Plasticity of rat motoneuron rhythmic firing properties with varying levels of afferent and descending inputs

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MacDonell CW, Button DC, Beaumont E, Cormery B, Gardiner PF. Plasticity of rat motoneuron rhythmic firing properties with varying levels of afferent and descending inputs. J Neurophysiol 107: 265–272, 2012. First published September 28, 2011; doi:10.1152/jn.00122.2011.—Hindlimb motoneuron excitability was compared among exercise-trained (E), sedentary (S), and spinal cord transected (T) Sprague-Dawley rats by examining the slope of the frequency-current (F/I) relationship with standard intracellular recording techniques in rats anesthetized with ketamine-xylazine. The T group included spinal transected and spinal isolated rats; the E animals were either spontaneously active (exercise wheel) or treadmill trained; and rats in the S group were housed in pairs. An analysis of motoneuron initial [1st interspike interval (ISI)], early (mean of 1st three ISIs), and steady-state (mean of last 3 ISIs) discharge rate slopes resulting from increasing and decreasing 500-ms injected square-wave depolarizing current pulses was used to describe rhythmic motoneuron properties. The steepest slope occurred in the S group (55.3 ± 22.2 Hz/nA), followed by the T group (35.5 ± 15.3 Hz/nA), while the flattest slope was found in the E group (25.4 ± 10.9 Hz/nA). The steepest steady-state slope occurred in the S group but was found to be similar between the T and E groups. Furthermore, a spike-frequency adaptation (SFA) index revealed a slower adaptation in motoneurons of the E animals only (~40% lower). Finally, evidence for a secondary range of firing existed more frequently in the T group (41%) compared with the S (12%) and E (31%) groups. The lower F/I slope and lower SFA index of motoneurons for E rats may be a result of an increase in Na+ conductance at the initial segment. The results show that motoneuronal rhythmic firing behavior is plastic, depending on the volume of daily activation and on intact descending pathways.

SEVERAL INVESTIGATIONS have illustrated plasticity of motoneurons by decreasing neuromuscular activity, eliminating supraspinal (spinal transected) and afferent (spinal isolation) activity, or increasing activity through exercise. Decreased neuromuscular activity tends to decrease excitability (Cormery et al. 2000, 2005) and shift the frequency-current (F/I) relation to the right (Cormery et al. 2005), while spinal transection and isolation result in motoneurons that show changes that lead to both increases and decreases in excitability. While the response of motoneurons to reduced activity is complex and may be mediated partly by descending drive and reduced neuromuscular activation, an increased activity paradigm (by way of exercise) results in adaptations that tend to increase the excitability of the motoneuron (Beaumont and Gardiner 2002, 2003). The mechanisms for changes to motoneuron properties may be due to changes in ionic conductance, as suggested when the Dai et al. (2002) motoneuron model was applied to findings in the Cormery et al. (2005) study.

Motoneuron models implicate altered ionic conductance as a potential mechanism for the adaptations associated with decreased or increased motoneuron input/output relation (Cormery et al. 2005; Gardiner et al. 2006). While it is clear that motoneuron properties are altered by increased/decreased activity, rhythmic properties in exercised animals have only been described with the use of a motoneuron model. The purpose of this research, therefore, was to identify changes in rhythmic hindlimb motoneuron properties in exercised rats and compare them to motoneurons of inactive (spinal transected and spinal isolated) and sedentary rats. Additionally, since changes in motoneuron function are influenced by changes in ionic mechanisms, spike-frequency adaptation (SFA; defined as the time-dependent decline in frequency of firing during sustained excitation) was examined, as SFA may be influenced by sodium becoming less available via a change in Na+ channel kinetics (Miles et al. 2005). However, there is no evidence available in the literature describing whether this property is plastic. Na+ channel conductance has also been proposed as a possible mechanism for adaptations seen with both decreased and increased levels of motoneuron activity, whereby Na+ conductance increases or decreases as a result of increases or decreases in activity (Cormery et al. 2005; Gardiner et al. 2006). As such, it was hypothesized that exercised animals would show a leftward shift in the F/I relationship and a decreased SFA owing to potential changes in Na+ conductance. These adaptations would favor excitability and prolonged discharge, which may attenuate fatigue in exercise-trained animals.

In the present study, an analysis of the F/I relation evoked by 500-ms current steps was undertaken using motoneuron data from Button et al. (2008), in which only the steady-state F/I slope was analyzed. Along with the steady-state F/I slope, the mean of the three early interspike intervals (ISIs) and the instantaneous rate of the initial ISI F/I slope are presented. Furthermore, the slopes of the F/I relationship from hindlimb motoneurons of spontaneously active and treadmill-trained rats were examined to make a comparison across animals with diverse activity levels. Finally, an index created from the ratio...
of the averaged last three ISIs to the initial ISI allowed an examination of the spike-frequency early adaptation rate among the groups. In light of previous results indicating that exercise leads to adaptations that favor increased excitability and that severe reductions in activity (as a result of spinal cord transection) tend to favor a decrease in cell excitability, it was hypothesized that motoneurons of exercise-trained rats would discharge at a lower stimulus intensity and demonstrate increased F/I slopes compared with those of nonexercised animals. In addition, it was hypothesized that running activity in the exercised rats would lead to adaptations that favor prolonged discharge, resulting in an attenuated decrease in discharge frequency (less SFA) during the 500-ms constant-current injection.

METHODS

Experimental Animals

Female Sprague-Dawley rats were separated into three groups: 1) the T group consisted of spinal transected and spinal isolated (spinal transected and dorsal rhizotomy) rats; 2) the S group consisted of sedentary rats; and 3) the E group consisted of spontaneously active and treadmill-trained rats (see below for more details on each group). The T and S group data were from a sample of the animals published by Button et al. (2008). All animals except those that were spontaneously active (confined in voluntary wheel cages with 24 h of access to the wheel) were housed in standard-sized cages in a room with a 12:12-h light-dark cycle and had access to food and water ad libitum.

Spinal transected animals. The T group consisted of spinal transected (250–350 g; n = 8) and spinal isolated (225–300 g; n = 10) rats. Animals were obtained from the University of California at Los Angeles, where surgery (spinal transection and spinal isolation) and postoperative 30-day maintenance were completed. After 30 days the animals were shipped to the University of Manitoba (cf. Button et al. 2008). The spinal cord of the spinal transected rats was completely transected at a midthoracic level (~T8) as described previously (Talmadge et al. 2002). Rats in the spinal isolated group had complete spinal cord transections at midthoracic (~T8) and upper sacral (~S1) levels with a bilateral dorsal rhizotomy between the two transection sites. Postsurgical care of spinal transected and spinal isolated rats involved manual expression of the bladder. The hindlimbs of spinal isolated animals were completely flaccid and exhibited no reflex activity (no muscular spasms, withdrawal reflexes, or toe spread responses) at any point during the 1-mo recovery period, indicating that the transections and dorsal rhizotomies were complete. The spinal isolation surgery and postoperative care procedures have been published previously (Button et al. 2008; Grossman et al. 1998; Hyatt et al. 2003; Roy et al. 1992). The T group animals were housed at the University of California at Los Angeles approved the procedures, which met the guidelines set forth by the Canadian Council of Animal Care and the American Physiological Society’s Guiding Principles for the Care and Use of Vertebrate Animals in Research and Training.

Exercise-trained animals. The E group consisted of female Sprague-Dawley rats that were chronically active for 16–20 wk via spontaneous activity in running wheels or daily endurance treadmill training. The cages for the spontaneously active group (250–350 g, n = 8) were equipped with a running wheel. These animals had 24-h access to wheels and ran a mean daily distance of 13.6 km in the week preceding data collection. The treadmill-trained animals were confined to standard plastic cages except during treadmill running for 60 min in the morning and evening (250–350 g, n = 6). Rats ran at a speed of 30 m/min and an incline of 10% 5 days a week. The Sprague-Dawley rats used for the E group were obtained from Charles River (St-Constant, QC, Canada). Exercise training and data collection occurred at l’Université de Montréal. The animal ethics committee for l’Université de Montréal approved the procedures, which met the guidelines set forth by the Canadian Council of Animal Care and the American Physiological Society Guiding Principles for the Care and Use of Vertebrate Animals in Research and Training.

Sedentary animals. The S Sprague-Dawley rats (280–350 g; n = 20) were obtained from Animal Care at the University of Manitoba. Data collection occurred at the University of Manitoba. The animal ethics committee for the University of Manitoba approved the procedures.

Surgical Procedures for Electrophysiology

Surgical procedures for all electrophysiological experiments have been previously published in detail (see Beaumont and Gardiner 2002, 2003; Button et al. 2008) and were similar for all groups. In summary, animals were anesthetized initially with an intraperitoneal ketamine and xylazine injection (90 and 10 mg/kg, respectively), followed by intraperitoneal administration of atropine (0.05 mg/kg atropine within 5% dextrose physiological saline) to minimize airway secretions during the subsequent tracheotomy. The depth of anesthesia was verified continuously via heart rate, bilateral toe pinch, and eye reflex. Once it was ascertained that the animal was initially anesthetized, a series of nonsurgical and surgical procedures were performed. The procedures included 1) insertion of a rectal probe to monitor animal temperature and maintain temperature near 37°C with a feedback Homoeothermic Blanket Control Unit (Harvard Apparatus); 2) insertion of a tracheal tube for ventilation (Harvard Apparatus; rate: 60–80 strokes/min; tidal volume range 2.0–2.5 ml) and to ensure that expired CO2 levels ranged between 3% and 4% (CAPSTAR 100 CO2 analyser, CWE); 3) catheterization of the femoral artery to maintain anesthesia through constant infusion of ketamine and xylazine (90 and 10 mg·kg−1·h−1, respectively; Pump 11, Harvard Apparatus) and to provide feedback for the maintenance of mean arterial pressure (MAP) at 80–100 mmHg throughout the duration of the experiment; 4) exposure of the left hindlimb sciatic nerve for antidromic stimulation; and 5) laminectomy from T12 to S1. The pia mater was incised lateral to the entry zone of dorsal roots to allow penetration of the glass microelectrode for intracellular recording. After the initial anesthetic, depth of animal anesthesia was monitored throughout the entire experiment by expired CO2 levels, MAP, heart rate, and bilateral toe pinch. Prior to the search for motoneurons, respiratory movement was minimized by performing a pneumothorax on the left side. To reduce blood pressure and respiration-related movement, several additional solutions were administered intravenously: a solution of 100 mM NaHCO3 (Fisher Scientific) and 5% dextrose (Fisher Scientific) in double-distilled water and pancuronium bromide (0.2 mg/kg). Pancuronium bromide was injected prior to the start of the electrophysiological recordings and readministered as needed to maintain paralysis in order to eliminate movement associated with peripheral nerve stimulation. A surgical plane of anesthesia was ensured by continual heart rate and MAP monitoring. Increased MAP and heart rate greater than 100 mmHg and 350 beats/min, respectively, were used as indicators to increase the depth of anesthesia by providing additional ketamine-xylazine via intravenous injection.

Intracellular Recordings

Intracellular recordings were obtained (Kopf Vertical Pipette Puller, David Kopf Instruments) with glass micropipettes (1.0 mm thin walled, World Precision Instruments) filled with 2 M K+ citrate. Electrodes had tip diameters between 1 and 2 μm and a resistance of ~10 MΩ. The tip of the electrode was positioned over the incision in the pia mater and lowered into the cord with an inchworm microdrive system (Burleigh Instruments) in steps of 5–10 μm. Motoneurons were antidromically identified through stimulation of the sciatic nerve.
with a bipolar silver-chloride hook electrode at a frequency of 1 pulse/s (0.1–0.2 mA for 0.1 ms). The field potential produced by the antidromic stimulation was monitored continuously while the micro-electrode was advanced through the cord. Evidence of successful impalement of a motoneuron was indicated by 1) a sudden increase in membrane potential to at least 50 mV; 2) an antidromic action potential (AP) spike amplitude >55 mV with a positive overshoot; and 3) a reproducible latency of <2.5 ms from the stimulation artifact. Intracellular motoneuron records created by increasing and decreasing 500-ms current steps (see Fig. 1 for current pulse and intracellular recording) were collected by an Axoclamp intracellular amplifier system (Axoclamp 2B, Axon Instruments) used in either a bridge (Beaumont and Gardiner 2003) or a discontinuous current-clamp (DCC; 2- to 10-kHz switching) mode, with capacitance maximally compensated (Button et al. 2008). Upon completion of recording from the motoneuron, the resting membrane potential was affirmed by monitoring the membrane potential as the microelectrode was removed from the cell in steps of 5 μm. Motoneurons recorded from had similar resting membrane potentials and did not vary considerably. Typically, experiments yielded a range between one and four motoneurons with complete and acceptable complements of data. Upon conclusion of data collection, animals were euthanized by an intravenous injection of potassium chloride (KCl) followed by a bilateral pneumothorax, in accordance with the regulations set forth by the ethics committees of the University of Manitoba and l’Université de Montréal.

500-ms Current Pulse and Minima and Maxima Data

A square-wave intracellular current injection lasting 500 ms was used to determine the rhythmic properties of hindlimb motoneurons (Fig. 1). For every neuron, a series of current steps was administered to ascertain the F/I relation. The 500-ms current pulse began at the lowest current required for discharge, increased in amplitude every 1–2 s until the motoneuron failed to fire during the entire 500-ms period, and was then decreased back to the minimum level required for motoneuron discharge (see Fig. 1 in Cormery et al. 2005). Although some electrode rectification occurred at high levels of current injection, electrodes were capable of passing high levels of current without marked rectification, and when this did occur, firing failure always occurred late in the 500-ms current pulse, after rectification had plateaued. Maximal motoneuron discharge rate and current amplitude were evaluated from the pulse directly before the failure of cell discharge occurred, while the minimum values were assessed from the lowest-amplitude current pulse where discharge continued for the duration of the 500-ms pulse ("sustained discharge"). If the motoneuron did not discharge throughout the pulse duration, or failure of action potentials occurred during the 500-ms pulse duration, these data were not included. The minimum and maximum current step and discharge rates represent the average amount of current used to initiate discharge and the amount needed to reach peak discharge for the entire pool within the group. Minimum/maximum discharge rate and current amplitudes presented in Table 1 are the average values of the initial ISI for each group.

Frequency-Current Analysis

Assessment of motoneuron rhythmic firing properties was performed by comparing the slope derived from the linear relationship between instantaneous discharge rate (reciprocal of the ISI value) and current amplitude. The following F/I slopes were calculated: the initial ISI slope (the slope of the relation between the discharge frequency calculated from the reciprocal of the first ISI and current); the early-state firing frequency slope (ESFF; slope of the relation between the discharge rate calculated from the reciprocal ISI mean of the first 3 intervals and current); and the steady-state firing frequency slope (SSFF; slope of the relation between the discharge rate calculated from the reciprocal ISI mean of the last 3 intervals and current) (see Fig. 1A for an illustration of the Initial ISI and ESFF, and SSFF). No fewer than four data points were used to establish an F/I relation for the primary range, and a 2 (slope) × 5 (group) analysis of variance was used to determine differences among groups (see Statistics).

The relation between the first ISI and current amplitude was used to determine whether a secondary range was present by visual inspection. If distinct primary and secondary ranges occurred, the F/I slope for that motoneuron was calculated from the primary range data. The slope was calculated from data generated by both increasing and decreasing 500-ms current steps (i.e., after the motoneuron failed to discharge, see above) for the majority of motoneurons. In six motoneurons, the slope was calculated from ascending current steps because of an adapting pattern of discharge, whereby a higher current was needed to generate the same frequency of spiking during descending amplitude current steps.

Fig. 1. Intracellular current injection and resulting action potentials for 3 different current amplitudes. Panels are representation of data collected at the current step to produce minimum motoneuron discharge rate (A), a discharge rate between minimum and maximum (B), and the maximum discharge rate (C). A also depicts the initial interspike interval (Initial ISI), the instantaneous discharge rate from the reciprocal of the first ISI; the early-state firing frequency (ESFF), the instantaneous discharge rate from the reciprocal ISI mean of the first 3 ISIs; and the steady-state firing frequency (SSFF), the instantaneous discharge rate from the reciprocal ISI mean of the last 3 ISIs depicted. The slope of the frequency-current (F/I) relation for each of these variables was calculated for each motoneuron.
Table 1. Frequency-current slopes and discharge properties of hindlimb motoneurons

<table>
<thead>
<tr>
<th>Measure</th>
<th>Groups</th>
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<tbody>
<tr>
<td></td>
<td>T</td>
<td>S</td>
<td>E</td>
<td></td>
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<tr>
<td>Initial ISI slope, Hz/nA</td>
<td>35.5±15.3</td>
<td>55.3±22.2**</td>
<td>25.4±10.9§</td>
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<tr>
<td>ESFF slope, Hz/nA</td>
<td>18.0±6.3</td>
<td>28.3±10.2*</td>
<td>13.9±4.7†</td>
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<tr>
<td>SSFF slope, Hz/nA</td>
<td>4.7±1.9</td>
<td>6.5±2.4*</td>
<td>5.3±1.7</td>
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<tr>
<td>SSFF:Initial ISI</td>
<td>0.16±0.11</td>
<td>0.12±0.06</td>
<td>0.25±0.14‡</td>
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<tr>
<td>Min discharge rate of first ISI during sustained discharge (mean), Hz</td>
<td>60.6±47.1</td>
<td>92.8±49.7*</td>
<td>38.8±36.3†</td>
<td></td>
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<tr>
<td>Max discharge rate of first ISI during sustained discharge (mean), Hz</td>
<td>426.0±170.7</td>
<td>451.8±125.3</td>
<td>259.1±102.0‡</td>
<td></td>
</tr>
<tr>
<td>Current mean min, nA</td>
<td>8.9±4.5</td>
<td>10.6±4.9*</td>
<td>8.0±4.1</td>
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<tr>
<td>Current mean max, nA</td>
<td>17.5±7.4</td>
<td>17.4±4.9</td>
<td>15.6±5.0</td>
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<tr>
<td>IR, MΩ</td>
<td>2.6±1.5</td>
<td>1.7±0.6</td>
<td>2.1±1.6</td>
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<tr>
<td>Rheobase, nA</td>
<td>7.4±4.6</td>
<td>9.8±3.7</td>
<td>6.2±2.8</td>
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<tr>
<td>Number</td>
<td>46</td>
<td>32</td>
<td>32</td>
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Values represent mean ± SD frequency-current (F/I) slopes and discharge properties of hindlimb motoneurons: initial interspike interval (ISI) slope, early-state firing frequency (ESFF), steady-state firing frequency (SSFF), mean minimum and maximum discharge rates, mean minimum and maximum current pulse amplitudes, input resistance (IR), and motoneuron number. Note that minimum discharge and maximum discharge do not reflect the absolute discharge capacity of the motoneuron but rather the minimum and maximum rates of the first interval achieved provided the motoneuron sustained discharge throughout the 500-ms pulse. T, transected; S, sedentary; E, exercise trained, Symbols indicate significantly different from all other groups at **α of P < 0.001 or *α of P < 0.01, significantly less than T group at †α of P < 0.03 or §α of P < 0.001, and ‡significantly less than all other groups at α of P < 0.001.

Adaptation Index

Upon injection of a constant current, motoneuron discharge frequency decreases in a time-dependent manner. The decrease in discharge rate can be separated into two (Granit et al. 1963; Kernell 1965; Kernell and Monster 1982a) or three (Sawczuk et al. 1995) phases, which are indicated by the rate of change in spike frequency. A 500-ms current pulse includes the initial (first few spikes) and a portion of the early phase of adaptation. The Initial ISI and SSFF were used to estimate SFA. The slopes of the current versus Initial ISI and SSFF outlined above describe the initial-to-early and early-late discharge patterns, respectively of a 500-ms pulse. The ratio of SSFF to Initial ISI, therefore, is an index of the magnitude of the SFA over the 500-ms intracellular current injection. The plasticity of the SSFF: Initial ISI was determined with a one-way independent ANOVA with three (groups) levels (see Statistics). In addition, the maximum voltage versus time rate of change (dV/dt) was calculated as a proxy for Na⁺ channel inactivation, since the initial rise of the action potential is a result of Na⁺ conductance (Hodgkin and Huxley 1952) and the rate of rise of the action potential has been shown to change during SFA (Powers et al. 1999).

Statistics

A custom program using MATLAB (R2007b; MathWorks) aided in the calculation of the slope derived from plotting current amplitude against initial slope and ESFF. An ANOVA using Statistica (v7.0; Statsoft) examined whether the dependent variable, slope, or ESFF: SSFF slope index differed among T, S, and E animals. The first step of the analysis determined whether the two groups within the T group and the E group differed. The 2 (slope: initial interval slope, ESFF slope) × 5 (group: spinal isolated, spinal transected, sedentary, spontaneously active, treadmill trained) independent-measures ANOVA used to determine whether a difference existed in slope between the groups revealed similar slopes within these two groups. Therefore spinal isolated and spinal transected data, as well as the spontaneously active and treadmill trained data, were pooled and made up the T and E groups, respectively. A 2 (slope: ISI, ESFF) × 3 (group: T, S, E) independent-measures ANOVA then determined whether a difference existed between activity levels. The degree of SFA between the groups and maximum dV/dt was tested with a main-effects independent-measures ANOVA, and a multivariate analysis of variance (MANOVA) was used to determine whether minimum/discharge rate of the first ISI and intracellular injected current differed to avoid running a separate ANOVA for each dependent variable. Finally, a χ² determined whether the incidence of a secondary range differed as a result of differences in activity levels, and the standardized test of residuals for each group aided in identifying which group contributed to a significant χ² result. Significant effects between group means were determined with planned comparisons. An α of 0.05 determined significance, and all data are reported as means ± SD.

RESULTS

Rhythmic firing properties of motoneurons of voluntary wheel- and treadmill-trained rats showed no difference for the F/I slope, i.e., were not exercise type dependent. Similarly, further isolating the cord, by removing ascending and peripheral afferent feedback in addition to spinal transection, did not further impact motoneuron rhythmic behavior compared with the spinal transected animals, i.e., was not isolation dependent. As outlined in Statistics above, the voluntary wheel and treadmill trained animal data were therefore combined to create an E group and the spinal transected and spinal isolated data were combined to create a T group. The results outlined hereafter make reference to three groups: T, S, and E. The following basic motoneuron properties did not covary with the F/I slope: 1) afterhyperpolarization (AHP) amplitude and AHP half-decay (collected in bridge mode, by challenging the cell with supramaximal 0.5-ms pulses); 2) input resistance (calculated from 60, or more, 1-nA hyperpolarizing 150-ms pulses); and 3) rheobase (data not shown). Since previous work (Cormery et al. 2005) found that the steady-state F/I slope did not covary with motoneuron type, this result was not unexpected. Table 1 outlines the means ± SD for the initial ISI slope, ESFF, SSFF; SSFF:ESFF; and the maximum and minimum mean values for discharge rate and injected current of the Initial ISI for the T, S, and E groups. Figure 2 illustrates the linear relation derived from plotting the initial ISI and ESFF against current for a motoneuron with two linear ranges and a motoneuron with a single range.
PLASTICITY OF RAT MOTONEURON FIRING PROPERTIES

Initial Interspike Interval Discharge and Slope and Early-State Firing Frequency Slope

Differences in the instantaneous frequency of the first ISI and current amplitude were present among E, S and T groups \( [F_{(2,103)} = 7.8, P < 0.0001] \) (Fig. 2A). The E group had a higher slope for the last three ISIs (5.3 \( \pm \) 1.7 Hz/nA) than the T group (4.7 \( \pm \) 1.9 Hz/nA). The significant difference between the S and T groups \( (P < 0.01) \) was not unexpected, since Button et al. (2008) reported that hindlimb unweighting resulted in a shift in the F/I relationship to the right and a decreased slope. To resolve whether the SFA rate during the 500-ms pulse differed between the groups, a ratio of SSFF versus current slope and Initial ISI versus current was calculated. A significant effect for the SSFF:Initial ISI was found \( [F_{(2,102)} = 11.84, P < 0.0001] \). The E group (0.25 \( \pm \) 0.14) showed the only difference, having a higher index \( (P < 0.0006) \), and therefore less adaptation, than the S (0.12 \( \pm \) 0.06) and T (0.16 \( \pm \) 0.11) groups. The maximum dV/dt, however, was similar among the groups.

First Interspike Interval Secondary Range

The first ISI was plotted as a function of current to determine whether a secondary range could be determined in rat hindlimb motoneurons (see Fig. 2A). Determination of a secondary range occurred through visual inspection and was confirmed by examining the point-by-point difference of the frequency data in order to identify a point of inflection that would indicate the beginning of an increased slope (i.e., \( y_2 - y_1, y_3 - y_2, y_{n+1} - y_n \)). For a linear relation with an \( R = 1.0 \), the point-by-point difference equals the magnitude of change in the independent variable for each point. A sizeable change in the point-by-point difference, therefore, would help identify whether the dis-
charge rate increased disproportionately to injected current. The threshold difference score of two times greater than the standard deviation was used to assist in the identification of a secondary range. [Figure 2A, inset, illustrates the difference score from a cell with two different linear ranges, and Fig. 2B, inset, displays the difference score from a different cell with a single linear range. Note the sharp change in difference score for Fig. 2A compared with the difference score data in Fig. 2B. The value of two times the standard deviation was used as the threshold value to indicate that two linear ranges may be present. The x-axis of the insets indicates the current step evaluated to create the difference score. Please note that a positive difference score (bars above zero) is associated with an increasing series of 500-ms current pulse amplitudes, and a negative difference score (bars below zero) is associated with a decreasing series of 500-ms current pulse amplitudes.

The incidence of a secondary range was low for all groups, with the highest incidence occurring in the T group (19/46 motoneurons), followed by the E group (10/32) and then the S group (4/32). A \( \chi^2 \) analysis revealed that a significant difference in the incidence of a secondary range was present \( (\chi^2 = 7.6, \text{df} = 2, P < 0.03) \), and an analysis of the standardized residuals (std. residual) indicated that the T group (std. residual = 1.4; secondary range more often than expected) and S group (std. residual = -1.8; secondary range less often than expected) contributed most to the significant outcome.

**DISCUSSION**

Rhythmic firing properties of rat hindlimb motoneurons differ among activity levels, whereby motoneurons of physically active rats tend to discharge rhythmically at lower levels of stimulation, have lower initial ISI firing rates and F/I slopes, and display less SFA. These features may allow trained motoneurons to summate forces of slower-contracting muscle fibers more effectively and discharge at higher rates for longer epochs. This report is the first to present data on the issue of what happens to the slope of the early and steady-state (SSFF) spike discharge frequency during a 500-ms intracellular current injection in motoneurons of animals with varying afferent and descending inputs and also whether early SFA differs under these conditions. In addition to this, the secondary range of the F/I relationship, often cited in the cat motoneuron literature, is more common in inactive rat motoneurons, although a low incidence overall was found.

Motoneurons with similar resting membrane potentials were identified by sciatic nerve stimulation and therefore included flexors and extensors as well as motoneurons innervating different muscle types in the lower hindlimb. The significant treatment effects indicate that, despite variation in the function and fiber type composition of the muscles innervated by these motoneurons, the plasticity in the measured properties is most likely a phenomenon common across the motoneuron pool. The possibility that differences between the groups were a result of a sampling bias in motoneuron type, either by activation threshold (high or low) or action (flexor or extensor), should be considered. However, the F/I slope, which was significantly altered in this study, has been shown here, and in previous work (Cormery et al. 2005), to be independent of basic properties and to be similar between “fast” and “slow” motoneurons. In addition, F/I slope is similar between flexor and extensor motoneurons in the decerebrate rat preparation (Chopek et al. 2010). Given these findings, the F/I data presented here likely represent a real difference between the groups, despite the potential for nonhomogeneous sampling of motoneurons between the groups.

**Exercise-Trained Group**

The a priori hypothesis, an increased slope and a leftward shift in the F/I relationship in this group, was not completely supported by the data. Although a modest leftward shift in the F/I curve occurred, the slope calculated from the relation between current and the first ISI and mean of the first three ISIs was found to be less steep than slopes for both the S and T groups, and the motoneuron discharge achieved slower maximum discharge rates. A possible explanation for these surprising findings is that the training (wheel and treadmill running) led to changes in \( \text{Na}^+ \) channel dynamics.

In addition to a small leftward shift in the F/I curve, the E group displayed smaller slopes and a decreased rate of SFA (as indicated by the adaptation index) compared with the other two groups. The finding of a reduced slope and SFA may partly be explained by altered \( \text{Na}^+ \) channel expression or \( \text{Na}^+ \) channel kinetics, either of which would affect \( \text{Na}^+ \) conductance. A change in \( \text{Na}^+ \) channel conductance is suggested for several reasons. Miles et al. (2005) showed the rate of SFA to covary with increased deactivation of fast, inactivating \( \text{Na}^+ \) channels over the duration of a current stimulus, limiting \( \text{Na}^+ \) availability. Dai et al. (2001) simulated a decrease in F/I slope via increased initial segment \( \text{Na}^+ \) conductance (5-compartment model with conductance from 10 channels), and Gardiner et al. (2006) reported that increased initial segment \( \text{Na}^+ \) conductance may result in a leftward shift of the F/I curve in a motoneuron model (5 compartment). Although neither \( \text{Na}^+ \) conductance nor channel kinetics were measured in the present investigation, both the changes in F/I slope and decreased SFA in the E group support a modified \( \text{Na}^+ \) channel kinetics. In addition to this, Woodrow et al. (2010) showed that treadmill training downregulates the expression of \( \beta_1 \)- and \( \beta_2 \)-subunits of the Na,1.6 sodium channel, a channel that has been shown to be instrumental in AP initiation and control of rhythmic firing (Lee and Heckman 2001). This preliminary work shows that \( \text{Na}^+ \) channel expression is modified via exercise. In an attempt to implicate changes in \( \text{Na}^+ \) channel function as the mechanism leading to the lower rate of motoneuron discharge decline, the maximum rate of membrane AP voltage change was assessed. The reason the rate of voltage change was examined was because a change in \( \text{Na}^+ \) channel kinetics may be reflected in the shape and rate of rise of the AP (Hodgkin and Huxley 1952; Powers et al. 1999). The analysis revealed no difference among the groups in the rate of change of the membrane potential both initially and throughout the duration of the 500-ms pulse, despite group differences in SFA. The relationship between ion conductances and SFA is obviously complex (Miles et al. 2005; Zeng et al. 2005), and the role of altered \( \text{Na}^+ \) conductance, either alone or in combination with other events, remains to be established.

Even though alteration in \( \text{Na}^+ \) channel kinetics may play an important role in the findings described above, two alternative explanations may be considered. First, summation of the AHP has been suggested as an important contributor to early SFA
(Baldissera et al. 1971), but Miles et al. (2005) showed that blocking the AHP (apamin) had no effect on adaptation in mouse motoneurons. Thus the larger degree of adaptation in the S and T animals is not likely due to a difference in the AHP (especially since AHP amplitude in chronically E animals has been shown to be larger than that in control animals; Beaumont and Gardiner, 2002, 2003). Since the motoneurons in each group were challenged with similar maximal magnitude current steps, current-dependent adaptation rates are an unlikely prospect. Another possibility is the influence of the initial discharge rate. Even though the pattern of adaptation appears to be independent of initial firing rates (Button et al. 2008), it may be that the slower frequencies found in the E group influenced the rate of SFA as calculated in this experiment. The instantaneous frequency of the initial ISI has been reported to covary with the rate of adaptation (Kernell and Monster 1982a; Sawszuck et al. 1995). Finally, although it is possible that persistent inward currents (PICs) may affect adaptation, there is no evidence to support an increased incidence of PICs or plateau potentials in the E group. As such, the decreased adaptation rate seen in the E group may be explained partly by a lower initial instantaneous frequency and altered Na⁺ entry through a decrease in the inactivation kinetics of fast inactivating Na⁺ channels. Nonetheless, it is worth noting that control rat motoneurons show an inverse relationship between SFA and the amplitude of estimated PICs (Button et al. 2007).

**Transected and Sedentary Groups**

The greater F/I slope of the T and S groups compared with the E group were not predicted. It is thought that the slope of the F/I relation in the S group was the steepest for the Initial ISI and ESFF because these animals are undergoing disuse adaptations as a result of imposed inactivity (via confinement to a standard cage). If one considers the spectrum of motoneuron use, the animals used in this study range from an extreme form of inactivity resulting from spinal cord transection to inactivity imposed through confinement to a standard plastic cage and animals that were exercised on a treadmill or housed in a cage with an exercise wheel. The E group, especially those animals housed with an exercise wheel, may actually represent the “normal” model, with the S group representing restricted activity, despite being able to move freely within their caged environment. Therefore, spinal cord transection and isolation may not be an appropriate model of inactivity alone, as they also include malfunction, not simply a decrease in activity. Furthermore, the sedentary model used here may be the appropriate model for decreased neuronal activation. In light of rats’ innate exploratory behavior (Barnett 1963), the voluntary exercise paradigm may be the closest model we have to “normal” motoneuronal activation.

The difference seen between the T and S groups may be attributable to intact descending drive. Using a hindlimb unweighting model, Cormery et al. (2005) reported a shift in the F/I relation to the right (decreased excitability) but identified no change in slope. The present results showed that the F/I relation of the T group did not shift to the right, but did decrease, compared with the S group. The discrepancy in the F/I curve between the data presented here and the Cormery investigation may be related to a fundamental difference between the models of hindlimb unweighting versus spinal cord transection, which differ in more than motoneuron activation alone. The hindlimb unweighted group had intact supraspinal input, whereas the T group had no supraspinal input. Other investigations on rats with eliminated supraspinal input (Beaumont et al. 2004, 2008; Button et al. 2008) also reported reductions in the F/I slope.

The rate of SFA was similar between the S and T groups, despite having different F/I slopes. The similar adaptation rate between S and T groups is comparable to the result reported by Button et al. (2008) when a 30-s current step was used.

**Secondary Range**

We also examined whether a secondary range of motoneuron firing, which is well documented in the cat literature, exists in the rat (Fig. 2A, for example). Indeed, neurons showing distinct linear ranges were found in rat hindlimb motoneurons. A comparison between the groups showed a higher incidence of a secondary range in T animals compared with the S and E groups. This may be due to increased estimated PICs in spinal isolated and spinal transected animals compared with control (i.e., sedentary) animals (Button et al. 2008). Activation of PICs drastically increases the firing frequency of motoneurons during ramp currents, and perhaps there is an underlying link to increased incidence of a distinct secondary range of firing and the incidence and amplitude of estimated PICs. The presence of estimated PICs in the E group could not be estimated, as the data did not include slope ramp current injections. As an aside, the secondary range slope calculated from the initial ISIs from the secondary range data of the T and S groups were much steeper than the E group, which is consistent with the primary range findings (the pooled slopes calculated from the initial ISI were 77.1 Hz/nA, 85.5 Hz/nA, and 37.2 Hz/nA for the T, S, and E groups, respectively).

**Conclusion**

This paper is the first to provide an analysis of rhythmic motoneuron firing across a spectrum of use. The spectrum included spinal transected and spinal isolated animals, sedentary animals (i.e., able to move freely in a standard cage), and exercised animals. Exercised animals show decreased rates of SFA, slopes, and discharge, which may be indicative of alterations in Na⁺ conductance. The decreased rate of SFA may be functionally significant in the sense that the motoneuron is able to maintain discharge over longer epochs. The sedentary (S) group showed the steepest motoneuronal F/I slope out of all the animals, which may be a result of their inactivity, since the transected (T; spinal transected and spinal isolated animals) group also showed steep slopes.

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