Spike frequency adaptation of rat hindlimb motoneurons

Duane C. Button, Jayne M. Kalmar, Kalan Gardiner, Farrell Cahill, and Phillip F. Gardiner

*Spinal Cord Research Center, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada*

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**METHODS**

The decline in motoneuron (MN) firing rate during a continuous stimulus is known as spike frequency adaptation (SFA). MN SFA is characterized by at least three phases: 1) initial adaptation or a linear decrease in spike discharge over the first two or three spikes (35, 37, 41), 2) early adaptation or an exponential decrease in spike discharge over the first couple of seconds (16, 21, 27, 34, 40), and 3) late adaptation or an exponential decrease in spike discharge over several seconds to minutes (16, 21, 27, 40). Each phase involves several possible mechanisms that contribute to the decrease in spike discharge rate, which are discussed in detail by Sawczuk et al. (37). The most likely mechanisms underlying SFA in MNs include 1) summation of the medium afterhyperpolarization (AHP) amplitude (2, 5, 24, 35), which is mediated by the Ca$^{2+}$-activated K$^+$ channel, and 2) slow (persistent Na$^+$ conductance) and fast (transient Na$^+$ conductance) inactivation of the voltage-gated Na$^+$ channel (5, 34, 35, 39).

MN SFA has been mathematically defined by 1) the time constant of the exponential decay in spike discharge rate during the early and later phases of SFA (15, 16, 27, 40) and 2) the decrease in the instantaneous discharge frequency or number of spikes over a given time period (4, 15, 19, 27, 40). However, there are limitations to both of these approaches. The difficulty in measuring the rate of MN SFA using time constants lies in determining the best number of exponential decays to fit the frequency-time curve. We have found this to be especially problematic in the late phase of adaptation in rat hindlimb MNs (unpublished observations). The alternative approach of quantifying the change in firing frequency from the onset to the end of the current injection is easier to execute, but not necessarily more accurate. For example, Kernell and Monster (27) defined the time course of MN late adaptation as the decline in peak discharge frequency from the 2nd s to the 26th s, a method that was later employed by Spielmann et al. (40). Although this method was successful in determining the rate of adaptation over this time period, it does not reflect the diverse patterns of spike frequency that may occur between 2 and 26 s. Furthermore, because SFA patterns vary among MNs and because spike frequency changes rapidly in the first 2–3 s of firing (initial and early SFA), a 1-s epoch may not provide an accurate estimate of SFA.

The first purpose of this study was to develop a simple method of quantifying SFA that is weighted toward late adaptation with the idea that we could develop an SFA index that would correlate well with membrane properties and be sensitive to a variety of SFA patterns. The second purpose of this study was to determine whether SFA is dependent on current amplitude. Thus, for each MN, SFA was recorded in response to three different current amplitudes near the threshold for rhythmic firing.

Because MN SFA is lowest in slow-type motor units and greatest in fast, fatigable motor units (24, 40), a good estimate of MN SFA should significantly correlate with properties that determine MN excitability, such as rheobase current and input resistance (IR). Another property of MNs that modulates membrane excitability is the amplitude of the persistent inward current (PIC). Our group (10) has demonstrated that hindlimb MNs of rats anesthetized by a ketamine-xylazine mixture demonstrate the presence of PICs. A recent review by Brownstone (5) suggested that the inactivation of PIC may relate to MN SFA. Therefore, our in vivo rat preparation has allowed us to determine whether MN PIC correlates with MN SFA. Previously, this was not possible in in vivo experiments because animals were anesthetized with pentobarbital sodium (27, 40), which abolishes PICs. Some of the data reported here have been presented elsewhere in abstract form (9).

**RESULTS**

Treatment of animals. Thirteen female Sprague-Dawley rats (275–325 g) were obtained from the University of Manitoba (Winnipeg, Canada).
Canada) and housed in plastic cages situated in an environmentally controlled room maintained at 23°C and kept on a 12:12-h light-dark cycle. Animals were provided water and food ad libitum and were experimented on within 7 days of receipt. All procedures were approved by the animal ethics committee of the University of Manitoba and were in accordance with the guidelines of the Canadian Council of Animal Care.

Surgery. Animals were anesthetized with ketamine (N-methyl-t-aspartate receptor antagonist)-xylazine (α2-adrenergic receptor agonist) (90 and 10 mg/kg ip). Once anesthetized, atropine (containing saline, 5% dextrose, and 0.05 mg/kg atropine) was administered (volume 6.6 ml/kg ip) to minimize airway secretions during the subsequent tra- cheotomy. The surgical procedures included 1) insertion of a tracheal tube to ventilate the rat (Harvard Apparatus) with oxygen-enriched and humidified room air, 2) catheterization of the femoral artery, which allowed continuous monitoring of mean arterial pressure and constant infusion of anesthetic (Pump 11, Harvard Apparatus), 3) exposure of the left hindlimb sciatic nerve for stimulation, and 4) exposure and isolation of the spinal vertebrae and laminectomy from T12 to S1 in a stereotaxic unit.

Physiological saline solution containing ketamine-xylazine (9 and 1 mg/h) was infused via the femoral artery to maintain anesthesia, and depth of anesthesia was verified continuously via heart rate, mean arterial pressure, CO2 levels, and bilateral toe pinch. Blood pressure was maintained between 80 and 110 mmHg, and respiration was kept at a tidal volume of 2.0–2.5 ml and a ventilation rate of 60–80 strokes/min. Expired CO2 levels were measured via a CAPSTAR 100 CO2 analyzer (CWE) and were maintained between 3.0 and 4.0% by adjusting tidal volume or ventilation rate. Rectal temperature was monitored and maintained near 37°C using a feedback homeothermic blanket control unit (Harvard Apparatus). The head, thoracic and lumbar vertebrae, hips, and left foot were immobilized with clamps, and the open leg and back incisions were used to make an oil bath around the sciatic nerve and spinal cord, respectively. The dura mater covering the spinal cord was incised, and the large dorsal roots composed of afferents from the left hindlimb were cut and reflected over the right side of the cord. An opening was made in the pia mater (lumbar spinal cord segments L2-L4) just lateral to the entry zone of these roots into the cord, in preparation for introduction of the glass microelectrode. Before the search for MNs, a pneumothorax was performed on the left side of the thorax.

Drugs and solutions. To reduce blood pressure and respiration-related movement artifacts and to stabilize the animal for optimal electrophysiological recordings, several solutions were administered intravenously. The rat received 1) a solution of 100 mM NaHCO3 (Fisher Scientific) and 5% dextrose (Fisher Scientific) double-distilled H2O and 2) pancuronium bromide (0.2 mg/kg). Pancuronium bromide was injected intravenously in the rat before the start of electrophysiological recordings to induce paralysis and was readministered as necessary to maintain paralysis of the respiratory and/or hindlimb muscles.

Measurement of MN properties. Glass microelectrodes (1.0 mm thin walled; World Precision Instruments) were pulled with impedances of ~10 MΩ (Kopf vertical pipette puller; David Kopf Instruments) and filled with 2 M potassium citrate. The tip of the electrode was positioned at an incision in the pia mater and was lowered with an inchworm microdrive system (Burleigh Instruments) into the cord in steps of 5–10 μm. The sciatic nerve was stimulated with a bipolar silver electrode at a frequency of 1 pulse/s while the microelectrode was advanced through the cord and the field potential was continuously monitored. Evidence of successful impalement of an MN was a sudden decrease in membrane potential to at least ±55 mV with an antidromic action potential spike amplitude of >55 mV and with a positive overshoot and a reproducible latency of <2.5 ms from the stimulation artifact. During recording, an axoclamp intracellular amplifier system (Axoclamp 2B, Axon Instruments) was used either in bridge or in discontinuous current-clamp (DCC; 2–10 kHz switching) mode, with capacitance maximally compensated. Passive MN properties recorded in bridge mode included antidromic action potential (from an average of 10 spikes) and orthodromic action potential in response to a 0.5-ms current pulse of supramaximal intensity (from an average of 40 spikes). Rheobase current (the amplitude of a 50-ms square-wave current resulting in spikes 50% of the time) and cell IR (from an average of 60, 1-nA hyperpolarizing pulses each lasting 100 ms) were recorded. From these recordings, we determined antidromic spike height and time course, amplitude, and half decay time of the AHP following an action potential evoked by a 0.5-ms current pulse, resting membrane potential (RMP), rheobase current, voltage threshold, and cell IR.

Measurement of frequency-current relationship. After we completed recording of MN passive properties, the frequency-current (f-I) relationship of MNs was measured in two different ways. First, cells were challenged with slow triangular current ramps (range of 0.5–6.0 nA/s), and the voltage response was measured in DCC mode (see Ref. 10 for a complete description of this measurement). Peak amplitude of the ramp depended entirely on the rhythmic threshold of the MN, and an attempt was made to evoke trains of impulses containing 10–75 spikes over 0.5–2.5-s duration. Ramps were used to determine the MN f-I relationship, to evoke voltage-dependent plateaus, and to measure the underlying PIC as previously described (3, 20, 30). During the current ramps, the estimated PIC (ePIC) producing the plateau and sustaining firing was estimated from the difference in injected current at spike recruitment compared with spike derecruitment [see Fig. 1 in Button et al. (10)]. Second, cells were challenged with 500-ms square-wave pulse current injections of increasing and then decreasing amplitudes every 1 s, and the voltage response was measured in DCC mode. Current was increased in steps until blocking occurred before the end of the 500-ms period, after which current steps of decreasing intensity were administered [see Fig. 1 in Cormery et al. (11) for more details]. Current injections of 500-ms square-wave pulse were used to determine minimum and maximum steady-state firing frequencies, and current used at those frequencies was used to determine the MNs f-I slope.

Measurement of MN SFA. Cells were challenged with three 30-s square-wave pulses of current, and the voltage response was measured in DCC and bridge mode. The magnitude of current injected into the cell depended on rhythmic firing threshold (Rth; the minimum current at which the MN would fire for at least 10 s) of the MNs, and an attempt was made to evoke trains of impulses for 30-s duration. Once the threshold current for Rth firing was determined, three separate square-wave pulses of current at 1.5, 3, and 5 nA above Rth (>Rth) were injected into the MN for 30 s. Time was allotted after each of these 30-s current injections for the MNs to repolarize back to RMP. From these recordings, we determined the change in spike frequency over time. Thirty-second current pulses were chosen because very little MN SFA is seen after this time point (36).

At the end of these measurements, the microelectrode was backed out of the MN in 5-μm steps, and the extracellular voltage was recorded. Typically, experiments yielded one or two MNs with complete and acceptable complements of data. At the end of the experiment, the animal was euthanized by an overdose of KCl and bilateral pneumothorax.

Analysis of SFA. For each cell, we used three different current amplitudes (1.5, 3, and 5 nA > Rth) to analyze MN SFA by the following process: 1) we counted the number of discharges in 1-s bins, beginning at the onset of firing such that we had 30 bins (Fig. 1B), 2) we normalized these bin counts such that the final bin in the 30-s period of firing always contained 5 spikes, 3) we expressed MN SFA as the percent decline in the number of spikes discharged between two bins, and 4) we repeated this process using 15 bins of 2-s epochs (Fig. 1C) as well as 6 bins of 5-s epochs (Fig. 1D). These estimates of MN SFA are more sensitive to late adaptation than the initial and early phases of SFA compared with estimates of SFA derived from instantaneous frequency. Many combinations of bins were used to find an
index of SFA that 1) correlated the most significantly with other MN properties and 2) demonstrated a wide range of SFA. For example, with 2-s bins, one possible index of SFA is $1 - (\text{bin 13}/\text{bin 1})$. This index expresses the percent decline in the number of spikes discharged in bin 13 (25–26 s of firing) relative to bin 1 (first 2 s of firing) (Fig. 1C). Finally, we correlated each index of SFA to other electrophysiological properties, including the basic properties listed above as well as the f-I relationships that determine f-I slope and demonstrate the presence of PICs.

Statistics. Only MNs that responded rhythmically for at least 26 s during the 30 s of current injection and passed all electrophysiological requirements were used in the analysis. To determine whether MN SFA was stimulus current dependent, we performed a two-way ANOVA (current intensity × time) on the 1-s bin counts. When a significant interaction was present, Tukey’s post hoc tests were used to detect significant differences among individual means. All data are expressed as means ± SD.

RESULTS

We recorded data from 18 MNs (with all properties listed in METHODS) in 13 rats that were anesthetized with ketamine-xylazine. Only MNs with RMPs ≤ 55 mV and spike amplitudes (>55 mV) with positive overshoots were used in the following results. Hereafter, all MN SFA data reported are normalized so that the minimum number of MN spikes discharged in the final second always equaled five. Although we indexed MN SFA using many different bin combinations (see METHODS; Fig. 1, B–D), we report only those indexes that
estimate a wide range of SFA and that correlate well with other MN properties [i.e., 1 - (bin 15/bin 25); that derived from 1-s bins is not an index that we would report because MN firing rate does not decline significantly between the 15th and 25th s of current injection].

**MN passive and active properties.** MN properties are shown in detail in Table 1. Some of these data were used in previously published work (10). The data include values that are typically seen in a wide range of MN types. MNs that did not discharge for 30 s had a 65% (P  = 0.03) higher AHP amplitude than MNs that rhythmically discharged for 30 s. Aside from AHP amplitude, there were no differences in properties between those cells that fired for 30 s and those that did not.

**Relationship between MN SFA and current.** A two-way ANOVA test revealed a significant interaction (P  < 0.001) between injected current amplitude and number of spikes discharged during 30 s of rhythmic discharge (Fig. 2). Post hoc analysis revealed that this interaction could be attributed to the first 2 s of MN firing when the number of spikes discharged was greater with a current that was 5 nA > Rth compared with the 1.5 nA > Rth (P  < 0.001) and 3 nA > Rth (P  < 0.001) current injections. There were no differences between the 1.5 nA > Rth and 3 nA > Rth at any time point. After the first 2 s of current injection, the number of spikes discharged no longer differed between current amplitudes for the duration of the MN rhythmic discharge (Fig. 2). Because the 5 nA > Rth current injection resulted in increased MN frequency over the first 2 s of MN SFA, these data were excluded and all SFA data reported hereafter are based on an average of 1.5 and 3 nA > Rth trials.

**Determination of the amount of MN SFA.** We used several epochs (1, 2, and 5 s) of the number of spikes discharged that were near and/or included the time points 2 and 26 s [originally defined by Kernell and Monster (27)] to devise an index of SFA. For simplicity, in Table 2, we show only five indexes of SFA calculated with different bin combinations using 1-, 2-, or 5-s bins. These five indexes showed the greatest range of MN SFA and were significantly correlated with other MN properties (Table 3). We found that SFA (the percent decline in the number of spikes discharged from initial to final bin) was significantly (P  < 0.01) higher for 1-s 1 - (bin 26/bin 1) and 2-s 1 - (bin 13/bin 2) than for all other indexes.

**MN SFA indexes correlate with other MN properties.** Linear regression was conducted to determine whether MN SFA indexes were correlated (via Pearson’s product moment correlation procedure) with several MN active and passive properties. Correlation coefficients and levels of significance are presented in Table 3. Interestingly, all indexes of SFA were significantly correlated with MN ePIC amplitude, and the following indexes were correlated with rheobase: 1 - (bin 26/bin 1) using 1-s bins, 1 - (bin 13/bin 1) using 2-s bins, and 1 - (bin 5/bin 1) using 5-s bins. There was a weak but significant correlation between IR and the SFA index of 1 - (bin 26/bin 2) derived from 1-s bins, and a trend for correlation between IR and SFA indexes derived from the 2- and 5-s bins. No significant correlations between any index of SFA and f-I slope, RMP, voltage threshold, AHP amplitude, AHP half decay time, or spike height (not shown) were found. Correlation coefficients were not improved by using a multiple regression analysis.

We found that the percent decline in the number of spikes discharged from 5-s bin 1 to 5-s bin 5 was the best index of
Table 2. Distribution of MN SFA

<table>
<thead>
<tr>
<th>MN</th>
<th>1-s bins</th>
<th>2-s bins</th>
<th>5-s bins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 – (bin 26/bin 1)</td>
<td>1 – (bin 13/bin 1)</td>
<td>1 – (bin 5/bin 1)</td>
</tr>
<tr>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>10.84</td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>3</td>
<td>0.71</td>
<td>0.68</td>
<td>0.71</td>
</tr>
<tr>
<td>4</td>
<td>0.83</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td>5</td>
<td>0.78</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>0.72</td>
<td>0.66</td>
<td>0.71</td>
</tr>
<tr>
<td>8</td>
<td>0.82</td>
<td>0.78</td>
<td>0.78</td>
</tr>
<tr>
<td>9</td>
<td>0.57</td>
<td>0.65</td>
<td>0.62</td>
</tr>
<tr>
<td>10</td>
<td>0.76</td>
<td>0.72</td>
<td>0.73</td>
</tr>
<tr>
<td>11</td>
<td>0.84</td>
<td>0.76</td>
<td>0.81</td>
</tr>
<tr>
<td>12</td>
<td>0.82</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>13</td>
<td>0.86</td>
<td>0.81</td>
<td>0.84</td>
</tr>
<tr>
<td>14</td>
<td>0.83</td>
<td>0.76</td>
<td>0.80</td>
</tr>
<tr>
<td>15</td>
<td>0.80</td>
<td>0.75</td>
<td>0.79</td>
</tr>
<tr>
<td>16</td>
<td>0.55</td>
<td>0.54</td>
<td>0.55</td>
</tr>
<tr>
<td>17</td>
<td>0.53</td>
<td>0.49</td>
<td>0.52</td>
</tr>
<tr>
<td>18</td>
<td>0.81</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean</td>
<td>0.76*</td>
<td>0.72</td>
<td>0.73</td>
</tr>
<tr>
<td>SD</td>
<td>0.11</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>Range</td>
<td>0.54–0.86</td>
<td>0.49–0.82</td>
<td>0.52–0.84</td>
</tr>
</tbody>
</table>

Summary of different indexes of spike frequency adaptation (SFA) derived from the normalized number of spikes in 1-, 2-, and 5-s bins. For example, for MN 9, the index of SFA derived from bin 13 relative to 2-s bin 1 = 0.62, indicating that there was 62% decline in the number of spikes fired from bin 1 (time 0–2 s) to bin 13 (time 25–27 s). Indexes shown were selected because they were sensitive to a wide range of MN SFA and were significantly correlated with other MN properties. MN 1 and MN 6 are not applicable (NA) because they demonstrated SFA patterns typical of those seen when a MN is stimulated extracellularly; thus they were excluded from the data set. *Significantly different (P < 0.001) from all other columns.

SFA. This index (1 – (bin 5/bin 1)) was significantly correlated to ePIC amplitude and rheobase current and tended to correlate to IR and f-I slope and overall showed the best correlation coefficients and P values. Figure 3 illustrates the relationship between this index and the four properties listed in Table 3. The correlations predict that MNs with lower rheobases and larger ePICs exhibit less MN SFA. Note that the correlation coefficient for SFA and ePIC (−0.76) in Fig. 3D is based on all data points, including the eight MNs that demonstrate zero PIC. When these eight data points are removed and a linear regression is performed, the correlation coefficient remains very similar (−0.77). The strength of correlations between MN SFA indexes and MN properties was strongest when the high discharge numbers of the initial bins were included in the index of SFA. For example, with the use of indexes of SFA derived from 1-s bins, the correlation between these SFA and ePIC decreased from −0.79 to −0.74 to −0.73 to −0.69 when SFA was calculated as 1 – (bin 26/bin 1), 1 – (bin 26/bin 2), 1 – (bin 13/bin 1), and 1 – (bin 5/bin 4), respectively (results not shown for the latter two).

Range of MN types that demonstrate SFA. Only 18 MNs discharged continuously for 30 s or more in response to each of the three different currents. Therefore, we wanted to compare the MN properties of those cells to the properties of cells that did not discharge rhythmically for 30 s to ensure that our sample of rhythmically active cells was representative. In Fig. 4, the three MN properties (ePIC, rheobase, and IR) that correlated strongly with the SFA indexes (see Table 3) are shown as percentiles for the MNs that could and could not fire rhythmically for 30 s. The group of MNs that rhythmically discharged for 30 s showed a wide range of ePIC amplitudes, rheobase currents, and IRs that are comparable to those of a much larger number of MNs that could not rhythmically discharge for 30 s. Furthermore, there were no significant differences between the mean values (see Table 1) for these three properties. The only noticeable difference between the

Table 3. Correlations between SFA indexes and other MN properties

<table>
<thead>
<tr>
<th>Bin Size</th>
<th>SFA Index</th>
<th>Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ePIC</td>
</tr>
<tr>
<td>1 s</td>
<td>1 – (bin 26/bin 1)</td>
<td>−0.79, P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>1 – (bin 26/bin 2)</td>
<td>−0.74, P &lt; 0.001</td>
</tr>
<tr>
<td>2 s</td>
<td>1 – (bin 13/bin 1)</td>
<td>−0.77, P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>1 – (bin 13/bin 2)</td>
<td>−0.72, P &lt; 0.01</td>
</tr>
<tr>
<td>5 s</td>
<td>1 – (bin 5/bin 1)</td>
<td>−0.76, P &lt; 0.001</td>
</tr>
</tbody>
</table>

Summary of the relationship between different indices of SFA and other MN properties. Correlation coefficients and level of significance are presented for each of the correlations between SFA ratio and ePIC, rheobase current, input resistance (IR), and f-I slope.
two groups of MNs is in the distribution patterns at the very low end for rheobase current (Fig. 4B) and very high end of IR (Fig. 4A). It appears that our MN SFA data set does not include the smallest MNs.

Not only do the rhythmically active MNs have a wide variety of rheobase currents and IRs, they also have a wide range of SFA. This is evident in Fig. 5, which plots the number of spikes in 1-s bins for seven of the MNs that fired for 30 s.
Intracellular injections of different current amplitudes have been used for years to study MN SFA. However, it is not clear whether the overall pattern of MN SFA is dependent on stimulus current amplitude. Furthermore, a standardized method of quantifying SFA that is sensitive to the wide range in SFA patterns among cells and correlates with motoneuronal properties is lacking in the present literature. In the present study, we found that rat hindlimb MNs injected with 30-s square-wave currents at amplitudes 1.5, 3, and 5 nA >Rth elicit similar patterns of MN SFA. Using the index of MN SFA described here, we found that SFA is only stimulus current dependent in the first 2 s of MN firing, when the number of discharges is significantly greater with the 5 nA >Rth than at the two lower current amplitudes. After normalizing the number of spikes discharged in 1-, 2-, or 5-s bins to a minimum bin count of five spikes for each MN, we found that, using 5-s bins, the SFA index 1 − (bin5/bin1) provided the greatest range of MN SFA between cells and was best correlated with other MN properties. These correlations predict that MNs with lower rheobase and larger ePICs exhibit less MN SFA.

Our observation that MN SFA is stimulus current dependent only in the first 2 s of firing is consistent with previous literature. Similar results can be seen in the sixth figure presented in an MN SFA study by Granit et al. (16). In this figure (16), the patterns of MN SFA in response to current intensities ranging from 5.7 to 32.2 nA are similar, whereas the highest current (48 nA) evokes a pattern of MN SFA that seems to differ from the others. Consistent patterns of MN SFA throughout a range of current intensities can also be seen in cat hindlimb (27) and forelimb (4) hypoglossal (36) and rat facial nucleus (33) MNs. If the data from these earlier studies were analyzed with the normalized method that we propose in the present study, the frequency-time curves generated at different current amplitudes near the threshold for rhythmic firing would likely overlap. Furthermore, it has been shown that higher currents evoke greater initial instantaneous firing frequencies (2, 14, 16). In the present study, the initial phase of MN SFA adds only one to three extra spikes to the first bin count; thus the drastic change in instantaneous firing frequency that occurs within the first two to three spikes does not have a major influence on the overall pattern of MN SFA.

In the cat, MN I-f relationships can be observed by recording the firing frequencies evoked by a series of 500-ms square-wave current injections of increasing amplitude. The resulting f-I curve is characterized by a primary, secondary, and tertiary range of firing frequencies (22, 23, 38). Briefly, as the intracellularly injected current increases, there is an initial linear increase in the MN firing frequency (primary range) followed by a sudden and sharp increase in slope (secondary range). There is also evidence that rat MNs can also fire in primary and secondary ranges when subjected to 500-ms current pulses (12) and ramp currents (3, 10). Thus the current dependence of SFA in the first few seconds seen in Granit et al. (16) and the present study could be due to a shift from the secondary to primary range during the first few seconds of a prolonged current injection. The lower current amplitudes (1.5 and 3 nA above Rth) may not have been high enough to induce the secondary range of MN firing in the present study.

Once we determined a range of intracellularly injected current intensities that did not influence the pattern of MN SFA, we used these currents to develop a method of “quantifying” MN SFA. The method employed here was a modified version of that used by Kernell and Monster (27) and Spielmann et al. (40). Indexes of SFA were used to reflect the decline in the number of spikes within the first 26 s of current injection using bins of several sizes (1, 2, and 5 s). Indexes of SFA derived from small bins (1 or 2 s) and that include the first second of firing yield the greatest estimate of SFA. This is not unexpected, since the majority of MN SFA occurs during the initial and early phases (16, 22, 24, 27), which combined can last up to ~2 s (16, 21, 27, 34, 40). Thus one would expect that an index that includes the first second of firing should provide...
the highest estimate of SFA. However, with the use of 5-s bins, an SFA index of 1 − (bin 5/bin 1) provides not only a high magnitude of SFA magnitude (81%) but the greatest range (42–81%) of MN SFA compared with other indexes. This is most likely because this estimate of SFA includes the first 5 s of MN firing (all of the early adaptation phases and some of the late adaptation phase), which captures the greatest time block (all three phases) of MN SFA.

An increased rate of adaptation has been previously shown to correlate with motor unit properties that are seen in MNs with higher rheobase currents and lower IRs (42). On a recruitment continuum from type slow to type fast, the conduction velocity increases, fatigue index decreases, twitch contraction times decrease, and peak tetanic forces increase (7, 8), all of which have been found to also correlate with the rate of MN SFA (25, 26, 40). Kernell and Monster (26) also showed that fast motor units demonstrate much greater rates of SFA and fatigue than slow motor units. All of these factors are consistent with our finding that MNs with higher rheobases and lower IRs demonstrate faster rates of SFA.

All five indexes of SFA were significantly correlated with ePIC amplitude, an MN active property. The finding that MNs with greater ePIC had less SFA was surprising since Zeng et al. (41) recently demonstrated that blocking the persistent Na⁺ channel during 30 s of sustained constant-current steps had no effect on the pattern of MN SFA. They suggested that ion channels other than the persistent Na⁺ channel work concomitantly in MNs to ensure maintenance of sustained firing. Furthermore, persistent Na⁺ channels need to be active in order for spinal MNs to sustain rhythmic firing (18, 31). In earlier work, Lee and Heckman (29) demonstrated that MN bistability (a property facilitated by PIC) was more pronounced in low vs. high rheobase current MNs, but there was no difference in the initial PIC conductance. The lack of bistability in high rheobase current MNs may be attributed to a faster inactivation of the PIC channels. In the present study, ∼50% of the MN ePIC amplitude may be due to the Na⁺ PIC channel (32). As the persistent Na⁺ channel inactivates, the MNs ability to sustain rhythmic firing decreases (31). Perhaps the MNs with lower ePIC amplitudes reported here have faster inactivation rates of the persistent Na⁺ channel, which possibly leads to differences in SFA patterns among the MNs. In addition, during constant current stimulation, MNs that innervate fast-twitch muscle fibers have high initial firing frequencies and tend to adapt the most (25). The high initial firing frequencies occur because of very fast AHP time courses (23) and adapt quickly because of a summation of these AHPs over the first few spikes (2, 5, 24, 35). However, we now have some evidence suggesting that these MNs may also have faster inactivation of PIC channels, which along with the AHP time course contributes to greater SFA.

On the other hand, MNs with high PIC amplitudes have less inactivation and therefore less SFA. Decreased PIC inactivation may be extremely important during behavioral tasks such as posture (1) and fictive locomotor activity (6). For instance, during fictive locomotion, MN repetitive firing does not exhibit SFA. Actually, the rate of repetitive firing is high and constant throughout each burst, suggesting that in this situation PIC channels may be turned on and off in an all-or-none fashion. All PIC channels are activated, leading to a continuous non-SFA burst of activity during excitation and are inactivated between excitation episodes or they are somehow modulated in a different way (5, 6).

The SFA index 1 − (bin 5/bin 1), derived from 5-s bins, was better correlated to ePIC, rheobase current, IR, and f-I slope than other indexes. Estimates of SFA that excluded the earliest phases of adaptation did not correlate well with ePIC, rheobase, or IR, illustrating the importance of using the first 1–2 s of MN discharge when correlating SFA to other MN properties. On the other hand, none of the indexes correlated with the AHP amplitude, AHP half decay time, or the f-I slope. We would not expect a relationship between MN SFA rates and f-I slopes because the f-I slope is not dependent on MN type (11). In addition, the cat model but not the rat model has been used to show the relationship between SFA and motor unit type. Cat motor unit type can be predicted from MN passive properties much more easily (42) than rat (13). Therefore, our finding of a lack of rat MN AHP amplitude and AHP half decay time correlating to our SFA indexes was not surprising.

We used percent distributions to compare rheobase, IR, and ePIC amplitude in cells that discharged rhythmically for 30 s vs. cells that did not and found that the distributions did not differ between the two groups. Also, there were many different patterns of SFA (see Fig. 5) and a wide range SFA among the MNs, providing further evidence that our sample of MNs is representative of a wide variety of MN types and sizes. However, the least SFA that we report for any cell was 42%. In comparison, Kernell and Monster (26) demonstrated that MNs innervating slow-twitch muscle had very little adaptation, much less than shown here. Therefore, it is possible that the absolute slowest types of MNs, with the lowest rheobase and highest IR, may not be completely represented in our sample of rhythmically active MNs (see Fig. 4). Another possibility is that the rat may not have hindlimb MNs that demonstrate very little SFA.

Two motoneurons (MNexcluded) had a frequency-time relationship that showed an increase in firing frequency at the onset of current injection followed by a slight decline in firing rates (i.e., no SFA by our measure). Furthermore, these MNs had high ePIC amplitudes and low rheobase currents. Interestingly, the SFA profiles of the MNexcluded conform very similar patterns to fatigue myogram patterns of rat fast-fatigue-resistant motor units (14), suggesting that these MNs innervate fatigue-resistant motor units. Because excitable cells tend to be more bistable (10), or capable of toggling between active and quiescent states, than low excitable cells (29), SFA may be related to MN bistability. The PIC may have activated a plateau potential that in turn allowed MNexcluded to express self-sustained rhythmic firing at a fairly constant frequency and remain firing for a long period of time (17, 28). The MNexcluded SFA patterns can be seen in a turtle interneuron that has a plateau potential (15). Furthermore, Sawczuk et al. (36) also showed that some hypoglossal MNs have an MN SFA pattern similar to our MNexcluded (see Fig. 2B in Sawczuk et al. (36)). However, an explanation for this pattern of SFA has not been provided. Perhaps, some bistable rat hindlimb MNs show a SFA pattern similar to our MNexcluded, but this is speculative at present.

The method of Kernell and Monster (27) to quantify MN late adaptation assumes that MN late adaptation patterns are similar across all MNs and that SFA is negligible on 2 s of rhythmic discharge. Our method of using normalized bin counts to index
MN SFA has illustrated that SFA patterns are widely disseminated among MNs and that these SFA patterns vary quite extensively even after 3 s or more of rhythmic discharge (Fig. 5). The 5-s bin SFA index \( [1 - (\text{bin } \frac{5}{\text{bin } I})] \) results in the greatest range of MN SFA values, which is probably due to capturing a greater time course of the different MN SFA phases. Using longer duration bin counts to estimate SFA demonstrates that significant changes in MN output do indeed occur after 2 s of a 30-s rhythmic discharge. SFA (measured by injecting current pulses intracellularly) patterns probably vary among MNs because of differences in their 1) relative number, density, and distribution of ion channels; 2) relative number, density, and distribution of membrane receptors; 3) ion channel and receptor subunit type; 4) the muscle fiber type that they innervate; and 5) dendritic and somata morphology. All of these factors may play a role in determining the onset time and duration of each of the MN SFA phases (initial, early, and late), thus providing a nonuniversal MN SFA pattern that requires a longer as opposed to shorter duration spike discharge bin count for its measurement.

In conclusion, rat hindlimb MNs show similar SFA patterns when injected with prolonged intracellular square-wave current injections of various intensities. The magnitude of SFA is only stimulus current dependent within the first 2 s of current injection, perhaps due to a shift from the secondary to primary range of the f-I relationship. As such, any current near threshold may be used to elicit SFA, rendering the time-consuming process of measuring SFA at several currents unnecessary. We estimated the amount of SFA using the decline in the normalized number of spikes discharged in 1-, 2-, and 5-s bins over 26 s of firing. In so doing, we found that quantifying SFA as the percent decline in number of spikes discharged from the first 5 s (bin 1) to the last 5 s (bin 5) during 26 s of sustained firing was a good index of SFA. This index \( [1 - (\text{bin } \frac{5}{\text{bin } I})] \) produced the widest range of SFA among MNs and the most significant correlation between SFA and ePIC amplitude, rheobase current, IR, and f-I slope. These correlations are consistent with the notion that larger MNs exhibit greater SFA. Lastly, and perhaps most importantly, we have developed a simple method of estimating MN SFA rates that may be applied to our altered activity models to study the adaptability of MN properties.

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