Bistability in Spinal Motoneurons In Vivo: Systematic Variations in Persistent Inward Currents

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Lee, R. H. and C. J. Heckman. Bistability in spinal motoneurons in vivo: systematic variations in persistent inward currents. J. Neurophysiol. 80: 583–593, 1998. Bistable behavior in spinal motoneurons consists of self-sustained firing evoked by a brief period of input. However, not all motoneurons possess an equal capacity for bistable behavior. In the companion paper, we found that self-sustained firing was present for long periods only in motoneurons with low rheobases and slow axonal conduction velocities. High rheobase, fast conduction velocity motoneurons tend to be only partially bistable in that self-sustained firing lasts at most 1–2 s. The mechanisms underlying these differences between fully and partially bistable motoneurons were investigated by measuring their current voltage (I-V) relationships in the decerebrate cat preparation after administration of the noradrenergic α agonist methoxamine. Slow (8 mV/s) triangular voltage commands were applied using the discontinuous single-electrode voltage-clamp technique. Both fully and partially bistable cells exhibited a region of negative I-V slope due to activation of a strong, persistent inward current. The peak amplitude of the total persistent inward current (I_{PIC}) was equally large in fully and partially bistable cells, but there were substantial differences in how I_{PIC} was activated and deactivated. In fully bistable cells, the onset of I_{PIC} on the descending phase of the triangular voltage command occurred at a substantially more hyperpolarized voltage than its onset on the rising phase. Thus the I-V function of fully bistable cells exhibited marked hysteresis. Partially bistable cells had significantly less hysteresis. The lack of hysteresis in partially bistable cells was due to a greater decay of I_{PIC} with time than that seen in fully bistable cells. Furthermore, the range over which activation and deactivation of I_{PIC} occurred was more depolarized in partially than in fully bistable cells. The I-V functions were compared with frequency-current (F-I) functions from the same cells, the characteristics of which were presented in the companion paper. The strong onset-offset difference in I_{PIC} in fully bistable cells corresponded to a similarly large hysteresis for the thresholds of their F-I functions. The reduced onset-offset difference for I_{PIC} in partially bistable cells corresponded to a lack of hysteresis in F-I thresholds. Thus the properties of I_{PIC} accounted for the main differences in the F-I behavior seen between fully and partially bistable cells.

INTRODUCTION

The monoamines norepinephrine and serotonin can cause spinal motoneurons to generate long-lasting plateau potentials and, consequently, to exhibit self-sustained firing (Conway et al. 1988; Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988). Self-sustained firing can be eliminated by a brief hyperpolarizing pulse of sufficient magnitude to deactivate the underlying plateau potential. Thus motoneurons are said to be bistable in the absence of input, in that they can be either be quiescent or firing tonically. However, not all motoneurons have an equal capacity for bistable behavior (Hounsgaard et al. 1988; Lee and Heckman 1996b). In the companion paper, we found that fully bistable motoneurons, which can generate self-sustained firing for long periods, tend to have low current thresholds for firing action potentials and slow conduction velocities in their axons (Lee and Heckman 1998). This makes good functional sense in that these motoneurons are most likely to innervate fatigue-resistant muscle fibers (Binder et al. 1996). In contrast, partially bistable motoneurons, which exhibit at most 1–2 s of self-sustained firing, have high thresholds and fast conduction velocities. Thus they likely innervate fatiguable muscle fibers.

Fully and partially bistable motoneurons also differ in their frequency-current (F-I) functions (Lee and Heckman 1998). The primary difference is that, in fully bistable cells, the threshold current level for rhythmic firing decreases once firing has begun. In partially bistable cells, the current levels for initiation and cessation of rhythmic firing are similar. In addition to these differences in F-I hysteresis in fully and partially bistable cells, differences in the firing acceleration due to the onset of plateau potential also were noted. The goal of this paper is to investigate the characteristics of the current-voltage (I-V) relations that generate these distinctive F-I behaviors.

Mammalian motoneurons in the adult cat exhibit a persistent inward current that can impart a negative slope region to the steady-state I-V function (Schwindt and Crill 1977, 1980). A persistent inward current is also evident in turtle motoneurons in spinal slice preparations. This current is mediated by L-type calcium (Ca^{2+}) channels and, in the presence of serotonin, generates plateau potentials and bistable behavior (Hounsgaard and Kiehn 1989; Svirskis and Hounsgaard 1997). In neonatal mouse brain stem motoneurons (Rekling and Feldman 1997) and in crab stomatogastric motoneurons (Zhang and Harris-Warrick 1995), Ca^{2+}-mediated nonselective cation channels play essential roles in sustaining the inward current during plateau potentials. Thus in motoneurons in the adult cat, the total persistent inward current (I_{PIC}) generating the plateau potential and bistable behavior may involve sustained activation of more than one type of channel.

An essential issue for understanding the hysteresis in the F-I function is how I_{PIC} activates and deactivates with changes in voltage. The persistent inward current in bistable motoneurons in the turtle exhibits a high degree of hysteresis between its activation and deactivation, with deactivation occurring at a much lower voltage and current than activation (Svirskis and Hounsgaard 1997). A similar behavior for I_{PIC} in cat motoneurons could account for the hysteresis in the
threshold current level in the $F-I$ function in fully bistable cells (Lee and Heckman 1998). The lack of $F-I$ hysteresis in partially bistable cells then might reflect a lack of $I-V$ hysteresis due to lack of a significant difference in the onset and offset $I_{\text{hyst.}}$.

To evaluate these possibilities, we used the discontinuous single-electrode voltage-clamp technique to obtain $I-V$ functions in cells in which $F-I$ behaviors were characterized by injected currents (see Lee and Heckman 1998 for the $F-I$ data). Our results showed that, as predicted, partially bistable cells have much less $I-V$ hysteresis. This was found to be due to a tendency for a greater decay in $I_{\text{hyst.}}$ with time. An unexpected finding was that $I_{\text{hyst.}}$ was activated at a substantially higher voltage in partially bistable cells than in fully bistable cells. A portion of these data has been presented in abstract form (Lee and Heckman 1996a, 1997).

METHODS

Measurements of $I-V$ relations

Single-electrode voltage-clamp data were obtained in 27 of the sample of 49 cells characterized during current clamp presented in the companion paper (Lee and Heckman 1998). All details of the decerebrate cat preparation and the current-clamp protocols are given there. Briefly, all studies were carried out after ventral topical application of the noradrenergic $\alpha_1$ agonist methoxamine. Thus the electrical properties of motoneurons were influenced by both this agent and the tonic activity in reticulospinal monoaminergic tracts, a characteristic of the decerebrate preparation (see Lee and Heckman 1998). The key current-clamp protocols were measurement of the duration of self-sustained firing, which we used to classify cells as either fully (duration $> 3$ s) or partially ($< 3$ s) bistable, and measurement of the $F-I$ function, which was done by applying triangular-shaped injected currents in bridge balance mode. Once these data were obtained, we changed to discontinuous, single-electrode voltage-clamp mode (Axoclamp 2A amplifier, Axon Instruments). Switching rates varied between 6 and 15 kHz. Headstage output was monitored at all times to assess settling of electrode transients. Data with inadequate settling were rejected. The clamp feedback gains ranged from 10 to 40 nA/mV. In addition, an external low-frequency feedback loop (gain of 100, $-3$ dB at 3 Hz) was used to virtually eliminate baseline offsets in voltage. Voltage-clamp data were filtered at 3 kHz, digitized at 5 kHz, and stored on removable hard disks for off-line analysis. The relatively high filter cutoff meant that the stored records were noisy, but this had the advantage of revealing any rapid spikes due to momentary loss of clamp control (see further text). Before any analyses were done, data records were further smoothed by digital filtering (typical $-3$ dB point of $\sim 0.3$ kHz).

The main characterization of $I_{\text{hyst.}}$ was accomplished with slow triangular voltage commands with a rise time of 5 s, a total duration of 10 s, and amplitude of 40 mV. The effect of changing the rate of rise of voltage was assessed in six cells (see RESULTS). In addition, 10-s duration depolarizing voltage steps were applied in seven cells to assess the rate of decay of $I_{\text{hyst.}}$. The holding potentials prior to the depolarizing steps were adjusted to $\sim 5$ mV below the onset of $I_{\text{hyst.}}$, and their amplitudes were such that the step reached the voltage range of peak activation of $I_{\text{hyst.}}$.

For the triangular inputs, the slow rate of change of membrane potential coupled with the relatively high switching rates and feedback gains were sufficient to prevent somatic fast sodium spikes from occurring as the cell passed through its spike threshold. However, in about one-half of the cells in our sample, a brief series of 1–5 “spikes” occurred at or just above the peak of the large current generated by $I_{\text{hyst.}}$ (see Fig. 2 for the characteristics of $I_{\text{hyst.}}$). These were very brief, usually being brought back under control within 5–10 switching cycles (i.e., $\sim 1–2$ ms), and were not associated with obvious afterhyperpolarizing currents. We suspect that they originated as sodium spikes in dendritic regions (cf. Larkum et al. 1996) under relatively poor space clamp. Data with more than a few breakthrough spikes were rejected. As long as the number of these spikes was less than about five, they did not produce significant distortion of the measured current. We compared records with and without a few breakthrough spikes in four cells where slight increases in feedback gain eliminated the spikes in a repeat trial. Smoothed current in the two cases differed by $<10\%$, which was about the same as the variance of associated with repeated trials lacking any spikes. Breakthrough spikes also occurred at the onset of the voltage steps, distorting the first 10–20 ms. This was not a problem because the peak current did not occur until $\geq 200$ ms, and our primary concern was the subsequent slow decay over the following 10 s.

Input conductance was measured from the slope of the $I-V$ function in a 5–10 mV range the upper boundary of which was $\pm 10$ mV below the onset of $I_{\text{hyst.}}$. As pointed out in the DISCUSSION, the onset and offset of $I_{\text{hyst.}}$ are important determinants of the shape of the $F-I$ function. Because our primary goal was to understand why $F-I$ functions differ between fully and partially bistable cells, the voltage and current levels for the onset and offset of $I_{\text{hyst.}}$ were assessed from the $I-V$ function without subtracting the leak conductance. However, leak conductances were subtracted from the measurements of the amplitude and time course of $I_{\text{hyst.}}$. For the amplitude measurements, this was done by fitting a regression line to the subthreshold region of the $I-V$ function and then subtracting this line from the whole function (Fig. 5 shows $I-V$ functions after this leak subtraction). Time course measurements were taken from 10-s voltage steps. The effect of the leak conductance was compensated by subtracting the measured clamp current from a waveform obtained by multiplying the voltage command step by the input conductance. Rate of decay of $I_{\text{hyst.}}$ was defined as the current remaining at the end of a 10-s step expressed as a percentage of the peak current.

Measurements of spike voltage thresholds

To provide a reference level for the voltage levels on the $I-V$ functions, we also measured the voltage threshold for action poten-
FIG. 2. Behavior of $I_{\text{PIC}}$ during triangular voltage commands. A: record of current (bottom) vs. time for a fully bistable cell when subjected to a slow (10-s total duration) triangular voltage command (top, labeled line). Onset, first 0 slope point on ascending phase denoting the onset of $I_{\text{PIC}}$. Initial peak, 2nd 0 slope point on ascending phase denoting peak of $I_{\text{PIC}}$. Sustained peak, 1st 0 slope point on descending phase denoting peak $I_{\text{PIC}}$ after prolonged depolarization. Offset, 2nd 0 slope point on descending phase denoting offset of $I_{\text{PIC}}$. B: same data as in A but plotted as current vs. voltage. Onset can be seen to occur at a substantially higher voltage and current level than offset, whereas the sustained peak occurs at a slightly lower voltage and is slightly smaller than the initial peak. C: record of current vs. time for a partially bistable cell. D: same data as in C but plotted as current vs. voltage. Onset and offset voltage and current levels are closer than for the fully bistable cells, and the sustained peak is substantially smaller than the initial peak.

TABLE 1. Onset and offset levels for persistent inward current, $I_{\text{PIC}}$

<table>
<thead>
<tr>
<th></th>
<th>Fully Bistable</th>
<th>Partially Bistable</th>
<th>$P$ Value for t-Test</th>
<th>$P$ Value for Slope of Regression With Input Conductance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset voltage, mV</td>
<td>$-50.7 \pm 4.5$</td>
<td>$-43.8 \pm 3.7$</td>
<td>$&lt;0.001^\dagger$</td>
<td>$&lt;0.0001^\dagger$</td>
</tr>
<tr>
<td>Offset voltage, mV</td>
<td>$-60.0 \pm 5.3$</td>
<td>$-47.3 \pm 5.7$</td>
<td>$&lt;1 \times 10^{-4}^\dagger$</td>
<td>$&lt;1 \times 10^{-4}^\dagger$</td>
</tr>
<tr>
<td>$\Delta$Voltage (onset, offset), mV</td>
<td>$9.1 \pm 2.2$</td>
<td>$3.5 \pm 2.9$</td>
<td>$&lt;1 \times 10^{-5}^\dagger$</td>
<td>$&lt;0.001^\dagger$</td>
</tr>
<tr>
<td>Onset current (nA)</td>
<td>$1.1 \pm 3.6$</td>
<td>$11 \pm 5.2$</td>
<td>$&lt;1 \times 10^{-4}^\dagger$</td>
<td>$&lt;0.0001^\dagger$</td>
</tr>
<tr>
<td>Offset current (nA)</td>
<td>$-4.5 \pm 4.2$</td>
<td>$9.0 \pm 6.3$</td>
<td>$&lt;1 \times 10^{-4}^\dagger$</td>
<td>$&lt;0.0001^\dagger$</td>
</tr>
<tr>
<td>$\Delta$Current (onset, offset), nA</td>
<td>$5.6 \pm 2.1$</td>
<td>$2.1 \pm 2.7$</td>
<td>$&lt;0.001^\dagger$</td>
<td>$&lt;0.03$</td>
</tr>
<tr>
<td>Sustained peak current (nA)</td>
<td>$-9.0 \pm 3.8$</td>
<td>$4.8 \pm 6.4$</td>
<td>$&lt;1 \times 10^{-4}^\dagger$</td>
<td>$&lt;0.0001^\dagger$</td>
</tr>
</tbody>
</table>

Number of cells: 10 fully bistable, 17 partially bistable. See definitions in Fig. 2 and the text. $\Delta$, tabulated values are the differences between the two values listed in parentheses. * Values are means $\pm$ 2SD. † Statistically significant for $P < 0.005$ ($= 0.05/10$; see METHODS).
preacceleration spike threshold. In 11 cells with at least four to five spikes before onset of firing acceleration, comparison of pre- and postacceleration spike thresholds showed that the latter were 4.3 ± 1.0 mV (means ± SD) more depolarized than the former. This finding is consistent with the tendency of spike threshold to increase with increasing firing rate (Schwindt and Crill 1982). On the basis of these considerations, spike threshold for the initiation of steady firing was determined as the average spike threshold from 10 to 20 spikes immediately after the completion of acceleration minus 4.3 mV.

Statistical analyses

Statistical procedures were identical to those in the accompanying paper. We used t-tests assuming unequal variances to compare the properties of fully and partially bistable cells and linear regression analyses to assess relations between variables. The significance level, alpha, was set at $P = 0.05$. Where results from multiple t-tests were compared (see Tables 1–3), we chose a conservative alpha level of $0.05/10 = 0.005$.

RESULTS

Of the 27 cells in which $I-V$ functions were measured, 10 were fully bistable and 17 were partially bistable. In 10 of these 27 cells, only data on the duration of self-sustained firing was obtained in addition to the $I-V$ function data. In the other 17 cells (6 fully bistable, 11 partially bistable), we also obtained data for $F-I$ functions (see Lee and Heckman 1998 for the main results on $F-I$ functions).

Input conductance

Previous studies of motoneurons have shown that input conductance is correlated closely with rheobase, which is a
TABLE 2. Spike voltage threshold compared to \( I_{\text{PIC}} \) onset and offset voltages

<table>
<thead>
<tr>
<th>Measured Threshold Value*</th>
<th>Fully Bistable</th>
<th>Partially Bistable</th>
<th>( P ) Value for ( t )-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage (spike voltage threshold, ( I_{\text{PIC}} ) onset, mV)</td>
<td>(-49.4 \pm 5.4)</td>
<td>(-47.4 \pm 4.5)</td>
<td>(=0.35)</td>
</tr>
<tr>
<td>( \Delta ) Voltage (spike voltage threshold, ( I_{\text{PIC}} ) offset, mV)</td>
<td>(1.2 \pm 3.7)</td>
<td>(-4.0 \pm 3.5)</td>
<td>(&lt;0.005)</td>
</tr>
<tr>
<td>Voltage (spike voltage threshold, ( I_{\text{PIC}} ) offset, mV)</td>
<td>(10.6 \pm 4.1)</td>
<td>(-1.2 \pm 4.4)</td>
<td>(&lt;0.00001)</td>
</tr>
</tbody>
</table>

Number of cells: 10 fully bistable, 17 partially bistable. See text for definition of value names. \( \Delta \), tabled values are the differences between the two values listed in parentheses. * Values are means \pm SD. † Statistically significant for \( P < 0.005 \) (=0.05/10; see METHODS).

Basic I-V relationships

Figure 2 illustrates the responses of a fully and a partially bistable cell to the standard triangular shaped voltage command. In the fully bistable cell, the current initially increases in proportion to voltage, but a departure from this linearity leads to a region of approximately zero slope (Fig. 2A). This is followed by a negative slope region, a second zero slope region, and then a return to a positive slope. This is the classic “N” shape produced by a strong inward current (e.g., Schwindt and Crill 1977; Svirskis and Hounsgaard 1997). An N shape is also evident as the voltage command descends back to baseline. Figure 2B shows the same data plotted as an I-V function, with ascending and descending functions superimposed. Rates of rise and fall for the voltage command were slow (8 mV/s), so that these N shapes could not be due to a rapidly inactivating inward current such as the fast sodium current underlying the action potential. Thus the negative slopes must necessarily be due to the activation and deactivation of a highly persistent inward current, denoted here as \( I_{\text{PIC}} \). On the ascending I-V function derived from depolarizing phase of the voltage command, the two zero slope points are referred to hereafter as the onset (see labels on Fig. 2) and initial peak of \( I_{\text{PIC}} \). On the descending (repolarizing) phase the zero slope points are referred to hereafter as the sustained peak and offset of \( I_{\text{PIC}} \). In the voltage range traversed between the initial and sustained peaks of \( I_{\text{PIC}} \), the ascending and descending slopes of the I-V (Fig. 2B) function markedly increased, presumably due to the activation of outward currents. It is likely too that \( I_{\text{PIC}} \) undergoes both a voltage- and time-dependent inactivation during this period because the sustained peak of \( I_{\text{PIC}} \) is smaller than its initial peak. In spite of its reduced amplitude, \( I_{\text{PIC}} \) has a substantially lower offset voltage than its onset voltage. These differences result in a strong hysteresis to the I-V function.

The ascending and descending current-time and I-V relationships for a partially bistable cell are shown in Fig. 2, C and D. Although the same general features seen in the fully bistable cell of Fig. 2, A and B, are evident, the hysteresis in the I-V function was diminished markedly. Consequently, the offset of \( I_{\text{PIC}} \) occurred only slightly below its onset. In addition, note that the onset and offset of \( I_{\text{PIC}} \) were shifted to more depolarized voltage and current levels compared with the fully bistable cell in Fig. 2, A and B.

Activation/deactivation of \( I_{\text{PIC}} \) in fully versus partially bistable cells

The examples shown in Fig. 2 suggest that the behavior of \( I_{\text{PIC}} \) differs markedly between fully and partially bistable cells. Quantitative support for this conclusion is provided in Table 1, where the behaviors of \( I_{\text{PIC}} \) in fully and partially bistable cells are compared. Table 1 also shows the results for regression analyses of how the properties of \( I_{\text{PIC}} \) vary with input conductance. There was a significantly greater difference along both the voltage and current axes between the onset and offset of \( I_{\text{PIC}} \) in fully bistable cells than in partially bistable cells (see Table 1). In addition to these differences in hysteresis, there was a clear trend for the onset and offset of \( I_{\text{PIC}} \) to be more hyperpolarized in fully bistable cells than in partially bistable cells. Figure 3 illustrates the relationship between \( I_{\text{PIC}} \) onset (A) and offset (B) voltages and input conductance. On average, the onset of \( I_{\text{PIC}} \) was

TABLE 3. Magnitude of \( I_{\text{PIC}} \)

<table>
<thead>
<tr>
<th>Measured Threshold Value*</th>
<th>Fully Bistable</th>
<th>Partially Bistable</th>
<th>( P ) Value for ( t )-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial peak magnitude, nA</td>
<td>(18.9 \pm 7.8) nA</td>
<td>(20.9 \pm 4.2) nA</td>
<td>(=0.24)</td>
</tr>
<tr>
<td>Sustained peak magnitude, nA</td>
<td>(11.9 \pm 4.7) nA</td>
<td>(10.4 \pm 3.5) nA</td>
<td>(=0.20)</td>
</tr>
<tr>
<td>( \Delta ) Current (initial-sustained), nA</td>
<td>(7.1 \pm 4.1) nA</td>
<td>(10.5 \pm 4.1) nA</td>
<td>(&lt;0.03)</td>
</tr>
</tbody>
</table>

Number of cells: 10 fully bistable, 17 partially bistable. See text for definition of value names. \( \Delta \), tabled values are the differences between the two values listed in parentheses. * Values are means \pm SD. † Statistically significant for \( P < 0.005 \) (=0.05/10; see METHODS).
~7 mV more hyperpolarized in fully than in partially bistable cells (Table 1). The analogous difference for offset voltages was even larger, about ~13 mV (Table 1).

This more hyperpolarized activation voltage for $I_{\text{PIC}}$ in fully bistable cells resulted in correspondingly lower current levels for onset and offset than those in partially bistable cells (see Table 1). This meant that the sustained peak of $I_{\text{PIC}}$ occurred at a net negative current level in all fully bistable cells, whereas it was net positive in all but two partially bistable cells (see Table 1). Consequently, $I_{\text{PIC}}$ can remain activated in fully bistable cells in the absence of applied inputs. This is likely to be the fundamental behavior allowing the fully bistable cells in our sample to exhibit tonic repetitive firing (see DISCUSSION).

Relationship between characteristics of $I_{\text{PIC}}$ and rhythmic firing behaviors

To investigate how the properties of $I_{\text{PIC}}$ influence rhythmic firing behaviors, data from cells in which both $F-I$ and $I-V$ characterizations had been made were analyzed. Figure 4 shows that the current levels for the onset and offset of $I_{\text{PIC}}$ exhibited a near one-to-one correspondence with the current levels for ascending (Fig. 4A) and descending thresholds (Fig. 4B) of the cells’ $F-I$ functions (determined from slow triangular current inputs) (see Lee and Heckman 1998). This suggests that the hysteresis between $I_{\text{PIC}}$ onset and offset largely accounts for the analogous hysteresis in the $F-I$ function (see DISCUSSION). However, the slope of the regression line in Fig. 4A was not exactly 1.0 because $I_{\text{PIC}}$ onset tended to be slightly below ascending $F-I$ threshold in fully bistable cells, whereas the opposite tended to be true in partially bistable cells. These results suggested the existence of differences between fully and partially bistable cells for the position of $I_{\text{PIC}}$ onset and offset voltages with respect to spike voltage threshold.

Spike voltage threshold was similar in fully and partially bistable cells (Table 2). However, because the onset voltage of $I_{\text{PIC}}$ differed between fully and partially bistable cells (Fig.

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** Effect of voltage ramp rate of rise on $I_{\text{PIC}}$. Leak current has been subtracted from all records. A portion of the gradual onset of $I_{\text{PIC}}$ may be due to closing of subthreshold H channels. A: direction of the voltage ramp. A: ascending ramp at 3 different rates of rise in a fully bistable cell. Slowest ramp (---, 2 mV/s) gives behavior is similar to the standard rate ramp (--, 8 mV/s). Fastest ramp (-----, 40 mV/s) gives a delayed onset and peak for $I_{\text{PIC}}$, indicating that activation of $I_{\text{PIC}}$ was not keeping up with the depolarization. B: descending ramp at same rates and in the same fully bistable cell as in A. Although sustained peak currents are similar, onset is delayed as ramp speed increases. C: ascending ramp at same speeds as in A in a partially bistable cell. Slow ramp initial peak slightly precedes the standard ramp but is diminished, indicating that decay of the current is already underway. Peak of $I_{\text{PIC}}$ is delayed in the fast ramp is delayed substantially, much as in the fully bistable cell in A. D: descending ramp at same rates and in same cell as in C. Slow ramp sustained peak exhibits substantially more decay than the standard and fast ramps that have similar peak levels. However, as in the fully bistable cell in B, the offset of $I_{\text{PIC}}$ is more delayed during the fastest ramp than during the standard ramp.
Amplitude and kinetics of $I_{\text{PIC}}$

Although a natural assumption would be that $I_{\text{PIC}}$ is larger in fully bistable cells than in partially bistable cells, this was not the case. Instead, there was a slight, nonsignificant tendency for the initial peak of $I_{\text{PIC}}$ to be larger in partially bistable cells (Table 3), and the magnitude of the sustained peak did not differ in the two cell types. However, the reduction in the amplitude of $I_{\text{PIC}}$ (defined as the difference between the initial and sustained peaks) was positively correlated with input conductance (Table 3). This pointed to a time-dependent decay of $I_{\text{PIC}}$ as the main factor affecting hysteresis and so the kinetics of $I_{\text{PIC}}$ were examined further.

The first step in studying the kinetics of $I_{\text{PIC}}$ was to determine if the standard rate of rise of the triangular voltage command used (8 mV/s) was slow enough to allow $I_{\text{PIC}}$ to approach steady state behavior. Two additional rates of rise (40 and 2 mV/s) were applied in six cells (2 fully bistable, 4 partially bistable). Figure 5 illustrates the resulting leak-subtracted $I-V$ functions for two of these cells, one fully bistable (A and B) and one partially bistable (C and D). On the ascending phase $I-V$ function of the fully bistable cell (Fig. 5A), slowing the rate of rise from 8 to 2 mV/s did not much alter the onset of $I_{\text{PIC}}$, but increasing rate to 40 mV/s shifted $I_{\text{PIC}}$ onset to a more depolarized voltage. The other fully bistable cell behaved similarly. The partially bistable shown in Fig. 5C also had similar onset voltages for the two slower rates. However, the peak of $I_{\text{PIC}}$ was smaller during the slowest rate, suggesting that $I_{\text{PIC}}$ had already begun to decay. As for the fully bistable cell, the fastest speed resulted in a depolarizing shift for $I_{\text{PIC}}$ onset. The other three partially bistable cells behaved similarly. These data indicate that the standard rate of rise used in our studies (8 mV/s) allowed $I_{\text{PIC}}$ to approach its steady-state activation behavior.

The deactivation kinetics of $I_{\text{PIC}}$ can be seen in Fig. 5, B and D, for the same two cells. In comparison with the activation kinetics in the fully bistable cell, deactivation appears to be slower (Fig. 5B), with a marked difference in offset voltage between all the ramp speeds. This also can be seen in the partially bistable cell (Fig. 5D), but the overriding feature of this panel is the dramatic reduction in the sustained peak as ramp speed decreases from 8 to 2 mV/s. Thus the time-dependent decay in $I_{\text{PIC}}$ was likely greater in partially than fully bistable cells.

The rate of decay of $I_{\text{PIC}}$ also was studied while holding voltage constant by applying long duration (10 s) voltage steps in seven cells (2 fully bistable, 5 partially bistable). Figure 6 shows examples for a fully bistable cell (A) and partially bistable cell (B). It is clear that $I_{\text{PIC}}$ cannot maintain a steady state in partially bistable cells but instead undergoes a continual decay. On average, current at the end of the step was $35 \pm 19\%$ of the peak current in the partially bistable cells as compared with $87 \pm 3\%$ in fully bistable cells. This difference was statistically significant ($t$-test, $P < 0.005$). Thus while $I_{\text{PIC}}$ was highly persistent in both types of cells, it was especially resistant to decay in fully bistable cells.

**Discussion**

In the presence of the $\alpha_1$ agonist methoxamine and tonic activity in reticulospinal monoaminergic tracts (see the discussion of Lee and Heckman 1998), the $I-V$ relationships of the motoneurons observed in this study revealed a negative conductance region due to activation of a persistent inward current, $I_{\text{PIC}}$. An important finding was that $I_{\text{PIC}}$ exhibited systematic differences in fully bistable motoneurons compared with partially bistable motoneurons. In fully bistable cells, $I_{\text{PIC}}$ had a greater difference between onset and offset for both voltage and current, a lower range of voltages and currents over which it is activated, and less decay with time. The following discussion considers how these differences in $I_{\text{PIC}}$ relate to the differences in the $F-I$ behaviors between fully and partially bistable cells. The origin of the differences in $I_{\text{PIC}}$ also are discussed.

**Effect of $I_{\text{PIC}}$ on rhythmic firing**

The negative slope imparted to the $I-V$ function by the activation of $I_{\text{PIC}}$ plays a major role in determining the form
of the $F-I$ function. Figure 7 illustrates the sudden shifts in voltage that tend to occur with negative $I-V$ slope (Hille 1992). As the cell is depolarized past point a in Fig. 7, the negative slope causes voltage to rapidly jump to point b. The same process occurs as voltage then is hyperpolarized, but note that the jump occurs from point c to d. The key to understanding the relationship between the $I-V$ functions presented here and the $F-I$ functions presented in the companion paper (Lee and Heckman 1998) is the position of the negative $I-V$ slope with respect to the spike voltage threshold. Figure 8 summarizes our $I-V$ and spike threshold data and is used as the basis of the following discussion. For convenience, Fig. 8C provides a summary of the $F-I$ relationship data from the companion paper. In the following, it is assumed that the dynamics of the afterhyperpolarization are too fast to significantly change the activation of $I_{\text{PIC}}$ and that the average membrane potential during firing is the key variable.

In fully bistable cells (continuous lines in Fig. 8, A–C), the negative slope due to the onset of $I_{\text{PIC}}$ occurs slightly below the spike threshold. Therefore as the injected current used to generate the $F-I$ function reaches the onset of $I_{\text{PIC}}$, the subsequent negative slope generates a strong depolarization (region a on Fig. 8A) that reaches spike threshold and initiates rhythmic firing. Because most of the most of the activation of $I_{\text{PIC}}$ is above spike threshold, $I_{\text{PIC}}$ also generates a strong acceleration in firing rate. Thus the ascending threshold and the onset of firing acceleration coincided in fully bistable cells (at 1 in Fig. 8C). Because $I_{\text{PIC}}$ decays very little with time (see Fig. 6A), it continues to provide a steady inward current as injected current increases, giving positive postacceleration gain (2 in Fig. 8C).

Figure 7 predicts that, during the descending phase of the $F-I$ relation, rhythmic firing should cease when the injected current reaches the sustained peak of $I_{\text{PIC}}$. Instead, it was the offset of $I_{\text{PIC}}$ that correlated with the descending $F-I$ threshold (Fig. 4B), while the sustained peak was ~4 nA lower than both of those parameters (see Table 1). The reason for the lack of correspondence between the sustained peak of $I_{\text{PIC}}$ and firing offset in fully bistable cells is not clear. Possibly, this discrepancy reflects an exception to the assumption made above that only the average membrane potential is important. The change in membrane potential between spikes at the very low rates in this threshold region may be sufficient to produce small changes in the activation of $I_{\text{PIC}}$, such as a slight reactivation as the hyperpolarization from the afterhyperpolarization (AHP) fades. Overall, in fully bistable cells, it is clear that the onset and offset of $I_{\text{PIC}}$ determine the ascending and descending thresholds of the $F-I$ function. Therefore the hysteresis in $I_{\text{PIC}}$ generates the hysteresis in the $F-I$ function.

In partially bistable cells, the onset of $I_{\text{PIC}}$ also imparts a very steep negative slope to the $I-V$ function—but, in contrast to fully bistable cells, this occurs slightly above spike voltage threshold. This provides a region where steady rhythmic firing can be maintained (b in Fig. 8A) before the onset of acceleration. Thus the ascending $F-I$ threshold and acceleration onset are separate events (see 4 and 5 in Fig. 8C). The time-dependent decay in $I_{\text{PIC}}$ (see Fig. 6B) results in negative postacceleration gain (6 in Fig. 8C) and allows $I_{\text{PIC}}$ to largely subside before voltage again reaches spike threshold on the descending phase of the $F-I$ function (7 in Fig. 8C). The correspondence between $I_{\text{PIC}}$ offset and descending $F-I$ threshold in partially bistable cell occurs because $I_{\text{PIC}}$ offset is placed only slightly above the spike threshold. This means that the $F-I$ functions in partially bistable cells lack hysteresis because $I_{\text{PIC}}$ onset is above spike threshold and because it lacks sufficient offset hysteresis to overcome this depolarized activation range.

Although the lack of threshold hysteresis in partially bistable cells is striking, it is also clear that their $F-I$ threshold currents are much higher than those of fully bistable cells (cf. 1 and 3 with 4 and 7 in Fig. 8C). Because spike voltage thresholds are the same in both types of cell, the difference in input conductance between the two cell types (see Fig. 1) accounts for a substantial portion of these differences in $F-I$ threshold currents. However, if this were the only factor, rhythmic firing would commence where a line extrapolated from the slope of the subthreshold $I-V$ function crossed the spike voltage threshold. Because $I_{\text{PIC}}$ is activated at a much lower voltage in fully than in partially bistable cells (see Fig. 8A), onset of $I_{\text{PIC}}$ is below spike threshold in fully bistable cells and therefore initiates firing at a lower current than predicted by input conductance alone. Similarly, the hysteresis in $I_{\text{PIC}}$ in fully bistable cells results in a very hyperpolarized offset voltage, which adds considerably to the difference in descending $F-I$ thresholds between fully and partially bistable cells.

Finally, the activation of $I_{\text{PIC}}$ with respect to spike voltage threshold also accounts for the subtle distinctions in fully and partially bistable cells for the relationships between $I_{\text{PIC}}$ onset current, ascending $F-I$ threshold current, and rheobase current. As noted earlier, $I_{\text{PIC}}$ is activated slightly below the spike threshold in fully bistable cells and thus in Fig. 4A, the $I_{\text{PIC}}$ onset current is slightly lower than the ascending $F-I$ threshold. Rheobase tends to be higher than the ascending $F-I$ threshold in fully bistable cells because the short current pulse used to test rheobase (50 ms) is ineffective in...

![Fig. 7](http://jn.physiology.org/)
activating $I_{\text{PIC}}$. Consequently, the spike voltage threshold determines rheobase not the onset threshold for $I_{\text{PIC}}$.

**Origin of low rheobases**

The range of rheobases and rhythmic firing thresholds in the present sample of motoneurons was shifted \(\sim 10\) nA downward in comparison with the range seen in pentobarbital-anesthetized preparation (e.g., Gustafsson and Pinter 1984; Kernell and Monster 1981), where neuronal activity is suppressed and there is no tonic activity in bulbospinal monoaminergic fibers. Previous in vitro studies of various types of motoneurons have shown that both serotonin and norepinephrine depolarize motoneurons and lower rheobase values, an effect in part mediated by reduction of a barium-sensitive resting potassium conductance (Larkman and Kelly 1992; Lindsay and Feldman 1993; Parkis et al. 1995). By itself, this would tend to decrease input conductance, but at least in the case of serotonin, this tendency can be offset by a depolarizing shift in the voltage sensitivity of the H channel (Larkman and Kelly 1992). This may well have occurred in our preparation, because the marked reduction in rheobase was not accompanied by an equally large reduction in input conductance (see Fig. 1). However, the preceding mechanism would not likely produce the negative rheobases seen in some cells. This appeared to occur because the onset voltage for $I_{\text{PIC}}$ was at a sufficiently hyperpolarized level in these cells to be activated even at the resting membrane potential.

**Origin of $I_{\text{PIC}}$**

A persistent inward current was first documented in cat spinal motoneurons by Schwindt and Crill (1977). They suggested that it was primarily generated by a Ca\(^{2+}\)/channel (Schwindt and Crill 1980). Consistent with this, Hounsgaard and colleagues have demonstrated that, in turtle motoneurons in a slice preparation, activation of a L-type Ca\(^{2+}\)/channel was necessary for generation of a persistent inward current and the resulting plateau potentials (Hounsgaard and Kiehn 1989; Svirskis and Hounsgaard 1997). Although L-type Ca\(^{2+}\)/channels usually are considered to be high-voltage activated, the data of Hounsgaard and colleagues clearly show it can be activated at low voltages. Furthermore, Magee and colleagues (1996) recently showed that L-type Ca\(^{2+}\)/channels were not significantly different for the 2 cell types—see Table 2).

![Graphs A, B, and C](http://jn.physiology.org/)

**FIG. 8.** Summary of I-V functions and how they relate to the F-I functions presented in the companion paper (Lee and Heckman 1998). All functions were constructed to represent the sample averages for the behaviors of either fully bistable cells (continuous lines in all parts) or partially bistable cells (interrupted lines). A: I-V functions for the ascending phase of the triangular voltage command. Points indicated by the triangles show the average values for the onset and initial peak of $I_{\text{PIC}}$ for the fully (filled triangle) and partially bistable (inverted triangle) cell samples. Vertical dashed line shows the average value for spike voltage threshold for the entire sample of cells, both fully and partially bistable (spike thresholds were not significantly different for the 2 cell types—see Table 2). I-V functions for each cell type were created by fitting 3 measured points (the average values for onset and peak of $I_{\text{PIC}}$ and the resting membrane potential) and 2 slope values using the technique of cubic splines. Fitted slope in the subthreshold range was specified by the average input conductance. Slope of the most depolarized portion of the function was not measured and was arbitrarily set to give a conductances of 3.0 and 4.0 \(\mu\)S in the fully and partially bistable cells, respectively. Labels with arrows and the 2 regions denoted a and b are important for the genesis of rhythmic firing and are explained in the text. B: I-V functions for the descending phase of the triangular voltage command. Inverted and filled triangles, average sample values for the offset and sustained peak of $I_{\text{PIC}}$. Functions constructed as in A to fit these average values. C: representative F-I functions for fully bistable cells (solid line) and partially bistable cells (dashed line) based on the results of the companion paper (Lee and Heckman 1998). Symbols as in A. Functions were constructed by connecting the average values (denoted by triangles) for ascending threshold, acceleration onset, and peak acceleration firing by straight lines. Transition points not denoted by triangles are for portions of the F-I functions that were not quantified and thus should be considered qualitative estimates.
currents in CA1 pyramidal cells were activated at very low voltages as long as extracellular Ca\(^{2+}\) concentration was kept within its physiological range.

The properties of \(I_{\text{PIC}}\) in our fully bistable cat motoneurons are very similar to those of the persistent inward current in turtle motoneurons (Hounsgaard and Kiehn 1989; Svirskis and Hounsgaard 1997), having similar voltage ranges for activation and deactivation and a similar degree of onset-offset hysteresis. Consequently it is very likely a L-type Ca\(^{2+}\) channel plays a fundamental role in generating \(I_{\text{PIC}}\) in cat motoneurons. Consistent with this, plateau potentials can be generated in motoneurons in the decerebrate cat preparation when an intracellular sodium channel blocker is present (Brownstone et al. 1994). However, L-type Ca\(^{2+}\) channels may not be the sole source of \(I_{\text{PIC}}\). The studies done thus far have not ruled out a role for Ca\(^{2+}\)-mediated currents. In fact, a Ca\(^{2+}\)-mediated nonspecific cation current plays an important role in plateau potential generation in esophageal motoneurons in neonatal rats and in stomatogastric motoneurons in the crab (Rekling and Feldman 1997; Zhang and Harris-Warrick 1995). Future investigations with intracellular Ca\(^{2+}\) chelators and also intracellular K\(^+\) blockers are needed to help clarify the mechanisms responsible for generation of the persistent inward current in adult spinal motoneurons.

**Mechanism of hysteresis in \(I_{\text{PIC}}\)**

What properties of the channels generating \(I_{\text{PIC}}\) produce the hysteresis? One possibility is extremely slow kinetics for the channels involved, so that \(I_{\text{PIC}}\) activation and deactivation fail to keep up with the rate of change of voltage. One source of slow kinetic behavior is that L-type Ca\(^{2+}\) channels exhibit voltage-dependent facilitation, which is a slow activation process that could contribute to hysteresis (Svirskis and Hounsgaard 1997). Voltage-dependent facilitation was not directly investigated here, but it is clearly present in both turtle and cat motoneurons (Svirskis and Hounsgaard 1997, 1998) (D. J. Bennett, H. Hultborn, B. Fedirchut, and N. Gorassini, unpublished data). However, slow activation and deactivation of \(I_{\text{PIC}}\) may not be sufficient to fully account for our results. Slowing the rate of change of our triangular voltage commands from 8 to 2 mV/s did not seriously alter the rate of onset of \(I_{\text{PIC}}\), suggesting that its activation kinetics were near steady state (see Fig. 5). Voltage steps with sufficient amplitude to reach the region of peak activation of \(I_{\text{PIC}}\) were associated with a time to onset of peak current on the order of 1 s and a time to offset of 1–2 s (Fig. 6). These lags do not appear to be sufficient to fully account for the hysteresis for triangular inputs lasting a total of 10–40 s (corresponding to rates of change of 8–2 mV/s).

An alternative source of hysteresis arises from the location of the channel. Channels in dendritic locations would experience a different steady-state voltage than somatic channels due to imperfect space clamp. It is clear that at least some of the channels that produce plateau potentials reside in dendritic regions (Hounsgaard and Kiehn 1993; Lee and Heckman 1996b). Furthermore, computer simulations with simple two compartment models of motoneurons show steady-state \(I-V\) hysteresis very similar to that observed here (Baginskas et al. 1993; Booth et al. 1997; Lee and Heckman 1996a). Finally, a steady-state hysteresis is likely to be necessary to achieve truly long-term self-sustained firing. This is because the voltage swings due to the AHP eventually would eliminate firing if the current were only slow to deactivate. However, these considerations do not rule out an important role for slow kinetics, and it seems likely that the hysteresis arises from a combination of kinetics and dendritic location.

Differences in kinetics probably account for the difference in hysteresis for \(I_{\text{PIC}}\) onset and offset in partially versus fully bistable cells. Figure 5 illustrates the finding that the offset of \(I_{\text{PIC}}\) in partially bistable cell was influenced more strongly by the rate of change of voltage-clamp command than was the offset in fully bistable cells. As rate of change of voltage slowed, hysteresis decreased markedly in the partially bistable cells. This occurred because \(I_{\text{PIC}}\) decayed more rapidly in these cells (see Fig. 6). Thus this tendency for decay of \(I_{\text{PIC}}\) in partially bistable cells appears to account for their lesser degree of hysteresis.

**Origin of decay of \(I_{\text{PIC}}\)**

Two possible explanations for the decay of \(I_{\text{PIC}}\) with time (Fig. 6) are inactivation of \(I_{\text{PIC}}\) and activation of outward currents. Schwindt and Crill (1980) found that the potassium AHP channel obscured the persistent inward current, and Hounsgaard (Hounsgaard and Mintz 1988) showed that apamin, a Ca\(^{2+}\)-dependent potassium channel blocker, enhanced plateau potentials. Although serotonin reduces the amplitude of the AHP (Bayliss et al. 1995; Hounsgaard and Kiehn 1989), it is not entirely suppressed and may still play a role in the time-dependent decay of \(I_{\text{PIC}}\). However, it is not clear how this would account for the difference in decay magnitudes for partially versus fully bistable cells.

The other possibility for the decay of \(I_{\text{PIC}}\) is inactivation. The low voltage activated (containing both T type and L type) calcium currents characterized by Avery and Johnston (1996) had an inactivating component with inactivation time constants of 60 and 200 ms. \(I_{\text{PIC}}\) did sometimes show an initial decay with a similar time course in some cells (not shown), however, the partially bistable cell illustrated in Fig. 6B makes it clear that a much slower time constant also is present. Moreover, \(I_{\text{PIC}}\) in fully bistable cells decayed very slowly, with a time constant of about \(\approx 10\) s. If the inactivation is at least partly voltage dependent, then a greater tendency toward inactivation might be expected in partially bistable cells. This is because the activation of \(I_{\text{PIC}}\) is shifted to more depolarized levels in partially bistable cells as compared to fully bistable ones (see Fig. 4). The sources of these differences in voltage range for \(I_{\text{PIC}}\) are unclear at present. However, computer simulations indicate the onset of \(I_{\text{PIC}}\) can be shifted to more hyperpolarized current levels either by reducing the AHP conductance or by increasing the L-type Ca\(^{2+}\) conductance, or both (Booth et al. 1997).

An alternative possibility is that the voltage thresholds for activation of the persistent inward current in partially and fully bistable cells only differ at the soma and are actually similar in the poorly clamped dendritic regions. The present results do not exclude this possibility. There is in fact a tendency for the dendritic tree to have a greater extent in fast conduction velocity motoneurons (Burke et al. 1982; Kernell and Zwaagstra 1980). Thus the persistent inward current could be generated at a greater electrical distance.
from the soma in partially as compared with fully bistable cells. We are presently investigating these issues via computer simulation.

Finally it should be noted that the decay of $I_{\text{PIC}}$ in partially bistable cells has a time course that is similar to the long-term adaptation in firing rate seen in motoneurons in the absence of monoamines (Kernell and Monster 1982; Sawczuk et al. 1995). Furthermore, $I_{\text{PIC}}$ in our preparation appears to simply an enhanced version of the persistent inward current seen in motoneurons in pentobarbital-anesthetized preparations (cf. Schwindt and Crill 1977, 1980). The amount of decay of firing in our results for partially bistable cells (see Fig. 5 in Lee and Heckman 1998) is greater than that seen in the pentobarbital-anesthetized preparation or in the slice preparation, but this difference may simply reflect a much larger amplitude of the persistent inward current in our preparation. Thus slow decay in the persistent inward current may be a major factor underlying long term rate adaptation in several different preparations. This leads to the prediction that agents that affect $L$-type $Ca^{2+}$ channels will have a strong impact on long-term rate adaptation.

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