Influence of Active Dendritic Currents on Input-Output Processing in Spinal Motoneurons In Vivo

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Lee, R. H., J. J. Kuo, M. C. Jiang, and C. J. Heckman. Influence of active dendritic currents on input-output processing in spinal motoneurons in vivo. J Neurophysiol 89: 27–39, 2003; 10.1152/jn.00137.2002. The extensive dendritic tree of the adult spinal motoneuron generates a powerful persistent inward current (PIC). We investigated how this dendritic PIC influenced conversion of synaptic input to rhythmic firing. A linearly increasing, predominantly excitatory synaptic input was generated in triceps ankle extensor motoneurons by slow stretch (duration: 2–10 s) of the Achilles tendon in the decerebrate cat preparation. The firing pattern evoked by stretch was measured by injecting a steady current to depolarize the cell to threshold for firing. The effective synaptic current ($I_{ON}$, the net synaptic current reaching the soma of the cell) evoked by stretch was measured during voltage clamp. Hyperpolarizing holding potentials were used to minimize the activation of the dendritic PIC and thus estimate stretch-evoked $I_N$ for a passive dendritic tree ($I_{N,pass}$). Depolarizing holding potentials that approximated the average membrane potential during rhythmic firing allowed strong activation of the dendritic PIC and thus resulted in marked enhancement of the total stretch-evoked $I_N$ ($I_{N,TOT}$). The net effect of the dendritic PIC on the generation of rhythmic firing was assessed by plotting stretch-evoked firing (strong PIC activation) versus stretch-evoked $I_{N,pass}$ (minimal PIC activation). The gain of this input-output function for the neuron ($I_{O_N}$) was found to be ~2.7 times as high as for the standard injected frequency current ($F-I$) function in low-input conductance neurons. However, about halfway through the stretch, firing rate tended to become constant, resulting in a sharp saturation in $I_{O_N}$ that was not present in $F-I$. In addition, the gain of $I_{O_N}$ decreased sharply with increasing input conductance, resulting in much lower stretch-evoked firing rates in high-input conductance cells. All of these phenomena (high initial gain, saturation, and differences in low- and high-input conductance cells) were also clearly apparent in the differences between stretch-evoked $I_{N,TOT}$ and $I_{N,pass}$, and thus could be accounted for by the activation of the dendritic PIC. As a result, stretch-evoked $I_{N,TOT}$ and $F-I$ provided an accurate prediction of the overall change in stretch-evoked firing. However, in about half of the low-input conductance cells, the rate of rise of firing in response to stretch was not smoothly graded but instead consisted of a rapid surge. Stretch-evoked $I_{N,TOT}$ was always smoothly graded. This suggests that although stretch-evoked $I_{N,TOT}$ can be used to predict the overall change in firing, prediction of the dynamics of firing may be less accurate.

INTRODUCTION

Voltage-sensitive conductances in dendrites have a major impact on the integration of synaptic inputs (Haussler et al. 2000; Johnston et al. 1996; Yuste and Tank 1996). These active dendritic currents can have a profound influence on the amplitude of the synaptic current reaching the soma and initial segment, where action potentials are primarily generated. Thus active dendritic currents should have a strong impact on the neuron’s net input-output processing.

The classic measure of the neuronal input-output processing during steady-state conditions is the relationship between firing frequency and current injected via the microelectrode (Granit et al. 1963; Kernell 1965; see Binder et al. 1996 for a review). These frequency-current ($F-I$) functions have been obtained in many types of neurons (e.g., Bao et al. 1995; Gustafsson et al. 1978; Jahnsen and Linhas 1984; Lacaille and Williams 1990; Lanthorn et al. 1984; Manis 1990; Minami et al. 1986). Studies in spinal motoneurons showed that the net synaptic current reaching the soma (the effective synaptic current, $I_N$) (Heckman and Binder 1988; Redman 1976) can be used as the input to the $F-I$ function to predict the actual change in firing rate produced by a variety of synaptic input systems with a reasonable degree of accuracy (Powers and Binder 1995, 2000). This $I_N$ and $F-I$ representation has provided the basis for biologically realistic steady-state input-output models of the motoneuron pool and the muscle it controls (Heckman 1994; Heckman and Binder 1991, 1993a,b).

The studies of $I_N$ and its coupling to the $F-I$ function were largely carried out in preparations where active dendritic currents are suppressed. However, in the presence of the neuromodulators serotonin and norepinephrine, the dendrites of spinal motoneurons generate a strong persistent inward current (PIC) that is highly voltage dependent (Carlin et al. 2000a,b; Hounsgaard and Kiehn 1993; Lee and Heckman 1996; Lee and Heckman 2000; Steverink and Hounsgaard 1997) and that sometimes results in sustained plateau potentials and bistable behavior (Hounsgaard et al. 1988; Lee and Heckman 1998b). An $L$-type calcium current plays a major role in generating this dendritic PIC (Carlin et al. 2002b; Hounsgaard and Kiehn 1989; Perrier and Hounsgaard 1999).

The dendritic PIC has the potential to markedly alter the relation between $I_N$ and the $F-I$ function. During voltage clamp, the amplitude of $I_N$ generated by a constant stimulation of a synaptic input becomes strongly voltage dependent (Lee and Heckman 2000). At hyperpolarized levels (~60 to ~80 mV), $I_N$ is similar in amplitude to preparations where the dendritic PIC is suppressed. However, at depolarized levels...
near the voltage threshold for spike initiation during rhythmic firing (approximately −50 mV), $I_N$ undergoes a remarkable two- to sixfold enhancement. Equally striking, further depolarization reveals a strong reduction in the amplitude of $I_N$, causing it to become smaller than at hyperpolarized levels. During rhythmic firing, excitatory synaptic inputs have recently been shown to increase firing rate to a much greater extent than what would be predicted from the $I_N$ measured for the same synaptic input at hyperpolarized levels multiplied by the gain of the $F-I$ function (Prather et al. 2001; see also Bennett et al. 1998). This enhanced firing is consistent with the enhancement of $I_N$ seen during voltage clamp.

However, no study has as yet directly compared depolarized $I_N$ to its impact on firing. There are two important questions in this regard. The first question is whether the activation of the dendritic PIC is all-or-none or smoothly graded. During voltage clamp, the depolarization-dependent enhancement and subsequent saturation in $I_N$ are a smooth function of the holding potential (Lee and Heckman 1996, 2000). During rhythmic firing, the large increases in firing generated by two separate synaptic inputs on their own sum linearly (Prather et al. 2001). These results suggest graded activation occurs both during rhythmic firing and voltage clamp. However, in all previous studies, the amplitude of each synaptic input was held constant. A truly graded activation implies proportional increases in both firing and $I_N$ as activation level of a synaptic input is gradually increased. The second question is whether firing is influenced by the reduction in $I_N$ that occurs as the membrane potential is depolarized above the range for peak enhancement. This saturation in $I_N$ tends to occur ~5–10 mV depolarized with respect to voltage threshold for spike generation, but spike voltage threshold depolarizes along with the average membrane potential between spikes as firing rate increases (Lee and Heckman 2001; Schwindt and Crill 1982). Thus the saturation in $I_N$ may produce a sharp limitation in firing rate as the amplitude of $I_N$ is gradually increased.

In the present study, a smoothly increasing synaptic input was generated by linear muscle stretch in the decerebrate cat preparation, which has tonic activity in axons descending from the brain stem that release the monoamines serotonin and norepinephrine (Hounsgaard et al. 1988; Lee and Heckman 1998b) into the lumbar spinal cord. Consequently, motoneurons in this preparation have strong dendritic PICs (Lee and Heckman 2000). The synaptic input evoked by muscle stretch is due to strong activation of group Ia and II afferents from muscle spindles (Matthews 1972). In addition, as the stretch reaches longer muscle lengths, it activates muscular free nerve endings and Golgi tendon organ afferents, so that stretch input may combine excitation and inhibition (Cleland and Rymer 1990; Matthews 1972). We measured both the firing pattern and the $I_N$ generated by stretch at various holding potentials as well as the $F-I$ relation. The results revealed that the dendritic PIC had a strong effect on stretch-evoked firing that was often nicely proportional to stretch but then exhibited strong saturation before stretch ended. Two surprising results were encountered: stretch had a much stronger effect on low as opposed to high-input conductance cells and some low-input conductance cells exhibited highly variable firing responses to stretch. A portion of the results has been presented in abstract form (Heckman et al. 2000).

**METHODS**

Measurements of input-output functions

OVERALL APPROACH AND BASIC TERMINOLOGY. The goal of this work was to assess the impact of the dendritic PIC on how motoneurons integrate synaptic input and convert this input into firing outputs. Figure 1 illustrates a simplified three-component model of this transformation. The leftmost component (1) is defined by the passive properties of the dendrites. The rightmost component (3) is defined by the active currents generating the $F-I$ function that are mainly located in the soma and the initial segment but probably also extend into the proximal dendrites (Safronov et al. 1997). The middle component (2) represents the dendritic PIC (although it may be partially somatic as well) (see Lee and Heckman 2000). The bidirectional arrows indicate the fact that these components interact. For example, current injected at the soma (during either current or voltage clamp) affects the activation of the dendritic PIC as well as the passive driving force for synaptic input, whereas activation of the dendritic PIC further alters driving force and can also alter the gain of the $F-I$ function. Nonetheless, all three components can be reasonably estimated from measurements, as follows.

**Component 1 (passive dendritic properties).** Previous studies in our lab (Lee and Heckman 2000) have shown that voltage-clamping the neuron at a hyperpolarized level (~65 to ~80 mV) largely prevents synaptic input from activating the dendritic PIC and thus provides an

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**FIG. 1.** Conceptual model underlying the influence of the dendritic persistent inward current (PIC) on input-output processing in motoneurons. The 2 active components are generated by voltage-sensitive currents. The passive component represents the classic influence of anatomy and geometry on the flow of synaptic current in the dendritic tree. Each term is defined more fully in the 1st section of METHODS.
estimate of the \( I_N \) generated in a passive dendritic tree (\( I_{N,\text{pass}} \)) (as labeled in Fig. 1, top left).

Component 2 (active dendritic properties). Our previous work (Lee and Heckman 2000) also established that voltage clamp at about \(-50\) mV allows for strong activation of the dendritic PIC by synaptic input. During rhythmic firing, the spike voltage threshold is about \(-50\) mV, and the average membrane potential (excluding the spikes) hovered reasonably close to this depolarized level (e.g., Lee and Heckman 1998a, 2001). Therefore application of synaptic input to the cell during voltage clamp near spike threshold provides an estimate of the \( I_N \) generated by combined effects components 1 and 2 (\( I_{N,\text{TOT}} \)) during rhythmic firing. The contribution from component 2 (due to the active dendritic processes, \( I_{N,\text{ACT}} \)) is then estimated by comparing \( I_{N,\text{PASS}} \) to \( I_{N,\text{TOT}} \). (Note that it is not correct to consider \( I_{N,\text{ACT}} \) as being generated only by voltage-sensitive currents because any change in dendritic voltage necessarily alters synaptic driving force and dendritic conductance. Thus \( I_{N,\text{ACT}} \) is perhaps more properly considered the net enhancement in \( I_N \) due to the interaction of active currents with passive properties).

Component 3 (active properties for generating spike trains). The measurement of this component is the standard F-I function generated by current injected via the microelectrode.

A significant problem with the preceding measurements is that the dendritic PIC is not restricted in its effects to component 2. Even with hyperpolarization, the dendritic PIC appears to have a small influence on \( I_{N,\text{PASS}} \) (Lee and Heckman 2000). More importantly, the dendritic PIC can have a large impact on the F-I function (Bennett et al. 1998; Hornby et al. 2002a,b; Hougsaard et al. 1988; Lee and Heckman 1998b). One way of estimating the magnitude of these errors is to compare results obtained in this study with those in previous studies in pentobarbital-anesthetized preparations where the dendritic PIC is strongly suppressed (see Discussion). The influence of the dendritic PIC on the F-I function can also be estimated by comparing two different combinations of the components as diagramed in Fig. 1.

In this study, we primarily assessed input-output processing using the components grouped as shown across the top of Fig. 1. Thus \( I_{N,\text{PASS}} \) was the input parameter and the I-\( O_N \) function was constructed by plotting the stretch-evoked firing rate generated in unclamped conditions versus the stretch-evoked \( I_{N,\text{PASS}} \) obtained in a subsequent trial during voltage clamp at a hyperpolarized level (see following text).

There are several advantages to using I-\( O_N \) to assess the net impact of the dendritic PIC on stretch-evoked input: 1) \( I_{N,\text{PASS}} \) is somewhat easier to measure than \( I_{N,\text{TOT}} \) because voltage clamp using the discontinuous single electrode method is more difficult to attain at depolarized than hyperpolarized levels. 2) I-\( O_N \) includes the effects of all voltage-sensitive currents, thus grouping them together as a factor modifying synaptic input. This grouping is in accord with one of the original goals for the formulation of \( I_N \), minimizing the influence of postsynaptic factors in the measurement of synaptic input (Heckman and Binder 1988, 1990). 3) I-\( O_N \) makes no assumption about the relative contribution of the dendritic PIC to \( I_{N,\text{TOT}} \) as compared with the F-I function because it measures the net influence of PIC on both factors simultaneously. 4) I-\( O_N \) provides a direct measure of the firing pattern evoked by stretch, including such factors as saturation at longer lengths (see Results).

The measurements of \( I_{N,\text{TOT}} \) at depolarized holding potentials also allowed us to evaluate an alternative formulation of input-output processing, which was to use \( I_{N,\text{TOT}} \) as the input to the F-I Function (Fig. 1, bottom labels). This formulation is equivalent to that of \( I_{N,\text{PASS}} \) and I-\( O_N \) if two assumptions are correct: that the dendritic PIC does not significantly influence the F-I function and that activation of the dendritic PIC is similar during rhythmic firing and during voltage clamp at an appropriately depolarized level (1 factor that might alter PIC activation is the afterhyperpolarization between spikes). The validity of these assumptions is tested in these experiments.

Surgical Preparation. All procedures were approved by the animal care committee at Northwestern University. Full details are available in a previous publication (Lee and Heckman 1998b). Briefly, all surgical preparations of the spinal cord and hindlimb were done under deep gaseous anesthesia (1.5–3.0% isoflurane in a 3:1 mixture of \( O_2 \) and \( N_2O \)). The preparation included a laminectomy to expose the L7 and S1 segments of the spinal cord for intracellular recording. The Achilles tendon was surgically isolated [the plantaris tendon was cut, leaving only the tendons to the triceps surae: medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LGS)]. The nerves to MG and LGS were isolated and placed on stimulating electrodes. The gaseous anesthesia was discontinued after a precollricular decerebration in which all forebrain anterior to the colliculi was removed. All preparations were then paralyzed with gallamine triethiodide (Flaxedil) and artificially respired. In addition, a bilateral pneumothorax was done to enhance intracellular recording stability. At the end of the experiment, the animals were killed with a lethal dose of pentobarbital.

Intracellular Recording Methods. Intracellular recordings of motoneurons antidromically activated by stimulation of the MG and LGS nerves were obtained in the lumbar cord with sharp microelectrodes. Microelectrode tips were broken back under microscopic observation and control. Because of the large currents required for successful single-electrode voltage-clamp techniques in spinal motoneurons, resistances of the electrodes were kept low—typically \(-3–4\) M\( \Omega \) in saline before entering the cord. Electrodes were filled with a solution combining potassium citrate (1.5 M) and potassium chloride (1.5 M). All currents were applied in the discontinuous current clamp mode (Axoclamp 2A amplifier, Axon Instruments; switching frequency of 8–10 kHz; data with inadequate settling of electrode transients were rejected). Voltage clamp was applied using the single electrode discontinuous mode [as for current clamp, switching rates were 8–10 kHz; low-frequency gain (\(-3\) dB point of 30 Hz) was enhanced 11-fold by an external circuit, resulting in gains that ranged from \(-100 \) to \(-300 \) nA/mV] (see Lee and Heckman 1998a for details).

Generation of Synaptic Input. The Achilles tendon was attached to a computer-controlled muscle puller. Muscle stretch was used to generate a slowly rising synaptic input to the MG and LGS motoneurons. These muscles were held at a length \(-8–10\) mm short of physiological maximum for all measurements of input conductance and frequency-current relations (procedures for these measurements are described below). A 10-mm stretch was applied by first shortening the muscle by 5 mm, stretching it by 10 mm, and then returning to the rest position (see Fig. 2D).

Measurements of Stretch-Evoked Firing Patterns and Effective Synaptic Currents. The cell was depolarized with injected current to threshold levels for rhythmic firing, as judged by the presence of either slow rhythmic firing (\(-10–20\) Hz) or a slower irregular pattern (i.e., the subprimary range [Kudina and Alexeeva 1992]). The 10-mm stretch was then applied, with the initial shortening silencing the firing. Firing was re-initiated as the muscle was progressively stretched (see Fig. 2). In a separate trial, the motoneuron was voltage clamped. The 10-mm stretch was applied at a hyperpolarized holding potential (typically 10 mV hyperpolarized with respect to the resting potential, resulting in holding potentials ranging from \(-65 \) to \(-85 \) mV) to measure stretch-evoked \( I_{N,\text{PASS}} \). Note that the amplitude and pattern of stretch-evoked \( I_{N,\text{PASS}} \) was found to be reasonably consistent from trial to trial within a single cell (trial to trial variability, assessed in 8 cells, was \(-10–15\%) \). Stretch \( I_{N,\text{TOT}} \) was defined as the stretch-evoked \( I_N \) measured within 2–3 mV of the voltage threshold for initiation of spikes in unclamped conditions (this typically occurred at about \(-50 \) mV). This spike threshold level was considered a reasonable approximation of the average membrane potential during steady rhythmic firing.

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MEASUREMENTS OF FREQUENCY-CURRENT FUNCTIONS AND INPUT CONDUCTANCES. A slow triangular current ramp (5 s to reach peak and 5 s to return to baseline), 10–30 nA in amplitude, was applied to the cell. If necessary, a steady depolarizing current was applied to bring the cell near threshold (for the highest input conductance cells). A few low-input conductance cells exhibited tonic firing in the resting state, requiring application of steady hyperpolarizing currents to eliminate firing before initiating the triangular current. Input conductance was usually assessed from the subthreshold region of the response to the triangular current used to generate the F-I function. A 10-mV region whose upper limit was ≥5 mV below the onset of firing was used to calculate input conductance.

Experimental protocols

The order of measurement protocols varied. However, current-clamp data (for stretch-evoked firing and F-I) tended to be taken before voltage-clamp data (stretch \( I_{\text{N,pass}} \) and stretch \( I_{\text{N,tot}} \)). In cells where recording quality remained stable, one or more of these basic protocols were repeated. In some cells, these repeat protocols included measurements of stretch \( I_{\text{s}} \) at various membrane potentials between those used to obtain stretch \( I_{\text{N,pass}} \) and stretch \( I_{\text{N,tot}} \).

Data analysis

GAIN OF THE NEURON’S NET INPUT-OUTPUT FUNCTION. \( I_{\text{ON}} \) was constructed by plotting stretch-evoked firing versus stretch \( I_{\text{N,pass}} \). Onset of firing to stretch was defined as the point during stretch where the instantaneous firing frequency began to consistently exceed 5 Hz. The gain of \( I_{\text{ON}} \) (in spikes s\(^{-1}\) nA\(^{-1}\)) was only measured from the slope of the regression relation between frequency and current while firing increased following this onset point so that the saturation in firing in response to stretch was excluded (see Fig. 2). At minimum, this gain calculation was based on ≥1 s of firing data, which never included <10–15 spikes. Cells in which stretch only generated a rapid surge in firing lasting <1 s were also fitted with regression relations to allow calculation of the net change in firing rate (see following text), but these cells were not included in the analyses of \( I_{\text{ON}} \) (see RESULTS). Regression relations for all cells had \( r \) values that were statistically significant (\( P > 0.05 \)). In some cells where multiple records of firing were obtained, firing patterns were variable in response to repeated stretches (see RESULTS). The record chosen for the calculation of gain in these variable cells was the one in which rate of increase of firing was steepest, subject to two further criteria: the increase had to occur over at least a 1-s period (as noted in the preceding text) and the pattern of firing had to be reasonably smooth compared with the other records. In addition to gain, we also measured the net change in firing frequency during stretch. This change was defined by the difference between the firing rates at the beginning and the end of the regression line fit to the firing rate versus current relation. Cells that only generated a rapid surge in firing were included in this analysis.

GAIN OF THE F-I FUNCTION. Firing frequency was plotted versus injected current. The slope of this relationship defined F-I gain. In some cells (see RESULTS), a significant acceleration in firing occurred once firing rate exceeded ~30–50 Hz. F-I gains were only calculated from the data points before acceleration occurred.

STATISTICS. Linear regression models were used for assessing trends in the data. In many cases, the cells were divided into two samples, those with low-input conductances (<1.0 \( \mu \)S) and those with high-input conductances (>1.0 \( \mu \)S). This division was made for convenience in certain analyses and does not imply a bimodal distribution of the measured parameters. All parameters were continuously distributed with respect to input conductance (e.g., Figs. 5 and 7). The \( t \)-test used to compare the averages of these two samples assumed that sample variances were unequal, providing a more conservative test than the standard equal variance assumption. The significance level for all statistical tests, alpha, was set at 0.05. If multiple comparisons were made, alpha was divided by the number of comparisons (i.e., the Bonferroni correction for multiple comparisons).

RESULTS

The effect of muscle stretch on motoneuron firing patterns was studied in 41 cells (21 cells had low-input conductances (<1.0 \( \mu \)S); 20 had high-input conductances (>1.0 \( \mu \)S)). In most cells (\( n = 24 \)), the speed of the applied 10-mm stretch was 2 mm/s, but 12 cells were also studied at a faster speed (4 mm/s) and 5 at a slower speed (1 mm/s). Figure 2 shows examples of stretch-evoked firing patterns and their corresponding \( I_{\text{N,pass}} \) patterns in six different cells, grouped in pairs according to stretch speed. As noted in METHODS, the stretch-evoked \( I_{\text{N,pass}} \) was measured at hyperpolarized levels to minimize activation of the dendritic PIC. In each pair, the dark traces illustrate the firing pattern and \( I_{\text{N,pass}} \) for a low-input conductance cell and the lighter traces illustrate the same behaviors for a high-input conductance cell. Firing rate initially increased approximately in proportion to the stretch and then tended to saturate at a relatively steady rate as stretch continued (the regression lines were only fit to the data before saturation occurs). In some cells, the firing pattern was highly variable about an overall increasing trend (e.g., cell 4) or included brief periods where the firing rate leveled off before continuing to increase (cell 5).

In each pair, stretch generated higher firing rates in the lower input conductance cells. In all six cells, \( I_{\text{N,pass}} \) progressively increased with stretch. There were no significant differences in the peak amplitude of \( I_{\text{N,pass}} \) in low- versus high-input conductance cells in the full sample of cells (\( t \)-test, \( P > 0.05 \)); 20 low- and 20 high-input conductance cells; current measurements were not obtained in 1 low-conductance cell; see Fig. 7). Finally note that the progressive increase in firing evoked by stretch lasted ≥1 s. In cells 3, 5, and 6, the progressive increase lasted 2–3 s.

Input-output functions in response to muscle stretch

To quantify how stretch affected generation of firing in each cell, the stretch-evoked firing rate was plotted as a function of the stretch-evoked \( I_{\text{N,pass}} \) to reveal \( I_{\text{ON}} \), the input-output function of the neuron for stretch. \( I_{\text{ON}} \) assesses the net effects of the dendritic PIC on both \( I_{\text{s}} \) and the F-I function (see METHODS). Figure 3 shows \( I_{\text{ON}} \) for each of the six cells illustrated in Fig. 2 (the saturating portion of these functions has been removed to allow clear illustration of gain differences). In each case, the gain of \( I_{\text{ON}} \) was greater for the low-input conductance cells. The response of each cell to injected current was also assessed to allow for comparison of F-I and \( I_{\text{ON}} \). Figure 4 illustrates the typical finding that the initial gain of \( I_{\text{ON}} \) tended to be greater than that of F-I in both low- and high-input conductance cells. The increase in F-I slope seen in the low-input conductance cell at higher current levels was due to an acceleration in firing (see following text).

VARIATION IN I-ON WITH INPUT CONDUCTANCE. Figure 5 presents a statistical analysis of the trends illustrated in Figs. 2–4. There were no significant differences between the data sets for
different stretch speeds, so the primary analyses were performed on the combined data set. The gain of I-O N tended to decrease with increasing input conductance ($r = -0.78$, $n = 34$, $P < 0.001$; 6 cells were excluded because they exhibited only sudden accelerations to stretch—see METHODS; 1 cell lacked data for stretch currents). The preceding calculation of gain of I-ON (see METHODS) excludes the saturation in which firing rate stayed constant as length and $I_{N, PASS}$ both increased (see Fig. 2). To assess the gain to stretch including this saturation in firing rate, the net change in firing frequency generated by stretch was divided by the amplitude of $I_{N, PASS}$ achieved at the longest stretch length. This measure of overall stretch gain was also negatively correlated with input conductance ($r = -0.58$, $n = 40$, $P < 0.01$), showing that differences in saturation were not the primary cause of the gain versus input conductance relationship. Overall, these results show that stretch, combined with the effects of the dendritic PIC, was a more potent input to low- than to high-input conductance motoneurons.

COMPARISON OF I-O N TO F-I FUNCTIONS. $F$-$I$ functions were obtained in 34 of the 41 cells. Figure 5 includes the relationship between gain of $F$-$I$ and input conductance (5 of the 34 cells were in the group with sudden acceleration to stretch; these were excluded from the comparison with I-O N in Fig. 5 and following text). Just as for I-O N, $F$-$I$ tended to be larger in low-input conductance cells ($r = -0.44$, $n = 29$, $P < 0.05$). However, the slope of the gain-input conductance relation was significantly steeper for I-O N than for $F$-$I$ ($t$-test for slope parallelism, $P < 0.05$). As a result, the gains of I-O N were, on average, ~2.3-fold larger than gains than for $F$-$I$ ($5.2 \pm 2.4$ vs. $2.3 \pm 1.0$ Hz/nA; this analysis only includes cells in which both $F$-$I$ and I-O N were obtained, $n = 29$; paired $t$-test, $P < 0.0001$). When this comparison was restricted to low-input

**FIG. 2.** Patterns for rhythmic firing and $I_{N, PASS}$ (see Fig. 1) evoked by stretch of the triceps surae muscles in 6 different triceps surae motoneurons. The traces in A–C each contain the behavior for a pair of cells. In each pair, the filled symbols and thick traces indicate the firing patterns and currents, respectively, for a low-input conductance motoneuron ($< 1$ $\mu$S), whereas the open symbols and thin traces indicate the same behaviors for a high-input conductance cell ($> 1$ $\mu$S). Baseline offsets for the currents have been removed. Regression functions (thick straight lines) have been fit to the data for each cell during the phase when firing increased in proportion to stretch. A: stretch applied at 4 mm/s. Cell 1: input conductance ($G_N$): 0.71 $\mu$S; baseline injected current for firing: 5.4 nA. Cell 2: $G_N$: 1.9 $\mu$S; baseline current: 21.9 nA. B: stretch applied at 2 mm/s. Cell 3: $G_N$: 0.49 $\mu$S; baseline current: 2.6 nA; cell 4: $G_N$: 1.4 $\mu$S; baseline current: 15.6 nA. C: stretch applied at 1 mm/s. Cell 5: $G_N$: 0.82 $\mu$S; baseline current: 5.8 nA; cell 6: $G_N$: 1.40 $\mu$S; baseline current: 12.9 nA. D: changes in muscle length for A–C.
conductance cells, the difference between gains of I-O_N and F-I was slightly larger: 2.7-fold (7.9 ± 1.9 vs. 2.6 ± 1.1; \( P < 0.0001 \), paired \( t \)-test, \( n = 14 \)).

**ACCELERATION IN F-I FUNCTIONS.** In 9 of the 34 total cells with an F-I function, a marked acceleration in firing was present, presumably due to activation of the dendritic PIC (Bennett et al. 1998; Hounsgaard et al. 1988; Lee and Heckman 1998b). In eight of these nine cells, the acceleration in the F-I function occurred at or above the range at which the stretch-evoked firing saturated (as in Fig. 4) (cf. Bennett et al. 1998). F-I gain in these cells was measured from the preacceleration portion of the function. In one cell, there appeared to be F-I acceleration right at threshold. (Note that F-I data from this cell and 4 of the 8 other cells with acceleration were excluded from the preceding comparison with I-O_N gain because they also only responded to stretch with a strong acceleration in firing.) Overall,

![Figure 3](image-url)  
**FIG. 3.** The I-O_N function, which plots stretch-evoked firing vs. stretch-evoked effective synaptic currents (\( I_{N,\text{PASS}} \), which was measured at hyperpolarized levels), for the 6 cells shown in Fig. 1. •, the function for the low-input conductance cell; ○, the function for the high-input conductance cell. Note that the baseline injected currents to bring the cells to threshold for firing before stretch application are not included within the x-axis values. The lines indicate the linear regression relations, which were only calculated while firing rate was increasing (the data points for the regression lines in Fig. 2 are identical to those in Fig. 1). A: stretch applied at 4 mm/s; B: 2 mm/s stretch; C: 1 mm/s stretch. Regression data: cell 1: slope = 6.1, \( r = 0.85, n = 38 \); cell 2: slope = 1.9, \( r = 0.65, n = 16 \); cell 3: slope = 5.7, \( r = 0.91, n = 61 \); cell 4: slope = 3.5; \( r = 0.51, n = 25 \); cell 5: slope = 10.3, \( r = 0.91, n = 35 \); cell 6: slope = 2.9, \( r = 0.63, n = 33 \). All regression relations in these and all cells in the sample were statistically significant at \( P < 0.05 \).

![Figure 4](image-url)  
**FIG. 4.** Comparison of frequency-current (F-I) functions generated by stretch (I-O_N) and injected (F-I) currents in 2 different cells. For I-O_N, the x-axis current is a combination of injected current, to reach baseline for firing, and synaptic current (\( I_{N,\text{PASS}} \), for modulation of firing. •, low-input conductance cell (cell 5 from Figs. 2C and 3C). ○, high-input conductance cell (cell 6 from Figs. 2C and 3C). —, the linear regression relations for each function. Note: as in Figs. 2 and 3, the regressions were only fit to the portion of each record where firing was monotonically increasing.
These results show that a stretch-evoked input capable of activating the dendritic PIC was much more effective at generating rhythmic firing than an equivalent amount of injected current that did not activate the dendritic PIC.

Depolarization-dependent changes in stretch $I_N$

The most likely explanation for the higher gain of $I_{ON}$ as compared with $F-I$ is that the stretch input activated the dendritic PIC to a much greater extent than did the injected current at the soma (cf. Bennett et al. 1998; Prather et al. 2001). To assess the impact of the dendritic PIC on stretch-evoked $I_N$, we evaluated how $I_N$ changed as the holding potential was shifted in a depolarizing direction, including the range traversed by the membrane potential during rhythmic firing where $I_{N,\text{TOT}}$ was measured (see METHODS).

Effects of Membrane Potential on $I_N$

Figure 6A shows how stretch $I_N$ changed as a function of holding potential in a low-input conductance cell. Stretch $I_{N,\text{TOT}}$ (recorded at $-49$ mV) was markedly enhanced as compared with that recorded at hyperpolarized levels to measure $I_{N,\text{PASS}}$ ($-71$ mV for this example). Figure 6B illustrates another low-input conductance cell, which was exceptional in that it had the most abrupt increase in firing in response to stretch (it was 1 of the 6 cells noted in the preceding text as being excluded from Fig. 5). This cell was also exceptional in that stretch-evoked $I_N$ reached its maximum amplitude at a membrane potential ($-57$ mV) between the potentials used to measured $I_{N,\text{TOT}}$ and $I_{N,\text{PASS}}$. Nonetheless, $I_{N,\text{TOT}}$ was larger than $I_{N,\text{PASS}}$ until near the end of the stretch. In the high-input conductance cell in Fig. 6C, stretch $I_{N,\text{TOT}}$ was slightly smaller than stretch-evoked $I_{N,\text{PASS}}$. Stretch-evoked $I_{N,\text{TOT}}$ in this cell also had a tendency to exhibit bursts of net outward current. Presumably these inhibitory bursts are less apparent in $I_{N,\text{PASS}}$ because the cell is much closer to the inhibitory reversal potential (cf. Powers et al. 1993).

Variation in $I_N$ with input conductance. $I_{N,\text{TOT}}$ was successfully measured in 26 of the 41 cells. Figure 7 shows that $I_{N,\text{TOT}}$ tended to decrease with increasing input conductance, although there is considerable scatter in the data. Figure 7 also

![Figure 5](image.png)  
**FIG. 5.** Gains of $I_{ON}$ and $F-I$ plotted as a function of input conductance for the full data sample. Thick line: regression relation for the $I_{ON}$ ($y = 9.6 - 3.9x$, $r = -0.78$, $n = 34$, $P < 0.001$). Thin line: regression line for $F-I$ ($y = 3.4 - 1.1x$, $r = -0.45$, $n = 29$, $P < 0.01$).

![Figure 6](image.png)  
**FIG. 6.** Enhancement of stretch-evoked $I_N$ as a function of membrane holding potential in 3 different cells. **A–C**, top: the firing pattern (symbols) and length changes (line) generated by the stretch. The thick lines in A and C are the regression relations for this increased period of increasing firing (not shown for B, as the increase in firing was too abrupt). **Bottom**: stretch-evoked $I_N$ at the indicated holding potentials. A: low-input conductance (0.5 $\mu$S) motoneuron. B: low-input conductance (0.6 $\mu$S) motoneuron. Triangles indicate high-frequency "double" firing. C: high-input conductance (2.0 $\mu$S) motoneuron.
FIG. 7. The relationship among the 3 measures of stretch-evoked \( I_N \) defined in Fig. 1 plotted as a function of input conductance. Filled diamonds and thick regression line: \( I_{N,\text{TOT}} \), \( y = 14.3 - 5.1x, n = 26, r = 0.45, P < 0.05 \). Open diamonds and thin regression line: \( I_{N,\text{ACT}} \) (which equals \( I_{N,\text{TOT}} \) minus \( I_{N,\text{PASS}} \); \( y = 6.2 - 4.8x, n = 26, r = 0.49, P < 0.05 \)). Crosses and dotted regression line: \( I_{N,\text{PASS}} \) (not significantly related to input conductance; \( P > 0.6 \)). Dashed line: the 0 level on the y axis.

shows that \( I_{N,\text{ACT}} \) (\( I_{N,\text{TOT}} \) minus \( I_{N,\text{PASS}} \); see Fig. 1) exhibited a similar negative correlation with input conductance. \( I_{N,\text{TOT}} \) was smaller than \( I_{N,\text{PASS}} \) in eight cells (1 low- and 7 high-input conductance cells), resulting in negative values for \( I_{N,\text{ACT}} \). In the high-input conductance cells, the negative value for \( I_{N,\text{ACT}} \) appeared to in part be due to either activation of outward currents or the presence of stretch-evoked inhibition (as in Fig. 6C). The one low-input conductance cell in the negative group is the cell in Fig. 6B where peak enhancement of \( I_N \) occurred hyperpolarized to spike threshold used to assess \( I_{N,\text{TOT}} \). As noted in the preceding text, there was no significant relation between \( I_{N,\text{PASS}} \) and input conductance. The approximately constant difference between \( I_{N,\text{TOT}} \) and \( I_{N,\text{ACT}} \) was consistent with the finding, also noted in the preceding text, that \( I_{N,\text{PASS}} \) was not significantly related to input conductance (average value: \( 5.7 \pm 1.7 \) nA). Both \( I_{N,\text{TOT}} \) and \( I_{N,\text{ACT}} \) correlated with the change in firing evoked by stretch \( (r = 0.71 \) and 0.73, respectively, \( P < 0.01 \) in both cases). Because \( I_{N,\text{ACT}} \) reflects the activation of the dendritic PIC (see DISCUSSION), it appears that differences in this activation correlate with the differences in stretch-evoked firing patterns in low- and high-input conductance cells.

**SATURATION IN \( I_{N,\text{TOT}} \).** Figure 6, A and B, also illustrate the typical finding that, approximately halfway through the stretch, \( I_{N,\text{TOT}} \) reached a more or less steady level. At levels depolarized to that for \( I_{N,\text{TOT}} \), \( I_N \) amplitude tended to declined and saturation occurred earlier in the stretch (tested in 7 cells). Saturation occurred in \( I_{N,\text{TOT}} \) in most of the cells it was measured in (19 of the 26 or \( \sim 73 \% \)). Saturation also occurred in the stretch-evoked firing patterns (see Fig. 2) in most cells (37 of 41 or \( \sim 90 \% \)). The time points for saturation in firing and in \( I_{N,\text{TOT}} \) correlated reasonably well (0.75, \( P < 0.01, n = 26 \)). These results suggest that saturation in \( I_{N,\text{TOT}} \) could account for the saturation in firing.

**Predictions of stretch firing using \( I_N \) and \( F-I \).**

One advantage of using \( I-O_N \) for the analysis of the input-output function in response to stretch is that it includes a direct measurement of the stretch-evoked firing pattern. Consequently, it predicts firing perfectly and the only sources of error are in the estimations of \( I_{N,\text{PASS}} \) (see DISCUSSION). However, the alternative model of Fig. 1, \( I_{N,\text{TOT}} \) and \( F-I \), is also a valid analysis of the input-output function if two assumptions are correct: that \( I_{N,\text{TOT}} \) fully accounts for the effect of stretch on firing rate and that the \( F-I \) function does not significantly activate the dendritic PIC.

**PREDICTION OF NET CHANGE IN FIRING.** To test the \( I_{N,\text{TOT}}/F-I \) model, the overall change in \( I_{N,\text{TOT}} \) during the time period for which firing steadily increased in response to stretch was multiplied by the gain of the \( F-I \) function (\( I_{N,\text{TOT}} \) was smoothed to remove fluctuations such as those in Fig. 6C). This result for predicted change in firing to stretch was then compared with the actual change in firing (which, as noted in METHODS, was measured over the same time period). Figure 8 shows that the predicted firing values were indeed close to the actual values \( (r = 0.87, n = 18) \); only cells with measurements of both \( F-I \) and \( I_{N,\text{TOT}} \) were included. The predicted regression line was not significantly different from 1.0 (\( t \)-test for parallelism, \( P > 0.4 \)). The tendency for underestimation in the cells with low rates probably reflects the increased fluctuations in \( I_{N,\text{ACTIVE}} \) compared with \( I_{N,\text{PASS}} \) in some high-input conductance cells (see Fig. 6C). Note also that a model that fails to take depolarization-dependent enhancement of \( I_N \) into account, i.e., \( I_{N,\text{PASS}} \) times \( F-I \) gain, gave predicted firing rates that correlated with the actual rates \( (r = 0.75) \) but fell well short of the actual values (open symbols in Fig. 8A). The slope of the regression line (0.33; significantly different from 1.0; \( t \)-test for slope parallelism, \( P < 0.01 \)) indicates that failure to take the dendritic PIC into account underestimates the impact of synaptic input by a factor of \( \sim 3 \). This value for estimation of the effect of the dendritic PIC on stretch input compares reasonably well with the 2.7-fold greater gain of \( I-O_N \) versus \( F-I \) cited in the preceding text with respect to the low-input conductance cells in Fig. 5. These results suggest that both models of Fig. 1 provide a reasonable model of motoneuron input-output processing in the presence of a strong dendritic PIC (cf. Schwindt and Crill 1996) (see DISCUSSION).

**PREDICTION OF THE DYNAMIC TIME COURSE OF FIRING.** The similarity between the temporal patterns of stretch-evoked firing and stretch-evoked \( I_{N,\text{TOT}} \) in Fig. 6A shows an example where saturation occurs in both current and firing at about the same point in the stretch, suggesting that accurate predictions of the full dynamic firing pattern during stretch from \( I_{N,\text{TOT}} \) might be possible. To evaluate this possibility, each time point in \( I_{N,\text{TOT}} \) before, during, and after stretch was multiplied by the gain of the \( F-I \) function. The resulting pattern of predicted firing rates were compared with the actual stretch-evoked rates by plotting the two against each other and applying regression analysis. Figure 8B shows that the correlation coefficients from this analysis (open circles) were reasonably good for cells in
which stretch generated a reasonably high firing rate (>10 Hz). Moreover, the slope of the regression relations (filled circles) tended to be near 1.0, showing that the magnitude of the predicted firing matches the actual reasonably well. There were, however, several cells in which the firing rates were underestimated (slope > 1.0) or overestimated (slope < 1.0). These errors are consistent with the scatter of values for net change in firing in Fig. 8A. The low values for correlations and slopes for cells with low firing rates appeared to result from a lack of modulation of firing—stretch-evoked changes in both $I_{N,TOT}$ and firing were small and noisy in these cases (see the example in Fig. 6C).

**Amplification or addition?**

Figure 6B provides a counter example to the generally good predictions of the time course of stretch-evoked firing documented in Fig. 8B. In this cell, the smooth modulation of all stretch currents, including those at depolarized levels with substantial saturation, was not matched by a similar smooth modulation in firing, which tended to exhibit doublets (small arrows) and a sharp stepwise increase in firing midway through stretch. Correlation between the firing pattern predicted by $I_{N,TOT}$ and the actual pattern was very poor (trials like this in which firing rate exhibited a sudden acceleration were not included in either Fig. 8A or B). This step increase in stretch-evoked firing illustrated in Fig. 6B suggests that the dendritic PIC is providing a stepwise addition in synaptic input instead of a proportional input amplification. Sudden accelerations in firing rate are in fact the standard form that activation of the dendritic PIC, with the natural synaptic input provided by muscle stretch generated an increase in firing before saturation that was longer than 1 s in most cells. In the 34 of the 41 totals cells in which gain for $I_{O-N}$ was calculated average durations of graded firing rate increases were as follows: slowest stretch, $3.7 \pm 1.0$ s; medium, $1.6 \pm 0.8$ s; fastest, $1.3 \pm 0.6$ s. The slowest stretch was significantly different from both the medium and fast speeds [$t$-test, $P < 0.0167 (\approx 0.05/3)$; the medium and fast speeds did not significantly differ; $P > 0.2$]. This

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** FIG. 8. Predictions of stretch-evoked firing frequencies using stretch $I_N$ and the gain of $F-I$ function. A: prediction of net change in firing, with the predicted on the $y$ axis and the actual on the $x$ axis. Filled symbols: predicted frequencies using stretch $I_{N,TOT}$, which is recorded at holding potential within the range traversed during rhythmic firing. Open symbols: predicted frequencies using stretch $I_{N,PASS}$, which is recorded at a hyperpolarized level to minimize the activation of the dendritic PIC. Dashed line: slope of 1.0, indicating perfect prediction. Thin lines: regression relations, with equations as shown. B: prediction of the time course of firing before, during, and after the stretch. The $y$ axis shows the scale for both correlation coefficients and slopes from the regression relation in each cell for predicted firing plotted as a function of actual firing. The $x$ axis shows the actual firing rate for each cell. Filled symbols: correlation coefficients. Open symbols: regression slopes. Solid line: 0 level on the $y$ axis. Dashed line: 1.0 indicating a perfect correlation and a perfect slope match.**

** FIG. 9. Variability of the firing pattern in a single low-input conductance (0.52 μS) cell in response to stretch. The 2 firing patterns shown here with symbols are the extreme behaviors from a set of 5 repeats. The other 3 patterns (thin dotted lines) fell between these extremes.**
longer duration suggests that the natural input from stretch induces graded activation of the dendritic PIC in the majority of cells during rhythmic firing.

**VARIABILITY IN FIRING PATTERNS.** As noted in the preceding text, six cells exhibited only a rapid acceleration in firing to stretch, which lasted <1 s (as in the example provided by Fig. 6B). To explore variability in firing behavior, repeated stretch trials ($n = 2$ to $5$) were applied in 21 cells (11 low-input conductance cells, 10 high). The majority (14 of 21) of these cells exhibited consistent firing patterns without sudden accelerations, but in seven cells, the firing patterns sometimes increased progressively with stretch and sometimes underwent a rapid surge. Figure 9 illustrates such a case, where repeated trials range from rapid acceleration to smoothly graded. The total sample of 21 low-input conductance cells was also split about evenly between cells with progressive increases (11 cells) and with sudden surges (10 cells, including 4 that exhibited surges in the only stretch trial applied). In contrast, the majority of high-input conductance cells had consistent, progressive increases (9 of 10 of the cells with multiple trials, 18 of 20 in the total sample). No obvious factors correlated with the presence of variable firing behavior in low-input conductance cells—for example, higher levels of baseline firing were associated with a tendency for acceleration in one cell but had no apparent effect in two other cells. These results indicate that activation of the dendritic PIC by stretch input sometimes includes a relatively rapid, nongraded event, much as often occurs for its activation by injected currents.

**TIME COURSE OF GRADED $I_N$ DURING STRETCH.** In contrast to the case for firing, we have never observed sudden acceleration in the stretch-evoked $I_N$ during voltage clamp (the example in Fig. 6B provides a clear distinction between abrupt firing and smooth current). Figure 6A shows a cell with one of the more rapid rates of rise of stretch $I_N$—but even here, the smooth increase to onset of saturation in $I_{N,TOT}$ occurred over the course of ~3 s. In those cells in which $I_{N,TOT}$ was enhanced compared with $I_{N,PASS}$ ($n = 18$), the average duration of the increase in $I_{N,TOT}$ for the 2.5-s stretch was $1.6 \pm 0.5$ s and for the 5-s stretch was $2.9 \pm 0.9$ s (there were only 2 cells for the 10-s stretch, both increased for >3 s). Thus during voltage clamp, stretch $I_N$ is always smoothly graded, but in about half of the low-input conductance cells, stretch firing sometimes exhibited a rapid acceleration.

**DISCUSSION**

The dendritic PIC generated a strong depolarization-dependent enhancement in stretch-evoked $I_N$. The properties of this enhancement were consistent with several key features of the effects of stretch on rhythmic firing in comparison to the effects of injected current. These stretch effects include the relatively higher gain of I-O$N$ than F-I in the initial phase of the stretch, the strong saturation in stretch-evoked firing, and the greater effect of stretch on low- versus high-input conductance cells. Overall, the dendritic PIC enhanced the effect of synaptic input on firing by two- to threefold. Figure 1 illustrated two ways of summarizing this enhancement in input-output processing. The $I_{N,PASS}$ and I-O$N$ model had perfect prediction of firing rates built in, as actual firing patterns were used in its construction. The predictions for the $I_{N,TOT}$ and F-I model were subject to the variability of both main parameters. In particular, the strong dependence of $I_{N,TOT}$ on holding potential probably induced significant variability. Differences of just 2–3 mV could substantially change the time course and amplitude of $I_{N,TOT}$ (see Fig. 6, A and B). Nonetheless, the predictions for this model were reasonably good. However, prediction of the time course of firing in general was subject to a significant limitation. Firing behavior in response to stretch was highly variable in about half of the low-input conductance cells, and this variability was not present in stretch-evoked $I_{N,TOT}$. This firing variability suggests that the activation of the dendritic PIC during rhythmic firing can be variable.

**Potential sources of error in estimates of the effect of the dendritic PIC on rhythmic firing**

$I_{N,PASS}$ may be influenced by a significant degree of activation of the dendritic PIC in spite of the hyperpolarized holding potential. Consistent with this, our previous study (Lee and Heckman 2000) has demonstrated that $I_{N,PASS}$ in the decerebrate evoked by selective activation of Ia afferents is ~30–40% larger in the decerebrate preparation than in the pentobarbital-anesthetized preparation where the dendritic PIC is suppressed. Furthermore, although portions of the F-I function with overt acceleration were excluded from our analyses (see RESULTS), it is possible that a small degree of activation of the PIC might have occurred during current injection without overt acceleration. This is especially true because the rest length between stretches produced steady muscle afferent input, which tends to lower the threshold for PIC activation via current injection (Bennett et al. 1998) (note the reduction in $I_N$ during the initial shortening phase preceding the stretch in Figs. 2 and 6A). In fact, the average F-I gain for this study (2.2 Hz/μA) was greater than the average obtained with similar methods in pentobarbital-anesthetized animals (~1.6 Hz/μA) (Lee and Heckman, unpublished data). Either of these effects of the dendritic PIC would cause a substantial underestimation in its impact on input-output processing. However, there exist reasonable alternative explanations for the preceding differences between decerebrate and pentobarbital preparations that do not involve the dendritic PIC. $I_{N,PASS}$ may be larger in the decerebrate due to differences in excitability of presynaptic inhibitory circuits. F-I gain may be larger because the monoaminergic inputs active in the decerebrate can increase F-I gain by shortening the duration of the afterhyperpolarization (e.g., Lee and Heckman 1999; reviewed in Powers and Binder 2001). Given these uncertainties, our estimate of the dendritic PIC enhancing the impact of synaptic input on firing generation by a factor of 2–3 should be considered a lower limit with a larger effect being possible.

**Mechanisms of enhanced input-output gain**

During voltage clamp, the enhancement of stretch-evoked $I_N$ at depolarized levels is likely due to actions of the stretch input on portions of the motoneuron outside the region under good clamp control. As pointed out previously (Lee and Heckman 1996, 2000; Schwindt and Crill 1995), the voltage clamp at the soma prevents changes in the activation of voltage-sensitive conductances in this region. However, the dendrites are not well controlled by the clamp and therefore the stretch input can activate dendritic voltage-
sensitive conductances. This interpretation assumes that the stretch-evoked synaptic input is purely ionotropic and that it does not activate N-methyl-d-aspartate (NMDA) receptors to any significant extent. Much of the stretch-evoked input is due to activation of muscle spindle Ia afferents, which monosynaptically excite motoneurons. In the adult cat, the Ia input does appear to be ionotropic (Brownstone et al. 1994; see also the discussion in Lee and Heckman 2000). Moreover, administration of antagonists for NMDA receptors has no effect on the reflex force generated by Ia afferents in the decerebrate preparation (Miller et al. 1997).

Stretch activates several other muscle afferents in addition to Ia’s, including group II spindle afferents and, to some degree, Golgi tendon organ Ib afferents and muscular free nerve endings (Matthews 1972). It is not known whether synaptic input reaching motoneurons via these other pathways utilize metabotropic glutamate or NMDA receptors. In fact, the proportion of the total stretch current due to the monosynaptic Ia input as compared with these other inputs still remains uncertain. However, just as for selective activation of Ia afferents, the reflex force generated by stretch is unaffected by NMDA antagonists in the decerebrate preparation (Miller et al. 1997). Thus although a contribution from metabotropic glutamate receptors has not been ruled out, most of the depolarization-dependent enhancement of stretch $I_N$ is probably due to activation of the dendritic PIC.

Saturation in synaptic efficacy and comparison to human motor unit firing patterns

The high gain to stretch in low-input conductance motoneurons comes with an important limitation: once the cell reaches a firing rate of $\sim 20–40$ spikes/s, it becomes completely insensitive to further stretch. This saturation is consistent with our previous study of the depolarization-dependent amplification of the $I_N$ generated by selective activation of Ia afferents (Lee and Heckman 2000). Presumably, once the dendritic PIC is fully activated, the dendrites are so strongly depolarized that subsequent input encounters both a sharply reduced driving force and, probably, voltage-dependent outward currents. In high-input conductance cells, saturation also occurs, but at a substantially lower firing rate—in fact, the stretch-evoked increase in firing was only a few spikes/second in some high-input conductance cells (e.g., cell 6 in Fig. 1). The reason for this difference is unclear but may reflect the presence of inhibition in the stretch-evoked input (see the next section). Further work is required to determine if the saturation seen here only applies to stretch or if different sources of input can overcome the saturation and increase firing. However, it is striking that the saturation in firing patterns evoked by graded stretch in the low-input conductance cells in this study looks similar to the marked decrease in slope of the firing rate versus force curve seen in low-threshold motor units recorded in human subjects during graded increases in voluntary force. In both cases, the firing during the saturation is noisier than during the increase in firing to reach that level (compare Fig. 1 to Fig. 4 in Kiehn and Eken 1997 and to Figs. 5 and 6 in Romaine et al. 1989). This comparison provides further support for a major role of the dendritic PIC in generating firing patterns in humans (e.g., Collins et al. 2001, 2002; Gorassini et al. 1998, 1999, 2002; Kiehn and Eken 1997).

Differences in low- and high-input conductance motoneurons

The lack of amplification of stretch-evoked input in the highest input conductance cells could result from several mechanisms, including a lack of dendritic PIC, a lack of metabotropic glutamate receptors evoked by the non-Ia component of the stretch input, or inclusion of stretch-evoked inhibition. The first possibility can be ruled out because our previous studies using selective activation of Ia afferents showed that the amplification of this input actually tended to be larger in high- rather than low-input conductance cells (Lee and Heckman 2000). Several of the high-input conductance cells in the present study exhibited irregular surges of outward currents at depolarized holding potentials (see Fig. 6B). Free nerve endings activated by stretch can produce inhibition of extensor motoneurons (“clasp knife” inhibition; Cleland and Rymer 1990). When the cord is intact, as in the present experiments, this inhibitory pathway is suppressed. However, it may be that the inhibitory component of the stretch input is relatively less suppressed in high- versus low-input conductance cells, resulting in a failure to activate the dendritic PIC in high-input conductance cells. This inhibitory input appears to be active right at stretch onset (note the bursts of outward current at stretch onset in Fig. 6C) but may not be tonically active during the maintained stretch present in other protocols. Free nerve endings tend to fire only in response to movements and do not maintain a tonic discharge (Cleland et al. 1990). Overall, it appears likely that synaptic integration in motoneurons with active dendrites is highly sensitive to the presence of inhibition in the synaptic input.

Can the dendritic PIC undergo graded activation?

An important recent study by Prather et al. (2001) investigated the interaction of two independent synaptic inputs in spinal motoneurons with strong dendritic PICs, using the same experimental paradigm as in the present work. As for the stretch input studied here, both inputs underwent depolarization-dependent amplification in which the firing rates they generated were much greater than predicted by the product of their $I_N$s at hyperpolarized levels and their $F-I$ gains. Further, just as in the present study, this was about a threefold enhancement. Prather et al. also showed that these inputs summed linearly in that the firing generated by simultaneous activation of the two inputs was, on average, equal to the algebraic sum of the firing effects of each input when activated independently. This striking result implies that the dendritic PIC can exist in partially activated states. Previous studies in our lab (Lee and Heckman 1996, 2000) also support the conclusion that the dendritic PIC can undergo graded activation. Ia $I_N$ underwent smooth increases in amplitude as the holding potential was slowly and continuously increased from hyperpolarized to depolarized levels. Nonetheless in the present study during current clamp, about half of the low-input conductance cells exhibited sudden surges in firing rate to stretch, suggesting that activation of the of at least part of the dendritic PIC can also occur in a nongraded manner. The resolution of this paradox awaits further study, but it may be that stable but partial activation of the dendritic PIC may be sensitive to the particular organization of the applied synaptic input, or it may be that two separate PIC mechanisms/currents exist. In addi-
tion, Prather et al. did not encounter saturation in the summation of their two inputs. This again suggests that different inputs can be processed differently by the dendritic PIC.

**Stretch input and motor outflow**

The classic study by Burke (1968) of stretch-evoked firing patterns showed that only motoneurons with medium- to low-input conductances would fire rhythmically in the absence of baseline injected currents to bring the cells near threshold. Baseline currents were applied in the present study. Our results for both firing patterns and effective synaptic currents evoked by stretch indicate that this input has a greater impact on low- as opposed to high-input conductance cells. Thus during stretch, not only would the low-input conductance cells have the lowest intrinsic thresholds but they would also receive a relatively stronger input. This input organization (Heckman and Binder 1993b) greatly enhances the tendency of motor units to be recruited in the normal, size principle sequence in which low-input conductance type S units are always activated first (Henneman and Mendell 1981). If, as argued in the preceding text, the presence of inhibition in the stretch-evoked I_N to high-input conductances is the primary reason this input fails to activate the dendritic PIC, then differences in the distribution of inhibition to low- and high-input conductance cells can have an especially strong impact on motor output.

**Input-output functions and motoneuron models**

Our previous models of the steady-state input-output structure of the mammalian motoneuron pool assumed that synaptic input could be represented by I_N and the conversion of I_N to frequency by F-I gain (Heckman and Binder 1991, 1993a). This assumption has been strongly supported by experimental studies in pontobulbar anesthetized preparations, where monoaminergic input is suppressed (Powers and Binder 1995; see Powers and Binder 2000 for exceptions). The effects of the dendritic PIC have been estimated in these models by decreasing the threshold and increasing the gain of F-I (Binder et al. 1993; Heckman 1994). This procedure is consistent with the model of Fig. 1 where I_O-N has a high gain in its processing of I_N,PASS. The same effect could be captured by increasing the synaptic weight for stretch input—i.e., using the model in Fig. 1 based on I_N,TOT and F-I gain. However, strong acceleration in F-I functions is present at low frequencies in our decerebrate preparation when a noradrenergic agonist is present (Lee and Heckman 1998b). This presents no problem for the I_N,PASS and I_O-N gain model, but some sort of modification would be required for the I_N,TOT and F-I gain model. The occurrence of rapid surges in stretch-evoked firing in many low-input conductance neurons is a problem that could probably be incorporated in either model. Finally, the decrease in I_O-N gain with increasing input conductance suggests that models will have to seriously consider the impact of inhibition on integration in dendrites with active conductances.

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