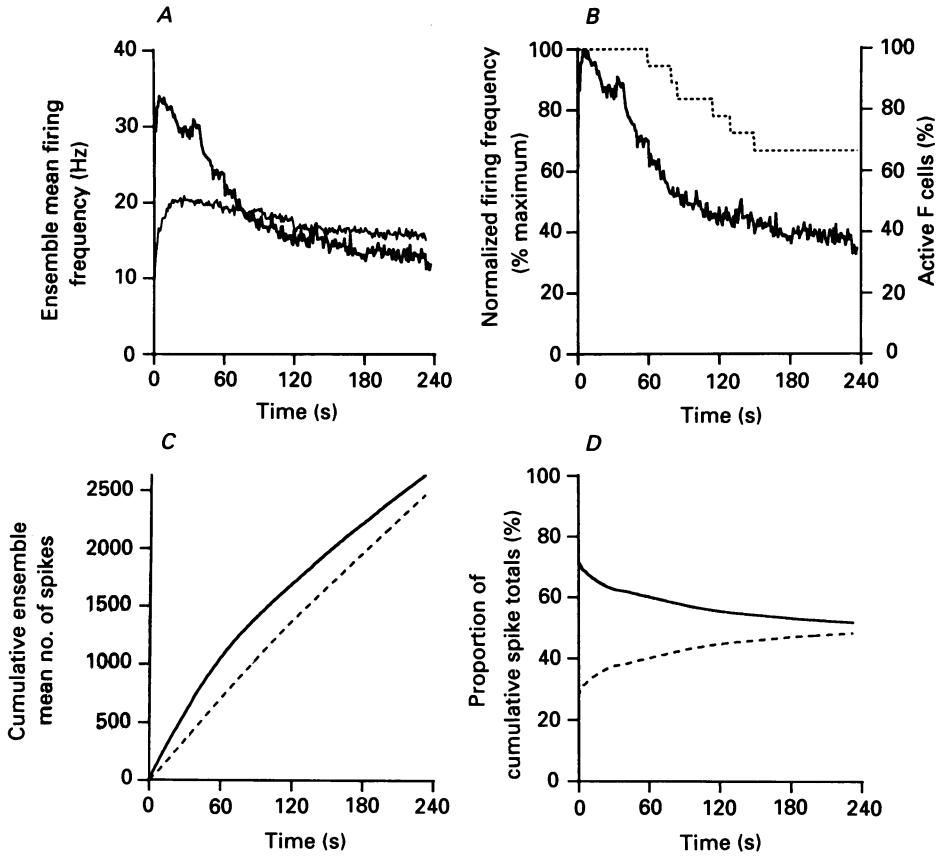

Fig. 13. Population mean responses to intermittent stimulation; F *vs.* S cells. *A*, mean ensemble frequencies (averaged over 1 s epochs) for all motoneurons of the same type (F, $n = 18$, thick line; or S, $n = 14$, thin line) plotted for 240 s of intermittent stimulation. *B*, normalized ensemble mean firing frequencies for the F cell population (continuous line) and the proportion of active F cells (dashed line) are plotted *vs.* time. *C*, cumulative ensemble mean number of spikes *vs.* time. F cell population, continuous line; S cell population, dashed line. *D*, proportion of cumulative total number of spikes produced by the two cell types at times following the onset of spiking. F cell population, continuous line; S cell population, dashed line. In *C* and *D*, at all time points, the relative contribution of F cells to the total number of spikes produced exceeded that of S cells.

$P < 0{\cdot}05$). The latter correlation was also significant when the F cells were considered separately ($n = 18$, $r = 0{\cdot}62$, $P < 0{\cdot}05$).

Magnitude of between-train adaptation. Relationships for between-train adaptation and various motor unit type (size)-related measurements are shown in Fig. 12. Significant correlations were observed between the extent of adaptation (from either

the peak firing frequency per train to 24 s later (Fig. 12*A*), or the peak to 58 s later, (Fig. 12*B*) and conduction velocity, when the full population (F+S) was used. However, no significant correlations were observed when the two cell types were considered separately. A significant correlation was not found between the extent of adaptation (measured at 24 s, Fig. 12*C*; or 58 s, Fig. 12*D*) and contraction time. However, there appeared to be a tendency for the data in Fig. 12*D* to show an inverse association between the drop in frequency from peak to 58 s later and contraction time, as one might predict based on present knowledge of motoneuron type (size)-related properties (cf. Kernell & Monster, 1982*b*). Significant correlations were found between the extent of adaptation (measured at 24 s, Fig. 12*E*; or 58 s, Fig. 2*F*) and peak tetanic force. In Fig. 2*E* and *F*, significant correlations were present for both the entire population and the F cells alone.

Population adaptation responses with intermittent activation: F vs. S cells

We have shown that between-train adaptation was greater in F than S cells. In contrast to the situation with sustained activation, the duration of discharge of the two cell types was not significantly different with intermittent stimulation. In order to illustrate the contribution of the two cell types to muscle activation (neural drive) over the entire period of stimulation under conditions of constant-intensity intermittent stimulation, we used the population ensemble mean previously described (cf. Fig. 9).

The results of this procedure for the population averages for intermittent stimulation are shown in Fig. 13*A*. The firing frequency was averaged (including doublets) over each 600 ms train. The mean response of the F cells (thick line) increased from 29 to 33 Hz per train in the first 5 s of spiking, and, from this point, showed a relatively rapid decay to a final mean firing frequency of 12 Hz/train. In contrast, the ensemble mean for the S cells (thin line) increased from 12 Hz/train at the onset of spiking to a peak of 20 Hz/train at 25 s. This was followed by a slow decay. However, the ensemble mean firing frequency for the S cells was still 16 Hz/train at 240 s. In further distinction between the F and S cells, the firing frequency per train of the F cell ensemble mean was significantly greater than that for the S cell mean during the first 40 s of stimulation (unpaired *t* test, $P < 0{\cdot}05$), after which point the means were not significantly different.

For the type S population the curve in Fig. 13*A* is predominantly influenced by changes in firing frequencies of the active cells; only one S cell ceased firing before the end of stimulation (at 120 s). In contrast, the F cell mean was influenced by the reduction in the number of active cells, in addition to firing frequency changes in the individual cells. The normalized F cell ensemble mean firing frequency is plotted *vs.* time in Fig. 13*B*, with the proportion of active F cells in each 1 s epoch indicated by the dashed line. In the first 60 s, all cells were active, yet the normalized ensemble firing frequency declined by 35% from the peak values, indicating the importance of adaptation in this period. At later time points, the ensemble mean is influenced by cessation of discharge in some cells, yet at all points the decline in the ensemble mean was steeper than the reduction in the number of active cells, indicating that reduction in individual cell firing frequencies continued to have an important effect on the population response throughout the 4 min period of stimulation.

These analyses provided a contrast in the adaptive behaviour of F *vs.* S cells. over the 240 s course of intermittent stimulation, the normalized contribution of the F cells declined from a peak of 33 Hz/cell to 12 Hz/cell, a 64% reduction in the ensemble firing frequency (Fig. 13*A*). In other words, the F motor unit population

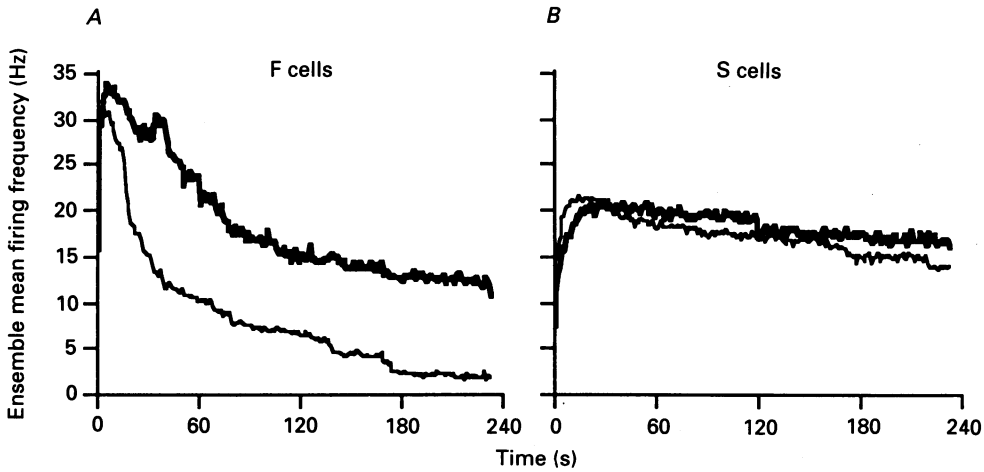

Fig. 14. Comparison between population mean responses during intermittent and sustained stimulation; F *vs.* S cells. The ensemble mean firing frequency (averaged over 600 ms for intermittent stimulation and 1 s epochs for sustained stimulation, including doublets) for F and S cells were averaged and plotted *vs.* time for both intermittent and sustained stimulation. For each cell contributing to the mean, time was referenced from the onset of spiking in that unit. The records were truncated at 234 s, which corresponds to the minimum stimulation time received by all cells following their onset of spiking. *A* and *B*, thick lines correspond to data from intermittent stimulation; the thin lines correspond to data from sustained stimulation. *A*, ensemble mean of F cells ($n = 17$). The mean values for the two sets of data were significantly different from 17 to 234 s of discharge (paired t test, $P < 0{\cdot}05$). *B*, ensemble mean of S cells ($n = 13$); their ensemble mean firing rate profiles were not significantly different at any point during the 240 s period of stimulation (paired t test, $P > 0{\cdot}05$).

could only make approximately 36% of its original contribution to total muscle force after 240 s of intermittent stimulation (disregarding non-linearities in the stimulus frequency–force relationship and the effects of contractile fatigue which would reduce this contribution even further). In contrast, the S cell population was much less affected; the reduction in ensemble firing frequency per train from the peak was 20% over the same 240 s period of stimulation.

An interesting finding emerges when the population responses of the F and S cells are compared from the perspective of their contribution to the total number of spikes produced during constant-intensity, intermittent stimulation (Fig. 13*C*). At all time points, the cumulative total number of spikes produced by the F cell population (continuous line) was higher than that of the S cells (dashed line). The proportion contributed by each cell type to the cumulative number of spikes at times following the onset of spiking is shown in Fig. 13*D*. The relative F cell contribution (continuous line) decreased as the duration of stimulation increased, from 70% of total spikes

after 1 s, to 52% at 234 s, yet at all time points the relative contribution of F cells remained higher than that of S cells.

Comparison of intermittent vs. sustained stimulation: F and S cell populations

The effects of intermittent *vs.* sustained stimulation were compared for the populations of F and S cells. In Fig. 14, the ensemble mean firing frequency for the F (Fig. 14*A*) and S (Fig. 14*B*) cells are shown for both intermittent (thick lines) and sustained (thin lines) extracellular stimulation. The F cells were much less susceptible to the combined effects of adaptation and cessation of discharge with intermittent stimulation: their ensemble mean firing frequency was significantly higher from 17 s to the end of the 240 s stimulation period for intermittent *vs.* sustained stimulation. In sharp contrast, the ensemble mean firing frequencies of the type S neurons (Fig. 14*B*) were not significantly different with either stimulation protocol at any point during the same 240 s period of stimulation.

A double exponential equation was fitted to the ensemble responses for F and S cells activated with intermittent and sustained stimulation. For the population ensemble responses, the τ_2' values provide an index of mean cell activity which is a composite of reduction in firing frequency (adaptation) and cessation of firing. The τ_2' values from the intermittent data were greater for both F and S cells compared to the values obtained from the sustained stimulation data (F, 170 *vs.* 60 s; S, 704 *vs.* 492 s). In contrast, the τ_1' values for both F and S cells were very similar for both stimulation protocols (F, 2·2 *vs.* 2·0 s; S, 5·0 *vs.* 3·7 s). The τ_1' values essentially quantify the initial increase in firing frequency of the cells responding to extracellular activation, as cessation of firing was not a factor in the first few seconds of activity.

Cumulative after-effects of spike production in adaptation

The experiments offered the opportunity of comparing the adaptation of the same cell in response to two different stimulation protocols. We were interested in evaluating the importance of the cumulative after-effects of spike production on adaption. The total number of spikes produced by the cell accumulated at a slower rate with intermittent activation because of the 400 ms 'off' time per cycle of stimulation. If adaptation were simply a function of the cumulative after-effects of the number of spikes produced (irrespective of the rate of accumulation of spikes), the drop in frequency with sustained and intermittent stimulation should be similar after an equal number of spikes had been produced in each case. That is, the plot of firing frequency *vs.* number of spikes should be similar for the same cell responding to sustained *vs.* intermittent stimulation. Twelve type F cells and seven type S cells were chosen for comparison of adaptation under each stimulation protocol. The criteria for inclusion in this analysis were: (a) the cell received both the sustained and intermittent stimulation protocol), (b) their spiking duration was > 24 s following the attainment of the peak firing frequency for sustained stimulation, and (c) there were no large discrepancies in the proportion of doublet discharge under the two conditions (which would have distorted the comparisons).

For each cell, the mean firing frequency was calculated (including doublets; averaged over 1 s epochs for sustained activation and 600 ms epochs for intermittent activation) and plotted *vs.* the number of spikes produced. An example of data for

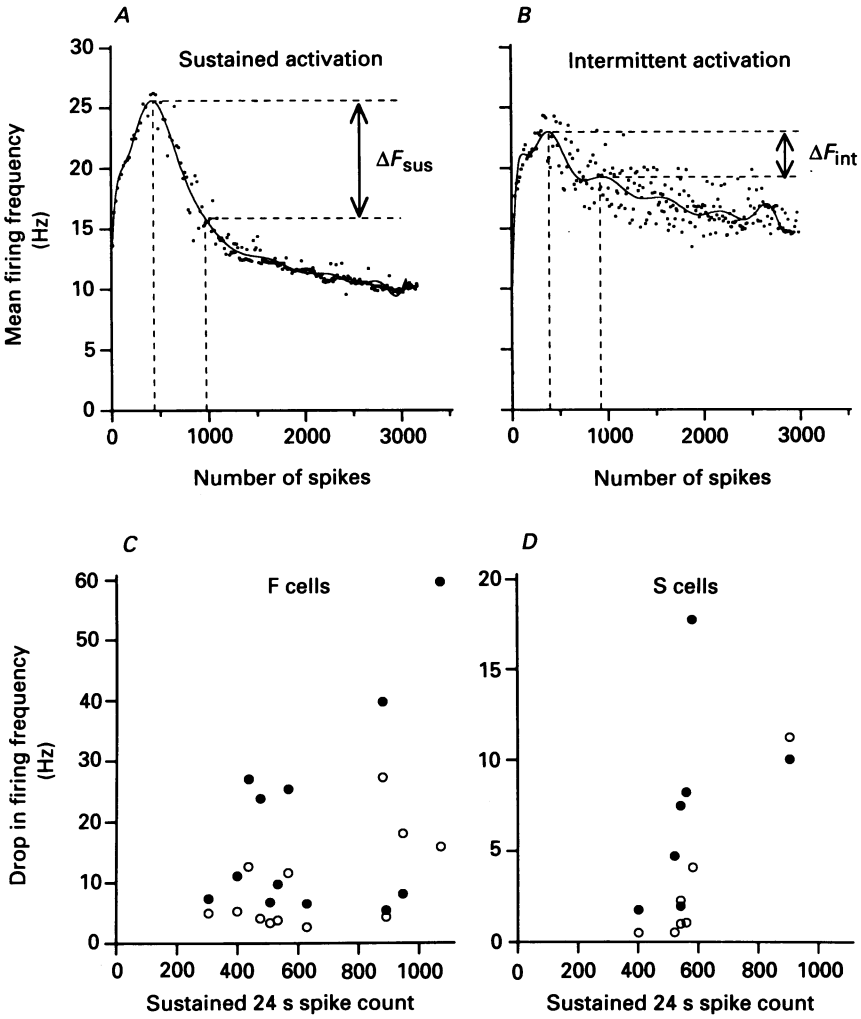

Fig. 15. Effect of the rate of accumulation of spikes on adaptation; sustained *vs.* intermittent stimulation. *A*, mean firing frequency *vs.* the number of spikes produced with sustained stimulation for a representative F cell. The vertical dashed lines indicate the number of spikes produced at the peak frequency, and at the time point 24 s later. In this example, the sustained 24 s spike count was 533 spikes. The data were fitted with a high-order polynomial (continuous line) to interpolate values for comparison. The change in frequency (ΔF_{sus}) from the peak to 533 spikes later was estimated from the fitted curve as 9·7 Hz. *B*, mean firing frequency *vs.* the number of spikes produced with intermittent stimulation for the cell shown in *A*. The change in frequency (ΔF_{int}) from the peak to 533 spikes later was estimated from the fitted curve (continuous line) as 3·7 Hz. This point occurred 39 s after the peak) hence the greater number of data points between the dashed lines in *B*). The drop in firing frequency associated with the production of the 533 spikes after the peak was greater with sustained stimulation. In this panel (and to a lesser extent, panel *A*) note that the continuous line provides a more temporal (wavy) fit of the data points than is evident in earlier figures (e.g. Figs 3 and 5). The difference is attributable to the method of curve fitting: higher-order polynomial in the present figure, and double-exponential in the preceding ones. *C*, drop in firing frequency *vs.* the sustained 24 s spike count for twelve F cells. Each cell has a data point for sustained (ΔF_{sus}; ●) and intermittent stimulation (ΔF_{int}; ○) which is plotted *vs.* the corresponding sustained 24 s

the same cell under the two stimulation conditions is presented in Fig. 15*A* and *B*. The data were fitted with a high-order polynomial in order to interpolate values for comparison. Comparison of discharge under the two conditions was performed by first calculating, for the sustained stimulation case (Fig. 15*A*), the peak firing frequency and the change in frequency between this point and the time point 24 s later (ΔF_{sus}). The total number of spikes produced by the cell in this 24 s epoch (the sustained 24 s spike count) was recorded, and this became the reference for comparison of the adaptation under the two conditions. The change in frequency with intermittent stimulation (ΔF_{int}) was calculated from the peak to the later point at which the cell had produced the same number of spikes following the peak as were produced in the sustained 24 s spike count (Fig. 15*B*). The result of this analysis for individual cells is summarized in Fig. 15*C* and *D*. For each cell, ΔF_{sus} (●) and ΔF_{int} (○) are plotted *vs.* the sustained 24 s spike count (which was unique for each cell). For both F (Fig. 15*C*) and S cells (Fig. 15*D*) the usual result was that ΔF_{sus} was larger than ΔF_{int}. The mean reduction in firing frequency of the twelve F cells was 9·7 Hz greater in the sustained than in the intermittent trial. This difference was statistically significant (paired *t* test; $P < 0{\cdot}03$). The mean drop in firing frequency of the seven S cells was 4·5 Hz greater in the sustained trial, but this difference was not significant (paired *t* test; $P < 0{\cdot}05$). These results suggest that for F cells the rate of accumulation of spikes is important for adaptation, in addition to the cumulative number of spikes produced, while this appears to be less important for type S cells.

DISCUSSION

An action potential is initiated by altering the transmembrane potential to a sufficient degree to depolarize the cell to its threshold for firing. The current needed to produce this depolarization may be from an intracellular microelectrode, synaptic events, or extracellular stimulation. In the present study, extracellular stimulation was chosen over the more traditional intracellular technique because it was thought that extracellular stimulation should reduce the possibility of impalement-induced injury associated with the use of intracellular microelectrodes. With regard to this point, the firing threshold of resting spinal motoneurons is particularly sensitive to the intracellular insertion of a microelectrode (Goldberg & Clamann, 1977). For active cells, the repetitive-firing literature has a few brief comments on the possibility that electrode penetration compromises cell firing (e.g. Kernell, 1965*a*; Kernell & Monster, 1982*b*; Schwindt & Crill, 1982). Kernell & Monster (1982*a*) also provided a quantitative demonstration of a significant correlation between the duration of repetitive firing and action potential amplitude, thereby giving rigorous credence to the electrode penetration issue. Several authors have used extracellular stimulation to elicit single, as well as repetitive discharges in motoneurons (Barron & Matthews, 1938; Gustafsson & Jankowska, 1976; for review of the biophysics of extracellular stimulation, see Ranck, 1979). Most germane to the present work is a report of Gustafsson & Jankowska (1976). They investigated the qualitative similarities between intracellular and extracellular motoneuron stimulation, albeit

spike count (i.e. the same number of spikes produced after the peak in each case). *D*, data arranged as in *C* for seven S cells. For most cells in *C* and *D*, the reduction in firing frequency was greater with sustained activation.

for single action potential generation. They concluded that the effects of the extracellular stimuli are exerted primarily via spread of current to the initial segment of the axon and its depolarization (i.e. the *same* mechanism as occurs during intracellular stimulation). Their results provided the key incentive for developing the presently used extracellular stimulation technique.

Mechanisms underlying the initial increase in motoneuron firing during extracellular activation

The initial slow increase in firing frequency to a step change in depolarizing current reported in the present study has not been observed previously during stimulation of motoneurons in the anaesthetized cat. Previous experiments on motoneurons from anaesthetized (intact or spinal) cats have shown that during intracellular stimulation with square current pulses, the initial discharge frequency is high and then very rapidly decreases to a lower firing rate after the first few spikes (i.e. *initial adaptation*; Kernell, 1965*a*). It would appear that the extracellular technique precludes study of initial adaptation, by virtue of the time course of the response to excitatory current of the cells under these conditions.

The reason for the delay between current onset and the first spike, and the slow increase in discharge frequency seen for all cells, with extracellular stimulation is unclear. There will be some delay due to capacitative charging of the extracellular tissue (which includes glial cells, and other neurons), before the entire magnitude of the stimulus becomes available to the test cell (cf. Ranck, 1979). It must be conceded, however, that the time course observed is too slow to be explained by capacitance alone. The variable distance between the electrode and the test cells may evoke an unknown interaction between the stimulus and the spike-generating mechanism of the cell. For example, the extracellular field may activate voltage-sensitive channels, perhaps in the dendrites, that augment depolarization of the initial segment (cf. Chan, Hounsgaard & Nicholson, 1988; Hounsgaard & Kiehn, 1990). Another interesting consideration is the possible role of diffusing microelectrode ions in the relatively slower initial response of the cells to extracellular stimulation. Repetitive firing was elicited with microelectrodes containing potassium citrate, but efforts were largely unsuccessful with electrodes containing sodium chloride. Citrate ions have a high affinity for free calcium, which may have resulted in a lowered extracellular free calcium concentration with stimulation. This would be expected to enhance motoneuron excitability (lower threshold) by effects on the voltage dependence of their sodium permeability.

Extrinsic (i.e. synaptic) effects evoked by the sustained extracellular stimulation were not considered to be of relevance in this study, because the dorsal roots were cut and the animal was deeply anaesthetized. Influence from direct stimulation of interneuronal components was also assumed to be of little consequence since the somata of most interneurons lie outside spinal lamina IX, the area in which we stimulated our test cells. Furthermore, it has never been demonstrated, to our knowledge, that these axons would be activated in a repetitively discharging manner during the application of sustained depolarizing current. Another issue is the number of motoneurons that could possibly be activated with the extracellular technique, and the possibility that their activation might lead to effects on the test cell via the Renshaw cell pathway. We did not assess whether motoneurons in other muscles were being activated during our test, however, based on estimates of cell density (1–2 cells per 100 μm^3; Aitken & Bridger, 1962), the careful positioning of the microelectrode near the site of lowest threshold for each test cell, and the relatively low-

amplitude currents used in the study, it seems unlikely that we activated many, if any, additional motoneurons. We concede that one or two additional motoneurons innervating other muscles *may* have been activated, but it is very unlikely that their activity could have resulted in a significant influence onto our test cell because the combined Renshaw cell influence as a result of activity in a few motoneurons would be insufficient to have any substantial effect in the test motoneuron, particularly in the deeply anaesthetized barbiturate animal (Hamm, Sasaki, Stuart, Windhorst & Yuan, 1987). Another possibility to be considered is primary afferent depolarization (PAD). It is conceivable that some transmitter could be released from presynaptic terminals very close to the electrode tip in response to sustained stimulation at current strengths ($\leqslant 1$ μA) used in the present study (cf. Fig. 1 in Ranck, 1975). However, the release would be transitory, as repetitive firing cannot be evoked in axons by this route, and limited to the arborizations of but a few axons.

Mechanisms underlying the initial and peak firing frequencies measured during sustained stimulation: F vs. S cells

A striking feature of this work was that although the mean values of stimulus strength (at 1·25 × the threshold for sustained firing for 2 s) were similar for F and S cells (Table 2), the F cells fired at *almost double* the rate of S cells, both at the onset of firing and at the time of their peak firing (Table 2). This pronounced difference in firing rate behaviour for the two cell types immediately prompts the question of whether this finding, obtained with extracellular stimulation, can be explained on the basis of previous work, which used intracellular stimulation.

Background information: extra- vs. intracellular activation

Kernell & Monster (1981) have reported different mean threshold intracellular currents for maintained repetitive firing for different cell types (FF, 21 nA; FR, 11 nA; S, 6·5 nA; cf. their Fig. 2). The present failure to demonstrate a significant difference in current strength required to activate F *vs.* S cells with extracellular current is at least partly explained by the complicating effect of uncontrolled distance of the extracellular electrode tip from the cell. Although every attempt was made to optimize electrode position for each cell, it could not have been uniform with the presently used technique. The steep relationship between current and distance with extracellular stimulation (Gustafsson & Jankowska, 1976) means that our threshold current measure is an approximate estimate of the true threshold to extracellular current. Furthermore, in the case of extracellular stimulation, although both cell groups may be subjected to the same current (i.e. similar distance from the electrode tip), the amount of depolarizing current that crosses their cell bodies is determined by differences in size and specific membrane resistance: the exact value is dictated by the combination of these two counteracting effects (Ranck, 1979). An analogy of this argument involves the extra-axonal activation of myelinated axons of large *vs.* small diameter. In this instance, firing threshold varies inversely with axon diameter, the large diameter axons 'see' more current, either on the basis of their size alone (Ranck, 1979), or, in addition, because of complexities of the myelin sheath (cf. Jack, Noble & Tsien, 1975).

Firing frequency: extra- vs. intracellular activation

It had to be recognized at the outset that initial firing frequencies could not be compared for the two routes of activation. Using intracellular stimulation, the initial frequency is subject to an initial adaptation to a *constant* stimulation. In contrast,

using extracellular stimulation, the initial frequency presumably gives an indication of the responsiveness of the cell to a stimulus of *progressive developing* strength rather than a constant strength.

It is none the less intriguing that the initial firing frequency obtained from the *Y*-intercept of the exponential fit (initial firing frequency in Table 2) *differed significantly* for the F and S cells (16·9 ± 6·7 Hz *vs.* 9·6 ± 2·4 Hz). It was almost double for F cells, in a fashion similar to the intracellular study of Kernell & Monster (1982*a*, *b*), i.e. F, 26·3 ± 4·6 Hz *vs.* S, 17·4 ± 3·4 Hz. However, the latter values, obtained 2 s after the onset of firing (i.e. to exclude the initial adaptation effect) bear relation to the *peak* firing frequencies observed in the present study, rather than the initial ones.

Peak firing frequency: extracellular vs. intracellular activation

When stimulated extracellularly with a current 1·25 × the threshold for repetitive firing, our cells exhibited similar peak firing frequencies to those of the corresponding intracellular study (Kernell & Monster, 1982*a*, *b*), when stimulated at 5 nA above their threshold for maintained repetitive firing. As mentioned in the Results section, this suggested that the present use of an extracellular stimulus strength set at 1·25 × the threshold for sustained firing was quite similar to an intracellular setting 5 nA above the threshold for repetitive firing. The finding that peak firing frequencies tend to be larger for F than S cells with both intracellular and extracellular stimulation is related to the fact that both cell types have different minimum repetitive discharge rates (Kernell, 1979). In the absence of systematic differences in the slope of the current–frequency relationship and provided that cells were stimulated with about the same amount of suprathreshold current (as seen intracellularly), then the peak rates should differ in the same manner as the minimum rates (i.e. higher in F than S cells).

Singlet vs. doublet firing; mechanisms of manifestation

Many (79%; 11 of 14) of the type S cells reported in this study displayed doublet firing (interspike interval $\leqslant$ 10 ms) with extracellular activation. In contrast, 45% (9 of 20) of the F cells showed doublet firing. Doublet discharge in motoneurons has been reported in a variety of experimental preparations including (1) healthy humans during weak voluntary contractions (Bawa & Calancie, 1983), (2) reflexive motor unit recruitment in decerebrate cats (Cordo & Rymer, 1982), (3) controlled locomotion in decerebrate cats (Zajac & Young, 1980), (4) intracellular current injection in cat motoneurons (Calvin, 1975), and (5) various pathological conditions in humans (Koenig & Stoehr, 1986). Interestingly, doublets were not observed in the intracellular study of Kernell & Monster (D. Kernell, personal communication). However, they were a prominent feature of the present work, particularly in the S cell discharge.

Currently, two theories can be entertained to explain the origin of the doublet. The first suggests that the second spike in a doublet discharge is generated in the motor axon rather than in the soma. Previously published values on the delay between extra-axonal spikes and the preceding (soma-dendritic) spike varied between 1·1 and 1·4 ms (Gogan, Gustafsson, Jankowska & Tyc-Dumont, 1984). Doublets from the present study had interspike intervals that varied between 4 and 10 ms, much longer intervals than those of axonal origin.

The second theory suggests that the second spike in a doublet discharge originates

from a delayed depolarization of either dendritic (Nelson & Burke, 1967) or initial segment origin (Baldissera, 1976) that appears as a 'hump' on the falling phase of an action potential. It is thought that under appropriate conditions (i.e. minimal firing rates) this delayed depolarization may reach threshold and bring about an additional spike (doublet).

For the present study, the second theory has particular appeal. It is conceivable that doublet spiking was brought about by the positioning of the extracellular microelectrode in close proximity to either a primary dendrite or the initial segment such that additional 'focal' depolarization near a secondary spike-generating site (Nelson & Burke, 1967) could have brought that region to threshold to produce a series of doublets. However, the present experimental arrangement did not allow for the testing of this particular hypothesis. One curious note is that S cells produced more doublet discharge than F cells. Since the input resistance of S cells (at least in the *passive* state) is greater than that for F cells it is conceivable that the local depolarization caused by the extracellular microelectrode was greater in the type S cells. This could have resulted in a greater percentage of the delayed depolarizations reaching threshold in the S cells.

Duration of firing: extracellular vs. intracellular stimulation

When sustained extracellular rather than intracellular current was used to activate motoneurons, a greater proportion of the cells were able to discharge for the entire 240 s period of stimulation (Fig. 4). The main difference between the intra- and extracellular techniques was found in the S cells, which were more likely to fire for longer periods during extracellular stimulation. It is likely that the expected superior capacity of S cells for sustained firing was obscured in the intracellular studies of Kernell & Monster (1982*a*, *b*) because of the greater susceptibility of this cell type to impalement-induced damage. It is reasonable to expect that S cells with their *slightly* smaller soma diameter (for review see Stuart & Enoka, 1983) are more susceptible to deleterious effects of electrode penetration than are the larger F cells.

Late adaptation with sustained activation

Several mechanisms have been proposed to account for late adaptation in cat spinal motoneurons, yet a precise description remains to be elucidated. Based on voltage clamp experiments, it has been suggested that late adaptation might be attributable to changes in after-hyperpolarization (AHP) of motoneurons (Barrett, Barrett & Crill, 1980). Several other possibilities have been suggested, including hyperpolarizing currents generated by an electrogenic sodium pump (Sokolove & Cooke, 1971; Grafe, Rimpel, Reddy & Ten Bruggencate, 1982), and/or a slowly inactivating potassium permeability (Llinás & Lopez-Barneo, 1988). Recent data from other laboratories reiterates the need for consideration of the AHP and its contribution to adaptation. Studies on decerebrate cats have demonstrated a significantly reduced AHP when motoneurons were activated during fictive locomotion (Brownstone, Jordan, Kriellaars, Noga & Shefchyk, 1992). In addition, when the Brownstone data were replotted (Spielmann, Laouris, Nordstrom, Reinking & Stuart, 1991) it was clear that the motoneurons activated during fictive locomotion did not display the typical adapting pattern (although excitatory drive may not have been constant), as compared to activation during current injection.

This is a striking demonstration of the powerful effect of extrinsic influences (i.e. via pathways involved in the elaboration of fictive locomotion) on an intrinsic motoneuron property.

The late adaptation observed in the present study was qualitatively similar to that found previously with sustained intracellular stimulation by Kernell & Monster (1982*a*, *b*). In the present study, the extent of adaptation was quantified by two separate methods. The first method provided a time constant (τ_2) of late adaptation. It was obtained by fitting the instantaneous firing frequency plots with a double exponential equation. This method is more rigorous than the method described below in that the double exponential fit provided the parameters that described the entire profile of singlet firing rather than describing only the discharge occurring between arbitrary time points in the record. In the intracellular study of Kernell & Monster (cf. their Fig. 4, 1982*a*) it was not possible to fit the data with a single time constant. Clear differences were seen in τ_2 values for F and S cells (Table 2). The mean magnitude of this difference (5·7 ×) is the largest reported to date for any property of F *vs.* S motoneurons (cf. Stuart & Enoka, 1983). This allows the prediction that τ_2 will prove particularly useful in subsequent attempts to unravel the cellular mechanisms underlying the differences in the properties of these two cell types.

The second method measured the drop in firing frequency from the peak of the double exponential fitted curve (cf. Fig. 3) to a value 24 s later. This measure was analogous to the drop in mean frequency between the 2nd and 26th seconds of firing, as reported by Kernell & Monster (1982*a*, *b*) for intracellular stimulation. These indices of the drop in firing frequency were nearly identical in both studies (see Fig. 7*A* and *B*) although the technical aspects of the two experiments differed significantly. In the sustained intracellular stimulation study of Kernell & Monster (1982*a*, *b*), cells were stimulated at 5 nA above rhythmic threshold regardless of unit type. In the present sustained extracellular stimulation study, cells were stimulated at 1·25 × the threshold for repetitive firing. However, absolute values of the firing frequency at 2 s (intracellular) and the mean peak singlet firing frequency (extracellular) were very similar in the two studies. With extracellular activation, F cells showed a greater decrease in rate than S cells.

Adaptation with intermittent activation

This study is the first to investigate adaptation in spinal motoneurons using intermittent current pulses applied extracellularly. Although there is an abundance of reports in the literature using intracellularly applied brief current pulses to activate moto- and other CNS neurons (for review see Burke & Rudomin, 1977; Binder & Mendell, 1990), none have studied both within- and between-train adaptation to the extent presented here. Similarly, while there are smatterings of intriguing examples of motoneuron adaptation during treadmill (Zajac & Young, 1980) and fictive (Hoffer, O'Donovan, Pratt & Loeb, 1981) locomotion of high decerebrate cats, and the treadmill locomotion of conscious cats (Hoffer, Sugano, Loeb, Marks, O'Donovan & Pratt, 1987), it must be recognized that the adaptation so observed was attributable to a combination of *intrinsic* and *extrinsic* (synaptic input) properties of the test cells.

Within-train adaptation

Motoneurons in deeply anaesthetized cats respond to 1·0–2·0 s intracellular current pulses with a discharge pattern that is generally characterized by a few very brief (4–5 ms) interspike intervals at current onset, with each successive interspike interval being longer than the preceding one (Kernell, 1965*a*). These first few short intervals are followed by progressively longer intervals, although the rate of this interspike interval lengthening slows dramatically after the first second or so of discharge (Kernell & Monster, 1982*a*). Initial, or within-train, adaptation has been attributed to the summation of conductances contributing to after-hyperpolarization (Baldissera & Gustafsson, 1974). In the present study, ten out of thirty-two cells showed within-train adaptation, but this was not a prominent feature beyond the first few interspike intervals. When present, within-train adaptation occurred very rapidly; it was most clearly evident as a prolongation of the second interspike interval compared to the first, in each stimulation cycle. Its effects were generally not cumulative beyond the second or third interval in the train, as joint-interval distributions for the second and subsequent intervals did not reveal a tendency for lengthening of the $(i+1)$th interval. These results are strikingly different to those from the intracellular studies of Kernell (1965*a*) and Llinás & Lopez-Barneo (1988), who described a form of within-train adaptation in tectal neurons of the guinea-pig slice preparation. The present spinal motoneuron work involved use of a cell-activation technique that precluded analysis of initial adaptation by virtue of the time course of the delivery of the excitatory current to the cells under these conditions. Viewed from this perspective, the within-train adaptation reported in this study may have some biophysical interest with regard to mechanisms underlying the extracellularly activation of CNS cells (cf. Ranck, 1975).

Between-train adaptation

The present results showed a decrease in the mean firing rate per train over the entire stimulation period. We have termed this *between-train adaptation*, and it was similar in many respects to the *late* adaptation seen with sustained activation. It is likely that the mechanisms of between-train and late adaptation are similar. The main significance of the present results on between-train adaptation was that the presently used technique (1) provided a clear-cut demonstration of the phenomenon, and (2) showed that it resembled late adaptation to sustained stimulation in several respects. For example, units with the higher peak frequency tended to show the most late (Fig. 11*B*) and between-train adaptation (Fig. 11*C* and *D*) with extracellular stimulation. With both sustained and intermittent extracellular activation, F cells showed more adaptation than S cells. This latter difference should prove of particular interest in the continuing evaluation of Henneman's size principle (see Binder & Mendell, 1990).

The present comparison of the response of the same cell to two different stimulation protocols is unique in the literature. Although an attractive feature of the present study, it none the less has the attendant possibility that the cell response to later stimulation protocols may have been influenced by prior activation. At present, we have no quantitative data on the effects of prior activity on the response of the cell to activation. We attempted to maximize cell recovery by allowing 2 min rests between each stimulation protocol (cf. Llinás & Lopez-Barneo, 1988; their Fig. 4). The present study actually incorporated four stimulation protocols: (1) and (2) sustained

stimulation with and without the superimposition of pink noise, and (3) and (4) intermittent stimulation with and without superimposed noise. Although not a direct test, an indication of the likely effects of prior activation of the cell can be gained by comparing the responses to 'similar' stimulation (i.e. with or without noise) following several periods of activation and rest. The raw interspike interval time plots for each cell were qualitatively similar under the two conditions, which would support the view that the repetitive discharge properties of the cells were not seriously impaired by preceding periods of extracellular activation.

Relation of the present result to Henneman's size principle

Kernell & Monster (1982*a*, *b*) have shown correlations between the magnitude of the late adaptation and: (1) the threshold for rhythmic firing, (2) the firing rate after 2 s of sustained discharge, and (3) muscle unit twitch contraction time. The extracellular approach used in the present study precluded precise assessment of correlations between the threshold for repetitive firing and other motor unit properties (see above). The present study is the first to show clear relationships between two *active* properties of motoneurons and some other well-known type (size)-related properties of motoneurons and motor units during sustained and intermittent stimulation. For sustained stimulation, both peak singlet firing frequency and the magnitude of late adaptation were shown to be significantly associated with each other (Fig. 7), and each was also significantly associated with axonal conduction velocity, twitch contraction time and peak tetanic force (Fig. 8). Virtually the same results were obtained for intermittent stimulation (Figs 11 and 12) except that in this instance the association between the magnitude of between-train adaptation and twitch contraction time was not significant. This may be attributable to the fact that intermittent stimulation reduced the magnitude of between-train adaptation in F cells as shown in Fig. 11.

The above associations were shown for the full (F + S) population of motor units. In work of this kind, it is more common to find a type (size)-related association between the parameters when the unit population includes both F and S cells (Stuart & Enoka, 1983). However, in the present study, several such associations were also observed *within* the F and S cell groups. For sustained stimulation (Fig. 8) they included: S cells, conduction velocity *vs.* peak singlet firing frequency, contraction time *vs.* τ_2. For intermittent stimulation, these associations included: F cells, peak tetanic force *vs.* peak firing frequency, and peak tetanic force *vs.* between-train adaptation (Fig. 12). All of the demonstrated correlations in the present work were as one would predict, based on the rather extensive body of literature relating either directly or indirectly to the size principle and to the generally accepted patterns of usage of the motoneuron and motor unit types during movement (Binder & Mendell, 1990). All in all, the present results, together with those of Kernell & Monster (1982*a*, *b*) provide convincing evidence that *active* firing-rate properties are as tightly coupled to the neuromechanical properties of motor units as the more conventionally studied *passive* (e.g. input resistance) and *transitional* (e.g. rheobase) properties (cf. Burke, 1981; Stuart & Enoka, 1983; Gustafsson & Pinter, 1984*a*, *b*, 1985). The close associations shown between peak firing frequency and late adaptation during sustained stimulation, peak firing frequency per train and between-train adaptation during intermittent stimulation and several well-known type (size)-related properties of motor units (i.e. axonal conduction velocity, twitch contraction time, peak tetanic

force) all point to a new opening in size principle studies – the search for the biophysical properties that underlie the type (size)-related *active* properties of motoneurons.

Ensemble responses of F and S cell populations

The population ensemble firing frequency was used to compare F and S cell responses with sustained (Fig. 9) and intermittent (Fig. 13) stimulation. For both stimulation protocols, the ensemble response of F cells declined more rapidly than S cells. The difference in responses of a particular cell type with sustained *vs.* intermittent stimulation can be seen in Fig. 14. The main difference found in the discharge profiles with intermittent *vs.* sustained stimulation was that F cells were able to maintain a higher ensemble mean firing frequency during the activation period. The time constant (τ_2') of the reduction in the ensemble mean firing frequency was longer with intermittent stimulation for both F (170 *vs.* 60 s) and S cell (704 *vs.* 492 s) populations. The long time constants for S cells meant that the curves of the ensemble mean firing frequency were very similar over the 240 s stimulation period. This emphasized the intrinsic ability of type S cells to discharge continuously for extended periods without displaying much adaptation. The steeper reduction in the F cell ensemble mean with sustained stimulation is due in part to the significantly shorter mean firing durations with sustained stimulation (123 ± 88 s *vs.* 194 ± 69 s; also compare the proportion of active F cells during sustained (Fig. 9*B*) and intermittent (Fig. 13*B* stimulation). For F cells, there was also a tendency for reduced adaptation of firing frequency with intermittent stimulation (cf. the mean drop in firing frequency from the peak to 24 s later in Table 3), but this difference was not significant. As further contrast between the two activation protocols with intermittent activation the ensemble mean number of spikes produced by the F cell population was greater than the S cell population at all times following the onset of spiking (Fig. 13*C* and *D*). These findings are of particular interest in that intermittent activation is probably a more physiologically relevant pattern of activation than sustained (continuous) activation, especially for type F cells.

Cumulative effects of spike production on adaptation

Although this study did not directly attempt to uncover the intrinsic mechanisms governing late adaptation, an inference can be made from the present data regarding the cumulative effects of repetitive discharge. The present results show that cells with a higher peak firing frequency adapt more rapidly than those with lower peak frequencies. Kernell & Monster (1982*a*) reported similar results and attributed their findings to 'some kind of cumulative after-effects of many consecutive spikes'. The after-effects, and presumably adaptation, would be greater as the number of preceding spikes increased. This was investigated in the present study by examining the reduction in firing frequency as a function of the number of spikes produced, for the same cell in response to the different stimulation protocols. The number of spikes accumulated at different rates in the two protocols (because of the 400 ms rest period with intermittent stimulation), even though mean firing frequencies during the periods of activation were similar. Comparison shows that the sustained stimulation

produced significantly more adaptation than intermittent stimulation in type F cells, when referenced to a similar number of accumulated spikes in each condition. This suggested that for F cells the rate of spike production was important for adaptation, as well as the total number of spikes produced. Adaptation in S cells was not significantly different between the two stimulation protocols when examined in this way, suggesting that the rate of spike accumulation was less important for adaptation in this cell type.

In summary, the present study has introduced a new technique to the study of the repetitive discharge properties of motoneurons and provided new data on several firing rate properties of motoneurons that contribute to the continuing investigation of mechanisms underlying the differences in the functional behaviour of F and S cells (for review see Binder & Mendell, 1990). Most of the previous work addressed passive properties of motoneurons (i.e. mechanisms underlying resting potentials, EPSPs and IPSPs), whereas the present work expands on that of Kernell & Monster (1982*a*, *b*) by adding information on the properties of actively firing cells.

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