Bistability in Spinal Motoneurons In Vivo: Systematic Variations in Rhythmic Firing Patterns

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Lee, R. H. and C. J. Heckman. Bistability in spinal motoneurons in vivo: systematic variations in rhythmic firing patterns. J. Neurophysiol. 80: 572–582, 1998. In the presence of the monoamines serotonin and norepinephrine, spinal motoneurons can exhibit bistable behavior, in which a brief period of excitatory input evokes prolonged self-sustained firing. A brief inhibitory input returns the cell to the quiescent state. To determine whether motoneurons differ in their capacity for bistable behavior, intracellular recordings were obtained in the decerebrate cat preparation. To enhance the likelihood of encountering bistable behavior, the noradrenergic α1 agonist methoxamine was applied to the ventral surface of the cord. The capacity of the cells to produce bistable behavior was assessed from the duration of self-sustained firing evoked by a brief (1.5 s) excitatory synaptic input from muscle spindle Ia afferents. About 35% (17 of 49) of the cells produced steady self-sustained firing for >3 s and were considered fully bistable. The other 32 cells (~65%) were partially bistable, with self-sustained firing lasting <3 s. Fully bistable cells tended to have lower current thresholds for spike initiation and slower axonal conduction velocities than did partially bistable cells. This suggests that fully bistable motoneurons innervate fatigue resistant muscle fibers. The frequency-current (F-I) relations of the motoneurons were characterized with slow triangular current ramps. Fully bistable cells displayed an acceleration in firing rate immediately on initiation of rhythmic firing. The F-I gain after completion of the acceleration was positive. Fully bistable cells also displayed a hysteresis in the current level for firing threshold with the ascending threshold occurring at substantially higher current level than the descending one. Additionally, these current thresholds usually were centered about zero current, so that the ascending current threshold was positive while the descending current threshold was negative. This negative offset meant that fully bistable cells could exhibit tonic firing without depolarizing injected current. Partially bistable cells exhibited very different F-I characteristics. Firing rate acceleration was just as large as in fully bistable cells but did not occur until well above the current level needed to initiate rhythmic firing. F-I gain after acceleration was negative, there was little to no hysteresis between the ascending and descending firing thresholds, and both thresholds were above the zero current level. These properties of partially bistable cells suggest their functional role is in tasks requiring relatively brief, high forces. The low thresholds of fully bistable cells mean they will be readily recruited in low force tasks like posture, where their prolonged self-sustained firing would be advantageous.

INTRODUCTION

The electrical behaviors of motoneurons are subject to modification by several neuromodulators, and generally speaking, these neuromodulators tend to enhance motoneuronal excitability (reviewed in Binder et al. 1996). The monoamines serotonin and norepinephrine have an especially profound effect on adult spinal motoneurons, allowing brief periods of synaptic input to generate sustained depolarizations known as plateau potentials (Conway et al. 1988; Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988). Input from either synaptic activation or current injected via the micro electrode can initiate a plateau potential. This is manifest as a sudden depolarization if the cell is subthreshold or as a sudden acceleration in discharge frequency if the cell is already firing. When the input is terminated, the plateau potential can persist for long periods, allowing the cell to maintain a tonic firing rate in the absence of applied input. A brief hyperpolarizing input can eliminate this self-sustained firing and return the cell to the quiescent state. Thus the cell is said to be “bistable” because it has two stable states for the same input condition, quiescent and self-sustained firing (Hounsgaard et al. 1984). Bistable behavior has been seen in several different types of cells (e.g., Hounsgaard and Kiehn 1989; Hounsgaard et al. 1988; Lechner et al. 1996; Morisset and Nagy 1996; Rekling and Feldman 1997; Russo and Hounsgaard 1996; Zhang and Harris-Warrick 1995).

The duration of self-sustained firing is very short in some motoneurons (Hounsgaard et al. 1988; Lee and Heckman 1996b), a phenomenon that may be linked to the well-known differences in motoneuron electrical properties (reviewed in Binder et al. 1996). Motoneurons requiring only a small amount of synaptic or injected current to reach threshold for rhythmic firing also tend to have low input conductances and slow axonal conduction velocities. These low-threshold motoneurons tend to innervate muscle fibers with slow conduction speeds and low forces but with very high resistances to fatigue. In contrast, the high-threshold motoneurons with fast conduction velocities innervate fast, high-force muscle fibers with poor fatigue resistances. Motoneurons with intermediate-valued properties are distributed in a continuum between these two extremes. This continuum of motor units usually is divided into three classes based on the differences in muscle fiber properties: type S (slow), FR (fast, fatigue resistant), and FF (fast, fatigable) (Burke et al. 1973). From a functional viewpoint, the fatigability of the motor units is a particularly important consideration for bistability in motoneurons because prolonged firing in FF motor units would rapidly lead to severe fatigue. Therefore a reasonable hypothesis is that long-term self-sustained firing is most likely to occur in motoneurons with low thresholds and slow conduction velocities, whereas it may be weak or absent in motoneurons with high-thresholds and fast conduction velocities.

The goal of this study was to evaluate this hypothesis by quantifying bistable behavior in motoneurons with a wide...
range of thresholds and conduction velocities in a preparation where there was a strong likelihood of encountering plateau potentials and self-sustained firing. We used the decerebrate cat preparation, which is known to exhibit tonic activity in reticulospinal serotonergic fibers (Crone et al. 1988; Engberg et al. 1968) and also may have tonic activity in reticulospinal noradrenergic fibers (Sastry and Sinclair 1976) (see DISCUSSION). In addition, to increase the probability that motoneurons would exhibit bistable behavior, we applied the pharmacological agent methoxamine, a noradrenergic \(\alpha_1\) agonist (cf. Lee and Heckman 1996b). The tendency for bistable behavior was assessed by applying a short period of steady synaptic input and by measuring the relationship between discharge frequency and the amplitude of current injection via the microelectrode (i.e., the \(F-I\) relationship). Our results were consistent with the proposed hypothesis that the duration of self-sustained firing was longest in cells with slow conduction velocities and low thresholds. In addition, these same cells exhibited marked hysteresis in their \(F-I\) relations (cf. Hounsgaard et al. 1988) (D. J. Bennett, H. Hultborn, B. Fedirchuk, and M. Gorassini, unpublished data). A portion of these results has been presented in abstract form (Lee and Heckman 1996a, 1997).

**METHODS**

**Preparation**

Data were obtained from 14 cats (average weight \(\approx 2.5 \) kg). All procedures were approved by the animal care committee at Northwestern University. Initial surgical preparations were done under deep gaseous anesthesia (1.5–3.0% isoflurane in a 3:1 mixture of \(O_2\) and \(N_2O\)), according to standard procedures in our lab (Heckman et al. 1994). In the left hindlimb, the nerves to medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LGS) muscles were isolated carefully and left in continuity. The entire Achilles tendon was attached to a muscle puller via a bone chip from the calcaneus. Before the tendon was freed, the foot was dorsiflexed manually, and a small piece of suture was tied to the tendon and to the underlying shank of the lower leg. After the soleus was attached to the puller, alignment of the two threads was defined as maximum physiological length. The surgically exposed areas of the hindlimb were covered with a pool of mineral oil that was formed within the pulled-up skin flaps. A precordial decerebration was performed by transecting the midbrain with an ophthalmic spatula and aspirating the entire forebrain. The calvarium was packed with saline-soaked cotton wool. The gaseous anesthesia was then discontinued, and the animal was allowed to breathe room air. Radiant heat was used to maintain hindlimb and core temperatures within physiological limits. At the end of the experiment, the animals were killed with a lethal dose (100 mg/kg iv) of pentobarbital sodium.

**Drug application and effects**

Methoxamine, a noradrenergic \(\alpha_1\) agonist (obtained from Sigma), was applied via a modified intrathecal method. A fine-diameter catheter (PE10 polyethylene) was slid gently under the lumbar enlargement after the dura of the cord was opened. The entry point for the catheter was at \(L_1\) and the catheter reached to approximately \(L_6\) or \(L_7\). The catheter was secured by suturing it to the pinned-out dura, and its distal end was taped to the spinal frame. Mineral oil then was applied to form a pool over the exposed spinal cord, with care being taken to avoid disturbing the CSF and saline that remained underneath the cord after the dura was cut. Thus when the drug was applied via this ventral topical method, it diffused into the CSF/saline already present under the cord.

The purpose of the ventral topical application of methoxamine was to maximize the probability that motoneurons would exhibit bistable behavior, not to systematically investigate the effects of this agent. However, of the 14 animals used for preliminary experiments to find a dose of methoxamine that produced strong, long-lasting effects on motoneuron excitability. To do this, a steady monosynaptic input in triceps surae motoneurons was evoked by steady activation of muscle spindle Ia afferents via high-frequency, low amplitude vibration (a 160-Hz sinusoid with 80-\(\mu\)M peak-to-peak amplitude) applied longitudinally to the Achilles tendon (Matthews and Stein 1969). The animals for these four experiments were not paralyzed, and the reflexive muscle force produced by the Ia input was assessed via a strain gauge. Muscle length was set at \(\approx 10\)-mm short of physiological maximum.

These reflex experiments established that ventral topical application of 5 \(\mu\)mol of methoxamine dissolved in 100 \(\mu\)l of distilled water resulted in a substantial increase in motoneuron excitability (i.e., a 30–100\% increase in steady force produced by the vibration as compared with predrug levels). The concentration of drug in the motor pool was of course unknown but was likely to be very much \(< 50\) mM concentration of the applied methoxamine solution due to dilution in the CSF/saline already present under the cord and the limited diffusion up into the cord. Typically, diffusion of the drug into the cord was a slow process, with the maximal drug effect occurring \(\approx 15–45\) min postapplication. Systemic blood pressure changed by no more than 5–15 mmHg. The duration of the drug effect was assessed by repeated measurements of the reflex force produced by the Ia input for 3–5 h after the initial application. In all four experiments, the enhanced excitability was maintained throughout this time. Fluctuations in reflex excitability, which are characteristic of the decerebrate preparation even in the absence of methoxamine, did occur. However, the reflexive force, averaged during 1-h periods, stayed within \(\approx 10–20\%\) of the initial level. Two experiments exhibited a small trend toward increasing excitability with time, whereas the other two exhibited a slightly decreasing trend. Note that the exogenous effects of methoxamine were likely superimposed on endogenous effects of tonic activity in serotonergic reticulospinal axons (Crone et al. 1988; Engberg et al. 1968), and there also may be tonic activity in noradrenergic reticulospinal axons (see DISCUSSION). The bistability seen in this study then resulted from both noradrenergic and serotonergic effects.

**Intracellular recording**

Intracellular recordings were obtained with sharp microelectrodes filled with potassium citrate (IM). In all 10 experiments involving intracellular recordings, the preparation was paralyzed with gallamine triethiodide (Flaxedil; 10 mg initial dose, supplemented at regular intervals with additional doses of 1–2 mg) and artificially respired. End tidal \(CO_2\) was monitored and kept within acceptable limits by adjusting respiration rate and volume. In addition, a bilateral pneumothorax was done to enhance intracellular recording stability. Microelectrode tips were broken back under microscopic observation and control. Because of the large currents needed to evoke rhythmic firing and because some of the cells were studied using single-electrode voltage-clamp techniques (see Lee and Heckman 1998), resistances of the electrodes were kept low—typically \(\approx 4–6\) M\(\Omega\) in saline before entering the cord.

Based on the preliminary studies of the effect of methoxamine on reflexes (see preceding text), the following procedures were used in the intracellular studies. An interval of 30 min was allowed to elapse before any data were taken to allow the drug effects to reach full potency. Intracellular recordings then were obtained in MG and LGS motoneurons in a 3–to-4-h time period after this 30-
min waiting interval. As noted above, the enhanced motoneuronal excitability mediated by methoxamine was well maintained during this recording period. In each cell, the standard protocol began with the measurement of a spike antidromically evoked by single shocks to either the MG or LGS nerve. The electrode resistance was compensated via a bridge circuit and then rheobase, defined as the amplitude of a 50-ms current pulse required to elicit a single spike, was measured. Next, a 1.5-s period of high-frequency, low-amplitude tendon vibration (using the same parameters as used in the reflex studies described earlier) was applied to evoke steady Ia afferent input. The response to the steady Ia input was assessed at three to six different levels of steady but subthreshold membrane depolarizations. These levels were produced by steady current injection in bridge balance mode. The maximum duration of self-sustained firing evoked by the Ia input was assessed when the cell was depolarized to within 2–5 mV of the voltage level at which rhythmic firing commenced. This level was estimated to be the membrane potential at which the sustained Ia excitatory postsynaptic potential (EPSP) first began to evoke spikes. Baseline depolarizations within 2 mV of the voltage threshold for rhythmic firing were avoided as these often were associated with the “subprimmy” range (Kudina and Alexeeva 1992), in which synaptic noise evokes spikes (Calvin 1974). Hyperpolarizing baseline currents were used to eliminate the self-sustained firing and confirm that the Ia input had a crisp offset. Next, the frequency-current (F-I) function of the cell was assessed via injection of a current waveform with a triangular shape. The rates of rise and fall were equal at 5 s each. The amplitude of the triangular-shaped current was adjusted so that each cell reached a firing rate of ≈60–80 Hz. This resulted in the rate of rise varying from 4 to 8 nA/s (the F-I behaviors obtained for this range of rates were similar). In most cells, the amplitude of the triangular-shaped current was 30 nA, resulting in a rate of rise of 6 nA/s, and a steady DC bias current was used to control the peak current reached during the input. In some cells, this was followed by a series of current steps lasting 1–3 s.

Note that a period of ≳30 s was allowed to elapse between each of the protocols. This was done to avoid the effects of voltage-dependent facilitation (Bennett et al., unpublished data; Svirskis and Hounsgaard 1997). This phenomenon was not quantified in our studies but was readily apparent. For example, if the current pulses to assess rheobase were applied with −0.5-s intervals, the depolarization produced by each pulse increased and a burst of spikes often developed by the third or fourth pulse.

**Data analysis**

**DATA ACCEPTANCE CRITERIA.** The main acceptance criteria were that the amplitude of the antidromic spike exceeded 70 mV and the resting membrane potential did not vary by more than ±5 mV during the course of the data collection. However, eight cells exhibited tonic firing in the absence of any input, requiring the application of a steady hyperpolarizing current for the duration of the impalement. After compensating for the electrode resistance with the bridge balance circuit, these eight cells met the same membrane potential stationarity and spike height requirements as the other cells. Additionally, most of these cells had free running spike heights greater than the 70-mV requirement even while firing tonically at zero current. A total of 49 cells met the above stability requirements.

**ASSESSMENT OF BISTABLE BEHAVIOR.** Data with sustained firing patterns generated in response to tendon vibration and injected current ramps were digitized at 5–10 kHz. Antidromic spikes were digitized at 58 kHz to allow accurate measurement of conduction time. All data were stored on removable hard disks for off-line analyses.

The strength of bistable behavior was defined as the duration of self-sustained firing after Ia input ceased. Due to limitations for on-line data storage capacity, firing data were only digitized for a maximum of 3 s after Ia input. All the cells that exhibited steady self-sustained firing for this 3-s period also continued to fire for at least another 5–10 s (as monitored on an oscilloscope; we did not monitor longer time periods because we wished to maximize data collection before the inevitable deterioration in cell recording quality). Thus the cells in which the duration of self-sustained firing >3 s were categorized as **fully bistable**. In the remaining cells, firing either abruptly stopped or steadily declined to zero during the 3-s period after Ia input ceased. These cells were categorized as **partially bistable**.

**CONDUCTION VELOCITY AND ESTIMATION OF MOTOR UNIT TYPE.** In preparations where activity in monoaminergic axons is suppressed, rheobase is an excellent predictor of motor unit type (Zengel et al. 1985). However, both serotonin and norepinephrine are likely to reduce rheobase (Larkman and Kelly 1992; Lindsay and Feldman 1993; Parkis et al. 1995) (see RESULTS). In contrast, conduction velocity is unlikely to be altered. To confirm that conduction velocity remained unchanged, we monitored the latency and shape of the extracellular field produced by antidromic stimulation of the MG and LGS nerves in two experiments before intracellular data were taken. No differences were found in either parameter before, during, and for 30 min after ventral topical application of the standard dose of methoxamine. We did not measure conduction distances in our experiments, preferring to primarily rely onwithin-animal comparisons of conduction times in the two experiments with the largest sample sizes (n = 9 and n = 10). This analysis was supplemented with a comparison of conduction times across experiments where the conduction times within each experiment were normalized by that experiment’s average. These across-experiment analyses were only carried out in experiments with data from at least two fully and two partially bistable cells. This gave a total of four experiments, the two with the largest samples and two further experiments where n = 7 and n = 4. Conduction time was measured from the difference between stimulation onset in the nerve and the initiation of the antidromic action potential above baseline.

**STATISTICAL ANALYSES.** The electrical properties of fully versus partially bistable cells were compared using t-tests, assuming unequal sample variances. In addition, linear regression analyses were used to assess the relationships between variables. The significance level, alpha, was set at P = 0.05. Where results from multiple t-tests were compared (as in Table 1), we chose a conservative alpha level of 0.05/10 = 0.005.

**TABLE 1. Comparison of F-I behaviors in fully and partially bistable cells**

<table>
<thead>
<tr>
<th>Measured Behaviors*</th>
<th>Fully Bistable</th>
<th>Partially Bistable</th>
<th>P Value for t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending threshold, nA</td>
<td>1.0 ± 3.6 (11)</td>
<td>10.9 ± 4.6 (20)</td>
<td>&lt;0.000001*</td>
</tr>
<tr>
<td>Descending threshold, nA</td>
<td>5.0 ± 4.7 (11)</td>
<td>11.8 ± 4.6 (20)</td>
<td>1 × 10⁻⁴</td>
</tr>
<tr>
<td>Δ (Ascending, descending, nA)</td>
<td>−5.9 ± 2.8 (11)</td>
<td>0.9 ± 3.4 (20)</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>Acceleration onset, nA</td>
<td>1.8 ± 3.9 (10)</td>
<td>17.7 ± 9.0 (16)</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>Acceleration gain, Hz/nA</td>
<td>13 ± 11 (10)</td>
<td>30 ± 25 (16)</td>
<td>&lt;0.015</td>
</tr>
<tr>
<td>Postacceleration gain, Hz/nA</td>
<td>1.3 ± 0.9 (10)</td>
<td>−5.0 ± 6.2 (14)</td>
<td>&lt;0.0002*</td>
</tr>
<tr>
<td>Descending gain, Hz/nA</td>
<td>2.2 ± 0.4 (11)</td>
<td>3.0 ± 1.4 (20)</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Behaviors are defined in Fig. 4 and the text. Δ, the difference between the behaviors in parentheses. * Values are means ± SD with number of cells in parentheses. t Statistically significant at P < 0.005 (=0.05/10; see METHODS).
RESULTS

Bistable behavior in motoneurons

All studies were carried out after application of a standardized dose of the noradrenergic α₁ agonist methoxamine via the catheter placed under the lumbar cord (see METHODS). The steady monosynaptic input from Ia afferents generated by tendon vibration (1.5-s duration) was used to assess the capacity of motoneurons to generate self-sustained firing. Of the 49 cells, 17 (or ~35%) were fully bistable, i.e., they exhibited self-sustained firing for >3 s (see METHODS). Figure 1A shows typical data for a fully bistable cell. When this cell was depolarized to within ~5 mV of its threshold for steady firing (Fig. 1A, top), it responded with a high discharge frequency (~50 Hz) during the Ia input. This was followed by steady, self-sustained firing at lower rate (~20 Hz) after the Ia input ceased. In the absence of injected current (Fig. 1A, middle), the self-sustained firing only lasted ~1 s. Hyperpolarization (Fig. 1A, bottom) eliminated firing and revealed a 1.5-s Ia EPSP with a sharp onset and offset.

In contrast, 32 of the 49 cells (~65%) were partially bistable, in that self-sustained firing faded within 3 s. Figure 1B shows results for a partially bistable cell that could only produce ~0.5 s of self-sustained even when the cell depolarized to within 2 mV of firing threshold. A reduction in depolarizing bias further reduced the duration of self-sustained firing (Fig. 1B, middle). Removal of depolarizing injected current (Fig. 1B, bottom) revealed a steady Ia EPSP with a sharp onset and offset.

Variation in bistable behavior with motoneuron electrical properties

To test the hypothesis that motoneurons with low rheobases and conduction velocities were more likely to exhibit strong bistable behavior, we compared the duration of self-sustained firing following Ia input in each cell to its electrical properties. In pentobarbital-anesthetized preparations, rheobase is well correlated with motor unit type, with type S units having the lowest values (Fleschman et al. 1981; Zengel et al. 1985). Thus a low rheobase implies a high fatigue resistance. Figure 2 illustrates the relationship between rheobase and the duration of the self-sustained firing after the Ia input. The average rheobase of the fully ((Mock)) and partially bistable cells (Mock) was 1.4 ± 4.1 nA and 8.8 ± 3.3 nA, respectively, and this difference was statistically significant (t-test, P < 0.000001).

However, motoneuronal rheobase is known to be decreased by norepinephrine and serotonin (Larkman and Kelly 1992; Parkis et al. 1995). Consistent with this, the range of rheobases in our sample (~5 to +14 nA) is shifted downward in comparison to previous studies in pentobarbital anesthetized preparations (+3 to +30 nA) (e.g., Gustafsson and Pinter 1984), which lack neuromodulatory input from the brain stem. Therefore we assessed axonal conduction velocity for the antidromic spike, which also is correlated with motor unit type (e.g., Emonet-Denand et al. 1988) but was not altered by methoxamine (see METHODS).

Conduction velocity was assessed from measurements of conduction time (see METHODS). Analysis of conduction time versus rheobase within the two experiments with the largest samples produced correlation coefficients of -0.90 (n = 9) and -0.74 (n = 10), resulting in P values of 0.0005 and 0.007, respectively. The across experiment analysis, which was based on the four experiments in which data from at least two fully and two partially bistable cells were obtained, also resulted in a significant correlation between normalized conduction time (see METHODS) and rheobase (P < 0.001). Thus the relationship between rheobase and conduction velocity seen in the pentobarbital-anesthetized preparation (e.g., Fleschman et al. 1981; Zengel et al. 1985) was preserved, suggesting that the overall range of rheobases...
rate in the otherwise clearly decreasing trend. Also note that steady firing existed below the current level require to evoke acceleration.

To systematically evaluate differences between fully versus partially bistable cells in response to varying input levels, the frequency-current (F-I) relationships of the cells were evaluated with slow triangular current inputs (4–8 nA/s; 5 s up and 5 s down). F-I data were obtained in 31 of the total sample of 49 cells. Of these 31, 11 were fully bistable and 20 were partially bistable. Figure 4 illustrates the two different types of F-I relationships seen in fully bistable cells (left) and partially bistable cells (right). Both columns in Fig. 4 show the change in membrane potential (A and B), the pattern of firing rate versus time (C and D), and the F-I relationship (E and F) produced during both the ascending and descending phases of the triangular input. The was lowered without seriously distorting the S versus FR versus FF hierarchy.

A similar analysis was done using t-tests to evaluate whether conduction times were longer in fully than partially bistable cells. The two within-experiment t-tests resulted in P values of 0.006 (n = 9) and 0.001 (n = 10). The combined across-experiment t-test for normalized conduction time was also significant (P < 0.005). Thus conductance times for fully bistable cells were longer than those for partially bistable cells.

In summary, our data support the hypothesis that the tendency for bistability is strongest in low rheobase and slow conduction velocity motoneurons. This finding supports the idea that prolonged self-sustained firing only occurs in fatigue resistant type S or FR motor units (see DISCUSSION for a consideration of the limitations imposed on this conclusion by the overlap in conduction velocities in FR and FF cells).

**Frequency-current relationships**

The effect of the plateau potential underlying the bistable behavior also was evaluated while systematically varying the input to the cell. Figure 3 shows the firing patterns of a fully and a partially bistable cell in response to three injected current steps of increasing amplitude. The fully bistable cell (Fig. 3A) began firing with the first step and then immediately underwent a relatively rapid acceleration in firing frequency to reach ~40 Hz. This acceleration likely was produced by the onset of the plateau potential (cf. Hounsgaard and Kiehn 1989). The subsequent two current steps gave further small increases in frequency, each coupled to a very slight degree of adaptation in firing rate during the course of the 3-s step duration. The partially bistable cell shown in Fig. 3B also showed a very strong acceleration in firing due to plateau potential onset, reaching ~36 Hz during the second step. However, the subsequent adaptation was much more dramatic than in the fully bistable cell, with firing rate decreasing steadily after the peak of the acceleration. The final step increase in current elicited a small increase in firing rate in the otherwise clearly decreasing trend. Also note that steady firing existed below the current level require to evoke acceleration.

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features labeled on the $F-I$ relationships in Fig. 4, E and F, were used to provide quantitative comparisons (see further text and Table 1).

The fully bistable cell begins firing at a relatively low current and then exhibits an immediate acceleration in firing frequency (Fig. 4, A and C). Thus the ascending threshold and acceleration onset for this cell’s $F-I$ function (Fig. 4E) occurred at the same input current level, 2.7 nA. The acceleration produced a region of apparently high gain (acceleration gain) on the $F-I$ relationship (Fig. 4E). It should be emphasized that this is not a steady high gain state but instead primarily reflects the kinetics of the onset of the plateau potential (cf. firing patterns in Fig. 4C to Fig. 3A). Of the 11 fully bistable cells, 8 had ascending thresholds and acceleration onsets that were the same (see Table 1). The other three cells exhibited only minor variations on this pattern.
One exhibited no acceleration, which on careful examination of the record revealed a strong surge in membrane voltage before the onset of firing (not shown), indicating that this cell had its plateau potential activated below threshold for firing. The other two exceptions in the fully bistable category were cells in which high gain firing was delayed until slightly after the onset threshold (not shown).

The $I-F$ gain after acceleration was completed likely reflects the response of the cell to increases in input while the plateau potential is fully active. In the fully bistable cell in Fig. 4E, the postacceleration gain of the $I-F$ slope was decreased in comparison to the acceleration gain but it was still positive. The average postacceleration gain ($1.3 \text{ Hz}/\text{nA}$; see Table 1) in fully bistable cells was only slightly lower than the average primary range $I-F$ gain seen in motoneurons in pentobarbital-anesthetized preparations ($\sim1.5 \text{ Hz}/\text{nA}$) (Hounsgaard et al. 1988). The $I-F$ slope on the descending phase (i.e., the descending gain) is slightly greater than the postacceleration gain (see Table 1). Firing occurs over a much wider current range on the descending phase because the descending threshold (i.e., the current at which firing ceases) did not occur until current reached a strongly hyperpolarized level ($\sim7 \text{nA}$). In Fig. 4A, it is clear that the plateau potential persists beyond the end of the triangular input, producing occasional spikes as it slowly decayed during about a 5-s period (mostly of which is shown). This threshold hysteresis was seen in all bistable cells (cf. Bennett et al., unpublished data; Hounsgaard et al. 1988), with the average difference between descending and ascending thresholds being $\sim6 \text{nA}$ (Table 1).

Figure 4, right, illustrates several ways in which the $I-F$ behavior of a partially bistable cell differed from the fully bistable cell discussed above. First, as expected from the differences in rheobase between partially and fully bistable cells (Fig. 2), the ascending threshold in partially bistable cells occurred at a significantly higher current than in fully bistable cells (Table 1). Furthermore, the partially bistable cell exhibits a clear region of moderate gain ($\sim2 \text{ Hz}/\text{nA}$ in Fig. 4F) before the onset of firing acceleration, indicating that the activation of the plateau potential did not occur until well above the ascending threshold. In general, acceleration onset occurred at a significantly higher current in partially bistable cells than in fully bistable cells (Table 1). These data suggest that the plateau potential is activated at a higher voltage level in partially bistable cells compared with fully bistable cells (see Lee and Heckman 1998).

A strong acceleration in firing rate does occur in partially bistable cells, so that acceleration gain is not significantly different from in fully bistable cells (Table 1). However, the postacceleration gain was negative for the cell in Fig. 4F (note that when the postacceleration gain had two different phases, as in this cell, we only measured the gain for the phase immediately after the acceleration) and in most partially bistable cells. This gave an average value that was significantly less than the postacceleration gain in fully bistable cells (see Table 1 and Fig. 5). Finally, descending threshold tends to be slightly above the ascending threshold in partially bistable cells (average difference of $\sim1 \text{nA}$; Table 1), which is opposite to the pattern in the fully bistable cell. Overall, the foregoing data indicate that $I-F$ behavior is strikingly different in fully versus partially bistable cells.

### Rate adaptation

The tendency for the firing rate to decay in partially bistable cells (see Figs. 3B and 4E) may be related to the long-term rate adaptation seen motoneurons in deeply anesthetized preparations (see the discussion in the companion article). A significant factor in long-term rate adaptation is that higher initial firing rates are associated with greater adaptation (Kernell and Monster 1982; Sawczuk et al. 1995). A relationship of this type was evident on the ascending phase of our triangular current inputs. The peak firing rate at the end of the high gain phase (i.e., after acceleration had run its course) was correlated negatively with the subsequent gain of the $I-F$ function within the partially bistable group ($P < 0.001$; see Fig. 5). However this relationship was not significant in the fully bistable group ($P = 0.12$), and the slope of this relationship was much steeper for the partially bistable group than the fully bistable group despite the extensive overlap in peak frequency ranges. This suggests that the greater tendency of the partially bistable group to exhibit rate adaptation is not solely due to higher frequencies during the acceleration. The increased tendency for decay in firing rate in partially bistable cells presumably reflects the decay characteristics of the persistent inward current that produces the plateau potential (see Lee and Heckman 1998).

#### Threshold for sustained rhythmic firing versus rheobase

In spinal motoneurons in pentobarbital-anesthetized preparations, the threshold current for sustained rhythmic firing (referred to above as the ascending threshold for the $I-F$ function) is usually greater than the threshold current required.
quired to generate a single spike (i.e., rheobase) (Kernell and Monster 1981). In Fig. 6 the ascending (A) and descending (B) current thresholds are plotted versus rheobase. Although the partially bistable cells conform to expectation that their ascending thresholds are greater than their rheobases, the fully bistable cells have ascending thresholds that tend to be lower than their rheobases. The slope for the descending threshold—rheobase relationship is even steeper because the descending threshold in bistable cells is even lower than the ascending threshold. The probable basis for this result is that the rheobase current is too brief to activate the plateau potential. Thus if the voltage threshold for the plateau potential is lower than the voltage threshold for the action potential, rhythmic firing can commence below rheobase (see Lee and Heckman 1998).

Figure 6B also illustrates the finding that 9 of 11 fully bistable cells have offset thresholds that are below zero. In contrast, all of the partially bistable group have offset thresholds well above zero. Thus most of the fully bistable cells in our preparation are capable of tonic firing without injected depolarizing current.

DISCUSSION

Summary

Approximately one-third of the cells in our sample could generate prolonged self-sustained firing and were considered fully bistable. This behavior resulted from a combination of the endogenous effects of tonic activity in reticulospinal serotoninergic and noradrenergic axons (see further text), and the exogenous actions of the noradrenergic α₁ agonist methoxamine. The fully bistable cells tended to have lower conduction velocities and lower current thresholds than the partially bistable cells, which lacked the capacity for prolonged self-sustained firing. Consequently, it is likely that the fully bistable cells are type S or perhaps type FR. The limitations imposed on this conclusion by the noise in the relation between conduction velocity and motor unit type are discussed later.

The fully bistable group also exhibited systematic differences in the $F$-$I$ relationships in comparison with the partially bistable group. The plateau potential in fully bistable cells was activated near $F$-$I$ threshold, persisted throughout the subsequent acceleration in firing rate, and only deactivated when current on the descending threshold reached a level well below that for the ascending threshold (cf. Bennett et al., unpublished data; Hounsgaard et al. 1988). In partially bistable cells, the plateau potential was activated well above $F$-$I$ threshold and then appeared to rapidly decay, resulting in negative postacceleration gain and a lack of threshold hysteresis.

Methodological considerations

Previous work has established that serotoninergic fibers that originate in the brain stem are tonically active in the decerebrate cat preparation (Crone et al. 1988; Engberg et al. 1968). In addition, it seems likely that there was some tonic activity in reticulospinal noradrenergic fibers in our preparation. Engberg et al. (1968) concluded that noradrenergic pathways did not play a significant role in decerebrate excitability, but Sastry and Sinclair (1976) found evidence for a tonic noradrenergic drive to Renshaw cells. Both of these studies were done in intercollicular decerebrate preparations. An intercollicular transection may damage the upper portion of the locus coeruleus, which is a primary source of descending noradrenergic fibers. Our studies used precollicular decerebrates, in which the locus coeruleus was likely left entirely intact. Therefore the effects of the applied methoxamine probably were superimposed on at least some endogenous release of both serotonin and norepinephrine.

Two potential limitations of our approach should be considered. Our comparisons of self-sustained firing in partially and fully bistable cells were, by definition, confined to testing whether baseline current injections that were subthreshold for firing allowed the Ia input to evoke prolonged afterdischarges. This did not exclude the possibility that larger,
suprathreshold baseline currents in partially bistable cells might allow the Ia input to evoke long duration increases in firing rate. We feel this possibility is unlikely because our results with triangular inputs showed that once firing acceleration was complete, partially bistable cells exhibited a decrease in firing rate even though injected current continued to increase (i.e., negative postacceleration gain; see Fig. 5). It is thus probable that the inward current producing the plateau potential has a greater tendency to decay in partially as compared with fully bistable cells (see Lee and Heckman 1998).

In addition, F-I behavior in the present study was only assessed via injected current. Because a substantial portion of the plateau potential occurs in dendritic regions (Kiehn and Hounsgaard 1993; Lee and Heckman 1996), it is likely that the form of the F-I function will be somewhat different when synaptic current is the primary source of input. For example, Bennett et al. (unpublished data) showed that synaptic input lowers the frequency and current at which firing acceleration begins. We suspect that addition of synaptic current will have approximately equal effects on partially and fully bistable cells so that the two cell types would maintain distinctive F-I behaviors. However, systematic comparisons of the effect of synaptic input on F-I behavior in fully and partially bistable cells clearly are needed.

**Motor unit type and bistable behavior**

As noted in the INTRODUCTION, a capacity for long-term, self-sustained firing in type FF motoneurons does not make good functional sense because of their very poor resistance to fatigue. The finding in the present study that fully bistable cells tend to have low current thresholds and slow conduction velocities is supportive of the conclusion that these are type S and FR cells. However, current thresholds are clearly lower in our sample than in the studies that established threshold-type correlations (Bakels and Kernels 1993; Fleshman et al. 1981; Gardiner 1993; Zengel et al. 1985), which were done in preparations were neuronal activity is suppressed by pentobarbital and thus lacked neuromodulatory input form the brain stem. Although conduction velocity is unlikely to be altered by the exogenous and endogenous neuromodulatory actions in our preparation (see METHODS), conduction velocity ranges for FF and FR cells overlap considerably (e.g., Emonet-Denand et al. 1988). Given these uncertainties, we cannot make a firm conclusion as to the motor unit types in our sample.

Conduction velocities can be estimated from our measurements of conduction times by assuming the average conduction time within each of our four experiments where the number of cells was at least four was equal to the average conduction velocity in MG motoneurons (97 m/s in the data of Zengel et al. 1985). By this estimate, our range of conduction velocities in fully bistable cells was from 80 to 103 mm/s and from 95 to 107 m/s in the partially bistable group. S motor unit conduction velocities range from ~70 to 100 m/s and FR conduction velocities from ~84 to 114 m/s (Zengel et al. 1985). Thus it is quite possible that some of the fully bistable cells in our sample were FR motoneurons. In addition, the relatively high minimum estimated conduction velocity implies the smallest cells were under-

represented in our sample. This was likely to occur because of the relatively large electrodes and strict criteria for recording stability that we used.

Despite these limitations, our data make it clear that fully bistable cells tend to have lower conduction velocities than partially bistable cells, which does support the existence of a relationship between duration of self-sustained firing and fatigue resistance. Direct demonstration of this relationship would require experiments in unparalyzed preparations where fatigue resistance can be directly assessed.

**Functional implications**

The bulbospinal tracts that release the monoamines nor-adrenaline and serotonin are tonically active in the waking state (Aston-Jones et al. 1991; Kiehn et al. 1996; Veasey et al. 1995). Indeed, it is quite possible the CNS makes continual adjustments in the activity of these tracts to match the electrical properties of motoneurons to the task at hand (cf. Jacobs and Fornal 1993). Thus motoneurons can be said to have a range of electrical behaviors. At one extreme are the properties measured in deeply anesthetized preparations when activity in the monoaminergic tracts is suppressed. In this study, we sought to quantify motoneuron electrical properties at the other end of the spectrum, the fully bistable state when monoamines have near maximal actions.

In the initial studies of bistability in motoneurons, it was noted that the fully bistable state makes considerable sense for the control of posture (Hounsgaard et al. 1988). Because S units are likely to be heavily used in postural tasks (Wamsley et al. 1978), the data presented here strongly supports the hypothesis (Kiehn et al. 1996) that bistability is an integral part of postural control. Furthermore, our systematic comparisons of F-I relations provide a picture of how fully bistable motoneurons might be advantageous for steady posture and partially bistable motoneurons advantageous for corrections for large postural disturbances.

First, it should be emphasized that monoaminergic effects on motoneuron electrical properties will not disrupt the normal hierarchy of recruitment, which progresses from S to FR to FF units [i.e., the size principle of Henneman and colleagues (Binder et al. 1996; Henneman and Mendell 1981)]. Rheobase is the primary determinant of motor unit recruitment order because low rheobase cells require only a small amount of synaptic current to reach threshold. Although rheobase values were lower in our data sample than in previous studies in the pentobarbital-anesthetized preparation, there was still a good correlation between rheobase and conduction velocity. Thus cells with the slowest conduction velocities, which are likely to be type S, will tend to be recruited first (cf. Bawa et al. 1984; Cope and Clark 1991).

In fact, it is likely that a substantial portion of the type S cells in our preparation fire tonically. This is because most of the fully bistable cells had negative descending thresholds for their F-I functions (e.g., Fig. 4E). This would provide a strong baseline force for maintained posture because ~70–80% of maximum force in S units can be achieved by firing rates of only 20 Hz (Botterman et al. 1986; Kernels et al. 1983) (the average self-sustained firing rate of fully bistable cells in our sample was 21 ± 10 Hz).

Consider the case where a strong postural correction is
required. As input levels increase relatively rapidly to meet this demand, there would be little increase in firing in type S units because they already would be above the region of firing rate acceleration where F-I gain is modest (Fig. 4E). This is appropriate, as there would be little further force available in these units. In contrast, the higher threshold, partially bistable cells would undergo a strong acceleration in discharge and consequently provide a large surge in force to counteract the disturbance. The plateau potential in these cells is prone to decay and withdrawal of synaptic input after a successful correction will result in an appropriately rapid reduction in firing rate. Throughout, baseline force is maintained by the plateau potentials in the type S units.

The above scenario is speculative, but some evidence does exist in its favor. Tansey and Botterman (1996) have shown that electrical stimulation near the mesencephalic locomotor region in the decerebrate cat preparation produces firing patterns much like those described above. That is, S units maintain a steady firing rate, while F units rapidly peak and decline. Kiehn et al. (1996) have found that elimination of monoamines in the spinal cord by neurotoxins virtually eliminates tonic electromyographic patterns in chronic recordings in the rat. Moreover, recent evidence suggests the motor units in humans do exhibit self-sustained firing (Gorassini et al. 1998; Kiehn and Eken 1997). All these data are consistent with the hypothesis that monoaminergic input to motoneurons plays an essential role in postural control. The question of how motoneuron properties are modulated in this and other tasks is clearly essential one for understanding the genesis of movement.

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