

# Firing Rate Modulation of Motoneurons Activated by Cutaneous and Muscle Receptor Afferents in the Decerebrate Cat

J. F. PRATHER,<sup>1</sup> B. D. CLARK,<sup>2</sup> AND T. C. COPE<sup>1</sup>

<sup>1</sup>*Department of Physiology, Emory University, Atlanta, Georgia 30322; and* <sup>2</sup>*Department of Physical Therapy, Temple University, Philadelphia, Pennsylvania 19140*

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**Prather, J. F., B. D. Clark, and T. C. Cope.** Firing rate modulation of motoneurons activated by cutaneous and muscle receptor afferents in the decerebrate cat. *J Neurophysiol* 88: 1867–1879, 2002; 10.1152/jn.01037.2001. The aim of this study was to investigate whether activation of spinal motoneurons by sensory afferents of the caudal cutaneous sural (CCS) nerve evokes an atypical motor control scheme. In this scheme, motor units that contract fast and forcefully are driven by CCS afferents to fire faster than motor units that contract more slowly and weakly. This is the opposite of the scheme described by the size principle. Earlier studies from this lab do not support the atypical scheme and instead demonstrate that both CCS and muscle stretch recruit motor units according to the size principle. The latter finding may indicate that CCS and muscle-stretch inputs have similar functional organizations or that comparison of recruitment sequence was simply unable to resolve a difference. In the present experiments, we examine this issue using rate modulation as a more sensitive index of motoneuron activation than recruitment. Quantification of the firing output generated by these two inputs in the same pairs of motoneurons enabled direct comparison of the functional arrangements of CCS versus muscle-stretch inputs across the pool of medial gastrocnemius (MG) motoneurons. No systematic difference was observed in the rate modulation produced by CCS versus muscle-stretch inputs for 35 pairs of MG motoneurons. For the subset of 24 motoneuron pairs exhibiting linear co-modulation of firing rate ( $r > 0.5$ ) in response to both CCS and muscle inputs, the slopes of the regression lines were statistically indistinguishable between the two inputs. For individual motoneuron pairs, small differences in slope between inputs were not related to differences in conduction velocity (CV), recruitment order, or, for a small sample, differences in motor unit force. We conclude that an atypical motor control scheme involving selective activation of typically less excitable motoneurons, if it does occur during normal movement, is not an obligatory consequence of activation by sural nerve afferents. On average and for both muscle-stretch and skin-pinch inputs, the motoneuron with the faster CV in the pair tended to be driven to fire at slightly but significantly faster firing rates. Computer simulations based in part on frequency-current relations measured directly from motoneurons revealed that properties intrinsic to motoneurons are sufficient to account for the higher firing rates of the faster CV motoneuron in a pair.

## INTRODUCTION

The CNS directs movement by recruiting motoneurons and adjusting the rate and pattern of their firing. Organization in this behavior among motor units gains particular interest because even within muscles, there is wide diversity in the contractile force, speed, and fatigue properties of the units. This functional diversity suggests a mechanism by which the differential activation of motor units might be adjusted to meet the unique

requirements of different movement tasks. Differential activation of functionally distinct motor units would be achieved presumably through diversity in the distribution of excitation and inhibition from different sources of synaptic input to motoneurons (Burke 1991; Kanda et al. 1977; Windhorst et al. 1991).

If differential activation was expressed in a population of motoneurons, it would be reasonable to expect that it might be most easily detected by examining the response of the population to inputs with disparate synaptic organizations. A frequently cited example of disparate synaptic organization involves the input from caudal cutaneous sural (CCS) afferents onto medial gastrocnemius (MG) motoneurons in cat. The majority of studies show a characteristic pattern whereby CCS synaptic potentials are primarily inhibitory in the most electrically excitable motoneurons and excitatory in less excitable ones (Burke et al. 1970; Kanda et al. 1977; Pinter et al. 1982; Powers and Binder 1985). Exceptions to this pattern have been reported (Heckman et al. 1994; LaBella et al. 1989; Rosenberg 1970), but the distribution of synaptic potentials from CCS afferents onto MG motoneurons is consistently found to differ from that for group Ia muscle spindle afferents. Group Ia afferents generate excitatory potentials across all MG motoneurons with greater excitation provided to the most excitable cells (Burke et al. 1976; Fleshman et al. 1981; Harrison and Taylor 1981; Heckman and Binder 1988). This difference in the synaptic arrangement of CCS afferents and group Ia spindle afferents suggests that these inputs should activate MG motoneurons in different ways. However, this expectation has not been borne out in motoneuron recruitment studies, wherein CCS and muscle afferent inputs evoke indistinguishable sequences of recruitment (Clark et al. 1993; Cope and Clark 1991).

Similarity of motoneuron recruitment sequence in cutaneous and muscle-afferent evoked reflexes may indicate that the synaptic organizations of these sources are not as different as has been reported. One cause of this discrepancy may be that studies of synaptic potentials were performed under different experimental conditions than the recruitment studies (see Heckman et al. 1994). Alternatively, the assay of recruitment sequence may be unable to resolve differences in synaptic organization, because properties intrinsic to motoneurons themselves (e.g., electrical excitability) predominate in determining recruitment order (Haftel et al. 2001). Recruitment threshold measured as rheobase current in cat MG motoneurons spans a 30-fold range (ca. 1–30 nA) (Zengel et al. 1985), and this wide range may obscure even substantial differences

Address for reprint requests: J. F. Prather, Physiology Dept., Whitehead Bldg., 615 Michael St., Suite 635, Emory University, Atlanta, GA 30322 (E-mail: jprathe@emory.edu).

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in synaptic input among motoneurons. Thus detection of differences in synaptic organization may require a more sensitive assay than recruitment sequence. For this purpose, we propose measures of motoneuron firing rate modulation. The contribution of motoneuron intrinsic properties to firing rate, measured as the increment in firing rate with injected current ( $f$ - $I$  relation), spans a fivefold range at maximum (ca. 0.5–2.5 pps/nA, see RESULTS of this work) for cat MG motoneurons. Therefore differences in synaptic drive onto motoneurons should be better reflected in firing rate modulation than in recruitment sequence.

Some data are available on the modulation of MG motoneuron firing evoked by stimulation of CCS afferents in the cat. Kanda et al. (1977) report two examples in which skin pinch in the receptive field of the CCS nerve decelerated firing in MG motoneurons with slow conduction velocity, while accelerating the rates of cells with faster conduction velocity. From this observation, Kanda et al. propose that CCS synaptic input “acting alone can activate preferentially some of the otherwise high-threshold motoneurons that innervate fast twitch, large force muscle units.” A more recent study does not support this proposal. Although Clark et al. (1993) found instances where skin pinch decreased the firing rate of low-conduction-velocity MG motoneurons, these instances were rare (2 of 32 motoneurons studied). Therefore it remains controversial whether synaptic drive from the CCS nerve, or from any source, can preferentially activate motoneurons that would otherwise be least excitable. Here we report a new attempt to resolve this issue.

The primary approach taken here was to compare the effects of CCS and muscle stretch inputs on the relative firing rates of motoneurons sampled in pairs. In these comparisons, properties of each motoneuron in the pair (e.g.,  $f$ - $I$  relations), whether similar or not, make a fixed contribution to firing produced by either input (Prather et al. 2001). With the motoneuron contribution common in these comparisons, relative firing rates of motoneuron pairs are direct indices of the relative amounts of synaptic current generated by each input in the two cells. In response to synaptic excitation, each motoneuron in a pair began firing repetitively and modulated firing rate according to changes in stimulus intensity. The pattern of rate modulation expressed by each pair of motoneurons was examined using rate-rate plots, in which the instantaneous firing rate of each motoneuron was plotted, one against the other (see also, Monster 1979; Monster and Chan 1977; Tansey and Botterman 1996). Differences in the rate-rate plots generated by CCS and muscle stretch inputs were used to detect differences in the distribution of synaptic drive from these two sources.

Our findings for MG motoneurons indicate that the synaptic organization of CCS afferents, while not identical to that for homonymous muscle stretch afferents, is neither systematic nor significant in its differences and is not related to differences in motoneuron conduction velocity or recruitment threshold. These indications were consistent across all measures made in this study, including recruitment order and several alternative analyses of firing rate modulation. An additional finding was the unexpected tendency for the modulation of firing rate, regardless of input, to be greater in the faster than in the slower conduction velocity motoneuron in a pair. These findings were reproduced in a computer simulation that incorporated motoneuron  $f$ - $I$  relations, measured from intra-somatic recordings of motoneurons, and synaptic current amplification, determined in earlier studies (Lee and Heckman 2000; Prather et al.

2001). These results indicate that the synaptic effects of homonymous muscle stretch and CCS skin pinch on rate modulation of physiologically diverse MG motoneurons are functionally indistinguishable. Portions of these results have been presented in abstract form (Prather et al. 1997).

## METHODS

### *Surgical procedures*

Data were collected from 70 MG motoneurons recorded in nine adult cats of either sex ranging in mass from 2.5 to 3.0 kg. Surgical and recording techniques used in these studies have been described in previously published reports from this laboratory (Cope and Clark 1991). Cats were studied in single experiments. Anesthesia was induced by enclosing the cat in a sealed chamber ventilated with Halothane in a 1:1 mixture of N<sub>2</sub>O and O<sub>2</sub>. When deep anesthesia had been achieved, the animal's trachea was intubated and connected to a respirator that was used to deliver anesthetic throughout the remainder of the dissection. Respiratory tidal volume was initially normalized to the animal's mass (10 ml/kg) and was adjusted as necessary to maintain end-tidal CO<sub>2</sub> between 3 and 4%. The right carotid artery was cannulated to monitor blood pressure, and the left carotid was ligated to prevent excessive bleeding during the subsequent decerebration. The right external jugular vein was cannulated to permit delivery of drugs throughout the course of the experiment. Drugs were administered in Ringer solution, which was occasionally delivered alone to replace fluid volume lost during surgery. The sympathomimetic amine, metaraminal bitartrate (Aramine), was infused as needed to help maintain blood pressure above 60 mmHg. Data were not recorded from preparations in which blood pressure remained lower than 60 mmHg for longer than 1 min. A laminectomy was performed to expose spinal segments L<sub>4</sub>–S<sub>1</sub>, and the left hindlimb was dissected to permit access to the triceps surae muscles, the MG muscle nerve, and the caudal cutaneous sural nerve. After dissection of the back and hindlimb, the cat was fixed in a rigid recording apparatus using clamps attached to the exposed spinous processes. The skin flaps at the site of each incision were tied to the rigid support, creating wells around the exposed back and hindlimb tissue, which were then filled with mineral oil to prevent tissue desiccation. The temperature of the mineral oil pools, as well as the body core, was monitored and maintained around 37°C by radiant heat. Decerebration was performed by removing all brain tissue rostral to an intercollicular midbrain transection. Following decerebration, delivery of anesthetic was discontinued. Ventilation was maintained as necessary, although the animals were often able to breathe well without assistance. At the conclusion of each recording session, cats were euthanized using barbiturate overdose.

### *Intra-axonal recording procedures*

Records of MG motoneuron repetitive firing were obtained intraxonally by driving glass micropipette electrodes (2 M K-acetate, 15–20 MΩ) into the ventral roots until antidromic action potentials, generated by electrical stimulation of the MG nerve using hook electrodes (0.04-ms pulses, 1 Hz), were identified in the micropipette voltage records. Reflex movements of the cat sometimes made it difficult to maintain stable axonal penetrations. Therefore a paralytic agent (pancuronium bromide) was usually administered intravenously, and paralysis was maintained throughout the experiment. In one experiment, no paralytic agent was administered; data recorded from this preparation were used to investigate the relation between firing rate behavior and motor unit contractile properties. Current pulses (0.04 ms, 5 Hz) were then injected into the motoneuron axon to generate single action potentials, which were recorded from the MG hook electrode (digitized at 40 kHz) and spike-trigger averaged online. A single sharp peak in the averaged record served as verification that only a single axon was being stimulated. This procedure was repeated so that two motor axons were simultaneously penetrated, one each by two

independent micropipettes. An advantage of this approach is that penetration of motor axons permits reliable recording of the firing expressed by single motoneurons without disturbing the site of synaptic integration. Action potentials were recorded and digitized at 22 kHz (Spike2, Cambridge Electronic Design). Passive tension on the MG muscle was maintained at 100 g and was recorded from the force transducer and digitized at 1 kHz. Data were stored onto disk and videotape, and action potentials were discriminated into event markers off-line.

### Stimulation procedures

The aim of the present experiments was to describe the functionally relevant features of the synaptic drive from muscle stretch and CCS nerve afferents onto MG motoneurons. All motoneuron pairs were stimulated to fire reflexively in response to synaptic activation by stretching the MG muscle and/or by pinching the skin overlying the lateral aspect of the ipsilateral ankle with toothed forceps. Muscle stretch stimuli were administered using either ramp-hold-release (4–8 mm, 0.2- to 1.0-s stretch/release time, 1-s hold) or sinusoidal (4–8 mm, 0.35–0.70 Hz) templates. The MG tendon of insertion was sectioned and tied to a force transducer using a short length of braided polyester cord (Dacron). The force transducer was rigidly attached to a servomotor, which was driven by custom-designed software to stretch the muscle according to user-specified waveforms. Sine and ramp waveforms were used interchangeably, as their effects on firing rate comodulation were indistinguishable (paired *t*-test,  $P = 0.88$ ). In addition, in pairs where multiple speeds of ramp or frequencies of sine stimulus were presented, variation in those parameters also had no significant effect on rate comodulation (ANOVA,  $F = 0.27$ ,  $P = 0.77$ ). Therefore in RESULTS, data obtained using either ramp or sinusoidal stretch are collectively referred to as stretch input data.

The data of this paper are taken from periods of sustained coactivity in pairs of MG motoneurons when firing rate was being modulated by a changing synaptic stimulus. The magnitude of that input was deeply modulated to strongly emphasize its influence as the driver of variations in motoneuron firing rate. For the analysis described in the following text, stimuli were repeated many times for each motoneuron pair. In the majority of pairs, muscle stretch (42/47) and CCS skin pinch (43/47) elicited trains of repetitive firing in both motoneurons for at least 4 s. In the remaining few pairs, one or both of the synaptic inputs produced only sporadic spikes but not repetitive firing and were therefore excluded from further analysis. Because the decerebrate cat is subject to fluctuations in motoneuron excitability over time, records sampled far apart in time could be influenced by fluctuating physiological conditions. To minimize this possibility, CCS and muscle stretch were compared using only contiguous recordings.

### Data analysis

Conduction delay for the averaged action potential of each cell was measured to the nearest 0.025 ms (SigAve, Cambridge Electronic Design) and compared with the distance between stimulation and recording sites to determine axonal conduction velocity (CV) for each motoneuron. Paired motoneurons were distinguished by their difference in CV, a parameter that correlates with motor unit properties and with motoneuron recruitability (Burke 1981; Henneman and Mendell 1981; Cope and Clark 1991). The difference in recruitment time was also measured across motoneuron pairs for the muscle stretch stimulus, which was constant across trials, but not for the pinch stimulus, which was not the same from one trial or from one motoneuron pair to the next. Finally, the sequence of recruitment both for muscle stretch and skin pinch was noted. The following procedures were repeated on firing rate data for each motoneuron pair and stimulus type.

### Rate-rate analytical techniques

Spike times were converted to firing rates using the algorithm of Berger et al. (1986; see also Kamen and Du 1999). That algorithm permits the instantaneous firing rate of each motoneuron to be sam-

pled simultaneously. Rates were collected at 50 samples/s throughout the interval in which both cells were simultaneously active. Sampled firing rates above 200 or below 5 pps were excluded to avoid overemphasis on doublets (Gorassini et al. 2000; Hennig and Lomo 1985), which were occasionally expressed during the first few interspike intervals of some firing responses (Cope and Clark 1991), and to ensure that long interspike intervals due to the period between stimulus presentations did not influence the result. "Rate-rate" plots were constructed by plotting the firing rate of the cell with the faster CV against the firing rate expressed simultaneously by the cell with the slower CV (Monster 1979; Monster and Chan 1977; Tansey and Botterman 1996). The collection of points in each rate-rate plot usually resembled an elliptically shaped cluster. As the firing rate data were not always normally distributed for each motoneuron of a pair, the nonparametric Spearman's rank-order correlation coefficient was computed for each set of rate-rate data. Data sets that were significantly correlated, and for which correlation explained at least 25% of the variance ( $r > 0.5$ ), were considered for further analysis. A reduced major axis regression (geometric mean regression) was used to quantify the slope of each rate-rate relation (Babu and Fiegelson 1992; Sokal and Rohlf 1995). Slope is a measure of the relation between rate modulations expressed by each motoneuron. A slope  $>1$  indicates that the rate of the motoneuron with faster CV was modulated more strongly than that of the slower CV motoneuron, whereas a slope  $<1$  indicates the opposite.

### Additional data analysis techniques

**SYNCHRONY ANALYSIS.** Shared influence of either muscle stretch or CCS skin pinch on MG motoneurons might result in synchronous discharge among the motoneurons in each pair. This possibility was examined by generating cross-correlograms of the action potentials expressed by each motoneuron in a pair during each stimulus trial (e.g., Nordstrom et al. 1992). The train of action potentials generated by the motoneuron with the faster axonal CV was designated the trigger, and those from the motoneuron with slower CV were designated the data. Care was taken to ensure that the same spikes were considered in both rate-rate and synchrony calculations. A cross-correlogram of the action potentials was generated for each stimulus trial. Bin size of each cross-correlogram was computed based on the number of trigger spikes and the average firing rate of the data spikes, so that an average of 5 data spikes fell into each bin. Bin sizes ranged from 0.5 to 18.2 ms ( $4.0 \pm 3.8$ , mean  $\pm$  SD). Synchrony was assessed using the maximum counts present in any bin within  $\pm 10$  ms from *time 0* in the cross-correlogram. This multiple-bin assessment allowed for possible synchrony at times other than exactly *time 0*. The counts present in control bins where synchrony was absent were computed by summing the counts of all bins between 10 and 20 ms before the trigger in the cross-correlogram. Control and peak bin values were not subject to significant contamination by the properties of the trigger auto-correlogram, as firing rates only transiently approached 50 pps (interval of 20 ms). The significance of the peak synchrony bin was assessed by comparing its magnitude against the counts present in control bins using the *z* statistic of Cox and Lewis (1966; see also Clark and Cope 1998; Cope et al. 1987; Garnett and Stevens 1980). Synchrony was considered significant at the 5% level if  $|Z| > 1.96$ .

**ANALYSIS OF SHARED FREQUENCY DOMAIN MODULATION.** Two additional tools were used to assess the possibility that firing rates were differentially modulated at particular frequencies by different synaptic inputs. First, we evaluated the "common drive" coefficient (DeLuca et al. 1982; Semmler et al. 1997), a time-domain measure of shared, low-frequency modulation of two spike trains. The common drive coefficient was computed from 10-s samples of band-pass filtered firing data as described by Semmler et al. (1997). Although this analysis is intended to be applied to data from motoneurons firing in a relatively steady fashion, we used it on data from motoneurons undergoing deep, but relatively slow fluctuations in firing rate. We reasoned that the 0.75-Hz high-pass filter applied as part of the analysis (Semmler et al. 1997) would attenuate most of these fluctu-



ations and result in a cross-correlogram that reflected low-frequency common drive. Second, using the same 10-s samples, we calculated coherence spectra from the spike trains as described by Rosenberg et al. (1989). These spectra estimate the strength of common modulation between two spike trains as a function of frequency. The 95% confidence level for the coherence spectra (Rosenberg et al. 1989) was used to determine significance. We examined each trial using a spectral resolution (frequency binwidth) of 0.25–4 Hz (cf. “window closing”) (Jenkins and Watts 1968); peaks were considered significant only if they consistently fell above the 95% level across multiple frequency bins. The results presented below are based on coherence spectra calculated from 0 to 50 Hz in 2-Hz bands of frequency.

### Intrasomatic stimulus and recording procedures

In an additional set of experiments performed using intrasomatic stimulus and recording procedures, motoneuron firing rates in response to microelectrode current injection were used to measure the gain of the motoneuron frequency-current relation ( $f$ - $I$  slope). The methods of these experiments have been described in detail previously (Prather et al. 2001). Briefly, glass micropipette electrodes (2 M K-acetate, 5–10 M $\Omega$ ), connected to an Axoclamp-2A amplifier operated in bridge mode, were driven into the ventral spinal gray matter until antidromic action potentials were identified in the voltage records. When resting membrane potential was steady and action potential amplitude exceeded 80 mV, records were collected (DC–10 kHz band-pass) and digitized at 22 kHz (Spike2). Rheobase current and axonal CV were measured using the protocols of Zengel et al. (1985). Repetitive firing was generated by injecting a 1-s current pulse through the microelectrode. The resulting firing rate was averaged over the final 500 ms of stimulation to obtain the firing rate corresponding to the current in the soma. Pulses of several different amplitudes were applied, and using least squares linear regression, the resulting data were used to construct an  $f$ - $I$  relation for each cell.

## RESULTS

### Correlated motoneuron firing in response to muscle stretch

Instantaneous firing rate was measured for 35 pairs of MG motoneurons coactivated by muscle stretch. The observed range in axonal conduction velocities (70–107 m/s) spanned a large portion of the total range previously reported for MG motoneurons (Zengel et al. 1985). Several different patterns of rate modulation were observed in response to muscle stretch, and representative cases are presented in Fig. 1. Instantaneous firing rates obtained in trials of muscle stretch (either sinusoidal or ramp-and-hold stimulus waveforms) are plotted in the *top portion* of each panel as  $\circ$  for the motoneuron with the slower CV in the pair and as  $\blacksquare$  for the motoneuron with faster CV. The rate-rate plots in the *bottom panels* of A and B, depict monotonic comodulation of firing rate that represents correlated increases and decreases in firing rate over the course of the stretch stimulus. Most pairs exhibited comodulated firing in response to muscle stretch stimulus, and the correlation coefficient exceeded 0.5 in 25 of 35 pairs (71%). Figure 1, A and B, also shows that firing rate could be comodulated whether the difference in recruitment time between motoneurons in the pair was relatively short (21 ms in Fig. 1A) or long (312 ms in Fig. 1B).

In 10 of the 35 pairs, (29%), the stretch stimulus evoked repetitive firing that was not comodulated with  $r < 0.5$ . Two such cases are depicted in Fig. 1, C and D. In Fig. 1C, the firing rate of the slower CV was modulated at the beginning and end of the stimulus, but fired steadily during the peak of the muscle stretch when firing rate was modulated in the faster CV motoneuron. This phenomenon has been referred to as “rate

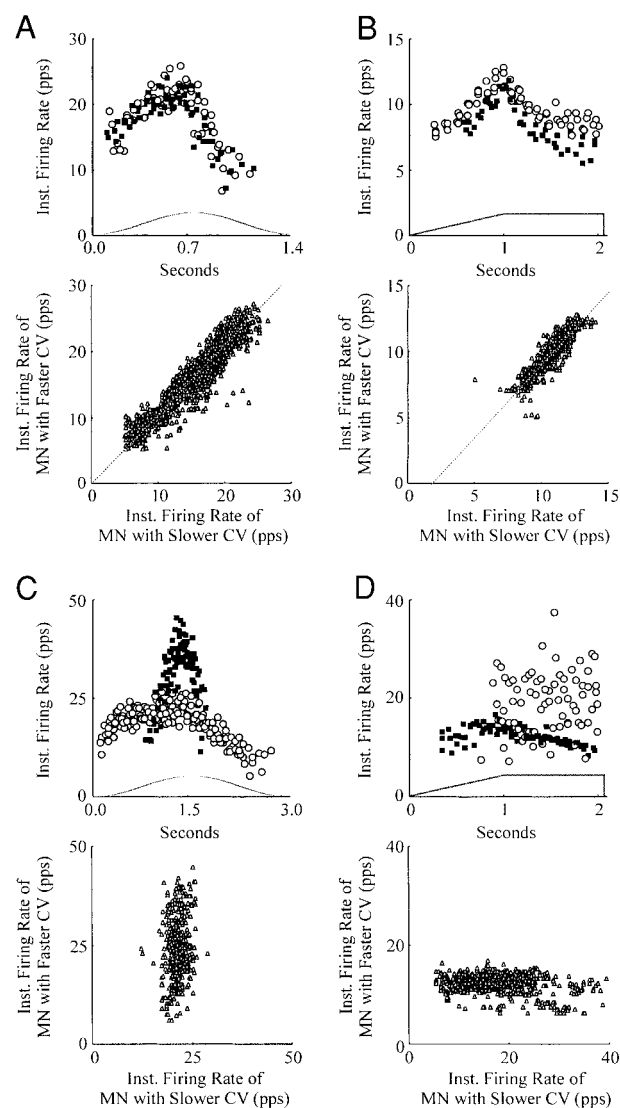


FIG. 1. Firing rate modulation for 4 motoneuron pairs recruited by muscle stretch. *Top*: the instantaneous (Inst.) firing rate superimposed over 4 stimulus trials for each motoneuron (MN) in a pair ( $\circ$ , motoneuron with slower axonal conduction velocity (CV);  $\blacksquare$ , cell with faster CV). Corresponding muscle stretch waveforms (either sinusoidal or ramp-and-hold stretches) are indicated as faint traces along the time axis. *Bottom plot* in each panel shows the rate-rate plot (see METHODS) for all stimulus trials. Firing rate co-modulation with Spearman correlation coefficient  $r > 0.5$  was observed for the majority of motoneuron pairs (25/35). Motoneuron pair in (A) exhibited a relatively large range in firing rate and similar firing onset (21 ms), while the pair in (B) had a smaller range in firing rate and larger difference in firing onset (312 ms). Geometric mean regression (---; A,  $r = 0.95$ ,  $P < 0.01$ ) and B ( $r = 0.92$ ,  $P < 0.01$ ). Firing rate co-modulation was not evident in all cases: in C co-modulation was weak ( $r = 0.39$ ,  $P < 0.01$ ), apparently the result of rate limiting (see text) for the low CV motoneuron ( $\circ$ ), and in D, the large variability in firing rate of the low CV motoneuron ( $\circ$ ) contributes to the absence of co-modulation ( $r = -0.07$ ,  $P = 0.05$ ).

limiting” (reviewed in Binder et al. 1996). In Fig. 1D, the firing rate of the faster CV motoneuron accelerated and then slowed during the hold phase of muscle stretch, while the firing rate of the slower CV motoneuron exhibited wide variation. Pairs that displayed comodulated firing in response to stretch input ( $n = 25$ ) were indistinguishable in their CV differences from pairs where firing was not comodulated ( $n = 10$ , Mann-Whitney  $U$  test,  $P = 0.33$ ). Patterns of firing rate comodulation evoked by

muscle stretch served as the standard of comparison with the subsequently described firing responses to CCS stimulation.

### Correlated motoneuron firing in response to CCS skin pinch

The same 35 pairs examined in response to muscle stretch were also studied in contiguous trials of firing evoked by CCS skin pinch. All 35 pairs responded to both stretch and pinch with at least 4 s of steady firing. In response to CCS stimulation, most motoneuron pairs displayed comodulated firing with correlation coefficients  $>0.5$  (31 of 35 pairs, 89%). This percentage of pairs displaying firing rate comodulation ( $r > 0.5$ ) was not significantly different between muscle stretch and CCS skin pinch (Yates corrected  $\chi^2$  test,  $P = 0.14$ ). Representative cases of firing evoked by CCS skin pinch are presented in Fig. 2. In Fig. 2A *top*, firing rates of a pair of motoneurons are modulated in concert with the intensity of pinch applied to the skin innervated by CCS (gray traces along the  $x$  axis). This comodulation is clearly evident in Fig. 2A, *bottom*, in a rate-rate plot constructed using all stimulus trials for the motoneuron pair. Intensity of skin pinch applied to the CCS innervation site was not monitored in every experiment, however comodulation of firing rates was clearly evident in cases where stimulus intensity was not recorded (e.g., Fig. 2B). In 4 of 35 pairs (11%), CCS stimulation evoked repetitive firing that was not comodulated between motoneurons of a pair. These pairs were indistinguishable in their CV differences from pairs for which firing was comodulated ( $n = 31$ , Mann-Whitney  $U$  test,  $P = 0.09$ ).

### Relative effects of muscle stretch and CCS skin pinch on motoneuron firing behavior

The central aim of this study was to determine whether the net effect on MG motoneuron spike output differs between muscle stretch and CCS skin pinch inputs. This determination was made through numerous analyses of motoneuron firing

activity. In comparison of recruitment order across the entire sample of 35 pairs of MG motoneurons, 31 pairs (89%) were composed of motoneurons with axonal CVs that differed by at least 2 m/s, a condition necessary to permit reliable assessment of recruitment order by CV (Clark et al. 1993; Cope and Clark 1991; Haftel et al. 2001) and therefore by the rubric of the size principle (Henneman et al. 1965; reviewed in Binder et al. 1996). Within the population of 31 pairs where order could be resolved, muscle stretch input evoked recruitment from low to higher CV in 25 of 31 pairs (81%) and reversed order in 6 pairs (19%). In response to CCS skin pinch, recruitment proceeded from low to higher CV in 22 of 31 pairs (71%) and in reverse order in 9 pairs (29%). Each input evoked the same recruitment sequence in 27 of 31 pairs (87%), and recruitment proceeded in agreement with the size principle in 21 of those 27 cases (78%). This tendency toward recruitment order in agreement with the size principle for both muscle stretch and CCS afferents is significantly different from random ( $\chi^2$  test,  $P = 0.02$ ) and is consistent with three previous studies from this laboratory (Clark et al. 1993; Cope and Clark 1991; Haftel et al. 2001) showing that CCS nerve stimulation does not systematically reverse the recruitment order of cat MG motoneurons.

The effects of muscle stretch and CCS skin pinch were also compared using the rate-rate relations they evoked in each pair of motoneurons. The premise of this analysis was that the rate-rate relation for a pair of motoneurons should not be the same if the relative strengths of synaptic drive were different for muscle stretch versus CCS skin pinch. The rate-rate relations in Fig. 3 represent the range of observations made. For the motoneuron pair in Fig. 3A, the patterns of comodulation of firing rate by muscle stretch (●) and skin pinch (△) were indistinguishable over the range of rates achieved. Therefore we suggest that the distribution of net synaptic excitation onto this motoneuron pair is similar for muscle stretch and CCS skin pinch inputs. The other three panels of Fig. 3 illustrate the types of differences in firing rate modulation evoked by each input. Both sources of synaptic drive yield comodulation of the motoneuron pair illustrated in Fig. 3B, but the relations are not the same. Figure 3C illustrates a case in which firing rate is coordinately modulated by muscle stretch but not significantly so by CCS skin pinch. Relative to the effect of muscle stretch, the firing evoked by skin pinch is much more variable. Figure 3D illustrates the opposite case in which firing rates are comodulated by CCS skin pinch but not by muscle stretch. Figure 3, B–D, suggests differences in the synaptic organization of afferents activated by muscle stretch and skin pinch.

Muscle stretch and CCS skin pinch could not be compared for 11 of 35 pairs (31%) because the criterion that firing rates were correlated between the motoneurons of the pair ( $r > 0.5$ ) was not fulfilled for one or both inputs (e.g., Fig. 3, C and D). In 1 of those 11 pairs (9%), firing evoked by CCS skin pinch failed the criterion but firing due to muscle stretch passed (Fig. 3C). In 7 of 11 pairs (64%), firing evoked by muscle stretch failed the criterion but firing due to CCS skin pinch passed (Fig. 3D). In 3 of 11 pairs (27%), both inputs failed to evoke significant comodulation. Obvious rate limiting (e.g., Fig. 1C) was not the cause of data exclusion in any of these 11 pairs, and there was no significant difference in the CV differences between the 11 pairs for which rate-rate analysis was not possible and the 25 pairs for which further analysis was possible (Mann-Whitney  $U$  test,  $P = 0.19$ ).

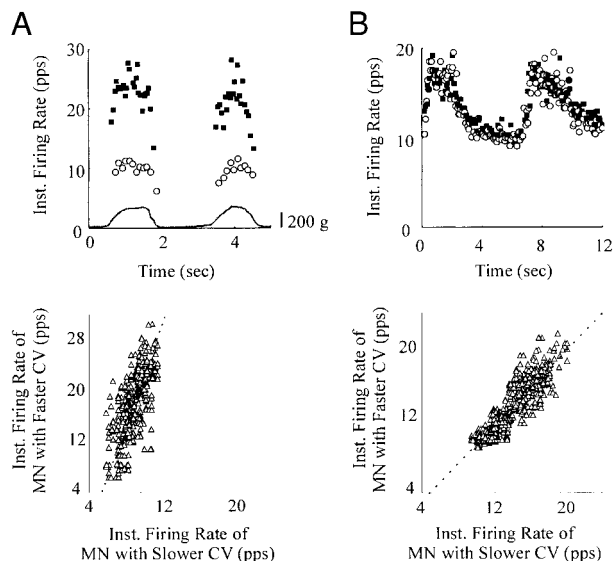


FIG. 2. Firing rate modulation for 2 motoneuron pairs recruited by skin pinch applied to the caudal cutaneous sural (CCS) nerve innervation site. Organization of A and B and symbols as described in Fig. 1. *Top*: instantaneous (Inst.) firing rates in 2 sequential trials of skin pinch (faint gray trace along  $x$  axis of A, scale bar indicates manual force applied to skin by forceps). *Bottom*: rate-rate plots for all stimulus trials [geometric mean regressions shown as dashed lines in A ( $r = 0.95$ ,  $P < 0.01$ ) and B ( $r = 0.92$ ,  $P < 0.01$ )]. Firing rate co-modulation with Spearman correlation coefficient  $r > 0.5$  was observed for 31/35 motoneuron pairs.

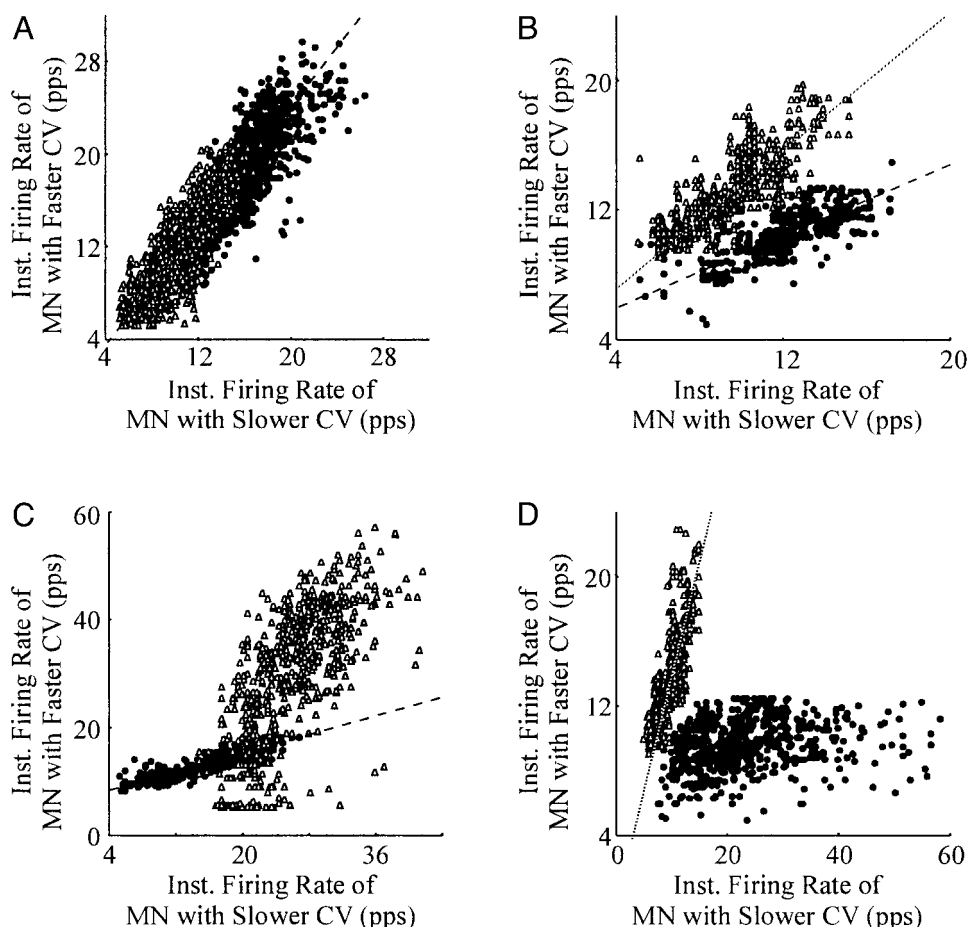


FIG. 3. Comparison of rate-rate plots evoked by muscle stretch (●) and CCS skin pinch (Δ) in 4 pairs of motoneurons. *A*: rate-rate plots for both inputs were indistinguishable over the range of rates achieved (dashed regression lines overlaid one another). *B*: rate-rate relations were correlated for each input ( $r > 0.5$ ), but the slopes were different. *C*: firing rate comodulated by muscle stretch but not by CCS skin pinch. *D*: firing rate comodulated by CCS skin pinch but not by muscle stretch.

Within the population of 24 pairs that did meet all criteria for analysis, slopes evoked by each input in the same pair were directly compared as illustrated in Fig. 4. Rate-rate slopes

elicited by muscle stretch and CCS inputs varied randomly around the line of identity. Pairs that were recruited by both inputs in the order from low to higher CV as predicted by the size principle (reviewed in Henneman and Mendell 1981) are represented by ○. Pairs that were recruited in reverse order by both inputs are represented by ●, and two pairs that were recruited in order by muscle stretch input and reverse order by CCS input are represented by +.

Considering rate-rate behavior for CV rank (Fig. 4A), the slopes elicited by muscle stretch and CCS inputs in the same pair were statistically indistinguishable by two measures. First, a paired *t*-test revealed no differences ( $P = 0.91$ ). Second, to examine the relation over the full range of firing rates, the geometric mean regression was computed. The slope of this regression is 0.96 (---), and the 95% confidence limits for that estimate (0.74–1.18) span a slope of 1. There does not seem to be any systematic difference from linearity. Thus these results demonstrate that when slopes were computed according to the CV of each motoneuron in a pair, the effects of muscle stretch and CCS inputs on firing were not systematically different across the sample of motoneuron pairs.

It is possible, however, that a difference in effect does exist between muscle stretch and CCS inputs, but it is obscured when motor units are organized by their physiological properties. As an alternative test, Fig. 4B shows the same data as A except the rate-rate slopes were computed with members of the pair are ranked by recruitment order instead of CV. As was the case for Fig. 4A, rate-rate slopes for muscle stretch and CCS

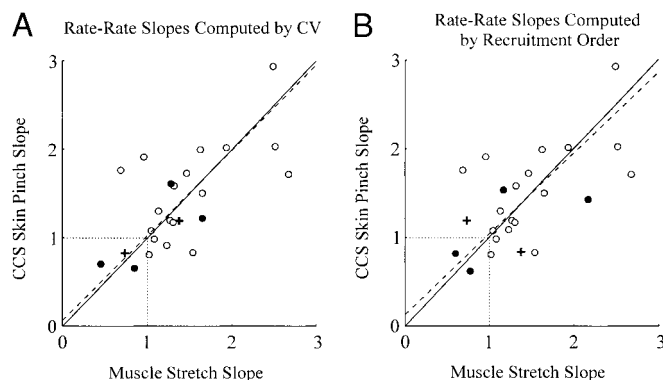


FIG. 4. Rate-rate slopes for muscle stretch and CCS skin pinch were not systematically different among motoneuron pairs. Each symbol represents a single motoneuron pair: 18/24 pairs (75%) recruited by both inputs in agreement by the size principle [low before higher axonal CV; ○]; 4/24 pairs (17%) recruited in reverse order by both inputs (●); 2/24 pairs (8%) recruited in order by muscle stretch and reversed order by CCS skin pinch (+). —, the line of identity; ---, the geometric mean regression. *A*: rate-rate slopes computed according to CV rank (faster CV on y axis, slower CV on x axis) were indistinguishable (paired *t*-test,  $P = 0.91$ ) and correlated ( $r = 0.59$ ,  $P < 0.01$ ) for muscle stretch and CCS inputs. Regression slope is 0.96. *B*: rate-rate slopes computed according to recruitment order (2nd recruited cell on y axis, 1st recruited cell on x axis) were also indistinguishable ( $P = 0.93$ ) and correlated ( $r = 0.53$ ,  $P < 0.01$ ). Regression slope is 0.91. Box formed by --- at a slope of 1 on each axis emphasizes the number of pairs in which rate-rate slope exceeded unity.



skin pinch in Fig. 4B were indistinguishable across pairs (paired *t*-test,  $P = 0.93$ ). Also similar to Fig. 4A, the regression slope for these points was 0.91 (—), and the 95% confidence limits (0.68–1.14) spanned unity. Together, these results indicate that muscle and cutaneous inputs elicited rate-rate slopes that were generally indistinguishable, regardless of how rate-rate slope was computed. Throughout the remainder of this paper, rate-rate results have been computed using CV rankings.

#### Rate-rate slope was not correlated with differences in axonal CV or recruitment time

Tansey and Botterman (1996) reported that in response to activation of MG motoneurons by descending midbrain input, the slope of rate-rate plots was closer to one for motoneuron pairs with similar excitability, as assessed by motor unit force, than for motoneurons with different excitability. This possibility was tested here using motoneuron CV and recruitment time difference as measures of cell excitability. We found that there was no trend in the present data for the 24 pairs that displayed firing rate comodulation to have smaller CV differences than the 11 pairs that failed to display comodulation (Mann-Whitney *U* test,  $P = 0.19$ ). Further, rate-rate slopes did not significantly covary with CV difference between the members of a pair (ANCOVA; Fig. 5A, *muscle stretch*: □,  $r = 0.03$ ,  $P = 0.87$ ; *CCS skin pinch*: ●,  $r = -0.11$ ,  $P = 0.61$ ). Motoneuron firing evoked by 1-s ramp stretch was used to investigate the relation between rate-rate slope and recruitment time difference in 11 motoneuron pairs (Fig. 5B), and slope did not systematically vary across differences in recruitment time ( $r = -0.25$ ,  $P = 0.45$ ). In six pairs sampled from one animal, motor-unit contractile properties were recorded along with CV of the motor axons. In these pairs, no correlation was evident between rate-rate slope and either CV difference (*muscle stretch*:  $r = 0.75$ ,  $P = 0.08$ ; *CCS skin pinch*:  $r = 0.61$ ,  $P = 0.20$ ) or difference in motor unit tetanic force (*muscle stretch*:  $r = 0.37$ ,  $P = 0.47$ ; *CCS skin pinch*:  $r = 0.77$ ,  $P = 0.07$ ). Thus the magnitude of rate-rate slope was independent of differences in both intrinsic properties, as assessed by CV difference, and motoneuron excitability, as assessed by recruitment time difference. The difference between the present results and those of Tansey and Botterman (1996) may reflect differences between descending and segmental inputs.

#### Rate-rate slope values often exceeded unity

Rate-rate slope values often exceeded unity. In 21 of the 24 pairs that met all criteria for further analysis (88%), rate-rate

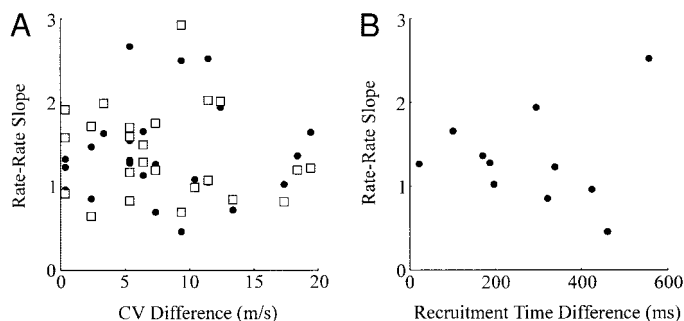


FIG. 5. Rate-rate slopes were not correlated with the differences between motoneurons in each pair for either axonal CV (A; *muscle stretch*: □,  $r = 0.03$ ,  $P = 0.87$ ; *skin pinch*: ●,  $r = -0.11$ ,  $P = 0.61$ ) or recruitment time (B, *muscle stretch*: □,  $r = -0.25$ ,  $P = 0.45$ ).

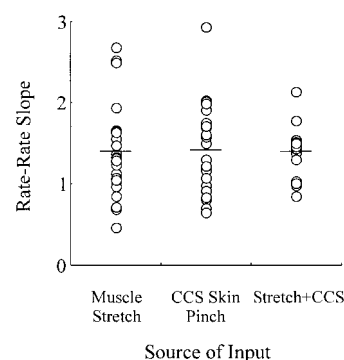


FIG. 6. Distributions of rate-rate slopes overlap for motoneuron pairs activated by muscle stretch, CCS skin pinch, and the sum of these inputs. Slopes for muscle stretch ( $n = 24$  pairs), CCS skin pinch ( $n = 24$ ), and their combination ( $n = 10$ ) were indistinguishable (ANOVA,  $F = 0.002$ ,  $P = 0.99$ ). Each pair represented as a single point in each column, and mean values indicated by horizontal bars.

slopes were  $>1$  for at least one input, and both inputs evoked slopes  $>1$  in 15 of 24 pairs (63%). In only 3 of 24 pairs (13%) did both inputs elicit slopes  $<1$ . Across all 24 pairs, the tendency for slope to be greater than one was significant (*muscle stretch*:  $1.39 \pm 0.57$ , mean  $\pm$  SD, *CCS skin pinch*:  $1.40 \pm 0.55$ , paired *t*-test,  $P < 0.01$  in both cases). A slope  $>1$  occurs when the cell with the faster CV, plotted on the y axis, modulates its firing rate more than the cell with slower CV. Such a result could be due to rate limiting, where the firing rate of one motoneuron remains nearly constant while the firing rate of another motoneuron systematically increases over the same period. To investigate the possible influence of rate limiting, each trial was visually inspected using rate-time plots like those in the top of each panel in Fig. 1. Evidence of rate limiting (e.g., Fig. 1C) was very rarely observed and was present in none of the trials used to compare muscle stretch and CCS skin pinch. Further, in 10 motoneuron pairs, firing rate comodulation was elicited by simultaneous activation of muscle stretch and CCS inputs, in addition to each input individually. In 8 of those 10 pairs, summed input generated faster mean firing rates in both motoneurons than was observed for either individual stimulus, indicating that the firing rates of those motoneurons were not rate-limited. In the remaining two pairs, the mean firing rates with summed input were approximately equal to the rates observed for either input alone. Across all 10 pairs, rate-rate slopes elicited by summed input were indistinguishable from those due to stretch input alone (paired *t*-test,  $P = 0.97$ , Fig. 6). These data indicate that the patterns of comodulation evoked by muscle stretch and CCS skin pinch were evident across a wide range of stimulus strengths and firing rates, and rate limiting was clearly not the primary cause of the tendency for slopes to be  $>1$ .

#### Motoneuron f-I relations

Aside from rate limiting, the motoneuron with faster CV modulates its firing rate more than the cell with slower CV, possibly because the faster CV cell receives more synaptic drive, possesses greater gain in its input-output relation, or both. Using intrasomatic recording, we found that motoneuron input-output gain (*f-I* slope) was not significantly correlated with either CV (Fig. 7A,  $r = -0.46$ ,  $P = 0.06$ ) or rheobase (Fig. 7B,  $r = -0.27$ ,  $P = 0.15$ ). Even if these trends had been

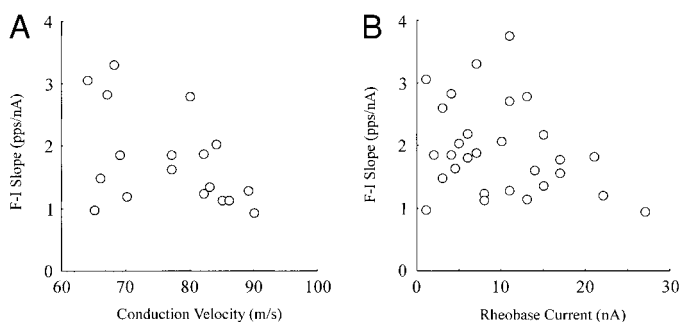


FIG. 7. Relations of axonal CV (A) and rheobase current (B) with  $f$ -I slopes among MG motoneurons in decerebrate cats.  $f$ -I slope was not significantly correlated with either CV ( $r = -0.46$ ,  $P = 0.06$ ) or rheobase current ( $r = -0.27$ ,  $P = 0.15$ ).

significant, their arrangement is the opposite of that required to generate rate-rate slopes systematically  $>1$ . To further investigate possible causes of the tendency for rate-rate slopes to exceed unity, rate-rate data were modeled using computer simulation. The results and implications of that simulation are considered further in the APPENDIX and DISCUSSION.

#### Additional analyses of firing rates

Cross-correlation of the action potentials generated by each motoneuron in a pair revealed that synchronous discharge was very rare, expressed in 1 of 24 pairs (4%) in response to muscle stretch and 2 of 24 pairs (9%) in response to CCS nerve input. In no case did both inputs evoke synchrony in the same pair. The firing rates evoked by synaptic drive from CCS and MG muscle inputs were also compared using two additional analyses, as described in the following sections.

#### "Common drive" analysis was unable to resolve differences between muscle stretch and CCS skin pinch

Analysis of "common drive" revealed that there was usually a strong shared synaptic drive affecting the two motoneurons in each pair, as expected under the present experimental paradigm. Common drive coefficients ranged from 0.31 to 0.98 ( $0.74 \pm 0.17$ , mean  $\pm$  SD). However, common drive coefficients were not related to difference in CV between the two motoneurons in each pair (*muscle stretch*:  $r = 0.04$ ,  $P = 0.87$ ; *CCS skin pinch*:  $r = 0.28$ ,  $P = 0.19$ ) or recruitment order (Mann Whitney  $U$  test, *muscle stretch*:  $P = 0.06$ ; *CCS skin pinch*:  $P = 0.41$ ) and were not significantly different for muscle stretch and CCS inputs (Wilcoxon matched pairs test,  $P = 0.35$ ). Within each stimulus type, the coefficients were not correlated with rate-rate slope (*muscle stretch*:  $r = -0.02$ ,  $P = 0.94$ ; *CCS skin pinch*:  $r = -0.07$ ,  $P = 0.73$ ). Furthermore, the coefficients for muscle stretch and CCS inputs were not correlated with each other ( $r = -0.04$ ,  $P = 0.85$ ). In summary, this analysis merely confirmed that the low-frequency stimulation provided via muscle stretch or CCS inputs drove the two motoneurons in concert but provided no additional insight into the synaptic organization of these inputs across the population of MG motoneurons.

#### Coherence spectra typically exhibited significant peaks at low frequencies

Significant coherence between the spike trains of two motoneurons was common at low frequencies (up to 4–6 Hz), especially for the muscle stretch stimulus. An example is

shown in Fig. 8A. There was a weak but significant correlation between coherence in the 0- to 2-Hz band for muscle stretch and CCS skin pinch ( $r = 0.34$ ,  $P < 0.05$ ). Coherence in this frequency band was also correlated with the common drive coefficients for each input (*muscle stretch*:  $r = 0.78$ ,  $P < 0.001$ ; *CCS skin pinch*:  $r = 0.59$ ,  $P < 0.01$ ). In particular, 20 of 24 pairs (83%) activated by stretch and 11 of 24 pairs (46%) activated by CCS stimulus showed significant peaks in the frequency band from 0 to 2 Hz. The incidence of significant coherence in the 0- to 2-Hz band is significantly lower for CCS skin pinch than it is for muscle stretch ( $\chi^2$  test,  $P < 0.001$ ). This difference is probably due to characteristics of stimulus presentation that were present during muscle stretch input but not during CCS skin pinch. For example, muscle stretch was administered rhythmically (0.35–0.70 Hz) using a computer-controlled servomotor. In contrast, CCS input was activated using manual pinch applied to the skin at variable intervals. Therefore this difference, although significant, likely reflects characteristics of stimulus presentation for muscle stretch inputs, rather than differences in the synaptic organization of these inputs.

Other significant coherence peaks at higher frequencies (8–38 Hz) were occasionally observed for both stimuli, but they were generally present only as single frequency bins, and they were absent in analyses using slightly shifted spectral bandwidth. These additional peaks probably represent the 5% of values expected to exceed the 95% confidence level by chance alone. An unusual case is shown in Fig. 8B, where a large, robust peak in coherence centered around 10 Hz was prominent over roughly half of a 31-s recording of firing in response to CCS stimulus. The other records that showed significant higher-frequency peaks over part or all of the trial included all three of the records exhibiting discharge synchrony.

#### DISCUSSION

The present study was designed to test the mechanism proposed to underlie a hypothetical motor control scheme. In this scheme, cutaneous stimulation preferentially excites motor units that contract quickly and forcefully over those that contract more slowly and weakly. This arrangement is widely proposed as a mechanism by which cutaneous feedback might facilitate tasks requiring rapid and forceful muscle contraction (Burke 1991; Floeter 1999; Ghez 1991; Grimby and Hannerz 1977; Kanda et al. 1977; Kernell and Hultborn 1990; Nardone et al. 1989; Nielsen and Kagamihara 1993; Rothwell 1994; Windhorst et al. 1991). Because supporting evidence is inconsistent across studies of both humans and cats (Clark et al.

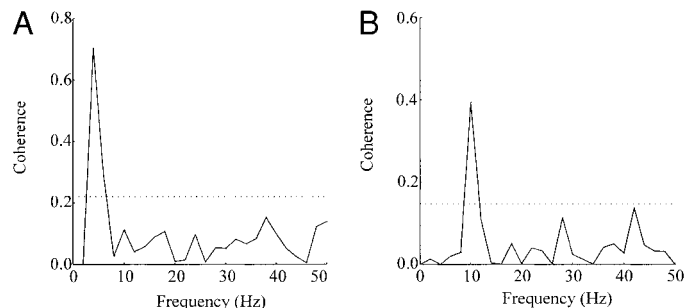


FIG. 8. Examples of significant peaks in coherence spectra. In each panel, the dotted line indicates the 95% confidence level for unrelated signals. A: coherence spectrum for 2 motor units brought to fire by muscle stretch; significant peak from 1 to 4 Hz. B: example of significant peak at higher frequency (10 Hz).



1993; Garnett and Stephens 1981; Gossard et al. 1994; Haftel et al. 2001; Kanda and Desmedt 1983; Kanda et al. 1977; Nielsen and Kagamihara 1993; Stein and Bertoldi 1981), a new experimental approach was taken here to study motor unit pairs in the MG muscle of acutely decerebrated cats. Possible uniqueness in the modulation of motor unit firing rate by CCS nerve activation was assessed through comparison with firing modulation by muscle stretch, a stimulus that excites motoneurons in the usual pattern, where the slow, weak-contracting motor units are excited more strongly than the fast, forceful ones as described by the size principle (Henneman et al. 1965; reviewed in Binder et al. 1996). The results show that modulation of motor unit firing rate by these muscle and cutaneous sources was not statistically distinguishable for most pairs of MG motor units, and the remaining pairs that did exhibit differences in response to these two inputs were not distinguishable by their CV or recruitment order. From these observations we conclude that the unusual motor control scheme described in the preceding text, if it does occur during normal movement (see Cope and Clark 1995), is not an obligatory consequence of cutaneous afferent excitation of motoneurons.

On first inspection, similarity in the input-output relations described here for muscle stretch and skin pinch stimuli seems incompatible with earlier descriptions of the synaptic organization of these inputs. Several studies of subliminal potentials recorded intracellularly from MG motoneurons in cat demonstrate clear differences in synaptic organization: the largest excitatory postsynaptic potentials (EPSPs) are produced by group Ia afferents in motoneurons with the slowest CV (Burke et al. 1976; Fleshman et al. 1981; Harrison and Taylor 1981; Heckman and Binder 1988) and by CCS afferents in motoneurons with the fastest CV (Burke et al. 1970; Kanda et al. 1977; Pinter et al. 1982; Powers and Binder 1985). Even the different patterns observed in a few reports on the distribution of CCS postsynaptic potentials among MG motoneurons (LaBella et al. 1989; Rosenberg 1970) are unlike those consistently observed for Ia postsynaptic potentials. Recent studies give clear evidence, however, of factors that might make each of these inputs more uniform among MG motoneurons. For Ia afferent input to motoneurons, Mendell and colleagues (1990) show that EPSPs that are large at low presynaptic firing frequencies grow smaller while those that are small at low frequencies are enlarged as frequency is increased during repetitive firing at rates in the physiological range. Furthermore, the postsynaptic potentials produced by CCS afferents in MG motoneurons with slow CV change from inhibitory to excitatory during repetitive stimulation (Heckman et al. 1992). These studies suggest that the synaptic organization is more uniform among MG motoneurons for Ia and CCS inputs when studied under the more physiologically relevant conditions of repetitive firing at frequencies exceeding 10–20 Hz than it is when examined with low-frequency stimulation.

#### *Correlated firing among motoneuron pairs*

Our primary method of examining the distribution of net excitatory drive onto MG motoneurons was to compare the instantaneous firing rates of motoneuron pairs. The firing rates of two motoneurons should covary in the same way for all sources of synaptic input that distribute net synaptic excitation in the same way. Conversely, the rate-rate relations should be different when there is a substantial difference in distribution

of net excitatory drive of the sort expected for muscle stretch versus skin pinch afferents. In these determinations, the effects of motoneuron excitability on rate modulation can be ignored, assuming that the contribution from the motoneuron is the same across different synaptic inputs. This assumption is well supported in the report by Prather et al. (2001) showing that MG motoneurons in the decerebrate cat increment firing rate by the same amount in response to equal amounts of synaptic current produced by stimulation of CCS and group Ia afferents. In addition, motoneurons did not reach maximum firing rate in response to either cutaneous or muscle afferent input, so rate limiting (see Monster and Chan 1977) was not a factor in determining the rate-rate relations studied here. Finally, it was very unlikely that firing rates were influenced by widespread induction of plateau potentials (reviewed in Kiehn and Eken 1998) that would result in a sudden increment in the firing rate of the affected motoneuron, apparent as a discontinuity in the rate-rate plot. Only one case of such a discontinuity was observed (Fig. 9), indicating that our results were not influenced by activation of plateau potentials during firing. Therefore the rate-rate relation provides an assessment of the distribution of synaptic input.

Limits in the ability of the rate-rate relation to resolve different patterns of synaptic drive were not determined here. We cannot rule out the possibility that unique patterns in the synaptic drive from cutaneous versus muscle afferent sources went undetected by the rate-rate analysis, although detection of differences in some cases establishes that the technique is not insensitive. Notwithstanding the ability of the rate-rate analysis to detect differences in synaptic organization, the utility of this analysis lies in its assessment of the functional consequence of synaptic input from different sources. Measurement of the motoneuron firing response to synaptic input provides the most direct and functionally relevant measure of the motor pool's input-output relation. Thus two synaptic inputs can be judged functionally indistinguishable with respect to their effects on motor pool activation if the rate-rate relations for motoneuron pairs are the same for the two inputs.

Additional analyses of shared firing behavior among motoneurons also revealed no differences in the functional organization of muscle stretch and CCS inputs. Large common drive coefficients and significant low-frequency coherence peaks were often evident in response to both inputs but were likely related to characteristics of stimulus presentation (e.g.,

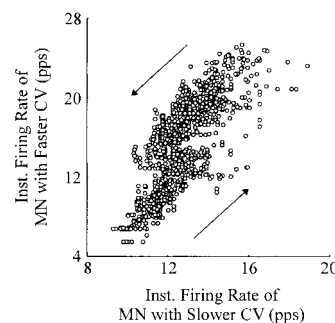


FIG. 9. Discontinuity in rate-rate plot for 1 motoneuron pair recruited by CCS skin pinch. Firing rates of the 2 motoneurons increased together as pinch intensity increased ( $\uparrow$ ). Around 14 pps, the motoneuron (MN) with faster axonal CV shifted to higher firing rates and continued to co-modulate rate with the other motoneuron over the remainder of increased and decreased ( $\downarrow$ ) stimulus intensity.

the repetition rate of the stimulus) rather than features of synaptic input organization. At higher firing frequencies, the incidence of coherence peaks was very low (cf. Marsden et al. 1999) and was not obviously different between muscle stretch and CCS stimuli. Perhaps these higher frequency peaks reflect the transient activity of neurons that affect both motoneurons of the pair at some dominant frequency, as suggested by the expression of high-frequency coherence (8–38 Hz) in all three of the records that showed clear discharge synchrony. Together, these results lend support to our previous conclusion that the functional organizations of homonymous muscle stretch and CCS inputs are indistinguishable across MG motoneurons.

#### *Interpretation of rate-rate slopes systematically greater than unity*

Rate-rate slopes were significantly greater than unity in response to either muscle stretch or skin pinch stimuli. Slopes greater than unity indicate that the firing rates of less excitable motoneurons are modulated more than rates of more excitable cells. Intracellular recordings revealed that this pattern of differential rate modulation was not due to systematic differences in input-output gain ( $f$ - $I$  slopes) across cells (Fig. 7, *A* and *B*). However, it was not clear whether  $f$ - $I$  slopes might nonetheless have some systematic effect on the pattern of coordinated firing rate modulation expressed by a pair of motoneurons. In addition, synaptic current amplification (Lee and Heckman 2000; Prather et al. 2001) can modulate the effects of synaptic input on repetitive firing. The term amplification refers to the enhancement of synaptic current generated by a stimulus during repetitive firing compared with the current generated by the same stimulus at the resting potential (Prather et al. 2001). Amplification of both group Ia and CCS input during repetitive firing is very common across MG motoneurons in decerebrate cats, and studies of repetitive firing have indicated that the magnitude of amplification is not systematically different across MG motoneurons (Prather et al. 2001). However, studies performed using voltage clamp have suggested that the magnitude of amplification is not uniform across MG cells with less excitable motoneurons expressing greater amplification (Lee and Heckman 2000). Therefore computer simulations of motoneuron firing rate were used to determine if  $f$ - $I$  slopes and possible differential expression of amplification could account for the tendency for rate-rate slopes to be greater than unity (see APPENDIX for details of the model).

Computer simulations revealed that in response to equal synaptic excitation to all motoneurons, a random arrangement of  $f$ - $I$  slopes yielded an average rate-rate slope of  $1.19 \pm 0.66$  (mean  $\pm$  SD). This average slope was significantly greater than unity but could not account for the experimentally observed slopes of  $1.39 \pm 0.57$  for stretch input and  $1.40 \pm 0.55$  for CCS input (all statistical comparisons reported in the APPENDIX). Simulation of the effects of systematic differences in amplification current also revealed an average model slope ( $1.14 \pm 0.27$ ) that significantly exceeded unity. However, this effect was also insufficient to explain experimentally observed rate-rate slopes. When simulations were performed to consider the combined effects of  $f$ - $I$  slopes and amplification, the average model slope ( $1.33 \pm 0.30$ ) was significantly greater than unity and was indistinguishable from experimental results for

either muscle stretch or CCS input. Therefore these model results indicate that the combined influences of variance in  $f$ - $I$  slopes and amplification currents are sufficient to account for the tendency for experimental slopes to be greater than unity. The results of these simulations are consistent with the idea that both muscle stretch and CCS inputs have uniform synaptic strengths across all motoneurons of the MG motor nucleus.

The results of these simulations reconcile our experimental results involving rate modulation and recruitment. Greater rate modulation in less excitable motoneurons could have been interpreted as an indication that the less excitable cell received greater synaptic excitation. This would lead to the prediction that threshold might be more easily achieved in less excitable cells, resulting in a recruitment sequence opposite that predicted by the size principle. Many authors have considered the implications of an input arrangement that would lead to systematically size-reversed recruitment (e.g., Burke 1991; Garnett and Stephens 1981; Grimby and Hannerz 1977; Kanda et al. 1977; Pinter et al. 1982; Windhorst et al. 1991). However, the sequence of recruitment reported here was generally in agreement with the size principle, as has been reported previously for these inputs (Clark et al. 1993; Cope and Clark 1991; Haftel et al. 2001). The present simulations reveal that greater synaptic excitation onto less excitable motoneurons is not necessary to account for rate-rate slopes greater than unity. The observed pattern of rate modulation is consistent with synaptic input that is uniformly excitatory but not systematically arranged across MG motoneurons.

#### *Earlier studies of comodulation of motoneuron firing*

Comodulation of firing rates has been observed in humans performing voluntary movements using muscles in the hand (DeLuca et al. 1982; Monster 1979; Monster and Chan 1977) and arm (DeLuca et al. 1982; Kanosue et al. 1979) and in cats during brain-stem-evoked contraction of the MG muscle (Tansey and Botterman 1996). In electromyographic recordings from single motor units in human extensor digitorum communis muscle (EDC), coordinated modulation of firing rate among motoneurons spanned a range of firing rates very similar to that reported in these experiments (approximately 5–25 pps) (see Monster and Chan 1977). When examined in a rate-rate plot, the higher threshold EDC motor unit generally increased its firing rate more rapidly than the lower threshold unit, yielding an average rate-rate slope of  $1.4 \pm 0.2$  (Monster and Chan 1977). Here we report rate-rate slopes of  $1.39 \pm 0.57$  for muscle stretch input and  $1.40 \pm 0.55$  for CCS input. These values can be simulated quite well using a model involving equal synaptic input onto every motoneuron, random variance in  $f$ - $I$  slope and systematic variance in amplification current across the motoneuron pool (average model slope  $1.33 \pm 0.30$ , see APPENDIX for details). The agreement between rate-rate data sampled from reflexively driven motoneurons of the decerebrate cat and data sampled from single motor units during volitional finger movement in humans (Fig. 10) suggests that the processes underlying synaptic integration may be similar in the two conditions. Tansey and Botterman (1996) report that rate-rate slopes evoked by stimulation of the midbrain locomotor region that apparently have greater variance than those of this and other studies. However, this difference may arise from activation of undetermined brain stem locations. Finally, it is interesting to note that in the same report in which human EDC rate-rate slopes were

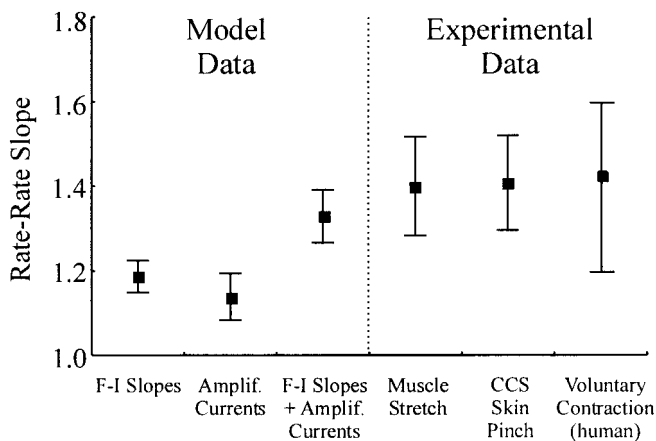


FIG. 10. Rate-rate slopes obtained from computer simulation (model data) and from measurements made in vivo (experimental data). For model data (see APPENDIX), synaptic currents were uniformly distributed and rate-rate slopes [means (filled squares)  $\pm$  SD (bars)] were computed for motoneurons having variable  $f$ - $I$  slopes (column 1), amplification (amplif.) currents (column 2), or both (column 3). The rate-rate slopes simulated by varying both  $f$ - $I$  and amplification currents (column 3) were not significantly different (Kruskal-Wallis ANOVA,  $\chi^2$  statistic,  $P = 0.85$ ) than the rate-rate slopes observed in vivo for muscle stretch (column 4) or for skin pinch (column 5) obtained in the present study. Data are shown in column 6 for rate-rate slopes obtained during voluntary contraction for human extensor digitorum communis muscle motor units (Monster and Chan 1977).

like those observed here (Monster and Chan 1977), the rate-rate correlation coefficient between EDC motor units was greater than 0.9 in 80% of trials. The correlation coefficient in the present data were greater than 0.9 in only 1/24 (4%) trials for muscle stretch and 4/24 (17%) trials for CCS input. Perhaps this difference in correlation quality reflects differences in the variance of input magnitude between descending volitional inputs and segmental reflex inputs (Gydikov and Kosarov 1973; Monster and Chan 1977; reviewed in Binder et al. 1996).

#### Implications for movement control

Skeletal muscles are composed of motor units that collectively express a wide range of physiological properties, represented as multiple types of motor units. This observation leads to the reasonable and widely accepted proposal that motor unit types within muscles might be differentially activated to achieve motor tasks with differing demands (see opening discussion). However, our studies identify only one systematic scheme in the activation of the physiologically diverse motor units in the medial gastrocnemius muscle of the decerebrate cat. We show here that the majority of MG motor units increase and decrease firing rate in a coordinated fashion that is not systematically different in response to synaptic drive from muscle stretch and skin pinch. Those motor unit pairs that did exhibit differences in firing rate co-modulation, and even those that failed to express co-modulation were not distinguishable on the basis of physical (axonal CV) or behavioral (recruitment order) properties. We extrapolate from these observations to hypothesize that systematic activation of motor unit types in reversed order of that predicted by the size principle is not a strategy used by the motor system to control movement. We suggest instead that the motor system regulates the parameters of limb and body movements by varying the participating motor units but not the relative activity levels among motor units of an ensemble.

#### APPENDIX

The purpose of this simulation was to evaluate whether variance in the frequency-current gain ( $f$ - $I$  slope) and the magnitude of synaptic amplification current expressed among MG motoneurons could account for the tendency for rate-rate slopes to be systematically greater than one, as reported in RESULTS. In all these simulations, each motoneuron received the same magnitude of synaptic current from muscle stretch and CCS inputs. In the absence of any differences between motoneurons, such an input arrangement would evoke the same firing rate modulation in every cell, resulting in rate-rate relations with slope of one in every pair. However, each model motoneuron was not the same in its intrinsic influence on firing; motoneurons expressed variance in both their  $f$ - $I$  relations and the synaptic amplification currents that they generated. These simulations investigate whether a random arrangement of  $f$ - $I$  slopes can account for the observed tendency for rate-rate slopes to be greater than one. Lee and Heckman (2000) showed that synaptic amplification current is greater in large motoneurons than in small ones as determined through measurements of input conductance. This difference could account for the observed tendency for the cell with faster CV in each pair to modulate its firing rate more than the cell with slower CV, resulting in rate-rate slopes greater than one. The effects of variance in amplification currents and  $f$ - $I$  slopes are considered separately and together in the following simulations.

#### Variance in $f$ - $I$ slopes generated rate-rate slopes greater than unity

The pool of simulated motoneurons consisted of 30 motoneurons. This number was chosen because 30  $f$ - $I$  relations were recorded experimentally (range: 0.94–3.76 pps/nA, see Fig. 7B), and each was represented uniquely in the model. Each model motoneuron was assigned an observed  $f$ - $I$  slope and was randomly grouped into 1 of 15 pairs. Because model motoneurons were assigned no intrinsic properties other than  $f$ - $I$  slope and  $f$ - $I$  slope is not systematically arranged with respect to either CV or rheobase current, each motoneuron was randomly designated to be the cell with faster or slower CV in its respective pair. Because each model motoneuron received the same amount of synaptic input, the firing rate expressed by each cell was specified entirely by its  $f$ - $I$  slope. This exclusion of variance in  $f$ - $I$  intercept is valid because the intent is to model rate-rate slopes expressed between motoneurons, and rate-rate slope is dependent only on changes in firing rate ( $f$ - $I$  slope) and not on the magnitude of rates expressed (intercept). Model rate-rate slopes were computed by dividing the  $f$ - $I$  slope of the faster CV motoneuron by the  $f$ - $I$  slope of the slower CV motoneuron. One simulation was performed with the pairing of  $f$ - $I$  slopes described in the preceding text, then the values were randomly grouped into new pairs, and the simulation was performed again. This sequence was repeated to generate a total of 20 simulated rate-rate slopes in each of the 15 pairs of model motoneurons.

Considering the effects of  $f$ - $I$  slopes alone, the average model rate-rate slope was  $1.19 \pm 0.66$  (mean  $\pm$  SD), and the 95% confidence interval for that mean did not include one (1.11–1.26). The average experimental slope was  $1.39 \pm 0.57$  for stretch and  $1.40 \pm 0.55$  for CCS input. When rate-rate slopes were compared between model and experimental data, differences were evident (Kruskal-Wallis ANOVA,  $\chi^2$  statistic,  $P = 0.04$ ). Further analysis revealed that while experimental rate-rate slopes evoked by muscle stretch and CCS inputs are not different (Mann-Whitney  $U$  test,  $P = 0.97$ ), model slopes are different from those of either input alone (muscle stretch:  $P = 0.03$ ; CCS:  $P = 0.02$ ). Together, these data reveal that random variance in  $f$ - $I$  slopes generates rate-rate slopes that are systematically greater than 1, but this trend is insufficient to account for the magnitude of experimentally observed rate-rate slopes.

The tendency for model rate-rate slopes to exceed unity is a numerical consequence of computing rate-rate slopes using ratios of randomly paired  $f$ - $I$  slopes. The ratio of two  $f$ - $I$  slopes can be either greater than unity or less than unity; given the random pairings used in these simulations each should occur with equal frequency. Thus the final set of



rate-rate slopes should consist of an approximately equal number of rate-rate slopes greater than unity and less than unity. However, in the condition where the ratio (rate-rate slope) is greater than one, the magnitude of the ratio is more different from unity than is its reciprocal. This is true for any two separate positive  $f$ -I slopes ( $X$  and  $Y$ ) because

$$(X/Y) - 1 > 1 - (Y/X)$$

as can be derived from

$$(X - Y)(X - Y) > 0$$

Therefore repeated generation of rate-rate slopes using randomly paired  $f$ -I slopes will result in an average model slope that is systematically greater than unity.

#### *Variance in amplification currents generated rate-rate slopes greater than unity*

In this simulation, the pool of model motoneurons consisted of 48 cells grouped into 24 pairs. This number was chosen because 24 motoneuron pairs passed all criteria for analysis in the accompanying RESULTS. Each model motoneuron was assigned the CV value of a cell in the experimental group, with each cell uniquely represented. An input CV was assigned to each model cell based on its CV value and a perfectly linear correlation between input conductance and CV (cf. Zengel et al. 1985). The input conductance of each model motoneuron was used to assign it a magnitude of synaptic amplification current (Lee and Heckman 2000). Each of the 48 model cells contributed some amount of intrinsic amplification current, ranging from 10 nA (motoneuron with CV of 70 m/s) to 13.72 nA (motoneuron with CV of 107 m/s). The increment in firing rate generated by this current (range: 19.1–26.2 pps) was computed by multiplying the simulated amplification current by 1.91 pps/nA, the average  $f$ -I slope recorded experimentally (see Fig. 7). Rate-rate slopes were computed by dividing the range of rates spanned by the cell with faster CV by the range of rates spanned by the cell with slower CV.

Considering the effects of amplification alone, the average model rate-rate slope was  $1.14 \pm 0.27$ , and the 95% confidence interval for that mean did not include one (1.02–1.25). The modeled and observed slopes were significantly different for muscle stretch input (Mann-Whitney  $U$  test,  $P = 0.04$ ) but not for the CCS input ( $P = 0.06$ ). However, the  $P$  value for CCS input is not far from the level of significance ( $P = 0.05$ ), and the mean observed slope exceeded that of the modeled data for both inputs. Therefore assuming equal synaptic drive to each motoneuron, greater amplification in faster CV motoneurons than in slower CV cells cannot fully account for the observed tendency for rate-rate slopes to be greater than one.

#### *Inclusion of both $f$ -I slopes and amplification generated rate-rate slopes indistinguishable from experimental data for muscle stretch and CCS inputs*

The parameters of this simulation were identical to those used to consider the influence of amplification alone except  $f$ -I slopes were also included in this analysis. The rate-rate slope of each pair due to differences in amplification currents was computed in the same way as when amplification was considered alone.  $f$ -I slopes were randomly assigned to each motoneuron from the collection of 30  $f$ -I values observed experimentally. Because 30  $f$ -I slopes were distributed to 48 motoneurons,  $f$ -I slopes were not uniquely represented. In each simulation, each experimental  $f$ -I slope was assigned to one of the first 30 simulated motoneurons. Then the  $f$ -I slopes were randomized and the first 18  $f$ -I slopes were assigned to the remaining 18 motoneurons. In this way, 18  $f$ -I slopes were represented twice in each simulated motoneuron pool; however, the values that were repeated and the arrangement of those values were random in each simulation. In each pair,  $f$ -I slopes were randomly assigned to each motoneuron in 20 separate trials. The rate-rate slope of each trial due to differences in  $f$ -I slope was computed by taking

the ratio of the  $f$ -I slope in the motoneuron with faster CV divided by that of the motoneuron with slower CV.

Simulation of variance in  $f$ -I slopes was performed multiple times, while the arrangement of amplification currents across the 24 model pairs remained fixed in each simulation. This is valid given the clear relation between motoneuron properties and amplification magnitude (Lee and Heckman 2000) and the absence of any relation between motoneuron properties and  $f$ -I slope. In each pair, the rate-rate slope due to amplification alone was multiplied by the slope due to  $f$ -I slopes alone for each of 20 trials. The result was a rate-rate slope for each trial that represented the influence of both  $f$ -I slopes and amplification currents for a model system where synaptic input was equal to all cells. At the conclusion of this analysis, a single rate-rate slope, generated by computing the average slope of the 20 trials run using different  $f$ -I slopes, was computed to represent each of the 24 pairs of model motoneurons. The collection of these 24 representative rate-rate slopes was then compared against the experimentally observed rate-rate slopes for muscle stretch and CCS inputs.

Considering the combined effects of variance in  $f$ -I slopes and amplification currents, the average model rate-rate slope was  $1.33 \pm 0.30$ , and the 95% confidence interval for this mean did not include one (1.20–1.46). Rate-rate slopes were indistinguishable between simulated and experimental data for muscle stretch and CCS inputs (Kruskal-Wallis ANOVA,  $\chi^2$  statistic,  $P = 0.85$ ). Further, model slopes are not different from those of either individual input (muscle stretch:  $P = 0.81$ ; CCS:  $P = 0.77$ ). Therefore these data indicate that the combined influences of variance in  $f$ -I slopes and amplification currents are sufficient to account for experimental observations (Fig. 10).

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