Characterization of motor units in behaving adult mice shows a wide primary range

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A recent study, however, suggested that mice might use a strategy of force recruitment very different from that observed in humans (Manuel and Heckman 2011). Using intracellular recordings of motoneurons in anesthetized mice in vivo, they showed that motoneuron firing rates jumped to very high levels (>30 Hz) as input current was graded. These rates were near to full tetanic force for motor units, such that further increases in firing rates by the motoneuron produced only minimal additional force. Low firing rates could only be observed within a “subprimary” range, in which firing rates of motor units were highly variable and not well related to the input current. These observations suggested that, in contrast to humans, mice might primarily rely on motor unit recruitment rather than frequency modulation for grading force output.

Although those experiments were performed in vivo without isolating spinal circuitry from descending systems, the use of anesthetics in those experiments might have significantly altered the processing of inputs by motoneurons. Anesthesia suppresses the neuromodulatory input from the brain stem that controls motoneuron intrinsic excitability and is essential for normal input-output processing (Heckman and Enoka 2012). In addition, normal synaptic input is much noisier than the injected currents used to generate firing by Manuel et al. (Manuel and Heckman 2011).

To evaluate the role of rate modulation in motor output during natural behaviors in mice, we developed techniques for recording the activity of motor units in mice during natural behavior. Although motor unit activity has been recorded in several animal models (Gorassini et al. 2000; Hoffer et al. 1987; O’Donovan et al. 1983), no studies have recorded motor units in behaving mice, presumably because of their small size. The ability to record motor unit activity during behavior in mice would be broadly significant because of the increasing use of mice as models of neurodegenerative diseases and for studying the functional consequences of various genetic manipulations. Using modifications of existing techniques for motor unit recordings in other species, we show here that it is possible to make reliable recordings of single motor units during quiescent behaviors in awake mice. These techniques allowed us to examine the strategies for graded motor output in mice. We show rate modulation across a wide range of firing rates and conclude that the strategies suggested by intracellular
motoneuron recordings were not observed in intact, unanesthe-
tized mice during natural behavior.

Some of these results were previously published in an
abstract at the 2012 Society for Neuroscience conference.
(Tysse1ing et al. 2012).

MATERIALS AND METHODS

Single-motor unit and gross electromyographic (EMG) recordings
were performed on 15 female CD-1 mice aged between 65 and 80
days. Fine-wire recording electrodes were percutaneously implanted
into the lateral gastrocnemius (LG) muscle of either the left or right
hindlimb, and mice were allowed to behave normally during all
recordings. This acute, percutaneous design worked very well for
quantifying motoneuronal activity in quiet stance. The method is fast,
does not require extensive surgeries, and is virtually pain-free for the
mice. Because we focused on quiet stance, very few motor units were
active at one time, giving us good isolation of single units.

Although recordings were performed in all 15 mice, good motor
unit activity meeting our selection criteria (detailed below) was only
found in 8 of the 15 mice. From those 8 mice, 28 different single
motor units were analyzed, with 13 of those units having accompa-
nying gross EMG data. All animal procedures were approved by the
Northwestern University Animal Care and Usage Committee.

Electrode design. Single-motor unit and gross EMG recording
electrodes were constructed with dual-core nylon-coated nickel/chro-
mium wire in a duplex configuration (California Fine Wire, material
no. 100189) for differential recordings. The diameter of each wire was
25 μm for single-motor unit recordings and 50 μm for the gross EMG
electrodes. Ground electrodes were constructed with single-core stain-
less steel 0.002-in.-diameter wire with a Teflon coating (A-M Sys-
tems, product no. 790700) (Fig. 1).

To construct the single-motor unit recording electrodes, an ~12-in.
segment of the 50-μm-diameter dual-core wire was cut and threaded
through a 30½-gauge hypodermic needle. With a scalpel blade, the
two wires were cut and the insulation was stripped so that there were
two 0.5-mm recording surfaces roughly 2 mm apart (Fig. 1B). Both
ends were hooked just as with the single-motor unit electrodes to
secure the electrode in the muscle. The opposite end of the dual-core
wire was separated and soldered to pins for connecting to the ampli-
fiers. The impedances for gross EMG electrodes were typically
between 30 and 150 kΩ.

Ground electrodes were made by cutting ~12 in. of the single-core
stainless steel wire that was then threaded through a 30½-gauge
hypodermic needle. At the recording end of the electrode, insulation
was carefully stripped with a sharp scalpel blade to expose roughly 0.5
in. of wire. This exposed wire was then bent to form a hook.

Implantation and data collection. For electrode implantation during
experiments, mice were anesthetized with isoflurane (2–3%) and
placed on a heating pad. The area of implantation on the hindlimb was
shaved, and both the fine-wire gross and single-motor unit recording
electrodes were percutaneously implanted in LG. The single-motor
unit electrode was inserted such that the line connecting the two
recording surfaces would be oriented perpendicular to the direction
of the muscle fibers (Andreassen and Rosenfeld 1978). The gross electrode
was inserted prior to the single-motor unit electrode in order to
minimize possible damage to the finer, more fragile single-motor unit
electrode. The ground electrode was implanted subcutaneously on the
back. All hypodermic insertion needles were pulled back and
secured at the far end of the electrode with a small amount of tape.
Recordings were taken once the animal had fully awakened and was
mobile, at least 5–10 min after discontinuation of isoflurane, and
continued until either the electrode was displaced by the animal or
sufficient data were collected (typically 15–30 min).

Animals were placed in a small closed-off area. A Mouse Igloo
(Bio-Serv) enclosure was placed in the area to encourage the animals
to rest quietly during recordings. We found that isolation of motor
units was very difficult during vigorous behaviors, such as locomotion,
because of interference with other motor units recorded on the
same electrode. We therefore focused on motor unit activity during
postural maintenance during rest. We recorded periods of spontaneous
activity in single motor units during this postural maintenance. In
some cases when units were not well isolated or when we had
recorded from a particular unit for an extended period of time, we
gently tugged the implanted electrode in order to move the electrode
within the muscle.

Signals from both the single-motor unit electrode and the gross
EMG electrode were recorded differentially. Single-motor unit activity
passed first through a differential amplifier (World Precision
Instruments, DAM-50-H) placed close to the animal that amplified
100× and filtered between 300 Hz and 10 kHz. Single-motor unit
activity was then passed through a second differential amplifier (A-M
Systems, 1700), where it was again amplified 100× and filtered
between 100 Hz and 5 kHz. Gross EMG was amplified (A-M
Systems, 1700; 10,000×), filtered between 100 Hz and 5 kHz, and
stored on a single channel. Both the single-motor unit and gross EMG
signals were digitized to the computer (PCI-6229, 16-bit, National
Instruments) and sampled at 20 kHz. National Instruments LabVIEW
software was used to visualize, record, and store all EMG signals.
Recordings were taken in roughly 2-min segments, with up to 10
recordings done per session for intervals of time ranging

Data analysis. All data analysis was performed with custom-
designed routines in MATLAB. The single-motor unit activity was
manually screened for areas of at least 5 s of continuous motor unit
activity with good isolation. Candidate waveforms were identified by
voltage threshold crossings, and the extracted waveforms were visu-
alized. The waveforms of potentially well-isolated units were then
characterized according to several features so that clustering could be
performed. Clustering was performed over windows of time ranging

Fig. 1. Schemata of single-motor unit and gross EMG recording electrode
designs. A: diagram of the recording surface for the single-motor unit recording
electrode showing the configuration of the dual-core wire. Wire was cut to
achieve the smallest possible recording surface, each measuring 25 μm. B:
diagram of the design for the gross EMG recording electrode. The 2
recording surfaces were each 50 μm in diameter by 0.5 mm in length and were
separated by 2 mm.
anywhere from 3 to 50 s, with typical windows between 5 and 10 s long. The duration of the window was chosen in order to be able to continuously track the activity of single units over time, even in the presence of slow drifts in the shape of the recorded waveform. Windows overlapped by 0.5–1 s to ensure that all instances of a unit were accounted for. Single motor units were first clustered on the basis of maximum and minimum voltages. Initial clustering was further refined by examining individual waveforms, interspike intervals (ISIs), and other waveform features such as time to largest peak, time to largest valley, and the first and second principal components. Any individual waveforms that were ambiguously identified on the basis of features were examined subsequently by the experimenter. Once all instances of a unit in a given window of data were found and clustered, the individual clustering windows described above were combined into one consolidated stretch of data, giving the spike times for a single recorded motor unit. In most cases, only a single, well-isolated motor unit was identified from each recording in order to avoid confusion in identifying waveforms during near-synchronous activity.

In addition to the close inspection of isolated waveforms during the process of clustering waveforms described above, we also performed a quantitative analysis of clustering quality. For each window used to cluster action potentials, we calculated the $L_{\text{ratio}}$ as described elsewhere (Schmitzer-Torbert et al. 2005; Schmitzer-Torbert and Redish 2004). Briefly, the $L_{\text{ratio}}$ assesses cluster separation as the probability that each unclustered spike should actually be included in a given cluster. $L_{\text{ratio}}$ values below 0.05 have been interpreted as indicating good cluster separation in previous studies (Anikeeva et al. 2012; Schmitzer-Torbert and Redish 2004). The $L_{\text{ratio}}$ value for each window was calculated with the first two principal components of each extracted waveform. In a small number of clustering cases (4) two large but distinguishable units were simultaneously active. In these cases, separation was achieved by individual waveform examination, allowing for the possibility of superpositions of waveforms. Since such superpositions obscure the parameters identified by principal component analysis, for these cases we assessed the quality of cluster separation using minimum and maximum voltages.

To characterize the firing behavior of a motoneuron, we first found the firing rate distribution of each unit. ISIs were calculated and used to find instantaneous firing rates. Average firing frequency for each unit was taken as the average instantaneous firing rate. Any ISI larger than 500 ms was considered a stop in firing and was not included in the calculation of average firing frequency (Hennig and Lomo 1985).

A second analysis of firing frequency was performed to characterize the lowest firing rate at which each motor unit could fire tonically. Periods of firing were first inspected visually to identify periods of at least 5 s over which the firing rate of neurons appeared not to vary substantially. For those units with simultaneous EMG recordings, we also examined EMG activity in the same periods, excluding periods with obvious transient changes in EMG levels. To confirm that the motoneuron firing rate did not change systematically over the identified period, we then calculated the correlation between the motoneuron firing rate and time over the period. Only periods for which there was no significant correlation ($P < 0.05$) were included in further analyses. For each unit, we performed the above analysis repeatedly, searching for the lowest tonic firing rate observed over a 5-s period. It is unlikely, however, that we were able to catch, in a given recording session, the absolute lowest rate possible for a motoneuron. Our analysis therefore most likely yielded an overestimation of the lowest firing rate. To examine whether motoneuron firing variability changed with firing rate, we also found the highest tonic firing frequency over a 5-s window, using the procedure and criteria just described. To guarantee that there was a substantial difference in firing rate between the low- and high-firing rate periods, we only included a high-firing rate period for a unit if the rate was at least one standard deviation (as calculated from the period of lower firing rate) above the low tonic frequency. Because of this condition, units for which a small range of firing rates was observed might not have both a high and a low firing rate. We then calculated the coefficient of variation (CV) in instantaneous firing rate over each 5-s period. The relationship between change in firing rate and CV was then assessed with a two-tailed Student’s t-test comparing the CV for high and low firing rates ($\alpha = 0.05$) and a Pearson correlation coefficient between CV and firing rate ($\alpha = 0.05$). Of note, we considered that our use of the CV as a measure of firing regularity is potentially flawed because it does not take into consideration the temporal relationship between ISIs. Previous work has tried to deal with this limitation by calculating the differences between consecutive ISIs ($\Delta$ISI) and using the interquartile range of the resulting distribution to determine the regularity of motor unit firing (Eken et al. 2008). However, we determined that this technique contained a bias when used across a large range of frequencies (by way of a test using a Poisson random variable) and thus was not applicable for comparing regularity of firing at high versus low frequencies.

Of the 28 units analyzed, 13 had simultaneous gross EMG data. For these 13 units, we analyzed the relationship between gross EMG activity and single-unit firing frequency. Raw gross EMG was high-pass filtered (2nd-order Butterworth, high-pass cutoff 50 Hz) to remove movement artifact and then rectified. Both the average level of gross EMG activity and average single-motor unit firing frequency were calculated over corresponding, nonoverlapping 500-ms windows during periods of tonic activity in unit firing. Correlation between EMGs and unit firing rates was assessed with the Pearson correlation coefficient and a one-tailed Student’s t-test with $\alpha = 0.05$.

Occasionally high-amplitude phasic events were seen in the gross EMG during tonic motor unit firing. Eken et al. reported similar observations in their study of spontaneous EMG activity in conscious unrestrained rats (Eken et al. 2008). They noted that these events were sometimes present at the initiation and termination of motor unit firing and during almost undetectable startle responses and postural adjustments. Although it is difficult during these high-amplitude phasic events to describe system input, they were not excluded from our analysis as these bursts of gross EMG activity help characterize mouse motor units across a range of motor output. Only cases for which we were able to maintain good unit isolation during these transients were included in the analyses described here. We also repeated the analyses between unit firing and EMGs after removing these events and found that the results described here were not substantially altered.

Isolation of single-motor unit waveforms during resting postures in mice. Figure 2A shows an example of activity in a single LG motor unit recorded along with gross EMG in LG. As can be seen in the figure, this unit was well isolated, with waveform amplitudes much larger than the background noise level and waveforms clearly distinguishable from other units. Note also the tonic firing of this unit across the recording window. Similar periods of tonic activity were used for analyses described below. The good isolation of this unit was confirmed by examination of extracted waveforms (Fig. 2B), showing the consistency of the recorded waveform during this window of activity and its separation from background noise. Figure 2C shows the values of the first two principal components calculated for the waveforms in Fig. 2B, illustrating again the good separation between the identified unit and background noise.

We quantified the isolation of single motor units with the $L_{\text{ratio}}$ (see above). The $L_{\text{ratio}}$ for the unit illustrated in Fig. 2 was $5.49 \times 10^{-12}$. The distribution of $L_{\text{ratio}}$ observed for the single motor units analyzed in this study is shown in Fig. 3. Note that the $L_{\text{ratio}}$ was calculated for each window over which single-unit waveforms were clustered, so an individual unit could contribute multiple $L_{\text{ratio}}$ values in this histogram. As seen in the histogram, the majority of clusters obtained had very small $L_{\text{ratio}}$ values, confirming that the units analyzed in this study were well-isolated single motor units.
RESULTS

Mouse motor units display wide range of firing frequencies during behavior. We obtained tonic firing data from 28 different motor units in the LG muscle of adult mice. Although the mice were free to move during recording sessions, they primarily remained in the enclosure during these recordings. An example of a typical distribution of instantaneous firing rates for a single motor unit observed during behavior is shown in Fig. 4A. The average firing frequency for this motor unit was 21 Hz. Figure 4B shows the average firing frequencies obtained from the 28 motor units. Note that these firing rates were calculated over all periods of activity for each individual motor unit. Figure 4C shows the histogram of these average firing frequencies; motor units are ordered in the figure from lowest to highest average firing frequency (9–68 Hz) to show the entire range of observed firing rates. Note that we observed both units with low firing rates and units with high rates, clearly demonstrating the existence of a broad range of rate modulation with units. Each of the ranges of 10–20 Hz, 20–30 Hz, 30–40 Hz, and 40–50 Hz were well represented in our sample, with one unit <10 Hz and one unit >60 Hz. Further examination of the recordings for this high-firing unit confirmed that it was well isolated throughout the recording. We also observed other units with lower firing rates from the same animal in the same recording session.

Comparison to previously published firing behavior evoked by intracellular current injection. The range of firing rates observed during quiet standing in the mouse is remarkably wide and appears to exceed that of larger animals (rats, cats, humans; see DISCUSSION). The previous intracellular studies showed that the lowest transition frequency from the subprimary to the primary range occurred in the slowest units and was ~30 Hz (Manuel and Heckman 2011). Because it is likely that quiet standing strongly recruits slow, low-threshold units (see DISCUSSION), the wide range of 10–70 Hz would be ex-
pected to require utilization of both the subprimary and primary ranges.

In the intracellular studies, the transition to the primary range is associated with a sharp drop in variance (this transition varied with motor unit contraction speed, from a minimum of 30 Hz in the slowest units to a maximum of 70 Hz in the fastest) (Manuel and Heckman 2011). To determine whether a transition in variance occurred in the motor unit firing patterns, we first examined the lowest rate at which the motor units were capable of firing tonically for periods of at least 5 s. In this analysis, 24 of the 28 units had 5 s over which the firing rate did not systematically vary with time (Pearson correlation coefficient between firing rate and time, $P > 0.05$). Figure 4D shows the range of these lowest tonic firing rates for each unit. The majority of recorded units (14 of 24) were capable of firing at rates lower than 30 Hz (horizontal dashed line), showing that low firing rates were commonly used by mouse motoneurons.

We then examined the relationship between motoneuron firing rate and EMG activity. EMG activity reflects the intensity of a motor command and thus provides an indirect estimate of the overall synaptic input to a motor pool. Gross EMG was recorded simultaneously with single-motor unit firing for 13 units. We found that the firing rate of 8 of the 13 single units had positive correlations to the rectified gross EMG. Two examples of units with positive correlations are shown in Fig. 5. Good correlations could be observed for units with both low (Fig. 5A; almost the full range is $<30$ Hz) and high (Fig. 5B; mostly $>30$ Hz) firing rates. No evident transition in the variability of firing was evident in any of the eight units with positive rate vs. EMG correlations. To examine this issue further, we calculated the CV of single-motor unit firing during periods of tonic firing. Consistent with the results shown in Fig. 5, we found no significant correlation between firing rate and variability [neurons with lower tonic firing rates were not associated with higher variability in firing ($r = 0.0033$, $P > 0.05$)] (Fig. 6A). We examined this same issue in individual motor units, comparing the variability of firing in a motor unit during its period of lowest tonic firing to the variability of firing during its period of highest tonic firing. We found no tendency for lower firing rates to be associated with higher variability in individual motor units. ($r = -0.1115$, $P > 0.05$) (Fig. 6B). Taken together, these results suggest that low firing

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Fig. 4. Mouse motor units have a wide distribution of average firing frequencies. A: histogram showing the instantaneous firing rate distribution for a motor unit with a low average firing frequency (21 Hz). B: average firing frequency, ordered from lowest to highest (9–68 Hz), calculated for each of 28 motor units. For each motor unit, the average was calculated across all firing. Bars show SD. C: histogram of average firing frequencies over the same 28 motor units. D: distribution of lowest tonic firing frequencies for each motor unit over a 5-s window. Dashed line indicates 30 Hz, below which motor units are expected to be in the subprimary range. More than half of the motor units had low tonic firing rates below 30 Hz. Motor units are displayed in ascending order of lowest tonic frequency.
rates were not associated with the high variance that is characteristic of the subprimary range. Thus the systematic occurrence of low firing rates in quiet standing in the mouse probably involves effects of synaptic noise and neuromodulatory inputs.

The intracellular study showed that the transition frequency from subprimary to primary range generated \(90\%\) of maximum force in all motor units (Manuel and Heckman 2011). As a result, this transition frequency varied from 30 to 70 Hz and was tightly correlated with motor unit contraction speed. If the units in the present study were predominantly slow, then units with firing rates above 30–40 Hz would exceed their tetanic maximum. Figure 4 shows that 16 of the 28 units recorded (\(57\%\)) had firing rates that averaged 30 Hz or above and 9 units (\(32\%\)) had average firing rates at 40 Hz or above. Moreover, the standard deviations of the average firing rates in Fig. 4 exceeded 40 Hz in 17 units (\(60\%\)), indicating that well over half the sample of units spent at least a portion of their activity at firing rates that exceed tetanic maximum for slow-twitch motor units. As force generation above tetanic maximum would be energetically inefficient, this result suggests that quiet standing in the mouse involves the recruitment of fast motor units, presumably the fast fatigue-resistant category.

**DISCUSSION**

This study presents data characterizing the activity of single motor units in conscious freely behaving mice. While motor unit studies have been performed in numerous species to date (Heckman and Enoka 2012), the development of transgenic mouse models of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) motivates an increase in motor unit studies in mice (Herron and Miles 2012). Briefly, our findings show rate modulation across a wide range of firing rates that is inconsistent with predictions from previous studies in anesthetized preparations.

**Applicability of fine-wire percutaneous electrodes to record single-motor unit activity in mice.** This study used percutaneous electrodes to record single-motor unit activity in mice. The advantage of a percutaneous electrode over an implantable electrode is that minimal surgery and recovery time are required. Additionally, the percutaneous electrode can be adjusted to sample from a different set of muscle fibers, allowing the recording of multiple single motor units with only one insertion. This technique is commonly used to record single-motor unit activity in humans with much success (Duchateau and Enoka 2011). However, there is a considerable difference in the size of mouse versus human muscles; therefore, use of this technique in mice requires careful consideration of the selectivity of the electrodes. Also, the particular quiet stance...
behavior targeted in this study is well suited to the percutaneous technique since very few motor units are active at once. As evidenced by waveform inspection (e.g., Fig. 2) and by the low $I_{\text{ratio}}$ values (Figs. 2 and 3), we were able to obtain well-isolated single-motor unit recordings using this approach.

We note that there may be some limitations to the types of muscles in which this technique can be applied for the mouse. Without surgically visualizing a muscle, it is more difficult to determine that the electrode has been successfully implanted. In the experiments described here, we examined units in a large muscle close to the skin surface, in order to minimize these concerns. We confirmed the appropriate placement of our electrodes in pilot experiments in cadavers, confirming that electrodes were placed in the targeted muscle. While it would be more difficult to apply this technique to smaller and more deeply located muscles, for studies of larger muscles it is a convenient alternative to chronic surgical techniques.

**Mouse motor units in comparison to other species.** In our study of mouse motor units during quiet standing, we observed a wider range of tonic firing frequencies (average values ranged from 9 to 68 Hz) than seen in other species during similar behavior. Eken (1998) reported median firing frequencies in the range of 16–25 Hz for tonically active motor units in the soleus muscle of rats. This was similar to results seen by Hennig et al. (Hennig and Lomo 1985). Higher rates have been seen in the LG muscle of the rat but only during locomotion (Gorassini et al. 1999). Cats during locomotion have been reported to have motor unit firing of the anterior thigh only in the range of 10–30 Hz (Hoffer et al. 1987). During slow ramp contractions, human motor units from tibialis anterior, the hand, and soleus were found to start firing at as low as 5 Hz but reached maximums of around only 30 Hz (Desmedt and Godaux 1977; Moritz et al. 2005; Oya et al. 2009). In ballistic contractions, human motor units from tibialis anterior, the hand, and biceps brachii muscles vary only from 8 to 15 Hz (Gorassini et al. 2002; Mottam et al. 2005). Similar to the results from slow ramp contractions, low-threshold single motor units in human short toe extensors during walking at normal speeds were found to only have firing frequencies in the range of 12–25 Hz (Grimby 1984). It is clear that there are differences between mouse and human motor unit recordings. Nonetheless, we anticipate that the relative changes in mouse and human motor unit firing will be reasonably similar in disease states like spinal injury and ALS, but this is yet untested. In addition, understanding the characteristic of mouse motor units will allow the power of the genetic approaches available in the mouse to be brought to bear on understanding the distortions of these patterns.

Our recordings came from LG, a muscle containing a heterogeneous mix of muscle fiber types. However, it is likely that we only observed activity in fatigue-resistant units, since we limited our recordings to quiet stance and focused on tonic firing of units. Hennig et al. documented that FF-type fibers in the extensor digitorum longus were only active for ~1–2 s (Hennig and Lomo 1985), and Eken et al. noted only one abnormally high-firing motor unit in the soleus (~47 Hz) that was active for >8 s at a time (Eken 1998). Our high-frequency motor units were active for much longer than 8 s; therefore, the prolonged motor unit firing that we observed likely did not come from highly fatigable fast motor units.

**Comparison of conscious behavioral studies to intracellular experiments.** Previous studies in mice have focused primarily on intracellular recordings of motoneurons (Alstermark and Ogawa 2004; Carp et al. 2008; Iglesias et al. 2011; Manuel and Heckman 2012; Meehan et al. 2010b) or decerebrate preparations (Nakanishi and Whelan 2012), which provide valuable information but are limited by the lack of information from natural behaviors. In our study, quiet stance produced steady motor unit firing rates that varied over a remarkably wide range, from <10 to >60 Hz. Broadly speaking, the wide range of firing rates seen in quiet stance is consistent with the wide range of firing rates generated by current injection in low-threshold motoneurons during intracellular recordings (Manuel and Heckman 2011). The intracellular recordings showed that low-threshold motoneurons that innervate slow fibers generate near-tetanic forces at 30–40 Hz (Manuel and Heckman 2011). Thus units at the upper end of the firing range in our data were either at supratetanic levels where rate modulation has little impact or they were fast fatigue-resistant units, which probably generate near-tetanic forces at ~40–60 Hz (Manuel and Heckman 2011). Based on Burke’s classification of S (slow), FR (fast fatigue resistant), and FF (fast fatigable) (Burke 1981), it seems reasonable to suppose that quiet standing in the mouse involves not only S but also significant FR recruitment. Presumably the higher rates are not due to recruitment of FF units, which fatigue easily (Clark et al. 1993). Perhaps the relatively fast motor behavior of this small animal or the faster metabolism from the higher surface area to body volume requires more frequent activation of fast units than in larger animals. It would be interesting to study smaller animals and test whether this difference continues, but our experience suggests that we are near our technical limit for this method of recording. Another potential explanation for such a wide range of rates would be abnormal motor unit recruitment due to stress. It seems unlikely that the mice are under much stress as they are acclimatized to the environment and to handling, and also have enclosures during testing. Also, recent work indicates that discharge rate of motor units is not effected by stress (Stephenson and Maluf 2010).

Although transition from the subprimary range to the primary range in the intracellular data is clearly associated with a marked reduction in variance of firing, no such transition in variance was observed from low to high motor unit firing rates during conscious behavior. The average CV in our data was 0.35; in the study of Manuel and Heckman the CV was also ~0.3 in the subprimary range but in the primary range only 0.12 (unpublished data). One explanation for this lack of transition in variance is that the intracellular recordings were obtained in anesthetized adult mice. This state lacks neuromodulatory inputs both from the brain stem and within the spinal cord that likely characterize normal motor behaviors and synaptic noise (Heckman and Enoka 2012). An important effect of neuromodulatory input, especially that mediated by the serotoninergic and noradrenergic brainstem systems, is to facilitate persistent inward currents (PICs). It is likely that PICs occur in mouse motoneurons (Meehan et al. 2010a) just as in cats and rats (Bennett et al. 2001; Hamm et al. 2010; Lee and Heckman 1999). The Na component of the PIC (NaPIC) plays a fundamental role in spike generation (Kuo et al. 2006; Lee et al.
and Heckman 1999; Li and Bennett 2003). In fact, the presence of the subprimary range in the anesthetized state may reflect an abnormally low-amplitude NaPIC (Iglesias et al. 2011; Manuel et al. 2012). A larger NaPIC that would occur with normal neuromodulation in the intact mouse may extend the primary range downward in the intracellular experiments and altogether avoid a subprimary range. In addition, the presence of synaptic noise in normal behavior would significantly affect variability through the firing range and may further obscure the transition between primary and subprimary ranges. Thus the presence of a wide range of firing rates without any transition from high to low variability in our intact mouse data could readily be accounted for by both noise and neuromodulatory input in the synaptic drive.

In summary, the wide range of firing seen in mouse LG motor units during quiet stance probably has two basic mechanisms. The low frequencies arise from PICs and neuromodulation, potentially extending the primary range downward. The high frequencies may be accounted for by recruitment of FR motor units. The implications of this wide firing range for mouse models of disease states like ALS and spinal injury are not as yet clear, requiring motor unit measurements in a wide range of disease models.

REFERENCES


