Exp Brain Res (2003) 150:497–505
DOI 10.1007/s00221-003-1475-8

RESEARCH ARTICLE

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Conduction velocity is inversely related to action potential threshold in rat motoneuron axons

Received: 9 December 2002 / Accepted: 10 March 2003 / Published online: 25 April 2003

Abstract Intra-axonal recordings were performed in ventral roots of rats in vitro to study the conduction velocity and firing threshold properties of motoneuron axons. Mean values ± SD were 30.5±5.6 m/s for conduction velocity and 11.6±4.5 mV for the depolarization from the resting potential required to reach firing threshold (threshold depolarization). Conduction velocity varied inversely and significantly with threshold depolarization (P=0.0002 by linear regression). This relationship was evident even after accounting for variation in conduction velocity associated with action potential amplitude, injected current amplitude, or body weight. Conduction velocity also varied inversely with the time to action potential onset during just-threshold current pulse injection. These data suggest that the time course of depolarization leading to action potential initiation contributes to the speed of conduction in motoneuron axons.

Keywords Myelinated axon · Threshold · Action potential · Conduction velocity · Intra-axonal recording

Introduction

Monkeys and rats can gradually increase (up-conditioning) or decrease (down-conditioning) the H-reflex when reward is contingent upon behavioral change (see Wolpaw 1997 for review). In monkeys, down-conditioning produces a positive membrane potential shift in motoneuron firing threshold that largely accounts for the smaller H-reflex. In addition to the effect on somatic firing threshold, down-conditioning is also associated with a decrease in conduction velocity (assessed by the latency from antidromic stimulation to somatic excitation; Carp and Wolpaw 1994). Down-conditioning in the rat produces a similar decrease in conduction velocity that is clearly due to a change in the axon, rather than just to delayed action potential transmission within the axosomatic transition region (Carp et al. 2001). The most parsimonious interpretation of these data is that down-conditioning alters excitability throughout the motoneuron (soma and axon) by a single mechanism. In the axon, the positive shift in firing threshold would increase the total conduction time between adjacent nodes by extending the time required to reach threshold at each node, thereby accounting for the decrease in axonal conduction velocity.

Down-conditioning-induced modification of motoneuronal sodium channels could account for the dual effects on axonal conduction velocity and somatic firing threshold. Sodium channels play an essential role in action potential initiation in the motoneuron soma and axon due to the time- and voltage-dependence of their activation and inactivation (Ritchie 1995; Vogel and Schwarz 1995). That their properties are subject to neuromodulation suggests the possibility that such a mechanism may be invoked by down-conditioning (Cantrell and Catterall 2001). Application of the intrasomatic data to a myelinated axon model suggests that a positive shift in sodium channel activation voltage in motoneuron soma and axon best explained the effects of H-reflex down-conditioning (Halter et al. 1995).

Determination of the dependence of the axonal conduction velocity decrease on firing threshold after down-conditioning requires knowledge of the relationship between these properties. Activity- and drug-dependent variation in charge or current thresholds for axonal excitation have been rigorously evaluated and related to axonal conduction time or velocity (Bullock 1951; Raymond and Lettvin 1978; Raymond 1979, 1992; Bostock and Grafe 1985). On the other hand, the contribution of intrinsic variation in axonal voltage threshold to the range of observed conduction velocities

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has not been systematically evaluated. The present study uses data recorded intra-axonally from unconditioned adult rat ventral roots in vitro to investigate axonal excitability and conduction velocity. These data demonstrate an inverse relationship between firing threshold and conduction velocity. They suggest that intrinsic variation in firing threshold (and presumably Na^+ channel properties) contribute to the range of motor axonal conduction velocities. Furthermore, these results are consistent with the hypothesis that modulation of firing threshold by down-conditioning of the H-reflex could account for the observed changes in axonal conduction.

Materials and methods

Experiments were performed with 20 male Sprague-Dawley rats weighing 380–570 g. All procedures adhered to the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, D.C., 1996) and had been reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center.

Surgical and recording procedures were based on and modified from those of Bostock and Grafe (1985). Rats were anesthetized with pentobarbital (70 mg/kg, i.p.) and their spinal cord and roots were exposed by dorsal laminectomy and longitudinal dural incision. L4–6 spinal roots were excised and maintained at room temperature (22–24°C) in artificial cerebrospinal fluid (aCSF) containing: 118 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO4, 1.2 mM $MgCl_2$, and 10 mM glucose, and bubbled with 95% O_2:5% CO_2. Ventral roots were transferred individually to a tissue bath and superfused with aCSF at 29–30°C. Each ventral root was draped across the long axis of a small convex plastic platform (6 mm long, 2 mm wide, 1.5 mm high at midpoint), surrounded by a silicone elastomer and gently strapped down to both ends of the platform with a pair of perpendicularly oriented dorsal roots that were secured to the silicone by fine pins. Suction electrodes held the proximal root end for stimulation and the distal root end for recording. The maximal ventral root compound action potential was monitored periodically to assess preparation viability.

Ventral root axonal recording was done with 30–70 MΩ micropipettes (3 M K^+ acetate). To maximize the length of the conduction path, recordings were made as far from the stimulating suction electrode as was feasible (mean distance between suction and recording electrodes ± SD = 18.7±4.7 mm). Recordings were considered intra-axonal if the height of the antidromic action potential increased with hyperpolarizing current and decreased with depolarizing current passed through the electrode (Stys and Kocsis 1995). Only axons with action potentials greater than 60 mV were included in the data pool.

In action potentials evoked by just-threshold stimulation applied to the whole ventral root (median number per axon, 12; range, 3–41), we measured amplitude, maximum rate of rise, and latency to onset. Axonal conduction velocity was calculated as the ratio of conduction distance to nerve stimulus-evoked action potential latency. Conduction velocity was only determined for axons in which action potentials were elicited at low nerve stimulation intensity to minimize current spread-induced underestimation of action potential latency. Conduction velocity values were lower than previously reported for Sprague-Dawley rats (Birren and Wall 1956; Sato et al. 1985; Chen et al. 1992). This reflects, at least in part, the lower temperature (i.e., 29–30°C) used in the present in vitro study. In addition, the low conduction velocity values may also reflect a slight underestimation of interelectrode distance due to the minimal tension applied to the ventral root and/or a small overestimation of conduction time due to inclusion of the time from stimulus onset to action potential onset at the suction electrode. Assuming that the latter error was no more than the internodal conduction time (about 0.03 ms for our data), our present estimate of conduction velocity would underestimate the true mean value ± SD by no more than of 6±2%.

To characterize axonal current threshold, we applied a series of 10-ms current pulses (median number per axon 40, range 16–103) at 2-s intervals at intensities that bracketed the firing threshold. Figure 1A shows examples of the membrane potential trajectory from one axon during repeated, just-threshold current injection. The same current stimulus elicited waveforms that followed a very similar time course over the first several tenths of a millisecond. By about 0.8 ms after current pulse onset, response variability increased dramatically, particularly in recordings during which action potentials were elicited. The initial period of low variability is likely to reflect primarily the passive properties of the electrodes and the axonal membrane. Few voltage-dependent conductances that activate rapidly enough to contribute to the early part of the response are active in this membrane potential range (Waxman 1980; Poulter et al. 1993; Ritchie 1995). Sodium channels could potentially contribute to the subthreshold membrane potential. However, the low variability of the initial membrane potential trajectories and the subsequent increase in variability (indicative of nondeterministic behavior associated with channel activity) suggest that the contribution of voltage-dependent conductances to the subthreshold membrane potential are modest during the early part of the response. Thus, we applied nonlinear curve fitting to the initial 0.5–0.75 ms of the membrane potential responses in which action potentials were not elicited to determine the membrane time constant. The fitting range was determined for each axon to focus analysis on the low-variability component (mean duration of fitted data segment ± SD=0.62±0.04 ms, which ended a mean ± SD of 0.83±0.58 ms before the mean action potential latency in suprathreshold trials). The initial low-variability portion of the membrane potential responses to just-subthreshold current injection was well-described by the equation:

$$MP_t = MP_{final} + A_{elec}e^{\left(-t/\tau_{elec}\right)} + A_{mem}e^{(-t/\tau_{mem})} \quad (1)$$

where MP_t is the membrane potential at time t after current pulse onset, MP_{final} is the asymptotic value of membrane potential during current passage, and A_{elec} and A_{mem} are the initial amplitudes and τ_{elec} and τ_{mem} are the time constants of two exponential components reflecting the voltage time course of the electrodes (τ_{elec}, typically 0.04–0.09 ms) and the axon (τ_{mem}). That our values for τ_{mem} are comparable with those reported previously using hyperpolarizing current pulses (Richter et al. 1974) supports our assumption that the low-variability component reflects primarily passive axonal properties.

To determine the firing probability for different levels of stimulation, we calculated the fractions of trials in which action potentials were elicited for narrow current ranges (typically 0.02- to 0.05-nA widths). The relationship between applied current and probability of firing is illustrated for one axon in Fig. 1B. Nonlinear fitting to these data of the sigmoidal relationship (dashed line in Fig. 1B) described by:

$$firing\ probability = \frac{1}{1 + e^{(rheobase - I)\cdot slope}} \quad (2)$$

estimated the rheobase (i.e., the amplitude of the injected current pulse, I, necessary to elicit an action potential 50% of the time; i.e., firing probability 0.5) and the steepness of the relationship between I and firing probability (i.e., slope).

The axonal action potential voltage threshold was measured as the difference between the resting membrane potential and the membrane potential just sufficient to elicit an action potential (i.e., threshold depolarization). Determination of threshold depolarization directly from recordings of action potentials elicited during current injection is suspect due to uncertainty in compensation for electrode resistance. To avoid this error, we estimated threshold depolarization indirectly as the difference between the mean action potential amplitude elicited by nerve-evoked activation and that elicited by 10-ms depolarizing current pulses of just-threshold

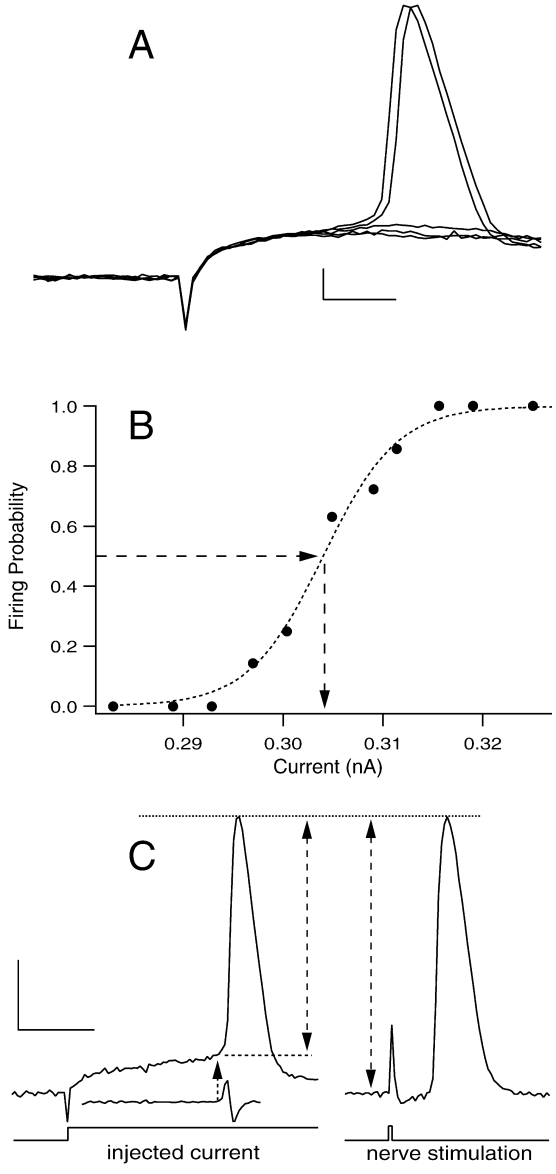

Fig. 1A–C Methods used to measure current and voltage thresholds. **A** Membrane potential recordings from one axon in which injection of a just-threshold current pulse elicits or does not elicit an action potential (shown truncated here). Membrane potential variability at rest and during the initial 0.8 ms after current pulse onset is low, but subsequently increases, even when a spike is not elicited. *Calibration bars:* time, 0.5 ms; membrane potential, 10 mV. **B** Firing probability (defined as the % of trials in which current injection elicited an action potential) in one axon increases with current. Nonlinear sigmoid curve fitting (*dotted line*) to Equation 1 (see Methods) estimates the current at which an action potential is elicited in 50% of the trials (i.e., rheobase, indicated by *dashed arrows*). **C** Method for determining threshold depolarization (modified from Gustafsson and Pinter 1984). Action potentials (*upper traces*) elicited from the same resting potential by current pulse injection (*bottom left trace*) or nerve stimulation (*bottom right trace*) achieved the same peak level (*dotted line at top*). During current injection, the onset of the positive peak (*upward arrow*) in the second derivative of membrane potential (*middle trace*) defines the onset time and voltage (*dotted line*) of the action potential. The difference between nerve-evoked and current-evoked action potential amplitudes (*double-headed dashed arrows*) represents the depolarization required for the nerve to reach threshold from the resting potential (i.e., threshold depolarization). *Calibration bars:* time, 1 ms; membrane potential, 20 mV; second derivative of membrane potential, 15×10^3 V/ms^2

intensity (median number per axon, 18; range, 6–66). This method, illustrated in Fig. 1C, has been used previously for determining motoneuron somatic threshold (Gustafsson and Pinter 1984; Carp 1992).

At the conclusion of recording, the potential difference recorded upon retraction of the electrode from the axon was used to determine resting potential. Unambiguous assessment of resting potential could only be performed in this way in a limited number of axons, because small movements of the electrode were associated with abrupt potential shifts, presumably resulting from electrode resistance changes during withdrawal of the electrode from the axon and myelin. Determination of which withdrawal potential represented the true extracellular value was often problematic. Action potential amplitude, an easily and reliably quantifiable measurement, varied with resting potential with a near-unity slope (r^2=0.37; slope, 1.05 mV/mV; P=0.0003, for linear regression of action potential amplitude on resting membrane potential). Thus, action potential amplitude was used in regression analyses that assessed the possible contribution of impalement quality to relationships between other axonal properties.

Results

Description of data pool

Recordings from 52 ventral root axons (median of two per rat; range of 1–11) provided data on resting and action potential properties. Figure 2 shows the distributions of action potential amplitude, threshold depolarization, and rheobase. In addition, 31 of these axons provided data on conduction velocity; their distribution is shown in Fig. 2D.

Table 1 shows the mean values ± SD of these and other axonal properties. The properties of the axons with conduction velocity measurements were similar to those of the entire data pool in mean value (Table 1) and in their distributions (Fig. 2A–C). The mean value of threshold depolarization is similar to that for monkey motoneuron cell bodies (i.e., 11±4 mV; Carp 1992).

For action potentials elicited by current, the mean currents (normalized to each axon's rheobase) used for studying threshold depolarization ranged from 0.90 to 1.07 (overall mean normalized current, 0.99±0.05). Neither threshold depolarization nor rheobase exhibited any dependence on the mean magnitude of the injected current used in the axon (r^2=0.00 and 0.01, P=0.75 and 0.45 for linear regressions of threshold depolarization and rheobase on mean normalized current, respectively).

Relationship between conduction velocity and threshold depolarization

Figure 3A shows that axonal conduction velocity varies inversely with threshold depolarization. Details of this and other simple regression analyses are shown in Table 2. This relationship was evident over the entire range of conduction velocities observed. It did not depend solely on the two axons that had the lowest conduction velocities and the highest threshold depolarizations, since it persisted when these outlying data were excluded (r^2=0.33; slope, -1.02 m·s^{-1}·mV^{-1}; P=0.002, illustrated by the dotted line in Fig. 3A).

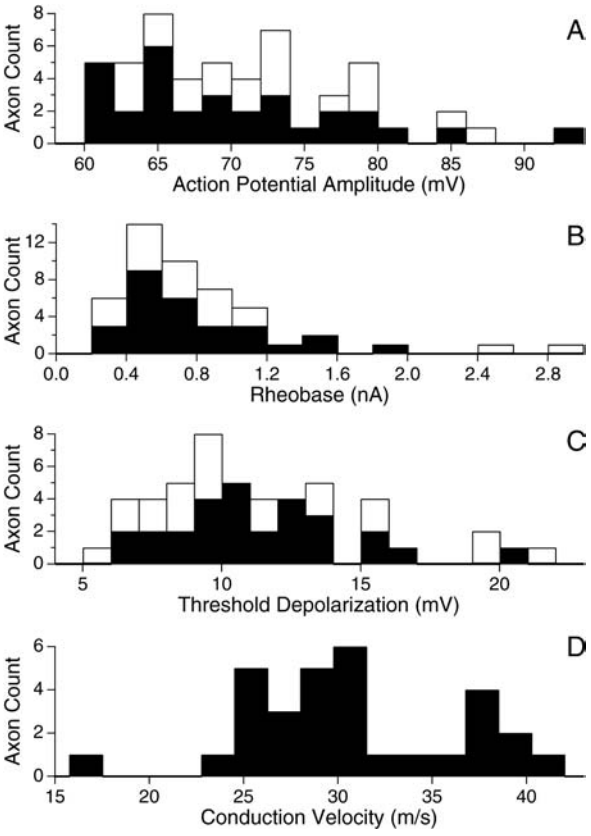

Fig. 2A–D Histograms show the distributions of **A** nerve-evoked action potential amplitude, **B** rheobase, **C** threshold depolarization, and **D** conduction velocity. The *white bars* show the distributions of the entire data pool. The *black bars* show the distributions for axons in which conduction velocity was measured

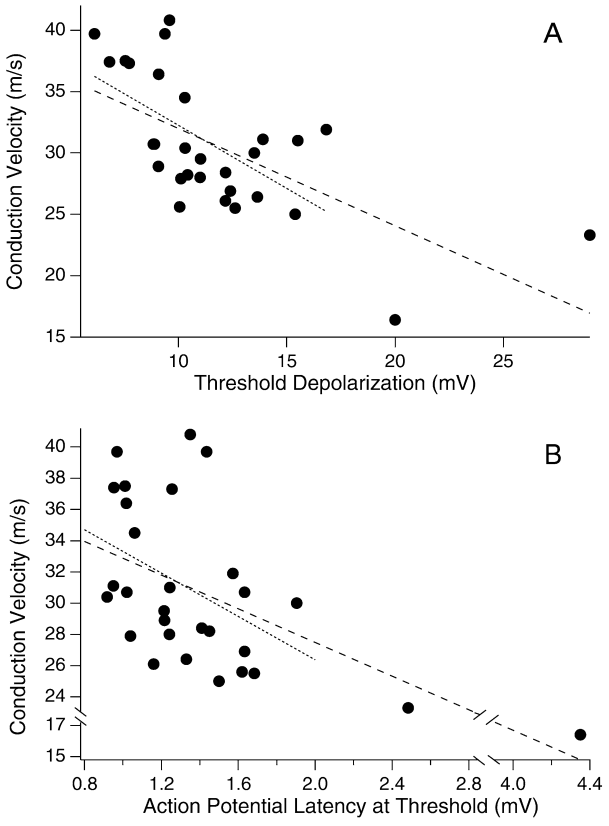

Fig. 3A, B Relationships between conduction velocity and **A** threshold depolarization and **B** action potential latency (i.e., time difference between current pulse onset and action potential onset). The *dashed lines* show the relationship predicted by linear regression of conduction velocity on threshold depolarization or action potential latency for the entire data pool. The *dotted lines* show the same relationships excluding the data from the two axons with the lowest conduction velocities. See Results and Table 2 for regression values

The relationship between conduction velocity and threshold depolarization could potentially reflect their covariation with other factors. For example, motoneuron somatic firing threshold properties vary with resting membrane potential (Gustafsson and Pinter 1984). Similarly, in the present data, action potential amplitude varied directly with threshold depolarization and inversely with conduction velocity (Table 2). Thus, intrinsic and/or impalement-related differences in the resting recording conditions contributed to the wide range of threshold depolarization and conduction velocity values measured here. However, the codependence on action potential amplitude of threshold depolarization and conduction velocity was not the basis of their relationship. Multiple regression analysis revealed that the relationship between conduction velocity and threshold depolarization was independent of action potential amplitude (for overall multiple regression: r^2=0.41, P=0.001; for component unique to regression of conduction velocity on thresh-

Table 1 Summary of axonal properties

Property	All Axons		Axons with conduction velocity measurements	
	Mean ± SD	*n*	Mean ± SD	*n*
Action potential amplitude (mV)	70.7±7.5	52	69.9±7.8	31
Action potential max rate of rise (mV/ms)	551±98	52	552±100	31
Action potential latency (ms)	1.44±0.56	50	1.44±0.66	29
Membrane time constant (ms)	0.38±0.16	41	0.43±0.20	23
Resting potential (mV)	–64±4	31	–64±4	18
Rheobase (nA)	0.80±0.52	48	0.77±0.40	28
Threshold depolarization (mV)	11.6±4.5	50	11.8±4.5	29
Conduction velocity (m/s)			30.5±5.6	31

Table 2 Simple linear regressions between axonal properties

Dependent variable	Independent variable	r^2	Slope	P
Conduction velocity	Threshold depolarization	0.41	−0.79 m/s/mV	0.0002
	Action potential amplitude	0.22	−0.34 mV/mV	0.006
	Rheobase	0.02	−2.0 m/s/nA	0.45
	Action potential latency	0.40	−5.4 m/s/ms	0.0002
	τ_{mem}	0.00	1.4 m/s/mV	0.82
Threshold depolarization	Action potential amplitude	0.32	0.34 mV/mV	<0.0001
	τ_{mem}	0.06	6.6 m/s/mV	0.13

old depolarization: r^2=0.20; slope, −0.76 m·s^{-1}·mV^{-1}; P=0.007).

In some axons, action potential amplitude varied significantly with current pulse amplitude (P<0.05 for linear regressions of action potential amplitude on injected current, normalized to each axon's rheobase, in 19 of 48 axons). Thus, any differences in the mean current (relative to the rheobase) used to evoke action potentials could potentially influence the action potential amplitude and, consequently, the value calculated for threshold depolarization. Based on the regression slopes and intercepts for the relationships between action potential amplitude and injected current (normalized to each axon's rheobase), we calculated the expected value for the action potential amplitude elicited by a current equal to rheobase, and then used this value to determine the rheobase-adjusted threshold depolarization. The relationship between conduction velocity and the rheobase-adjusted threshold depolarization was still evident (r^2=0.39; slope, −0.69 m·s^{-1}·mV^{-1}; P=0.0004 by linear regression), indicating that differences in the mean level of injected current did not account for the relationship between conduction velocity and threshold depolarization.

Age-dependent differences among animals could potentially contribute to the relationship between conduction velocity and threshold depolarization. For example, axonal conduction velocity increases with body weight in rats (Stanley 1981; Chen et al. 1992). Thus, the data could be affected if some animals contributed more axons to the data pool than did others. However, use of mean values of conduction velocity and threshold depolarization for each animal did not change the relationship between conduction velocity and threshold depolarization (for linear regression: r^2=0.51; slope, −1.05 m·s^{-1}·mV^{-1}; P=0.006). Furthermore, inclusion of body weight as a factor in a multiple regression analysis did not affect the relationship between conduction velocity and threshold depolarization (for overall multiple regression: r^2=0.42, P=0.0008; for regression of conduction velocity on threshold depolarization independent of body weight: r^2=0.39; slope, −0.77 m·s^{-1}·mV^{-1}; P=0.0003).

Previous studies have demonstrated relationships between conduction velocity or time and axonal current threshold (Raymond and Lettvin 1978; Raymond 1979, 1992). An alternative explanation for the relationship between conduction velocity and firing threshold could be that apparent differences in firing threshold simply reflect variation in axonal current threshold. However, conduction velocity displayed no linear dependence on rheobase (Table 2). In addition, inclusion of rheobase in a multiple regression analysis did not preclude the inverse relationship between conduction velocity and threshold depolarization (for overall multiple regression: r^2=0.45, P=0.0005; for regression of conduction velocity on threshold depolarization independent of rheobase: r^2=0.43; slope, −0.86 m·s^{-1}·mV^{-1}; P=0.0002). Thus, the relationship between conduction velocity and firing threshold is independent of changes in axonal current threshold.

Relationship between action potential latency and conduction velocity

The data described above are consistent with the hypothesis that the depolarization required to achieve firing threshold is a determinant of conduction velocity in myelinated axons. If this is true, then axons with larger threshold depolarizations would be expected to have lower conduction velocities, because they need more time to reach firing threshold at each node. Figure 3B shows that the action potential latency determined during just-threshold current pulse injection (see Materials and methods, and Fig. 1C) varies inversely with conduction velocity (Table 2). Although the two axons with the lowest conduction velocities and longest latencies are strong contributors to this relationship, the relationship persists even when these data are excluded from the analysis (for linear regression without the axon with the lowest conduction velocity, r^2=0.23; slope, −6.9 m·s^{-1}·ms^{-1}; P=0.01; without the two axons with the lowest conduction velocities, r^2=0.15; slope, −6.9 m·s^{-1}·ms^{-1}; P=0.04, illustrated by the dotted line in Fig. 3B). Excluding the two largest latency values, the remaining 1-ms range of latencies accounts for a 7-m/s range of conduction velocities, or 29% of the 24-m/s range recorded in this study. The association of lower conduction velocity with longer delay to firing threshold is consistent with the dependence of conduction velocity on the time course of action potential initiation in the axon.

The mean action potential latency ± SD during injection of just-threshold current pulses was 4.3±2.0 times τ_{mem}. Although, on average, the electrotonic component of the voltage response had reached more than 98% of its asymptotic value, there was considerable variability among axons in normalized action potential latencies (range 1.3–9.5 times τ_{mem}). Subthreshold active

conductances are likely to underlie this high level of variability in action potential initiation, given that there is no background activity in the axon that could be responsible for it.

Relationships between conduction velocity or threshold depolarization and other axonal properties

The rising phase of the action potential is largely determined by sodium channel density and by the voltage- and time-dependence of sodium channel activation and inactivation (Ritchie 1995; Vogel and Schwarz 1995). Thus, we performed multiple regression of conduction velocity or threshold depolarization on the maximum rate of rise and amplitude of the nerve stimulus-evoked action potential (the latter factor was included to account for variance that was due simply to the intrinsic dependence of rate of rise on action potential amplitude). Conduction velocity varied directly (r^2=0.14; slope, 0.028 m/V; P=0.02) and threshold depolarization inversely (r^2=0.08; slope, –0.017 ms; P=0.02) with the maximum rate of rise of the action potential. These modest but significant relationships are consistent with the hypothesis that sodium channel density and/or kinetics are determinants of conduction velocity and firing threshold. In addition, the fact that the data show opposite relationships is consistent with systematic variation in sodium channel properties underlying the inverse relationship between conduction velocity and firing threshold.

Neither conduction velocity nor threshold depolarization varied significantly with τ_{mem} (Table 2). This is consistent with variations in membrane resistivity and/or capacitance not being major determinants of the range of axonal conduction velocities or firing thresholds in axons (Moore et al. 1978).

Discussion

The data presented here demonstrate a relationship between axonal conduction velocity and depolarization threshold. This relationship is not merely the consequence of weight- or sampling-dependent differences among animals. It persists even after the contribution of these factors has been taken into account. It is also independent of interaxon differences in impalement quality (to the extent that this is reflected in action potential amplitude). Furthermore, the tendency of conduction velocity to be slower in axons with larger action potentials argues against preferential impalement-related damage to smaller, slower conducting axons.

The relationship between conduction velocity and threshold depolarization is unlikely to reflect an intrinsic difference between α- and γ-motoneurons. One axon had a conduction velocity of 16 m/s, which corresponds to 23 m/s after accounting for the effects of temperature (assuming a Q_{10} for conduction velocity of 1.6; Paintal 1978). This axon was probably from a γ-motoneuron, most of which conduct more slowly than 32 m/s at 37°C (Andrew and Part 1972; Andrew et al. 1978). Since a small fraction (less than 8%) of soleus γ-motoneuron axons conduct faster than 32 m/s (Andrew et al. 1978), other slowly conducting axons in our sample could also be from γ-motoneurons. However, the two most slowly conducting axons did not account for the inverse relation between conduction velocity and threshold.

The conduction velocity of a myelinated fiber depends on the complex interactions among the structural features of the axon and its myelin sheath, and on the nature and distribution of the conductance mechanisms of the axon (Waxman 1980). The time course of axonal conduction from node to node has two components. The first is the time required after action potential initiation at a given node for longitudinal current flow to arrive at the next node. This depends on internodal geometry (e.g., axon diameter and myelin thickness) and electrophysiological properties (e.g., myelin and membrane resistivity and capacitance). The second component is the time from arrival of this current at the second node until the nodal membrane is sufficiently depolarized to elicit an action potential. This latency until action potential initiation depends on nodal structure and passive membrane properties (e.g., nodal area, resistivity, and capacitance), and nodal voltage-dependent properties (e.g., channel density and kinetics). Thus, a dependence of conduction velocity on firing threshold is implicit in the present understanding of axonal excitation (Jack et al. 1975; Ritchie 1995) and forms the basis of a relationship between an axon's speed of conduction and the depolarization necessary for it to reach its firing threshold.

Previous studies on the activity- and drug-dependence of axonal excitability support a relationship between current threshold and conduction velocity in myelinated fibers (Raymond and Lettvin 1978; Raymond 1979, 1992). On the other hand, voltage threshold has been considered to be similar among myelinated axons of a given species (Brinley 1980). Although there have been no prior reports of relationships between axonal conduction and voltage threshold, two studies give insight into this issue. First, in a study of axonal properties of a branched lobster axon (Grossman et al. 1979), the voltage thresholds of the smaller daughter branches were more depolarized than those of their larger parent branches. Although these findings were reported as population data instead of as within-preparation paired data, they provide indirect support for an inverse relationship between axonal firing threshold and conduction velocity (to the extent that the latter property is reflected by axon diameter). Second, in a study of frog myelinated fibers (Vallbo 1964), an inverse relationship was detected between voltage threshold and the voltage at which steady-state sodium channel inactivation was half-maximal (i.e., the more negative the membrane potential at which inactivation occurs, the higher the voltage threshold). To the extent that accommodation contributes to axonal conduction, this relationship is consistent with the dependence of conduction velocity on firing threshold.

The present data extends these findings by demonstrating that conduction velocity varies inversely with threshold depolarization during low-frequency activation and under drug-free conditions independently of variations in current threshold. These findings suggest that current and voltage thresholds contribute to axonal conduction in different ways. This is consistent with theoretical arguments that current and voltage thresholds can vary independently (Noble and Stein 1966; Jack et al. 1975).

The contribution of firing threshold to the latency to action potential discharge during just-threshold current injection is likely to differ from that during action potential propagation. During intra-axonal current injection, the full range of motoneuron conduction velocities (excluding the one presumptive γ-motoneuron) is associated with a 1.6-ms range of latencies. The use of just-threshold currents, while necessary for the determination of threshold properties, magnifies differences in latency to action potential onset between fast and slow axons. As membrane potential approaches the maximum of its asymptotic time course, small differences in potential are associated with large differences in action potential onset. In addition, the gradually decreasing rate of change of voltage in response to the current pulse is likely to elicit a corresponding increase in firing threshold due to accommodation (Vallbo 1964). The associated preferential increase in firing threshold with lower voltage slope would further disperse the already wide range of action potential latencies.

During action potential propagation, axial current flow is many times larger than rheobase, providing a large safety factor for saltatory conduction. Larger currents would increase the rate of depolarization and shorten the latency to firing threshold. In a simple model, a just-threshold current step produces an exponential rise in voltage to threshold that occurs at a latency 4.3 times the membrane time constant (as described in the Results above). Assuming a 7-fold safety factor (Tasaki 1959), the fastest and slowest axons would reach threshold in no more than 228 μs, one-seventh the time required for just-threshold activation. This time span would be even further abbreviated by the reduction in accommodation resulting from the more rapid current influx than during just-threshold current injection (Tasaki 1959; Vallbo 1964). Determination of how much the decrease in accommodation would reduce the conduction time difference between the fastest and slowest fibers would require application of numerical models beyond the scope of this paper. Nevertheless, given that variation in action potential latency accounts for 29% of the full range of conduction velocities (see Results), variation in threshold would only be expected to account for about 5 μs of the expected 18-μs difference in internodal conduction times between our fastest and slowest α-motoneurons (assuming a mean internodal distance of 1 mm). Thus, even very small threshold-related differences in action potential latency could contribute to the intrinsic variation in conduction velocity among fibers.

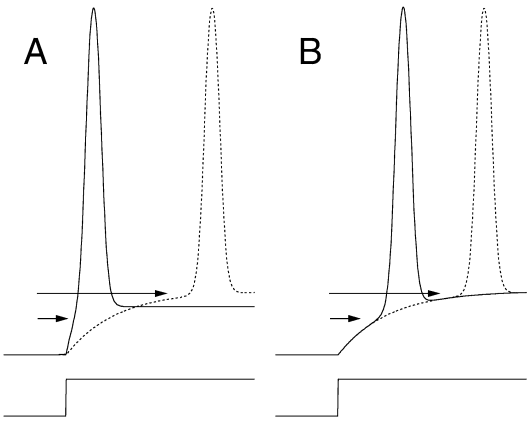

Fig. 4A, B Schematic illustrations of the effect of intrinsic differences in nodal axonal properties on membrane potential trajectory (*upper traces*) during current pulse injection (*lower traces*). *Horizontal arrows* indicate firing threshold in the membrane potential traces. **A** An axon with a long membrane time constant (*dashed trace*) attains firing threshold more slowly than does an axon with a short time constant (*solid trace*). Firing threshold is elevated by accommodation due to the lower rate of change of membrane potential, which contributes additionally to the difference in action potential latency. **B** An axon with a higher sodium channel density and/or more hyperpolarized activation voltage (*solid trace*) has a similar initial time course, but it then reaches a more hyperpolarized firing threshold at a shorter latency than does an axon with a lower sodium channel density and/or more depolarized activation voltage (*dashed line*)

The relationship between conduction velocity and threshold depolarization, and between conduction velocity and action potential latency, could arise in one of two ways (illustrated in Fig. 4). First, axons with intrinsically longer time constants exhibit a more gradual depolarization during just-threshold level current injection. Figure 4A illustrates how a lower rate of increase in membrane potential could itself delay the onset of the action potential. In addition, it also shows that an accommodative increase in firing threshold could further delay action potential initiation. Our results and other data do not support this hypothetical mechanism. In the present study, conduction velocity was independent of both τ_{mem} and the slope of the relationship between action potential onset voltage and latency (which reflects the degree of accomodation-like threshold change). Furthermore, our previous modeling study showed that unrealistically large changes in factors contributing to τ_{mem} would be required to produce even modest changes in firing threshold (Halter et al. 1995).

A second way in which the relationship between conduction velocity and threshold depolarization, and between conduction velocity and action potential latency, could arise is by variation in nodal sodium channel properties. Figure 4B illustrates how axons with lower sodium channel density and/or increased activation threshold could delay action potential onset and elevate firing threshold. Large reductions in the magnitude of the sodium current by application of local anesthetics or by decreasing extracellular Na^+ concentration do decrease

conduction velocity/increase conduction time and increase current threshold (Fink and Cairns 1985; Raymond 1992). However, theoretical analyses favor changes in sodium channel kinetics over changes in channel density. Our previous modeling study predicted that very large changes in channel density would be required to produce even modest changes in conduction velocity, while small changes in sodium channel kinetics (particularly in the activation voltage) would have more substantial effects on conduction velocity (Halter et al. 1995). Other modeling studies support our observation that the conduction velocity is relatively insensitive to modest changes in sodium channel density (reviewed in Jack et al. 1975). Thus, we hypothesize that intrinsic variation in sodium channel kinetics provides the most plausible explanation for the observed relationship between conduction velocity and threshold depolarization.

This study was undertaken to describe the relationships among the time course of axonal depolarization, firing threshold, and conduction velocity in normal animals for future comparisons with data from conditioned animals. These control data show that there is wide variation in axonal firing threshold. This variation is substantially larger than the 2- to 3-mV positive shift in somatic firing threshold seen in down-conditioned monkey motoneurons (Carp and Wolpaw 1994). If there were no dependence of conduction velocity on firing threshold over this wide range of values, then it would seem unlikely that such dependence could exist for the smaller conditioning-induced shift in firing threshold. That there is substantially more variability in firing threshold among control axons than between motoneurons of control and conditioned animals is consistent with our hypothesis that conditioning-induced change in sodium channel kinetics underlies both the depolarized somatic firing threshold and slower axonal conduction velocity. Assessment of this hypothesis awaits comparison of these data with those from conditioned animals.

Sodium channel properties are modulated by protein kinases A and C (Schreibmayer 1999; also see discussion in Halter et al. 1995). The fact that the phosphorylation state of the sodium channel is under neural control (Cantrell et al. 1996, 1999; Carr et al. 2002) suggests a possible mechanism by which a systematic variation in firing threshold could be established. This mechanism is also consistent with our hypothesis that activity-dependent modulation of Na^+ channels throughout the motoneuron contributes to the increase in somatic firing threshold and to the decrease in axonal conduction velocity produced by H-reflex down-conditioning. It also provides further support for the hypothesis that plasticity in neuronal properties (in addition to plasticity in synaptic properties) is a mechanism of learning (Spitzer 1999).

Acknowledgements This work was supported in part by NIH grants NS22189 and HD36020. We thank Mr. David Smith for his expert technical assistance.

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