

# Recruitment of Cat Motoneurons in the Absence of Homonymous Afferent Feedback

VALERIE K. HAFTEL,<sup>1</sup> JONATHAN F. PRATHER,<sup>1</sup> C. J. HECKMAN,<sup>2</sup> AND TIMOTHY C. COPE<sup>1</sup>

<sup>1</sup>*Department of Physiology, Medical School, Emory University, Atlanta, Georgia 30322; and* <sup>2</sup>*Department of Physiology, Northwestern University Medical School, Chicago, Illinois 60611*

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**Haftel, Valerie K., Jonathan F. Prather, C. J. Heckman, and Timothy C. Cope.** Recruitment of cat motoneurons in the absence of homonymous afferent feedback. *J Neurophysiol* 86: 616–628, 2001. This study provides the first test in vivo of the hypothesis that group Ia muscle-stretch afferents aid in preventing reversals in the orderly recruitment of motoneurons. This hypothesis was tested by studying recruitment of motoneurons deprived of homonymous afferent input. Recruitment order was measured in decerebrate, paralyzed cats from dual intra-axonal records obtained simultaneously from pairs of medial gastrocnemius (MG) motoneurons. Pairs of MG motor axons were recruited in eight separate trials of the reflex discharge evoked by stimulation of the caudal cutaneous sural (CCS) nerve. Some reports suggest that reflex recruitment by this cutaneous input should bias recruitment against order by the size principle in which the axon with the slower conduction velocity (CV) in a pair is recruited to fire before the faster CV axon. Recruitment was studied in three groups of cats: ones with the MG nerve intact and untreated (UNTREATED); ones with the MG nerve cut (CUT); and ones with the MG nerve cut and bathed at its proximal end in lidocaine solution (CUT+). The failure of electrical stimulation to initiate a dorsal root volley and the absence of action potentials in MG afferents demonstrated the effective elimination of afferent feedback in the CUT+ group. Recruitment order by the size principle predominated and was not statistically distinguishable among the three groups. The percentage of pairs recruited in reverse order of the size principle was actually smaller in the CUT+ group (6%) than in CUT (15%) or UNTREATED (19%) groups. Thus homonymous afferent feedback is not necessary to prevent recruitment reversal. However, removing homonymous afferent input did result in the expression of inconsistency in order, i.e., switches in recruitment sequence from one trial to the next, for more axon pairs in the CUT+ group (33%) than for the other groups combined (13%). Increased inconsistency in the absence of increased reversal of recruitment order was approximated in computer simulations by increasing time-varying fluctuations in synaptic drive to motoneurons and could not be reproduced simply by deleting synaptic current from group Ia homonymous afferents, regardless of how that current was distributed to the motoneurons. These findings reject the hypothesis that synaptic input from homonymous group Ia afferents is necessary to prevent recruitment reversals, and they are consistent with the assertion that recruitment order is established predominantly by properties intrinsic to motoneurons.

## INTRODUCTION

A stereotypical sequence of activation is observed among the motor units that are grouped together in an ensemble (Cope and Sokoloff 1999). The member motor units follow one another in progression, for example, from the weaker to the

stronger and from the slower to the faster contracting units (Binder et al. 1996; Burke 1981a; Cope and Clark 1995; Henneman and Mendell 1981; Stuart and Enoka 1983). This recruitment order, named the size principle (Henneman et al. 1965a), must emerge from systematic variation in the integration of synaptic input by the motoneuron component of motor units. However, the relative importance of the various pre- and postsynaptic contributors to recruitment order remains unclear despite extensive study and discussion (Binder et al. 1996; Burke 1981b; Cope and Pinter 1995; Pinter 1990).

Recruitment order depends to some extent on properties intrinsic to motoneurons. The most persuasive evidence comes from behavioral studies demonstrating that motor units recruited into activity are recruited in the same order by a wide variety of synaptic inputs (Henneman et al. 1965b; Somjen et al. 1965; reviewed by Henneman and Mendell 1981). For example, motor units belonging to the medial gastrocnemius (MG) muscle in decerebrate cats are recruited in the same sequence when activated in muscle stretch reflexes as they are when activated in a cutaneous reflex (Clark et al. 1993; Cope and Clark 1991). The common element underlying this conforming behavior in the face of dissimilar synaptic inputs is the motoneuron itself. Exactly which combination of intrinsic properties might establish the hierarchy in motoneuron excitability, whether the motoneuron's physical size, voltage threshold, and/or membrane resistivity, has not been definitively established (reviewed by Binder et al. 1996; Burke 1981a; Cope and Pinter 1995; Pinter 1990). Nonetheless, biophysical studies of barbiturate-anesthetized cats demonstrate that the intrinsic excitability of motoneurons, measured as current threshold, does covary, although imperfectly, with those motor unit properties that predict recruitment order (e.g., axonal conduction velocity and tetanic force) (Fleshman et al. 1981; Gustafsson and Pinter 1984). The inference drawn from these kinds of correlations is that orderly recruitment traces back, at least in part, to variation in the intrinsic excitability of motoneurons.

It is also evident that synaptic input has an important role in the recruitment process. First, synaptic input is required for recruitment and determines which motoneurons are selected into activity (Cope and Sokoloff 1999). Second, synaptic input has the potential to reverse the usual order in which motoneu-

Address for reprint requests: V. K. Haftel, Dept. of Physiology, 1648 Pierce Dr., Emory University, Atlanta, GA 30322 (E-mail: valh@physio.emory.edu).

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rons are recruited (Clamann et al. 1983; Davies et al. 1993; Masakado et al. 1991; Nielsen and Kagamihara 1993). For example, Garnett and Stephens (1980) reported that the order in which motor units in the human hand are recruited during voluntary contractions can be reversed by prolonged electrical stimulation of the skin. The question addressed in the present study is whether there is a third role, one in which synaptic input actually contributes to establishing or preserving recruitment order.

Results of computer simulations reported by Heckman and Binder (1993) provide an assessment of the relative contributions of intrinsic motoneuron properties and synaptic input to orderly recruitment. These investigators developed a computer model using data collected from MG motoneurons in barbiturate-anesthetized cats to test how synaptic current from different afferent sources influences recruitment order. Synaptic current from homonymous group Ia afferents was distributed among MG motoneurons such that motoneurons with low current threshold received greater excitatory current than those with higher thresholds (Heckman and Binder 1988). When introduced into the model, synaptic input from Ia afferents expanded the range of recruitment thresholds and reinforced recruitment order by the size principle. By contrast, synaptic current from the rubrospinal tract compressed the range in recruitment threshold by distributing excitation more strongly to motoneurons with intrinsically high thresholds than to motoneurons with lower thresholds (Powers et al. 1993). In the model, the rubrospinal input increased the number of reversals in recruitment order, but the addition of group Ia input counteracted this effect and sharply reduced the incidence of recruitment reversals. Based on these findings, Heckman and Binder (1993) hypothesized that "... one major role of the monosynaptic Ia afferent system is to preserve orderly recruitment across a wide variety of input combinations ..." (see also Grimby and Hannerz 1968, 1976; Harrison and Taylor 1981; Heckman and Binder 1990; Stein and Bertoldi 1981). The present study tests this hypothesis with data collected in vivo from the cat.

One test of this hypothesis is afforded by studying the recruitment order of motoneurons in the reflex initiated by stimulation of the caudal cutaneous sural (CCS) nerve in the decerebrate cat. Sural nerve stimulation, while able to differentially alter ongoing firing of MG motoneurons (Kanda et al. 1977), fails to disturb the usual sequence of recruitment order (Clark et al. 1993; Cope and Clark 1991). This failure is surprising given evidence that sural nerve input is distributed to MG motoneurons in much the same manner as that described above for the rubrospinal input (Burke et al. 1970; Pinter et al. 1982; however see LaBella et al. 1989). One possible explanation for this surprising result (Binder et al. 1996) is that Ia synaptic activity, which is generally present in intact animals and particularly high in decerebrate cats (Matthews 1972), acts to suppress recruitment reversals just as in the simulations and in accord with the hypothesis of Heckman and Binder (1993). This possibility was tested here in decerebrate cats for MG motoneurons recruited by sural nerve stimulation under the condition in which the MG nerve was cut and treated with lidocaine. This nerve treatment was found to completely eliminate homonymous afferent feedback but did not reduce the proportion of motoneuron pairs recruited in order from low to high axonal conduction velocity (CV), as predicted by the size

principle and found with the MG nerve intact. This finding demonstrates that the basic structure of orderly recruitment does not rely on homonymous group Ia afferent input. However, the increased number of pairs that demonstrate trial-to-trial switches in recruitment order suggests a role for homonymous afferents in stabilizing motoneuron excitability. Portions of these results were presented previously in abstract form (Haftel et al. 1997, 1998).

## METHODS

### *Surgical preparation*

Experiments were performed on 17 male and female cats (2.5–5 kg) as approved by the Emory University Institutional Animal Care and Use Committee. The surgical and recording procedures briefly described here were similar to those detailed in previous reports from this laboratory (e.g., Cope and Clark 1991; Dacko et al. 1996). Animals were anesthetized throughout surgery by a gaseous mixture of halothane in 1:1 O<sub>2</sub> and NO<sub>2</sub>, with the halothane level adjusted between 1 and 2.5% as required to completely suppress withdrawal reflexes. The volume and rate of artificial respiration were adjusted to maintain end-tidal CO<sub>2</sub> between 3 and 4%. Mean blood pressure was monitored via carotid artery catheter, and maintained above 70 mmHg as needed either by intravenous infusion of Ringer solution or, on a few occasions, by a sympathomimetic amine (Aramine). Radiant heat was adjusted to maintain rectal temperature at 37°C.

Laminectomy enabled recording from ventral or dorsal roots in spinal segments S<sub>1</sub> or L<sub>7</sub>, while dissection of the left hindlimb enabled stimulation and/or recording from the medial gastrocnemius muscle and nerve and the caudal cutaneous sural nerve. The cat was then fixed in a rigid frame to permit stable neural recording. The hip and knee angles were fixed at 140° each and the ankle at 90°. The MG tendon of insertion was cut and attached through a force transducer to a servomotor used to hold the muscle at a length producing 100 g passive tension. The MG nerve (intact or central cut end, see following section), as well as the CCS nerve (intact) were positioned on stimulating electrodes near the popliteal fossa. Exposed tissues were covered in warm (37°C) mineral oil.

Following decerebration by removal of all brain tissue rostral to intercollicular transection of the brain stem, gaseous anesthesia was discontinued to permit reflex activation of motoneurons as described in the following text. To assist with recording stability, cats were paralyzed by intravenous infusion of pancuronium bromide (0.04 mg/kg). Cats were killed by barbiturate overdose at the end of the recording session.

### *Nerve treatment*

The primary goal of this study was to test the dependence of recruitment order on homonymous afferent input. This goal was accomplished by comparing data collected from 17 cats in which the MG nerve was subjected to one, and in some cases, more than one of the following three acute treatments. In one treatment group (UNTREATED), data were collected from 10 cats with the MG nerve intact and untreated. In a second group (CUT), data were sampled with the MG nerve cut in the popliteal fossa during the terminal experiment. These data were collected from a total of 11 cats, 9 of which contributed data to both UNTREATED and CUT groups, being studied both before and after nerve cut. In a third treatment group (CUT+), the MG nerve was cut as just described, and the central end was drawn into the tip of a suction electrode containing a dilute solution of the fast sodium channel blocker, lidocaine (2% in isotonic saline, pH = 6.9). This condition was studied in six cats, including one examined both before and after the nerve was cut and treated with lidocaine. These three treatments proved the most practical means of

studying the dependence of recruitment order on homonymous afferent input under the conditions of these experiments. Early attempts to reversibly block action potential conduction in the intact MG nerve with lidocaine proved impractical under these conditions in which recording time is limited because blockade was slow to take effect and to reverse.

### Recording and stimulation procedures

Action potentials were recorded by penetrating individual axons with glass micropipettes (2 M K-acetate, 20–30 M $\Omega$ ) in either dorsal or ventral roots. For the purpose of determining recruitment order and firing rate, two motor axons were penetrated simultaneously, each by a separate micropipette, in intact ventral roots L<sub>7</sub> or S<sub>1</sub> (Fig. 1). Intra-axonal recording was also used to study the effectiveness of MG nerve treatments in eliminating homonymous afferent activity. Afferent axons supplying the MG muscle were penetrated by glass micropipettes in intact dorsal roots S<sub>1</sub> and L<sub>7</sub>. Motor or afferent axons supplying the MG muscle were identified from action potentials evoked by stimulation of the MG nerve. All action potential records were amplified (100 times), filtered (0.1 Hz – 10 kHz), digitized (22–35 kHz), and stored on computer for later analysis.

Axonal CV (in m/s) was calculated from the conduction distance and delay measured from a micropipette in the ventral (or dorsal) root to the bipolar electrode on the MG peripheral nerve. Extracellular action potentials recorded from the MG nerve were initiated by injecting suprathreshold current (50-ms pulses, 1 Hz) through the micropipette. Stimulus-triggered averages (50–100 records, digitized at 40 kHz) of the action potentials recorded extracellularly at the bipolar electrode were used to measure conduction delay off-line from stimulus onset to the peak of the extracellular action potential.

MG motoneurons were recruited in the segmental reflex initiated by stimulation of the caudal cutaneous sural nerve. The CCS reflex was used because it is very effective in recruiting MG motoneurons (Hagbarth 1952; Kanda et al. 1977; Sherrington 1910; Siegel et al. 1999). This reflex was also of interest because studies of synaptic potentials (Burke et al. 1970; Kanda et al. 1977; Pinter et al. 1982; Powers and Binder 1985; but see LaBella et al. 1989) have suggested that sural reflex pathways might recruit motoneurons in an order reversed from that predicted by the size principle. The CCS reflex was useful therefore in testing the hypothesis that input from MG afferents prevents reversals of recruitment order. The reflex was evoked by stimulation of the CCS nerve electrically (40- $\mu$ s pulse duration, 100 Hz) at strengths incremented manually and rapidly (typically within 1–2 s) to levels that recruited both MG motor axons under study. The stimulus was repeated until both MG motor axons in a pair were recruited to fire action potentials in eight separate stimulus trials. Trials were separated by  $\geq 30$  s to minimize the possible effects of activation history on the CCS. Repetition of the stimulus permitted determination of the stability of recruitment order, and eight trials were selected because this number was generally obtainable before axonal recording quality deteriorated. Whenever possible, motor axons were also recruited by firm pinch applied by rat-toothed forceps to the CCS receptive field on the lateral aspect of the ipsilateral ankle. Skin pinch was used to compare recruitment order in response to electrical versus natural stimulation. Pinch trials were also used to produce action potential trains from which motor axon firing rate could be measured without the contamination by stimulus entrainment that was often observed with electrical stimulation.

### Data analysis

Recruitment order was determined from the sequence in which paired axons began firing action potentials (Fig. 1). All axon pairs were categorized by the consistency of their recruitment behavior over all eight recruitment trials. Pairs in which recruitment order was the same across all eight trials were labeled consistent; pairs exhibiting

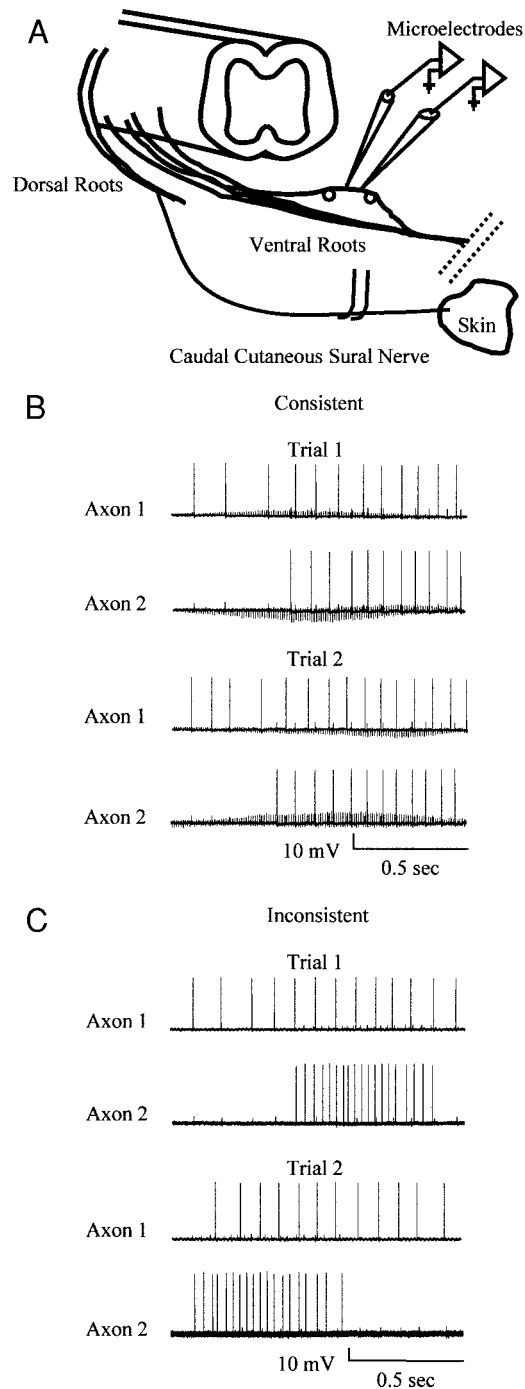


FIG. 1. Measuring recruitment order. A: schematic diagram of method used to record action potentials from pairs of medial gastrocnemius (MG) motor axons recruited in reflexes initiated by electrical stimulation of the caudal cutaneous nerve. B and C: action potentials shown in recruitment trials selected from a total of 8 trials for 2 pairs of MG motor axons. B: recruitment order was consistent and in order by the size principle with axon 1 [conduction velocity (CV) = 84 m/s] firing before axon 2 (CV = 93 m/s). C: recruitment order was inconsistent with axon 1 (CV = 76 m/s) firing either before (trial 1) or after (trial 2) axon 2 (CV = 94 m/s).

inter-trial variation were labeled inconsistent. Among pairs in the consistent category, recruitment order was designated either in accord with the size principle, when the axon firing first had the slower CV, or in reverse order, when the axon firing first had the faster CV. Pairs in which axonal CV differed by  $\leq 2$  m/s were not reliably distinguishable by this property, owing to variation of  $\leq 1$  m/s in repeated



measures for individual axons. Therefore those pairs were excluded from further examination.

In addition to firing sequence, firing rate was measured from those pairs in which both motor axons fired 10 or more action potentials in response to each of three separate trials of skin pinch. The mean firing rate was calculated and compared between axons during the time in which the axons were simultaneously active. The purpose was to estimate the organization of synaptic excitation from the CCS pathway by observing the relative firing rates in each member of a motoneuron pair. Since doublet firing (inter-spike intervals of  $\leq 10$  ms) (Nelson and Burke 1967) is thought to originate from a postsynaptic mechanism (Bawa and Calancie 1983; Spielmann et al. 1993; Zajac and Young 1980), the doublets that were observed in some motor axons were discarded from calculation of mean firing rates.

### Statistical analysis

The effects of homonymous afferent input on motoneuron behavior were tested statistically by comparing pooled data from each of the three treatment conditions of the MG nerve: UNTREATED, CUT, and CUT+. Recruitment data were examined using the *G* test (log likelihood ratio test) applied in tests of goodness-of-fit and in tests of independence (Sokal and Rohlf 1995). The goodness-of-fit test was used to determine whether the observed recruitment order was distinguishable from a random order. The *G* test of independence was used to test for differences in the observed frequency of pairs recruited according to the size principle among the three animal groups.

## RESULTS

### Eliminating homonymous afferent feedback

The present study was designed to reduce or eliminate homonymous afferent feedback to MG motoneurons to test the hypothesis that motoneuron recruitment order depends upon this feedback. Elimination was achieved by cutting the MG nerve in the periphery and treating the central cut end with lidocaine. Intra-axonal recording from dorsal roots in one CUT+ cat showed that 24/24 MG afferents within the group I range of axonal CV ( $>72$  m/s) and sampled within 90 min of lidocaine application were silent, exhibiting no occurrences of spontaneous action potentials. In addition, dorsal root volleys could not be evoked by electrical stimulation of the cut and lidocaine-treated nerve ending at strengths exceeding five times the threshold determined prior to lidocaine treatment. The re-emergence of an evoked dorsal root volley, typically 1–3 h after treatment, was taken as evidence that the action potential blockade was wearing off, and the recruitment study was discontinued until replenishing the lidocaine solution re-established complete blockade of the dorsal root volley. These procedures enabled study of motoneuron recruitment in the absence of feedback from homonymous afferents.

Under the condition in which the MG nerve was cut but not treated with lidocaine, spontaneous activity was observed in the intra-axonal records taken from several MG afferents. Spontaneous discharge in axotomized afferents occurring beyond the brief period of injury discharge has been reported by others (reviewed by Devor 1995; see also Seburn et al. 1999). In the present study, 21/47 (45%) MG afferents sampled from three cats fired spontaneously  $>5$  min after cutting the MG nerve. In comparison, ongoing firing was observed in 12/45 (27%) group I MG afferents in the same three cats with the MG muscle held at a slackened length before the MG nerve was cut (cf. Botterman and Eldred 1982). Spontaneous activity was observed in some afferents up to the longest postcut duration examined, ca. 7 h, a period that extends through the typical duration of a recruitment order study. The spontaneous discharge took the form of either high-frequency bursts or steady firing rates. In the case of steady firing, rates observed in the CUT condition [ $34 \pm 24$  (SD) pps] were not significantly different ( $P > 0.05$ , Student's *t*-test for independent samples) than for UNTREATED Ia fibers with the MG muscle held either at 100 g passive tension ( $42 \pm 48$  pps,  $n = 21$ ) or at a slack length ( $34 \pm 43$  pps). These findings established that simply cutting the MG nerve is not effective in eliminating group I homonymous afferent input to motoneurons. Nonetheless data from this CUT treatment group proved valuable for distinguishing the effects of cutting motor axons from the effects of eliminating afferent feedback.

### Recruitment behavior

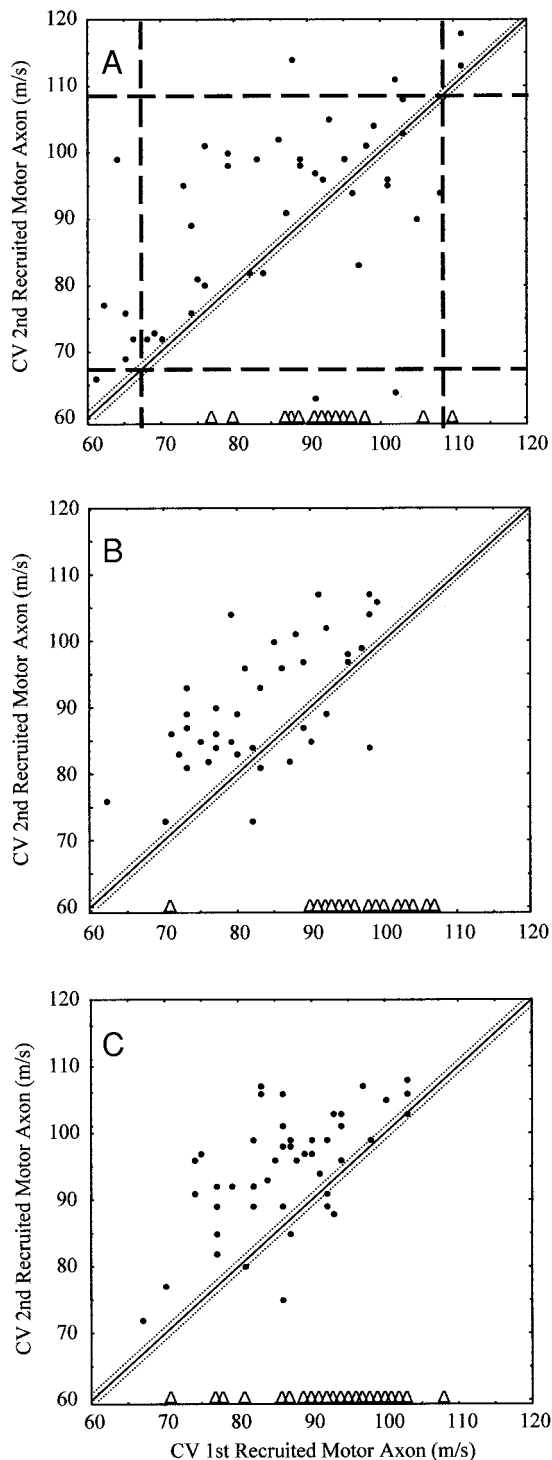
Axon pairs expressed one of three kinds of recruitment order. Order was either consistent across all eight trials, with either the low CV motor axon or the high CV motor axon recruited first, or inconsistent across trials, exhibiting at least one trial in which order switched. Table 1 lists the proportion of units falling into these three categories. Within each treatment group, the distribution of axon pairs across recruitment categories was significantly different from a random model in which percentages were distributed 33:33:33% ( $P < 0.005$ , *G* test). The nonrandom distribution that was observed reflects the majority of axon pairs that were consistently recruited from low-to-high CV and therefore in accord with the size principle in all three treatment groups. Two additional points of primary importance to the present study are apparent in Table 1. First, eliminating homonymous afferent input did not increase the number of recruitment reversals, as can be seen for the CUT+ group in which the percentage of axons recruited from high-to-low CV (6%) was numerically smaller than for the other two

TABLE 1. Recruitment order for pairs of medial gastrocnemius motor axons

MG Nerve Treatment	Low CV Axon Recruited First, %	High CV Axon Recruited First, %	Low or High CV Axon Recruited First, %
Untreated	70 (33)	19 (9)	11 (5)
Cut	70 (33)	15 (7)	15 (7)
Cut + Lidocaine†‡	61 (41)	6 (4)	33 (22)

Percentages of axon pairs distributed across three categories of recruitment order within each treatment group; numbers of axon pairs in parentheses. Distribution of percentages in each group was significantly different from a random distribution of 33:33:33% ( $P < 0.005$ ; *G* test for goodness-of-fit). Distribution of pairs across categories for the Cut + Lidocaine group was significantly different (*G* test of independence) than both the Untreated group (†,  $P < 0.005$ ) and the Cut group (‡,  $P < 0.05$ ). Untreated and Cut groups were not significantly different ( $P > 0.25$ ).

groups (19 and 15%). Second, eliminating homonymous afferent input yielded a substantial percentage (33%) of axon pairs expressing inconsistency in recruitment order. This increase in the proportion of axon pairs expressing inconsistency in the CUT+ group was associated with decreases in both categories of consistent order. Table 1 also shows that cutting the peripheral nerve alone, without lidocaine, had no detectable effect on recruitment behavior; UNTREATED and CUT groups were not significantly different.



### Consistent recruitment order

Figure 2 illustrates the recruitment order measured for pairs of MG motor axons sampled in each of the three treatment groups. Only those axon pairs exhibiting consistent order in all eight recruitment trials are presented; axon pairs expressing inconsistency are considered in the next section. Figure 2A for UNTREATED motor axons replicates earlier findings (Clark et al. 1993; Cope and Clark 1991) in showing that recruitment order is strongly biased to proceed from slower to faster CV motor axons during sural nerve reflexes. Data from two axon pairs in the UNTREATED group (both from the same cat) fall conspicuously below the line of identity in Fig. 2A, the result of axons with relatively slow CV ( $\leq 65$  m/s) being recruited after axons with much faster CV ( $\geq 91$  m/s). Reversals in recruitment order of this magnitude are rarely observed in this laboratory (see also Kanda and Desmedt 1983; Stein and Bertoldi 1981), although preferential activation of fast over slow CV axons on cutaneous stimulation has been emphasized by others (Garnett and Stephens 1980; Kanda et al. 1977; Nielsen and Kagamihara 1993).

The tendency observed in the UNTREATED group for the first recruited axon in a pair to have the lower CV was also observed for samples taken from CUT (Fig. 2B) and CUT+ groups (Fig. 2C). The bias toward recruitment order from low-to-high CV was significantly different from a random recruitment order for each of the three treatment groups ( $P < 0.005$ ;  $G$  test for goodness of fit). For the UNTREATED sample, the proportion of pairs recruited in order from low-to-high CV (33/42; 79%) was similar to the proportion of 30/36 (83%) reported earlier by this lab (Sokoloff et al. 1999). Most pertinent to the present study, comparison of data from the three treatment groups revealed no significant differences in the frequencies of axon pairs recruited in order from low-to-high CV ( $P > 0.1$ ;  $G$  test of independence). These findings demonstrate that eliminating MG afferent feedback was not sufficient to increase the proportion of MG motoneurons consistently recruited in reverse order of the size principle.

Further inspection of Fig. 2 shows that the group samples are comparable with respect to axonal CV. The ranges in CV overlap extensively and span  $\geq 40$  m/s, which is a large portion of the reported CV range (Zengel et al. 1985; cf. McDonagh et al. 1980). Note also the similarity in CV range for those axons sampled in each group that were not recruitable by sural nerve stimulation in these experiments (Fig. 2,  $\Delta$ ). However, closer inspection of Fig. 2 reveals that the CV range for axon pairs in the UNTREATED group exceeded that for the other two groups. Taking this difference into account by comparing

FIG. 2. Recruitment order in motor axon pairs. Plots of axonal CV of 1st vs. 2nd recruited axon for those pairs recruited in the same or consistent order across all 8 trials.  $\Delta$ , CV for single axons that were unrecruitable in the cutaneous reflex. A: data from 7 cats with UNTREATED MG nerve; 33/42 axon pairs recruited in order from low-to-high CV and 14 axons unrecruitable. B: data from 6 cats with MG nerve CUT; 33/40 pairs recruited in order from low-to-high CV and 16 axons unrecruitable. C: data from 6 cats with MG nerve CUT+ treated with lidocaine; 41/45 pairs recruited in order from low-to-high CV and 21 axons unrecruitable. The bias toward recruitment order by CV for all 3 treatment groups was significantly different from that predicted by a random distribution of 50% recruited from low-to-high and 50% recruited from high-to-low CV ( $P < 0.025$ ;  $G$  test of goodness-of-fit adjusted by Yates correction factor). Observed proportions of recruitment order by CV were not significantly different ( $P > 0.10$ ;  $G$  test of independence adjusted by Yates correction factor) among any of the 3 treatment groups.

treatment groups over the same range in CV did not alter the outcome obtained in comparison of the complete data sets. This conclusion is supported by Fig. 2A wherein the limits in CV range taken from the CUT+ group (Fig. 2C) are superimposed as --- on data taken from the UNTREATED group. Statistical comparison of the proportions of axons recruited in order from low-to-high CV over this restricted range in CV also revealed no significant differences among the three groups ( $0.25 > P > 0.10$ ;  $G$  test of independence).

Recruitment order was also assessed whenever both axons in a pair could be recruited by skin pinch applied to the sural receptive field. For all pairs that were recruited in consistent order over multiple trials of sural nerve electrical stimulation, skin pinch invariably yielded the same order. This was observed for 6/6 axon pairs in the UNTREATED group and 17/17 axon pairs in the CUT+ group (no pairs were tested in the CUT group). This finding establishes that our observations on recruitment order are not artifacts of electrical stimulation. It is also consistent with earlier observations from this laboratory (Cope and Clark 1991) that recruitment order does not depend on the strength of electrical stimulation of the sural nerve, which varied across trials and across pairs but which was not monitored in these experiments.

#### Inconsistent recruitment order

Some axon pairs in each group exhibited inconsistency in recruitment order over eight trials of sural nerve stimulation. The inter-trial variation in order gave no evidence of history dependence, i.e., there was no tendency for switches to occur in relation to trial number. Neither was there any tendency for inconsistencies to increase or decrease over the course of an experiment. Figure 3 shows the extent of inconsistency for all pairs that expressed switches in recruitment order. The percentage of the total sample for the CUT+ group (33% or 22/67 pairs) was significantly larger ( $P = 0.0021$ ;  $\chi^2$  test) than for the UNTREATED and CUT groups combined (13% or 12/94 pairs). Also, 11/22 pairs in the CUT+ group exhibited recruitment inconsistencies with order ratios of 3:5 or 4:4 trials, while only 3/12 pairs in the UNTREATED and CUT groups combined had this degree of variability in order. These findings demonstrate that removing homonymous afferent input promotes recruitment instability.

CV was not significantly different for axon pairs in the CUT+ group that exhibited inconsistent versus consistent recruitment order ( $93.0 \pm 10.4$  m/s,  $n = 44$  vs.  $90.7 \pm 9.4$  m/s,  $n = 90$ ;  $P > 0.1$ , Kolmogorov-Smirnov test;  $P = 0.21$ , Student's  $t$ -test for independent samples). In addition, Fig. 4 shows that inconsistent recruitment order was neither restricted to nor uniformly expressed by pairs with similar CV. For example, 26% (7/27) of the pairs for which the difference in CV was greater than or equal to 10 m/s expressed inconsistent order. For the remaining pairs with CV difference  $< 10$  m/s, 37% (15/40) or only  $\sim 1/3$  expressed inconsistency. These results give no support to the possibility that only those axon pairs for which recruitment thresholds were similar, as judged by similar CV, prior to the removal of homonymous afferent input express greater variability in order.

The effect of inconsistent recruitment on our assessment of the overall tendency of the MG population to express either size-ordered or -reversed recruitment was evaluated as follows.

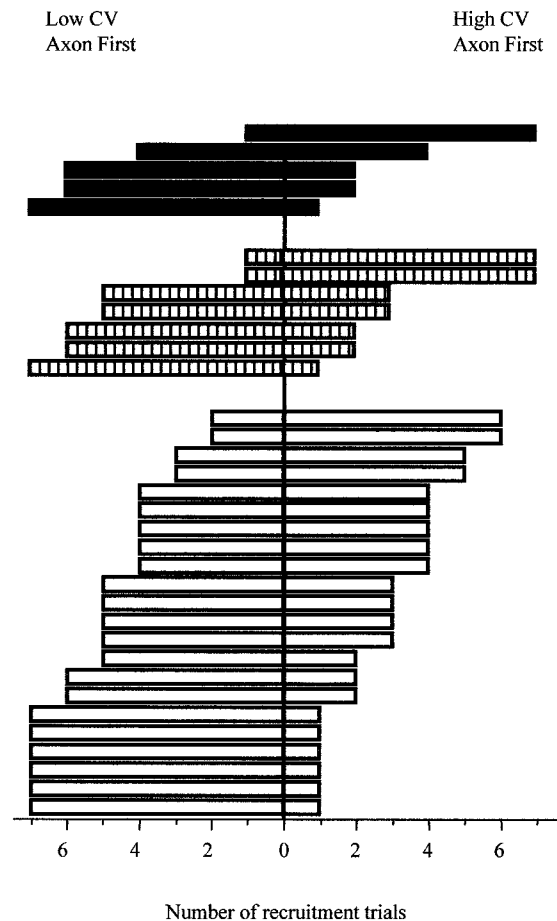


FIG. 3. Inconsistent recruitment order. Each horizontal bar represents a motor axon pair taken from the 3 groups: UNTREATED (black bars,  $n = 5$ ), CUT (hatched bars,  $n = 7$ ), or CUT+ (open bars,  $n = 22$ ). The number of trials in which the recruitment occurred for the low CV axon first extends the bars to the left of the vertical line at 0, and the number for the high CV axon first extends to the right.

Recruitment order was assigned to the 28 pairs from all three treatment groups for which either the low or the high CV axon in a pair was recruited first in a simple majority of trials ( $\geq 5/8$  trials; Fig. 3). Six pairs could not be assigned and were excluded from further analysis because order and reversed order were expressed in equal numbers of trials (4:4). When combined with data taken from the consistent pairs (Table 1), the frequency of pairs expressing recruitment order from low-to-high CV was 78% (36/46), 81% (38/47), and 87% (54/62), respectively for the UNTREATED, CUT, and CUT+ groups. Statistical analysis of these frequencies yields the same result as the previous analysis for the subset of axon pairs selected for consistency (Table 1), namely that all groups exhibited recruitment order that was significantly different from random ( $P < 0.01$ ) but not significantly different from one another ( $P > 0.05$ ). These observations demonstrate that whereas removal of homonymous afferent input increased the proportion of motor axons expressing inconsistency, it did not increase the incidence of reversed order.

#### Distribution of sural excitation among MG motoneurons

The expected increase in frequency of pairs exhibiting recruitment reversals following the elimination of homonymous

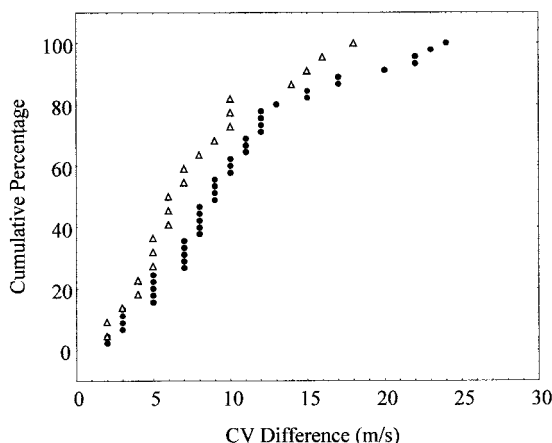


FIG. 4. Cumulative histogram for the difference in CV within motor axon pairs for CUT+ treatment group. Pairs recruited in inconsistent order ( $\Delta$ ,  $n = 22$ ) overlap with ones recruited in consistent order ( $\bullet$ ,  $n = 45$ ).

afferent input was based on the suggestion that sural reflex pathways have the potential to reverse recruitment order (Heckman and Binder 1993; Kanda et al. 1977). However, given the discrepancy in findings on the distribution of sural excitation onto MG motoneurons (Clark et al. 1993; Heckman et al. 1992; LaBella et al. 1989), it seemed necessary to estimate the distribution of sural input under the conditions of these experiments. This estimation was made from the simultaneous repetitive firing evoked in both motor axons of a pair in response to sural stimuli. An advantage of this pairwise estimation technique is that firing is measured simultaneously from two motor axons in response to the identical sural nerve stimulus.

Figure 5A illustrates the mean firing rates for those pairs driven by skin pinch to fire repetitively for a minimum of 10 spikes in each of three stimulus trials for pairs sampled from the CUT+ group. A clear tendency appears for the axon with faster CV to fire at a higher mean rate than the axon with slower CV in 7/8 pairs. Among these seven pairs, the increase in mean firing rate between member units of each pair ranged from 7 to 39 pps. This finding suggests that in the absence of homonymous afferent feedback, the sural reflex pathway distributes excitation more strongly onto fast CV than onto slow CV motoneurons and therefore might act to compress the range of recruitment threshold (see INTRODUCTION). If this tendency resulted solely from differences in intrinsic excitability of faster versus slower CV motoneurons rather than from differences in synaptic drive, then this same tendency for the faster CV axon to fire faster should occur regardless of the source of synaptic excitation. To the contrary, Fig. 5C shows a very different pattern, one in which stretch of the MG muscle resulted in slower mean firing rates in the faster CV motoneuron for 5/7 pairs. This result lends further support for the utility of this means of estimating the distribution of synaptic input from relative firing rates. Interestingly, the differential effects of skin pinch were also less regular with the MG nerve intact (Fig. 5B). In this group, firing rate was faster (2–21 pps) in 5/9 pairs, slower in 3/9 pairs (6–15 pps), and did not differ in 1 pair, for the faster versus the slower CV axon in each pair. This finding suggests that the distribution of synaptic excitation from the sural nerve onto MG motoneurons is modified by MG afferents.

## DISCUSSION

The present study provides the first direct test of the hypothesis that synaptic current from homonymous group Ia muscle afferents assists in establishing orderly recruitment among  $\alpha$ -motoneurons. The role of muscle stretch afferents was tested indirectly in a study by Henneman et al. (1965a) in which activity in muscle spindle afferents was presumably reduced as a consequence of eliminating gamma motoneuron drive to

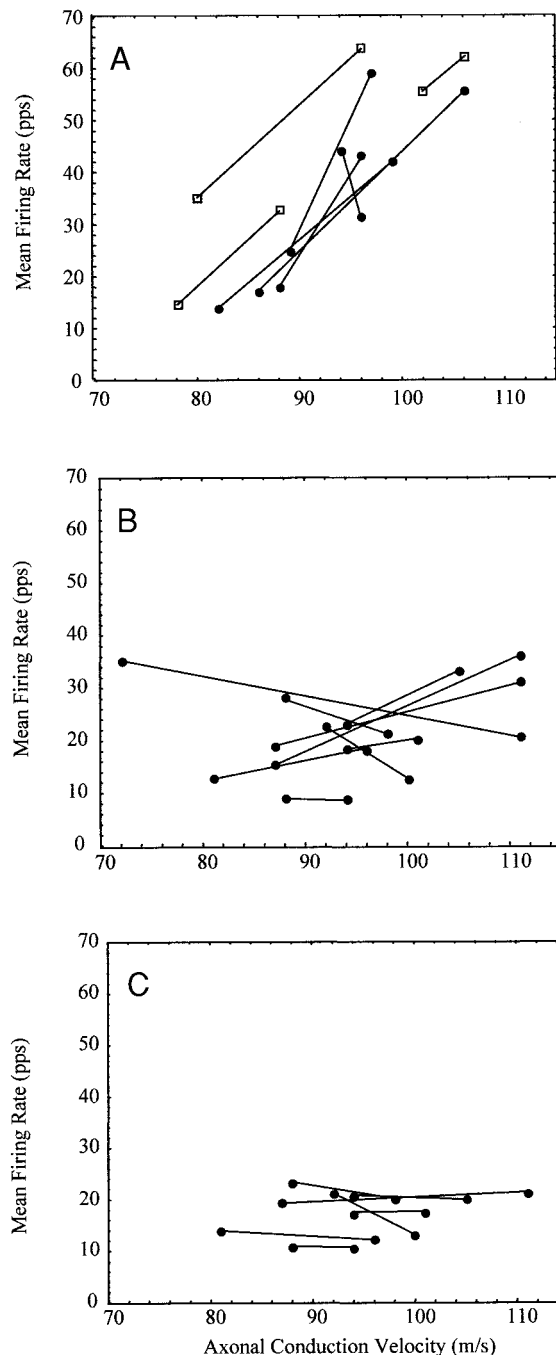


FIG. 5. Comparison of mean firing rates measured concomitantly in pairs of MG axons identified by CV. Data from 8 pairs in CUT+ group ( $\bullet$ , for axon pairs recruited in consistent order;  $\square$ , for pairs recruited in inconsistent order) (A) and from 9 pairs in UNTREATED group (B), with recruitment achieved by pinching caudal cutaneous sural dermatome. C: data from 7 pairs in UNTREATED group recruited by MG muscle stretch (ramp-hold-release stretches 4–6 mm).



spindles by cutting ventral roots. No change in recruitment sequence was observed under this condition. By contrast, some evidence was obtained for reversals among motor units recruited volitionally in human subjects in whom afferent impulses were blocked from reaching the spinal cord either by nerve compression or by lidocaine injection (Grimby and Hanerz 1968, 1976). However, the experimental procedures were acknowledged to leave uncertainty about which afferents were blocked and to what extent. In the present study, input to MG motoneurons from homonymous afferents including group I afferents was verifiably blocked, thereby permitting definitive conclusion that this input is not essential to the basic structure of orderly recruitment. On elimination of homonymous afferent input, most MG motor axon pairs were recruited in a consistent, orderly manner in accordance with the size principle, such that the lower CV axon of the pair was recruited first. There were exceptions to this order, but these size principle violations were no more frequent than with afferent input intact. Elimination of homonymous afferent input was not without effect, however, as evidenced by increased variability in recruitment order. In sum, we find that homonymous afferent input does not suppress consistent reversals of recruitment order as predicted, but its presence does check recruitment instability.

#### Potential mechanisms for changes in recruitment behavior

Removal of homonymous afferent feedback produced a threefold increase in the proportion of axon pairs exhibiting inter-trial variation in recruitment order. This increase occurred together with small decreases in frequency in both categories of consistent recruitment, i.e., order as well as reverse order of the size principle. None of these effects was attributable to injury sustained by the MG motor axons since cutting the MG nerve alone did not alter the normal recruitment pattern (Table 1). We have found no previous reports that lidocaine applied to the MG nerve in the periphery acts retrogradely on motoneurons to alter their central excitability. The possibility of a retrograde effect seems unlikely because inconsistent recruitment order occurred as frequently within hours after lidocaine treatment as it did later when potential retrograde effects might have been expressed. We conclude, therefore, that the recruitment instability is caused by the elimination of homonymous afferent feedback to the spinal cord.

To assist in identifying the plausible mechanism(s) by which elimination of afferent feedback produces inconsistency in recruitment order, we adapted the computer simulation of Heckman and Binder (1993) to perform repeated recruitment trials following procedures of the present study (see APPENDIX). Recruitment of MG motoneurons was achieved by activating synaptic currents modeled after rubrospinal inputs. Effective synaptic current from this input is much greater in the least excitable MG motoneurons than in the more excitable ones (Powers et al. 1993), resulting in a strong compression of the recruitment threshold range across the MG motor nucleus. This compression is thought to be similar to that produced by sural input (Binder et al. 1996; Heckman and Binder 1993).

Figure 6 illustrates the observed and simulated recruitment patterns under a variety of conditions. In the presence of homonymous Ia feedback, there is good correspondence between observed and simulated data (Fig. 6, ○ and ●, respec-

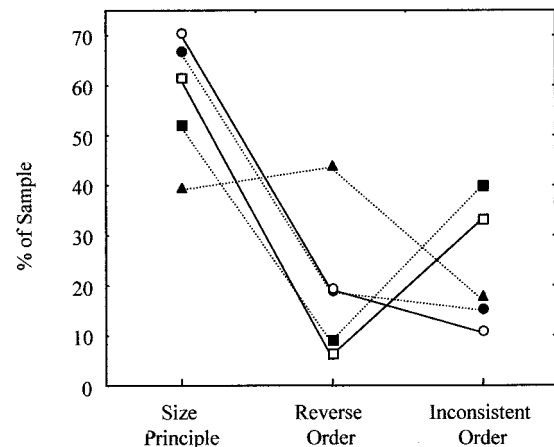


FIG. 6. Percent of axon pairs recruited in order by the size principle, i.e., from low-to-high CV, in reverse order, or in inconsistent order as determined in computer simulation (●, ■, ▲) or in vivo (○, □). Percentages observed in the UNTREATED group (○) for axon pairs recruited by caudal cutaneous sural nerve stimulation match well with those obtained through simulated recruitment by rubrospinal input (see APPENDIX). Percentages observed in the CUT+ group (□), in which homonymous afferent input is removed, are poorly matched by percentages obtained after removal of synaptic current from Ia afferents in the simulation (▲) but well matched when simulated recruitment by rubrospinal input (●) was introduced to increased amplitude of time-varying fluctuations in synaptic drive (■; see APPENDIX).

tively) in the percentages of axon pairs exhibiting size principle order, reverse order, and inconsistent order. This finding for recruitment obtained experimentally with sural input and simulated with rubrospinal input is consistent with the assertion that these inputs are similarly distributed across MG motoneurons (Burke et al. 1970). Evidence that sural nerve input resembles rubrospinal input in compressing the recruitment threshold range is also given by our confirmation of one earlier report (Kanda et al. 1977) that MG motor axons with faster CV fire faster than those with slower CV in sural nerve reflexes (Fig. 5). Most importantly the model simulates the normally observed pattern of recruitment by sural nerve input. From this position, we determined whether removal of homonymous Ia afferent input from the model could reproduce the effects of removing homonymous afferent input on recruitment behavior in vivo. Figure 6 shows that removal of Ia input in the model was unable to yield the observed result. With Ia input modeled to expand recruitment range as it does in the pentobarbital sodium (Nembutal)-anesthetized cat (Heckman and Binder 1988), its removal dramatically increased consistent reversals and had little effect on recruitment inconsistency (Fig. 6, ▲). This was not observed in the decerebrate cat (Fig. 6, □).

A recent report calls for additional examination of the discrepancy just described between simulation and observation. Lee and Heckman (2000) report that for the decerebrate cat, effective synaptic current from group Ia afferents *increases* together with motoneuron input conductance, a tendency that is the reverse of that observed in Nembutal-anesthetized cats (Heckman and Binder 1988). This distribution of effective synaptic current in decerebrate cats appears to involve amplification of the synaptic current, perhaps by voltage-sensitive conductances in MG motoneurons. However, Lee and Heckman (2000) also report that amplification in the high-input conductance cells begins at more depolarized membrane potentials and therefore later in time as the membrane potential



approaches threshold than in the low input conductance motoneurons. Thus the magnitude versus the onset of amplification presents competing influences on recruitment order, thereby leading to uncertainty about the ultimate effect of amplification on order. Notwithstanding this uncertainty, we went on to simulate the effect on recruitment order of Ia input distributed among MG motoneurons so as to compress the range in recruitment threshold. Similar to the result obtained upon removal of homonymous afferent input in the decerebrate cat, removal of Ia input in the simulation did not yield an increase in recruitment reversals. Unlike the result observed *in vivo*, however, the incidence of inconsistent recruitment order did not change in the simulation. These results demonstrate that regardless of how Ia input is distributed among MG motoneurons, whether it compresses or expands the threshold range, eliminating Ia input cannot reproduce the full range of effects observed *in vivo*.

By contrast, the changes in recruitment pattern observed following elimination of homonymous afferent input could be reproduced in the model by amplifying time-varying fluctuations in synaptic drive (see APPENDIX). Increasing the amplitude of these fluctuations in the model yields a large increase in pairs with inconsistent order (40%) at the expense of pairs with consistent size principle and reversed orders (Fig. 6, ■). This roughly matches the observations made in the decerebrate cat following elimination of afferent input (Fig. 6, □), suggesting that removal of homonymous afferent input acts to increase variance in either or both motoneuron excitability and synaptic drive. If this increased variance was uniformly distributed among MG motoneurons, then we should have observed inconsistency predominantly among motor axon pairs with similar excitability as assessed from CV. The latter pattern was not observed, however (Fig. 4), so we suggest that increased variance is distributed unevenly among MG motoneurons and not in relation to CV. This uneven distribution of variance might derive from fluctuations in membrane potential, originating pre- or postsynaptically, that are temporally uncorrelated or unequally distributed among MG motoneurons (Gossard et al. 1994).

#### *Recruitment by the size principle in the sural nerve reflex*

With the MG nerve intact or treated, the activation of sural reflexes either by skin pinch or by electrical stimulation has little effect in reversing recruitment order by the size principle (Fig. 2). It is important to the validity of these findings, however, to carefully consider the way in which the sural nerve was activated. Single electrical stimuli applied to the sural nerve can produce synaptic potentials that appear first as an inhibitory postsynaptic potential (IPSP) followed by an excitatory postsynaptic potential (EPSP), particularly in motoneurons with low CV (Kanda et al. 1977; Pinter et al. 1982; Powers and Binder 1985). In addition, Heckman et al. (1992, 1994) report that the early IPSP gives way to a steady-state EPSP during high-frequency stimulation of the sural nerve. These observations are relevant to the present work wherein recruitment achieved by progressively increasing stimulus strength may have allowed time for early inhibition to give way to excitation before either motoneuron in the pair was recruited. In this way, the potential effect of an early IPSP on recruitment order might have been artificially obscured. Al-

though we cannot discount this possibility entirely, it seems highly unlikely for the following reasons. In the present and in previous studies from this laboratory (Clark et al. 1993; Cope and Clark 1991), recruitment sequence was not sensitive either to the rate or to the strength of electrical stimulation of the sural nerve. In some instances, we studied recruitment by simply switching on the electrical stimulation of the sural nerve at strengths previously determined to recruit both motor units and found that the percentage of reversals by the size principle of recruitment was no greater than when the units were recruited by a ramp increase in stimulation strength. In addition, we report here that sudden skin pinch invariably yields the same recruitment sequence as does electrical stimulation. For these reasons, it seems reasonable to conclude that the tendency toward recruitment order by the size principle was not an artifact of the stimulus protocol.

#### *Pre- and postsynaptic dependence of recruitment order revisited*

Our study provides the first test of earlier assertions that group Ia input assists in establishing recruitment order by the size principle (Grimby and Hannerz 1968, 1976; Heckman and Binder 1993; Stein and Bertoldi 1981). The emergence of this orderly recruitment despite increased instability demonstrates that order among MG motoneurons is not dependent on synaptic input from either group Ia afferents or any of the other primary afferents in the homonymous nerve. However, it is possible that homonymous group Ia input is not the only synaptic source acting to expand the recruitment threshold range. For example, MG motoneurons receive group Ia input from the lateral gastrocnemius and soleus muscles, and this heteronymous input was not eliminated in these studies. However, in preliminary study, we determined that recruitment inconsistency in CUT+ cats was unaltered when heteronymous group Ia afferent input was manipulated by changing passive muscle stretch or by applying vibration to the lateral gastrocnemius and soleus muscles. It seems unlikely then that heteronymous stretch-sensitive afferent inputs produce any substantial expansion of recruitment threshold range. No afferents other than the homonymous group Ia afferents are known to expand the recruitment threshold range, but such afferent systems cannot be ruled out. Powers and Rymer (1988) found that cutting the dorsal spinal cord compressed recruitment threshold range, and reversed recruitment order in 4/14 pairs of motoneurons. An injury-induced change in motoneuron excitability cannot be excluded as the basis for this effect (see discussion in Carp et al. 1991). All considered, it remains possible that recruitment order is disrupted as a result of cutting some afferent system that distributes excitation onto MG motoneurons in a pattern that assists in establishing orderly recruitment.

Our findings are entirely consistent with the view that recruitment order is determined predominantly if not completely by intrinsic properties of motoneurons. The intrinsic excitability of MG motoneurons measured as rheobase current covers a 30-fold range in Nembutal-anesthetized cats (Zengel et al. 1985). Perhaps the computer simulations used here and earlier (Heckman and Binder 1993) overestimated the ability of synaptic input to reverse recruitment order because the range in intrinsic excitability was underestimated. It is possible that

threshold range is even greater in decerebrate cats as a consequence of voltage-dependent conductances. A motoneuron property that might expand the range of intrinsic excitability is the persistent inward current that is activated by much less depolarization in the most excitable motoneurons than in less excitable ones (Lee and Heckman 1998). Whatever they may be, the intrinsic properties of motoneurons seem most likely to account for the abundant observations (Calancie and Bawa 1990; Cope and Clark 1995; Henneman and Mendell 1981) that orderly recruitment occurs during virtually all naturally evoked reflexes and volitional contractions, and, as shown here, after removal of certain inputs. Synaptic inputs, by contrast, seem to play no part in establishing recruitment order, but rather they determine which motoneurons are selected into activity as well as the recruitment gain (Burke 1981a; Heckman 1994; Kernell and Hultborn 1990), i.e., the number of motoneurons recruited per increment in net excitatory synaptic drive.

## APPENDIX

The purpose of these simulations was to evaluate whether removal of Ia input could account for the findings presented in the accompanying paper. Previous simulations of motoneuron recruitment (Heckman and Binder 1993) tested the role of Ia input, but these studies did not consider the consistency of recruitment in repeated trials for individual pairs as was done in the foregoing work.

### Basic methods

Methods for the simulations were identical to those presented by Heckman and Binder (1991, 1993), with the exception that each pair of recruited motoneurons was tested for recruitment order in eight repeated trials. The pool of simulated motoneurons consisted of 100 motoneurons. The key motoneuron parameters for the present recruitment order simulations were the threshold current for generating an action potential ( $I_{\text{thres}}$ ) and the CV of the axon (CV). The distribution of threshold values was closely matched to the distribution for the cat medial gastrocnemius motoneuron pool (modeled in Heckman and Binder 1991 based on experimental results from Kernell and Monster 1981). CVs were assigned to each motoneuron with even spacing between 70 and 110 m/s, assuming a perfect correlation between  $I_{\text{thres}}$  and CV. Addition of random noise (see following text) typically expanded this range to ~50–130 m/s and produced  $I_{\text{thres}}$ -CV relationships with a considerable degree of scatter. The organization of synaptic inputs was based on weighting factors derived from the systematic studies of effective synaptic currents from Ia input and various descending systems by Powers, Binder, and colleagues (reviewed in Binder et al. 1996). The synaptic current weighting factors and the threshold currents together determined the recruitment threshold of the motoneuron.

### Techniques for specifying noise levels

Varying degrees of random noise were added to the  $I_{\text{thres}}$  and CV of each motoneuron to simulate normal biological noise. This noise was divided into two components. The first component ( $N_{\text{neuron}}$ ) was meant to mimic the biological variance inherent in the electrical properties of motoneurons. This variance is likely caused by factors such as the differences between motoneurons in density of voltage-sensitive channels.  $N_{\text{neuron}}$  thus specified the average scatter in the relationship between  $I_{\text{thres}}$  and CV. The second component of the noise ( $N_{\text{synaptic}}$ ) was meant to represent the time-varying fluctuations in membrane potential in motoneurons due to time-varying fluctuations in their synaptic drive. Both forms of noise had Gaussian

distributions and both were added to the  $I_{\text{thres}}$  and CV values via standard Monte Carlo techniques (Heckman and Binder 1993). Each Gaussian distribution was scaled so that three standard deviations fell within a specified percentage of each motoneuron's standard  $I_{\text{thres}}$  and CV values. For example, if  $N_{\text{neuron}}$  was set to 30% for  $I_{\text{thres}}$ , then a given motoneuron's value for  $I_{\text{thres}}$  was randomly chosen from a distribution in which 3 SDs encompassed a range of 0.7–1.3 times that motoneuron's standard  $I_{\text{thres}}$  value.  $N_{\text{neuron}}$  was usually set to 30% for both  $I_{\text{thres}}$  and CV. Correlations between  $I_{\text{thres}}$  and CV for  $N_{\text{neuron}} = 30\%$  averaged about  $r = 0.6$  to  $0.7$ , which falls at the upper end of the range seen in experimental data for correlations between these two parameters within individual animals (see the methods section of Lee and Heckman 1999). For each set of eight trials,  $N_{\text{neuron}}$  was applied only at the start of the set. In contrast,  $N_{\text{synaptic}}$  was reapplied for each of the eight trials to generate intertrial variability. Values for  $N_{\text{synaptic}}$  varied between 2 and 20%.

### Simulation procedures

Each simulated experiment was performed as follows. The synaptic organization and percentages for  $N_{\text{neuron}}$  and  $N_{\text{synaptic}}$  were specified. A specific value for  $I_{\text{thres}}$  and CV for each of the 100 motoneurons was set by adding the combined noise levels specified by  $N_{\text{neuron}}$  and  $N_{\text{synaptic}}$ . The recruitment threshold for each of the 100 motoneurons was calculated based on the  $I_{\text{thres}}$  values (as modified by  $N_{\text{neuron}}$  and  $N_{\text{synaptic}}$ ) and the synaptic weighting factors for the chosen synaptic organization (Heckman and Binder 1993). Each of the motoneurons was then randomly paired with another one with each motoneuron used in only one pair. The percentages of the 50 pairs showing normal recruitment (low CV motoneuron first) and reversed recruitment (low CV motoneuron recruited second) were calculated. The run was then repeated seven more times with precisely the same pairing of motoneurons and the same synaptic organization. However, time varying fluctuations from trial to trial were simulated by re-adding the noise level corresponding to the  $N_{\text{synaptic}}$  to the  $I_{\text{thres}}$  and CV values of the previous trial before each new trial began. Thus the values of  $I_{\text{thres}}$  and CV for each motoneuron set in the first trial by application of both  $N_{\text{neuron}}$  and  $N_{\text{synaptic}}$  then varied in subsequent trials only by the amount specified by  $N_{\text{synaptic}}$ . A new experiment consisting of eight repeated trials was initiated by selecting new values of  $I_{\text{thres}}$  and CV from the Gaussian distributions, by reapplying both  $N_{\text{neuron}}$  and  $N_{\text{synaptic}}$ , and by randomly setting new pairings between motoneurons.

Experiments were repeated 500 times for a given input organization and noise level. The results for a set of 500 experiments were expressed in terms of the percentage of consistent normal pairs (low CV motoneuron recruited first in all 8 trials), the percentage of consistent reversals (low CV motoneuron recruited second in all 8 trials) and the percentage of inconsistent pairs ( $\geq 1$  trial with a different recruitment order than the others). All percentages were averages of the values across all 500 experiments.

### Organization of synaptic input

To replicate the control conditions in the experimental data, three assumptions were made about the organization of simulated synaptic input. 1) The main excitatory drive during the sural input generated relatively larger effective synaptic currents in high- than low-threshold motoneurons. This input thus tended to compress the range of recruitment thresholds set by the ~10-fold range of  $I_{\text{thres}}$  values (3.5–40 nA before any noise was added) (Heckman and Binder 1993). This assumption is supported by the larger effect of sural input on firing rate of high-threshold units (see Fig. 5). 2) A small, constant inhibitory bias current existed. This inhibition was applied simply because it seemed unlikely that the sural drive was purely excitatory. While much of the inhibition during the sural input fades within the first 50 ms of steady stimulation (Heckman et al. 1994), recruitment of units in the present study occurred near the onset of sural input, where

inhibition is still likely to be important. In addition, there may exist some tonic baseline inhibition in the decerebrate due, for example, to tonic firing in antagonist Ia afferents. 3) All motoneurons received a steady bias of excitatory input from Ia afferents. This of course was the main assumption driving the experimental studies (see INTRODUCTION). The sensitivity of the simulations to these three assumptions was evaluated (see following text). The amplitude of the inhibitory bias was set to 1 nA and the excitatory bias from Ia afferents was set to 2 nA. The inhibition was given a uniform distribution for all motoneurons (the inhibitory systems studied thus far have uniform distributions) (Binder et al. 1996). As pointed out in the INTRODUCTION, the Ia input is distributed preferentially to low-threshold units and thus tends to expand the range of recruitment thresholds (Heckman and Binder 1988).

### Simulation results

The main results are shown in Fig. 6 in the DISCUSSION. An accurate recreation of the control data was achieved with  $N_{\text{neuron}}$  set to 30%,  $N_{\text{synaptic}}$  set to 4%, and the sural synaptic drive distributed according to the weighting factors used for the rubrospinal system in Heckman and Binder (1993). As presented in DISCUSSION, removal of Ia input without altering  $N_{\text{neuron}}$  and  $N_{\text{synaptic}}$  did not replicate the effect of removing the homonymous input (cf. Fig. 6,  $\blacktriangle$  and  $\square$ ). A good match to removal of homonymous input was achieved when  $N_{\text{synaptic}}$  was increased from 4 to 12% (Ia input was again eliminated;  $N_{\text{neuron}}$  remained at 30%; Fig. 6,  $\blacksquare$ ). This replicated both facets of the removal of homonymous input in the experimental data: slight decreases in both consistent size principle pairs and consistent reversed pairs coupled to a large increase for inconsistent pairs.

### Sensitivity analyses for synaptic organization

The rubrospinal input is much stronger in high than low-threshold cells (Binder et al. 1996). In the simulations, this differential action was captured by using a ninefold range of weighting factors (largest weights to high-threshold units, smallest to low-threshold units) (see Heckman and Binder 1993). Effective synaptic currents for sural input have not yet been measured, but the assumption that the sural excitation also favors activation of high-threshold units is reasonable (see DISCUSSION). However, the differential sural effect may not be as strong as for the rubrospinal excitation. We therefore systematically simulated the effects of reducing the preferential excitatory drive to high-threshold motoneurons by substituting vestibulospinal excitation (which has only a twofold range of weighting factors) for rubrospinal excitation. For the Ia input, the key issue is whether the simulated amplitude of 2 nA exaggerates the impact of the tonic firing of Ia afferents in the homonymous nerve in the decerebrate preparation used in this study. The amplitude of Ia input used in the simulation was based on activation by tendon vibration in the pentobarbital anesthetized preparation (Heckman and Binder 1988), which likely generated average firing rates of 180 Hz in Ia afferents. The lower firing rates of tonically active Ia afferents (see RESULTS) are at least somewhat offset by dendritic amplification of Ia actions that are present in the decerebrate (Lee and Heckman 2000; Prather et al. 2001). We tested the effect of both lowering and raising the amplitude of the Ia bias current. Finally, we also evaluated the effect of lowering and raising the inhibitory bias currents. None of these changes in input organization altered the main result of Fig. 6. In general, changes in input organization, whether from different input systems or changes in amplitudes of bias currents, impact recruitment order by compressing or expanding the 10-fold range of recruitment thresholds established by the intrinsic current thresholds of the motoneurons (i.e., the differences in  $I_{\text{thres}}$  in the simulation) (Heckman and Binder 1993). Changes in threshold range invariably had parallel effects on the percentages of consistent reversals and inconsistent pairs. A strong compression of the range produced a marked increase in consistent

recruitment reversals coupled with a small increase in inconsistent pairs (this was essentially what happened when the simulated Ia input was removed in Fig. 6). Expansion had the opposite effects. Neither change mimicked the experimental results for removing homonymous input in which consistent recruitment reversals and inconsistent pairs changed in opposite directions (see Fig. 6).

### Sensitivity analyses for noise

Changes in  $N_{\text{synaptic}}$  had the expected effects: the larger the amplitude of  $N_{\text{synaptic}}$  the greater the percentage of inconsistent pairs and the lower the percentage of both consistent size principle pairs and consistent reversed pairs. This is exactly what eliminating homonymous input did (Fig. 6,  $\circ$ ). In contrast, increases in  $N_{\text{neuron}}$  had inverse effects on consistent pairs, decreasing the size principle pairs while increasing the reversed pairs. The percentage of inconsistent pairs increased slightly with increased  $N_{\text{neuron}}$ . In other words, increasing  $N_{\text{neuron}}$  acted somewhat like compressing the range of recruitment thresholds, having parallel effects on consistent reversals and inconsistencies. Thus changes in  $N_{\text{neuron}}$  could not produce the decrease for consistent reversals and large increase for inconsistencies seen in the data and accurately simulated by changes in  $N_{\text{synaptic}}$  (Fig. 6).

One aspect of the experimental results that could not be simulated was the result illustrated in Fig. 5, which shows inconsistency in recruitment among motoneuron pairs expressing the full range of differences in CV. In the simulations, there was a strong tendency for inconsistent recruitment to occur more frequently among pairs with close CVs. The reason for the lack of this relation in the experimental results is unclear, as one would expect it to be easier to alter recruitment order in unit pairs with close thresholds, which presumably have close CVs. Perhaps this discrepancy can be accounted for by the dynamic characteristics of the synaptic noise—for example, occasional large noise peaks that also have fast rates of rise might be particularly effective at recruiting even a relatively high-threshold unit out of its normal order. Obviously, dynamic phenomena cannot be simulated using the present techniques, which assume steady conditions. However, these dynamics would only apply to  $N_{\text{synaptic}}$  not  $N_{\text{neuron}}$  because  $N_{\text{neuron}}$  is only meant to capture the variability in intrinsic differences in motoneuron properties. Thus the basic conclusion of this subsection, that changes in  $N_{\text{synaptic}}$  but not  $N_{\text{neuron}}$  could replicate the experimental results shown in Fig. 6, still holds. Similarly, the conclusion of the previous section, that changes in synaptic organization alone cannot produce the experiment results of Fig. 6, would not be altered by including dynamic properties in  $N_{\text{synaptic}}$ .

### Summary of sensitivity analyses

The sensitivity analysis presented in the preceding text indicates that the simulation results presented in Fig. 6 are representative examples of a wide range of simulations with different synaptic organizations and synaptic noise amplitudes. Thus the main conclusion from Fig. 6, that increases in  $N_{\text{synaptic}}$  provided the best simulation of removing homonymous input, is likely to be correct even if the specifics of the simulation did not precisely replicate biological reality.

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