Intrinsic Properties of Mouse Lumbar Motoneurons Revealed by Intracellular Recording In Vivo

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We have developed an in vivo model for intracellular recording in the adult anesthetized mouse using sharp microelectrode electrodes as a basis for investigations of motoneuron properties in transgenic mouse strains. We demonstrate that it is possible to record postsynaptic potentials underlying identified circuits in the spinal cord. Forty-one motoneurons with antidromic spike potentials (>50 mV) from the sciatic nerve were investigated. We recorded the intrinsic properties of the neurons, including input resistance (mean: 2.4 ± 1.2 MΩ), rheobase (mean: 7.1 ± 5.9 nA), and the duration of the afterhyperpolarization (AHP; mean: 55.3 ± 14 ms). We also measured the minimum firing frequencies (Fmin, mean 23.5 ± 5.7 SD Hz), the maximum firing frequencies (Fmax; >300 Hz) and the slope of the current–frequency relationship (f–I slope) with increasing amounts of current injected (mean: 13 ± 5.7 Hz/nA). Signs of activation of persistent inward currents (PICs) were seen, such as accelerations of firing frequency or jumps in the membrane potential with increasing amounts of injected current. It is likely that the particular anesthetic regime with a mixture of Hypnorm and midazolam is essential for the possibility to evoke PICs. The data demonstrate that mouse spinal motoneurons share many of the same properties that have been demonstrated previously for cat, rat, and human motoneurons. The shorter AHP duration, steeper f–I slopes, and higher Fmin and Fmax than those in rats, cats, and humans are likely to be tailored to the characteristics of the mouse muscle contraction properties.

INTRODUCTION

Most of what is known today concerning the intrinsic properties of adult mammalian spinal motoneurons has been obtained from electrophysiological studies in which the adult cat has traditionally been the model of choice. This has been due to the mechanical stability and longevity normally required for such investigations (Eccles 1957; Kernell 2006). Subsequently, these techniques were extended to investigations using rat in vivo preparations (Bakels and Kernell 1993a,b; Button et al. 2006, 2007, 2008; Gardiner and Kernell 1990; Grant et al. 1963). Changes in the intrinsic properties of motoneurons are a feature of a number of disorders for which transgenic mouse models are now available (e.g., amyotrophic lateral sclerosis [ALS]; Borries et al. 2007; Kuo et al. 2004, 2005; Pieri et al. 2003; Zona et al. 2006). This, together with the development of transgenic mice with mutations affecting the normal development of spinal cord circuitry, now make it crucial to be able to perform such experiments in the adult mouse vivo.

Despite early pioneering work using intracellular recording of neurons in vivo in the adult mouse spinal cord (Biscoe et al. 1975, 1977; Huizar et al. 1975; Martin et al. 1978) and some recent initiatives (Alstermark and Ogawa 2004; Graham et al. 2004, 2007; Manuel et al. 2009), the electrophysiological techniques necessary for this kind of investigations have not yet been developed for routine use in the mouse. This is due to the technical demands associated with the small size and the much greater sensitivity to the stress of invasive surgery of the mouse. Consequently, a number of laboratories have been developing in vitro models. However, such preparations are limited with respect to the age of the mice and thus do not allow for studies of developmental, compensatory, and degenerative changes.

The primary aim in this study was therefore to develop an adult in vivo mouse model in which the intrinsic properties of neurons and neuronal circuitry can be investigated. We demonstrate that it is possible to record the intrinsic properties of motoneurons intracellularly in the anesthetized adult mouse, as has been performed in the cat, and that these properties are consistent with the smaller size of mouse motoneurons and appear to be tailored to meet the characteristics of mouse muscle contraction properties. These data from the normal adult mouse will form a control for future comparisons with relevant transgenic strains. We also demonstrate that by using an anesthetic regime of Hypnorm and midazolam it is possible to see evidence of the plateau potentials that are mediated by persistent inward currents and that these appear to be a common feature of mouse motoneurons. Furthermore, in this model we demonstrate that it is also feasible to record postsynaptic potentials underlying identified circuits in the spinal cord such as monosynaptic excitatory postsynaptic potentials (EPSPs) and disynaptic Ia reciprocal inhibitory postsynaptic potentials (IPSPs).

METHODS

The results presented in this study originate from 15 adult (≥20 wk, 25 g) mice of the C57BL/6J strain (10 female, 5 male). We selected this particular strain of mouse because a number of transgenic mice that we seek to investigate (future experiments) were created from this strain. The experimental procedure was approved by the Danish Animal Experiments Inspectorate. Anesthesia was briefly induced with isoflurane for a duration sufficient to allow for a stress-free injection of a longer-lasting anesthesia. A longer-lasting anesthesia was induced using a cocktail of 1 part fentanyl (Hypnorm 0.315 mg/ml), 1 part midazolam (5 mg/1 ml), and 2 parts sterile water (0.15 ml of cocktail/25 g) injected subcutaneously. Three intraperitoneal cannulas were inserted for the drug delivery and anesthesia was maintained by 0.02-ml doses of this cocktail at 20-min intervals.
through one of the cannula. A tracheal cannula was inserted and atropine (0.02 mg) was administered subcutaneously to reduce mucous secretions.

Usually only the sciatic nerve was dissected, except in four experiments where it was further dissected into its common peroneal and main tibial branches. Laminectomies were performed at vertebral levels T13 and L1 and mice were placed in a stereotactic frame with vertebral clamps on the T12 and L2 vertebrae. The mice were paralyzed using the neuromuscular blocking agent Pavulon (diluted 1:10 with saline then 0.1-ml dose initially followed by 0.05-ml doses every hour) and artificially ventilated at 70 breaths/min (and a tidal volume of \( \sim 0.2 \) ml). Expired carbon dioxide levels were measured using a Capstar CO₂ analyzer (ITTC Life Science). The temperature was monitored using a rectal probe and maintained at 37°C using a heating pad underneath and a heat lamp above the mouse, controlled by the output from the temperature probe. The electrocardiogram (ECG) was monitored using clips placed on the ear and rear foot and small doses of ephedrine (dosage to effect) were given in response to slight drops in heart frequency. Due to possible central effects of ephedrine this was used only when necessary and usually this was restricted to a single dose when the mouse was initially transferred into the frame following surgery. Records were kept regarding the drugs given to each animal. There appeared to be no difference between those mice receiving the ephedrine and those that did not in terms of mean frequency–current relationships. Thus we believe that this limited dosage did not significantly affect our results.

Once paralyzed, the same dosages of anesthesia as previously used throughout the surgical preparation were given at 20-min intervals (as during the preparation). Additionally, the heart rate was monitored and in the event of any increase supplemental dosages of anesthesia were administered.

The dissected nerves were placed on bipolar stimulating electrodes and a silver ball recording electrode was placed on the dorsal part of the spinal cord to record the incoming volleys from stimulation of the peripheral nerves. The dura was gently torn away from a small region on the dorsal surface of the spinal cord using fine watchmaker forceps. A sharp glass microelectrode filled with 2 M potassium acetate (with resistances of 10–24 MΩ) was used to record intracellularly from spinal motoneurons identified by antidromic action potentials elicited by stimulation of one of the peripheral nerves. Using an electronic microdrive the microelectrode was slowly lowered into the spinal cord through the dorsal columns toward the ventral horn, tracking the extracellular field potentials of the motoneurons evoked by stimulation of the peripheral nerves. Successful impalement of a motoneuron was confirmed by the presence of an all-or-nothing antidromic action potential from stimulation of the sciatic nerve (or its common peroneal or tibial branches). Changes in membrane potential were recorded and amplified (×10) using the Axoclamp 2A amplifier (Axon Instruments, Union City, CA) using either bridge (for afterhyperpolarization [AHP] measurements) or discontinuous current-clamp (DCC) mode (×3 kHz). The output was further amplified (×10 for a low-gain view and ×100 for a high-gain view) and then filtered (high pass 5 kHz for low-gain view and low pass 1 Hz for high-gain view) using custom-made amplifiers and was digitized using the 1401 analog-to-digital converter (Cambridge Electronic Design [CED], Cambridge, UK) and recorded using Spike2 software (CED).

Only motoneurons with antidromic spike potentials of >50 mV, membrane potentials of \( \sim -55 \) mV or more hyperpolarized (confirmed on exit from the cell), and repetitive firing in response to long-lasting depolarizing current pulses were accepted for analysis (repetitive firing was usually not possible in cells with more depolarized resting potentials). Deterioration in the quality of the impalement (usually due to cardiovascular and respiratory associated movements) was evident as a clear reduction in the membrane potential below this level and failure to evoke repetitive firing. This was normally the point for termination of data collection and this is the reason that not all parameters were measured in every cell. Movements of the spinal cord were the biggest problem when trying to obtain stable intracellular recordings in the mouse. These were greatly reduced by raising the vertebral clamps until the spinal cord was kept at the same level as the head (or even slightly higher) and then gently stretching the cord by moving the rostral and caudal vertebral clamps apart from each other (without compromising the blood supply to the cord). By making only a hemilaminectomy over the side of the spinal cord from which recordings were made the contralateral part of the lamina acted as a type of pressure plate, further reducing movements.

### Measurement of the postspike afterhyperpolarization

The action potential in response to a short depolarizing current pulse (\( \sim 1 \) ms duration) was recorded and averaged using Spike2 (\( \geq 10 \) sweeps). The exact return of the AHP to baseline was not always easy to detect and thus we also measured the time for the membrane potential to return to one third of the peak amplitude of the afterhyperpolarization (AHP/3).

### Measurement of input resistance

The input resistance \( (R_m) \) values (using a 1- to 4-nA hyperpolarizing pulse of \( \sim 20 \)-ms duration) were recorded in DCC mode and averaged using Spike2 software.

### Frequency–current relation

This relationship was determined by creating frequency–current (=f) curves in one of two ways.

1) Square depolarizing pulses of 400 ms were given through the microelectrode evoking repetitive firing. This current was gradually increased for each pulse and the resulting increases in firing frequency were calculated by measuring the interspike intervals (ISIs) between the first and the second spikes, between the second and the third spikes, and between three consecutive spikes (and an average taken for the three) occurring at 100 and 300 ms after the onset of the pulse. These intervals were expressed as instantaneous firing frequencies \( (Hz) \) and the \( f/I \) relationship was given as the increase in firing frequency per nA current \( (Hz/nA) \).

2) Slow triangular ramps of current were given through the microelectrode and the response was measured. Changes in firing frequency (measured in spikes/s) were plotted as frequency–current curves and the \( f/I \) relationship could be determined.

### Muscle twitch contraction times

In two mice we recorded the muscle twitch contraction time (CT) of the triceps surae muscles. In these mice the sciatic nerve was dissected, ligated, and cut proximally. Distally the peroneal branch and the branches of the tibial nerve innervating muscles other than the triceps surae were sectioned; thus stimulation of the sciatic nerve resulted in a selective contraction of the combined triceps surae muscles. The knee region (femur and tibia) and the ankle were fixed by pins and the Achilles tendon was attached to a strain gauge.

### Immunohistochemistry for CaV1.3 channels

Following the electrophysiological investigations, some of the mice were perfused and subsequently the spinal cord was processed for immunohistochemistry to verify the presence of CaV1.3 immunoreactivity in the spinal cord and large ventral horn neurons. The protocol was the same as recently described (Sukiasyan et al. 2009). Here only a short description will be given, focusing on the antibody against CaV1.3 and relevant controls. The rabbit anti-CaV1.3 antibody, purchased from Millipore-Chemicon, is an affinity-purified, polyclonal antibody raised against amino acid residues 859–875 of the CaV1.3 subunit of the rat brain voltage-gated calcium channel (accession
number: P27732). It recognizes all forms of Ca$_{v}1.3$ subunits from the voltage-gated calcium channel and does not cross-react with any other calcium channel antigens tested so far (manufacturer’s data sheet). The specificity of the antibody was previously verified by Western blot on rat spinal tissue (Sukiasyan et al. 2009). Control staining was performed with Ca$_{v}1.3$ antibodies preadsorbed with a sufficient amount of the corresponding epitope peptides (supplied by the manufacturer) or with the primary antibody being omitted; no positive reaction was then detected.

**RESULTS**

The results of this study are based on intracellular recordings from 41 motoneurons that were antidromically identified from the sciatic nerve (or one of its branches—the tibial nerve or the peroneal nerve; Fig. 1B). For each of these motoneurons the following parameters were recorded: 1) the postspike afterhyperpolarization (AHP) following an action potential evoked by a short-lasting (~1 ms) current pulse, 2) the input resistance, 3) the rheobase, and 4) the repetitive firing evoked by an intracellular rectangular current pulse of ≥300-ms duration and/or a triangular current pulse. As mentioned earlier in **METHODS** it was not always possible to obtain all of these parameters for every cell. However, the motoneurons included in the data set all demonstrated repetitive firing following intracellular current injections. The resting membrane potentials for the cells included in the study ranged from −55 to −83 mV (mean: −66 mV, SD 7.7). Repetitive firing was usually not possible in cells with more depolarized membrane potentials. The resting membrane potential (at least within this range) was not correlated with either the AHP duration, AHP amplitude, the $f$–$I$ relationship, rheobase, or the input resistance.

Although a full systematic description of synaptic activity is beyond the scope of this study it should be noted that, in those experiments in which the common peroneal and tibial nerve were dissected and stimulated separately, the monosynaptic Ia EPSP could be seen following stimulation of the homonymous nerve at stimulation intensities lower than those required to achieve antidromic activation (Fig. 1A). Stimulation of the antagonist nerve produced disynaptic IPSPs (Fig. 1B; here polysynaptic IPSPs are seen in addition). The disynaptic Ia inhibition often required two shocks to appear (temporal facilitation) and was very small at membrane potentials more hyperpolarized than −50 mV; however, they were greatly enhanced if the motoneuron was depolarized by injecting current. Polysynaptic EPSPs and/or IPSPs were almost always also observed following stimulation of either homonymous or heteronymous nerves. Under this anesthesia spontaneous synaptic activity could also be recorded (Fig. 1C).

The AHP following a pulse-evoked spike is illustrated in Fig. 2, A and B. Even under the most stable recording conditions it can be difficult to judge with accuracy when the AHP returns to baseline. Furthermore, the precise return to baseline can sometimes be obscured by the presence of a late afterdepolarization as previously described in cats (Kernell 1965c). Since most of the recordings that we obtained were less stable than normally obtained in the cat and rat (Hultborn, personal observation), we decided to measure the duration from the start of the spike to the time at which the AHP amplitude had returned by two thirds toward the baseline (AHP$_{2/3}$), as indicated by the arrows in Fig. 2B, and where possible the estimated return of the AHP to baseline (AHP$_{b}$). Both measurements were performed from averaged records ($n$ ≥ 10 trials). The distribution of AHP$_{2/3}$ measurements for 38 motoneurons is shown in the frequency histogram in Fig. 2C. The relationship between the two different estimates of AHP duration from 20 motoneurons for which the AHP$_{b}$ could be measured (58.2 ± 17.2 [SD] ms) suggests that AHP$_{b}$ = 1.8(AHP$_{2/3}$) + 18 ms. This allowed us to estimate the return to baseline for all 38 motoneurons, giving an average of 55.3 ± 14 (SD) ms (see Table 1).

The input resistance was calculated from the voltage drop following a current pulse of 1–4 nA and ≥20 ms as illustrated in Fig. 3A. The mean was 2.4 ± 1.17 (SD) MΩ, with the distribution illustrated in Fig. 3B. Although not systematically investigated, we often noticed a sag following somewhat longer (and often larger) current pulses, as illustrated in Fig. 3C (cf. Ito and Oshima 1965).

With depolarizing current injections (rectangular or triangular pulses) it was possible to evoke repetitive firing in all of the 41 motoneurons reported here. In some (other) motoneurons with acceptable amplitude (≥50 mV) of single spikes evoked...
by antidromic activation from the sciatic nerve or by a short (1-ms) intracellular current pulse it was not possible to evoke repetitive firing by longer-lasting current pulses. In those cases there was often a short burst of action potentials of decreasing amplitude, suggesting a rapid inactivation of Na⁺ conductance; those motoneurons were not included in the present study. Several motoneurons initially responded with repetitive firing to long-lasting current pulses and later developed an inactivation of repetitive firing, while still able to support single spikes. We thus believe that the lack of repetitive firing to long-lasting current pulses reflects a pathological deterioration of the motoneuron. The frequency–current (f–I) relationship was determined from either rectangular current pulses of increasing amplitude (Fig. 4) or triangular current pulses (Fig. 5). With rectangular pulses it was possible to measure the f–I relationship for both the initial ISIs (first and second ISIs in Fig. 4, B and C) and the more adapted frequency at 100 or 300 ms from the start of the current pulse (Fig. 4, A and C). Note the much steeper slope for the first interval than that for the second and later intervals; it thus seems that much of the early adaptation occurs already following the first ISI. The f–I slope for the “steady-state” firing was 5.0 Hz/nA for the illustrated motoneuron.

With triangular current pulses it was possible to determine the f–I relationship from a single trial lasting only a few seconds (Fig. 5A). This current pulse injection could be repeated a few times to check the reliability and determine a mean value for the recorded motoneuron. It was important to determine how the f–I relationship obtained from a triangular pulse compares with the “adapted” (300 ms) f–I relationship obtained from a large number of rectangular current pulses of different amplitudes. The f–I slopes obtained by these different methods are illustrated for an individual motoneuron in Fig. 5B. The f–I slope with rectangular pulses is plotted (open circles) in relation to the injected current, a very similar slope is seen. This was true for all recorded motoneurons (a criterion for the inclusion), whereas the other parameters were not obtained for all motoneurons. The AHPd could be measured in 20 motoneurons, whereas the AHP2/3 was measured in an additional 18 motoneurons. From the relation between the AHPd and AHP2/3 in those 20 motoneurons [AHPd = 1.8(AHP2/3) + 18 ms; see text], it was possible to estimate the AHPd for the remaining 18 motoneurons (“AHPd calculated”).

### Table 1. Summary of the values obtained in the present study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>R∞, MΩ</td>
<td>2.4 ± 1.2</td>
<td>33</td>
</tr>
<tr>
<td>Rheobase, nA</td>
<td>7.1 ± 5.9</td>
<td>37</td>
</tr>
<tr>
<td>AHP2/3, ms</td>
<td>21.7 ± 6.0</td>
<td>38</td>
</tr>
<tr>
<td>AHPd measured, ms</td>
<td>58.2 ± 17.2</td>
<td>20</td>
</tr>
<tr>
<td>AHPd calculated, ms</td>
<td>55.3 ± 14.0</td>
<td>38</td>
</tr>
<tr>
<td>Fmin, Hz</td>
<td>23.5 ± 7.4</td>
<td>23</td>
</tr>
<tr>
<td>f–I slope, Hz/nA</td>
<td>13.0 ± 5.7</td>
<td>41</td>
</tr>
</tbody>
</table>

Values are means ± SD. Note that the f–I slope was obtained for all recorded motoneurons (a criterion for the inclusion), whereas the other parameters were not obtained for all motoneurons. The AHPd could be measured in 20 motoneurons, whereas the AHP2/3 was measured in an additional 18 motoneurons. From the relation between the AHPd and AHP2/3, it was possible to estimate the AHPd for the remaining 18 motoneurons (“AHPd calculated”).
the case for all five motoneurons for which both methods were used. We have therefore preferentially used the triangular pulses for estimating the \( f - I \) slopes. Since the \( f - I \) slopes were very similar, independent of which method was used, we have pooled the results. Figure 5, C and D illustrates different types of responses to triangular current pulses. Here we illustrate the most typical responses during both the ascending and the descending phases of the triangular pulses. In the motoneuron of Fig. 5C there is a linear increase in firing frequency during the ascending phase (17.7 Hz/nA; black squares). Here the slope is very similar for the descending phase (16.5 Hz/nA; gray triangles). Figure 5D illustrates the more common response with a significantly lower firing frequency for the same current intensities during the descending phase, thus reflecting an ongoing adaptation.

In some cells, in which higher currents were injected, a steeper slope (an acceleration) developed at the end of the ascending phase (Fig. 6A). This “secondary range” is assumed to reflect the recruitment of persistent inward currents (PICs; Heckman et al. 2005; Hultborn et al. 2004; Powers and Binder 2001). In addition to the clear secondary range in the \( f - I \) slopes we also observed a number of other responses to the current injections consistent with the activation of PICs. It was occasionally seen that the derecruitment occurred with lower current intensities than those for the initial recruitment (\( \Delta I = 1.5 \) nA in Fig. 6B). The difference in current at recruitment and derecruitment may reflect the size of the PIC (Bennett et al. 2001; Button et al. 2006; Hounsgaard et al. 1988a).

In some cells the firing suddenly ceased during the ascending part of the triangular current injection. This usually oc-
occurred at high firing frequencies and appeared to be accompanied by an apparent jump in membrane potential. The response pattern illustrated in Fig. 6C is likely to reflect the same recruitment of PICs. In this case there was a repetitive firing during the beginning of the ascending phase, but suddenly the firing was interrupted (spike inactivation) and the membrane potential “jumped” around 14 mV. It thus seems likely that activation of the PICs contributed to a sudden spike inactivation. As far as we are aware this pattern of response has not been described for cat or rat motoneurons, but is likely to reflect a transition from the state with full spikes without inactivation to the fully inactivated state. As in the present example, the firing sometimes returned during the descending phase. While the cell is firing spikes it is hard to determine the exact contribution of PICs to this apparent jump in membrane potential versus the return to baseline after the AHP. Illustrated in Fig. 6D is a “jump” in the membrane potential in a motoneuron in which the repetitive firing had vanished following a long-lasting intracellular recording. During the ascending phase of a triangular current pulse (at 12.5 nA) there is a sudden “jump” in the membrane potential (by 10.5 mV, from a membrane potential of −48 mV). A somewhat similar jump (down) is then seen during the descending phase. When the membrane potentials are superimposed for the ascending and descending phases—for the same amount of injected current (Fig. 6E)—the “counterclockwise” hysteresis of the membrane potential can be appreciated (i.e., the jump down occurs with a smaller current than that in the jump up). A similar jump could also be seen following a rectangular current pulse (Fig. 6F; same neuron as in Fig. 6, D and E) after a latency of around 140 ms. These findings are also interpreted as a sign of an activation of PICs (plateau potential) by the current pulse and the latency in Fig. 6F is likely due to the slow activation of the PIC conductance (cf. Carlin et al. 2000).

Since the secondary range was usually seen only at high firing frequencies (>150 Hz) and the jumps in membrane potential (together with spike inactivation) occurred at similarly high firing frequencies it was hard to retrospectively determine proportions of cells with PICs because not all motoneurons had been pushed to these extremes. However, in 13 motoneurons that were challenged with triangular current injections beyond the point at which cells fired at 150 Hz, 10 of 13 cells showed secondary range firing. The transition from primary to secondary range almost always occurred between 140 and 185 Hz (mean 162 Hz, SD 11.4 Hz) and this was usually consistent across multiple trials, regardless of speed of current injection. If firing persisted beyond this point into the secondary range the firing frequencies reached between 223 and 433 Hz (mean 327 Hz, SD 59.9 Hz) before the spikes finally inactivated. All 3 cells that did not enter the secondary range of firing ceased firing, with apparent jumps in membrane potential. The firing frequencies at which the spikes inactivated (150, 160, and 165 Hz) are consistent with the ranges at which the transition from primary to secondary range of firing occurred in the other cells. In the cells showing a secondary range of firing occasionally on some trials the spiking would fail with this apparent jump. This happened in 9 cells and always occurred within the same range of firing frequencies where the transition from primary to secondary range occurred on previous trials in the same cell. Thus we believe that this sudden cessation of firing (spike inactivation) accompanied by the apparent jump in membrane potential indeed represents the onset of a plateau potential mediated by persistent

FIG. 5. Repetitive firing evoked by triangular current pulses. A, top trace: the intracellular recording of the repetitive firing. Bottom trace: the injected current. B: the relationship between the instantaneous firing frequency of a motoneuron and the injected current (f–I) obtained by rectangular current pulses (filled and open circles) and the ascending part of a triangular current pulse (triangles). C and D: f–I relationships in 2 different motoneurons obtained by triangular current pulses.
inward currents. Thus in conclusion, all of these 13 motoneurons showed signs of persistent inward currents.

Because plateau potentials in motoneurons have been partly attributed to the activation of CaV1.3 channels we have also confirmed the presence of CaV1.3 immunoreactivity in the spinal cord of some of the mice in this study (not illustrated). Immunohistochemistry using the antibody directed against the voltage-gated calcium channel CaV1.3 produced widespread labeling in the gray matter of the spinal cord, including the dorsal horn, the intermediate zone, and the ventral horn. In the ventral horn both somata of the large ventral horn neurons (presumably motoneurons) and their dendrites were clearly labeled. This labeling pattern is similar to that recently described for the rat spinal cord (Sukiasyan et al. 2009) and also confirms the motoneuron labeling seen in another mouse strain (Balb/C) with a different antibody (Jiang et al. 1999).

It has been suggested that the duration of the AHP determines the minimal firing frequency during repetitive firing (see further in the DISCUSSION). We have therefore determined the lowest firing frequencies from rectangular current pulses (with the lowest possible amplitude for repetitive firing). Figure 7 illustrates the distribution of the “minimum firing frequencies” as a function of the AHPd. The variation in ISIs at the lower frequencies was indeed large and it was often difficult to determine whether a long final interval represents a “true” interval during repetitive firing or, rather, an interval after the end of repetitive firing and an accidental discharge due to a random synaptic input or a mechanical instability. We there-

![Diagram of AHP and firing frequency](http://jn.physiology.org/)

**FIG. 6.** Evidence for plateau potentials. A: the f-I relationship of a motoneuron showing a “secondary range” of firing (steeper slope) as the injected current was increased beyond 9 nA. B, top trace (AC-filtered in this example): the membrane potential. Bottom trace: the injected current. Note the lower current at derecruitment than that for recruitment of firing. C: arranged as in B, but from another motoneuron (here with DC recording). The response to triangular current injection in a motoneuron in which the spike suddenly inactivates and the membrane potential shows a step depolarization (top trace). D: the response to triangular current injection in a motoneuron in which the spike had spontaneously inactivated following a prolonged recording. The bottom trace shows the current injection. E: same records as in D, but with the ascending and descending phases of the triangular current injection superimposed (for the top trace the gray line is the ascending phase and the black is the descending phase). The bottom trace shows the current injection. F: responses to a square-pulse current injection in the same motoneuron as in D and E. The bottom trace shows the current injection.
of the next few hours. The ability to record postsynaptic potentials from defined spinal circuits make this a good model to investigate the final compensation in the adult mice, with mutations affecting the normal development of these circuits or specific neurotransmitter receptors.

Our results suggest that PICs are a common feature of mouse spinal motoneurons and are evoked at firing frequencies >145 Hz, i.e., where the secondary range firing is initiated. This is much higher than that seen for cat and rat and assists the motoneurons to fire at frequencies ≤430 Hz. PICs serve as a controlled “amplifier” of classical synaptic input to motoneurons and appear to be the mechanism underlying the steeper f-I relationship in the secondary range (Heckman et al. 2005; Hultborn et al. 2004; Powers and Binder 2001). These PICs are to a large extent mediated by nifedipine-sensitive voltage-gated L-type calcium channels (Hounsgaard and Kiehn 1989). Because the PICs could be triggered at relatively hyperpolarized levels, close to firing threshold (Hounsgaard and Mintz 1988; Hounsgaard et al. 1988a), they were attributed to a particular subgroup of L-type calcium channels, CaV1.3 (Alaburda et al. 2002). Although it has been firmly established that noninactivating components of Na+ channels also contribute to the PICs (Harvey et al. 2006), it has not yet been possible to identify which subgroup of Na+ channels is responsible. The CaV1.3 channel has been immunohistochemically labeled on mouse spinal motoneurons (Balb/C strain; Carlin et al. 2000; Jiang et al. 1999), which was confirmed for the C57BC strain in this study.

Our current observations of PICs with Hypnorm/midazolam anesthesia demonstrate the feasibility for investigations of PICs in anesthetized mouse preparations. This is important because other anesthetics, in particular barbiturates, have been demonstrated to reduce/abolish the PIC in rats (Button et al. 2006), cats (Hultborn and Kiehn 1992), and in vitro in turtle (Guertin and Hounsgaard 1999; Hounsgaard et al. 1986). However, in the rat Button et al. (2006) described PICs under ketamine and xylazine anesthesia being as strong as those in unanesthetized decerebrate preparation. Under ketamine/xylazine anesthesia, however, rat motoneurons had smaller AHPs than those in decerebrate preparations and required more current to reach firing threshold, although the f-I slopes, the presence of PICs, and their amplitude were not affected by the ketamine/xylazine (Button et al. 2006). The exact effect that our protocol of Hypnorm and midazolam has on the intrinsic properties of neurons is unknown. However, it is unlikely that mice will survive the associated stress and blood loss following a decerebration, rendering the decerebrate unanesthetized preparation impractical for routine recording in vivo. Thus we believe this anesthetic regime represents a good alternative for comparisons between experiments using the same anesthetic in transgenic mice in which PICs may be affected, such as the SOD-1 mouse mutants of ALS.

Our study provides data for comparisons not only with future data to be obtained in transgenic mice but also provides data for a cross-species correlation between motoneuron properties and their muscle unit contraction properties. In the present material of motoneurons supplying the sciatic nerve we found that the input resistance was around 2.4 MΩ (Table 1), which is far higher than that in the cat and rat (see references listed with Table 2). This is as would be expected from the size of the mouse spinal motoneurons (Hardesty 1902; McHanwell

**DISCUSSION**

The present study demonstrates the feasibility of intracellular recording in the adult mouse in vivo preparation. Using the present experimental protocol we were able to obtain intracellular recordings for 1–6 h after the surgical procedure (lasting 2–3 h). Typically we could impale two to eight motoneurons per animal with stable recordings (with membrane potentials better than −50 mv) for 5–10 min. The mechanical stability is certainly less than that in the cat or rat and this makes it more difficult to obtain recordings without artifacts originating from the pulse pressure and/or artificial respiration. The most stable recordings occur within the first 2 h after the mouse was placed in the frame and artificially ventilated. Movement artifacts, usually cardiovascular, then gradually worsen over the course
AHP duration and minimum firing frequencies in individual motoneurons matched with their twitch contraction properties in the mouse and other species. However, there are significant quantitative differences that depend on the relation between the AHP duration, minimum firing frequency, and muscle contraction properties in the mouse. All in all, these basic features are qualitatively the same as those given in Table 1.

Table 2. Comparison of motoneuron properties and twitch contraction among different species

<table>
<thead>
<tr>
<th>Species</th>
<th>( R_{m} ) M( \Omega )</th>
<th>AHP(_{m} ) ms</th>
<th>( F_{\text{min}} )</th>
<th>( F_{\text{max}} )</th>
<th>( f-I ) slope, Hz/nA</th>
<th>Twitch Contraction Time, ms</th>
<th>Twitch Duration, ms</th>
<th>MN ( \varphi ), ( \mu m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>NA</td>
<td>148 (a)</td>
<td>5.4 (b)</td>
<td>NA</td>
<td>56 (a)</td>
<td>131 (a)</td>
<td>62 (c)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>132 (b)</td>
<td>8.4 (d)</td>
<td>7.8 (e)</td>
<td>61 (b)</td>
<td>104 (b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>0.9 (j)</td>
<td>83 (j)</td>
<td>15.0 (o)</td>
<td>54–218 (o)</td>
<td>35 (k)</td>
<td>80 (p)</td>
<td>53 (c)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.1 (k)</td>
<td>79 (k)</td>
<td></td>
<td></td>
<td>31 (l)</td>
<td>100 (i)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.2 (l)</td>
<td>52 (l)</td>
<td></td>
<td></td>
<td>30 (p)</td>
<td>158 (r)</td>
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<td></td>
<td>105 (m)</td>
<td></td>
<td></td>
<td></td>
<td>43 (q)</td>
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<tr>
<td></td>
<td>77 (n)</td>
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<td></td>
<td>31 (l)</td>
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<tr>
<td></td>
<td>82 (dd)</td>
<td></td>
<td></td>
<td></td>
<td>41 (r)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Rat</td>
<td>2.2 (s)</td>
<td>59 (x)</td>
<td>24.0 (t)</td>
<td>104 (v)</td>
<td>17 (x)</td>
<td>63 (x)</td>
<td>35 (c)</td>
<td></td>
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<tr>
<td></td>
<td>2.2 (t)</td>
<td>50 (u)</td>
<td>35.0 (v)</td>
<td></td>
<td>14 (s)</td>
<td>56 (s)</td>
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<tr>
<td></td>
<td>49 (s)</td>
<td></td>
<td>6.5 (v)</td>
<td></td>
<td>16 (u)</td>
<td>63 (s)</td>
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<tr>
<td></td>
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<td>16.0 (t)</td>
<td></td>
<td></td>
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<tr>
<td>Mouse</td>
<td>3.1 (ee)</td>
<td>32 (aa)</td>
<td></td>
<td>&gt;300 (ff)</td>
<td>8.7 (bb)</td>
<td>32 (cc)</td>
<td>27 (c)</td>
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<tr>
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<td>2.4 (ff)</td>
<td>46 (ee)</td>
<td>24 (ff)</td>
<td>13.0 (ff)</td>
<td>6.9 (cc)</td>
<td>47 (ff)</td>
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<td>55 (ff)</td>
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</tbody>
</table>

All values are obtained for mixed lower limb muscles (with slow and fast units; values from the soleus muscle are not quoted), with the exception of the first interosseous muscle in humans (Gossen et al. 2003; Spiegel et al. 1996). Numbers in italics reflect that they are estimated from records or graphs in the cited reference, rather than specifically given in the text or in tables. The letters in parentheses refer to the references listed later in this footnote. Boldface entries (numbers and letters) refer to this study. The duration of the AHP in humans was estimated from the “interval death rate transform” of motor unit discharge ISI histograms (MacDonell et al. 2007; Matthews 1996; Powers and Binder 2000). The \( F_{\text{max}} \) is usually not given with absolute numbers, but are extrapolated from the text or illustrations in the cited reference. No references are given for \( F_{\text{max}} \) values in humans; the range from a large number of publications is large, partly depending on the difficulties in following single motor units during maximal contraction. Usually the cited estimates range from 20 to 40 Hz. The size of the motoneurons has been measured/estimated in many ways. Here we have given only a single reference, with the strength that all estimates (for all species summarized here and many others as well) were obtained in the same way (Hardesty 1902). Cited references: (a) Gossen et al. (2003); (b) MacDonell et al. (2008); (c) Hardesty (1902); (d) Van Cutsen et al. (1997); (e) Spiegel et al. (1996); (f) Oda et al. (2007a); (g) Oda et al. (2007b); (h) Vandervoort et al. (1983); (i) Gordon and Phillips (1953); (j) Zengel et al. (1985); (k) Hultborn et al. (1988); (l) Dum and Kennedy (1980); (m) Hammarberg and Kellerth (1975); (n) Kornell (1965a); (o) Kornell (1965c); (p) Burke (1967); (q) Emonet-Denand et al. (1988); (r) Denny-Brown (1929); (s) Bakels and Kornell (1993a); (t) Button et al. (2006); (u) Gardiner and Kornell (1990); (v) Button et al. (2008); (w) Granit et al. (1963); (x) Bakels and Kornell (1993b); (y) McPhedran et al. (1965); (z) Close (1967); (aa) Huizari et al. (1975); (bb) Bateson and Parry (1983); (cc) Luff (1981); (dd) Eccles et al. (1958); (ee) Manual et al. (2009); (ff) present study; values are the same as those given in Table 1.

The AHP–firing rate relationship: are the lowest firing frequencies in individual motoneurons matched with their AHP duration?

The original observations of a strong correlation between AHP duration and minimum firing frequency stem from the work in the cat by Kornell (1965c); see also Wienecke et al. 2009. The literature regarding motoneurons in the cat, rat, and turtle spinal cord all suggest that the firing rate is strongly influenced by the conductances contributing to the slow component of the AHP that follows the spike potential (for critical reviews with reference to all original work, see Kornell 2006; Powers and Binder 2001; Stauﬀer et al. 2007). In the present study (Fig. 7) there is a trend for an AHP–\( F_{\text{min}} \) relationship with higher \( F_{\text{min}} \) (shorter ISIs) with shorter AHP durations. Despite the large scatter, likely depending on the difficulty to...
determine the minimal firing frequency, this relationship reached statistical significance ($P < 0.05$). However, the ranges of AHP durations (41–76 ms) and the $F_{\text{min}}$ (14–41 Hz, corresponding to intervals of 24–73 ms) are certainly consistent.

The description of the relationship between the AHP and the f–I slope also originate from the work by Granit and Kernell (Granit et al. 1963; Kernell 1965a,b,c). Experiments in which the AHP amplitude was reduced by various blockers (application of 5-HT, or AHP K$^+$-conductance blockers) have indeed shown an associated increase of the f–I slope (Hounsgaard et al. 1988b; Hultborn et al. 2004; Zhang and Krnjević 1987a,b). The steeper f–I slopes in the mouse (present study) than those in other species (Table 2) may simply reflect that the total AHP current is smaller in the smaller-sized mouse motoneurons.

Altogether, the mouse motoneuron seems to be endowed with properties to sustain firing with frequencies higher than those seen for other species investigated previously. As discussed in the following section, the necessity for high firing rates may be related to the very fast contraction times of the innervated muscles.

**A comparison of motoneuron discharge properties and twitch contraction among different species**

It is well known that muscles are comprised of many different types of motor units and that the properties of the muscle units are matched with the motoneurons that innervate them (see Burke 1981). Eccles et al. (1958) described (in the cat) that slow and fast muscles were innervated by “tonic” and “phasic” motoneurons, respectively. The tonic motoneurons were found to have longer-lasting AHPs than the phasic motoneurons, thus being matched to the contraction times of the fast and slow muscles. Later, Kernell (1965a) described a strict correlation between the duration of the AHP and the $F_{\text{min}}$ across the spectrum of fast and slow motor units. Those studies focused on differences between fast and slow muscles/motor units within the same species. In this section we will discuss the information regarding the relationship between AHP duration, minimum firing frequency, and twitch duration across species—from mouse to man.

A number of studies reported by A. V. Hill have focused on the relationship between the size of an animal and the speed of muscle contraction (summarized in Hill 1950). One of the characteristics of movements (such as locomotion) in smaller animals is the rapidity compared with those of larger animals that would require considerable differences in muscle contraction speed/contraction time. In Table 2 we have summarized a number of parameters for the motoneurons ($R_m$, AHP, $F_{\text{min}}$, $F_{\text{max}}$, f–I slope, and the diameter of the largest motoneurons), as well as the muscle (units) (twitch CT and twitch duration) for the mouse, rat, cat, and human. The isometric contraction time (time to peak) and twitch duration indeed differ substantially, with the shortest times in the mouse and the longest in humans (mice < rats < cats < humans; for references see Table 2). Consequently, the firing rates needed for force gradation by summation of muscle unit twitches are the lowest for humans and the highest for mice. Our present findings (Table 1) are in accordance with this prediction. The shorter duration of the AHP as well as the steeper f–I slope in the mouse than those in the other species support the view of the AHP as a major factor in the control of motoneuronal discharge properties.

In Fig. 8 we illustrate the pivotal relationship between the duration of the AHP and the muscle twitch (contraction time as well as twitch duration) across the species. Using the median from several studies (specified in Table 2) there is indeed a striking relationship, suggesting that the relationship between muscle twitch and AHP duration exists for slow and fast muscles not only within the same species, but also between species.

Even though the results quoted in Table 2 all relate to “mixed” (fast and slow) hindlimb muscle (with one exception, the human first dorsal interosseus), there are several difficulties in finding comparable data. In some material the motor units are classified as fast, intermediate, and slow. In other publications (as in our present study) there was no grouping in fast and slow units. In most publications the sampling method of motoneurons would favor “fast” motoneurons. For Table 2 we have focused on the mean values for mixed hindlimb muscles (values for the slow soleus muscle have not been quoted; see further in the legend of Table 2). The AHP values for human subjects are estimated from the “interval death rate transform” of motor unit discharge ISI histograms (MacDonell et al. 2007; Matthews 1996; Powers and Binder 2000). Since the data have originated from several studies with different techniques there is indeed a large variation and thus we have also indicated the range of values in Fig. 8 (quoted in Table 2).

**Conclusions**

This adult intracellular in vivo model is a feasible model in which to study the basic circuitry of the spinal cord as well as the intrinsic properties in spinal motoneurons of adult mice. This makes this model viable for investigating such features in transgenic mice in which the development of normal circuitry has been interrupted or in which the intrinsic properties of neurons may be altered. The present data demonstrate that mouse spinal motoneurons share many of the same properties that have been demonstrated previously for cat, rat, and human motoneurons. The shorter AHP duration, steeper f–I slopes, and higher $F_{\text{min}}$ and $F_{\text{max}}$ values than those in rats, cats, and humans are likely to be tailored to the characteristics of the mouse muscle contraction properties.

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